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# Short-term Airway Clearance Management in people with stable Bronchiectasis



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Tesis Doctoral

# SHORT-TERM AIRWAY CLEARANCE MANAGEMENT IN PEOPLE WITH STABLE BRONCHIECTASIS


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**UNIVERSIDAD DE ZARAGOZA**
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# Short-term Airway Clearance Management in people with stable Bronchiectasis

**Doctoral Student**

Beatriz Herrero-Cortina

Universidad Zaragoza

*“I have no special talent.*

*I am only **passionately curious”***

*“No tengo ningún talento especial.*

*Sólo soy **apasionadamente curioso”***

*Albert Einstein*

# Acknowledgements

*Once upon a time*…la curiosidad despertó mi interés por el ámbito respiratorio al ser una disciplina escasamente abordada en mi formación de fisioterapia. A través del método PICO formulé mis primeras preguntas de investigación, ¿por qué la fisioterapia en España tiene menos protagonismo que otras disciplinas?; ¿la fisioterapia respiratoria es útil para pacientes con patologías respiratorias?...en realidad, se cumplían todos los criterios de selección para que mi primer estudio de investigación (con cuatro fases diferentes) pudiese comenzar: (i) excusa perfecta para conocer de primera mano cómo funcionaba la fisioterapia respiratoria en otros países; (ii) la poca información disponible sobre el tema fomentaba uno de mis factores de riesgo a la "adicción", el auto-aprendizaje; (iii) mi motivación por buscar lo inusual o poco corriente me animó a ir más allá de mis propios límites.

Mi reclutamiento fue sencillo, me enseñaron muy de cerca la importancia que tienen los pequeños detalles en la mejora de la calidad de vida de las personas con patología respiratoria crónica, y que, a pesar del alto nivel de incapacidad debido a la disnea y la expectoración crónica, [es lo que tiene un bronchiectasis-COPD overlap ; - ) ], siempre hay que ofrecer la mejor versión de uno mismo, cuidando los detalles, agradeciendo los cuidados y sin perder nunca la educación, ni la sonrisa. Gracias a ti, gracias a lo que me enseñaste, consigo alcanzar interacciones simbióticas con los pacientes que nos ayudan a avanzar de la mano[(1)].

La primera fase del estudio consistió en una prueba piloto en Chaumont-en-Vexin con acceso a pacientes respiratorios exacerbados, continuando con un acceso directo a la familia de Fiquis, tal y como ellos se denominan. De esta etapa, Elena, Beatriz, Enea, José Luis, Ana Cris, Irene (con

sus papis) y Álvaro fueron (y siguen siendo) outliers que me marcaron el camino hacia la resolución de preguntas, hacía la mejora del tratamiento y hacía las carcajadas aseguradas. ¿Cómo no iba a intentar ofreceros el mejor tratamiento posible? Así surgió la necesidad de incluir una intervención complementaria, que no estaba incluida en el protocolo, pero que aseguraba un efecto mínimo clínicamente relevante en los pacientes…

…Una formación especializada en fisioterapia respiratoria me confirmó que el camino que había elegido, seguía siendo el correcto. Esta formación me enseñó el poder del análisis crítico, la caducidad de los conceptos y, por tanto, la virtud del auto-conocimiento y la actualización constante. El "destino" hizo que el mismísimo Jordi Vilaró me asignara de forma aleatoria uno de los artículos que posteriormente más referenciaría a lo largo de mi trayectoria (Murray et al. Eur Respir J. 2009) y que el mismo día, Jordi nos informará de la existencia de una vacante de investigación en Barcelona (Hospital Clinic) relacionada con la temática del artículo. En otras palabras, en mi modelo lineal mixto incluí factores fijos tales como enfermedad huérfana e investigación en drenaje de secreciones (siendo ambos factores atípicos) y factores aleatorios como Barcelona y dejar a la familia Fiqui. En esta ocasión, la diferencia observada fue estadísticamente significativa a favor de los factores fijos, por lo que la decisión estaba tomada. Sin ti, Jordi, seguramente también habría realizado una tesis doctoral…pero no en bronquiectasias, no en esta compleja enfermedad donde lo que parece sencillo es complejo, y lo que parece que funciona en todos…, en realidad no se adapta a ellos, es decir, es la Queen disease for people who loves challenges ; - )

Como consecuencia de esta decisión, la segunda fase del estudio se desarrolló en Barcelona trabajando mano a mano con Eva y conseguimos generar, prácticamente de la nada, una novedosa línea de investigación enfocada en personas con bronquiectasias. Gracias Eva, me

guiaste de la mano al principio, pero enseguida me diste la total libertad para crear, diseñar e innovar; ingredientes esenciales para alimentar todavía más mi pasión. La presente tesis se engendró y maduró allí, siendo posible gracias al soporte del Dr. Torres, Medical Product Research (MPR) y Praxis Pharmaceutical. Además, de forma totalmente inesperada, una covariable se cruzó en mi camino; llego a mis manos un proyecto multicéntrico de una calidad metodológica que nunca había visto hasta ese momento. Lo comparé con mis trabajos previos (previsible…verdad, Elena?) y fue el complemento perfecto de esta gran adicción, no sólo investigar en drenaje de secreciones, si no intentar hacerlo con la mejor calidad metodológica posible. Finalmente, hay que reconocer que esta etapa no hubiera sido la misma sin mis aliadas italianas: Elena Prina y Marta Di Pasquale.

Quien me conoce, sabe que la sangre maña corre por mis venas y poder ver el Pilar cada mañana …no tiene grant. Así que, para comenzar la tercera fase del estudio, cogí mi billete de vuelta a casa. Eso sí, dejando en muy buenas manos los proyectos del Clinic (gracias Victoria por continuar con los proyectos). Fue una decisión con límites de concordancia muy alejados, ya que implicaba renunciar parcialmente a mi pasión a favor de los factores aleatorios. Y así fue, tan duro como la quinta rejection de un paper; sin embargo, me esperaban sorpresas estadísticamente significativas. Allí estaba Marta(2): el blanco, la letra A, el sí, el inicio, la luna creciente…es decir, la mejor versión de mi discrepancia, aquella en que los conflictos nunca vencen al crecimiento y el avance hacía una meta común (*How to improve the airway clearance management?*). Gracias por acompañarme a contracorriente durante toda esta etapa y me encantaría seguir contra-complementándonos durante la siguiente fase, ya que el Postdoc nos espera!. Por otro lado, hay que destacar la labor que han realizado el control de calidad durante esta tercera fase del estudio: (i) las lecciones de vida de Sara(3) y la importancia de las segundas oportunidades; (ii) el extraordinario iCardioRespi team formado por Marina(4),

Juanan y Marta[2], ya que gracias a ellos se ha conseguido una pequeña pero consolidada línea de investigación en fisioterapia cardiorrespiratoria muy enfocada a la práctica clínica en Zaragoza; (iii) los viejos reencuentros (Yas) y las nuevas incorporaciones (Rita) al equipo, ya que engrandecen el día a día; así como (iv) la última adquisición, la superheroína Almu[5], la antítesis de lo empírico y el fiel reflejo de la importancia de lo cualitativo frente a lo cuantitativo; Almu[5], contigo es imposible ir hacia el lado oscuro.

Finally, the fourth and last phase of the present study included my research stages. The first stage in Aveiro increased my knowledge about respiratory sounds, which is currently one of my preferred teaching topics. However, the best part was meeting incredible research girls, particularly Ana Oliveira, who helped me from the beginning in my little Portuguese adventure and still helps me today. It is a really pleasure work with you, Ana! After a wash-out period, I started my second research stage (Universidad San Jorge and CAI-Inmaculada, thanks for your support). Although I had the opportunity to play with sputum samples…the main lesson that I taught was that you can´t control everything…and the real challenge is not trying to find answers or blame…is to control the way you respond to what´s happening.

Overall, the main limitation of this study is my English skills; however, I have bet on my last challenge, being faithful to myself. Por otro lado, las fortalezas de este estudio son claras y concisas, y a continuación se describen más detalles sobre ellas:

- Todos y cada uno de las personas con bronquiectasias que han participado en esta tesis. Mi principal objetivo es buscar el método de drenaje de secreciones que mejor se adapte a vuestras necesidades clínicas, sin vosotros….esto no hubiera sido posible.

- Señorita Marta Almagro…un placer haber compartido la relación simbiótica más fructífera de mi carrera profesional contigo.

- The blinded assessors, yeahh…the peer reviewers of my manuscripts are also a strength point. You will never read this message (what a shame!), but thank you for your time, your questions, your discrepancies…You didn´t make it easy for me…but It has been the process in which I learned the most. I would also like to thank the external examiners (Dr. Annemarie Lee and Dr. James Chalmers) of the doctoral thesis, their suggestions increased the quality of the thesis

- El equipazo de investigación externo, compuesto por Ane (diseñadora de estudios), Vic (organizadora de estudios) Elena (metodóloga de estudios) Marian (estadística de los estudios) y Ana (difusora de resultados). Con este equipo, la financiación está asegurada.

- Una familia incondicional (mami(6), papi(7), tato(8), Silvia y sobris(9)) qué, aunque no comprenden del todo mis decisiones, las apoya y respeta, así como me protege y cuida cuando más lo necesito.

- Mi banda de rock & roll formada por los *Pedorros* del Silos. Los cantantes y protagonistas de esta banda, Lorena(10) y Javichu(11), consiguen darle un sentido muy especial a mi carrera extra-académica, que claramente se refleja en momentos inolvidables con alto factor de impacto.

- Mi banda del patio de Fisioterapia, Maria N(12), Laura(13), María G, Alba, Gracey, Edu, con la que, independientemente del tiempo que pase, seguimos teniendo un coeficiente de correlación intraclase excelente.

- La música…y que mejor manera de expresar sentimientos…que dedicar unas cuentas canciones a la gente que más quiero, espero que os gusten! Gracias!

- Ella[14]

| Referencia/Reference | Mi pequeño mensaje / My little message |
|---|---|
| 1 – Adapted from "Ojos de gata". Los Secretos. 1991 | *Loca por verte despertar en tu dormitorio…esa noche tomé tu mano al amanecer todo tu reservorio…con el "quiero Inhalar" el aire y acunarme entre sus mantas…y soñó con mis ojos de gata, pero no recordó que yo aquí le esperaba…desperté con hipercapnia y busqué pero allí ya no estabas…me dijeron que él me esperó, porque me ausenté y me usó como cuartada…comentó por ahí que yo era una chavala lumbraria,* ***pero cómo explicar que me volví ejemplar al cuidarte en cada escenario****…* |
| 2 – Adapted from "Back to black". Amy Winehouse. 2006 | *There is no time to regret…kept your dreams alive…with your different new unsafe bet…you and you head high…and your hopes fresh…get on without any fear…you won´t back to what you were, so far removed from all that you went through, and you tread a troubled track, your odds are increased,* ***you´ll go back to white.*** |
| 3 – Adapted from "Ain´t no mountain high enough". Marvin Gaye and Tammi Terrell. 1966 | *Remember the day you trusted me…I told you, you could always count on me Sarah…from that day on, I made a vow…I´ll be there when you need me…with some ropes, some thermos…´cause Sarah* ***there ain´t no mountain high enough****…ain´t no valley low enough…ain´t no river wide enough…****there is nothing you cannot achieve****…* |
| 4 – Adapted from "Copenhage". Vetusta Morla. 2008 | Ella corría, nunca le enseñaron a andar…venció las luces pálidas…ella corría en la montaña y horas de más…ilusiones unas vienen, otras se van, igual que Alicia sin ciudad, el valor para atreverse, el coraje a llegar…llueve en Edinbra…la amistad enseña el camino hacia el mar…todos duermen ya…dejarse llevar, suena demasiado bien…jugar al azar…**nunca saber dónde puedes terminar…o empezar…** |
| 5 – Adapted from "La mujer de verde". Izal. 2012 | *Sé que ella quisiera regular…****sus superpoderes****…kineseando a los demás…la mujer de verde…se ha vuelto a poner el traje…para rescatarme…¿Qué sucederá cuando el bloqueo no rebote…y lo empírico sea más fuerte…y el chakra no sea tan fácil…ya testaremos nuestros planes!!!* |
| 6 – Adapted from "Te quiero, te quiero". Nino Bravo. 1970 | *De por qué me estás apoyando, no te exijas la razón…pues yo misma no me entiendo con mi propia decisión…al llegar la siguiente aventura, tu mente desesperada, buscará una explicación…yo quiero ser siempre fiel, a mi pasión e intuición…aunque falle una y otra vez…mi voz igual que un niño, te pide con cariño "ven a mí y abrázame"…porque te quiero, te quiero, te quiero, te quiero, te quiero, te quiero…****y hasta el fin te querré****…* |

| | |
|---|---|
| 7 – Adapted from "Feo, fuerte y formal". Loquillo. 2001 | *No vino aquí para hacer amigos…pero sabes que **siempre puedes contar con Santi**…dicen de él que es un tanto animal…pero en el fondo es un sentimental…en el helador de la noche, y a plena luz del día…siempre dispuesto para llevarte en Taxi…es un hombre de bien…a carta cabal…y como el duque: **F**eo, **F**uerte y **F**ormal* |
| 8 – Adapted from "El coche no". Def con dos. 1995 | *Pero ándate con ojo…no te equivoques…hagas lo que hagas…no borres mi tesis…¡no, no la tesis no!...¡no, no, la tesis no!...puedes robarme todos mis papers y ridiculizarme delante de mis alumnos…aprópiate de mis mejores resultados…hazlos tuyos **y vente a mi calvario**…* |
| 9 – Adapted from "Busca lo más vital". El libro de la selva. 1967 | *Donde quiera que vaya, donde quiera que estoy…os echo de menos, I really miss you…la tita Bea os aguarda así…porque hace mil que no la veis…y **las cosquillas os esperan si**…para haceros al menos cien…¡de una primera sentada!* |
| 10- Adapted from "Sweet Child O´Mine". Guns N´ Roses. 1987 | *She´s got eyes of the bluest skies, as if they thought of sea...I like to look into those eyes…and **see a solid friendship…our laughs reminds me of a warm safe place**…where as a child we´d play…and dream for the future…and present…to closely pass together by…oh, oh, oh sweet child o´hood…oh, oh,oh sweet friend of mine….* |
| 11 – Adapted from "It´s my life". Bon Jovi. 2000 | *This is for the ones who stood their ground…It´s for Javier Monguilan who never backed down…tomorrow´s getting harder, make no mistake…luck ain´t enough…you´ve got to achieve your own goals…It´s your life…and it´s now and forever…you just want to live while you´re alive…my friendship is as an open highway…like Peter said…I did it my way…* |
| 12 – Adapted from "Pequeña gran revolución". Izal. 2015 | *Que **nuestra complicidad, no desaparezca**… y crezca como lo hacen, ahora tus metas…las que te llevarán, tan lejos como quieras…emprendiendo la vida, con manual therapies…bienvenida Elena, pequeña gran revolución…que con tus pasos marcas, un nuevo rumbo en dirección…a nuevas aventuras, aunque parezcan menos altas…* |
| 13 – Adapted from "Golfa". Extremoduro. 1998 | *¿Y por qué no Cierra bares?... Porque no le da la gana…dice que si no **se froda**… dice que no siente nada…¿Y por qué no Cierra bares?...Porque no le da la gana…dice que si no **se froda**…dice que no siente nada…Si hace sol, nos acercamos a la barra, y por el camino, las calles se levantan, y ahí vamos, a romper las telarañas de la juventud…veras como se apartan…¡Laura! ¡Laura! ¡Laura!* |
| 14 – Adapted from "Your soung". Elton John. 1970 | *Anyway the thing is…what I really mean…you give the sweetest hugs…I´ve ever had…and you can tell everybody…this is my grandchild´s thesis…It may be quite simple but…now that It´s done…I hope you don´t mind…I hope you don´t mind…That I put down in words…**how wonderful life was…while you´re in the world…*** |

# Short-term airway clearance management in people with stable bronchiectasis

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# Preface

This dissertation is submitted for the degree of Doctor of Health and Sports Science at the University of Zaragoza. The research described herein was conducted under the supervision of Dr. Eva Polverino (Hospital Val d´Hebron, Barcelona, Spain) and Dr. Eva María Gómez Trullén (University of Zaragoza, Zaragoza, Spain) between October 2013 and October 2019.

During this period, two short-term international research stays was completed at the University of Aveiro, Portugal under the supervision of Dr. Alda Marques (from July 2014 to August 2014) and at the Royal Infirmary of Edinburgh, UK under the supervision of Prof. Adam Hill (from Abril 2016 to October 2016). Both research stays increased the knowledge of the PhD candidate in relation to the analysis and interpretation of computerised respiratory sounds and the potential use of biomarkers from sputum samples, respectively. The PhD candidate won a grant from Caja Ahorros de la Inmaculada (CAI) for her research stage in Edinburgh.

The thesis consists of a compilation of scientific publications co-authored by the PhD candidate in accordance with the PhD program guidelines of the University of Zaragoza.

**Study 1**

***Herrero-Cortina B**, Vilaró J, Marti D, Torres A, San Miguel-Pagola M, Alcaraz V, Polverino E. Short-term effects of three slow expiratory airway clearance techniques in patients with bronchiectasis: a randomised crossover trial. Physiotherapy. 2016;102(4):357-364. DOI: 10.1016/j.physio.2015.07.005*

*Impact Factor (2016): 3.010. Quartil (Rehabilitation): 1*

**Study 2**

***Herrero-Cortina B**, Alcaraz V, Vilaró J, Torres A, Polverino E. Impact of hypertonic saline solutions on sputum expectoration and their safety profile in patients with bronchiectasis: a randomised crossover trial. J Aerosol Med Pulm Drug Deliv. 2018; 31:281-289. DOI: 10.1089/jamp.2017.1443*

*Impact Factor (2018): 2.866. Quartil (Respiratory System): 2*

**Study 3**

***Herrero-Cortina B**, Alcaraz-Serrano V, Torres A, Polverino E. Reliability and minimal important difference of sputum weight in people with bronchiectasis. Respir Care. 2019. Accepted.*

*Impact Factor (2018) 1.736. Quartil (Respiratory System): 4*

**Study 4**

***Herrero-Cortina B**, Oliveira A, Polverino E, Gómez-Trullén EM, Torres A, Marques A. Feasibility of computerized adventitious respiratory sounds to assess the effects of airway clearance techniques in patients with bronchiectasis. Physiother Theory Pract. 2019; 23:1-11 DOI: 10.1080/09593985.2019.1566945.*

*Impact Factor (2018) 1.158. Quartil (Rehabilitation): 4*

**Narrative review**

***Herrero-Cortina B**, Lee AL, O'Neill B, Bradley J. Airway clearance techniques, pulmonary rehabilitation and physical activity. In: Chalmers JD, Polverino E, Aliberti S, eds. Bronchiectasis (ERS Monograph). Sheffield, European Respiratory Society, 2018; pp. 331–352. DOI: 10.1183/2312508X.10017017.*

Apart from the work done for the present thesis, the PhD candidate has also been principal investigator of different projects: (i) a multicentre project entitled “Resistive inspiratory manoeuvres as an airway clearance technique in people with cystic fibrosis: a randomised crossover trial (NTC02261987)” funded by Cystic Fibrosis Spanish Society; (ii) a long term project entitled “ Long-term effects of a home-based airway clearance programme in people with bronchiectasis: a randomised controlled trial (NCT02324855)”; (iii) and a psychometric project entitled “Psychometric properties of computerised respiratory sounds in people with bronchiectasis” both funded by the Spanish Pulmonology and Thoracic Surgery Society (SEPAR).

The PhD candidate has also participated in other ongoing research projects, most of them related to airway clearance, which have resulted in additional manuscripts and congress contributions that the candidate has co-authored during the pre-doctoral period (all of which are listed at the end of this thesis as an appendix).

# Abstract

**Background** Daily sputum expectoration is one of the most common respiratory symptoms in people with bronchiectasis. It is associated with poor health outcomes and negative impacts on social life. Although the use of airway clearance techniques (ACTs) and hyperosmolar agents is recommended to more easily manage chronic productive cough, the quality of evidence is still low-moderate and the level of recommendation ranges from weak to strong in bronchiectasis. However, there is a need to evaluate in depth the short-term effects of airway clearance therapeutic approach that are so far under-investigated in bronchiectasis in order to design optimal long-term future trials in this field. Therefore, the aims of this thesis were to investigate what is the short-term effectiveness of airway clearance therapeutic approaches in adults with clinically stable bronchiectasis and how to correctly interpret the clinical benefits observed after these interventions.

**Methods** Two randomised, three-way crossover trials were conducted to compare the short-term effectiveness of three slow-expiratory ACTs (autogenic drainage, slow-expiration with glottis opened in lateral posture [ELTGOL] and temporary positive expiratory pressure [TPEP]) and three inhaled saline solutions (hypertonic saline [HS], hyaluronic acid + HS [HA + HS] and isotonic saline [IS]) in people with bronchiectasis. Wet sputum weight during sessions was selected as the primary outcome in both trials. Moreover, an ad hoc analysis was performed using the sputum samples of both studies to evaluate the reliability of 24-hour sputum weight and the minimal important difference (MID) after short-term airway clearance sessions in bronchiectasis. Finally, a feasibility study was conducted to examine the potential use of computerised adventitious respiratory sounds (ARS) as an outcome measure to assess short-term effects of airway clearance sessions in bronchiectasis.

**Results** (1) Autogenic drainage and ELTGOL enhanced greater sputum expectoration compared to TPEP during sessions in individuals with stable bronchiectasis; the participants preferred autogenic drainage; (2) the HA + HS solution was as efficacious as HS solution and greater than IS in improving sputum expectoration, but with a better safety profile than HS in people with bronchiectasis. Thus, they selected it as the preferred solution; (3) the wet sputum weight was an acceptable reliable measure over 24 hours, but the level of agreement was slightly wide, particularly for greater expectoration levels. Moreover, a reduction of at least 6.4 g in the amount of sputum expectorated during the 24 hours following an airway clearance intervention, or a relative change of approximately -17% from baseline, was the estimated MID; (4) computerised ARS was a feasible outcome; the expiratory coarse crackles appeared to be the most appropriate outcome for use in future studies.

**Conclusion** The findings of this thesis highlight which short-term airway clearance approach are more efficacious in people with clinically stable bronchiectasis. Therefore, the next step is to design and conduct long-term trials to explore these airway clearance therapeutic approaches.

# Resumen

**Introducción** La expectoración crónica es uno de los síntomas respiratorios más prevalentes en personas con bronquiectasias y se asocia con un peor estado clínico y un fuerte impacto en la vida social. A pesar de que el uso de técnicas de drenaje de secreciones (DS) y agentes hiperosmolares es recomendado para facilitar el manejo diario de la tos productiva en pacientes con bronquiectasias, la calidad de su evidencia es todavía baja-moderada y su grado de recomendación varía de débil a fuerte. Por este motivo, se requiere evaluar en profundidad nuevos enfoques terapéuticos a corto plazo que faciliten el DS y que no hayan sido investigados hasta la fecha, para poder diseñar futuros ensayos clínicos a largo plazo con mayor nivel de garantía. Por consiguiente, el objetivo de esta tesis fue investigar que enfoque terapéutico a corto plazo facilita en mayor medida el DS en personas adultas diagnosticadas de bronquiectasias en periodo de estabilidad clínica, al igual que averiguar cómo interpretar correctamente los beneficios clínicos de estas intervenciones.

**Métodos** Dos ensayos clínicos aleatorizados y cruzados se llevaron a cabo para comparar la efectividad a corto plazo de tres técnicas espiratorias lentas de DS (drenaje autógeno, espiración lenta con glotis abierta en decúbito infralateral [ETLGOL] y presión espiratoria positiva temporal [TPEP]) y tres soluciones salinas para inhalar (suero hipertónico [SH], suero hipertónico con ácido hialurónico [SH+AH] y suero isotónico [SI]) en personas con bronquiectasias. El objetivo primario en ambos ensayos clínicos fue el peso húmedo de la cantidad de esputo expectorada durante las sesiones. Además, se realizó un análisis ad-hoc utilizando las muestras de esputo de ambos ensayos clínicos para examinar la fiabilidad de la cantidad de esputo durante 24 horas, así como la diferencia importante mínima (DIM)

después de una sesión de DS en bronquiectasias. Finalmente, se examinó mediante un estudio de viabilidad/factibilidad el potencial uso de los ruidos respiratorios adventicios analizados de forma computacional como herramienta de evaluación de los efectos a corto plazo del DS en pacientes con bronquiectasias.

**Resultados** (1) Las técnicas de drenaje autógeno y ELTGOL favorecieron en mayor grado la expectoración durante las sesiones que la técnica TPEP en personas con bronquiectasias en estabilidad clínica; siendo el drenaje autógeno la técnica preferida por los participantes; (2) la solución SH+AH fue tan efectiva como la solución de SH y mejor que la solución de SI facilitando la expectoración; sin embargo presentó un mejor perfil de seguridad que el SH en personas con bronquiectasias en periodo de estabilidad clínica y por esto fue la técnica de elección por los participantes; (3) el peso húmedo de la cantidad de esputo expectorada durante 24 horas presentó una fiabilidad aceptable; sin embargo su nivel de concordancia fue ligeramente amplio, especialmente para niveles de expectoración elevados. Además, se estimó que una reducción de al menos 6,4 g. en la cantidad de esputo expectorada durante las 24 horas posteriores a una intervención de DS, o un cambio relativo de alrededor del -17% con respecto al nivel basal, es la DIM; (4) el uso de los ruidos respiratorios adventicios analizados de forma computacional fue una herramienta viable/factible y parece ser que el número de crujidos espiratorios graves es la mejor variable para utilizar en futuros estudios.

**Conclusión** Los hallazgos de esta tesis resaltan cuáles son los enfoques terapéuticos de DS a corto plazo más efectivos en personas con bronquiectasias en periodo de estabilidad clínica. Por lo tanto, el próximo paso es diseñar y realizar ensayos clínicos a largo plazo que analicen los efectos de estos enfoques terapéuticos en personas con bronquiectasias.

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# Acronyms

| | |
|---|---|
| **HRCT** | High resolution computed tomography |
| **COPD** | Chronic obstructive pulmonary disease |
| **HRQoL** | Health related quality of life |
| ***P. aeruginosa*** | *Pseudomonas aeruginosa* |
| **BSI** | Bronchiectasis severity index |
| **FACED** | Five dichotomised variables: $FEV_1$%(F), age (A), chronic colonisation by P. aeruginosa (C), number of affected lobes (E) and dyspnoea (D). |
| **E-FACED** | Exacerbations + FACED |
| **ABPA** | Allergic bronchopulmonary aspergillosis |
| ***H. influenza*** | *Haemophilus influenza* |
| **NTM** | Nontuberculous mycobacteria |
| **ASL** | Airway surface liquid |
| **PCL** | Periciliary liquid layer |
| **ENaCs** | Epithelial sodium channels |
| **CFTR** | Cystic fibrosis transmembrane conductance regulator |
| **CaCC** | Calcium-activated chloride channel |
| **ACTs** | Airway clearance techniques |
| **BTS** | British Thoracic Society |
| **ACBT** | Active cycle of breathing technique |
| **ELTGOL** | Slow expiration with glottis opened in lateral posture |
| **PEP** | Positive expiratory pressure |
| **TPEP** | Temporary positive expiratory pressure |
| **HS** | Hypertonic saline |
| **IS** | Isotonic saline |
| **$FEV_1$** | Forced expiratory volume in the first second |
| **MCID** | Minimal clinically important difference |
| **HA** | Hyaluronic acid |

| | |
|---|---|
| **SCC** | Sputum colour chart |
| **PRO** | Patient-reported outcome |
| **BHQ** | Bronchiectasis Health Questionnaire |
| **QoL-B** | Quality of Life-Bronchiectasis |
| **QoL-PCD** | Quality of Life-Primary ciliary dyskinesia |
| **SGRQ** | St George´s Respiratory Questionnaire |
| **CAT** | COPD Assessment Test |
| **CRDQ** | Chronic Respiratory Disease Questionnaire |
| **LCQ** | Leicester Cough Questionnaire |
| **CQLQ** | Cough Quality of Life Questionnaire |
| **CCIQ** | Chronic Cough Impact Questionnaire |
| **BCSS** | The Breathlessness Cough and Sputum Scale |
| **CASA-Q** | Cough and Sputum Assessment Questionnaire |
| **PPS** | Patient preference scale |
| **VAS** | Visual analogical scales |
| **GROC** | Global rating of change |
| **FVC** | Forced vital capacity |
| **RV** | Residual volume |
| **DLco** | Difussion capacity for carbon monoxide |
| **FRC** | Functional residual capacity |
| **TLC** | Total lung capacity |
| **LCI** | Lung clearance index |
| **CORSA** | Computerised Respiratory Sound Analysis guidelines |
| **ARS** | Adventitious respiratory sounds |
| **2CD** | Two cycle-duration |
| **IDW** | Initial deflection width |
| **SH+AH** | Suero hipertónico + ácido hialurónico |
| **SI** | Suero salino |

Acronyms only used in the tables, figures and manuscripts

| | |
|---|---|
| **BE** | Bronchiectasis |
| **CBC** | Complete blood count |
| **PFT** | Pulmonary function test |
| **CT** | Connective tissue disease |
| **PCD** | Primary ciliary dyskinesia |
| **CF** | Cystic fibrosis |
| **CFTR-RD** | Cystic fibrosis transmembrane conductance regulator-related disease |
| **A1ATD** | α1-antitrypsin deficiency |
| **IBD** | Inflammatory bowel disease |
| **YNS** | Yellow nail syndrome |
| **DPB** | Diffuse panbronchiolitis |
| **TB** | Tuberculosis |
| **GROD** | Gastro-oesophageal reflux disease |
| **ID** | Immunodeficiency |
| **ATP** | Adenosine triphosphate |
| **ADO** | Adenosine |
| **P2Y2** | Purinoceptor |
| **A2B** | Purinoceptor |
| **NE** | Not established |
| **O-PEP** | Oscillating positive expiratory pressure |
| **$FEF_{25-75}$** | Forced expiratory flow at 25-75% of the FVC |
| **IC** | Inspiratory capacity |
| **VC** | Vital capacity |
| **ISWT** | Incremental shuttle walk test |
| **ESWT** | Endurance shuttle walk test |
| **MIP** | Maximal inspiratory pressure |
| **MEP** | Maximal expiratory pressure |
| **ESR** | Erythrocyte sedimentation rate |
| **CRP** | C-reactive protein |

| | |
|---|---|
| **6MWT** | Six minute walk test |
| **IMT** | Inspiratory muscle training |
| **mMRC** | Modified medical research council |
| **IPV** | Intrapulmonary percussive ventilation |
| **IPPB** | Intermittent positive pressure breathing |
| **NPV** | Negative pressure ventilation |
| **SF-36** | Short form health survey questionnaire |
| **SVC** | Slow vital capacity |
| **Raw** | Airway resistance |
| **Gaw** | Airway conductance |
| **PEQ** | Patient evaluation questionnaire |
| **VDP** | Ventilation defect percent |
| **BSQ** | Bronchiectasis symptoms questionnaire |
| **LRTI** | Lower respiratory tract infection |
| **IFN** | Interferon |
| **HADS** | Hospital anxiety and depression scale |
| **Pr** | Posterior right |
| **Pl** | Posterior left |
| **Lr** | Lateral right |
| **Ll** | Lateral left |
| **Ar** | Anterior right |
| **Al** | Anterior left |
| **Tr** | Trachea |
| **N/A** | Not applicable |
| **SEM** | Standard error of measurement |
| **MDC** | Minimal detectable change with a 95% confidence level |
| **AD** | Autogenic drainage |
| **CI** | Confidence interval |
| **CONSORT** | Consolidated standards of reporting trials |
| **SD** | Standard deviation |
| **BMI** | Body mass index |

| | |
|---|---|
| **HFCWO** | High frequency chest wall oscillation |
| **AEs** | Adverse events |
| **QoL** | Quality of life |
| **NaCl** | Sodium chloride |
| **IQR** | Interquartile range |
| **MID** | Minimal important difference |
| **ICC** | Intraclass correlation coefficient |
| **COSMIN** | Consensus-based Standards for the selection of health status measurement instruments |
| **ROC curves** | Receiver operating characteristic curves |
| **ES** | Effect size |
| **RCT** | Randomised crossover trial |

# Background

## 1. Definition, epidemiology and prognosis of bronchiectasis

Bronchiectasis is an abnormal, permanent and progressive dilation of the bronchi usually diagnosed by axial images of high resolution computed tomography (HRCT) scans. The criterion for this radiological finding is an internal bronchus diameter that is larger than that of its accompanying vessel or the bronchus fails to taper in the periphery of the chest[1]. Therefore, radiological bronchiectasis is observed at any given level when the bronchial/arterial ratio is > 1. Moreover, bronchiectasis can be classified according to the pattern in ´tubular´ (smooth dilation of the bronchi), ´varicose´ (bronchial dilatations with multiple indentations) and ´cystic´ (bronchial dilatations finished in blind ending sacs)[2] (Figure 1). These radiological findings are observed in diverse chronic respiratory disease such as asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD)[3].

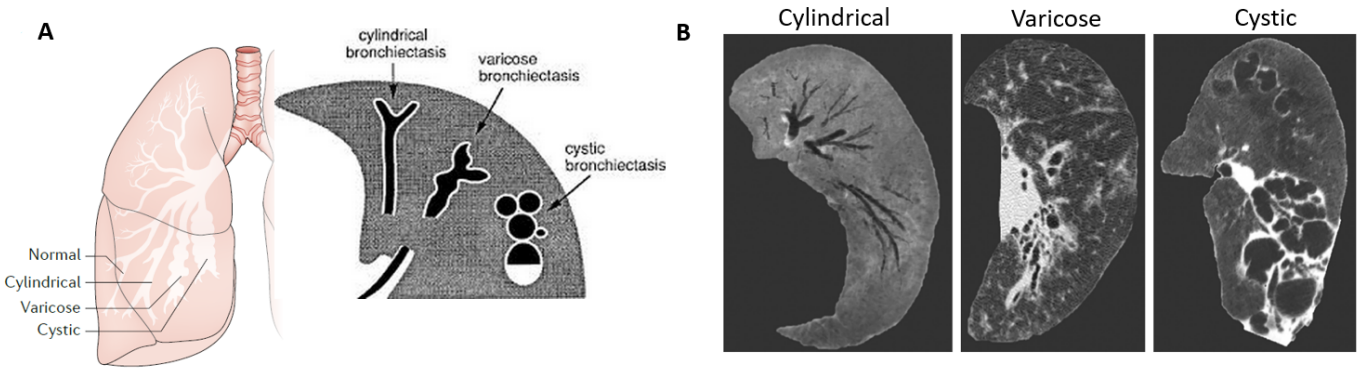


**Figure 1.** Basic morphologic types of bronchiectasis: cylindrical (smooth tubular contour), varicose (undulated irregular contour*)* and cystic (saccular dilatations) represented by (a) a schematic drawing reproduced and (b) an axial minimum intensity projection CT images; both reproduced from Milliron et al. and Chalmers et al.[4, 5].

The term bronchiectasis also refers to a clinical syndrome characterised by recurrent airway inflammation and infection with heterogeneous symptoms that range from chronic cough, daily sputum expectoration and recurrent exacerbations (all of which are the most frequent)[6] to lower exercise capacity[7]. Other symptoms include reduced physical activity[8, 9], fatigue[10], chest pain[11, 12], rhinosinusitis[13], haemoptysis[14], dyspnoea[15, 16], anxiety and depression[17] and sleep disorders[18], with markedly impaired health-related quality of life (HRQoL)[19].

The latest findings from a cohort study population in the UK[20] suggested that the incidence of bronchiectasis increases overall across all age groups, particularly in people over the age of 70 years (from 69.72 per 100,000 person-years in 2004 to 35.17 per 100,000 person-years in 2013). The prevalence of bronchiectasis across all age groups is higher in woman (566.1 per 100,000 person-years in 2013) compared to men (485.5 per 100,000 person-years in 2013); it affects more than 1% of people over 70 years old[20]. In fact, the prevalence of bronchiectasis in the 70-79-year-old group (including both sexes) was 125.74 per 100,000 person-years in 2013[20]. A lower prevalence rate was reported by Henkle et al.[21] in the US population, but they only analysed people over 65 years of age. However, these data are not comparable because Henkle et al.[21] used a stricter definition of bronchiectasis than the UK cohort, namely a required diagnosis by a pulmonologist[22]. These data are consistent with the findings reported by Monteagudo et al.[23] from a population database in Catalonia, Spain. The prevalence (36.2 per 10,000 inhabitants) and incidence (4.81 per 10,000 per year) increase with age in both sexes, with higher prevalence and incidence rates in woman (39.1 per 10,000 and 4.93 per 10,000 per year, respectively) compared to men (33.3 per 10,000 and 4.69 per 10,000 per year, respectively) [23].

The higher bronchiectasis prevalence and incidence rates with ageing is likely attributable to the increased access to HRCT scans, the frequently observed association between bronchiectasis and other common diseases (including COPD and asthma), improvement in health care and available treatments and increased life expectancy[24]. Despite these findings, COPD and asthma are underdiagnosed respiratory conditions. Therefore, it is believed that patients with overlapping syndromes (bronchiectasis-COPD or bronchiectasis-asthma) are also misdiagnosed[22].

The economic burden attributed to bronchiectasis is substantial in Europe. Recently, it was estimated that the direct expenditure of new patients diagnosed with bronchiectasis is €18,634.57 during a 3-year follow-up period, which is 33% higher compared to people matched for age, gender and comorbidities in Germany[25]. The annual incremental costs in the US for people with bronchiectasis compared with matched controls ranges from US$2,319 to 5,681 per patient[26].

Hospital admission in people with bronchiectasis represents a great economic impact. The mean annual age-adjusted hospitalisation rates this population range from 1.8 to 25.7 per 100,000 population, with an average hospitalisation duration between 2 and 17 days[27]. In a prospective and multicentre study in Spain, de la Rosa et al.[28] estimated a mean hospitalisation cost of €5,284.70 in people with bronchiectasis and found a higher overall cost for people with chronic infection by *Pseudomonas aeruginosa*, longer hospital admission and the completion of treatment at home. In the US, Seifer et. al[29] and Blanchette et al.[30] demonstrated that a bronchiectasis-COPD overlap diagnosis and the presence of *P. aeruginosa* infection markedly increase the healthcare cost and utilisation.

Although hospital admissions crucially impact the global management cost of people with bronchiectasis, the cost of outpatient treatment (antibiotics, bronchodilators, corticoids and mucoactive therapies) also represents a significance expenses in bronchiectasis management[31]. Moreover, it is crucial to highlight the importance of indirect costs in the management of people with bronchiectasis. Diel et al.[25] observed people with this condition take a mean number of 40.5 sick days, with a induced work-loss costs of €4,230.49.

People with bronchiectasis present higher mortality rates compared to the general population. Quint et al.[20] reported that the age adjusted mortality rate for woman with bronchiectasis in the UK was 1,437.7 per 100,000 population and for the general population (woman) was 635.9 per 100,000 population. The age adjusted mortality rate was 1914.6 per 100,000 population for men with bronchiectasis, whereas for the general population (men) it was 895,2 per 100,000 population[20]. In 2014, Goeminne et al.[32] found that the overall mortality rate in newly diagnosed people in Belgium was 20.4%. After a mean follow-up period of 5.18 years, it increased to 55% for people with bronchiectasis-COPD overlap syndrome[32]. Recently, Gaile et al.[33] determined the mortality rate of chronic respiratory diseases in England from 2005 to 2015 and the age-standardised mortality rate of people with bronchiectasis was 1,463 per 100,000 population, which is slightly lower than for people with COPD (1,503 per 100,000 population) and markedly higher than for people with asthma (856 per 100,000 population).

The independent risk factors for mortality in bronchiectasis observed in single studies include age, lung function impairment, poor gas transfer, *P. aeruginosa* infection, number of lobes affected, concomitant COPD diagnosis and air pollution[32, 34-36]. On the other hand, vaccination against influenza and pneumococci are associated with improved survival[37]. Currently, there are three different clinical tools to predict mortality in people with

bronchiectasis: the Bronchiectasis Severity Index (BSI), FACED score and the posterior E-FACED score version[38-40].

The BSI is a validated clinical predictive tool for people with bronchiectasis that classifies individuals according to their disease severity and determines the risk of mortality, hospitalisation and exacerbations during a 5-year follow-up[38]. It comprises nine variables, which involve anthropometric characteristics, lung function, extension of the disease, microbiological aspects and clinical condition (exacerbations, dyspnoea). The total score is calculated by summing the scores for all nine variables (0-26). It is then categorised into three severity levels: mild (0-4), moderate (5-8) and severe (≥ 9)[38]. An online calculator is accessible at http://www.bronchiectasisseverity.com.

The FACED score is a validated and multidimensional tool to predict mortality from all causes (including respiratory) and exacerbations[39]. FACED is the acronym of the five dichotomised variables that are measured: percentage of predicted forced expiratory volume in the first second ($FEV_1$%)(F), age (A), chronic colonisation by *P. aeruginosa* (C), extension of the disease measured by the number of affected lobes (E) and dyspnoea using the Medical Research Council (MRC) scale (D). The total score is obtained by adding the score from each variable (0-7) and divided into three severity levels: mild (0-2), moderate (3-4) and severe (5-7)[39]. In 2017, Martinez-Garcia et al. [40] developed a new version of the FACED score (E-FACED) by considering the frequency of exacerbations and their severity (hospital admission) to use this tool to predict future exacerbations and also mortality.

The capacity of BSI and FACED scores to predict long-term mortality was compared in a single-centre retrospective cohort analysis in UK[41]. The data suggested that both scoring systems predict long-term mortality (15 years) with high specificity, but the FACED score

shows slightly superior predictive power[41]. Conversely, BSI is more accurate than the FACED score to predict hospitalisations, exacerbations and clinical outcomes across all disease severity classifications[42]. Specifically, BSI shows a numerically higher area under the curve (AUC) to predict hospitalisations (BSI 0.893 versus FACED 0.809) and exacerbations (BSI 0.808 versus FACED 0.734) in a Spanish cohort[43]. The findings also suggest that BSI and FACED scores do not classify patients similarly according to their disease severity because FACED score tends to be more skewed to mild classification[43-45].

## 2. Aetiology diagnosis in bronchiectasis

Multiple aetiologies, coexisting disease and overlapping syndromes have been associated with bronchiectasis. Therefore, identifying the underlying cause of bronchiectasis is a challenge. Consequently, a high percentage of patients with bronchiectasis are eventually assigned an idiopathic diagnosis. Recently, Chalmers et al.[5] summarised the possible aetiologies and the recommended diagnostic tests to identify them (Table 1).

A systematic aetiology evaluation for all patients with a clinical history compatible with bronchiectasis and a positive HRCT scan is important because some of the causes require specific therapies and respond positively to the treatment. Guidelines recommend a bundle of aetiology tests, including: i) complete blood count; ii) immunoglobulin levels; iii) testing for allergic bronchopulmonary aspergillosis (ABPA); and iv) bacterial and mycobacterial sputum culture[24, 46-48]. If an aetiological diagnosis cannot be reached, additional tests are appropriate, according to specific features or in patients with severe or rapidly progressive disease[24, 46-48].

**Table 1.** Possible aetiologies of bronchiectasis. Adapted from Chalmers et al.[5]

| Key features | Examples of specific aetiology | Key diagnostic test(s) |
|---|---|---|
| **Post-infectious** | Tuberculosis | Medical History |
| | Pneumonia | |
| **Immunodeficiency** | Primary: agammaglobulinaemia and hypoglobulinaemia | Serology (IgG, IgA , IgM and IgE levels) |
| | Secondary: HIV infection and chemotherapy- induced immunosuppression | Serology and medical history |
| **Impaired mucociliary clearance** | Primary ciliary dyskinesia (PCD) | Nasal nitric oxide test, cilia biopsy and motility test and genetic tests |
| | Cystic Fibrosis | Sweat test and genetic test |
| **Previous lung injury** | Aspiration | Swallowing assessment, endoscopy and motility testing |
| | Bronchiolitis obliterans | Chest CT scan |
| | Interstitial lung disease | Medical history |
| **Airway lesions** | Congenital tracheobronchomegaly, tracheal stenosis or cartilage abnormalities | Chest CT and bronchoscopy |
| | Obstructive (for example, foreign body or external compression) | Medical history , chest CT scan and bronchoscopy |
| | Tracheobronchomalacia | Bronchoscopy |

| | | |
|---|---|---|
| **Concurrent other chronic respiratory diseases** | Chronic obstructive pulmonary disease (COPD) | Lung function and smoking history |
| | Asthma | Lung function — tests for airway reactivity |
| | Allergic bronchopulmonary aspergillosis (ABPA) | Serology. Elevated anti- Aspergillus spp. IgE levels, total IgE supported by raised IgG and/or Aspergillus spp. precipitin and eosinophil assessment |
| **Connective tissue disease and/or autoimmune** | Inflammatory bowel disease | Colonoscopy |
| | Rheumatoid arthritis<br>Systemic lupus erythematosus<br>Sjögren syndrome | Serum autoantibody assessment |
| **Other syndromes** | Ataxia- telangiectasia | α- Fetoprotein and genetic analysis (ATM gene) |
| | Marfan syndrome | Clinical characteristics (defined set exits) with or without the presence of fibrillin-1 (FBN1) mutation |
| | Polycystic kidney disease | Renal ultrasonography and genetic analysis |
| | Velocardiofacial syndrome | Genetic analysis (22q11 deletion) |
| | Yellow nail syndrome | Clinical characteristics. In some patients, mutations in *FOXC2* may be found |
| **Others** | Prematurity | Medical history |
| | α1-Antitrypsin deficiency | Levels and function of α1-antitrypsin |

In 2017, Araujo et al.[49] created an aetiology classification algorithm (Figure 2) to improve the aetiology diagnosis; they tested it on 10 different databases from the UK. Despite the limitations of the study (retrospective), the number of participants diagnosed clinically as idiopathic bronchiectasis was substantially lowered using this standardised algorithm across all centres. Although further research is needed, this algorithm represents a promising tool to improve the diagnosis of the underlying cause of bronchiectasis and could increase the possibility that patients receive the most suitable treatment. Moreover, a global standardised aetiological algorithm will allow the comparison of findings from different centres and/or countries with higher accuracy.

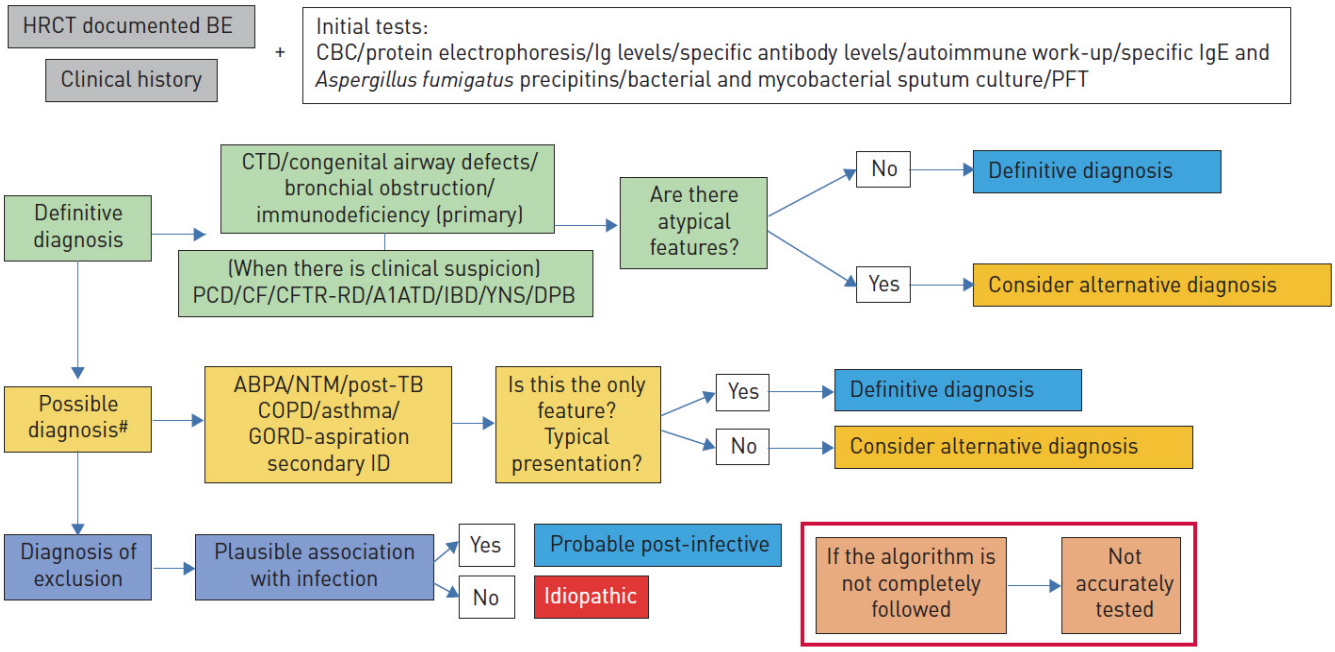


**Figure 2**. Algorithm to identify the cause of bronchiectasis proposed by Araujo et al.[49]. HRCT= high-resolution computed tomography; BE= bronchiectasis; CBC= complete blood count; PFT= pulmonary function test; CT=: connective tissue disease; PCD= primary ciliary dyskinesia; CF= cystic fibrosis; CFTR-RD= cystic fibrosis transmembrane conductance regulator-related disease; A1ATD= α1-antitrypsin deficiency; IBD= inflammatory bowel disease; YNS= yellow nail syndrome; DPB= diffuse panbronchiolitis; NTM= nontuberculous mycobacteria; TB= tuberculosis; COPD= chronic obstructive pulmonary disease; GORD= gastro-oesophageal reflux disease; ID= immunodeficiency.

## 3. Physiopathology of bronchiectasis

The pathophysiologic process of bronchiectasis is complex and not yet well understood, at least partly due to the lack of experimental and animal models. A large number of possible aetiologies have been identified (as previously described), and it is likely that the physiopathology depends on the underlying cause[5]. Despite this fact, most of the conditions that cause bronchiectasis have similar features[50].

It is proposed that an initial insult/event compromises the mucociliary clearance system and allows transient bronchial dilatation. At this stage, if the original stimulus is controlled or removed, the disease may be reversible[5]. However, this event may disrupt the immune response and predispose the individual to chronic airway infection and an increased inflammatory response. This phenomenon leads to pathological remodelling of the airways and generates bronchiectasis (Figure 3). This model was first described as the "vicious cycle hypothesis" by Cole[51]. Recently, Flume et al.[50] proposed changes to the vicious cycle by a vortex concept because all components of the physiopathology process can affect the others (Figure 3,b). This vertex model ratifies the importance of simultaneous multimodal treatments to break the cycle. Thus, to obtain substantial clinical benefits in people with bronchiectasis, addressing all aspects of the disease is necessary[50].

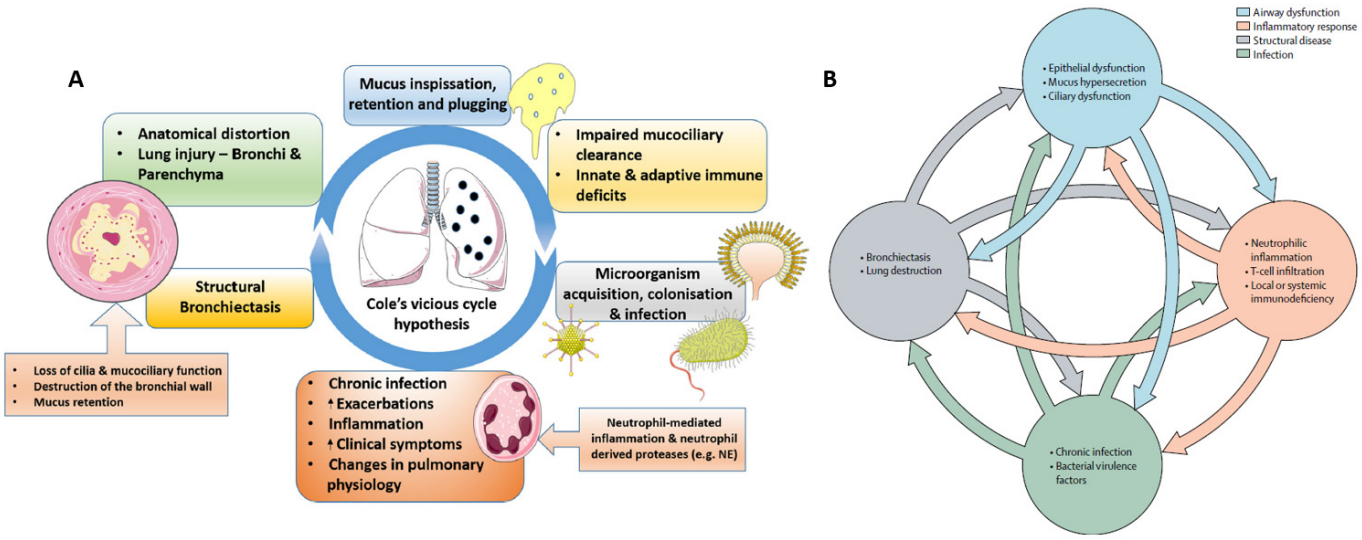


**Figure 3.** Two complement approach of Cole´s vicious cycle hypothesis of developing bronchiectasis reported by (A) Chandrasekaran et al.[52] and (B) Flume et al.[50]. All components (inflammation, infection, impaired mucociliary clearance and structural lung damage) are interrelated and are responsible for the development and progression of bronchiectasis.

As this doctoral thesis focuses on airway clearance management, from this point on, the impaired mucociliary clearance is considered the central point of this vicious cycle/vertex model. Therefore, independent of the first event, there are three essential and interrelated components that are responsible for the mucociliary clearance impairment in people with bronchiectasis: airway inflammation and immune dysfunction, airway infection and structural lung damage[53].

### 3.1. Impact of airway inflammation and immune dysfunction on mucociliary clearance in bronchiectasis

High levels of inflammatory cells in bronchoalveolar lavage and sputum are frequently observed in patients with bronchiectasis[54-56]. These findings represent an imbalance due to an excessive pro-inflammatory response and/or a failure of anti-inflammatory mechanisms[53].

Bronchiectasis is classically considered to be a chronic neutrophilic airway inflammation. After an insult/event, massive neutrophil recruitment occurs in the airways, particularly in the smaller ones[57], that is driven by chemoattractants such as interleukin-8 and leukotriene B4, among others[58]. The neutrophil activation is responsible for the antimicrobial defence by phagocytosis or through an extracellular pathway associated with neutrophil extracellular trap formation (NETosis)[53]. However, detrimental effects can appear during the neutrophil activation/degradation because several pro-inflammatory mediators (neutrophil elastase, metalloproteinase and myeloperoxidase) are released[53, 59].

These neutrophil-delivered products impair neutrophil phagocytosis, affect the immune response and damage the airway epithelium cells, all of which cause mucus gland hyperplasia, increase mucus production and slow the ciliary beating rate[58, 59]. In fact, the concentration of neutrophil elastase in sputum is considered to be a biomarker in bronchiectasis because it is correlated with disease severity and long-term clinical outcomes[54, 55].

### 3.2. Impact of airway infection on mucociliary clearance in bronchiectasis

The presence of bacteria in the airways inhibits ciliary function, stimulates mucus production, damages the airway epithelium and is the main factor responsible of airway inflammation, particularly in the small airways[57, 60]. Consequently, it stimulates the release of chemokines, which promote the presence of large numbers of neutrophils in the airways[60].

The European registry reports that the most frequent pathogen isolated in people with bronchiectasis are *Haemophilus influenzae*, followed by *P. aeruginosa*[38]. Similar data was recently observed for Australian people with bronchiectasis[61]; however, *H. influenzae* appears

be uncommon in the US, whereas the frequency of nontuberculous mycobacteria (NTM) isolation is higher in that population[62].

The disease burden is greater for people with chronic *P. aeruginosa* infection because it is related to higher exacerbation frequency and hospitalisations, worse HRQoL and increased mortality risk in people with frequent exacerbations, after adjustment for multiple possible confounding variables[34, 63]. Once *P. aeruginosa* has been established in the airway, the treatment becomes a challenge because this microbe can grow in a biofilm to protect itself from host defences and antimicrobial therapeutics. This phenomenon promotes greater airway inflammation and more severe damage to the underlying airway[60, 64, 65] and thus perpetuates the vicious cycle/vortex model. Additionally, Alcaraz-Serrano et al.[66] recently found that airway clearance management may be more difficult in people with chronic *P. aeruginosa* infection because worse viscoelastic properties of sputum (e.g. elasticity, viscosity and stiffness) have been observed in samples with *P. aeruginosa*. This factor leads to a deterioration of ciliary clearance and perpetuates the vicious cycle/vortex cycle.

Although the impact of *H. influenza* in people with bronchiectasis has been less explored, its presence in the airways may facilitate a process similar to that described above. Chemokine release and stimulation of mucus production can directly damage the airway epithelium[60].

### 3.3. The impact of structural lung damage on mucociliary clearance in bronchiectasis

The inflammatory mediators produced by neutrophils (elastase, metalloproteinase and reactive oxygen species) are responsible for the loss of elastin in large airways that cause bronchial dilatations. In advanced cases, these mediators even cause the loss of muscle and cartilage in the airways[53, 60]. These structural abnormalities lead to mucus stasis, which favours continued

chronic infection and the airway inflammatory response and, consequently, the cycle continues. As the disease progresses, the continuous infection-inflammation leads to small airway obstruction due to the cell-mediated inflammatory infiltrate and the presence of lymphoid follicles. This phenomenon potentiates mucus stasis. Finally, the inflammation beyond the airways causes interstitial pneumonia (Figure 4)[57].

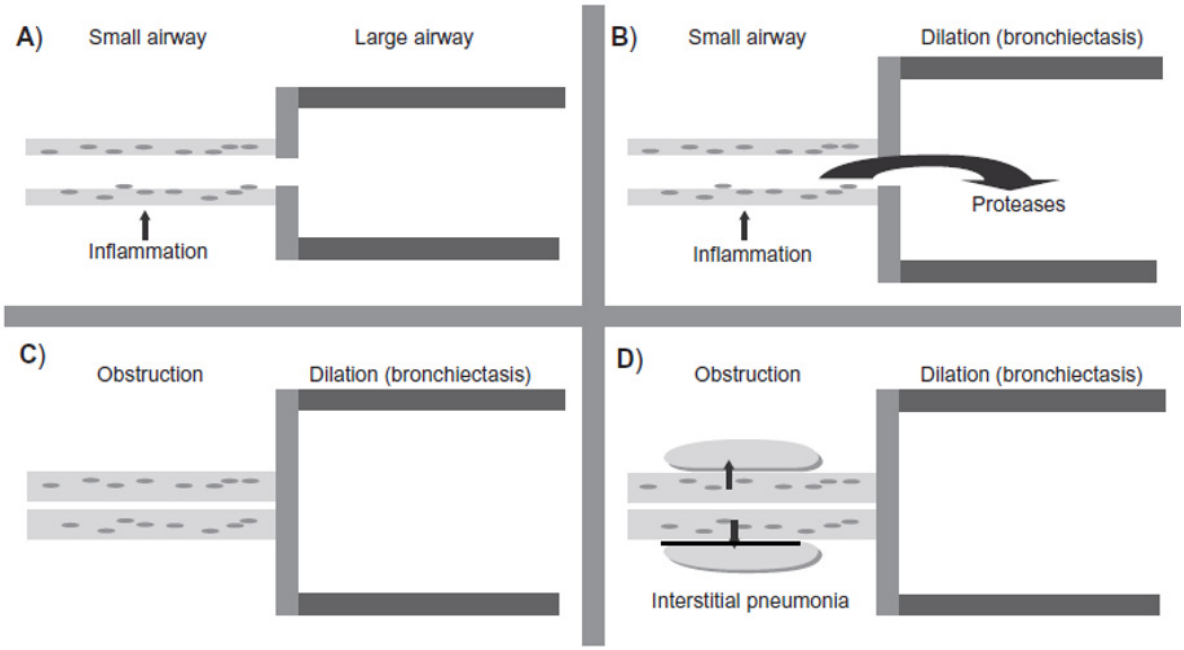


**Figure 4.** Pathologic changes in bronchiectasis as described by Whitwell and reported by King et al.[57] (A) the presence of an initial event (i.e. severe pneumonia) generates an excessive inflammatory response (B) that release inflammatory mediators which damage the large airways resulting in bronchiectasis; (C) progressively small airways are also damage which become thicker from a combination of cell-mediated inflammatory infiltrate and lymphoid follicles resulting in obstruction; (D) the final process involves the spread of inflammation beyond the airways resulting in interstitial pneumonia.

## 4. Mucociliary clearance system in healthy people

The mucociliary clearance system is the main mechanism to clean the lungs and the respiratory tract. The combined action of ciliary beating and coughing facilitates the transport of inhaled foreign particles entrapped in the airway surface liquid (ASL) layer out of the lungs[67].

The ASL layer lines the surface epithelium of intrapulmonary airways. It is composed of two different layers: the mucus layer and the periciliary liquid (PCL) layer (Figure 5)[68]. The mucus layer (approximately 2 to 5 µm in depth) is a viscoelastic gel composed of mucins, particularly MUC5AC and MUC5B, secreted from goblet cells and submucosal glands[67]. Its role is to bind and entrap all inhaled particles on airway surface during normal breathing[68]. This layer (and the entrapped particles and foreign pathogens) is propelled to the oropharynx area by the coordinated synchronised action of the ciliary system and coughing. However, its transportability depends on the composition and proportion of mucins and its hydration level[68].

The PCL is located under the mucus layer (Figure 5); it is in contact with the airway surface environment. This low viscoelastic gel layer is mostly composed of tethered mucins (MUC1, MUC4 and MUC16) and is approximately 7 µm in depth[68]. Its main role is to lubricate the area surrounding the cilia and facilitate their beating[69]. It also acts as a physical barrier to restrict the direct access of particles to airway surfaces[68]. Therefore, the PCL hydration level is important to ensure its functionality.

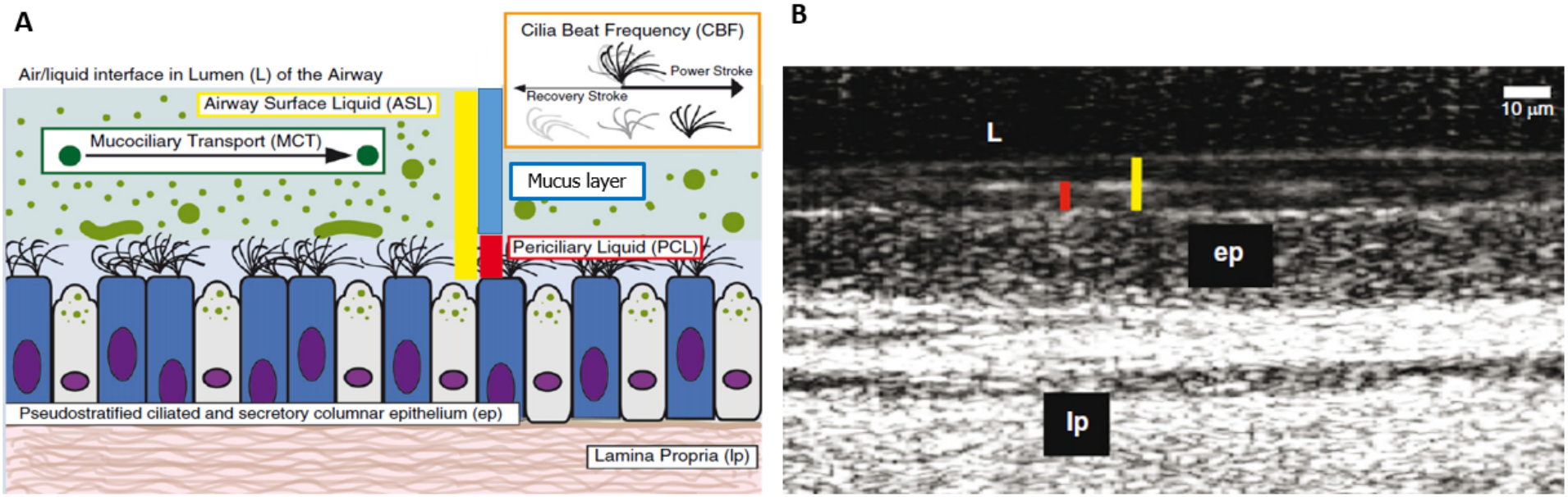


**Figure 5** (A) Representation of the airway surface layer, under normal conditions. Airway surface liquid (ASL) layer is represented by the yellow bar; periciliary Liquid layer (PCL) is indicated by the red bar; mucus layer depth is shown by the blue bar; ciliary beating is represented in the orange box. (B) Micooptical coherence tomography (*µOCT)* image of excised swine trachea. The epithelium (ep) and lamina propria (lp) are visible inferior to the airway surface liquid (ASL), as indicated by the yellow bar, and the PCL, as indicated by the red bar. Images reported by Shei et al.[(69)] .

In healthy lungs, the frequency of cilia beating is approximately 12 to 15 Hz, which generates waves of metachronal motion that propel only the mucus layer in cephalic direction. This action yields a mucociliary clearance velocity of 50 μm/sec[70, 71]. Cilia tips contact with mucus layer on the power forward stroke because their lengths are approximately 4 to 7 nm (depending on the airway region). However, during the slow return stroke, the cilia recede and are contained in the PCL[70, 71]. Cilia only beat in synchrony with cilia located perpendicularly and in a phase-shifted manner with other cilia located parallel along the axis of the effective stroke[71]. Research suggests that ciliary movement is also facilitated by a thin surfactant line located between mucus layer and PCL that prevents ciliary entanglement in the mucus layer[71].

The regulation of ASL hydration (absorption and secretion) is crucial for effective mucociliary clearance (Figure 6). Airway epithelial ion channels control the mass of salt and water on the airway surfaces[72, 73]. Amiloride-sensitive epithelial sodium channels (ENaCs) active $Na^+$ absorption from airway surfaces to the submucosal compartment and, therefore, reduce the ASL hydration status. On the other hand, the cystic fibrosis transmembrane conductance regulator (CFTR) and the calcium-activated chloride channel (CaCC) activate $Cl^-$ secretion to the airway surfaces that increases the ASL hydration status[72, 73]. The function of these channels depends on the concentration of nucleotides and nucleosides within the ASL, and thus they apparently play an important role in regulating the airway surface hydration[73].

In healthy people, when the PCL presents normal hydration, its osmotic pressure is higher than the mucus layer because the tethered mucins generate a “constrained” system[74]. Consequently, the mucus layer does not compress the PCL and the ciliary beating is normal. Moreover, the lower osmotic pressure of mucus layer is responsible for fluid balance changes (more absorption/secretion). The mucus layer depth will be the first to change (e.g., after

activation of $Cl^-$ secretion by the nucleotides, liquid secretion from the submucosal compartment is added to the mucus layer, a phenomenon that leaves the PCL unchanged)[74].

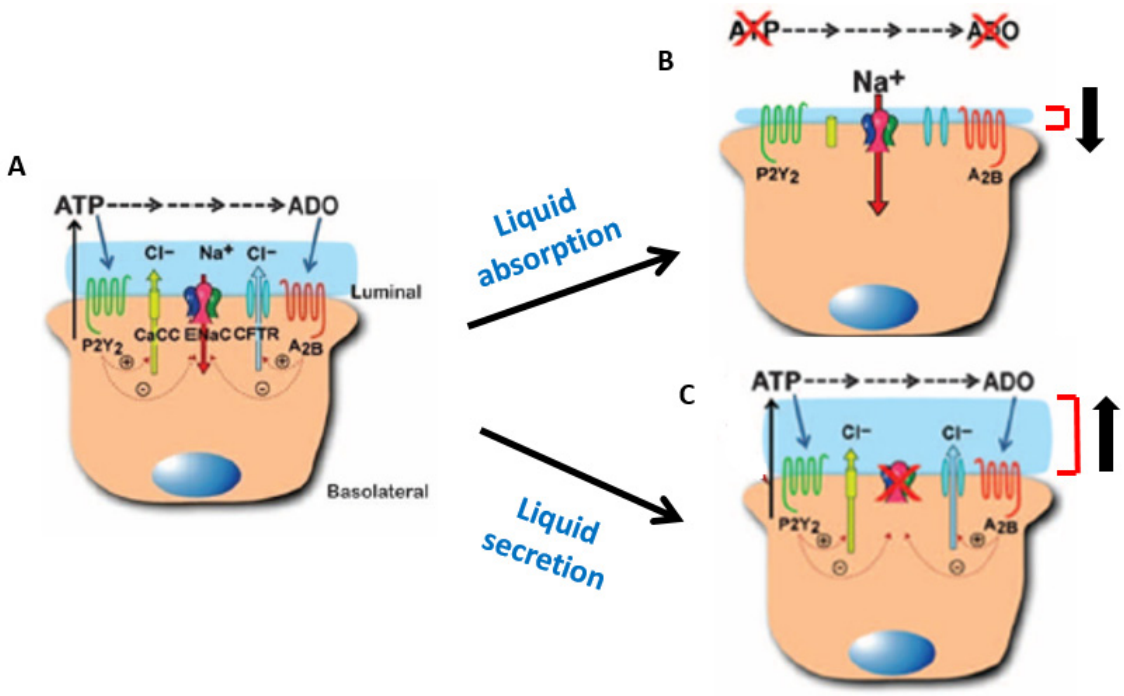


**Figure 6.** Airway surface liquid (ASL) layer regulation. (A) Airway epithelia channels controls ion transport ($Na^+$ and $Cl^-$) between airway surface and epithelium under normal concentrations of nucleotides; (B) Lower concentration of $Cl^-$ on the airway surface is detected when the CFTR and CaCC are inhibited by the absence of nucleotides. As a result, fluid from ASL (preferably from the mucus layer) is absorbed to the epithelium. (C) An increase in nucleotides release enhance $Cl^-$ secretions to airway surface and inhibit $Na^+$ absorption to the epithelium. Thus, the ASL depth increase (particularly the mucus layer). ATP= adenosine triphosphate; ADO= adenosine; CaCC= the calcium-activated chloride channel; ENaC= The amiloride-sensitive epithelial sodium channels; CRTF= the cystic fibrosis transmembrane conductance regulator; P2Y2 and A2B= purinoceptors. Obtained from Button et al.[73] .

Coughing is an alternative mechanism of mucociliary clearance, particularly from central airways. This forced expiratory manoeuvre partially reduces the cross-sectional area of central airways by generating a dynamic airway compression. Consequently, the velocity and airflow turbulence increase to generate higher shearing forces within airways[75]. Cough transportability

depends on the adhesion between the mucus layer and airway surface (adhesivity) and the tendency of mucus to stick to itself and form threads (cohesivity) (Figure 7)[76, 77]. Therefore, coughing is an effective method in healthy people because the normal PCL hydration status prevents the adhesion of the mucus layer to the airway surface and the mucus cohesive strengths are low.

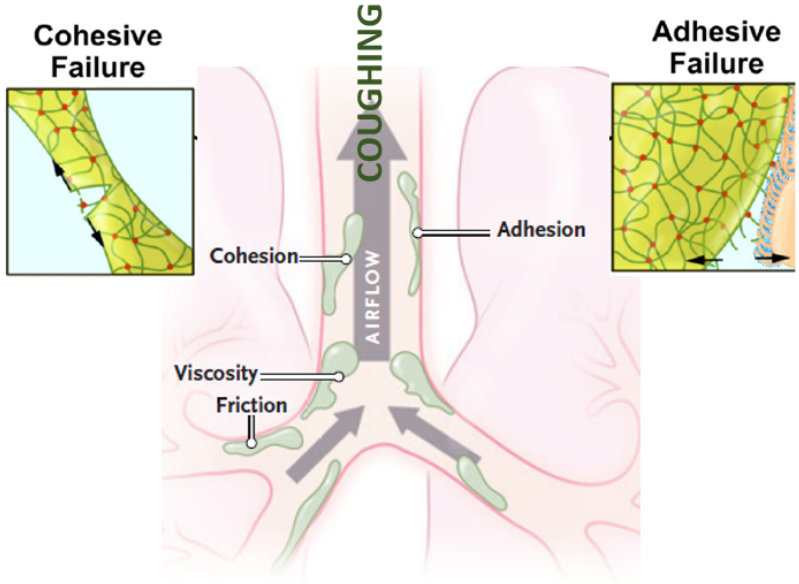


**Figure 7.** Cough transportability (larger airways) depends on tenacity (the sum of adhesivity and cohesivity). The higher airflow generated during coughing fractures intramucus cohesive forces, adhesive forces between mucus layer and the airway surface, or both to expectorate mucus as sputum. In more distal airways, mucus is sliced by frictional forces and propelled by the ciliary beating. Adapted from Boucher[78] and Button et al.[76].

## 5. Impaired mucociliary clearance in people with bronchiectasis

A productive cough or difficulty in expectorating sputum is a clinical symptom that reflects the presence of an impaired mucociliary clearance system in people with bronchiectasis. Abnormalities in mucus production, ciliary function and biophysical and surface mucus properties directly contribute to a decreased mucus clearance rate[77, 79, 80].

Unfortunately, there are limited data about the function of ASL in bronchiectasis. Nevertheless, the hypothesis that mucus layer dehydration impairs mucus transport[80-82] seems to also apply in people with bronchiectasis[83]. First, neutrophil elastase activity plays an important role in the pathogenesis and progression of bronchiectasis[54]. Excessive neutrophil elastase activity within the inflamed airway decreases ciliary beating and stimulates mucin secretion[54, 59]. Also like COPD and cystic fibrosis, MUC5B appear to be the most predominant mucin in bronchiectasis[83], and higher airway mucin levels are associated with disease severity[83, 84]. However, these data are based on few studies, and further research is needed to confirm these results.

An excess of secreted mucin leads to mucus layer dehydration and generates an osmotic imbalance between the mucus layer and the PCL. This phenomenon ultimately compresses the PCL and ciliary system (Figure 8)[85]. Consequently, ciliary beating is slowed down and mucus layer adhesion to the airway epithelial surface (adhesivity) is facilitated, therefore reducing mucus transport and enhancing mucus accumulation[80, 81]. Ciliary dysfunction can also result from a genetic disorder of the ciliary structure and function in people with bronchiectasis (primary ciliary dyskinesia).

Second, adhesivity appears to be the strongest dependent factor of cough clearance effectiveness[77, 79] when respiratory muscle strength is preserved. This action is independent of mucus viscoelastic properties[86]. Greater adhesivity appears when the interfacial tension is high between the mucus layer and the airway epithelium and/or the mucus wettability is low[79, 87]. The limited data that are available suggest that cough transportability is impaired in bronchiectasis[88, 89], although it is still more effective when compared to other diseases (e.g., cystic fibrosis and bronchitis)[90].

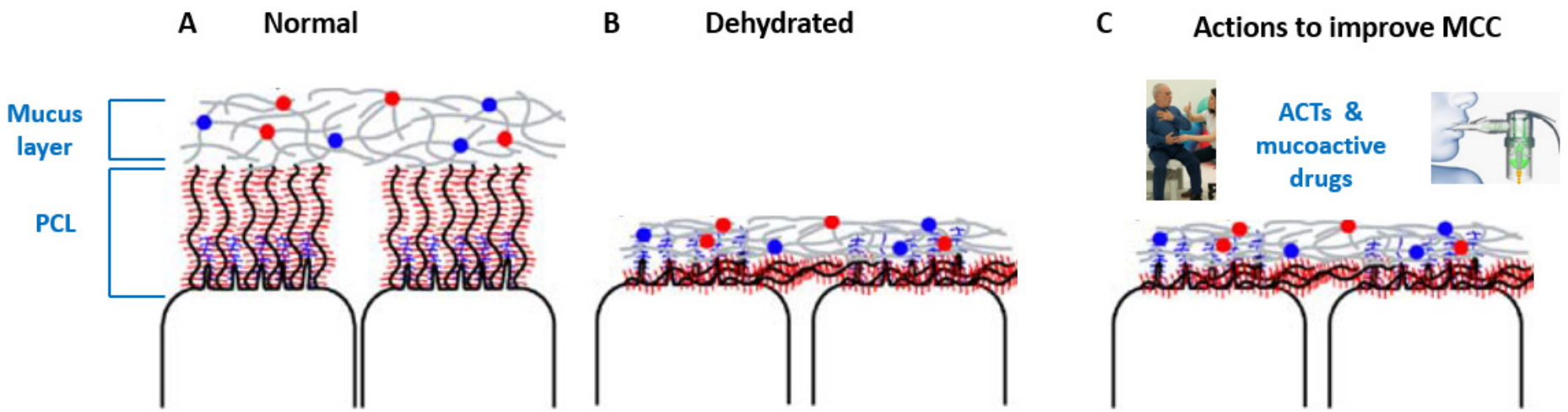


**Figure 8.** (A) Representation of the airway surface layer, including mucus layer and periciliary liquid layer (PCL), under normal conditions; (B) Representation of the airway surface layer under dehydration conditions. An excess of mucin concentration leads to mucus layer dehydration and collapse the PCL and ciliary system. As result, the mucus transport is impaired and produce mucus adhesion to airway surface (adhesivity); (C) Airway clearance techniques (ACTs) and hyperosmolar agents as therapeutic options to enhance sputum removal. Adapted from Randell et al.[85]

## 6. Impact of daily sputum expectoration in people with bronchiectasis

Daily productive cough is a common respiratory symptom experienced by approximately 65% of people with bronchiectasis[91]. It reflects the presence of an impaired mucociliary clearance system in this population. Mucus retention has also been associated with a major decline of lung function, more exacerbations and a higher risk of mortality in people with bronchiectasis and other chronic respiratory diseases[19, 92, 93].

The incidence of daily sputum expectoration is similar across all age groups[94], and it is independent of the time of productive cough onset (childhood or adulthood)[95]. The amount of sputum expectorated tends to increase over time in patients with bronchiectasis[96], and a change in daily sputum expectoration is recognised as an important factor to identify exacerbations in this population[97]. In fact, people with bronchiectasis perceive sputum as an important symptom burden[98]: it is difficult to manage it correctly[99], associated with a negative impact on social life (embarrassment in public)[100] and negatively impacts their HRQoL[19, 98]. Therefore, it is not surprising that one of main research priorities proposed by patients is how to implement and facilitate access to airway clearance management[99].

People with chronic sputum expectoration would like to control the need to expectorate in public[98]. They report that expectorating is disgusting, embarrassing and an unwelcome reminder about their health condition[100, 101]. They usually try to hide the need to spit in public because it may be considered a feature of their disease[100, 101]. For these reasons, people with bronchiectasis and adherent to airway clearance therapies admit to using these interventions before going out to better control the need to expectorate in public[101]. Consequently, airway clearance therapies are essential self-management strategies to better control the need to

expectorate in daily life in people with bronchiectasis and may prevent the negative impact of sputum-related symptoms during social interactions.

## 7. Physiological mechanisms to enhance airway clearance

Mechanical stress applied to the airways is a strategy to stimulate hydration of the mucus layer and, therefore, enhance airway clearance[68, 72]. During normal breathing, two physical stresses are generated during both respiratory phases that are essential for the normal regulation of airway surface hydration: the airflow and the trans-airway pressure gradient[68]. Previous studies reported that fluid shear stress, compression/stretch and osmotic shock are the main physical mechanisms that stimulate airway surface hydration[68]. Additionally, an *in vitro* flow model suggests two conditions that improve airway clearance[102-104]: i) the peak expiratory flow rate should be greater than the peak inspiratory flow rate (at least 10%) for mucus to move proximally; and ii) a peak expiratory flow rate of 30-60 L/min is required to break the adhesive bonds generated between the mucus layer and the airway epithelial surface. Accordingly, airway clearance strategies based on generating greater mechanical stress on the airways compared to normal breathing and the achievement of both conditions described above may play an important role to improve airway clearance in people with bronchiectasis.

## 8. Airway clearance approaches for people with clinically stable bronchiectasis

### 8.1. Airway clearance techniques (ACTs)

Based on international and national guidelines, ACTs should be taught to all people with bronchiectasis and chronic productive cough or inability to cough effectively[46-48, 105-107]. However, the level of recommendation for ACTs is heterogeneous across the guidelines (ranges from weak to strong) and supported by low-moderate quality evidence[108].

Indeed, there is a lack of long-term studies, and it is difficult to compare findings among studies to determine the main underlying reasons for the benefits of ACTs[109].

ACTs are included in the first-line treatment in patients with stable bronchiectasis[47] because it is believed that regular application of this treatment may help increase the clearance of inflammatory markers in the airways, prevent mucus accumulation and reduce the risk of exacerbations[110]. However, these potential benefits are still poorly explored in people with bronchiectasis.

A trained respiratory physiotherapist with expertise in all possible ACTs should be the person responsible for educating and teaching people with bronchiectasis how to manage sputum symptoms[46, 47]. According to the British Thoracic Society (BTS) guidelines[47], patients with stable bronchiectasis should be instructed in the performance of ACTs at a first evaluation; however, it has not been clearly reported what clinical criteria should be considered to refer patients to the physiotherapy department and when this first assessment should be performed. Moreover, a review session within 3 months after the first assessment is recommended, as well as a visit to the physiotherapist as part of their annual clinical evaluation[47]. Finally, if a patient suffers an increase of the number of exacerbations and/or a worsening of the symptoms, the ACT performance and adherence should be checked[47].

Although ACTs are often recommended twice per day in clinical practice, there is no evidence to support this frequency. Rather, it should be tailored according to patients´ characteristics[47]. The duration of each ACT should be long enough to enhance sputum expectoration but not too long so as to avoid fatigue and the feeling that this treatment is really time consuming[47].

Despite the guidelines recommendations, the access to airway clearance management in Europe is clearly suboptimal in people with bronchiectasis and differs across European countries. Data from the EMBARC registry showed that approximately 54% of European patients do not perform any ACT; however, the access to this treatment is greater in Northern Europe countries[92(111)]. Surprisingly, the main reason for not performing ACTs appears to be a physician's decision[(92)]. This phenomenon can be attributed to the lack of knowledge in relevant aspects in relation to airway clearance management, such as how to identify people who need this treatment, how to implement it and how to standardise it. Therefore, a further evaluation of ACTs benefits using high methodological standards is needed to enlighten the possible benefits of these treatments in people with bronchiectasis.

The main ACTs used in people with bronchiectasis are described in Table 2. Further information on each procedure is available on specific evidence-based multimedia web resources (including images and videos) for people with bronchiectasis[(112, 113)].

With respect to physiological or symptom effect or impact on quality of life, no single ACT is more beneficial than any other in people with clinically stable bronchiectasis[(109, 114)]. Therefore, it is recommended to select ACTs according to the patient's characteristics (level of autonomy, preference, breathlessness, tolerability, fatigue and even economic resources)[(110)]. However, recent guidelines showed a clear discrepancy with respect to the type of techniques recommended (particularly for manual ACTs), despite similarities in research methodology and the procedure applied to grade the evidence[(46, 47, 105)].

**Table 2**. An overview of the main airway clearance techniques used in people with stable bronchiectasis

| Technique | Procedure[112, 113] | Physiological basis to enhance sputum removal[72, 102] | Short-term clinical benefits | Long-term clinical benefits |
|---|---|---|---|---|
| **Active cycle of breathing technique (ACBT)** | ✓ Patient positioning: it is generally performed in sitting position; however, an alternative position (supine, side-lying) may be also used.<br>✓ Breathing: it is a combination of exercises including breathing control, thoracic expansions with breath hold after inspiration and finished by forced expiration technique (huff). | ✓ Thoracic expansion exercises with breath hold generate a greater trans-airway pressure gradient (mechanical stress) than normal breathing. This mechanism also uses interdependence and collateral ventilation to allow the presence of air behind obstructed lung units. | ✓ Enhances sputum removal during treatment[115]<br>✓ Slight improvement in lung function after treatment[115] | ✓ NE |
| **Autogenic drainage** | ✓ Patient positioning: it is generally performed in sitting position; however, an alternative position (supine, side-lying) may be also used (Figure 9).<br>✓ Breathing: commence breathing from lower lung volume levels in the expiratory reserve volume, through higher lung volume levels into the inspiratory reserve volume with the | ✓ Stage 1 ("Loosening phase"): the cross-sectional area of the medial and peripheral airways is reduced (mechanical stress) and the airflow velocity increases in these areas. This is achieved by breathing repeatedly using low lung volumes in the expiratory reserve volume. The slow expirations with an open glottis avoid dynamic compression during maneuvers and maintain the airway patency[116, 117].<br>✓ Stage 2 & 3 ("Collect and move up phase"): breathing progressively with high lung volumes towards the inspiratory | ✓ May improve ventilation homogeneity[118] | ✓ NE |

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| | glottis opened and including a breath hold after inspiratory phase. The sputum is cleared by cough or forced expiratory technique. Before starting autogenic drainage, patients should be taught how to exhale with glottis opened, using or not a mouthpiece. | reserve volume including a breath hold generate a greater trans-airway pressure gradient (mechanical stress) and also allow the air to move behind the obstructed lung units via collateral ventilation. | | |
| **ELTGOL** | ✓ <u>Patient positioning</u>: it is performed in the lateral decubitus position with the affected lung in the dependent position. Two lateral decubitus position should be recommended when both lungs are affected (Figure 9).<br>✓ <u>Breathing</u>: slow expirations from functional residual capacity to the end of expiratory reserve volume with the glottis opened. The sputum is cleared by cough or forced expiratory technique. Before starting ELTGOL, patients should be taught how to exhale with glottis opened, using or not a mouthpiece. | ✓ Placing the patient in the side-lying position, the airways in the dependent lung are stretched (mechanical stress) and, therefore, the airflow velocity increases in the medial and peripheral areas[117].<br>✓ Slow exhalations with an open glottis from the functional residual capacity to the end of the expiratory reserve volume maintain the airways slightly narrowed without dynamic compression[116, 117]. | ✓ Reduces pulmonary hyperinflation[119] | ✓ Reduces the frequency of exacerbations[120]<br>✓ Improves HRQoL[120]<br>✓ Reduces cough impact[120]<br>✓ Increases sputum removal[120] |
| **Non-oscillating PEP** | ✓ <u>Patient positioning</u>: it is generally performed in sitting position; however, | ✓ Active expiration against a mild expiratory resistance (10-25 $cmH_2O$) increasing the expiratory phase time and generating a greater trans-airway pressure gradient (mechanical stress) | ✓ NE | ✓ NE |

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| | an alternative position (supine, side-lying) may be also used.<br>✓ Breathing: slow inspirations with slightly greater volume than tidal volume followed by an end breath hold. After this breath hold, exhalation against a resistance occurs through the PEP device, using a mouthpiece or a mask. The sputum is cleared by cough or forced expiratory technique. A manometer may be used for education purposes to ensure a correct expiratory pressure (10-25 $cmH_2O$). | compared to normal breathing. This mechanism also uses collateral ventilation to enable the presence of air behind obstructed lung units.<br>✓ It may be useful to combine a PEP device with autogenic drainage or the ELTGOL technique in patients with higher airway resistance and/or lower elastic recoil pressure, moving the equal pressure point towards the cartilaginous airways[116]. | | |
| **Oscillating-PEP** | ✓ Patient positioning: it is generally performed in sitting position; however, an alternative position (supine, side-lying) may be also used.<br>✓ Breathing: slow inspirations with slightly greater volume than tidal volume followed by an end breath hold. After this breath hold, exhalation against a resistance occurs through the oscillating PEP device, using a mouthpiece or a mask | ✓ High frequency airflow oscillation (mechanical stress) during the expiratory phase improves the biophysical properties of the mucus (viscoelasticity) and stimulates the ciliary beat.<br>✓ Active expiration against a mild expiratory resistance (10-25 $cmH_2O$) increases the expiratory phase time and generates greater trans-airway pressure gradient (mechanical stress) than normal breathing. This mechanism also uses collateral ventilation to allow the presence of air behind obstructed lung units.<br>✓ It may be useful to combine an oscillating-PEP device with autogenic drainage or the ELTGOL technique in patients with | ✓ Enhances sputum removal during treatment in stable state[121]<br>✓ Reduces sputum viscosity[122]<br>✓ Decreases sputum adhesivity[123, 124]<br>✓ Increases cough transportability[123, 124]<br>✓ Decreases airway inflammation[124] | ✓ Improves HRQOL[126]<br>✓ Reduces cough impact[126]<br>✓ Improves exercise capacity[126]<br>✓ Increases sputum removal[126] |

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| (Figure 9). The sputum is cleared by cough or forced expiratory technique. | higher airway resistance and/or lower elastic recoil pressure, moving the equal pressure point towards the cartilaginous airways[116]. The vibration effect on the properties of the mucus may also improve the effectiveness of autogenic drainage or ELTGOL when both techniques are used in combination. | ✓ Reduces airway resistance[125]<br>✓ Slight improvement in lung function after treatment[119] |

ACBT= active cycle of breathing technique; ELTGOL = slow total expiration performed with the glottis opened in lateral posture; PEP = positive expiratory pressure; NE = not established

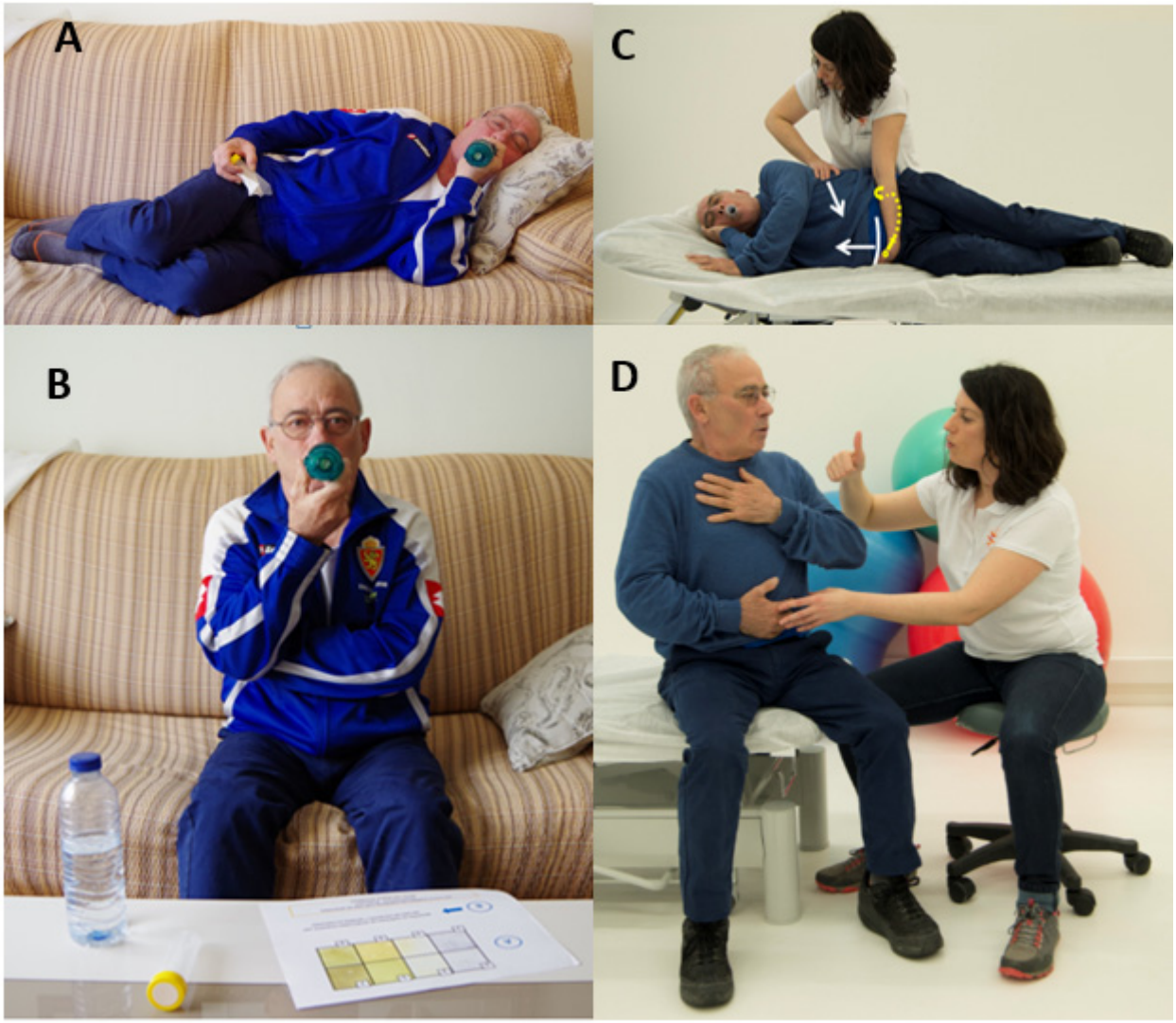


**Figure 9.** A patient with bronchiectasis and daily sputum expectoration is performing different airway clearance techniques. (A and B) ELTGOL combined with an oscillating-PEP device (Acapella) and autogenic drainage combined with an oscillating-PEP device (Acapella); He assesses the efficacy of the session at home using a volumetric container and a sputum colour chart validated in bronchiectasis[127]; (C and D) regular appointments at hospital/institution are encouraged to assist/educate/revise in the procedure of the different techniques (ELTGOL and autogenic drainage, respectively).

Traditionally, gravity-assisted drainage, passive chest manual techniques (percussion and vibration) and breathing control techniques (active cycle of breathing technique, ACBT) are more common in Anglo-Saxon countries[128]. However, slow-expiratory ACTs[117] (i.e., autogenic drainage or slow expiration with glottis open in lateral posture [ELTGOL]) are more frequently used in European francophone countries (France and Belgium) and adjacent countries (such as Spain[129]) and South American countries (such as Brazil) (Table 3). In Australia and New Zealand, the ACBT, positive expiratory pressure (PEP) devices and exercise are the most common techniques[130, 131]. Otherwise, instrumental ACTs seem to be more widely explored across the world. The preferences of specific countries or regions influence the clinical trials developed on this topic across the world (Table 3) and may influence the type of ACTs recommended in each guideline[47, 105].

While the Spanish Society of Pulmonology (SEPAR) guidelines highlight the beneficial effects obtained using slow-expiratory ACTs in patients with bronchiectasis and only recommend the use of gravity-assisted techniques in people with a low level of cooperation[105], the BTS guidelines advocated for the ACBT and gravity-assisted techniques as their preferred option[47]. In fact, no reference to the ELTGOL technique is included in this guideline, despite being the sole ACT which has demonstrated long-term benefits compared to no treatment in bronchiectasis[120]. The Brazilian and Saudi Thoracic guidelines recommend all ACTs but did not report any specific criteria for selecting them[106, 107] and the Australia and New Zealand Thoracic Society guideline did not mention any specific techniques[48] . Therefore, these discrepancies may also generate a heterogeneous airway clearance management approach within clinical practice across the world in people with bronchiectasis.

**Table 3**. Main characteristics of the studies evaluating the effects of airway clearance techniques in people with stable bronchiectasis published in the last 10 years

| Authors & Country | Study design | Participants | ACT used | Position | Frequency and duration | Outcomes |
|---|---|---|---|---|---|---|
| **de Souza et al.**[132] 2019 Brazil | Randomised crossover trial | 20 bronchiectasis 20 healthy | O-PEP (Flutter) *vs.* thoracic compressions *vs.* control | O-PEP= sitting Thoracic compression= sitting | ACT session=30 min Frequency=once per day Treatment duration=1 day | ✓ Sputum quantity during session + 30 min post intervention (wet and dry sputum weight)<br>✓ Sputum purulence (Murray scale)<br>✓ Biophysical sputum properties (adhesivity)<br>✓ Lung function (airway resistance using impulse oscillometry) |
| **Figueiredo et al.**[125] 2012 Brazil | Randomised crossover trial | 8 (≥ 25 mL of daily expectoration) | O-PEP (Flutter) *vs.* sham intervention | Sitting | ACT session=15 min + 5-min of cough Frequency=once per day Treatment duration=1 day | ✓ Sputum quantity during sessions (wet sputum weight)<br>✓ Lung function (airway resistance using impulse oscillometry)<br>✓ Patients´ feedback (acceptability and tolerability using a Likert scale) |
| **Guimarães et al.**[119] 2012 Brazil | Randomised crossover trial | 10 (daily productive cough) | ELTGOL *vs.* O-PEP (Flutter) *vs.* control | ELTGOL= right and left lateral decubitus (downward affected lung) O-PEP= sitting | ACT session=15 min + 5-min of cough Frequency=once per day Treatment duration=1 day | ✓ Sputum quantity during sessions (dry sputum weight)<br>✓ Lung function ($FEV_1$, FVC, $FEV_1$/FVC, $FEF_{25-75}$, IC,VC,TLC,FRC,RV, RV/TLC, IC/TLC) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | Control= sitting | | |
| **Mandal et al.**[133] 2012 UK | Randomised controlled trial | 30 (daily expectorating mucopurulent or purulent sputum) | O-PEP (Acapella) *vs.* Pulmonary Rehabilitation + O-PEP (Acapella) | O-PEP = sitting | ACT session=20-30 minutes Frequency=twice per day Treatment duration=8 weeks | ✓ Exercise capacity (ISWT, ESWT<br>✓ Questionnaires (LCQ, SGRQ)<br>✓ Lung function ($FEV_1$, FVC, $FEV_1$/FVC)<br>✓ Respiratory muscle strength (MIP, MEP)<br>✓ Systemic inflammation (cell count, ESR, CRP) |
| **Marques et al.**[134] 2012 UK | Quasi-experimental trial (Pre-Post) | 23 | ACBT | Unknown | ACT session= 24 min (15 to 30 min) Frequency= once per day Treatment duration= 1 day | ✓ Lung sounds (crackles, 2CD and the median number of crackles per respiratory phase)<br>✓ Lung function ($FEV_1$, FVC and peak expiratory flow)<br>✓ Vital signs (SpO2%, dyspnoea using modified Borg scale) |
| **Muñoz et al.**[120] 2018 Spain | Randomised controlled trial | 44 (≥10 mL of daily expectoration) | ELTGOL *vs.* placebo intervention (stretching) | ELTGOL = right and left lateral position (downward affected lung) | ACT session= 15 or 30 min Frequency= twice per day Treatment duration= 52 weeks | ✓ Sputum quantity for 24-hour (wet sputum volume)<br>✓ Sputum purulence<br>✓ Sputum bacteriology<br>✓ Exacerbations (frequency and time to first event)<br>✓ Questionnaires (LCQ and SGRQ)<br>✓ Exercise capacity (6MWT)<br>✓ Systemic inflammation (cell count, ESR, CRP, fibrinogen) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | ✓ Lung function ($FEV_1$)<br>✓ Safety (adverse events)<br>✓ Adherence (diary card) |
| **Murray et al.**[126]<br>2009<br>UK | Randomised crossover trial | 20<br>(daily expectoration) | O-PEP (Acapella) *vs.* control | Sitting | ACT session= 20-30 min<br>Frequency= twice per day<br>Treatment duration= 12 weeks | ✓ Questionnaires (LCQ, SGRQ)<br>✓ Sputum quantity for 24-h (sputum volume)<br>✓ Lung function ($FEV_1$, FVC, $FEV_1$/FVC)<br>✓ Respiratory muscle strength (MIP, MEP)<br>✓ Exacerbations (frequency)<br>✓ Exercise capacity (ISWT) /<br>✓ Sputum bacteriology |
| **Naraparaju et al**.[135]<br>2010<br>India | Randomised crossover trial (pilot study) | 30<br>(>30 mL of daily expectoration) | O-PEP (Acapella) *vs.* IMT | O-PEP =sitting<br>IMT = sitting | ACT session=5 repetitions + cough/huff + 5 repetitions + cough/huff<br>Frequency=once per day<br>Treatment duration=1 day | ✓ Sputum quantity (sputum volume during session and in 2-hour period after interventions)<br>✓ Patients´ feedback (PSS) |
| **Nicolini et al.**[136]<br>2013<br>Italy | Randomised controlled trial | 30<br>(>20 mL of daily expectoration) | HFCWO *vs.* group of techniques (ELTGOL, O-PEP, fixed-PEP, postural drainage, percussion) *vs.* control | HFCWO= sitting<br>Group of techniques= unknown | HFCWO session= 30 min<br>Group of techniques = 40 min<br>Frequency=twice per day<br>Treatment duration=2 weeks | ✓ Questionnaires (BCSS, CAT)<br>✓ Lung function ($FEV_1$, FVC, TLC, RV)<br>✓ Systemic inflammation (cell counts, CRP)<br>✓ Dyspnoea (mMRC)<br>✓ Sputum quantity for 1 hour since the session started (sputum volume) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | ✓ Respiratory muscle strength (MIP and MEP)<br>✓ Gas arterial (pH, $PaO_2$, $PaCO_2$)<br>✓ Airway inflammation (cell counts) |
| **Paneroni et al.**[137]<br>2011<br>Italy | Randomised crossover trial | 22<br>(>20 mL of daily expectoration) | IPV *vs.* postural drainage + percussion + vibration + forced expiration | IPV= sitting<br>CPT=prone, right and left lateral decubitus (upward affected lung) and sitting | ACT session=30 min<br>Frequency=once per day<br>Treatment duration=1 day | ✓ Sputum quantity during session and for up 4 hours after session (wet sputum weight and dry sputum weight)<br>✓ Patients feedback (VAS) |
| **Poncin et al.**[118]<br>2017<br>Belgium | Quasi-experimental trial (Pre-Post) | 26<br>(daily expectoration) | Autogenic drainage | Semi recumbent position (45° angle from the horizontal | ACT session= 30 min<br>Frequency=once per day<br>Treatment duration= 1 day | ✓ Lung function (LCI, $FRC_{multiple\text{-}breath\ washout}$, SVC, $FEV_1$, FVC, $FEV_1$/FVC, $FEF_{25\text{-}75}$, TLC, $FRC_{plethysmography}$,RV, RV/TLC, Raw, Gaw)<br>✓ Sputum quantity (wet and dry sputum weight) |
| **Powner et al.**[138]<br>2019<br>US | Retrospective cohort study | 65<br>(daily sputum production) | HFCWO | Unknown | ACT Session= 30 min<br>Frequency= once / twice per day<br>Treatment duration= 1 year | ✓ Exacerbations (frequency, number of hospitalisations, antibiotic use)<br>✓ Lung function ($FEV_1$, FVC, $FEF_{25\text{-}75}$ ) |
| **Ramos et al.**[139]<br>2015<br>Brazil | Randomised crossover trial | 22<br>(≥15 mL of daily expectoration) | Coughing *vs.* postural drainage + coughing *vs.* postural drainage + percussion + coughing *vs.* postural drainage + huffing | Right and left lateral position (upward affected lung) | ACT Session= 90 min<br>Frequency= once per day<br>Treatment duration= 1 day | ✓ Biophysical sputum properties (viscosity, elasticity, percentage of solids) |

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| **Ramos et al.**[122] 2009 Brazil | Randomised crossover trial | 15 (constant volume/aspect of the secretion) | O-PEP (Flutter) at 15cm$H_2O$ *vs.* O-PEP (Flutter) at 25cm$H_2O$ | Sitting | ACT session=30 min (10-min break included) Frequency=once per day Treatment duration=1 day | ✓ Biophysical sputum properties (mucociliary transport, cough transport, contact angle, viscosity) |
| **Semwal et al.**[140] 2015 Indian | Randomised crossover trial | 30 (daily expectoration) | O-PEP (Acapella) *vs*. Autogenic drainage | O-PEP= sitting Autogenic drainage= sitting | ACT session=20-30 min Frequency=once per day Treatment duration=1 day | ✓ Sputum quantity during session and 10 min after intervention (sputum volume, sputum weight)<br>✓ Lung function (peak expiratory flow)<br>✓ Vital signs (dyspnoea using modified Borg scale, SpO2%) |
| **Shabari et al.**[141] 2011 India | Randomised crossover trial | 40 (>30 mL of daily expectoration) | O-PEP (Acapella) *vs.* O-PEP (Rc-cornet) | O-PEP (Acapella) = sitting O-PEP (Rc-cornet) = patients´ preference | ACT session=15-20 min Frequency=once per day Treatment duration=1 day | ✓ Sputum quantity during session and for up 2 hours after session (sputum volume)<br>✓ Patients feedback (PPS) |
| **Silva et al.**[142] 2017 Australia | Randomised crossover trial | 40 (≥25 mL of daily expectoration) | O-PEP (Flutter) *vs.* O-PEP (Lung Flute) | Sitting | ACT session= Lack of secretions or 30 min Frequency=once per day Treatment duration= 1 day | ✓ Sputum quantity (wet and dry sputum weight)<br>✓ Patients´ perception of safety and tolerability (Likert scale)<br>✓ Patients´ preference |
| **Su et al.**[143] 2012 Taiwan | Randomised crossover trial | 26 (≥30 mL of daily expectoration) | IPPB + postural drainage + ACBT *vs.* NPV + postural drainage + ACBT | IPPB = sitting NPV = supine | ACT session=1 hour Frequency=once per day Treatment duration=4 weeks | ✓ Vital signs during sessions (SpO2%, pulse rate, dyspnoea using modified Borg scale)<br>✓ Lung function ($FEV_1$, FVC)<br>✓ Exercise capacity (6MWT) |

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| | | | | | | ✓ Recovery after an exercise test (SpO2%, pulse rate, Borg)<br>✓ Patients´ feedback (cough difficulty, VAS)<br>✓ Use of accessory muscles (modified manual muscle test) |
| **Svenningsen et al.**[144]<br>2017<br>Canada | Quasi-experimental trial (Pre-Post) | 15 bronchiectasis<br>15 healthy | O-PEP (Aerobika) | Unknown | ACT session 30 min<br>Frequency=once per day<br>Treatment duration= 3 weeks | ✓ Questionnaires (SGRQ, PEQ)<br>✓ Lung function ($FEV_1$, FVC)<br>✓ Exercise capacity (6MWT)<br>✓ Magnetic resonance imaging (VDP) |
| **Syed et al.**[115]<br>2009<br>India | Randomised crossover trial | 35<br>(>30 mL of daily expectoration) | Postural drainage + ACBT *vs.* postural drainage + percussion + vibration | Posture according to gravity assistance drainage | ACT session=20-30min<br>Frequency=thrice per day<br>Treatment duration=1 day | ✓ Sputum quantity for 24 hours including sessions (sputum volume and sputum weight)<br>✓ Lung function ($FEV_1$, FVC, FEV1/FVC)<br>✓ Patients feedback (therapy comfort, VAS) |
| **Tambascio et al.**[123]<br>2011<br>Brazil | Randomised crossover trial | 18<br>(able to provide a sputum sample) | O-PEP (Flutter) *vs.* PEP (Flutter without oscillation | O-PEP=sitting<br>PEP= sitting | ACT session=30min<br>Frequency=once per day<br>Treatment duration=4 weeks | ✓ Biophysical sputum properties (mucociliary transport, cough transport, contact angle) |
| **Tambascio et al.**[124]<br>2017<br>Brazil | Randomised crossover trial | 17<br>(> 0.5 mL of sputum sample) | O-PEP (Flutter) *vs.* sham intervention | Sitting | ACT session= 30 min<br>Frequency= once per day<br>Treatment duration= 4 weeks | ✓ Biophysical sputum properties (mucociliary transport, cough transport, contact angle, adhesiveness)<br>✓ Sputum colour (Murray scale) |

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|---|---|---|---|---|---|---|
| | | | | | | ✓ Sputum bacteriology<br>✓ Airway inflammation (total cell count) |
| **Üzmezoğlu et al.**[145]<br>2018<br>Turkey | Randomised controlled trial | 40 | ACBT + PD *vs.* O-PEP (Flutter) | ACBT + PD = posture according to gravity assistance drainage<br>O-PEP= unknown | ACT session= 15 or 20 min<br>Frequency= twice per day<br>Treatment duration= 4 weeks | ✓ Symptoms reported by participants (cough, fatigue, wheezing, loss of appetite)<br>✓ Sputum quantity (4-category scale)<br>✓ Lung function (not reported)<br>✓ Dyspnoea (MRC and Borg)<br>✓ Questionnaires (SF-36) |

O-PEP= oscillating positive expiratory pressure; ACT= airway clearance techniques; PEP=positive expiratory pressure; ELTGOL= slow expiration with glottis opened in lateral posture; $FEV_1$= forced expiratory volume in the first second; FVC= forced vital capacity; $FEF_{25-75}$= forced expiratory flow at 25-75% of the FVC; IC= inspiratory capacity; VC= vital capacity; TLC= total lung capacity; FRC=functional residual capacity; RV= residual volume; ISWT= incremental shuttle walk test; ESWT= endurance shuttle walk test; LCQ= leicester cough questionnaire; SGRQ= St. George´s respiratory questionnaire; MIP= maximal inspiratory pressure; MEP= maximal expiratory pressure; ESR=erythrocyte sedimentation rate ; CRP= C-reactive protein; 6MWT= six minute walk test; IMT= inspiratory muscle training; PSS= patient preference scale; HFCWO= high frequency chest wall oscillation; BCSS= breathlessness cough and sputum scale; CAT= COPD assessment test; mMRC= modified medical research council; IPV= intrapulmonary percussive ventilation; VAS= visual analogic scale; IPPB= intermittent positive pressure breathing; NPV= negative pressure ventilation; ACBT= active cycle of breathing technique; SF-36= short form health survey questionnaire; LCI= lung clearance index; SVC= slow vital capacity; Raw= airway resistance; Gaw=airway conductance; PEQ=patient evaluation questionnaire; VDP= ventilation defect percent. The manuscripts of the present thesis were not included in this table.

After reviewing all identified studies analysing the effects of ACTs in people with clinically stable bronchiectasis that had been published in the last 10 years[109, 114], the PEP devices (fixed or oscillating) are the most frequently explored ACTs, followed by gravity-assisted and breathing control (ACBT) techniques. Therefore, the number of studies that assess slow-expiratory ACT effects (i.e., autogenic drainage or ELTGOL, Figure 9) are still very limited in people with bronchiectasis despite the preference for these techniques in clinical practice/research in some regions, such as Spain[129] (Table 3).

The use of PEP devices offers advantages for people with chronic expectoration. These devices are used independently (self-administered), and the process to correctly learn the technique seems be easier when compared with other ACTs. Moreover, the use of an external device may help to avoid daily monotonous treatment. All these factors promote self-management[110] and may be related to the strong interest of these devices in people with bronchiectasis.

The physiological action of PEP devices (fixed and oscillating) are detailed in Table 2 and also nicely explained in the Cochrane systematic review conducted by Lee et al.[114]. Overall, if the main objective is to enhance sputum expectoration, the use of an oscillating PEP device may be more appropriate. Oscillating PEP devices combine the benefits of a fixed PEP device with high frequency oscillation during the expiratory phase, a design that improves the biophysical properties of mucus and stimulates ciliary beating. Murray et al.[126] demonstrated that twice daily airway clearance sessions using an oscillatory PEP device (Acapella) improves cough severity and HRQoL, increases 24-hour sputum volume and improves exercise tolerance after 3-month treatment in people with bronchiectasis. Tambascio and colleagues[123, 124] also found that a flutter device

(oscillating-PEP device) used over 4 weeks improves cough clearance, reduces sputum adhesivity and may decrease airway inflammation in people with bronchiectasis. Therefore, the use of an oscillating PEP device in our target population appears to improve the biophysical and surface mucus properties and promote clinical benefits at long term.

Moreover, a new PEP modality, namely temporary positive expiratory pressure (TPEP), has recently gained interest in some European countries. The TPEP modality generates a minimum positive pressure (1 $cmH_20$) with an intermittent vibration (42 Hz) during the first two thirds of expiration that quickly falls during the last third of expiration. The TPEP mechanism of action tries to avoid an airway collapse during prolonged expirations by using minimal resistance to avoid fatigue and maintain the benefits obtained from vibrations. Although TPEP appears to be a useful ACT to improve airway clearance in people with COPD[146, 147], its effectiveness in bronchiectasis has not been sufficiently explored.

Although autogenic drainage is one of the most frequent ACTs evaluated in research and used in clinical practice by people with cystic fibrosis[148, 149], it is rarely selected in studies that involve people with bronchiectasis (Table 3). In a quasi-experimental study, Poncin et al.[118] found a slight improvement in the ventilation distribution after a single session of the autogenic drainage technique. The findings also suggested that a greater change in ventilation inhomogeneity is associated with people who expectorated greater amount of sputum during the session. On the other hand, O´Connor et al.[150] reported that although autogenic drainage promoted higher sputum expectoration during a single session compared with a control period in people with bronchiectasis, there were no changes in airway resistance after the intervention. Finally, the randomised crossover trial conducted by Semwal et at.[140] showed that autogenic

drainage technique and an oscillatory PEP device (Acapella) enhance similar sputum expectoration after a single intervention.

On the other hand, the physiological short-term effects of the ELTGOL technique in bronchiectasis was analysed by Guimarães et al.[119] in 2012. They reported that this technique slightly reduces lung hyperinflation and enhances greater sputum expectoration compared to oscillatory PEP devices (flutter device). However, these findings were only focused on the immediate/acute effects of a single session without considering the possible post-intervention effects. Indeed, the use of repeated measures to assess the short-term effects of ACTs may be more appropriate than a single session due to the high variability observed for most commonly outcome measured used.

Recently, it was reported that twice daily ELTGOL treatment for 12 months enhances greater 24-hour sputum expectoration, improves cough severity and HRQoL and reduces the frequency of exacerbations in people with bronchiectasis. These data were from the first long-term ACT study in people with bronchiectasis[120]. Despite these promising benefits, some methodological concerns (i.e., sputum volume selection as the primary endpoint for a long-term study, the standard deviation selected for the sample size calculation may be underestimated, and between-group difference not reported for the primary outcome) may partially challenge the results. Independent of these methodological issues, further studies are needed to confirm the long-term benefits of slow-expiratory ACTs and improve the level of evidence of these interventions.

Slow-expiratory ACTs have not yet been compared in people with bronchiectasis. Thus, before conducting another long-term trial using slow-expiratory ACTs, it may be

appropriate to first compare different slow-expiratory ACTs (based on the same mechanism actions) over a short-term period to identify whether the clinical benefits and patients´ preference are comparable between the techniques. The issues to consider for improving the methodological quality of short-term trials in airway clearance field are: i) repeated sessions may be more appropriate than single session to maximise the accuracy of the findings[151, 152]; ii) a crossover trial is recommended when the primary endpoint shows higher variability (such as sputum quantity) because each patient will serve as her or his own control (intra-subject comparison)[153]; iii) the measurement periods may also assess the impact of the intervention after the airway clearance session in patients´ daily life[98, 100] to understand better the potential benefits of these treatments (long-lasting effects)[154].

## 8.2 Mucoactive treatments in people with bronchiectasis

Mucoactive therapies should be considered when people with bronchiectasis suffer a deterioration of their daily respiratory symptoms and an optimal ACT approach is not enough to manage these symptoms that negatively impact HRQoL[46, 47]. Therefore, it is recommended as the second-line treatment after reviewing the ACTs optimisation (appropriate frequency, level of adherence, correct ACT selected according to patients´ preferences and clinical characteristics)[47]. There is no consensus about the level of evidence of these treatments in people with bronchiectasis, but the majority of guidelines reported a low-moderate level of evidence with a weak-strong recommendation[46-48, 106-108].

Mucoactive drugs are used to enhance mucus clearance, improve coughing and/or reduce mucus hypersecretion[155]. According to the potential mechanism of action,

mucoactive therapies are classified in four groups: mucolytics, mucokinetic, mucoregulator and expectorants[156, 157].

Mucolytic medications decrease mucus viscosity because they can degrade or dissociate mucins or other mucus components. Examples include N-acetylcysteine (breaks disulphide bonds in mucins)[156] and DNase (degrades the copolymer network of DNA and filamentous actin)[157]. Mucokinetic drugs improve sputum clearance by increasing ciliary beating and airflow (β-2 agonist bronchodilators) or reducing adhesion between the mucus layer and ciliary tip (surfactants). Mucoregulator agents decrease stimulated mucus hypersecretion, either by their anti-inflammatory activity (e.g., glucocorticoids or macrolide antibiotics) or by inhibiting a particular aspect of mucus physiology (e.g., anti-cholinergic drugs)[156, 157]. Finally, expectorants increase mucus hydration and may lead to the secretion of mucins to a point where a sufficient volume of mucus is produced to enable it to be expectorated[156, 157]. These drugs are often irritants and stimulate coughing to facilitate expectoration[155, 156]. Hypertonic saline (HS) and mannitol are examples of expectorants.

A recent systematic review that analysed the mucolytic agents effects in people with bronchiectasis highlighted the negative impact of DNase on lung function and exacerbations in this population. In addition, although N-acetylcysteine or carbocysteine are widely used in clinical practice, there is limited evidence to support the routine use of these therapies[5, 158]. Therefore, the use of DNase should not be offered to patients with bronchiectasis[46, 47, 105], and further investigation is needed to clarify whether other mucolytic drugs may be a useful adjunct to enhance sputum clearance in combination with other treatments such as ACTs[158].

The use of mucokinetic or mucoregulator agents to promote sputum expectoration has been poorly explored in patients with bronchiectasis. However, Bennett et al.[82] described the potential applicability of new therapies such as ENaC inhibitors (reduces the reabsorption of fluid from the airway layer surface compartment) in combination with expectorants to enhance sputum clearance when mucus dehydration and mucus clearance impairment are relevant features for the disease progression, including in some people with bronchiectasis.

Moreover, the use of CFTR protein modulators may represent another alternative[82, 159]. Some individuals with bronchiectasis present a CFTR-related disorder that may play an important role in the disease pathogenesis[159]. The use of an appropriate CFTR modulator may be effective even in the absence of genetic mutations by increasing the activity of residual channels, stimulating anion secretion, improving mucus hydration and accelerating mucus clearance[82, 159]. New studies that explore these effects are expected over the next years. In fact, a two-phase crossover trial has already been completed in people with primary ciliary dyskinesia. It assessed the effects of an ENaC inhibitor (VX-371) in combination with HS or HS + CFTR modulator (ivacaftor; trial NCT02871778); however, their findings are not yet published.

Regarding expectorant drugs, HS and mannitol are the most studied hyperosmolar agents in people with bronchiectasis. These hyperosmolar drugs produce an osmotic shock in the airway that draws fluid from the airway epithelium onto the ASL to improve airway hydration. This extra volume decreases the concentration of mucus solids and thereby accelerate mucus transportability[82, 160]. Hyperosmolar agents may also break ionic bonds and change the biophysical properties of mucus[88, 161, 162]. The potential anti-inflammatory and antimicrobial effects of these agents in the airways remains

unclear[163]. Table 4 shows the main characteristics of the studies evaluating the hyperosmolar agent (HS and mannitol) effects in people with bronchiectasis published in the last 10 years.

Three clinical trials have analysed the long-term benefits of 6 or 7% HS in people with bronchiectasis[164-166]. The solution concentration was based on cystic fibrosis findings[166-168]. Globally, it appears that HS is not superior compared to isotonic saline (IS) solution in improving quality of life, lung function and exacerbation frequency in bronchiectasis[162]. These findings clearly contrast with the clinical effects observed in people with cystic fibrosis[169] and reflect that treatment extrapolation from studies in other respiratory diseases may not always be appropriate in bronchiectasis and reinforce the need for further research.

**Table 4.** Main characteristics of the studies evaluating the effects of hyperosmolar agents (HS and mannitol) in people with stable bronchiectasis published in the last 10 years.

| Authors & Country | Study design | Participants | Hyperosmolar solution | ACTs | Frequency and duration | Outcomes |
|---|---|---|---|---|---|---|
| **Bilton et al.**[170] 2014 Multicenter n=84 (USA, Europe, Australia, New Zealand, South America) | Randomised controlled trial | 461 ($FEV_1$≥ 40% and ≤85% pred and ≥ 1L; SGRQ ≥ 30; exacerbation frequency ≥ 2; >10 mL of daily expectoration) | Mannitol (40mg) vs mannitol (5mg) | O-PEP (Acapella) Not monitored | Frequency=twice per day<br>Treatment duration= 52 weeks | ✓ Exacerbation (frequency, time to first event, duration, number of hospitalisation and antibiotic use)<br>✓ Questionnaires (SGRQ)<br>✓ Sputum quantity over 24-hour (sputum weight)<br>✓ Lung function ($FEV_1$, FVC)<br>✓ Adherence (capsules count)<br>✓ Safety (blood count, renal and liver function, sputum bacteriology, physical examination, tolerance test, adverse events) |
| **Bilton et al.** [171] 2013 Multicenter n=22 (Australia, New Zealand and UK) | Randomised controlled trial | 343 ($FEV_1$≥ 50% and ≥ 1L; >10 mL of daily expectoration) | Mannitol (40mg) vs placebo capsules (10mg) | O-PEP (Acapella) Not monitored | Frequency = not clearly reported<br>Treatment duration= 12 weeks + optional extension over 52 week | ✓ Sputum quantity over 24-hour (sputum weight)<br>✓ Questionnaires (SGRQ, LCQ, BSQ)<br>✓ Lung function ($FEV_1$, FVC)<br>✓ Exacerbation (frequency, time to first antibiotic use)<br>✓ HRCT |

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| | | | | | | ✓ Exercise capacity (ISWT)<br>✓ Sputum bacteriology<br>✓ Airway inflammation (IL-6, IL-8, elastase, tumor necrosis factor-α)<br>✓ Adherence (not reported)<br>✓ Safety (tolerance test, sputum bacteriology, adverse events) |
| **Daviskas et al.** [172]<br>2010<br>Australia | Randomised crossover trial | 14 | Mannitol (160mg) vs mannitol (320mg) vs mannitol (480mg) vs control | 100 coughs | Frequency=once per day<br>Treatment duration= 1 day | ✓ Biophysical sputum properties (solids content, surface tension, contact angle, adhesion, viscosity and elasticity)<br>✓ Amount of mannitol in the sputum<br>✓ Safety (tolerance test) |
| **Kellet et al.** [165]<br>2011<br>UK | Randomised crossover trial | 28 | HS at 7% vs IS at 0.9% | - | Frequency=once per day<br>Treatment duration= 12 weeks | ✓ Lung function ($FEV_1$, FVC)<br>✓ Questionnaires (SGRQ)<br>✓ Exacerbations (frequency, antibiotic use)<br>✓ Biophysical sputum properties (viscosity using a 4-ordinal scale)<br>✓ Patients´ feedback (ease of expectoration, VAS)<br>✓ Safety (tolerance test) |
| **Máiz et al.** [173]<br>2018 | Longitudinal study | 137<br>($FEV_1$≥ 35% or ≥1L;<br>>30 mL of daily expectoration | HS at 7% or HA at 0.1% +HS at 7% | - | Frequency=twice per day | ✓ Tolerance (symptoms using Likert test, tolerance test)<br>✓ Questionnaires (QoL-B, LCQ) |

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| Spain | | | | | Treatment duration= 4 weeks | ✓ Adherence (Morisky-Green, Haynes-Sackett)<br>✓ Adverse events |
| **Nicolson et al.** [164]<br>2012<br>Australia | Randomised controlled trial | 38<br>($FEV_1$≥ 1L;<br>daily sputum expectoration;<br>exacerbation frequency ≥ 2) | HS at 6% vs IS at 0.9% | Most participants were adherent at the beginning of trial, but ACTs adherence during trial was not reported | Frequency=twice per day<br><br>Treatment duration= 52 weeks | ✓ Questionnaires (SGRQ, LCQ)<br>✓ Exacerbation requiring antibiotics or not (frequency, duration, hospitalisation)<br>✓ Cough frequency (VAS)<br>✓ Lung function ($FEV_1$, FVC, $FEF_{25-75}$)<br>✓ Aadherence (dairy)<br>✓ Safety (tolerance test) |
| **Paff et al.**[166]<br>2017<br>Netherlands | Randomised crossover trial | 22<br>(primary ciliary dyskinesia;<br>$FEV_1$≥ 40% | HS at 7% vs IS at 0.9% | - | Frequency=twice per day<br><br>Treatment duration= 12 weeks | ✓ Questionnaires (SGRQ, QoL-B)<br>✓ Symptoms (modified LRTI-VAS)<br>✓ Exacerbations (frequency, time to first event)<br>✓ Lung function ($FEV_1$, FVC, $FEF_{25-75}$)<br>✓ Airway inflammation (total count, IL-β, IL-6, IL-8, IL-10, elastase, tumor necrosis factor-α, myeloperoxidase, IFN- α and IFN- β)<br>✓ Systemic inflammation (total count, CRP, ESR)<br>✓ Adherence (capsules count) |

$FEV_1$= forced expiratory volume in the first second; SGRQ= St. George´s respiratory questionnaire; O-PEP= oscillating positive expiratory pressure; FVC= forced vital capacity; LCQ= leicester cough questionnaire; BSQ= bronchiectasis symptoms questionnaire; HRCT= high resolution computerised tomography scan; ISWT= incremental shuttle walk test; HS=hypertonic saline; HA=hyaluronic acid; QoL-B= quality of life of bronchiectasis; ACT= airway clearance techniques; $FEF_{25-75}$= forced expiratory flow at 25-75% of the FVC; LRTI= lower respiratory tract infection; VAS= visual analogic scale; IFN= interferon; ESR=erythrocyte sedimentation rate ; CRP= C-reactive protein. The manuscripts of the present thesis were not included in this table

The lack of clearly observed benefits with HS compared to IS in most of these clinical trial studies[164, 166] may be related to different factors. First, the response to HS may be better in people with more advanced disease[82, 162]. Therefore, greater benefits are expected in people with poor lung function; however, the impairment of lung function was only mild/moderate (forced expiratory volume in the first second, $FEV_1$% pred., from 66 to 80%) in the population included in these clinical trials. Second, the use of HS may be more suitable for people who do not have control of their daily sputum symptoms or who have a substantial daily sputum burden with a negative impact on their HRQoL[46, 82]. These clinical trial studies did not consider these factors as selection criteria (Table 4). Only Nicolson et al.[164] requested that subjects have daily expectoration, but they did not consider a minimum amount of sputum or a reduced HRQoL due to sputum symptoms. Third, the possible use of ACTs as a concomitant treatment was not correctly monitored in any of the trials (Table 4). This factor could play a major confounding factor of the findings observed.

It is also important to highlight that although Kellet et al.[165] observed an improvement in HRQoL, lung function and fewer number of exacerbations in favour of the HS group, their findings should be taken with caution. The authors did not show evidence about the impact of possible carry-over effects on their results and the sample size was not estimated. Thus, it remains unclear whether the power of the study was sufficient to ensure the accuracy of findings.

In 2013 and 2014, Bilton et al.[170, 171] conducted multicentre randomised controlled trials to analyse the long-term impact of the regular use of mannitol (twice daily) in people with bronchiectasis. Globally, their findings suggested that although the time to the first exacerbation was longer in favour of the mannitol group, the regular use of this

expectorant did not impact on the exacerbation rate after 52-week treatment in bronchiectasis (primary endpoint of the study)[170]. The mannitol group also improved their HRQoL, but did not reach the minimal clinically important difference (MCID), and there were no changes in lung function in either group[170]. Therefore, the clinical benefits observed are more inconspicuous compared to what was observed in people with cystic fibrosis[174]. Unlike the other studies about the use of HS, the selection criteria proposed by Bilton et al.[170] in 2014 required impaired HRQoL and at least 10 grams of daily expectoration (Table 4). Additionally, all participants received an oscillating PEP device at the beginning of the study; however, the adherence rate to this intervention was not monitored. Consequently, the participants included in this study were better adapted to the possible profile of patients with bronchiectasis who respond satisfactorily to mucoactive treatments.

The effects of mannitol and HS have not yet been compared in people with bronchiectasis. The main advantage of mannitol over HS is its easily administration using a dry-powder inhaler. Therefore, it is a treatment that requires little time and does not need to be cleaned or sterilised, being both essential factors to ensure long-term adherence[175]. Although inhaled mannitol has been approved for use in adults in Europe, it is unfortunately not yet available in Spain[105].

The presence of adverse events (bronchospasm, excessive coughing, throat irritation, chest tightness and/or salty taste) using hyperosmolar agents are risk factors for intolerance to these treatments or poor long-term adherence[163, 173]. Therefore, a tolerability test in hospital is required before starting this treatment in all patients[46]. Age and a greater lung function decline appear to be factors associated with lower rate of tolerance in people with bronchiectasis[173]; however, those people may be better

candidates for these treatments and they are often excluded from the studies because they fail the initial tolerability test.

Hyaluronic acid (HA) is a glycosaminoglycan that can mitigate bronchospasm induced by elastases and balance water homeostasis in airways[176, 177]. The addition of HA to HS solution has already explored in patients with cystic fibrosis. This combination improves tolerance and pleasantness in this population[178, 179]. Based on cystic fibrosis findings, the HA + HS solution is used in the clinical practice in Spain for patients with bronchiectasis and is even recommended in the national guidelines[105], even though its evidence remains scarce in this disease.

In 2018, Maíz et al.[173] found in a Spanish longitudinal study that almost 70% of patients intolerant to HS (7%) passed the initial tolerability test using the combined solution of HA (0.1%) + HS (7%), and almost 60% of the participants continued the treatment for 4 weeks. Therefore, it appears that the tolerance to the combined solution (HA + HS) is greater than HS solution in patients with bronchiectasis. However, before conducting a long-term study using the HA + HS solution in bronchiectasis, it may be appropriate to explore whether the short-term effects (sputum clearance, respiratory symptoms and HRQoL) are at least equal for both solutions in bronchiectasis patients.

Notably, a combined treatment that includes hyperosmolar agents and ACTs may be the most appropriate approach to correctly manage daily symptoms related to productive cough. Indeed, as mentioned above, the use of hyperosmolar agents is only recommended after the ACT treatment is optimised[47]. In clinical practice, ACTs are often applied after hyperosmolar agents and before inhaled antibiotics. Previous studies performed in patients with cystic fibrosis suggest that HS inhalation during ACT has similar

clinical benefits to HS inhalation before ACT, with the benefit of saving time[180, 181]. However, further research is needed in people with bronchiectasis to clarify this finding.

In subjects with clinically stable bronchiectasis and mild daily sputum expectoration (< 10 grams per 24 hours), greater sputum weight was obtained during combined sessions (hyperosmolar agents + ACTs) using HS rather than IS(182). However, Kellet et al.[182] did not clarify whether the benefit observed in favour of HS plus ACT in comparison with IS plus ACT is related to the inhalation solution itself (different osmolality) or to a greater potential effect of the posterior effectiveness of ACTs. For this reason, additional research to better understand the short-term action mechanism of the combination of hyperosmolar agents and ACTs in people with bronchiectasis is needed to improve the design of the future long-term studies in this field.

In summary, short-term trials are welcome to deeply analyse the clinical benefits in terms of enhancing expectoration, safety and patients´ preference for HA + HS treatment and to identify the possible synergistic mechanism of hyperosmolar agents with ACT in people with bronchiectasis. These trials should be designed considering the same methodological issues explained in the previous section of ACTs (repeated measurements, intra-subject comparison, assess the long-lasting effects)[151, 152, 154] to ensure an adequate quality.

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# 9. Outcome measures to assess short-term effects of airway clearance in people with bronchiectasis

## 9.1. Mucociliary and cough clearance in vivo

Assessment of the time required to clear a tracer from the airways is considered the most valid measure of mucociliary and cough transport[183, 184]. Findings are frequently reported as percentage of retention or percentage of clearance over time[185], and measurement periods are usually acquired at 2, 4-6 or 24 hours post-intervention[186]. Although this test is considered the gold standard outcome measure for short-term periods, its use is limited due to patients having to inhale a radioactive tracer and the low accessibility to the highly specialised equipment required (i.e., gamma camera). Few studies in people with bronchiectasis have included this outcome measure as part of their design. Specifically, the mucociliary clearance rate has been used to assess the short-term effects of mannitol[160, 187, 188] and the effect of different therapeutic strategies (humidification, nebulised saline and nebulised terbutaline) as an adjunct to ACTs[185, 189].

The *in vivo* mucociliary clearance rate is more suitable for intra-subject comparison due to the high variability[184, 190]. In order to minimise this factor, it is recommended to control the particle size and the inhalation patterns to ensure similar initial regional deposition on airways[184]. Moreover, the use of the regional deposition index as covariance in the statistical analysis is also suggested to ensure greater accuracy of the results[184]. Finally, the frequency and time of spontaneous coughs should be monitored (or even introduce a control treatment arm with voluntary coughs equal to the number of spontaneous coughs in the active treatment arm) to ensure that cough clearance does not influence the results[184].

Although it is known that the mechanism of action to enhance sputum clearance is different among ACTs[102], all of them apparently provide similar short-term benefits to people with bronchiectasis[109, 114]. However, it remains unknown whether ACTs with different physiological actions provide a similar impact on airway clearance, as assessed by mucociliary clearance rates, in this population.

Moreover, the controversial findings shown in trials with mucoactive treatments conducted in bronchiectasis[164, 170] may be because individuals with bronchiectasis present diverse mucociliary clearance impairments or heterogeneous responses to these treatments[191]. Therefore, the use of mucociliary clearance rate as an outcome measure is needed to better understand the physiologic respond to these treatments according to disease severity or other specific-characteristics in people with bronchiectasis and also to identify which population is more likely to response to airway clearance treatments. Consequently, future studies that use mucociliary clearance rates in people with bronchiectasis will allow the possibility to better understand the physiological response to airway clearance interventions and, thus, to design better future long-term studies.

### 9.2. Sputum samples

Sputum samples are useful to analyse surrogate markers of airway clearance effects (e.g., sputum quantity, biophysical and surface properties, inflammatory markers and bacterial load). Daily sputum expectoration is an essential selection criterion for the majority of trials that involve airway clearance approaches in people with bronchiectasis (Table 3 and 4), and it is a common respiratory symptom reported by around 65% of this population[91]. Thus, it is important to highlight as a potential limitation that not all people with bronchiectasis can provide spontaneous sputum samples.

### *9.2.1. Sputum quantity*

The main objective of airway clearance interventions is to enhance sputum clearance and, thus, the use of sputum quantity appears to be a reasonable outcome measure to analyse their effects. Sputum quantity is a feasible outcome measure that is inexpensive, easily implemented in clinical practice and considered relevant to people with bronchiectasis[99, 192]. These reasons explain the widespread use of sputum quantity for the management of people with bronchiectasis. Moreover, it is an important factor to identify exacerbations[97] and also for its use in clinical research assessing effects of ACTs[120, 193], mucoactive therapy[170, 194] and even antibiotics[195, 196].

Sputum quantity is usually measured as sputum weight (grams) or sputum volume (mL). Sputum weight is preferred used when a calibrated scale is available and participants are not involved in the measurement process[121]. Although sputum volume and weight may be comparable[121], both methods have not yet been adequately compared. Indeed, there is a tendency to overestimate the findings obtained using sputum volume compared to sputum weight[197]. Weighting the amount of sputum may be a more accurate method because it depends on neither the graduated scale on the containers nor on the assessors´ interpretation.

The sputum quantity ratio (i.e., sputum collected over a period of time relative to total sputum collected over 24 hours, expressed as a percentage) is another potential outcome measure to assess the efficacy of airway clearance interventions[198]. The sputum quantity ratio allows one to identify the impact of airway clearance interventions on sputum expectoration during the sessions as well as throughout the rest of the day.

Sputum quantity is widely considered to be a controversial outcome measure because the amount of sputum collected during or after an airway clearance intervention does not exactly reflect the impact on airway clearance; it is poorly correlated with mucociliary clearance rates *in vivo*[183]. Indeed, if sputum quantity is used for comparison between different periods, it must be assumed that mucus production in the airways is stable during the measurement times[183], and potential confounders (i.e., antibiotics) should be controlled[171]. Moreover, saliva contamination, involuntary swallowing and patient compliance may contribute to over- or underestimation of the effects of airway clearance interventions[183, 199]. For all these reasons, the use of sputum quantity is appropriate for intra-subject comparison (clinical practice or cross-over design), during short time periods and in stable conditions (to guarantee that the amount of mucus production remains stable) and to include sequential measurements due to its higher variability.

Drying the amount of sputum collected is one possibility to avoid salivary contamination (particularly when assessing hyperosmolar agents effect) to obtain more accurate findings. However, it is difficult to use this technique in clinical practice. Wet sputum weight is suggested to be a good predictor of dry sputum weight[200], and previous studies that assessed airway clearance interventions in people with bronchiectasis found similar findings using dry sputum and wet sputum weight[118, 142]. Thus, a deep comparative analysis to determine whether wet sputum weight is a useful method to use in clinical practice and research as predictor of dry sputum weight is needed.

Although sputum quantity is widely used to assess the airway clearance intervention effects, it is still a challenge to interpret the findings. There is a knowledge gap in the psychometric

properties of sputum weight, which is a mandatory factor for the correct interpretation of this outcome measure[201].

### *9.2.2. Surface and biophysical properties of sputum samples*

Airway clearance therapies enhance sputum clearance because they generate a greater mechanical stress on the airways (shear stress, pressure gradients, compression/stretch and/or osmotic shock) compared to normal breathing[72]. Therefore, these interventions can alter the biophysical properties of mucus and increase the hydration of mucus layer, ultimately changing its surface properties.

The effect of airway clearance interventions on cough transportability is measured *in vitro* using a cough machine and strongly depends on the tenacity (the product of cohesivity and adhesivity) of the mucus layer and weakly on viscoelasticity[202] (Figure 7). Sputum cohesivity is the tendency to remain attracted to itself and form threads when it is slowly stretched[87]. The ability to form filaments can be assessed through the distance required to break a sputum sample under conditions of attachment deformation, and it is a parameter required to calculate cohesivity[203]. Adhesivity is the attractive force of the mucus layer to airway epithelium and it is facilitated by a higher mucus concentration (dehydration of mucus layer). Adhesivity is calculated by assessing the wettability (measured by the contact angle) and the interface tension of sputum samples[202].

Although the viscoelastic properties of mucus have little impact on cough transportability, low viscosity may impair cough effectiveness[87]. The viscoelastic behaviour of sputum

samples during a cough manoeuvre could be measured *in vitro* using a rheometer setting at 100 rad/sec.

Airway clearance therapies also aim to impact mucociliary transport by increasing the rate of ciliary beating and decreasing mucus viscosity, whereas the elasticity is preserved in lower airways. A mucus flog palate is frequently used to assess *in vitro* the effects on mucociliary transportability and a rheometer simulating the ciliary movement (setting at 1 rad/sec) is usually chosen to evaluate the viscoelastic properties in lower airways. Finally, the possible impact of airway clearance interventions on mucus hydration can be estimated by measuring the solid content, mucin concentration and/or the partial osmotic pressure from sputum samples[81].

Overall, the above parameters are appropriate outcomes to assess the mechanical actions of airway clearance therapies on cough and ciliary transport in people with bronchiectasis. In fact, Valentine et al.[204] provided data for correctly estimating the sample size using these outcome measures in bronchiectasis. As described above, trained assessors and specific equipment are needed to estimate these outcome measures. Consequently, these measurements are almost exclusively used in the research field, a fact that makes it difficult to transfer the results into clinical practice.

Previous findings suggest that solids content, tenacity and cough transportability can change acutely after mannitol inhalation[88, 172] and after 4-week using an oscillatory PEP device in bronchiectasis[123, 124]. Daviskas et al.[172] also found that interfacial tension correlate inversely with mucus clearance by assessing scintigraphy in this population. Despite these promising

results, interpreting airway clearance effects using these parameters is a challenge because their psychometric properties are still unknown in bronchiectasis.

Recently, Radtke et al.[151] demonstrated high variability and poor test-retest reliability of sputum solid content and viscoelastic properties in a small sample of adults with cystic fibrosis. Thus, future studies to explore the psychometric properties of biophysical and surface properties of sputum samples in patients with bronchiectasis are needed before conducting investigations to analyse the effects of airway clearance interventions using these outcomes.

### *9.2.3 Airway inflammatory markers and bacterial load*

Airway clearance interventions that are performed regularly avoid mucus retention and could play a modulate role on bacterial load and/or airway inflammatory response. These outcomes are frequently used for studies that evaluate antibiotic effects[195, 205, 206] but rarely for airway clearance therapies (Table 3 and 4).

Previous findings suggest that bacterial load (reported as colony forming units) does not change after long-term mucoactive treatment[166, 171] or ACTs[126] in people with bronchiectasis and primary ciliary dyskinesia. In contrast, Nicolson et al.[164] showed a significant reduction of positive sputum culture after 12 months of HS and IS treatment.

Sputum neutrophil elastase, myeloperoxidase, interleukins and cell counts are the most commonly evaluated airway inflammatory markers. There are no clear changes in

inflammatory markers after hyperosmolar solution or ACT treatment in people with stable bronchiectasis[124, 136, 171] or other respiratory diseases[166, 176]. It is possible that those studies were unpowered for detecting small differences in airway inflammation in stable condition, as suggested by Brivio et al.[176].

Enhancing daily sputum clearance may play a preventive effect to avoid increases in bacterial density and/or airway inflammation over time. Thus, bacterial eradication or a clear reduction of bacterial load or airway inflammation markers are not expected outcomes for airway clearance approaches. In fact, a modulatory effect on airway infection and inflammation is more likely to identify those therapies by examining long-term clinical outcomes[207], such as exacerbations or need of extra medication, which would allow for specifically controlling potential cofounders such as antibiotics or corticosteroids.

### *9.2.4. Sputum colour*

Sputum colour is used to identify the level of purulence from sputum samples. Different scales have been used to analyse this outcome in bronchiectasis[208, 209]. However, it is widely recommended to use the sputum colour chart (SCC) developed and validated by Murray et al.[127] (Figure 9). Sputum colour is scored into three gradations: mucoid (clear), mucopurulent (pale yellow/pale green) and purulent (dark yellow/dark green). Sputum purulence assessed by SCC is associated with bacterial colonisation, airway inflammation and HRCT severity scored in bronchiectasis[127, 210]. This tool is easily implemented in clinical practice and can be used by patients/physicians to detect the possibility of developing an exacerbation[97]. However, previous experience or a training period is needed to guarantee the correct use of this tool[211]. A clear response on sputum purulence after airway clearance therapy is unlikely, therefore it may be more appropriate to use SCC only to characterise the

samples collected rather than as an outcome measure to assess the effectiveness of an intervention.

## 9.3. Patient-reported outcome (PRO) measures

PROs can provide a wide variety of health-related information (i.e., quality of life, symptoms, functional status, health-related behaviours and treatment side effects). They are reported directly by patients and without a clinician's interpretation of the patient´s response[212]. PROs are increasingly important for proper assessment of patients in research and clinical practice. These PROs are usually assessed using self-administered questionnaires (often referred as PRO measures) to ensure a standardised method for all patients. It is recommended to use PROs as endpoints in clinical trials to guarantee that the impact of an intervention from the patients´ perspective is also measured[212, 213].

### *9.3.1. HRQoL and specific respiratory symptom questionnaires*

Specific-HRQoL questionnaires are widely used in people with bronchiectasis[214]. Bronchiectasis Health Questionnaire (BHQ)[215] and Quality of Life-Bronchiectasis (QoL-B)[216] have been specifically developed for people with bronchiectasis. A specific questionnaire for primary ciliary dyskinesia (QoL-PCD) was also developed and validated by Lucas et al.[217]. Alternatively, there are other HRQoL questionnaires that were initially developed for other respiratory conditions (i.e., St George´s Respiratory Questionnaire [SGRQ][218]; COPD Assessment Test [CAT][219]; Chronic Respiratory Disease Questionnaire [CRDQ][220]) that have been correctly validated in people with bronchiectasis and, consequently, their use is appropriate.

Cough is one of the most prevalent symptom in people with bronchiectasis[6], and thus cough-specific questionnaires are appropriate tools to assess/monitor the impact of cough in daily life. The Leicester Cough Questionnaire (LCQ)[221] is the most common one used in this population. This questionnaire was validated in bronchiectasis by Murray et al.[221] in 2009 from its original version for individuals with chronic cough[222]. Other cough-specific questionnaires, including the Cough Quality of Life Questionnaire (CQLQ)[223] and Chronic Cough Impact Questionnaire (CCIQ)[224], have been developed for other populations (i.e., chronic cough or idiopathic pulmonary fibrosis) but not yet validated in patients with bronchiectasis. Furthermore, sputum-specific questionnaires are also available that may be excellent tools for assessing the effects of airway clearance therapies (i.e., the Breathlessness Cough and Sputum Scale, [BCSS][225] and Cough and Sputum Assessment Questionnaire [CASA-Q][226]). However, they have also not been validated in bronchiectasis. Therefore, these questionnaires should only be used after appropriate validation in bronchiectasis.

Specifically, there are five HRQoL or specific respiratory symptoms questionnaires translated and validated to Spanish people with bronchiectasis using standardised procedures: BHQ, CAT, LCQ, QoL-B and SGRQ[120, 215, 219, 227, 228]. The psychometric properties of those questionnaires are summarised in Table 5. From the 26 studies reviewed in Table 3 and 4, 12 had at least one end-point that used these questionnaires as PRO measures to assess the airway clearance therapies effects in people with clinically stable bronchiectasis. The SGRQ and LCQ are the most frequently used questionnaires, especially in studies designed to assess long-term effects. The LCQ was selected as a primary endpoint by Murray et al.[126]; they reported an improvement greater than the MCID for the total LCQ score after 3-month therapy. The SGRQ also

improved beyond its established MCID after treatment in this study. A similar positive outcome was observed for Muñoz et al.[120] after 1-year ACT. These data demonstrate that HRQoL and cough-specific questionnaires (LCQ and SGRQ) can change, to reach the MCID, after a long-term ACT intervention in people with bronchiectasis.

Conversely, the changes observed in LCQ and SGRQ after long-term treatment using hyperosmolar agents are unclear. There are opposing findings as to whether HS solution improves HRQoL/cough impact more than IS solution[164, 165]. Additionally, the study conducted by Bilton et al.[170] showed a significant change in SGRQ over a 12-month period in favour of twice daily mannitol treatment; however, this change did not achieve the MCID.

The use of specific HRQoL measures can provide more sensitive findings that are more relevant for assessing intervention effects. Unfortunately, the specific HRQoL questionnaires designed for people with bronchiectasis (QoL-B and BHQ) have been minimally used to assess the effects of airway clearance interventions (Table 3 and 4). However, some of the ongoing trials in this field (e.g., NCT02324855 and ISRCTN89040295) have included the QoL-B as secondary endpoint. Herrero-Cortina et al.[229] recently presented the preliminary results of a home-based ACT programme (NCT02324855) and found that the QoL-B improvement (respiratory domain) is apparently greater than the MCID in favour of the experimental group after 12-month follow-up. The definitive findings of these trials will provide researchers with the opportunity to better understand the behaviour of this questionnaire for airway clearance interventions in individuals with bronchiectasis.

Although the HRQoL/cough impact questionnaires are preferred outcome measures for assessing long-term effects of airway clearance interventions, they may be also useful for short-term periods (< 4 weeks) when participants are naïve or not adherent to those treatments or similar interventions. The inclusion of these questionnaires for short-term trials provide information about the immediate effect of interventions from patients´ perspective. It is important to transfer the results to clinical practice, particularly for patients´ opinions and feelings about airway clearance interventions. Indeed, the patients' outlook can greatly influence the future adherence rate[230].

**Table 5** Psychometric properties of health questionnaires validated in Spanish

| **Questionnaires** | **Reliability** | | | **Validity** | | **MID** |
|---|---|---|---|---|---|---|
| **(Scores)** | **Internal consistency** (Cronbach's α coefficient) | **Test-retest** (ICC with 95%CI) | **Level of agreement** (Bland-Altman method) | **Convergent** (r , instrument) | **Discriminant** (instrument) | **Score** (method used) |
| **BHQ**[215]<br>(0-100) | 0.85 | 0.89 (0.77-0.94) | Upper limit ≈ 10<br>Lower limit ≈ 10 | r= -0.82 (total SGRQ score)<br>r= -0.27 ($FEV_1$)<br>r= -0.49 (previous exacerbations /12 months)<br>r= - 0.70 (dyspnoea, VAS)<br>r= - 0.61 (cough, VAS)<br>r= - 0.48 (sputum, VAS) | $FEV_1$%, sputum colonisation, previous exacerbations / 12 months and previous hospitalisations / 12 months, HRCT (lobes) | NE |
| **CAT**[219]<br>(0-40) | 0.86 | 0.95 (0.92-0.97) | Upper limit ≈ 6<br>Lower limit ≈ 7 | r= -0.70 (BHQ)<br>r= 0.75 (total SGRQ score)<br>r= -0.68 (QoL-B, respiratory scale)<br>r= 0.22 (expectoration)<br>r= 0.48 (dyspnoea, mMRC)<br>r= 0.21 (Charlson index score)<br>r= - 0.29 (FEV1%)<br>r= 0.29 (Bhalla score)<br>r= 0.44 (fibrinogen)<br>r=0.31 (CRP) | No differences for bronchiectasis severity scores (FACED, E-FACED and BSI) | 3 points (distribution-based approach) |

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|---|---|---|---|---|---|---|---|
| | | | | | r= 0.57 (HADS-depression)<br>r= 0.46 (HADS-anxiety) | | |
| **LCQ**[231]<br><br>(3 to 21) | **Physical** | 0.87 | 0.87 (0.84-0.90) | NE | r= -0.67 (total SGRQ score) | Not evaluated / reported | NE |
| | **Psychological** | 0.87 | 0.82 (0.77-0.86) | | r=- 0.59 (total SGRQ score) | | |
| | **Social** | 0.86 | 0.79 (0.73-0.84) | | r= -0.65 (total SGRQ score) | | |
| | **Total** | 0.91 | 0.84 (0.79-0.87) | Upper limit = 4.6<br>Lower limit = -4.8 | r= -0.66 (total SGRQ score) | FACED (mild vs severe); BSI (mild vs moderate and mild vs severe) | NE |
| **QoL-B**[228]<br><br>(The scores are standardised across 8 scales, ranging | **Physical functioning** | 0.91 | 0.88 | NE | r= -0.81 (total SGRQ score) | $FEV_1$%, Bhalla score, haemoptysis, *P. aeruginosa, H. influenzae* | NE |
| | **Role functioning** | 0.84 | 0.86 | | r= -0.77 (total SGRQ score) | $FEV_1$%, Bhalla score, haemoptysis, *P. aeruginosa* | |
| | **Vitality** | 0.82 | 0.78 | | r= -0.67 (total SGRQ score) | $FEV_1$%, Bhalla score, haemoptysis, *P. aeruginosa* | |
| | **Emotional functioning** | 0.84 | 0.86 | | r= -0.64 (total SGRQ score) | $FEV_1$%, haemoptysis | |
| | **Social functioning** | 0.70 | 0.78 | | r= -0.53 (total SGRQ score) | $FEV_1$%, Bhalla score, haemoptysis | |
| | **Treatment burden** | 0.72 | 0.68 | | r= -0.34 (total SGRQ score) | Bhalla score | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| from 0 to 100) | **Health perceptions** | 0.71 | 0.83 | | r= -0.68 (total SGRQ score) | $FEV_1$%, Bhalla score, haemoptysis, *P. aeruginosa* | 8.2 points (distribution-based approach) |
| | **Respiratory symptoms** | 0.87 | 0.83 | | r= -0.69 (total SGRQ score) | $FEV_1$%, Bhalla score, haemoptysis, *P. aeruginosa, H. influenzae* | |
| **SGRQ**[227] (0 to 100) | **Symptoms** | 0.81 | NE | NE | r= 0.49 (dyspnoea, MRC)<br>r= 0.42 ($FEV_1$ mL)<br>r= 0.45 ($FEV_1$ % pred.)<br>r= 0.48 (cough)<br>r= 0.53 (expectoration)<br>r= 0.36 (wheezes)<br>r= 0.23 (previous exacerbations/6 months)<br>r= 0.41 (*P aeruginosa*) | NE | NE |
| | **Activity** | 0.87 | | | r= 0.62 (dyspnoea, MRC)<br>r= 0.61 ($FEV_1$ mL)<br>r= 0.56 ($FEV_1$ % pred.)<br>r= 0.27 (expectoration)<br>r= 0.26 (wheezes)<br>r= 0.34 (Bhalla score)<br>r= 0.26 (*P aeruginosa*) | | |

| | | | |
|---|---|---|---|
| **Impact** | 0.81 | r= 0.54 (dyspnoea, MRC)<br>r= 0.51 ($FEV_1$ mL)<br>r= 0.58 ($FEV_1$ % pred.)<br>r= 0.22 (cough)<br>r= 0.37 (expectoration)<br>r= 0.29 (Bhalla score)<br>r= 0.26 (*P aeruginosa*) | |
| Total | 0.90 | r= 0.65 (dyspnoea, MRC)<br>r= 0.59 ($FEV_1$ mL)<br>r= 0.59 ($FEV_1$ % pred.)<br>r= 0.32 (cough)<br>r= 0.47 (expectoration)<br>r= 0.29 (wheezes)<br>r= 0.20 (previous exacerbations / 6 months)<br>r= 0.33 (Bhalla score)<br>r= 0.41 (*P aeruginosa*) | Bhalla score, expectoration, $FEV_1$%, *P. aeruginosa,* exacerbations, dyspnoea (MRC) |

MID= minimal important difference; ICC= intraclass correlation coefficient; CI= confidence interval; BHQ= bronchiectasis health questionnaire; SGRQ= St. George´s respiratory questionnaire; $FEV_1$= forced expiratory volume in the first second; VAS= visual analogic scale; HRCT= high resolution computerised tomography scan; CAT= COPD assessment test; QoL-B= quality of life of bronchiectasis; mMRC= modified medical research council; CRP= C-reactive protein; HADS, hospital anxiety and depression scale; BSI= bronchiectasis severity index; FACED and E-FACED= bronchiectasis prognostic scores; NE= not estimated.

### *9.3.2 Patients´ feedback*

No one ACT has been shown to be more beneficial than any other[109, 114], and the effects of hyperosmolar agents are still unclear[162] in individuals with bronchiectasis. Thus, patient preference is an important factor to considerer when the most appropriate ACT and/or hyperosmolar agent is/are selected[110]. Long-term adherence is the main barrier to these treatments, and thus if the patients´ opinion is considered, the adherence rate may be improved. Consequently, patients´ feedback should be an essential outcome measure in studies that compare the effects of different ACTs.

The most appropriate study design for analysing patients´ feedback among interventions is the randomised crossover trial because participants receive all treatments[153]. However, this study design is more adapted for short-term intervention periods[153]. The patient preference scale (PPS), Likert scales and visual analogical scales (VAS) are the most common tools used to assess patients´ feedback (Table 3 and 4). Although the patients´ preference regarding ACTs has been previously analysed using the PPS[135], this scale was not specifically validated in bronchiectasis. Finally, the global rating of change (GROC) is a specific Likert scale that is often used to calculate the MCID of an outcome measure[232]. It is used as an anchor to determine the clinical magnitude of change perceived by patients after an intervention.

## 9.4. Lung function

There is now a worldwide consensus that forced spirometry ($FEV_1$, forced vital capacity [FVC] and $FEV_1$/FVC) is not a sensitive outcome measure to assess airway clearance treatment effects in people with chronic respiratory conditions, such as

bronchiectasis[192]. Indeed, there is a trend to use forced spirometry more as a safety endpoint rather than an effective endpoint in bronchiectasis clinical trials[192], a design that relegates this measure to a secondary outcome[120, 170, 233].

Recently, Radovanovic et al.[234] observed that air trapping (residual volume [RV] > 120% predicted) and diffusion ($DL_{CO}$) impairment are the most common lung function abnormalities. Moreover, the perception of dyspnoea seems to be more closely related to lung hyperinflation than lung obstruction severity[16]. Thus, these characteristics (air trapping, diffusion and lung hyperinflation) are not evaluated with forced spirometry. Guimarães et al.[119] observed a significant reduction of RV, functional residual capacity (FRC) and total lung capacity (TLC) after a single ELTGOL session and a flutter device (an oscillating PEP device) in adults with clinically stable bronchiectasis. In contrast, Poncin et al.[118] did not find any change after a single autogenic drainage technique session in the same population. Although the ELTGOL and autogenic drainage technique have a similar mechanism of action, the opposite results of both studies may be related to the small sample size (10 and 24 people, respectively) and the differences in the measurement process (basal lung volume after 5 min of coughing and basal plethysmography assessment before any intervention, respectively).

The lung clearance index (LCI), measured by multiple breath washout (MBW), is a gauge of ventilation inhomogeneity. It has been postulated as a superior physiological test to spirometry for monitoring peripheral airways or early changes in lung function in patients with chronic respiratory diseases, including adults with bronchiectasis[235, 236]. LCI is an apparently reliable measurement (intraclass correlation coefficient [ICC] from 0.95 to 0.97) in people with stable bronchiectasis[235, 236], with a coefficient of variation of approximately 4.5%[235, 236] and a mean bias according to Bland-Altman plot of

1.16[236]. However, LCI neither changes significantly after short-term ACT intervention in stable and exacerbated individuals with bronchiectasis[47, 236] nor after a course of intravenous antibiotic therapy[236]. Similar results were reported in people with cystic fibrosis[237-239].

Grillo et al.[236] and Pfleger et al.[238] nicely explained the main reasons as to why the LCI response to ACTs or other therapies is unpredictable. An improvement in the more affected areas (i.e., a reduction in mucus plugging) may lead to a paradoxical response in LCI because ACTs may partially open previously obstructed airways and therefore increase the time constants of lung units (worsening LCI)[236, 238]. However, a positive effect on gas mixing after ACT in less affected areas may lead to the absence of LCI changes; this test is strongly dependent on the most obstructed areas of the lung[236, 238]. Finally, hyperinflation reduction after treatment may decrease LCI values[238]. Altogether, LCI is a promising outcome measure, but the lack of a consistent response after treatments currently limits its use in analysing short-term ACT effects.

## 9.5. Promising outcome measures

### *9.5.1 Computerised respiratory sounds*

Computerised respiratory sound is an emerging outcome measure to assess the effects of respiratory therapies, including airway clearance interventions[134, 240-243]. It directly records respiratory sounds using microphones or electronic stethoscopes, following the Computerised Respiratory Sound Analysis (CORSA) guidelines[244]. Seven locations (trachea and right and left posterior, lateral and anterior chest) are the most common points to record respiratory sounds (Figure 10). Subsequently, the sound files are analysed using validated algorithms to detect and characterise both normal and adventitious respiratory sounds (ARS) for each respiratory phase (inspiration and expiration)[199]. Therefore, the use of the

computerised respiratory sounds solves the main limitation of conventional auscultation; respiratory sounds are detected objectively and do not depend on the health professionals´ interpretation[245, 246].

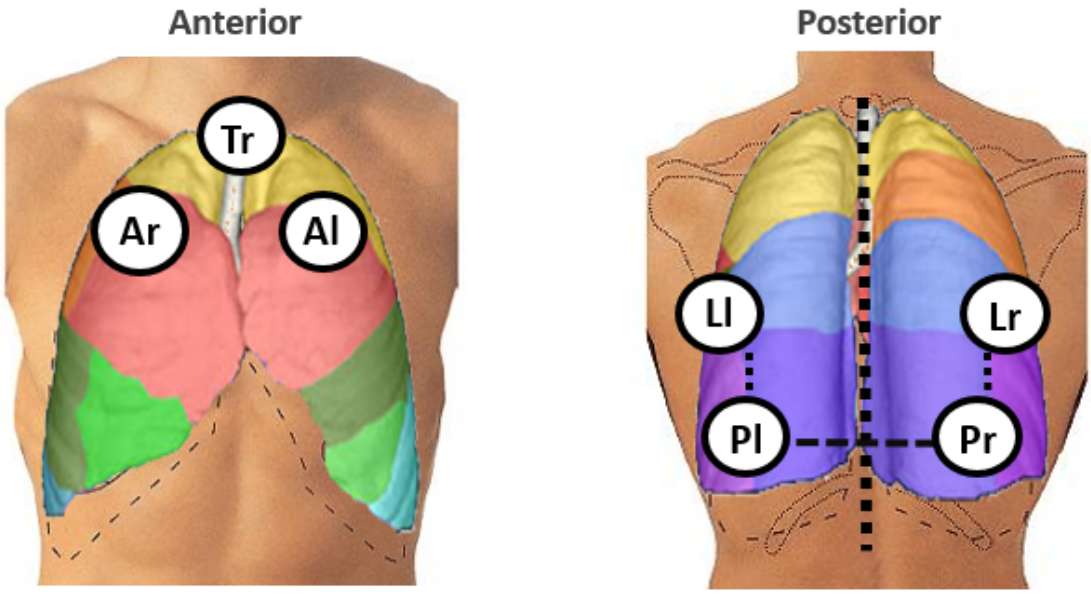


**Figure 10.** The seven anatomical chest points frequently recorded for adventitious respiratory sounds (ARS) are: posterior right (Pr); posterior left (Pl); lateral right (Lr); lateral left (Ll); anterior right (Ar); anterior left(Al); trachea (Tr)

Normal respiratory sound analysis (i.e., intensity and frequency) provides an indirect evaluation of the pulmonary regional ventilation[134], whereas ARS (such as crackles and wheezes) have been associated with the presence of excessive airway mucus and bronchial obstruction[247-251]. Crackles and wheezes are the most frequent ARS used to analyse the effects of respiratory therapies[243].

Crackles are short sounds heard when an airway is opened/closed suddenly. They indicate the loss of elastic recoil and bronchial support, the main mechanisms that underlie the abnormal airway closure during expiration in bronchiectasis[250]. Crackles are classified based on their frequency and duration. Coarse crackles present a lower frequency and longer

duration, and they usually appear in early-inspiration or expiration, a finding that indicates the mechanism is produced in larger airways[248, 250]. In contrast, fine crackles usually appear at the end of inspiration and are characterised by higher frequencies and shorter durations compared to coarse crackles. These features imply that smaller airways being affected. The number, frequency and/or time duration (two cycle-duration [2CD] or initial deflection width [IDW]) are the most frequent parameters reported for crackles[243].

Wheezes are continuous sounds that appear when there is a flow limitation in the airway; the air velocity increases at this point and generates an oscillation of the airway wall[252]. They can appear during inspiration or expiration and are associated with the presence of airway obstruction. Thus, they represent a frequent ARS in people with bronchiectasis[248]. The most often used parameters are the number, type (monophonic or polyphonic) and the occupation rate (%) of wheezes[243].

Interest in computerised respiratory sounds has increased recently in the physiotherapy field because the changes observed in normal respiratory sounds and ARS after an airway clearance session may reflect an improvement in regional ventilation/obstruction and/or the amount of sputum expectorated after those treatments[243]. Thus, auscultation is still a frequent tool used in clinical practice for physiotherapists to define the most appropriate airway clearance therapeutic approach and monitor the response to these treatments[199]. However, there is little evidence about its psychometric properties and the ability to change after airway clearance interventions in people with bronchiectasis.

Marques et al.[253] determined the within-day reliability of two crackle parameters in a single study that included bronchiectasis (n = 37) and cystic fibrosis (n = 17) patients. The within-day reliability for 2CD and IDW were ´good´ to ´excellent´ for all chest locations, with an ICC

from 0.76 to 0.95[253]. Although data that evaluate the validity, between-day reliability and responsiveness of computerised respiratory sounds in bronchiectasis are not yet available, the psychometric properties of this outcome measures observed in COPD are acceptable[254, 255]. These data suggest the possibility that this tool might be used in the future to assess the effects of airway clearance interventions in adults with bronchiectasis.

On the other hand, there are opposing findings with regards to computerised respiratory sounds changes after airway clearance interventions. In people with clinically stable bronchiectasis, Marques et al.[134] identified neither changes in the duration (2CD) nor number of crackles after a single ACBT session. However, when data were analysed individually, there were significant changes in the duration of crackles, but with an inconsistent direction of change. In contrast, Oliveira et al.[241] suggest that the major change after a single airway clearance session (using combined therapies) in people with an acute exacerbation of an obstructive disease is an increase in the number of crackles and the wheeze frequency. Therefore, it remains unclear which computerised respiratory sounds parameter(s) is (are) the most appropriate to assess the effects of airway clearance interventions and what direction and magnitude of change corresponds to a clinical improvement.

Consequently, before conducting an adequately powered clinical trial using computerised respiratory sounds as an outcome measure to assess airway clearance therapy effects in people with bronchiectasis, a preliminary feasibility study is recommended to ensure greater accuracy of the results achieved.

### *9.5.2 Cough monitors*

An observational study by Spinou et al.[256] indicated that self-reported sputum production is an independent factor of 24-hour cough frequency in 54 people with stable bronchiectasis. Therefore, if airway clearance interventions impact on the amount of sputum expectorated after sessions, the use of cough monitors to assess the cough frequency/severity may represent a useful tool for analysing their short-term effects. However, a specific study is needed to better understand the direction of change expected after sessions[192].

The ideal response is to reduce the cough frequency after airway clearance interventions in patients with chronic sputum expectoration. However, it remains unclear whether the positive effect of this treatment on enhancing expectoration only occurs during the sessions or after sessions (and perhaps increases the daily cough count). Therefore, clinicians require a better understanding of the short-term effects of airway clearance therapies on sputum expectoration/productive cough in people with bronchiectasis before selecting cough frequency as primary outcome in future trials.

# Rationale

Daily sputum expectoration is one of the most common respiratory symptoms in adults with bronchiectasis. It is associated with poor health outcomes, particularly lower quality of life[19, 98]. For these reasons, one of the research priorities encouraged by people with bronchiectasis is how to implement and facilitate the accessibility of airway clearance management[99]. Although international guidelines agree in recommending the use of airway clearance interventions to more easily manage chronic productive cough, the quality of evidence is still low-moderate in bronchiectasis[46-48, 105-107].

No ACT has been shown to be more effective than another in enhancing sputum expectoration in bronchiectasis, possibly due to methodological limitations[109, 114]. Slow-expiratory ACTs (i.e., autogenic drainage, ELTGOL and PEP) have gained more interest compared to traditional chest physiotherapy (i.e. postural drainage, percussion and vibration) in recent years. However, slow-expiratory ACTs have not been compared in bronchiectasis. Given that autonomy and patients´ preferences are factors that influence long-term ACT adherence[110], it is appropriate to first conduct a short-term trial that compares different slow-expiratory ACTs with distinct levels of autonomy in bronchiectasis and to evaluate whether the benefits are comparable among techniques.

The inhalation of hyperosmolar agents is a therapeutic option when individuals with bronchiectasis are unable to manage related sputum symptoms or obtain adequate/sufficient airway clearance despite an optimal airway clearance therapeutic approach[46, 105]. However, the

recommendation of its use ranges from weak to strong, and the quality of evidence for its effectiveness is low-moderate due to controversial or contrasting findings[46, 48, 105]. The methodology used to date to analyse the long-term effects of hyperosmolar agents in bronchiectasis is based on previous studies in cystic fibrosis, but findings from other respiratory diseases cannot always be extrapolated to bronchiectasis. Globally, the questions that remain unanswered are: i) what are the short- and long-lasting effects of hyperosmolar agents when used only once a day in bronchiectasis; ii) how can clinicians improve the tolerability and safety of hyperosmolar agents in people with bronchiectasis to ensure long-term adherence; ii) does the addition of ACTs after hyperosmolar solution administration generates greater benefits compared to hyperosmolar agents alone in our target population. Answering all of these questions will provide us with the possibility of correctly designing future long-term studies in this field.

The selection of the outcome measures to assess short-term effects of airway clearance interventions and the interpretation of their results, remains a challenge. Indeed, this factor may hamper establishing the evidence of these treatments in people with bronchiectasis. Wet sputum weight is one of the most common outcome measure selected to assess the short-term effects of airway clearance interventions; however, its use is still controversial due to possible cofounding factors (i.e., presence of saliva contamination and inadvertent swallowing)[183, 199, 245]. Despite its described limitations, the current widespread use of sputum weight can be attributed to it being a simple and feasible outcome measure, its relevant to people with bronchiectasis and its easy implementation in clinical practice[99, 192]. However, the knowledge gap about the psychometric properties of wet sputum weight (i.e., reliability and MCID) makes it difficult to correctly interpret this outcome measure.

On the other hand, conventional auscultation remains a frequent tool used in clinical practice for physiotherapists because the changes observed in respiratory sounds after an airway clearance intervention may reflect an improvement in regional ventilation/obstruction and/or the amount of sputum expectorated after those treatments[248, 250]. Thus, computerised ARS are becoming a potential outcome measure to assess the short-term effects of airway clearance interventions. The objectivity of this tool is a clear advantage over sputum weight; however, its potential use in clinical practice/research is still unknown. Consequently, a preliminary feasibility study is recommended before conducting an adequately powered trial that uses computerised ARS as an outcome measure to assess short-term effects of airway clearance therapies in bronchiectasis.

# Objectives

## General Objective

To investigate what is the most short-term efficacious airway clearance therapeutic approach in adult people with clinically stable bronchiectasis and how to correctly interpret the clinical changes observed after these interventions.

## Specific Objectives

1. To compare the short-term effectiveness of three slow-expiratory ACTs (autogenic drainage, ELTGOL and TPEP techniques) with different degrees of autonomy on enhancing sputum expectoration and identify the preferred technique in people with clinically stable bronchiectasis and daily expectoration. The secondary objectives are to determine whether these interventions impact on cough severity and lung function.

2. To evaluate whether HA+HS solution is as effective as HS solution alone and IS solution in improving short-term sputum expectoration, cough severity, lung function, tolerability and safety and also identify the preferred solution in adults with stable bronchiectasis and daily expectoration. The subsidiary objectives are to determine whether hypertonic solutions (HA+HS and HS) increase effectiveness of ACTs after inhalation and to analyse whether a combined session of saline solutions (HA+HS, HS, IS) plus ACT is better than saline solutions alone.

3. To examine the reliability of 24-hour wet spontaneous sputum expectoration (without performing any airway clearance intervention) in people with stable bronchiectasis and daily expectoration. The secondary objective is to estimate the MID for 24-hour wet spontaneous sputum expectoration after an airway clearance intervention in the same target population.

4. To determine the feasibility of computerised ARS as an outcome measure to assess the short-term effects of slow-expiratory ACTs in adults with clinically stable bronchiectasis and daily expectoration.

# Methodology

The research conducted within the framework of the doctoral thesis included two randomised crossover clinical trials conducted by me (the PhD candidate) in adults with stable bronchiectasis. This endeavour resulted in four original manuscripts and a narrative chapter that reviewed the evidence of ACTs in bronchiectasis. This section includes information on the general methodology applied for the four manuscripts in order to address the four specific objectives. Specific information included in the narrative chapter of ACTs in people with bronchiectasis has been used in the background section of this doctoral thesis. For further information, I encourage the reader to go to the specific methodology of each study[193, 194, 257, 258].

## General Methodology

### *1. Study design*

An open-label randomised three-way crossover trial with concealed allocation was conducted to compare the short-term effectiveness of three slow-expiratory ACTs (autogenic drainage, ELTGOL, TPEP) in order to response the first specific objective of the present thesis (Study 1; Figure 11). Each technique was applied in three non-consecutive sessions (once daily) during the same week with a 7-days washout period between the treatment arms. The primary endpoint was the amount of wet sputum obtained during sessions. Secondary outcomes included 24-hour sputum expectoration after sessions, cough impact, lung function and patients´ preference. Finally, computerised respiratory sounds were also recorded for only exploratory purposes.

After finishing the first study, a double-blind randomised three-way crossover trial with concealed allocation was conducted to response the second specific objective of the present thesis (Study 2; Figure 11). Participants inhaled three saline solutions (HA+HS, HS, IS) in a randomly order. Each solution was administrated during four consecutive sessions (once daily) in the same week. All sessions for the three treatment arms included 30 minutes of ACTs after inhalation, except for session 3. The ACT selected for this trial was based on findings of Study 1 in terms of effectiveness and preference during sessions. A 1-week of wash-out period was applied between the three treatment arms. The primary endpoint was the amount of wet sputum obtained during sessions. Secondary outcomes included 24-hour sputum expectoration after sessions, cough impact, lung function, safety, tolerability and patient´s preference.

The clinical interpretation of both studies in terms of enhancing sputum expectoration was difficult because the psychometric properties of wet sputum weight had rarely been explored. Therefore, an ad-hoc analysis was performed to evaluate the test-retest reliability of 24-hour wet spontaneous sputum expectoration (without any airway clearance intervention) using the sputum samples from the two previous randomised crossover trials and an ongoing parallel-group randomised trial (NCT02614300) and responding to the third specific objective of this thesis. The MID of 24-hour wet sputum weight after an airway clearance intervention was also estimated using an ad-hoc analysis from the previous randomised crossover trials (Study 3; Figure 11). The airway clearance therapeutic approach selected for this study was based on findings of Study 1 and Study 2 in terms of effectiveness and preference.

Finally, before conducting an adequately powered clinical trial using ARS as outcome measures to assess the short-term effectiveness of airway clearance therapies, a feasibility study of computerised ARS was conducted to examine the potential use of this tool (fourth specific

objective of the present thesis) (Study 4; Figure 11) using the exploratory approach conducted in Study 1. The ACTs selected for this study was based on findings of Study 1 in terms of effectiveness during sessions.

### *2. Participants*

Adult outpatients diagnosed with bronchiectasis using HRCT scan and followed in the Bronchiectasis Clinic of Hospital Clinic, Barcelona, Spain. Globally, the inclusion criteria included: evidence of daily spontaneous sputum expectoration, being clinically stable before the randomisation process and able to perform the airway clearance interventions. Patients were excluded if they were smokers, adherent to any airway clearance intervention and present severe lung function impairment. A pulmonary exacerbation during the study period was considered a withdrawal criterion. For more specific information, please go to the specific methodology of each study[193, 194, 257, 258].

### *3. Interventions*

One week before starting the trial, all candidates were instructed to breath with the glottis opened and coughing correctly. All participants performed the airway clearance sessions at the same time of day at the hospital over the study period and the interventions lasted 40 minutes for single sessions (slow-expiratory ACTs) or ≈ 50 minutes for combined session (inhalation plus slow-expiratory ACTs). Pauses during sessions were allowed if necessary, and cough manoeuvres were always spontaneous (no encouragement from the physiotherapist). Peripheral oxygen saturation and heart rate were continuously monitored during sessions.

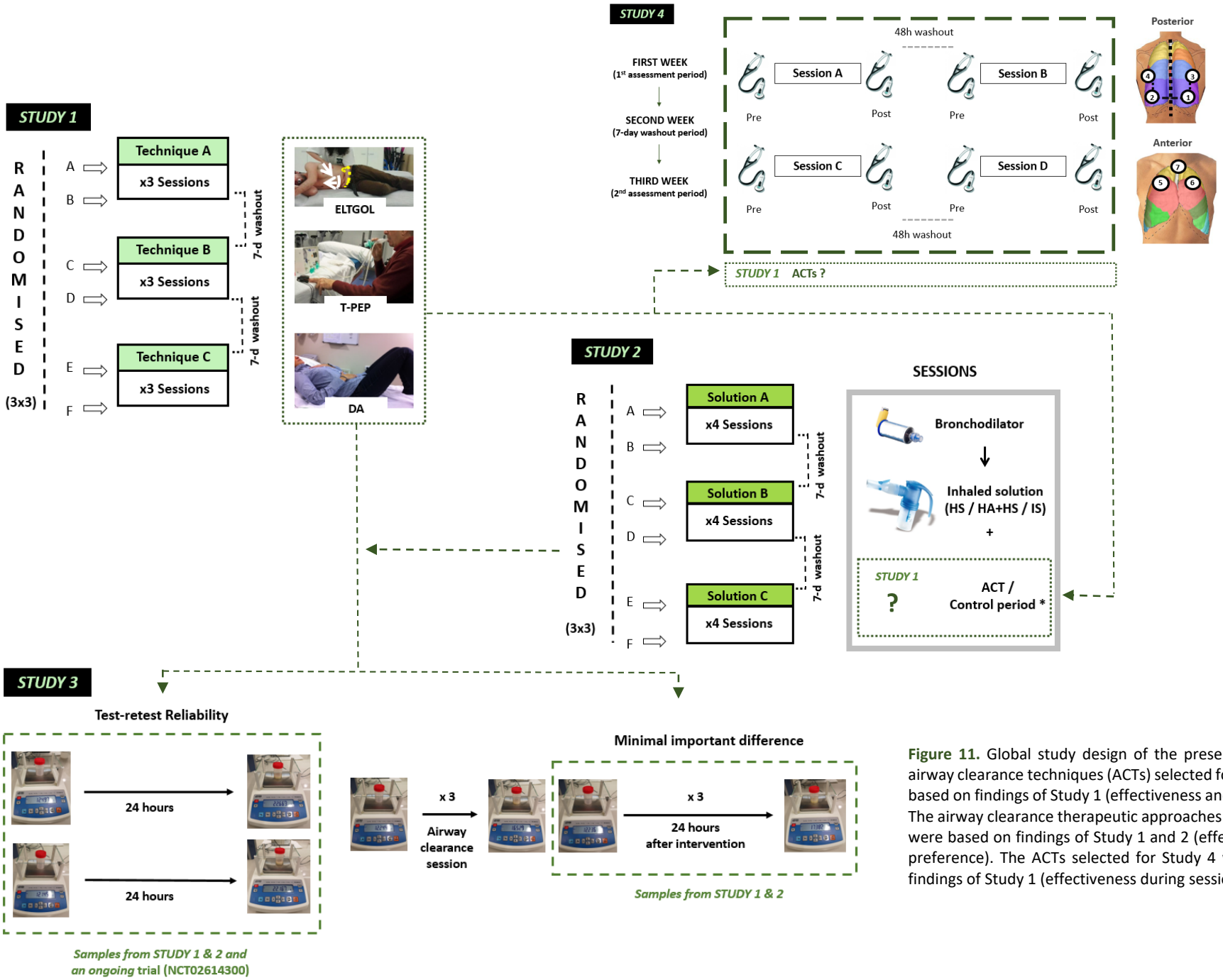


**Figure 11.** Global study design of the present thesis. The airway clearance techniques (ACTs) selected for Study 2 was based on findings of Study 1 (effectiveness and preference). The airway clearance therapeutic approaches in the Study 3 were based on findings of Study 1 and 2 (effectiveness and preference). The ACTs selected for Study 4 was based on findings of Study 1 (effectiveness during sessions)

The inhalation of the different saline solutions (HA+HS, HS and IS)[46, 259, 260] and the slow-expiratory techniques (autogenic drainage, ELTGOL, TPEP)[136, 261-263] were performed according to previous instructions. Participants were asked not to perform any airway clearance intervention by their own over the study period. Pharmacological treatment remained unchanged and participants were encouraged to take their long-term medications at the same time of the day over the study period. For more details, please go to the specific methodology of each study[193, 194, 257, 258].

*4. Outcome measures*

Two transparent pre-weighted containers were provided to all candidates to measure the total amount of spontaneous sputum expectorated in two days before starting the randomisation process. Sociodemographic, anthropometric and clinical data (i.e. aetiology of bronchiectasis, pharmacological treatment) were collected only from those candidates who met the selection criteria. The clinical stability criterion was assessed by an expert respiratory physician.

The outcome measures used to analyse the effectiveness and the safety of airway clearance treatments, the reliability and the MID of wet sputum weight and the feasibility of computerised ARS are summarise in Table 6.

**Table 6.** Outcome measures used for each study included in this thesis

| Outcome | | Study 1[193] | Study 2[194] | Study 3[258] | Study 4[257] |
|---|---|---|---|---|---|
| Amount of sputum | Purpose | Effectiveness (primary endpoint) | | Reliability (primary endpoint)<br>MID | Correlation (exploratory purpose, secondary endpoint) |
| | Time measurement point | | ✓ 24-hour (baseline point)<br>✓ During airway clearance sessions<br>✓ 24-hour post airway clearance sessions | | |
| | Material | | ✓ Transparent pre-weighed containers<br>✓ Calibrated scale (Acculab VIC 212, Germany) | | |
| | Calculation | ✓ Wet sputum weight (grams) | ✓ Wet sputum weight (grams)<br>✓ Sputum quantity ratio (%) | ✓ Wet sputum weight (grams)<br>✓ 24-hour percentage of change (%) | ✓ Sputum quantity ratio (%) |
| Cough impact | Purpose | Effectiveness (secondary endpoint) | | MID (Anchor) | NA |
| | Time measurement point | ✓ Before starting the treatment arms<br>✓ After finishing the treatment arms (1 week) | | ✓ Before starting the treatment period<br>✓ After finishing the treatment period (1 week) | |
| | Material | LCQ | | LCQ | |
| | Calculation | ✓ Total score (3 to 21)<br>✓ Physical score (1 to 7)<br>✓ Psychological score (1 to 7)<br>✓ Social score (1 to 7) | | ✓ Total score (3 to 21) | |

| | | | | | |
|---|---|---|---|---|---|
| **Lung function** | **Purpose** | Effectiveness/safety (secondary endpoint) | | NA | NA |
| | **Time measurement point** | ✓ Before starting the treatment arms<br>✓ After finishing the treatment arms (1 week) | | | |
| | **Material** | Spirometer (NDD EasyOne, Switzerland) | | | |
| | **Calculation** | ✓ $FEV_1$ (L)<br>✓ FEV (L)<br>✓ $FEF_{25-75}$ (L/s) | | | |
| **Patients´ perception preference** | **Purpose** | Effectiveness (secondary endpoint) | | MID (Anchor) | NA |
| | **Time measurement point** | ✓ After finishing the treatment arms<br>✓ At the end of the trial | | ✓ After finishing the treatment arms<br>Study 1 → AD<br>Study 2 → HA+HS + AD | |
| | **Material** | Likert scale | | Likert scale (GRC) | |
| | **Calculation** | Total score (0 to 7) | | Total score (-7 to 7) | |
| **Minor adverse events** *(bronchospasm, coughing, throat irritation, haemoptysis and desaturation)* | **Purpose** | NA | Safety (secondary endpoint) | NA | NA |
| | **Time measurement point** | | During inhalation periods | | |
| | **Material** | | Three-point ordinal scale | | |
| | **Calculation** | | ✓ Presence<br>✓ Total score (0 to 9) | | |
| | **Purpose** | NA | Safety (secondary endpoint) | NA | NA |

| | | | | | |
|---|---|---|---|---|---|
| **Tolerability** | **Time measurement point** | | First day of each saline solution | | |
| | **Material** | | Spirometer (NDD EasyOne, Switzerland) | | |
| | **Calculation** | | Fall ≥ 12% $FEV_1$ | | |
| | **Purpose** | Exploratory | | | Feasibility (primary endpoint) |
| | **Time measurement point** | Before and after the first two sessions of each treatment arm | | | Before and after four ACTs session using ELTGOL and AD |
| | **Material** | 3MTM Littman, Model 3200) | | | 3MTM Littman, Model 3200) |
| **Computerised ARS** | **Calculation** | The recordings were stored but not analysed | NA | NA | ✓ Suitability and safety<br>✓ Time required<br>✓ Equipment cost<br>✓ Direction and magnitude of change using validated algorithms<br>✓ Most appropriate ARS parameter to assess ACTs effects<br>✓ Sample size required for future trials |

MID= minimal important difference; LCQ= leicester cough questionnaire; $FEV_1$= forced expiratory volume in the first second; FVC= forced vital capacity; $FEF_{25-75}$= forced expiratory flow at 25-75% of the FVC; HA+HS= hyaluronic acid + hypertonic saline; ACTs= airway clearance techniques; ELTGOL= slow expiration with glottis opened in lateral posture; AD= autogenic drainage; ARS= adventitious respiratory sounds; NA= not applicable

### *5. Sample sizes calculation*

The parameters used to estimate the sample sizes for each study are summarised in the Table 7. For further information, please go to the specific methodology of each study[193, 194, 257, 258].

**Table 7.** Sample size estimation for each study included in this thesis

| | **Study 1**[264] | **Study 2**[193] | **Study 3**[201] | **Study 4**[265, 266] |
|---|---|---|---|---|
| **Primary endpoint** | Sputum weight during sessions (effectiveness) | Sputum weight during sessions (effectiveness) | 24-hour sputum weight (reliability) | Feasibility of ARS* |
| **α risk** | 0.05 | 0.05 | 0.05 | |
| **β risk** | 0.2 | 0.2 | | |
| **SD** | 8 | 5.7 | | |
| **Minimal difference** | 5 | 8.5 | | |
| **Two-sided / one-sided test** | Two | Two | Two | |
| **ICC** | NA | NA | 0.9 | Hypothesis testing was not undertaken |
| **IC width** | NA | NA | 0.2 | |
| **Total** | 23 | 20 | 21 | |
| **Drop-out rate (%)** | 20% | 20% | 20% | |
| **Minimal sample size recommended** | NA | NA | 50 | |
| **Total + drop-out rate** | 28 | 24 | 60 | |

SD=standard deviation; ICC=intraclass correlation coefficient; IC= interval confidence; NA= not applicable * The main objective of a feasibility study is to describe and estimate treatment effects for future purposes; thus, a sample size calculation was not estimated.

### *6. Data analysis*

Globally, baseline characteristics of participants in all studies and feasibility outcomes were summarised descriptively. Data were analysed according to the intention-to treat principle, except for safety analysis[267] in the Study 2. Parametric and nonparametric analyses were used according to Shapiro-Wilk test results. The statistical test used to analyse/ describe treatment effects are described in the Table 8. Missing data were not imputed[268, 269]. Difference between the treatment arms were reported as mean difference (95% CI) or median difference (95% CI)[270] and statistical significance was set at $p<0.05$ for all calculations. Data analysis was performed using SPSS v.19 (IBM, Chicago, IL, USA) and plots were created using GraphPad Prism version 5.01 (GraphPad Software, La Jolla, California, USA). For more specific information, please go to the specific methodology of each study[193, 194, 257, 258].

**Table 8.** Statistical test used for each study included in this thesis

| | Primary outcome | Secondary outcomes (I) | Secondary outcomes (II) |
|---|---|---|---|
| **Study 1**[193] | *Objective:* compare the three treatment arms (effectiveness)<br>*Test:* Linear mixed model (data were logarithmically transformed)<br>*Covariance:* baseline 24-hour sputum weight | *Objective:* compare the three treatment arms (effectiveness)<br>*Tests:* Friedman´s test with Wilcoxon´s rank sum test<br>*Post-hoc analysis*: Bonferroni correction | NA |
| **Study 2**[194] | *Objective:* compare the three treatment arms (effectiveness)<br>*Test:* Linear mixed model (data were logarithmically transformed)<br>*Covariance:* baseline 24-hour sputum weight | *Objective:* compare combined session (inhalation + ACTs) with control session (inhalation + control period) for each treatment arm (effectiveness)<br>*Test:* Wilcoxon´s rank sum test and ES using rank-biserial correlation¥ | *Objective:* compare the three treatment arms (safety)<br>*Test:* Linear mixed model |
| **Study 3**[258] | *Objective:* reliability of 24-hour weight sputum<br>*Sample:* ´24-hour test-retest cohort´<br>*Test:* $ICC_{3,1}$ (two-way mixed-effects, single measurement, absolute agreement)[271]<br>*Graphical representation:* Bland-Altman plot, including their 95% CI for bias and for the limits of agreement | *Objective:* MID of 24-hour sputum weight after intervention<br>*Sample:* ´airway clearance cohort´<br>*Method 1*: distribution-based approach<br>- 0.5 times SD<br>- Cohen´s effect size<br>- Empirical rule effect size<br>- SEM<br>- $MDC_{95\%}$<br>*Method 2*: anchor-based methods<br>- Total LCQ score | *Objective:* pre vs post comparison<br>*Sample:* ´airway clearance cohort´<br>*Tests:*<br>- Paired t-test with ES ¶<br>- Wilcoxon´s rank sum test with ES ¥ |

| | | |
|---|---|---|
| | | - GRC score<br>- *Graphical representation*: ROC curves (only if a sufficient correlation, r ≥0.4, was observed with the anchors)[272] |
| **Study 4**[257] | *Objective:* feasibility of computerised ARS<br>Test: NA<br>Differences were only explored and described using median difference (95%CI), correlations and ES | |

ES= effect size; ICC= intraclass correlation coefficient; CI= confidence interval, MID= minimal important difference; SD=standard deviation; SEM=standard error of measurement; MDC=minimal detectable change with a 95% confidence level; LCQ= leicester cough questionnaire, GRC= global rating of change; ROC= receiver operating characteristic; NA= not applicable. ¶ ES for paired-t test was calculated using this formula $r=\sqrt{t^2/t^2+df}$ ; ¥ ES for Wilcoxon signed-rank test was calculated using this formula $r = z/\sqrt{n}$ . The $ICC_{3,1}$ values were interpreted as excellent (>0.75), moderate-to-good (0.4–0.75) or poor (<0.4)[273]; correlation was interpreted as weak (r ≤ 0.29), moderate (0.30 < r ≤ 0.59 and strong (r ≥ 0.60)(274); effect size (ES) was interpreted as small effect (<0.3), moderate effect (≥0.3), and large effect (≥ 0.5)[275, 276].

# Results

## Manuscript 1

**Herrero-Cortina B,** Vilaró J, Martí D, Torres A, San Miguel-Pagola M, Alcaraz V, Polverino E.

Short-term effects of three slow expiratory airway clearance techniques in patients with bronchiectasis: a randomised crossover trial.

Physiotherapy. 2016;102(4):357-364.

DOI: 10.1016/j.physio.2015.07.005

Impact Factor (2016): 3.010. Quartil (Rehabilitation): 1

## Manuscript 2

**Herrero-Cortina B,** Alcaraz V, Vilaró J, Torres A, Polverino E.

Impact of hypertonic saline solutions on sputum expectoration and their safety profile in patients with bronchiectasis: a randomised crossover trial.

J Aerosol Med Pulm Drug Deliv. 2018; 31:281-289

DOI: 10.1089/jamp.2017.1443

Impact Factor (2018): 2.866. Quartil (Respiratory System): 2

**Manuscript 3**

**Herrero-Cortina B**, Alcaraz-Serrano V, Torres A, Polverino E

Reliability and minimal important difference of sputum weight in people with bronchiectasis.

Accepted in Respir Care (15-10-2019)

Impact Factor (2018) 1.736. Quartil (Respiratory System): 4

**Manuscript 4**

**Herrero-Cortina B,** Oliveira A, Polverino E, Gómez-Trullén EM, Torres A, Marques A.

Feasibility of computerized adventitious respiratory sounds to assess the effects of airway clearance techniques in patients with bronchiectasis.

Physiother Theory Pract. 2019; 23:1-11

DOI: 10.1080/09593985.2019.1566945

Impact Factor (2018) 1.158. Quartil (Rehabilitation): 4

**Narrative review**

**Herrero-Cortina B**, Lee AL, O'Neill B, Bradley J.

Airway clearance techniques, pulmonary rehabilitation and physical activity

In: Chalmers JD, Polverino E, Aliberti S, eds. Bronchiectasis (ERS Monograph). Sheffield, European Respiratory Society, 2018; pp. 331–352.

DOI: 10.1183/2312508X.10017017.

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**MANUSCRIPT 1**

# Short-term effects of three slow expiratory airway clearance techniques in patients with bronchiectasis: a randomised crossover trial

**Herrero-Cortina B,** Vilaró J, Martí D, Torres A, San Miguel-Pagola M, Alcaraz V, Polverino E.

Physiotherapy. 2016;102(4):357-364.

Physiotherapy 102 (2016) 357–364

# Short-term effects of three slow expiratory airway clearance techniques in patients with bronchiectasis: a randomised crossover trial

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[d] *University of Barcelona, Barcelona, Spain*

## Abstract

**Objective** To compare the efficacy of three slow expiratory airway clearance techniques (ACTs).
**Design** Randomised crossover trial.
**Setting** Tertiary hospital.
**Participants** Thirty-one outpatients with bronchiectasis and chronic sputum expectoration.
**Interventions** Autogenic drainage (AD), slow expiration with glottis opened in lateral posture (ELTGOL), and temporary positive expiratory pressure (TPEP).
**Main outcomes** Sputum expectoration during each session (primary endpoint) and in the 24-hour period after each session. Leicester Cough Questionnaire (LCQ) score and spirometry results were recorded at the beginning and after each week of treatment. Data were summarised as median difference [95% confidence interval (CI)].
**Results** Median (interquartile range) daily expectoration at baseline was 21.1 (15.3 to 35.6) g. During physiotherapy sessions, AD and ELTGOL expectorated more sputum than TPEP [AD vs TPEP 3.1 g (95% CI 1.5 to 4.8); ELTGOL vs TPEP 3.6 g (95% CI 2.8 to 7.1)], while overall expectoration in the 24-hour period after each session was similar for all techniques ($P = 0.8$). Sputum clearance at 24 hours post-intervention was lower than baseline assessment for all techniques [AD vs baseline −10.0 g (95% CI −15.0 to −6.8); ELTGOL vs baseline −9.2 g (95% CI −14.2 to −7.9); TPEP vs baseline −6.0 g (95% CI −12.0 to −6.1)]. The LCQ score increased with all techniques (AD 0.5, 95% CI 0.1 to 0.5; ELTGOL 0.9, 95% CI 0.5 to 2.1; TPEP 0.4, 95% CI 0.1 to 1.2), being similar for all ACTs ($P = 0.6$). No changes in lung function were observed.
**Conclusions** Slow expiratory ACTs enhance mucus clearance during treatment sessions, and reduce expectoration for the rest of the day in patients with bronchiectasis.
**Clinical Trial Registration Number** NCT01854788.


*Keywords:* Bronchiectasis; Respiratory therapy; Mucus; Quality of life; Crossover studies

## Introduction

Impaired mucociliary clearance in patients with non-cystic fibrosis bronchiectasis (henceforth referred to as 'bronchiectasis') [1] usually produces a continuous productive cough that affects patients' quality of life significantly

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0031-9406/

[2–4]. Mucus retention has also been associated with a major decline in lung function, more exacerbations and a higher risk of mortality in patients with bronchiectasis and other chronic respiratory diseases [3,5,6].

Current guidelines for the management of bronchiectasis and physiotherapy indicate that bronchial drainage could be a relevant non-pharmacological tool to avoid mucus retention [7,8] and possibly improve quality of life [9]. Nevertheless, evidence supporting this common belief remains scarce [10].

Slow expiratory airway clearance techniques (ACTs) [i.e. autogenic drainage (AD), slow expiration with glottis opened in lateral posture (ELTGOL), and positive expiratory pressure devices] are currently attracting more interest than conventional chest physiotherapy techniques (i.e. postural drainage, percussion and vibration) due to higher patient adherence and preference [11,12]. The main mechanism to improve mucus clearance is the generation of expiratory flow exceeding inspiratory flow [13], thereby making slow expiratory ACTs (flow-assisted/active drainage) an option to increase mucus transportation.

AD is the most popular self-administered technique in cystic fibrosis [14], but very limited information is available in patients with bronchiectasis [15]. Evidence supporting the use of ELTGOL in respiratory diseases has grown in the last few years [16–18]. Finally, temporary positive expiratory pressure (TPEP), a new positive expiratory pressure technique, has been proposed recently in some European countries for people with hypersecretion [19,20].

No ACT has been shown to be more effective than another in improving mucus clearance in bronchiectasis [21,22], possibly due to methodological limitations. Indeed, the short-term effects of chest physiotherapy are usually evaluated during a single session [22], and the effects of long-term mucus clearance are not considered after the intervention.

As adherence to treatment over time is a usual limitation for all ACTs [12,23], it is crucial to select the most appropriate technique according to patient autonomy and preferences in order to improve results [8]. However, the short-term effectiveness of slow expiratory ACTs with different degrees of autonomy (total autonomy, requiring physiotherapist assistance or device-dependent) has not been investigated to date in respiratory diseases. Therefore, a comparative trial assessing the efficacy of different techniques is needed to help physiotherapists to choose the most appropriate therapy for each patient.

Accordingly, three slow expiratory ACTs (AD, ELTGOL and TPEP) with different degrees of autonomy were studied to determine the effects of mucus clearance in stable adult patients with bronchiectasis and chronic expectoration. The secondary aim of this study was to evaluate differences between the techniques related to the impact on cough, lung function and patient preference.

## Methods

### *Patients*

Patients were recruited from the Bronchiectasis Clinic of Hospital Clinic, Barcelona, Spain from October 2010 to June 2013. The inclusion criteria were: (1) radiological diagnosis of bronchiectasis based on a high-resolution computed tomography chest scan; (2) mean daily production of spontaneous sputum $\geq$15 ml (measurements from two non-consecutive days during the week prior to starting the protocol); and (3) clinical stability over the previous 6 weeks (defined as no need for extra antibiotics or changes in usual therapy, no haemoptysis and no clinical features of exacerbation) [24].

Participants were excluded for the following reasons: (1) smokers or former smokers; (2) cystic fibrosis; (3) active interstitial lung disease or active tuberculosis [9]; (4) severe lung function impairment [forced expiratory volume in 1 second ($FEV_1$) $\leq$30% predicted, forced vital capacity (FVC) $\leq$45% predicted and peak expiratory flow <270 l/minute]; and (5) regular chest physiotherapy during the previous month (at least two sessions per week) [9]. Withdrawal criteria were: (1) pulmonary exacerbation during the study; or (2) any new medical or personal condition hindering study continuation. Patients only participated once in the study.

Written informed consent was obtained from the patients, and the study was approved by the Hospital Clinic Research Ethics Committee.

### *Study design*

Three slow expiratory ACTs were compared in an open-label, randomised three-way, crossover trial. Block randomisation was computer generated by an independent investigator, and the allocation was concealed. Each technique was applied in three non-consecutive sessions during the same week. A 7-day washout period was required between the different techniques to avoid carryover effects. Therefore, the three treatment arms lasted 5 weeks in total (Fig. 1). The study was performed in accordance with the CONSORT statement for non-pharmacological trials (Clinical Trial Registration Number NCT01854788).

### *Physiotherapeutic interventions*

Three slow expiratory ACTs with different levels of autonomy were used.

#### *Autogenic drainage*

Patients were instructed to breathe from lower lung volume levels in the expiratory reserve volume, through higher lung volume levels into the inspiratory reserve volume with the glottis open [13,25]. This technique was considered to have total autonomy as the physiotherapist only gave advice to patients during the performance of AD.

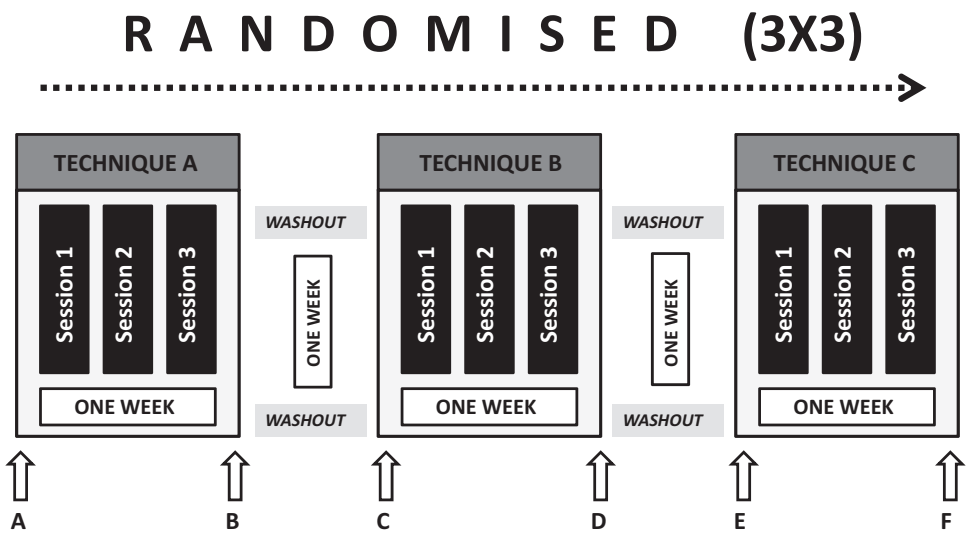


Fig. 1. Study design. Autogenic drainage (AD), slow expiration with glottis opened in lateral posture (ELTGOL) and temporary positive expiratory pressure (TPEP) were the airway clearance techniques (ACTs) performed in a randomised order. Global health outcomes (cough severity and pulmonary function test) were performed at the start and end of each treatment arm (arrows).

*Slow expiration with glottis opened in lateral posture*

Patients were encouraged to exhale slowly from functional residual capacity to the end of expiratory reserve volume with the glottis open to achieve maximum infralateral lung deflation, with active physiotherapist support [26]. Patients performed the technique in both lateral positions. ELTGOL was therefore considered to be an active-assisted technique.

*Temporary positive expiratory pressure*

Patients were instructed to breathe slowly through an electronic device (UNIKO Medical Products Research, Legnano, Italy). This tool generates a low positive pressure (1 cm $H_2O$) during the first two-thirds of the expiratory time through a pulsatile flow at a frequency of 42 Hz [19,20]. This technique was considered to be device-dependent.

All treatment sessions lasted for 40 minutes and were performed at the same time of day. The same physiotherapist supervised/assisted each session to ensure treatment standardisation. Pauses between the manoeuvres were allowed if necessary, and coughing manoeuvres were spontaneous (no encouragement from the physiotherapist). Due to the nature of the intervention, neither participants nor physiotherapist were blinded to the intervention.

One week before the trial, all the patients were instructed to breathe with the glottis open, achieve an adequate cough manoeuvre and practice the techniques. Pharmacological treatments remained unchanged over the study period.

*Outcome measures*

Baseline assessment (1 week before the trial) comprised anthropometric and clinical data, lung function, quality-of-life assessment (St George's Respiratory Questionnaire) and spontaneous daily sputum expectoration (without physiotherapeutic intervention).

*Sputum production [27]*

Two preweighed containers were used for each session to collect and weigh the sputum production. One was used to collect the wet sputum expectorated during each session (primary outcome), and the other was used to measure the total spontaneous expectoration over the 24-hour period following each session.

*Cough severity [28]*

The Leicester Cough Questionnaire (LCQ) was self-administered at the start and end of each week of treatment (1-week adapted version) (Fig. 1). The impact of cough is evaluated in three domains: physical, psychological and social. Low scores represent greater severity and a greater impact on quality of life. The intraclass correlation coefficient is 0.96 for patients with bronchiectasis, and the minimal clinically important difference is 1.3 units.

*Pulmonary function tests*

Forced spirometry was performed at the start and end of each week of treatment (NDD EasyOne Diagnostic Spirometry System, Zurich, Switzerland) following the recommendations of the American Thoracic Society/European Respiratory Society (Fig. 1). Device calibration was performed before each assessment.

*Patient preference*

At the end of each treatment arm, the patients completed a Likert questionnaire (self-administered) to indicate their preferred technique.

Pulse oximetry and heart rate were monitored during the sessions, and adverse events were noted by the same trained investigator.

*Sample size calculation*

A sample size of 23 subjects was deemed necessary to detect a minimal difference of 5 g of sputum expectoration during physiotherapy between the three techniques [27], with an $\alpha$ risk of 0.05 and a $\beta$ risk of 0.2 in a two-sided test, assuming a standard deviation (SD) of the difference in response to treatment by the same patient of 8. Considering a common drop-out rate of 20%, the final sample size required was 28 patients.

*Statistical analysis*

Data were analysed according to the intention-to-treat principle. Parametric and non-parametric analyses were performed using the Shapiro–Wilk test. Logarithmic transformation of data was performed to achieve normal distribution of the variables using linear mixed model analysis. Baseline spontaneous expectoration was incorporated into the model as a covariance. Allocation sequence, group and treatment were considered as fixed effects, and subjects within sequence were considered as a random effect. Friedman's test was employed for non-normally-distributed continuous variables with Wilcoxon's rank sum test and Bonferroni's correction for post-hoc analysis. Mean differences (95% CI) in clinical outcomes were reported. $P < 0.05$ was considered to indicate statistical significance, unless otherwise stated. SPSS Version 19 for Windows (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5.01 for Windows (GraphPad Software, La Jolla, CA, USA) were used for the analyses.

## Results

Fig. A (see online supplementary material) is a flow diagram of the patients studied. Thirty-one of the 49 subjects evaluated were recruited for the protocol. Two patients dropped out of the study due to acute low back pain and pulmonary exacerbation, respectively. Table 1 shows the patients' baseline characteristics.

There were no statistical differences in the total LCQ score and lung function between baseline of Session 1 and the end of the two washout periods (Fig. 1, Points A, C and E), indicating no carryover effects.

*Wet sputum expectoration*

*During physiotherapy sessions*

The AD and ELTGOL sessions led to similar expectoration [median difference AD vs ELTGOL 0 g (95% CI −2.2 to 0.8)], being greater than that for TPEP [AD vs TPEP 3.1 g (95% CI 1.5 to 4.8); ELTGOL vs TPEP 3.6 g (95% CI 2.8 to 7.1)]. These findings were not affected by order, week or baseline sputum production (Fig. 2), and were maintained by comparing each individual session of the three techniques (see Table A, online supplementary material).

Table 1
Patients' characteristics.

| | |
|---|---|
| Recruited patients | 31 |
| Patients lost during follow-up | 2 |
| Males (%) | 9 (29) |
| Age (years) | 59.6 (18.1) |
| Ex-smokers (%) | 5 (16) |
| Body mass index (kg/m$^2$) | 24.3 (3.8) |
| Aetiology of bronchiectasis (%) | |
| Idiopathic | 15 (48) |
| Post-infection | 6 (19) |
| Associated COPD | 5 (16) |
| Immunodeficiency | 3 (1) |
| Primary ciliary dyskinesia | 2 (1) |
| Chronic airway infection (%) | 21 (68) |
| *Pseudomonas aeruginosa* infection | 16 (52) |
| No. of lobes affected by bronchiectasis | 4.1 (1.2) |
| Inhaled antibiotic therapy[a] (%) | 18 (58) |
| Lung function | |
| $FEV_1$ (l) | 1.72 (0.82) |
| $FEV_1$% pred. | 63.3 (23.4) |
| FVC (l) | 2.62 (1.04) |
| FVC % pred. | 73.3 (22.7) |
| $FEV_1$/FVC % | 63.4 (15.1) |
| $FEF_{25–75}$ l/second | 1.08 (0.99) |
| $FEF_{25–75}$% pred. | 45.41 (32.43) |
| Baseline sputum expectoration[b] | |
| 24-hour sputum volume (ml) | 21 [15.8 to 36.5] |
| 24-hour sputum weight (g) | 21.1 [15.3 to 35.6] |
| Total baseline SGRQ score | 43.0 (12.5) |
| Physiotherapy-naive patients (%) | 24 (77) |

COPD, chronic obstructive pulmonary disease; $FEV_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; $FEF_{25–75}$ forced expiratory flow at 25% to 75% of the pulmonary volume; SGRQ, Saint George's Respiratory Questionnaire; pred., predicted.
Data presented as *n*, *n* (%), mean (standard deviation) and median [intercuartile range]
[a] Inhaled antibiotic therapy: aerosol tobramycin (29%), nebulised colistin (29%).
[b] Measured on two non-consecutive days during the week prior to initiation of the study.

*Twenty-four-hour period after physiotherapy*

Mean sputum clearance over the 24-hour period after each session (excluding treatment period) was similar for the three techniques ($P = 0.8$). The amount of sputum expectorated across the 3 days of the same treatment was similar for all techniques ($P > 0.05$). Otherwise, sputum clearance over the 24-hour period after each session was lower than the 24-hour baseline assessment (without physiotherapeutic intervention) [median difference AD vs baseline −10.0 g (95% CI −15.0 to −6.8); ELTGOL vs baseline −9.2 g, 95% CI −14.2 to −7.9; TPEP vs baseline −6.0 g (95% CI −12.0 to −6.1)] (Fig. 3).

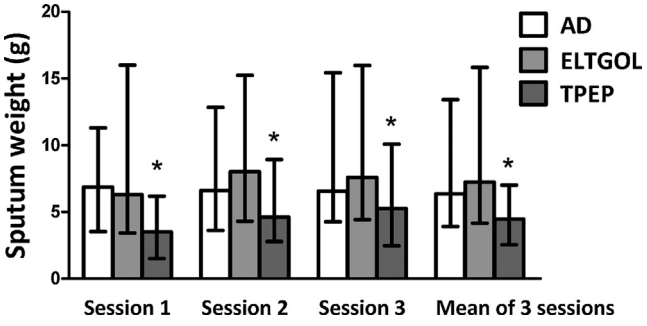


Fig. 2. Wet sputum expectorated during three physiotherapy sessions and mean values. Bar graphs express medians and ranges. *$P < 0.02$. AD, autogenic drainage; ELTGOL, slow expiration with glottis opened in lateral posture; TPEP, temporary positive expiratory pressure.

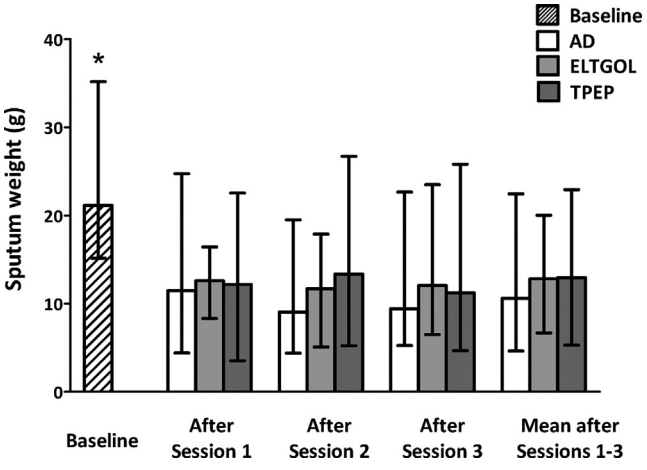


Fig. 3. Wet sputum expectorated spontaneously at baseline and during the 24-hour period following three physiotherapy sessions (excluding during treatment), and mean values. Bar graphs express medians and ranges. The amount of sputum collected over the 24-hour period after each physiotherapy session was significantly lower than the baseline sputum value for all three techniques (*$P < 0.001$) with no significant differences between the techniques ($P > 0.05$). Baseline sputum, two measures over 24 hours on two non-consecutive days before initiating the study; AD, autogenic drainage; ELTGOL, slow expiration with glottis opened in lateral posture; TPEP, temporary positive expiratory pressure.

*Total sputum quantity (including treatment)*

Overall sputum clearance did not differ between techniques ($P = 0.1$), being similar to baseline (without intervention) [median difference AD vs baseline −1.4 g (95% CI −5.1 to 1.2); ELTGOL vs baseline −1.6 (95% CI −4.8 to 1.0); TPEP vs baseline −2.5 (95% CI −8.6 to 0.1)].

The median expectoration obtained during each physiotherapy session represented, on average, 41.3%, 42.3% and 28.1% of the total 24-hour sputum collection for AD, ELTGOL and TPEP, respectively.

*Impact on cough severity*

All techniques induced a significant, albeit not clinically relevant, improvement in total LCQ score ($P < 0.05$) after 1 week of treatment. Only ELTGOL achieved significant changes in all domains of the LCQ score (Table 2).

The median change in total LCQ score after the intervention was similar for all techniques ($P = 0.6$).

*Lung function and patient preferences*

Lung function (FVC, $FEV_1$, $FEF_{25–75}$) remained stable after all ACTs ($P > 0.05$). Globally, 48.8% of participants ($n = 15$) described AD as their preferred technique, followed by ELTGOL (35.4%, $n = 11$). No adverse events associated with slow expiratory ACTs were recorded during or after the physiotherapy sessions.

## Discussion

The main findings of this study were as follows.

- In stable patients with bronchiectasis, AD and ELTGOL induced higher sputum expectoration than TPEP during physiotherapy sessions. However, the three techniques showed similar amounts of sputum expectoration in the 24-hour period after each session, demonstrating similar efficacy.
- Sputum clearance in the 24-hour period after each session was significantly lower than that for the 24-hour baseline assessment; however, overall sputum clearance (during and after physiotherapy sessions) was similar to baseline for all ACTs.
- All techniques showed a similar positive short-term effect on quality of life by increasing the total LCQ score over a 1-week period. Moreover, these increases did not differ between the techniques.

To facilitate daily expectoration, the use of different ACTs has increased recently in the management of patients with bronchiectasis. Self-management techniques are recommended to improve the level of treatment adherence [8,11], but no one technique to date has demonstrated greater efficacy than other techniques [22,29–31]. However, to the best of our knowledge, this is the first study to compare three slow expiratory ACTs with different levels of autonomy (total autonomy, requiring physiotherapist assistance and device-dependent).

In a quasi-experimental study, O'Connor and Bridge [15] reported that AD promoted higher sputum clearance during a single session compared with control sessions in patients with bronchiectasis. Moreover, in patients with cystic fibrosis, AD has been shown to induce similar or greater sputum expectoration compared with conventional chest physiotherapy with the benefit of no adverse events [8,32]. Recently, TPEP has been studied in patients with mucus hypersecretion as an adjunctive technique of ELTGOL, and has been found to have greater effects on expectoration compared with ELTGOL alone, although baseline patient expectoration was not reported [19].

Table 2
Change in Leicester Cough Questionnaire score after 1 week of treatment for each slow expiratory airway clearance technique.

| | Total score | | | Physical score | | | Psychological score | | | Social score | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Median difference | 95% CI | *P*-value | Median difference | 95% CI | *P*-value | Median difference | 95% | *P*-value | Median difference | 95% CI | *P*-value |
| AD | 0.5 | 0.1 to 0.5 | 0.01 | 0.1 | 0.0 to 0.3 | 0.1 | 0.1 | −0.1 to 0.4 | 0.1 | 0.0 | −0.1 to 0.5 | 0.2 |
| ELTGOL | 0.9 | 0.5 to 2.1 | 0.001 | 0.4 | −0.1 to 0.6 | 0.006 | 0.3 | 0.1 to 0.6 | 0.001 | 0.2 | 0.1 to 0.9 | 0.001 |
| TPEP | 0.4 | 0.1 to 1.2 | 0.04 | 0.1 | −0.1 to 0.3 | 0.3 | 0.1 | 0.0 to 0.5 | 0.06 | 0.1 | 0.0 to 0.5 | 0.02 |

AD, autogenic drainage; ELTGOL, slow expiration with glottis opened in lateral posture; TPEP, temporary positive expiratory pressure; CI, confidence interval.
Wilcoxon's tests were performed. $P < 0.05$ was considered to indicate statistical significance.

Unfortunately, the timing and duration of different effects of ACTs on mucus clearance are poorly known [9]. Measurements of mucociliary clearance *in vivo* are usually acquired 2 hours and 24 hours after inhalation [33]; therefore, the same timeline was used in this study for sputum measurements. Similarly, Martins *et al.* [18] reported the persistence of ELTGOL effects 2 hours after treatment using mucociliary clearance assessment.

Additionally, Guimarães *et al.* [16] demonstrated the greater effectiveness of ELTGOL compared with an oscillating positive expiratory pressure device only 15 minutes after the intervention in stable patients with bronchiectasis, but the effects of mucus clearance following the physiotherapeutic intervention were not measured. Unfortunately, the duration of ACT sessions has not been standardised, which may limit the comparison of study results.

Greater sputum expectoration was observed during AD and ELTGOL than TPEP sessions, with similar mucus clearance effects over the 24-hour period after each session for all techniques. The three slow expiratory ACTs were able to concentrate part of the sputum clearance during the intervention period, hence reducing the need to expectorate throughout the rest of the day, indicating slower action timing for TPEP than for AD and ELTGOL. Indeed, 28% to 42% of the total daily sputum was expectorated during the physiotherapy session, independently of the technique, thereby reducing the impact of daily symptoms related to mucus retention such as cough. Similarly, Osman *et al.* [27] studied wet sputum expectoration in cystic fibrosis exacerbations by comparing various ACTs in common use in cystic fibrosis with high-frequency chest wall oscillation (HFCWO). Their patients achieved approximately 28% (usual ACTs) and 13% (HFCWO) of total daily expectoration during the physiotherapeutic intervention ($P < 0.001$).

It is known that the learning time for AD is longer than for other techniques, which could potentially influence sputum clearance analysis [25]; however, all patients in the present study were highly motivated to collaborate, and were trained before the trial commenced. In addition, all sessions were supervised by a physiotherapist experienced in the management of mucus clearance using slow expiratory ACTs.

ACTs were found to have a positive impact on the quality of life of patients. The improvement in the total LCQ score may be due to: (1) more efficient expectoration, (2) reduced cough during the rest of the day, and (3) adequate tolerability of the manoeuvres. However, this amelioration was not clinically relevant, possibly due to the short duration of the intervention. Nevertheless, this positive effect disappeared after the washout period (no carryover effects), suggesting that patients could possibly benefit from regular chest physiotherapy to maintain clinical effects.

In agreement with other studies [9,10], forced spirometry did not show any significant change in lung function after the interventions, thereby supporting the safety of these ACTs in patients with bronchiectasis.

This study mainly recruited patients with low previous adherence to physiotherapy with the aim of assessing

intervention-related clinical changes more closely, and avoiding any potential intervention during the washout period.

Of the ACTs used in this study, the participants preferred AD, possibly due to the high quantity of sputum expectorated during the sessions and the fact that it is performed independently, reinforcing personal satisfaction. Indeed, the study population was cooperative and correctly understood the instructions to perform the techniques. Conversely, both ELTGOL (when a physiotherapist is available) and TPEP (for domiciliary treatment) may be preferred by poorly collaborative patients considering their similar profile of efficacy/tolerability.

A strength of this study was that the short-term effects of ACTs were analysed based on three different sessions, rather than a single session. The outcome measure of sputum quantity showed great variability between subjects. Therefore, the crossover design and repetitive sessions ensured both within-subject comparison and greater accuracy of the results. Moreover, the potential long-term effect of sputum clearance of ACTs was also measured.

Potential limitations of this study include the sample size calculation being based on patients with cystic fibrosis (due to similarities in clinical manifestations) because of the lack of data related to the primary endpoint in bronchiectasis. Participants and the physiotherapist were not blinded due to the characteristics of the interventions; however, the physiotherapist had lengthy experience with ACTs, reducing performance bias, while subjective outcomes were reported by patients, limiting detection bias. Although wet sputum quantity is considered to be a controversial outcome to assess mucus clearance (saliva contamination or secretion swallowing) [34,35], no study to date has evaluated the psychometric properties, and the crossover design of this study may reduce this potential bias. Moreover, the amount of sputum collected over the 24-hour period after each session depends on patient compliance. Nevertheless, 24-hour sputum containers were weighed immediately after the assessment period, and no differences were observed between the 3 days of the same treatment. Furthermore, the efficacy of slow expiratory ACTs in patients with limited expectoration (<15 g) remains unknown.

In conclusion, AD, ELTGOL and TPEP are effective, safe techniques for enhancing airway clearance. All three chest physiotherapy techniques were able to reduce cough and expectoration for the remainder of the day, and improve patients' quality of life even with a short-term intervention. However, an individualised approach based on patient characteristics (autonomy, preferences) should be performed in bronchiectasis in order to improve patient adherence to physiotherapy and optimise outcomes.

The positive results regarding short-term efficacy of ACTs support their regular use in clinical practice to reduce the impact of daily cough and improve overall quality of life. Moreover, assessment of the effects of ACTs was simple and may be easily extrapolated to clinical practice.

Nevertheless, these results also raise the question of whether longer-term use of these techniques will show similar efficacy or even reduce the risk of exacerbations, as suggested by Lee *et al.* [36] in an exercise training trial in bronchiectasis. Further studies are required to determine the longer-term effects of these techniques on airway clearance and major clinical outcomes (exacerbations, lung function decline and mortality).

*Ethical approval:* This study was approved by the Research Ethics Committee from Hospital Clinic, Barcelona, Spain (HCP/2010/215). All participants provided informed consent.

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*Conflict of interest:* BH and EP received a grant and non-financial support from MPR during the conduct of the study to support the overall research activity. Outside the study, EP received personal fees from Praxis Pharmaceutical, but without any competing interests with the trial. Ciber de Enfermedades Respiratorias, PII bronquiectasias and PII infecciones respiratorias (Area TIR) from SEPAR provided scientific support to the study but has no competing interests.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.physio.2015.07.005.

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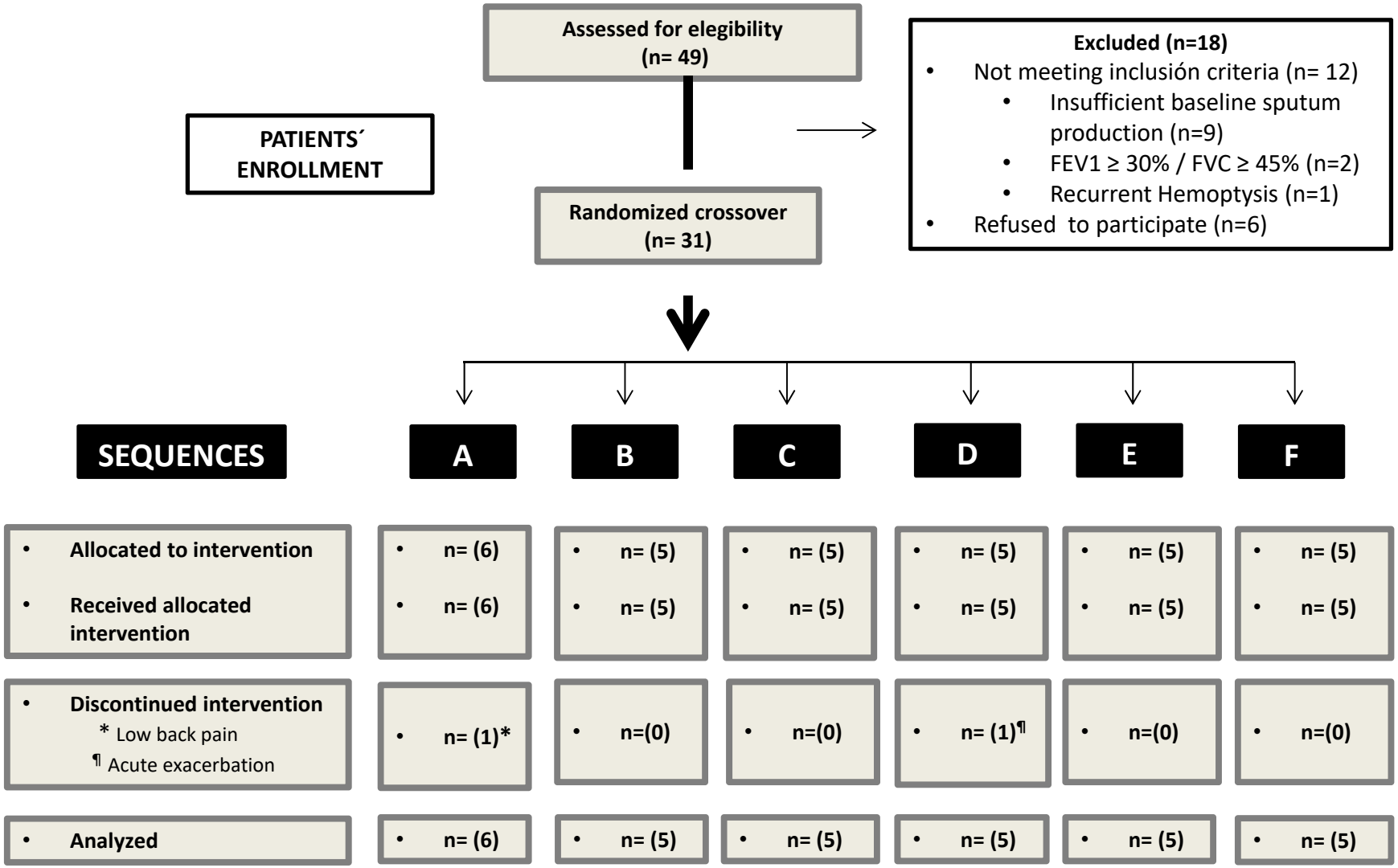


**Figure A** Consort flow diagram for non-pharmacological clinical trial. TreatmentSequences**: A** (AD, ELTGOL, TPEP); **B** (AD, TPEP, ELTGOL); **C** (ELTGOL, AD, TPEP); **D** (ELTGOL, TPEP, AD); **E** (TPEP, AD, ELTGOL); **F** (TPEP, ELTGOL, AD). Reasons for discontinued interventions: * low back pain (in the three week); ¶ acute pulmonary exacerbation (in the second week). All interventions were performed by the same researcher

## Supplementary material

**Table A** Wet sputum expectorated during physiotherapy treatment for each one of the sessions performed.

| | 1st session | | 2nd session | | 3th session | |
|---|---|---|---|---|---|---|
| | Median difference (95% CI) | p value | Median difference (95% CI) | p value | Median difference (95% CI) | p value |
| **AD vs. ELTGOL** | 0.0 ( -0.7 to 2.4) | 0.7 | 0.2 ( -0.6 to 3.2) | 0.1 | 0.5 ( -1.1 to 2.3) | 0.2 |
| **AD vs. TPEP** | -2.4 ( -5.6 to -2.1) | < 0.001 | -1.5 ( -4.0 to -0.4) | 0.01 | -1.8 ( -5.5 to -0.5) | 0.002 |
| **ELTGOL vs TPEP** | -3.4 ( -7.6 to -2.4) | < 0.001 | -3.5 (-7.3 to -1.8) | < 0.001 | -2.5 (-7.6 to -1.4) | < 0.001 |

Data had been presented as median difference [95% confidence interval]. A p value < 0.05 were estimated statistically significant; AD: autogenic drainage; ELTGOL: slow expiration with glottis opened in lateral posture; TPEP: temporary positive expiratory pressure.

**MANUSCRIPT 2**

# Impact of hypertonic saline solutions on sputum expectoration and their safety profile in patients with bronchiectasis: a randomised crossover trial

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# Impact of Hypertonic Saline Solutions on Sputum Expectoration and Their Safety Profile in Patients with Bronchiectasis: A Randomized Crossover Trial

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## Abstract

***Background:*** The role of hyaluronic acid plus hypertonic saline (HA+HS) as a mucoactive treatment in patients with bronchiectasis is still unknown. This study evaluated whether HA+HS solution enhances similar sputum quantity with better safety profile than HS alone in patients with bronchiectasis.

***Methods:*** In this double-blind randomized crossover trial, three solutions (7% HS; 0.1% HA +7%HS; and 0.9% isotonic saline, IS) were compared in outpatients with bronchiectasis and chronic sputum expectoration. Participants inhaled each solution across four consecutive sessions. All sessions, except on session 3, also included 30 minutes of airway clearance technique. A 7-day washout period was applied. Sputum weight was collected during the sessions (primary outcome) as well as during a 24-hour follow-up. The Leicester Cough Questionnaire (LCQ) and lung function were measured before/after each treatment arm. Safety was assessed by the monitoring of adverse events (AEs).

***Results:*** Twenty-eight patients with bronchiectasis (mean age of 64.0 (17.9) and $FEV_1$% 60.9 (24.6) of predicted) were recruited. HS and HA+HS promoted similar expectoration during sessions, both being greater than IS [median difference HS vs. IS 3.7 g (95% CI 0.5–6.9); HA+HS vs. IS 3.2 g (95%CI 0.5–5.9)]. Sputum expectorated exclusively during the ACT period was similar across all treatment arms [HS vs. IS −0.3 g (95% CI −1.7 to 0.9); HA+HS vs. IS 0.0 g (95% CI −1.3 to 1.4); HS vs. HA+HS 0.0 g (95% CI −1.2 to 0.4)]. Sputum collected over the 24-hour follow-up tended to be lower for HS and HA+HS compared with IS [HS vs. IS −1.7 g (95% CI −4.2 to 0.0); HA+HS vs. IS −1.1 g (95%CI −3.6 to 0.7)]. No differences in LCQ or lung function were observed. Most severe AEs were reported using HS.

***Conclusion:*** HS and HA+HS were more effective on sputum expectoration than IS in patients with bronchiectasis, reporting HA+HS better safety profile than HS.

**Keywords:** airway clearance techniques, bronchiectasis, hypertonic solutions, mucoactive treatment, sputum expectoration

## Introduction

According to the recent European guidelines, the hyperosmolar agents plus airway clearance techniques (ACTs) may be considered to further enhance sputum expectoration in adult patients with noncystic fibrosis bronchiectasis (henceforth referred to as ''bronchiectasis''[1]); however, scientific evidence supporting this statement is quite scarce.[2]

The inhalation of hypertonic saline (HS) produces an osmotic shock in the airway surface layer that improves

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airway hydration, accelerating mucus transportability.[3] This effect of HS could potentiate and prolong the posterior effectiveness of ACTs. Greater sputum weight has been observed during combined sessions (inhalation and ACT) using HS rather than isotonic solution (IS) in patients with bronchiectasis and mild daily sputum expectoration (<10 g/24 h).[4] However, the reasons why combined interventions (rather than individual) could be more efficient are unclear.

Overall, short and long-term use of HS has been demonstrated to be generally well tolerated by patients with bronchiectasis[5] and other respiratory diseases.[6,7] Nevertheless, minor adverse events (AEs) (e.g., throat irritation, excessive coughing, or airway narrowing) have been frequently reported with HS inhalation.[8–10] These minor AEs may appear after the first HS inhalation, negatively impacting long-term treatment adherence.[9,11]

Hyaluronic acid (HA) is a glycosaminoglycan that is able to mitigate bronchospasm induced by elastases and to balance water homeostasis in airways.[12,13] Therefore, the addition of HA to HS (HA+HS) may be beneficial to improve tolerance and efficacy of HS solution. Previous studies conducted in cystic fibrosis showed greater tolerance and pleasantness in favor of HA+HS compared with HS alone.[8,11,14] So far, no studies have compared the effectiveness and tolerability of HA+HS and HS in adult outpatients with bronchiectasis. Improved tolerability with at least equal efficacy of HA+HS may improve patients' adherence, while hopefully improving chronic respiratory symptoms and quality of life (QoL) for patients with bronchiectasis.

Therefore, the purpose of this study was to evaluate whether HA+HS is as efficacious as HS alone and better than IS in improving sputum expectoration (primary outcome), cough severity, and lung function in stable patients with bronchiectasis and daily expectoration (>10 g/24 h) naive to hyperosmolar agents. Also, this study aimed (i) to examine whether hypertonic solutions (HS, HA+HS) could increase effectiveness of ACTs in expectorated sputum compared with IS; (ii) to analyze whether a combined session of saline solutions and ACT is better than saline solutions alone in enhancing sputum quantity; (iii) to evaluate whether the short-term tolerability and safety of HA+HS is better compared with HS alone.

## Methods

### Design

A double-blind, randomized, crossover trial with concealed allocation was conducted. Participants each randomly inhaled one of the three solutions: HS solution (7% NaCl); HA + HS solution (0.1% sodium hyaluronate +7% NaCl), and IS solution (0.9% NaCl), during four consecutive sessions (once daily). A 7-day washout period was applied between the treatment arms (Supplementary Fig. S1; Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/jamp).

A block random list was computer generated and retained by a research nurse, not directly involved in the project. The nurse received a notification email confirming the eligibility criteria by the enrolling investigator and the randomization took place after baseline data collection. Immediately, the sequence of treatments was revealed to the pharmacist (also external to the project) to produce the masking of inhaled solutions. Patients and physiotherapists were thus blinded to the inhaled solutions throughout the study.

The study was approved by the Hospital Clinic Research Ethics Committee (HCP/2011/6401) and was performed in accordance with the CONSORT statement (Clinical Trial Registration Number NCT02392663). All participants gave written informed consent before enrolment.

### Participants

Patients were recruited from the Hospital Clinic, Barcelona, Spain. They were eligible to participate if over 18 years of age, diagnosed with bronchiectasis using high-resolution computed tomography, clinically stable over the previous 4 weeks,[15] producing spontaneous sputum expectoration (mean sputum ≥10 g/24 h), able to test the inhalation solutions, and to perform the ACT. The exclusion criteria included: smokers or former smokers (≥10-pack-years),[16] bronchial hyperresponsiveness diagnosis,[17] asthma[18] or allergic bronchopulmonary aspergillosis,[5] forced expiratory volume in the first second ($FEV_1$) <30% after bronchodilation, total lung capacity <45%, and inhalation of mucoactive agents before screening.

Finally, the withdrawal criteria were: pulmonary exacerbation during the study or any new medical/personal condition hindering study continuation.

### Intervention

First, participants were encouraged to inhale 200 $\mu$g of albuterol with a spacer chamber, or their usual bronchodilator,[19] and before starting the inhalations they had to wait 15 minutes for short-acting bronchodilators or 30 minutes for long-acting bronchodilators. Participants inhaled 5 mL of the assigned solution through a mouthpiece using a jet nebulizer (PARI BOYSX® device with PARI LC® Sprint nebulizer) in a seated position. They were instructed and advised to inhale slowly and deeply follow by a short breath-hold (2–3 seconds) to improve aerosol deposition.[20] Participants stopped compressor during coughing and all sessions were supervised by the physiotherapist to guarantee the correct mode of breathing during the inhalation.

All sessions for all treatment arms included 30 minutes of supervised ACT after inhalation, except for session 3. Autogenic drainage was the ACT chosen for the trial, based on our previous experience in terms of patient preference and short-term effectiveness[21] and was performed in a supine position following the authors' recommendations.[22,23] Physiotherapist gave oral advice and manual feedback during the performance. In each third session, participants remained 30 minutes in the same supine position after the inhalation period without performing any ACT (''control period'').

Cough maneuvers were always spontaneous during sessions, and if necessary pauses were allowed. Peripheral oxygen saturation and heart rate were monitored during intervention. All study visits were performed to the same schedule at the hospital. Patients were asked not to perform ACTs before the beginning of the sessions and for 24 hours after the intervention.

Pharmacological treatments remained unchanged and patients were encouraged to take their long-term medications at the same time of the day over the study period. All

participants were trained to breathe with the glottis open and coughing correctly before starting the trial.

### Outcome measures

The primary outcome was the wet *sputum expectoration (g)* collected during sessions. It was measured through two preweighed containers, one to collect the sputum expectorated during inhalation period and the other to measure the sputum collected during ACT period. For the primary outcome, session 3 (inhalation period + control period) was not included, but the sputum expectorated was also measured following the same procedure.

Secondary endpoints included the spontaneous sputum expectorated over a 24-hour follow-up after the end of sessions, collected in another preweighed container. The timeline chosen for evaluating the long-lasting effects of interventions was based on similar previous studies on sputum expectoration.[21,24,25]

Secretions from sinus after an inspiratory forced maneuver were not allowed to include in the containers. Participants were instructed to swallow saliva before coughing during inhalation periods and also to avoid salivary contamination over a 24-hour follow-up after intervention. In addition, most part of possible saliva was removed from the containers before weighing.

*Cough severity*, using the Leicester Cough Questionnaire (LCQ)[26,27] and lung function ($FEV_1$ and FVC),[28] was also assessed at the beginning and end of each treatment arm. At the end of the trial, participants selected their preferred treatment arm while also determining which solution was the saltiest.

*Safety* was assessed by monitoring AEs after each inhalation.[11] Perception of bronchospasm (wheezing or chest tightness), excessive coughing, and throat irritation were reported by participants and their severity was measured using a three-point ordinal score[11] (0 = absent, 1 = mild, 2 = moderate, and 3 = severe; from 0 to 9). The presence of hemoptysis and desaturation was assessed by the physiotherapist.

*Tolerability* was evaluated the first day of each saline solution tested. A spirometry was performed pre- and postbronchodilator and again 5 minutes after completing the inhalation.[29] Participants with inhalation-induced bronchospasm (fall ≥12% $FEV_1$)[30] were not allowed to continue the remainder sessions of the same treatment arm; however, they continued the study trying the next saline solution according to the same schedule and procedure.

### Data analysis

A sample size of 20 completing patients was calculated to be necessary to provide 80% power and 5% level of significance, in a two-sided test, to detect a minimum 5.7 g[21] of difference in sputum quantity during sessions, including inhaled and ACT periods, between the three treatment arms. This is based on the fact that the standard deviation of difference in the response variable for the same patient is 8.5.[21] Allowing for 20% early withdrawal, this study recruited a total of 24 patients.

Repeated-measures analysis was performed using linear mixed models to determine changes in expectorated sputum, cough severity, lung function, and safety score across the three treatment arms. Allocation sequence, group, and treatment were considered as fixed effects, and subjects within sequence were considered a random effect. The daily expectoration measured before starting each treatment period was incorporated into the model as a covariance (except for safety analysis). Difference between the treatment arms was reported as median difference (95% Confidence Interval [CI])[31] and statistical significance was set at $p < 0.05$ for all calculations.

All randomized participants were included in the analysis for effectiveness (intention-to-treat basis); however, those who never began treatment after the randomization process (dropouts before the first session), did not complete the study, or pass the tolerability test for all solutions, were not included in the safety analysis.[32] Missing data were not imputed.[33,34]

The sputum collected during the intervention and over the 24-hour follow-up was compared with a combined session, including ACT period (session 1, 2, or 4), and the single session, including a control period (session 3) using a Wilcoxon test for each of the treatment arms. To select only one of the three possible ACT sessions, a list of random numbers was generated. The sputum obtained was analyzed as sputum weight and sputum quantity ratio (%) (i.e., sputum expectorated during the session or during the 24-hour follow-up/total sputum collected × 100). Effect sizes were computed to estimate the magnitude of changes, using rank-biserial correlation (*r*) and interpreted as small effect (<0.3), moderate effect (≥0.3), and large effect (≥ 0.5).

## Results

From March to December 2015, thirty-eight volunteers were screened for the study, with 28 meeting the eligibility criteria, who were consented and randomized. Three participants dropped out of the study before the first session and two participants withdrew during the study (Supplementary Fig. S2). Accordingly, 23 patients completed the study protocol with 98% adherence to planned treatment sessions.

Participant characteristics are shown in Table 1. At the beginning of all treatment arms, participants were similar with respect to daily sputum expectoration, total LCQ score, and lung function, indicating no carryover effects.

### Treatment effect

Sputum expectoration. The primary outcome, sputum collected during sessions (including inhalation plus ACT periods) with HS and HA+HS treatments, was similar, both being greater than that obtained with IS (Table 2).

Similarly, the sputum obtained exclusively during the inhalation period was also similar to the HS and HA+HS treatments, both being greater than that obtained with IS. However, sputum expectorated exclusively during the ACT period was similar across all treatment arms. The sputum collected over the 24-hour follow-up showed a decreasing trend from IS (the highest), to HA+HS and HS, being the effects of hypertonic solutions being very similar (Table 2).

When comparing the random combined session, including ACT (session 1, 2 or 4) and that without it (session 3), we consistently observed more sputum in the combined session at the end of sessions, independently of the inhaled solution (Table 3). Consequently, the session sputum quantity ratio (%) obtained was always greater for the combined session

Table 1. Baseline Characteristics of Randomized Participants

| *Characteristics* (n = 28) | |
|---|---|
| Age (yr), mean (SD) | 64.0 (17.5) |
| Gender, *n* female (%) | 18 (64.3) |
| BMI (kg/m$^2$), mean (SD) | 24.3 (3.6) |
| Etiology of bronchiectasis, *n* (%) | |
| Unknown | 10 (35.7) |
| Postinfection | 12 (42.8) |
| Primary ciliary dyskinesia | 3 (10.7) |
| Others | 3 (10.7) |
| Chronic airway infection (%)[a] | 22 (78.6) |
| *P. aeruginosa* infection | 14 (50) |
| Lung function ($FEV_1$) | |
| Liters | 1.5 (0.8) |
| % pred. | 60.9 (24.6) |
| Long-term inhaled $\beta_2$ agonists, *n* (%) | |
| Short acting | 3 (10.7) |
| Long acting | 22 (76.6) |
| Long-term inhaled anticholinergics, *n* (%) | |
| Short acting | 2 (7.1) |
| Long acting | 14 (50.0) |
| Long-term inhaled steroid therapy, *n* (%) | 21 (75.0) |
| Long-term antibiotic treatment, *n* (%) | |
| Oral (macrolides) | 3 (10.7) |
| Inhaled | 3 (10.7) |
| Baseline sputum expectoration[b] | |
| 24-hour period, median (IQR) | 13.6 (10.8–21.4) |

Data presented as *n*, *n* (%), mean (SD, standard deviation) and median (IQR, interquartile range).

[a]Chronic airway infection was defined as pathogen organism cultured in at least two or more sputum samples, at least 3 months apart, in the preceding 12 months.

[b]Measured on two consecutive days during the week before the start of the study.

BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity,% pred, percentage of predicted.

than session 3 [HS *Inh+ACT* vs. *Inh+Control* 14.5% (95% CI 6.2–24.5); HA+HS *Inh+ACT* vs. *Inh+Control* 11.4% (95% CI 2.2–20.3); IS *Inh+ACT* vs. *Inh+Control* 24.1% (95% CI 11.1–35.9)] (Fig. 1).

On the contrary, the sputum quantity ratio obtained during the 24-hour follow-up period was consistently proportionally lower in the random combined session, including ACT (session 1 or 2 or 4), than in session 3, including control period for all solutions [HS *Inh+ACT* vs. *Inh+Control* −11.6% (95% CI −22.9 to −3.2); HA+HS *Inh+ACT* vs. *Inh+Control* −9.4% (95% CI −19.3 to −1.7); IS *Inh+ACT* vs. *Inh+Control* −20.7% (95% CI −34.4 to −8.6)] (Fig. 1).

Finally, the time spent during inhalation period was similar between the three solutions ($p = 0.06$), showing greater amounts of time spent with HS (20 minutes and 58 seconds) and HA+HS (18 minutes and 39 seconds) treatments compared with IS (16 minutes and 5 seconds) treatment.

Cough severity and lung function. By comparing the 3 treatments, the observed changes in total LCQ were not significantly different ($p > 0.05$). Lung function ($FEV_1$ and FVC) also remained unchanged after all interventions ($p > 0.05$) (Supplementary Table S1).

Preference and salty taste. Around 48% of participants who started all treatment arms selected HA+HS as their preferred solution to include in chronic treatment. As expected, most of the patients (69%) reported HS as the saltiest solution.

### *Safety and tolerability of the intervention*

HS showed the poorest safety profile [HS vs. IS 2.7 (1.6–3.9); HS vs. HA+HS 1.2 (0.3–2.4)]; whereas HA+HS showed an intermediate safety profile between HS and IS [HA+HS vs. IS 1.2 (0.3–2.0)] (Supplementary Table S2). Coughing and throat irritation were the most frequent minor AEs classified as moderate or severe by participants, particularly after the inhalation of HS and, to a lesser extent, after HA+HS (Fig. 2). Mild oxygen desaturation was detected in three patients, but the values increased to normal after the inhalation period. Three small sputum samples stained with blood from two participants (one during IS solution and the other during HS and IS solution) were observed.

While IS solution was well tolerated by all participants, the tolerability test of the HS solution failed in three patients. Two of these three patients also failed the tolerability test for the HA+HS solution (Supplementary Fig. S2). These three participants were elderly men (≥75 years) and presented a major impairment of lung function ($FEV_1 \leq 40\%$ of predicted) compared with the sample average.

Consequently, the three treatment arms were completed from 71% of randomized participants. Individually, 20

Table 2. Median (Interquartile Range) Values of Sputum Expectorated during Sessions 1, 2, and 4 at Different Time Points for Each Treatment Arm and Median Difference (95% CI) Between Treatment Arms

| | *Treatment arms* (n = 28) | | | *Median difference between treatment arms* (n = 28) | | |
|---|---|---|---|---|---|---|
| | *HS* | *HA + HS* | *IS* | *HS vs. HA + HS* | *HS vs. IS* | *HA + HS vs. IS* |
| Inhalation + ACT[a] | 11.3 (0.4–19.8) | 10.7 (4.2–18.8) | 8.4 (2.9–15.3) | 0.4 (−0.4 to 2.2) | 3.7 (0.5–6.9) | 3.2 (0.5–5.9) |
| Inhalation | 6.6 (0.4–11.2) | 5.8 (0.9–11.1) | 3.0 (0.9–5.2) | 1.6 (0.0–2.8) | 4.0 (1.6–6.7) | 2.6 (1.3–4.6) |
| ACT | 4.0 (0.0–7.8) | 3.9 (1.3–9.6) | 5.4 (1.7–8.4) | 0.0 (−1.2 to 0.4) | −0.3 (−1.7 to 0.9) | 0.0 (−1.3 to 1.4) |
| 24-hour follow-up[b] | 4.8 (0.4–11.0) | 7.7 (2.7–10.4) | 8.4 (3.6–12.6) | −0.2 (−2.2 to 1.4) | −1.7 (−4.2 to 0.0) | −1.1 (−3.6 to 0.7) |

The amount of sputum was measured in grams (g).

[a]Primary outcome.

[b]Twenty-four hour follow-up does not include the time of intervention. Comparisons were adjusted by the level of expectoration collected before starting each treatment arm.

HS, hypertonic saline; HA + HS, hyaluronate acid plus hypertonic saline; IS, isotonic saline; ACT, airway clearance technique.

Table 3. Median (Interquartile Range) of Sputum Expectorated During the Random Combined Session, Including ACT Period (Session 1 or 2 or 4) and the Single Session, Including Control Period (Session 3) at the Different Periods for Each One of the Treatment Arms, Median Difference (95% CI) Between the Sessions

| | *Treatment arms* (n = *28*) | | *Median difference (95% CI)* | |
|---|---|---|---|---|
| | *Session including ACT period* | *Session including control period* | *Session including ACT vs. Session including control period* | *Effect size* |
| HS | | | | |
| Inhalation + ACT/control | 12.1 (0.5–21.8) | 6.3 (0.0–11.7) | 4.5 (2.1–9.7) | 0.61 |
| Inhalation | 5.3 (0.5–12.7) | 5.4 (0.2–9.4) | 1.0 (0.0–3.0) | 0.37 |
| ACT/control | 4.6 (0.0–7.5) | 0.0 (0.0–2.0) | 3.3 (1.8–5.4) | 0.68 |
| 24-hour follow-up[a] | 3.4 (0.0–12.7) | 7.8 (0.0–19.7) | −2.7 (−7.5 to −0.3) | 0.57 |
| HA + HS | | | | |
| Inhalation + ACT/control | 11.5 (3.2–18.4) | 6.2 (0.0–13.3) | 4.0 (0.8–6.6) | 0.52 |
| Inhalation | 6.3 (0.8–11.4) | 4.5 (0.3–11.4) | 0.0 (−2.0 to 2.3) | 0.00 |
| ACT/control | 4.0 (0.8–8.7) | 0.0 (0.0–1.9) | 2.9 (1.7–5.3) | 0.67 |
| 24-hours follow-up[a] | 7.0 (1.6–12.5) | 8.1 (2.3–18.9) | −2.1 (−6.4 to −0.3) | 0.53 |
| IS | | | | |
| Inhalation + ACT/control | 8.6 (3.4–13.7) | 3.0 (0.0–4.7) | 5.1 (3.5–8.4) | 0.72 |
| Inhalation | 2.0 (0.1–4.6) | 1.6 (0.0–3.5) | 0.5 (−0.5 to 1.3) | 0.16 |
| ACT/control | 5.7 (1.6–8.3) | 0.5 (0.0–2.0) | 4.4 (2.7–6.9) | 0.76 |
| 24-hour follow-up[a] | 6.0 (2.1–13.1) | 10.0 (5.4–17.8) | −1.9 (−6.6 to −0.1) | 0.38 |

The amount of sputum was measured in grams (g).
[a]Twenty-four hour follow-up does not include the time of intervention.



(71%) patients completed the HS solution arm, 22 (79%) the HA+HS solution arm, and 23 (82%) the IS solution arm.

## Discussion

This is the first study to evaluate the impact of three different saline solutions (HS, HA+HS, IS) inhaled before ACT on sputum expectoration in adult outpatients with bronchiectasis and chronic expectoration (>10 g/24 h).

The main findings were: (i) both hypertonic solutions at 7% (HS and HA+HS) promoted greater sputum weight during sessions than IS. Contrarily, the sputum collected over the 24-hour follow-up after sessions showed a decreasing trend from IS to HA+HS and, finally, to HS (the lowest). No significant changes in cough severity and lung function were observed between the treatment arms after four sessions; (ii) effectiveness of ACT (using autogenic drainage) seemed not to be influenced by the previously inhaled solutions; in fact the sputum obtained during the ACT period was similar throughout all treatment arms; (iii) sputum collected at the end of a combined session (inhaled + ACT period) was always greater than a single session, including the control period (session 3), independently of the inhaled solution; conversely, the sputum expectorated in the combined sessions 24 hours hence was clearly lower than after session 3 (inhalation + control); (iv) globally, the

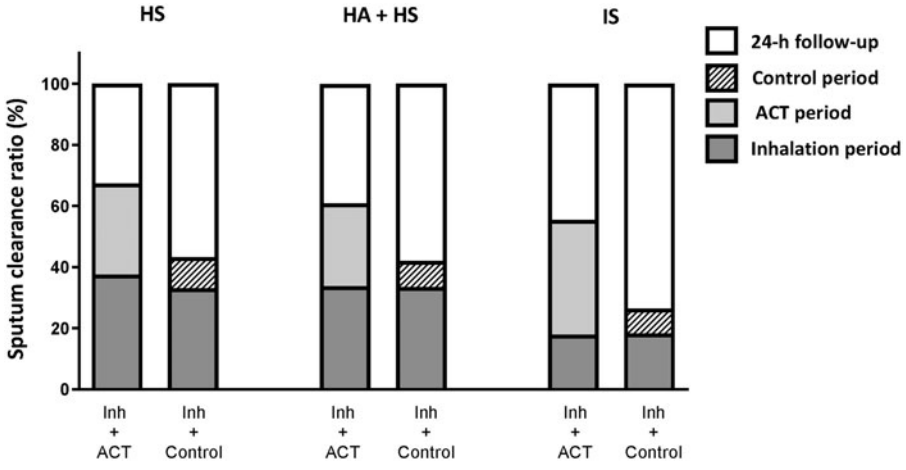


**FIG. 1.** Percentage (%) of sputum clearance collected at different time points during the random combined session (day 1 or 2 or 4), including ACT period and the single session (session 3), including control period. ACT, airway clearance technique; HS, hypertonic saline; HA+HS, hyaluronate acid plus hypertonic saline; IS, isotonic saline.

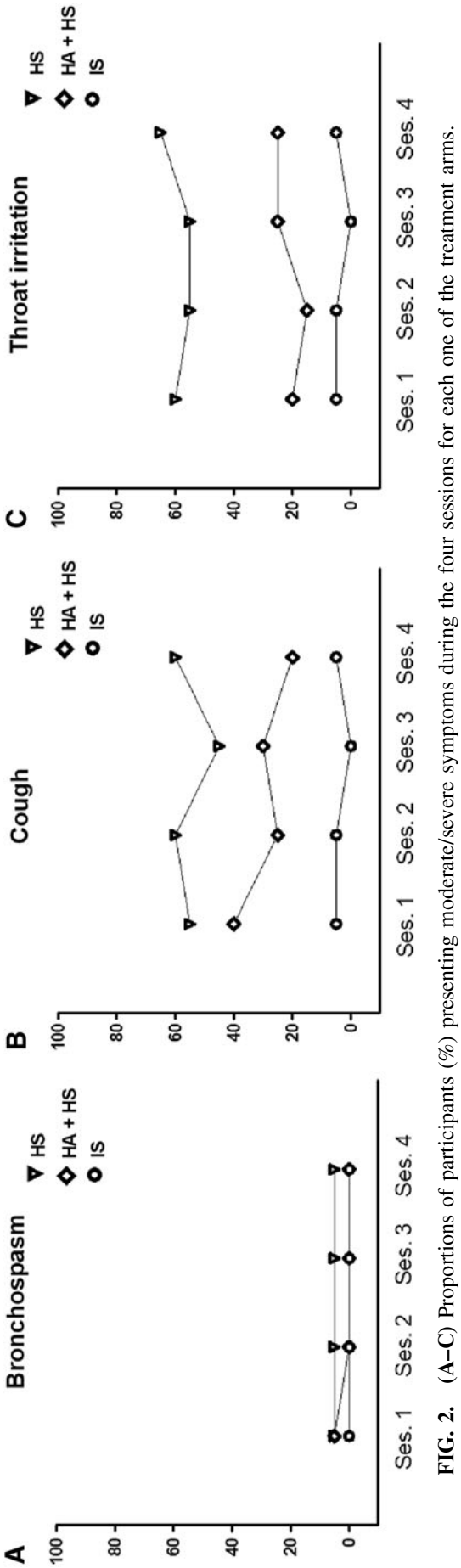


**FIG. 2.** (A–C) Proportions of participants (%) presenting moderate/severe symptoms during the four sessions for each one of the treatment arms.

solutions were well tolerated, but AEs during inhalation were more frequent and severe, from HS to HA+HS to IS (lowest).

The effect duration of hypertonic solutions (HS and HA+HS) is poorly known in bronchiectasis, but their short-term efficacy may be related to their improved biophysical sputum properties (lower adhesivity and greater cough transportability)[35,36] The major short-term efficacy of hypertonic solutions was also confirmed by the lower sputum expectoration observed in the 24 hours following HS and HA+HS, compared with IS. Hypertonic solutions were able to concentrate greater amounts of sputum during the treatment period and reduce the need to expectorate throughout the rest of the day, being one of the main short-term goals of airway clearance treatment.[37] A similar finding was described during the 24-hour period following the intervention in a previous trial comparing different ACTs in patients with bronchiectasis.[21]

However, this result should be taken with caution because the measure of sputum expectoration has some potential limitations (saliva contamination, involuntary swallowing, patient compliance) and does not necessarily reflect the impact on airway clearance.

Kellet et al.[4] did not clearly describe whether the increased sputum weight obtained using HS compared with IS was due to the osmolality of the different solutions or to the increased effectiveness of ACT after the inhalation of HS in patients with bronchiectasis and mild daily sputum expectoration (<10 g/24 h). In our study, no increased effectiveness during ACT was observed following hypertonic solutions, despite their higher osmotic impact on the airway surface layer compared with IS. Baseline sputum expectoration in our population (>10 g/24 h) was greater compared with Kellet' study, thus proving to be irrelevant to the efficacy of hyperosmolar solutions. Moreover, our findings confirm that the advantage of HS and HA+HS on sputum expectoration is more related to the inhalation than to ACT.

Unfortunately, the study design did not have a single ACT arm (no previous inhalations) that would have helped to better understand the interaction between inhalations and the efficacy of ACT. Thus, future studies are recommended to further investigate if the efficacy of ACTs is influenced by previous saline solutions in bronchiectasis.

Similarly, inhalation of HA alone was not evaluated in the present study. Previous studies suggest that inhalation of HA reduces elastin degradation in COPD and prevents bronchoconstriction in people with asthma without AEs reported.[13,38,39] To the authors' best knowledge, inhalation of HA alone has never been explored in bronchiectasis and further research is needed to evaluate the impact of this treatment on mucus clearance and airway inflammation.

Participants used bronchodilators before all inhalations to improve tolerability,[2] optimize pulmonary deposition,[2] and improve cough clearance of secretions by increasing expiratory flow.[40] It is known that $\beta$-agonist bronchodilators may stimulate ciliary beat frequency and anticholinergics can decrease volume of secretions that is triggered by airway inflammation.[41] In this study, participants were clinically stable throughout the study, their medication was unchanged, and the study design allowed intrasubject comparison,[42] therefore the impact of bronchodilators on sputum expectoration differences between treatment arms is limited.

The length of combined sessions (inhalation + ACT period) may be considered burdensome by patients. Considering our results, it might be recommended to include an ACT after inhalation to achieve a greater reduction of daily expectoration after intervention, independently of the solution previously inhaled. Studies conducted in patients with cystic fibrosis suggest that the timing of HS (before or during ACTs) had no impact on the clinical effectiveness,[43,44] although it clearly reduces the time burden associated with treatment and may promote future adherence. Future studies are required to explore if HS and HA+HS inhalation during ACT has similar results in patients with bronchiectasis as well as an equal reduction in the need for expectoration after the intervention.

Previous studies comparing the effects of HS and IS on QoL and lung function have demonstrated controversial findings.[5] No significant changes were observed in LCQ score and lung function across all treatment arms. Perhaps the short duration of our intervention did not enable us to observe a significant impact on the LCQ, a subjective assessment of cough. Thus, future studies may incorporate cough monitoring to detect objectively differences in cough frequency between interventions.[45]

Furthermore, it has been hypothesized that the response to hypertonic saline could increase with the severity of lung function impairment.[3] Therefore, it is likely that greater changes in cough severity and lung function could be expected in patients with more severe bronchiectasis and lung function impairment, unlike our population which showed a moderate lung obstruction ($FEV_1 = 61\%$ of predicted).

Globally, the saline solutions were well tolerated. The participants that did not pass the tolerability test for hypertonic solutions were elderly with severe lung obstruction. As expected, the main AEs reported were with HS, whereas HA+HS showed an improved safety profile, between those of HS and IS. These findings agree with previous studies conducted within cystic fibrosis,[8,11,14] and support the alternative use of HA+HS in bronchiectasis. The relatively low rate of AEs of HA+HS may also explain why it was chosen as the preferred saline solution by patients.

The present study has different strengths: despite the weighing wet sputum being controversial as an outcome measure[46] and only can be interpretable over a short-effect period,[37] the crossover study design with consecutive sessions improved the accuracy of our results, allowing intrasubject comparisons.[42] The repeated measurements of sputum at different points in time (inhalation, ACT, and 24 hours follow-up) also better describe immediate and short-term effects on sputum expectoration, such as frequency and severity of the AEs of the different saline solutions in patients with bronchiectasis. For future long-term trials, mucus dehydration (e.g., sputum percentage solids) or sputum inflammatory cells may be a useful measurement to assess the effect of hypertonic saline solutions on disease severity.

The main limitation was that most participants identified the HS solution by its salty taste; however, HA+HS and IS were not easily identifiable. It was decided not to use quinine sulfate as a blinding agent, as it is unknown whether it may influence the HA+HS properties and alter its effectiveness. However, our participants were naive to inhaled saline solutions and not informed about the different characteristics of the three treatment arms. A similar limitation was observed in the Nicolson et al.[47] study, demonstrating that salty taste does not consistently affect the blindness of participants.

Finally, the high dropout rate throughout the study may be another limitation. Nevertheless, the primary reason for withdrawal was related to the time burden perceived by participants. Although the research protocol could have been easily performed at home, we preferred the hospital setting to guarantee a close monitoring of AEs.

In conclusion, we found that inhaled HA+HS was as efficacious as HS in improving sputum expectoration, but with a better safety profile in patients with bronchiectasis. Moreover, the additional performance of ACT (autogenic drainage) after hypertonic solutions achieved the greater sputum weight, and a significant reduction in expectoration for the rest of the day. The daily use of hypertonic saline solutions, combined with ACT, may be useful in reducing the burden of daily symptoms, although further investigation is needed to assess long-term outcomes.

## Acknowledgments

The authors are grateful to the participants and the hospital staff for their assistance in conducting the trial, particularly to Rebeca Domingo and Montserrat Solá.

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## Authors' Contribution

BH-C was directly involved in the project design, study development, data collection, statistical analysis, and article draft. V-A contributed to data collection and article draft. JV and AT contributed to data interpretation and to critical revision of the article for intellectual concept. EP led the study, being responsible for the overall quality and content of the article. All authors have reviewed and approved the final version of the article.

## Author Disclosure Statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Supplementary material**

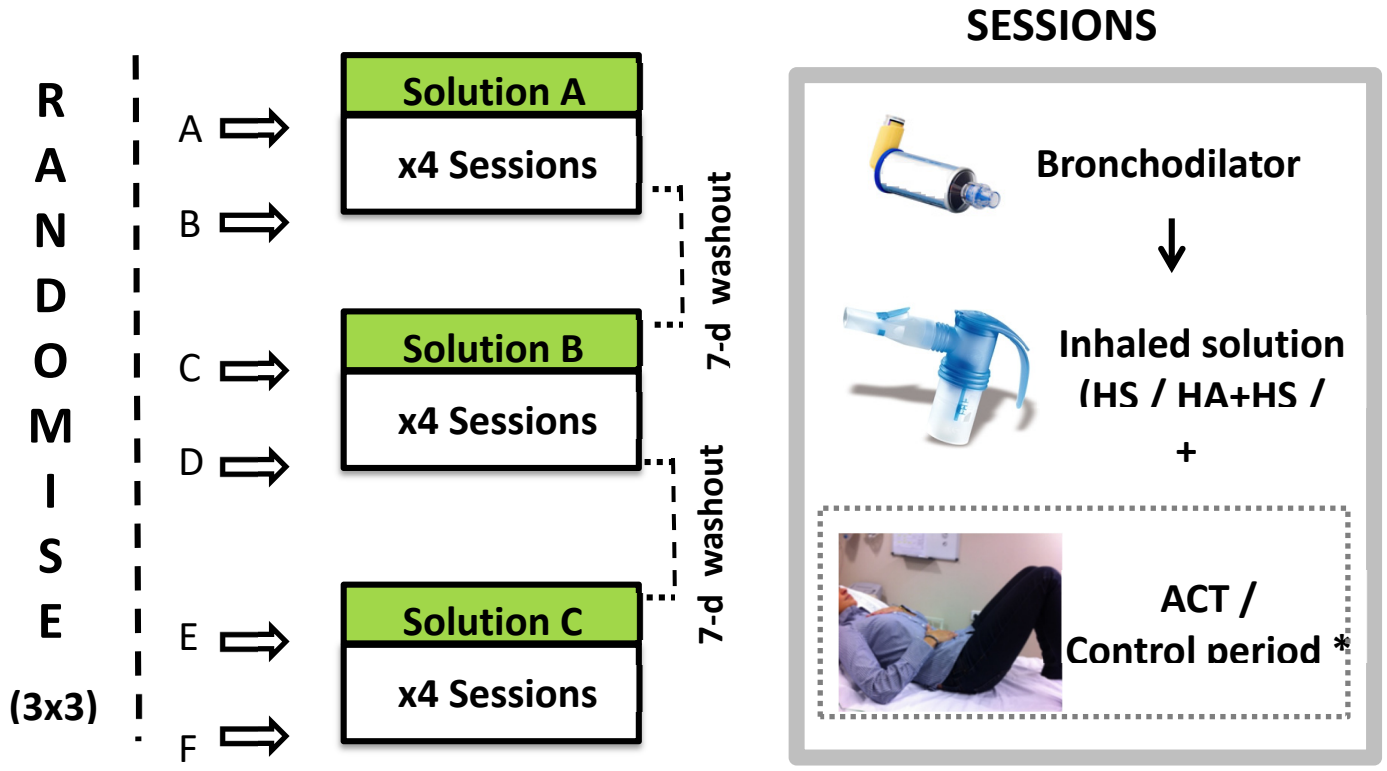
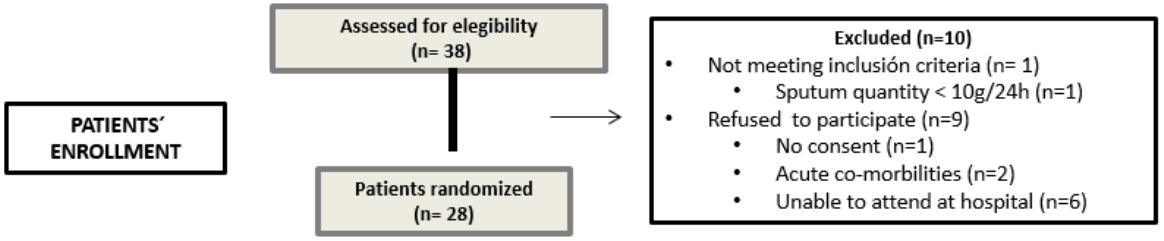



**Figure A.** Study design. The three arms of treatment (hypertonic saline, HS; hyaluronate acid plus hypertonic saline, HA+HS; isotonic saline, IS) were performed in a randomized order. Cough severity were assessed at the start and end of each treatment arm (arrows). Lung function was measured after the bronchodilator in the first session and 30-min after the ACT in the last session of each treatment arm. * All sessions for all treatment arms included 30-min of airway clearance technique (ACT), except for sessions 3 (control period).




| SEQUENCES | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| • **Allocated to intervention (n=28)** | n=5 | n=4 | n=5 | n=5 | n=5 | n=4 |
| • **Dropout prior to first session (n=3)** | n=0 | n=0 | n=1 | n=0 | n=1 | n=1 |
| • **Not tolerability** | | | | | | |
| • HTS (n=3) | n=1* | n=0 | n=0 | n=0 | n=1 | n=1* |
| • HA + HTS (n=2) | n=1* | n=0 | n=0 | n=0 | n=0 | n=1* |
| • IS (n=0) | n=0 | n=0 | n=0 | n=0 | n=0 | n=0 |
| • **Withdrawal (n=2)** | n=0 | n=1 | n=0 | n=0 | n=0 | n=1 |
| • **Analysed for effectiveness (n=28)** | n=5 | n=4 | n=5 | n=5 | n=5 | n=4 |
| • **Analysed for safety (n=20)** | n=4 | n=3 | n=4 | n=5 | n=3 | n=1 |

**Figure B** Flowchart of participants through the study. Three participants dropped out of the study prior to the first session (inability to attend the visits at the same schedule, herpes zoster diagnosis, and acute bronchiectasis exacerbation). Two participants withdrew during the study (one due to an acute bronchiectasis exacerbation at the beginning of the third treatment arm and one due to a work schedule incompatible with study visits after the third session of the first treatment arm). * Positive tolerance test for the same participant. HS, hypertonic saline; HA+HS, hyaluronate acid plus hypertonic saline; IS, isotonic saline.

**Table A** Median [interquartile range] values of the changes in total LCQ score and lung function (FEV1 and FVC) after 4 sessions of each one of the treatment arms and median difference (95% CI) between treatment arms.

| | Treatment arms (n=28) | | | Median difference between treatment arms (n=28) | | |
|---|---|---|---|---|---|---|
| | **HS** | **HA + HS** | **IS** | **HS *vs.* HA + HS** | **HS *vs.* IS** | **HA + HS *vs.* IS** |
| Total LCQ score | 0.00 [-0.61-0.87] | 0.19 [-0.29-1.17] | 0.00 [-0.31-0.78] | -0.39 (-0.95 to 0.23) | 0.32 (-0.64 to 0.96) | 0.38 (-0.45 to 1.29) |
| $FEV_1$ (L) | -0.01 [-0.07-0.04] | -0.03 [-0.13-0.02] | -0.02 [-0.12-0.08] | 0.01 (-0.02 to 0.09) | 0.00 (-0.12 to 0.08) | -0.01 (-0.13 to 0.04) |
| FVC (L) | 0.00 [-0.14-0.03] | -0.02 [-0.23-0.09] | -0.03 [-0-36-0.08] | 0.00 (-0.15 to 0.15) | 0.03 (-0.14 to 0.18) | 0.01 (-0.18 to 0.27) |

LCQ, Leicester cough questionnaire; $FEV_1$, forced expiratory volume in the first second; FVC, forced vital capacity; HS, hypertonic saline; HA + HS, hyaluronate acid plus hypertonic saline; IS, isotonic saline.

**Table B** Median [interquartile range] values of safety score for each treatment arm and median difference (95% CI) between treatment arms.

| **Outcome measure** | **Treatment arms (n=20)** | | | **Median difference between treatment arms (n=20)** | | |
|---|---|---|---|---|---|---|
| | **HS** | **HA + HS** | **IS** | **HS *vs.* HA + HS** | **HS *vs.* IS** | **HA + HS *vs.* IS** |
| Safety score | 3.4 [0.9-5.1] | 1.7 [0.1-3.2] | 0.0 [0.0-0.9] | 1.2 (0.3 to 2.4) | 2.7 (1.6 to 3.9) | 1.2 (0.3 to 2.0) |

Safety score assessed the severity of adverse events (bronchospasm, throat irritation and cough) by a three-point ordinal score (0=absent; 1= mild; 2= moderate; 3= severe). HS, hypertonic saline; HA + HS, hyaluronate acid plus hypertonic saline; IS, isotonic saline.

**MANUSCRIPT 3**

# Reliability and minimal important difference of sputum weight in people with bronchiectasis.

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Accepted in Respir Care. 2019 *(15-10-2019)*

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# Respiratory Care - Decision on Manuscript ID RC-07175.R1

1 message

**Respiratory Care** <onbehalfof@manuscriptcentral.com> 15 October 2019 at 11:22
Reply-To: dhess@aarc.org
To: Beafisiorespi@gmail.com
Cc: sara.moore@aarc.org, branson@aarc.org, gail.drescher@aarc.org

15-Oct-2019

Dear Miss Herrero-Cortina:

Your manuscript entitled "Reliability and minimal important difference of sputum weight in people with bronchiectasis" has been evaluated by 2 external consultants, whose comments are included at the foot of this letter. In accordance with their assessment and recommendations, I am pleased to accept it for publication in Respiratory Care in its current form.

Your paper will be scheduled for the next available issue of the Journal. We try to publish papers as soon as possible after they are accepted, but in some cases we experience a backlog of 6 to 9 months between acceptance and publication. You will receive page proof prior to final publication.

In the meantime, your paper will be uploaded as an Epub (paper in press) and will appear in PubMed. It usually takes several months for papers to move through production to Epub.

Please note that by this email notifying you that we have accepted your paper, copyright is automatically transferred to Daedalus Enterprises.

Thank you for submitting this work to the Journal.

Dean R Hess PhD RRT FAARC
Managing Editor
RESPIRATORY CARE

Reviewer(s)' Comments to Author:
Reviewer: 1

Comments to the Author
You have appropriately addressed my concerns and those of the other reviewers. There are a number of grammatical and punctuation errors that need to be corrected.

Reviewer: 2

Comments to the Author
Thank you to the authors for addressing all the comments made. The manuscript now reads better and is more transparent to readers. I look forward to seeing this work published.

**Title** Reliability and minimal important difference of sputum weight in people with bronchiectasis

**Authors** Beatriz Herrero (MSc)[1], Victoria Alcaraz (MSc)[2], Antoni Torres (MD, PhD)[3], Eva Polverino (MD, PhD)[4].

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**Location** The present study was performed at Hospital Clinic, Barcelona, Spain. The study was approved by the Hospital Clinic Research Ethics Committee (HCP/2011/6401; HCP/2011/6401; HCP/2013/8410).

**Preliminary results** BH-C presented preliminary results of this paper at the 47ª SEPAR (Sociedad Española de Neumología y Cirugía Torácica) Congress, held June 6–9, 2014, in Bilbao, Spain and at ERS (European Respiratory Society) International Congress, held September 6-10, 2014 in Munich, Germany.

**Author´s contribution** BH-C was directly involved in the project design, study development, data collection, statistical analysis and manuscript draft. VA contributed to data collection and revision of the manuscript. AT and EP contributed to data interpretation and to critical revision of the manuscript for intellectual concept. All authors have reviewed and approved the final version of the manuscript.

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**Title** Reliability and minimal important difference of sputum weight in people with bronchiectasis: ad-hoc analysis of three clinical trials.


## Abstract

**Background:** Despite sputum weight being widely used to assess the effect of airway clearance interventions, its psychometric properties have not been evaluated. The purpose of this ad hoc analysis was to determine the test-retest reliability of 24-hour sputum weight in clinically stable people with bronchiectasis. This study also aimed to estimate the minimal important difference (MID) of 24-hour sputum weight after an airway clearance session in people with bronchiectasis.

**Methods:** Sixty participants were included in the '24-hour test-retest cohort´, 42 of whom were part of the 'airway clearance cohort´. For the 24-hour test-retest cohort, spontaneous sputum expectorated was collected over 24-hour on two different days, without any airway clearance interventions. For the ´airway clearance cohort´, sputum expectoration was also collected during three airway clearance sessions and over the 24-hour following these interventions. Intraclass correlation coefficient ($ICC_{3,1}$) and Bland-Altman analysis were used to assess reliability. The MID was calculated using distribution-based and anchor-based methods. Cough impact assessed by the Leicester Cough Questionnaire and the global rating of change were chosen as anchors.

**Results**: The reliability was acceptable ($ICC_{3,1}$ =0.75) for sputum weight over 24 hours without any intervention. The agreement level was wide, particularly for high levels of sputum expectoration. The MID of the sputum collected in the 24-hour after the intervention from baseline was - 6.4 g (about - 17%), determined using distribution-

based methods. There was no correlation between sputum weight and the anchors, thus the anchor-based methodology could not be used.

**Conclusion**: Multiple measurements should be considered to increase the agreement when sputum weight is used as an outcome measure for short periods in people with bronchiectasis. A reduction of 6.4 g (or 17%) in sputum collected during the 24-hour after the airway clearance intervention may be considered the MID in people with bronchiectasis.

**Keywords:** bronchiectasis, sputum, airway clearance techniques, reliability, minimal important difference, psychometric properties.

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**Trial registration:** ClinicalTrials.gov Identifier (NCT02392663; NCT01854788; NCT02614300)

## Background

Bronchiectasis is a heterogeneous respiratory disorder characterised by recurrent airway inflammation and infection. Daily sputum expectoration is one of the most common symptom experienced by people with bronchiectasis[1] and the incidence of this symptom is similar across all age groups[2], and is independent of the time of onset of the productive cough (childhood or adulthood)[3]. The amount of sputum expectorated tends to increase over time in people with bronchiectasis[4], and a greater sputum quantity has a negative impact on patients' quality of life[5, 6]. In addition, a change in daily amount of sputum expectoration is recognised as an important factor to identify exacerbations in this population[7].

Strategies to assess and monitor sputum quantity in individuals with bronchiectasis have gained importance[8], as well as the recommendation to incorporate the use of mucoactive therapies and/or airway clearance techniques (ACTs) as part of daily treatment to improve symptoms related to the productive cough (sputum expectoration, uncontrollable cough, sore or irritated throat)[9, 10].

Qualitative studies reported that people with bronchiectasis and cystic fibrosis use airway clearance interventions as a strategy to manage sputum symptoms and improve self-confidence in social life[11, 12]. If they completed interventions prior to going out, the need to cough and/or expectoration is reduced and, thus, embarrassing situations related to sputum are less likely[11, 12]. Therefore, the patient´s perception might be

focused on the change of sputum expectoration experienced after intervention and not during the session itself.

Although sputum quantity is considered a controversial outcome measure because of the likelihood of the presence of salivary contamination or inadvertently swallowed secretions[13, 14], this outcome measure is often used to assess the short- and long-term effectiveness of interventions in people with bronchiectasis, such as antibiotic therapy[15], mucoactive treatment[16, 17] and ACTs[18, 19]. The current widespread use of sputum quantity can be attributed to it being a simple and feasible outcome measure, which is relevant to people with bronchiectasis[20] and is easily implemented in clinical practice[21].

Sputum quantity could be measured as sputum weight or sputum volume. The sputum weight (dry and wet) is most frequently used when a calibrated scale is available and participants are not involved in the measurement process[22]. Findings using sputum weight may be more accurate because they do not depend on the graduated scale of containers and the assessors´ interpretation. Despite of dry sputum weight is clearly preferred to wet sputum weight because saliva contamination is completely removed, it is difficult to assess in clinical practice. For that reason, wet sputum weight is a simple and safe outcome measure for monitoring the sputum quantity. However, interpreting the clinical significance of changes in wet sputum weight after an intervention remains a challenge[21].

There is a knowledge gap in the reliability and the minimal important difference (MID) of the wet sputum expectoration in people with bronchiectasis, which are prerequisites for the correct interpretation of this outcome measure[23], as well as to calculate an adequate sample size for future studies.

The primary outcome of this study was to examine the reliability of 24-hour wet spontaneous sputum expectoration (grams) without performing any airway clearance intervention in clinically stable people with bronchiectasis. This study also aimed to estimate the MID for 24-hour wet sputum expectoration after an airway clearance intervention in people with bronchiectasis. We hypothesised that the wet spontaneous sputum obtained over a 24-hour period would be an acceptable reliable measure in clinically stable patients with bronchiectasis and that patients´ response to an airway clearance intervention would be a reduction in the need to expectorate after the intervention (less amount of sputum collected over the 24-hour follow-up period after intervention).

## Methods

### Study design and participants

This study analysed the test-retest reliability and the MID of wet sputum weight using data from two previous crossover trials[16, 19] and a current parallel-group randomised controlled trial (NCT02614300) applying an ad-hoc analysis. All studies recruited individuals with bronchiectasis to assess the efficacy of physiotherapy interventions at

Hospital Clinic, Barcelona, Spain. The sputum collection process was similar for all studies.

The inclusion criteria comprised a confirmed diagnosis of bronchiectasis on CT scan, aged ≥18 years, clinical stability for 1 month prior to the start of the study (defined as no need for extra antibiotics or changes in usual therapy, no haemoptysis and no clinical features of exacerbation), and daily spontaneous sputum expectoration. The exclusion criteria were a diagnosis of cystic fibrosis, smoker or former smoker (< 2 years), and regular use of hyperosmolar agents or ACTs. Finally, the withdrawal criteria were pulmonary exacerbation during the study or any new medical/personal condition hindering study continuation. Written informed consent was obtained from all participants before data collection began, and all studies were approved by the research ethics committee of the Hospital Clinic.

## Procedures

At the baseline visit, all participants were instructed on the importance of collecting all sputum samples in a transparent pre-weighted container during the different assessment time points. All containers were weighed before and after the sputum collection using the same calibrated scale (Acculab VIC 212, Germany).

The baseline spontaneous sputum expectorated was collected over a 24-hour period (from the beginning of the day until the following day, including the night) on two non-

consecutive weekdays (24-hour apart) within the same week. These sputum samples were collected in two transparent containers during the recruitment period from the three trials, before starting any intervention (Figure 1). Despite of participants were not adherent or were naïve to airway clearance interventions, they were asked not to perform any of these treatments during this week.

Salivary contamination and secretions from the sinuses after an inspiratory forced manoeuver were not collected in the containers. However, if a small amount of saliva was detected in the containers, it was manually removed using a paper filter before being weighed. The wet sputum weight (grams) was chosen as the outcome. All participants who collected two samples at this time point (baseline) were considered the ´24-hour test-retest cohort´.

Participants from the two crossover trials, referred as the 'airway clearance cohort', performed three airway clearance sessions (once per day) during the same week[16, 19]. Each trial explored three different treatment arms; however, it was selected only data from one of them. The treatment arm selection was based on the study purpose which was to detect the minimal change in the 24-hour sputum expectoration after an airway clearance intervention that would likely be important from patients´ and clinician´s perspectives[24]. Consequently, the treatment arm was chosen according to its efficacy in enhancing sputum expectoration during sessions and the reported patient's preference by the entire group of participants[16, 19]. Participants recruited in the ongoing randomised

controlled trial were not included in ´the airway clearance cohort´ because the intervention in this study was not similar to the other two trials.

Therefore, there were two different airway clearance interventions, one chosen from each trial: (i) a combined intervention using a hyperosmolar agent inhalation plus ACTs (hyaluronic acid + hypertonic saline solution (7%) and autogenic drainage technique), or (ii) a single intervention with ACTs (autogenic drainage technique), as previously described[16, 19]. Participants were seated during the inhalation period and were lying in a supine position during the ACT intervention. In both studies, an experienced physiotherapist supervised the sessions to guarantee a correct performance of the inhalation and/or the autogenic drainage technique.

The time spend doing the combined intervention was ≈ 50 minutes (≈ 20 min for inhalation and 30 min for autogenic drainage technique) and the total duration of the single intervention was 40 minutes[16, 19]. Each participant received the same airway clearance intervention (combined or single intervention) in all sessions, were performed at the same time of day and, if a participant participated in both studies, only data from the first study to which they were recruited were used.

The ´airway clearance cohort´ was instructed to collect all sputum expectorated (grams) during three airway clearance sessions and over a 24-hour follow-up period after each intervention into different pre-weighed transparent containers, following the same

procedure described above (Figure 1). Participants were reminded of the importance of following the sputum collection instructions in each session.

The impact of the cough was assessed using an 1 week-adapted version of the Leicester Cough Questionnaire (LCQ)[25] at the beginning and end of the sessions (approximately 1 week later) in the ´airway clearance cohort´. The LCQ intraclass correlation coefficient (ICC) range between 0.87 and 0.96 and their MID is 1.3[26, 27].

The self-administered global rating of change (GRC) scale was used to evaluate the change in 24-hour sputum weight perceived by the ´airway clearance cohort´ after completing the week of airway clearance sessions. Participants were asked if the airway clearance sessions changed their need to expectorate in the 24-hour after the intervention (*"Has your amount of sputum changed over the 24-h follow-up after intervention compared to a day without airway clearance intervention?")*, which was scored using a Likert scale (scored from -7 to 7)[28]. Negative scores indicated a reduction in the need to expectorate, and positive scores indicated a greater need to expectorate. Neither end of the Likert scale was marked as better than the other and participants were not informed about the hypothesis of this study (the expected direction of the sputum weight change). The amount of change was classified as follows: ±0–1 is no change, ±2–3 is a small change, and ±4–7 is a substantial change[29].

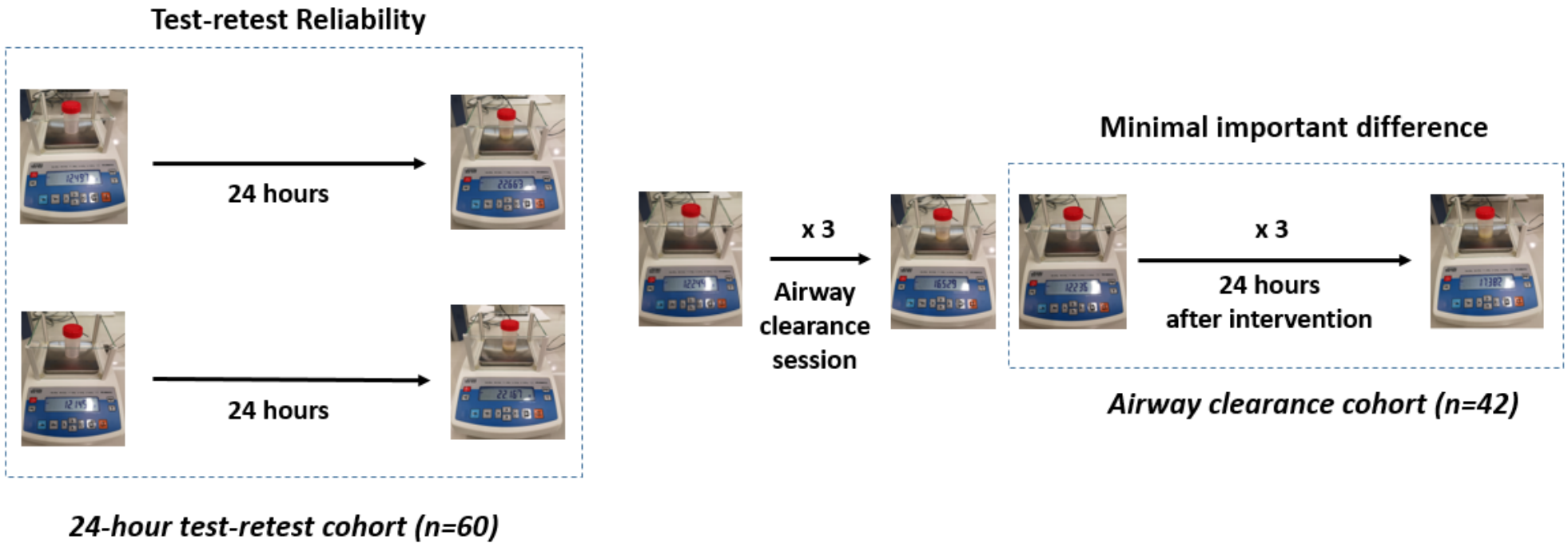


**Figure 1.** Overview of sputum collection design for reliability (spontaneous sputum expectorated over 24 hours without intervention) and minimal important difference (spontaneous sputum expectorated during the 24 hours after airway clearance sessions) based on repeated measures.

## Statistical analysis

A power analysis was performed to estimate the sample needed to achieve reliability. Considering a minimal ICC of 0.9 with a 95% confidence interval (CI) width of 0.2 ($\alpha$ = 0.05 and k = 2), a sample size of 21 participants was required. However, according to COSMIN recommendations, a 'good' sample size for reliability studies includes at least 50 participants[23]. Therefore, we attempted to include this larger number of participants.

The reliability of the amount of wet sputum collected was estimated using the $ICC_{3,1}$ (two-way mixed-effects, single measurement, absolute agreement)[30] with 95% CI at baseline for the spontaneous sputum collected over a 24-hour period without intervention in the ´24-hour test-retest cohort´. The $ICC_{3,1}$ values were interpreted as excellent (>0.75), moderate-to-good (0.4–0.75) or poor (<0.4)[31]. The agreement for these outcomes was represented using Bland-Altman plot, including their 95% CI for bias and for the limits of agreement[32]. A regression approach was also included when a relationship between differences and the size of measurement was identified[32].

The change in the amount of wet sputum collected during the 24-hour follow-up period after the airway clearance session was expressed as the absolute weight (grams) and as the change relative to the amount of sputum expectorated over the 24-hour baseline period (percentage). To estimate the MID, distribution-based and anchor-based methods were used with data from the ´airway clearance cohort´. The mean results for the three days were used to ensure greater accuracy of the results. The techniques used for the distribution-based approach are summarised in Table 1. For anchor-based

methods, two potential anchors were explored: the total LCQ score, as the MID has been established as 1.3 points and is known to change after airway clearance treatment[18]; and GRC score, as this is the recommended method for estimating the MID of an outcome[28].

A correlation of ≥0.4 between the change in the anchor and the change in the amount of sputum collected (grams or percentage) over the 24-hour follow period was considered necessary to calculate the MID using the anchor-based method[33]. In the presence of a sufficient correlation, sensitivity- and specificity-based approaches with receiver operating characteristic (ROC) curves would have been used. However, if the correlation requirements are not reached, the estimation of MID should only be calculated using a distribution-based approach.

Within-group differences in total LCQ score and 24-hour sputum weight were analysed using a paired t-test and Wilcoxon signed-rank test, and expressed as the mean difference and median difference along with the respective 95% CI. A p-value <0.05 was considered statistically significant in all analyses. Effect size (r) was also estimated and interpreted as either a small effect ($r < 0.3$), moderate effect ($r \geq 0.3$) or large effect ($r \geq 0.5$).

## Results

Sixty participants were recruited and completed the baseline assessment (´24-hour test-retest cohort´). Of these, 42 participants underwent airway clearance treatment (´airway clearance cohort´). The baseline characteristics of both cohorts are outlined in Table 2.

**Table 1.** Distribution-based approach to estimate the minimal important difference (MID) of wet sputum weight collected during 24-hour after intervention in the ´airway clearance cohort´ (n=42).

| Method | Formulas | Minimal Important Difference (MID) [#] | | | |
|---|---|---|---|---|---|
| | | Absolute value (g) | n (%) responders (≥MID) | Relative change from baseline (%) | n (%) responders (≥MID) |
| 0.5 times SD | $0.5 * SD_{baseline}$ | -5.7 | 28 (66.7) | NA | NA |
| Cohen´s effect size | $0.5 * SD_{\Delta}$ | -4.5 | 30 (71.4) | - 16.8 | 34 (80.1) |
| Empirical rule effect size | $0.08 * 6 * SD_{\Delta}$ | -4.4 | 32 (76.2) | - 17.5 | 34 (80.1) |
| SEM | $SD_{baseline} * \sqrt{(1 - ICC}$ | -5.4 | 28 (66.7) | NA | NA |
| $MDC_{95\%}$ | $1.96 * (2 \times SEM)^{1/2}$ | -6.4 | 28 (66.7) | NA | NA |

SD= standard deviation; baseline= mean of the spontaneous sputum expectorated over a 24-h period in two different days during the recruitment period (without airway clearance treatment); Δ difference in wet sputum weight collected (g) between the mean of the sputum expectorated over the 24-h follow-up period after intervention and baseline / Δ percentage of change from baseline; SEM= standard error of measurement; ICC= intraclass correlation coefficient of the sputum collected during the 24-h follow-up period after intervention; MDC: minimal detectable change with a 95% confidence level; [#] MID was estimated based on the mean value of three measurements; NA= not applicable.

**Table 2.** Baseline characteristics of participants.

| Characteristics | 24-h test-retest cohort (n= 60) | Airway clearance cohort (n=42) |
|---|---|---|
| Age *(yr)*, mean (SD) | 62.7 (15.9) | 61.0 (17.4) |
| Gender, n female (%) | 41 (68.3) | 28 (66.6) |
| BMI *(kg/m$^2$)*, mean (SD) | 24.5 (3.5) | 24.2 (3.7) |
| Aetiology of bronchiectasis, n (%) | | |
| – Idiopathic | 20 (33.3) | 18 (42.9) |
| – Post-infection | 18 (30.0) | 12 (28.5) |
| – Associated COPD | 10 (16.7) | 6 (14.3) |
| – Immunodeficiency | 5 (8.3) | 4 (9.5) |
| – Primary ciliary dyskinesia | 2 (3.3) | 2 (4.8) |
| – Others | 5 (8.3) | 0 (0.0) |
| Chronic airway infection, n (%)[#] | | |
| – *P. aeruginosa* infection | 26 (43.3) | 20 (47.6) |
| – *H. influenzae* infection | 6 (10) | 4 (9.5) |
| Long-term antibiotic treatment, n (%) | | |
| – Oral (macrolides) | 13 (21.6) | 7 (16.6) |
| – Inhaled | 19 (31.6) | 17 (40.5) |
| Lung function, mean (SD) | | |
| – $FEV_1$ (L) | 1.66 (0.8) | 1.79 (0.8) |
| – $FEV_1$ % pred. | 64.1 (19.3) | 67.3 (19.7) |
| – FVC (L) | 2.59 (0.9) | 2.75 (0.9) |
| – FVC % pred. | 76.0 (17.0) | 79.3 (17.1) |
| | 0.63 (11) | 0.64 (10) |

- $FEV_1$/FVC (%)

| | | |
|---|---|---|
| Baseline sputum expectoration*, (g) | | |
| – 24-h period, median [$P_{25}$-$P_{75}$] | 15.4 [11.4 – 26.1] | 15.6 [14.0 – 27.7] |
| – > 15g/24h, n (%) | 35 (58.3) | 29 (69.0) |

Data presented as *n*, *n* (%), mean (SD, standard deviation) and median [$P_{25}$-$P_{75}$, 25$^{th}$ and 75$^{th}$ percentile] # Chronic airway infection was defined as pathogen organism cultured in at least 2 or more sputum samples, at least 3 months apart, in the preceding 12 months *Measured on two different days within the week prior to start the study. BMI, body mass index; $FEV_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; % pred, percentage of predicted.

## Test-retest reliability

The reliability was found to be acceptable ($ICC_{3,1}$=0.85) for the two spontaneous sputum collected over a 24-hour period without intervention, with CIs from 0.76 to 0.91. No bias was identified using Bland-Altman plot [mean difference (95% CI) 1.2 (-0.7 to 3.0)]; however, the limits of agreement were wide showing larger ranges for greater weight of sputum expectorated (Figure 2). After modelling the relationship between mean differences and the magnitude of sputum weight, it was identified that limits of agreement fit greater for lower values of sputum weight (~ ≤15 grams) (Figure 2).

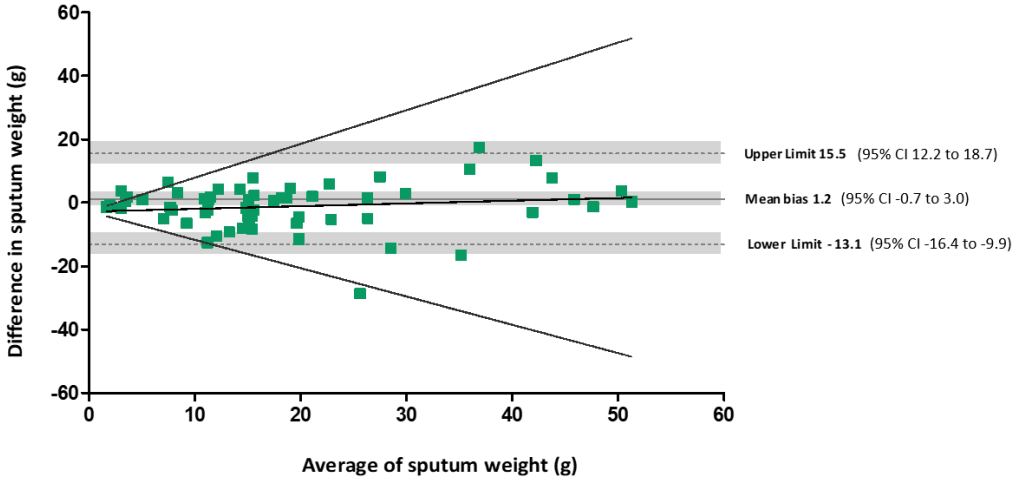


**Figure 2.** Bland-Altman plots for absolute reliability of the spontaneous sputum weight collected over 24 hours at baseline in the test-retest reliability cohort (n=60). The straight grey line represents the mean difference between both measurements, dotted grey lines represent the 95% upper and lower limits of agreement, shaded grey areas represent the confidence interval (95%) for mean and limits of agreement, the continuous black lines represent the limits of agreement using the regression approach.

## Estimation of the MID

### *Distribution-based methods*

The MID of the 24-hour sputum weight after airway clearance treatment compared to baseline ranged between -4.4 and -6.4 g (being the last value the minimal detectable change, $MDC_{95\%}$). Therefore, the MID estimate should be a reduction of at least 6.4 g to guarantee a change that exceeds the error of measurement[34]. In addition, a threshold of change from baseline of between -16.8% and -17.5% was also estimated using distribution-based methods (Table 1).

*Suitability of LCQ and GRC scale as potential anchors for the 24-hour sputum weight*

A reduction in the amount of wet sputum expectorated after the intervention was observed in 38 participants (90.4%). The sputum expectorated during the 24 hours following the intervention was lower than the 24-hour baseline assessment, with the effect size ranging from 0.71 to 0.79 (Table A, supplementary material). The median [$P_{25}$-$P_{75}$] relative change from baseline was -47.8% [-62.9 to -25.8]. Three participants (7.1%) showed a change in sputum weight score of ≥85% from baseline, and four participants (9.5%) scored ≤15%, indicating that there were no extreme changes.

Participants collected a similar weight of sputum during the treatment period, independent of the intervention performed (hyperosmolar agent inhalation plus ACTs *vs*. ACTs; all p-values >0.05) (Table B, supplementary material), showing that pooling the findings from the two crossover trials was appropriate.

The total LCQ score improved after 1 week of airway clearance treatment [mean difference (95% CI) 0.6 (0.0 to 1.3) and median difference (95% CI) 0.6 (0.3 to 1.0)] and the effect size ranged from 0.32 to 0.52 (Table A, supplementary material). Nevertheless, the change in total LCQ score did not correlate with the change in the 24-hour expectorated mean sputum (absolute weight) from baseline (r = 0.1, p = 0.5), nor with the relative change (r = -0.1, p = 0.3; Figure 3). Most of participants (83.3%) reported a substantial change using the GRC scale, with a median [$P_{25}$-$P_{75}$] of -6 [-7, -5]. No significant correlations were observed between patient GRC score and the change in 24-hour expectorated sputum (absolute) from baseline (r= 0.2, p = 0.2), nor with the relative change (r= 0.2, p = 0.2; Figure 3). Therefore, neither total LCQ score nor GRC could be used as reliable anchors.

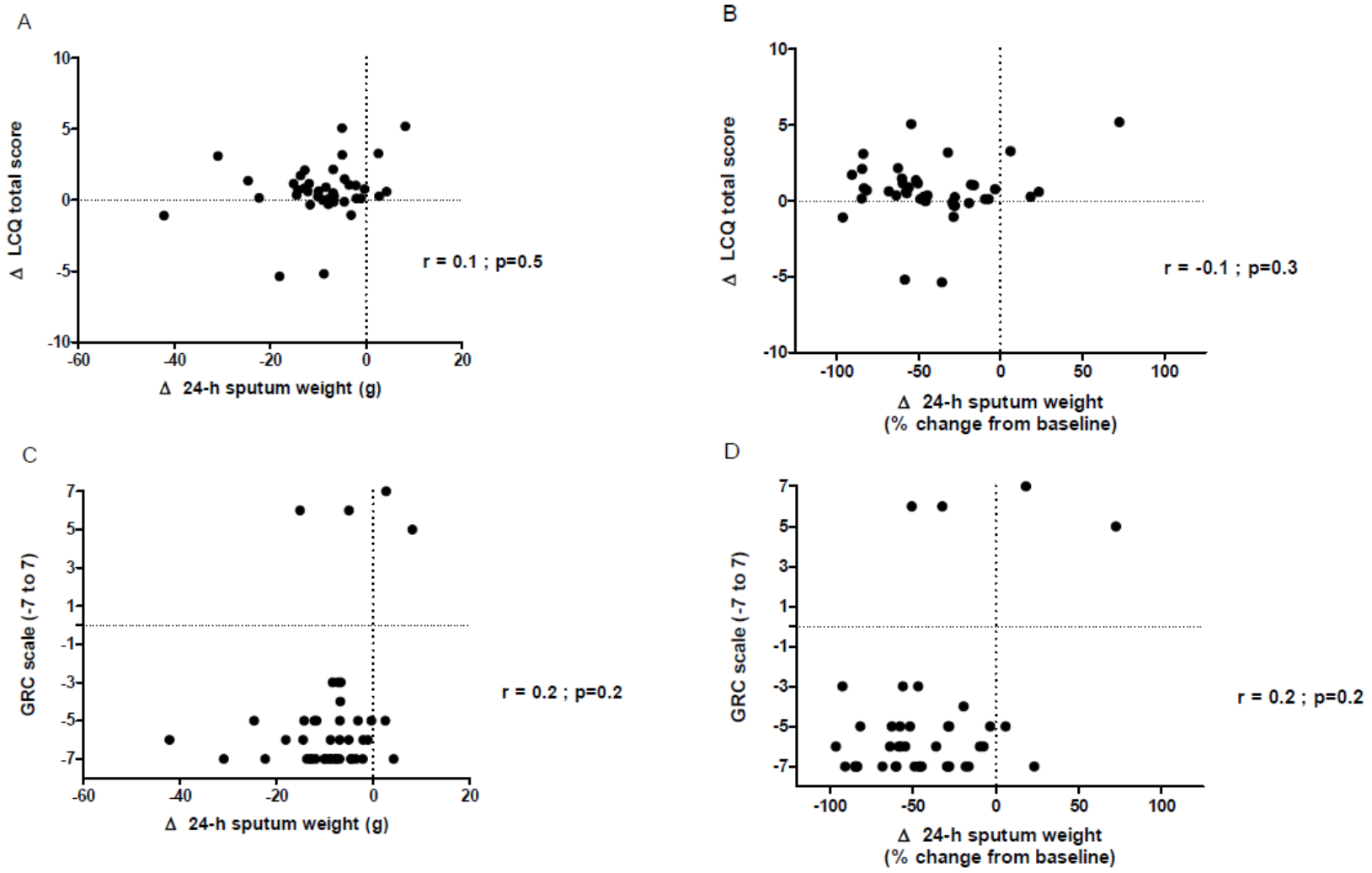


**Figure 3**. Correlation (Spearman´s rank correlation) between change in 24-h sputum weight and anchors (LCQ total score and GRC scale) using ´airway clearance cohort´(n=42); A and C= absolute change in sputum weight (g); B and D = percentage of change from baseline (%).

## Discussion

The present study provides evidence of the test-retest reliability of wet sputum weight as an outcome measure for short periods (24-hour period) in clinically stable individuals with bronchiectasis. This study also reports an estimate of the MID after an airway clearance session based on the mean value of three sessions.

Mucociliary clearance rates, assessed *in vivo* using gamma scintigraphy, is the most widely accepted outcome measure to assess the effects of airway clearance treatments[14, 35]. However, only a few previous trials have used this outcome to assess the effects of mucoactive agents and/or ACTs in people with bronchiectasis[36, 37], indicating that poor accessibility to the highly specialised equipment required and the need to inhale radiolabeled markers limits its use in research and, in particular, in clinical practice. Although the wet sputum weight is generally considered a controversial measure (saliva contamination, involuntary swallowing) to assess the effects of airway clearance[13, 14], its use to evaluate short-term efficacy is acceptable even though its psychometric properties have not been established for any specific disease.

Participants were constantly educated and encouraged in our study to avoid sputum swallowing during the assessment time period and the saliva contamination was manually removed from the sputum samples. Drying sputum samples before weighing is a recommended method for completely removing saliva mixed with sputum. However, wet sputum weight was used in preference to dry sputum weight in this study as i) it provides immediate information to patients and facilitates response to the GRC

score, as they could compare the amount of sputum expectoration between different periods of time; ii) the findings may be easily transferred to clinical practice because it is a more feasible measure; and iii) it was suggested that wet sputum weight is an acceptable predictor of dry sputum weight; however, further research is needed on this point, especially when hydrator therapies have been used.

Based on our data, spontaneous sputum collected over 24 hours presents acceptable reliability [ICC= 0.85 (95%CI 0.76 to 0.91)]. However, these results are lower to those described for other widely accepted outcomes in bronchiectasis, such as walking tests[38], impact of cough on quality of life[26] or lung clearance index[39] (all with a lower limit of 95%CI > 0.9).

The viscoelastic properties and solids content of sputum are alternative biomarkers used to analyse the effect on airway clearance. However, a recent study has reported poor reliability for both methods in sputum samples from people with cystic fibrosis ($ICC_{3,1}$ from 0.21 to 0.42)[40]. The higher reliability values obtained in this study using sputum weight may be explained by: (i) the short interval between sputum sample collections (within the same week); (ii) stable condition of all participants throughout the study; (iii) and the highly standardised sputum collection process.

Although no systematic difference has been found for the spontaneous sputum collected over a 24-hour period without intervention, the agreement intervals are not

sufficiently narrow, particularly for high levels of sputum weight expectorated (~ ≥15 grams). Therefore, the use of repetitive measurements to improve the reliability and agreement may be a solution, as have previously been recommended for sputum samples[40, 41]. Moreover, the ability to detect differences between groups using sputum weight is limited due to the high variability observed, thus only intrasubject comparisons are recommended (clinical practice or crossover designs).

Nevertheless, these results should be interpreted with caution[30] because the number of participants with lower levels of expectoration was low in this study (31% of participants in the ´airway clearance cohort´) and our sample size does not allow stratification of data according to the level of expectoration. Therefore, future research is needed to confirm this finding. In addition, more in-depth analysis of the reproducibility of sputum weight should be conducted by performing longitudinal studies with longer intervals between measurements (similar to clinical practice).

Although the anchor-based methodology is considered the best method to estimate the MID, the lack of correlation between the change in 24-hour wet sputum weight and the anchors selected impeded their estimation in this study[24]. One possible reason for the lack of correlation could be that the short duration of the airway clearance intervention in this study did not enable us to observe greater changes in LCQ than its MID, which is in contrast to previous long-term trial[18].

The potential impact of the physiotherapist and patients´ beliefs regarding the sputum weight on GRC score was minimised because: i) participants were not informed about the study hypothesis (the expected direction of change); ii) the question focused on a period of time in which participants had no contact with the physiotherapist, thus they did not receive any feedback; and iii) neither end of the Likert scale was marked as better than the other. However, the fact that participants did not regularly perform any airway clearance treatment before the study may explain why almost all patients classified their change in 24-hour sputum weight using the GRC score as 'substantial'. More research is needed in the future to assess real impact of airways clearance interventions on social life.

Most of participants (n= 38, 90%) presented a reduced need to expectorate after the airway clearance intervention, showing a clear direction of change. Using the distribution-based methodology, the MID for the 24-hour wet sputum weight was found to range between -4.4 to -6.4 g (absolute value), with a relative change of about -17%. Since this estimate was based solely on the statistical criteria (distribution values of our sample), the selection of the $MDC_{95\%}$ as the lower limit of MID estimation is strongly recommended[34]. For that reason, the MID estimated for the 24-hour sputum weight was at least -6.4 g, ensuring the selection of a minimum value that implies a real change and not a measurement error.

The majority of participants (n=28, 67%) achieved a reduction in sputum collected over 24 hours after the intervention of at least 6.4 g, and 80% of them achieved a relative

change equal or greater than -17.5% from baseline, showing that the MID is feasible for airway clearance treatment in stable people with bronchiectasis. However, this data should be interpreted with caution because distribution-based methods are not fully able to separate clinical importance from statistical significance.

The availability of a MID for sputum weight may assist in clinical practice and future research to assess the short-term efficacy of new treatments to enhance sputum expectoration in this target population, in addition to assisting sample size calculations for future trials. Nevertheless, future studies are needed to corroborate the MID estimated using other potential anchors such as the cough frequency, assessed using monitors[42], or computerised respiratory sounds[43]. Moreover, the validity of this MID estimate should be further evaluated with longitudinal studies which include a relevant clinical indicator such as exacerbation frequency or the severity of exacerbations.

The validity of sputum weight is not evaluated in this study. Previous findings showed that self-reported sputum production is an independent factor of cough frequency in people with bronchiectasis[42]. Therefore, if we consider that the main effect of airway clearance interventions is to reduce the need to expectorate after treatment, the number of coughs using objective cough monitors might be a good standardised outcome measure[42] to analyse the construct validity of sputum weight in future studies.

The present study has some limitations. Although the anchor-based method could not be used, the lower limit of the MID estimation was based on the $MDC_{95\%}$ to guarantee a real change[34]. Our population was not adherent or naïve to airway clearance treatment and the majority of participants had a moderate level of expectoration (≥15 g/24-hour), thus, it is not clear whether these results can be extrapolated to people adhering to airway clearance treatment or/and with lower levels of expectoration. Finally, while the direction of change was clear, as almost all participants experienced a reduction in the need to expectorate after the airway clearance intervention, the response to this treatment could differ over longer periods. Further research is needed to clarify these points.

Our study also has its strengths. Firstly, the interval time (24-hour) used to estimate the MID is in line with previous studies using mucociliary clearance rates or LCI as outcome measures to assess airway clearance interventions[35, 44]. As the timing and duration of airway clearance treatments are still unknown, measurements of 24-hour clearance have gained interest as a method to assess the possible cumulative clearance effects[16, 18, 19]. In addition, to improve the accuracy of the findings, repetitive measurements were included to estimate the MID. Finally, the wet sputum weight seems to provide more accurate findings than the sputum volume using a calibrated scale rather than a graduated scale of containers requiring the assessors´ interpretation. Indeed, there may be a tendency to overestimate the findings obtained using the sputum volume compared to the sputum weight[45]. However, both methods have not yet been adequately comparable.

In conclusion, wet sputum weight is an acceptable reliable measure over 24-hour after intervention, but the level of agreement is not narrow enough, particularly for greater level of expectoration (> 15 g/24 hours). Moreover, it is estimated that a reduction of at least 6.4 g in the amount of sputum expectorated during the 24 hours following the intervention, or a relative change of about -17% from baseline, was needed to achieve a real change in our population, assessed based on distribution-based methods. Therefore, the use of sequential measurements of sputum weight is recommended to assess the short-term effects of airway clearance treatments in stable individuals with bronchiectasis.

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## Quick lock

### *Current Knowledge*

Wet sputum expectoration is often used to assess the effects of airway clearance interventions in people with bronchiectasis. However, their correct interpretation is still a challenge because its reliability and the minimal important difference has not been evaluated yet.

### *What this paper contributes to our knowledge*

Wet sputum expectoration is an acceptable reliable measure over 24-hour after intervention in stable individuals with bronchiectasis, but the level of agreement is not narrow enough. Therefore, it is recommended to use this outcome measure only for intrasubject comparisons (clinical practice or crossover designs).

Most of participants presented a reduced need to expectorate after the airway clearance intervention. The MID estimated was a reduction of 6.4 g in the amount of sputum expectorated during the 24 hours following the intervention, or a relative change of about -17% from baseline, based on distribution-based methods.

## Supplementary material

**Table A**. Changes in LCQ and 24-hour sputum expectorated (g) after airway clearance sessions.

| | Baseline | Post Intervention | Mean difference | ES (r)¶ | Baseline | Post intervention | Median difference | ES (r)¥ |
|---|---|---|---|---|---|---|---|---|
| | Mean (SD) | Mean (SD) | (95% CI) | | Median [IQR] | Median [IQR] | (95% CI) | |
| **LCQ total score *** | 16.2 (4.1) | 16.9 (3.8) | 0.7 (0.0 to 1.3) | 0.32 | 17.2 [14.1 – 19.9] | 18.1 [15.1 – 20.0] | 0.6 (0.3 to 1.0) | 0.52 |
| **24-h sputum weight (g)** | 21.2 (11.5) | 12.1 (10.0) | -9.1 (-11.9 to -6.3) | 0.71 | 15.6 [14.0 – 27.7] | 8.8 [4.5 – 18.0] | -7.8 (-10.2 to -5.7) | 0.79 |

*LCQ= Leicester Cough Questionnaire assessed at the beginning and end of one week performing airway clearance sessions; SD= standard deviation; CI= confidence interval; ES= effect size; IQR= interquartile range; ¶ ES for paired-t test was calculated using this formula $r=\sqrt{t^2/t^2+df}$ ; ¥ ES for Wilcoxon signed-rank test was calculated using this formula $r = z/\sqrt{n}$

**Table B**. Median [$P_{25}$ - $P_{75}$] values of sputum expectorated (g) at different time points for each airway clearance intervention and median difference (95%CI) between interventions in the ´airway clearance cohort´(n=42)

| | **Airway clearance intervention** | | **Median difference (95% CI) (n=42)** |
|---|---|---|---|
| | **AD** | **[HA + HS] + AD** | **AD *vs.* [HA + HS] + AD** |
| 24-hour baseline | 18.2 [15.0 – 28.0] | 14.3 [10.1 – 24.8] | 4.4 (0.4 to 9.8) |
| Total (session + follow-up period) | 19.4 [13.4 – 30.5] | 19.3 [12.8– 25.2] | 1.7 (-9.9 to 4.8) |
| Airway clearance session | 6.4 [4.0 – 12.7] | 10.7 [8.3 – 15.3] | -3.8 (-7.3 to 0.1) |
| 24-hour follow-up after session ♯ | 10.6 [4.9 – 21.0] | 7.8 [4.1 – 10.3] | 4.4 (-2.0 to 9.4) |

The amount of sputum was measured in grams (g). ♯ 24-h follow-up does not include the time of intervention. AD, autogenic drainage; HA + HS, hyaluronate acid plus hypertonic saline; CI, confidence interval.

**MANUSCRIPT 4**

# Feasibility of computerized adventitious respiratory sounds to assess the effects of airway clearance techniques in patients with bronchiectasis

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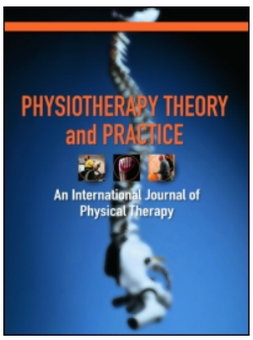

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# Feasibility of computerized adventitious respiratory sounds to assess the effects of airway clearance techniques in patients with bronchiectasis

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**ABSTRACT**

**Objective:** To examine the feasibility of adventitious respiratory sound (ARS) as an outcome measure to assess the effects of airway clearance techniques (ACTs) in outpatients with bronchiectasis.

**Methods:** ARS were registered pre/post four ACTs sessions. Clinical outcomes included: number of crackles (coarse and fine), number of wheezes (monophonic and polyphonic), wheezes occupation rate (%) and sputum quantity. Feasibility outcomes of ARS included: reasons for exclusion, suitability, safety, equipment and time required, magnitude of change after intervention and sample size estimation.

**Results:** Seven patients (49.7 ± 20.5 years; $FEV_1$ 69.3 ± 15.8% predicted) were included. Recordings from four patients were excluded due to excessive environment noise. All ARS measurements were completed without any adverse events. An electronic stethoscope was acquired and the time spent to complete each assessment was 6 ± 3.5 min. The largest changes were observed for number of expiratory coarse crackles [effect size (95%CI) ES = 0.40 (0.01–0.79)], which correlated moderately with sputum quantity (r = 0.56), and inspiratory monophonic wheezes [ES = 0.61 (0.22–1.00)]. The estimated sample size for a full crossover trial was 46.

**Conclusions:** ARS is feasible to assess the effects of ACTs in patients with bronchiectasis. Expiratory coarse crackles seem to be the most appropriate ARS parameter, but this finding needs to be confirmed in an adequately powered trial.

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Physical therapy; bronchiectasis; respiratory sounds; rehabilitation; airway clearance techniques

## Introduction

Airway clearance techniques (ACTs) are recommended for patients with bronchiectasis, by the recent European guidelines aiming at improving sputum expectoration (Polverino et al, 2017). Nevertheless, the level of evidence of ACTs is still poor (i.e. weak recommendation and low quality of evidence) (Lee, Burge, and Holland, 2017; Polverino et al, 2017), mainly due to the limitations of the available measures (Bradley, O´Neill, Vilaró, and Mcllwaine, 2018; Marques, Bruton, and Barney, 2006), such as subjectivity (e.g. conventional auscultation), unstandardized and challenge procedures (e.g. sputum volume) and lack of sensitivity to detect small changes (e.g. lung function). Therefore, the selection of outcome measures to assess ACTs effects and the interpretation of its results should be carefully performed, as they may hamper establishing the effectiveness of ACTs.

Computerized adventitious respiratory sounds (ARS), such as crackles and wheezes, are objective, simple and non-invasive outcome measures (Marques, Bruton, and Barney, 2006), that have been associated with the presence of excessive airway mucus and bronchial obstruction (Bohadana, Izbicki, and Kraman, 2014; Piirilä and Sovijärvi, 1995). Given the potential of ARS to be used as outcome measures to assess airway clearance or bronchial obstruction, previous studies have been exploring ARS responses to different interventions in respiratory diseases (Marques, Oliveira, and Jácome, 2014).

ARS have shown to be reliable and valid to be used in patients with bronchiectasis (Marques, Bruton, and Barney, 2009) and other respiratory conditions (Jácome and Marques, 2015; Oliveira, Lage, Rodrigues, and Marques, 2017a). However, it is still unclear what parameter of crackles and wheezes are the most appropriate to

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Supplemental data for this article can be accessed here.

evaluate the effects of ACTs and what direction and magnitude of change corresponds to a clinical improvement in patients with bronchiectasis. Moreover, according to the authors' best knowledge, the correlation of computerized ARS after ACTs with changes in other clinical outcomes, such as the amount of sputum collected, has never been explored, limiting the interpretation of the results achieved (Mokkink et al, 2009). Thus, before conducting an adequately powered definitive clinical trial using computerized ARS as an outcome measure for ACTs in patients with bronchiectasis, a preliminary study assessing the feasibility of this outcome measure is needed to ensure greater accuracy of the results achieved.

This study aimed to determine the feasibility of computerized ARS as outcome measure in patients with bronchiectasis by: 1) exploring the suitability and safety of ARS measurement procedures; 2) assessing the time required to complete the ARS registration; 3) describing the equipment required and their cost; 4) exploring the direction and magnitude of changes after four sessions of slow-expiratory ACTs; 5) evaluating the correlation between changes in ARS and sputum expectorated after slow-expiratory ACTs; and 6) estimating the parameters required to calculate the sample size for a future definitive randomized crossover trial (RCT). The authors hypothesized that the mean number of crackles, the mean number of wheezes and wheezes occupation rate (%) per respiratory phase (inspiratory and expiratory phase) will change significantly following the ACTs treatment (Marques, Oliveira, and Jácome, 2014; Oliveira, Pinho, and Marques, 2015), and these changes will have a positive and moderate correlation with the amount of sputum expectorated during ACTs treatment in patients with bronchiectasis.

## Methods

### Study design

A prospective repeated measures feasibility study, part of a randomized crossover trial (NCT01854788) (Herrero-Cortina et al, 2016), was conducted. Ethical approval was obtained from the Hospital Clinic Research Ethics Committee (HCP/2010/215).

### Participants

Adult outpatients diagnosed with bronchiectasis by high-resolution computed tomography (HRCT) scans were recruited from a community hospital in Barcelona (Spain) between October 2011 and June 2013. The inclusion criteria were: evidence of moderate daily sputum production (≥15 ml) based on classification previously proposed by King et al. (2006); being clinically stable for 6 weeks before data collection defined as no need for extra antibiotics or changes in usual therapy (Murray et al, 2011); and having training in the performance of slow-expiratory ACTs (i.e. slow expiration with glottis opened in lateral posture – ELTGOL and autogenic drainage – AD). Patients were excluded if they were smokers, had severe lung function impairment (forced expiratory volume in one second percentage predicted – $FEV_1 \leq 30\%$ predicted and forced vital capacity percentage predicted – $FVC \leq 45\%$ predicted), were not allocated to receive ELTGOL and AD at the beginning of the main study, experienced an exacerbation of their respiratory condition during the study period and presented poor quality of ARS recordings (i.e. artefacts or environment noise) (Rossi et al, 2000), which negatively affects the analysis. Prior to any data collection, written informed consents were collected from all participants.

### Intervention

The intervention consisted in 4 airway clearance sessions performed in two non-consecutive weeks at hospital. The first two sessions were performed in the first week at least 48-h period apart, and the remained sessions were performed in the third week. During the second week, no physiotherapy treatment was performed (a 7-day washout period). For the purposes of this study, repetitive sessions were analyzed to ensure greater accuracy of the results (Figure 1).

All patients performed ELTGOL and AD techniques two times in the same week in a random order (ELTGOL/AD or AD/ELTGOL) (Figure 1) according to recommendations (Agostini and Knowles, 2007; Martins et al, 2012;). In the current study, the ELTGOL and AD techniques were both chosen to assess the feasibility of computerized ARS to slow-expiratory ACTs because both are based on the same physiological action (i.e. decrease of the cross-sectional ratio of medial and peripheral airways without dynamic compression to increase the airflow velocity in these areas) (McIlwaine, Bradley, Elborn, and Moran, 2017; Wong, Sullivan, and Jayaram, 2018), and have shown equal efficacy (i.e., similar level of expectoration after the application of each technique) in patients with bronchiectasis (Herrero-Cortina et al, 2016). Sessions lasted 40 minutes and during ELTGOL sessions, participants spent approximately 20 minutes in each decubitus, and treatment was applied by one trained physiotherapist in a standardized schedule.

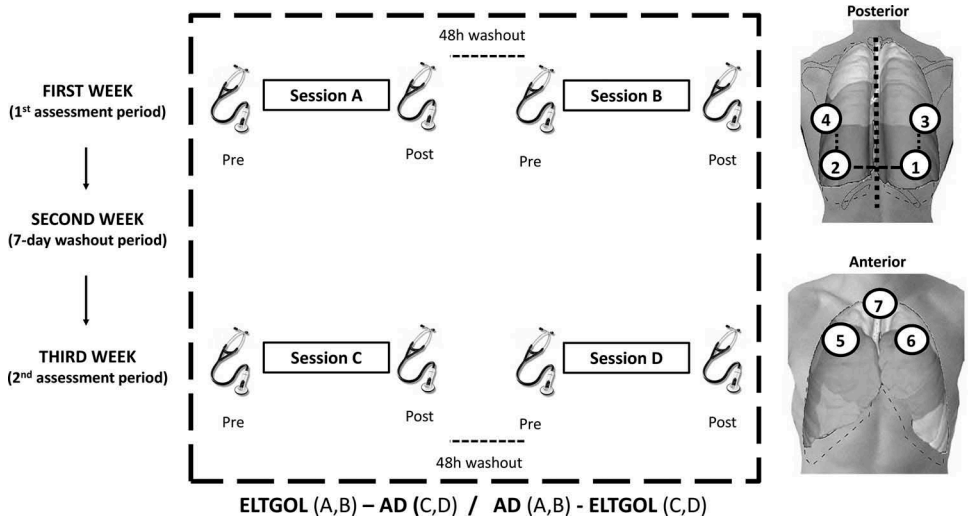


**Figure 1.** Study design. The feasibility of adventitious respiratory sounds (ARS) to assess the effects of airway clearance techniques was analyzed with the sound files recorded immediately before and after each of the 4 airway clearance sessions. The anatomical chest points recorded for ARS were: posterior right (1); posterior left (2); lateral right (3); lateral left (4); anterior right (5); anterior left (6); trachea (7). ELTGOL= slow expiration with glottis opened in lateral posture; AD = autogenic drainage.

### *Clinical data collection*

A trained physiotherapist conducted all data collection. One week prior to the intervention, patients' sociodemographic, anthropometric and clinical data (i.e. etiology of bronchiectasis, radiological severity and lung function and quality of life) were collected. Computerized ARS were recorded immediately before and after each of the four airway clearance sessions (Session A, B, C and D) (Figure 1) in a single room at hospital. Recordings were performed according to the Computerized Respiratory Sound Analysis (CORSA) guidelines for short-acquisition (Rossi et al, 2000). Participants were in a seated-upright position and respiratory sounds were collected with a hand-held electronic stethoscope (3MTM Littmann®, Model 3200). Sequential 15-second recordings were performed in seven chest locations (i.e. right and left: posterior, lateral, anterior chest and trachea) (Figure 1). During data collection, the sounds were transmitted, via Bluetooth®, and stored in a computer in .wav format.

All sound files were analyzed using automatic validated algorithms (Huq and Moussavi, 2010; Pinho et al, 2016; Taplidou and Hadjileontiadis, 2007) implemented in Matlab 2009 (The MathWorks, Inc, Natick, MA, USA) to detect and characterize respiratory phases and ARS.

The parameters extracted from crackles were: mean number of crackles (total, coarse, and fine) per respiratory phase (inspiration and expiration). Trachea was excluded from the crackles analysis due to its poor reliability observed in previous data (Jácome and Marques, 2015; Oliveira, Lage, Rodrigues, and Marques, 2017a). Mean number (i.e. total, monophonic, and polyphonic) and occupation rate of wheezes (%) per respiratory phase were extracted from wheezes, including trachea point in the analysis (Jácome and Marques, 2015).

The amount of sputum obtained (g) was assessed using two pre-weighted containers, one to weigh the wet sputum expectorated during each airway clearance session and the second to collect the spontaneous sputum obtained over the 24 h period after the sessions (Herrero-Cortina et al, 2016). All Participants were instructed to avoid salivary contamination and secretions from sinus were not allowed to include in the containers.

### *Feasibility of computerized ARS*

The suitability of ARS assessment was evaluated based on completion rate, rate of missing data and reasons for exclusion or dropouts due to the procedure. The cost of the additional equipment required was also calculated (expressed in Euros) to complete the feasibility analysis for clinical practice. Safety was explored by describing the number and type of adverse events which occurred during recordings, and the time needed to complete the assessment including instructions was measured in minutes. With no clear existing criteria, the feasibility criteria for computerized ARS were: completion rate assessment ≥80%, less than 20% of missing data from data extracted, no dropouts nor adverse events due to the procedure, and the total time pre and post measure did not exceed the airway clearance session.

### Statistical analysis

This feasibility study was not powered to determine differences in computerized ARS after ACTs, thus, hypothesis testing was not undertaken (Lancaster, Dodd, and Williamson, 2004; Orsmond and Cohn, 2015). Accordingly, the results were only focused on describing and estimating the treatment effects to offer insights to guide the future definitive RCT.

Baseline characteristics of participants and feasibility outcomes were summarized descriptively. The ARS characteristics were described for each of the sessions performed and global ARS findings were stratified for each one chest location recorded (trachea, anterior, lateral and posterior). For this purpose, right and left locations were pooled (Jácome, Oliveira, and Marques, 2015; Oliveira et al., 2017b). The pre and post findings of the four sessions were included in the analysis to increase the accuracy of the results. Differences in crackles and wheezes parameters pre and post sessions were explored and results were expressed as median difference and 95% confidence interval (95%CI) (Altman, Machin, Bryant, and Gardner, 2000). Effect sizes (ES) were also estimate using rank-biserial correlation (Wendt, 1972) and 95%CI (Nakagawa and Cuthill, 2007).

To establish the most appropriate ARS parameters to assess airway clearance, the ARS presenting the highest ES (i.e. one specific acoustic parameter of crackles and one of wheezes, to avoid multiple correlations that increase the risk of Type I error) (Feise, 2002) were selected and correlated with the sputum quantity ratio (%) (i.e., sputum expectorated during the session/24 h overall sputum obtained x 100) using Spearman´s rank correlation. Correlation values were interpreted as: weak ($r \leq 0.29$); moderate ($0.30 < r \leq 0.59$); and strong ($r \geq 0.60$) (Domholdt, 2000). Finally, the variability and the change observed from these ARS parameters selected were used to estimate the sample size needed for a definitive trial. Data analysis was performed using SPSS v.19 (IBM, Chicago, IL, USA) and plots were created using GraphPad Prism version 5.01 (GraphPad Software, La Jolla, California, USA).

## Results

From the 31 participants randomized in a larger trial (Herrero-Cortina et al, 2016), 11 were allocated to receive ELTGOL-AD or AD-ELTGOL at the beginning of the trial. All participants accepted and completed all ARS measurements without the occurrence of adverse events. Only one participant, who presented the major lung function impairment ($FEV_1$% predicted = 41), needed pauses between the recordings. Data rates extracted from the recordings were excellent (100%) without missing data; however, the quality of data from four participants was low due to excessive environmental noise and had to be excluded. Thus, only seven participants and their characteristics are shown in Table 1. Three participants started with ELTGOL and four started with AD. The sputum quantity ratio obtained during sessions was 39% (Supplementary Material, Table A).

The additional equipment required was only a handheld electronic stethoscope because the computer used belonged to the physiotherapy department. The cost of the stethoscope was estimated around 380€ (based on 2011 prices). The physiotherapist spent 6 ± 3.5 min to complete the seven chest locations recordings for each evaluation session and a total of 392 respiratory sound files from all anatomical locations were analyzed. Table 2 shows the descriptive characteristics of ARS for each of the four sessions, including all chest locations recorded. Table 3 presents the global ARS findings stratified by each chest location recorded.

### Crackles findings

After slow-expiratory ACTs, the mean number of inspiratory and expiratory crackles increased, except in the first session, with coarse crackles the main ARS responsible for these changes (Table 2). Inspiratory coarse crackles increased mainly in anterior and posterior regions while expiratory coarse crackles decreased in anterior regions and increased in lateral and posterior regions, after the sessions (Table 3).

Considering participants' individual results, after the airway clearance session, four participants experienced

**Table 1.** Participants' socio-demographic, anthropometric and clinical characteristics (n = 7).

| Patients' characteristics | n = 7 |
|---|---|
| Gender (male) | 1 (14%) |
| Age (years) | 49.7 ± 20.5 |
| BMI ($Kg/m^2$) | 24.1 ± 3.8 |
| Etiology of bronchiectasis | |
| – Primary ciliary dyskinesia | 2 (28%) |
| – Associated COPD | 1 (14%) |
| – Secondary immunodeficiency | 2 (28%) |
| – Idiopathic | 2 (28%) |
| No. of lobes affected by bronchiectasis | 4 ± 1.7 |
| Chronic airway infection | |
| – *Pseudomonas aeruginosa* infection | 3 (42.8%) |
| Lung function | |
| – $FEV_1$% predicted | 69.3 ± 15.8 |
| – FVC % predicted | 85.2 ± 18.0 |
| – $FEV_1$/FVC | 66.5 ± 4.5 |
| St George´s Respiratory Questionnaire total score | 44.6 ± 9.4 |

Data are presented as number (percentage %) or mean ± standard deviation

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; $FEV_1$%predicted, forced expiratory volume in one second percentage predicted; FVC % predicted, forced vital capacity percentage predicted.

**Table 2.** Descriptive characteristics of adventitious respiratory sounds for each one of the four airway clearance sessions.

| | Session 1 | | Session 2 | | Session 3 | | Session 4 | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Number of Crackles[†] | | | | | | | | |
| *Inspiratory phase* | | | | | | | | |
| Total | 1.62[1.27–2.52] | 2.31[1.43–3.00] | 1.56[1.19–2.78] | 1.80[1.25–1.97] | 1.77[1.37–2.83] | 2.06[1.43–2.19] | 1.83[1.25–2.61] | 2.08[1.36–2.47] |
| Coarse | 1.48[1.13–1.75] | 1.98[1.36–2.19] | 1.43[0.97–1.91] | 1.55[0.94–1.77] | 1.58[1.20–1.73] | 1.44[1.33–1.90] | 1.58[1.13–2.23] | 1.47[1.33–2.27] |
| Fine | 0.16[0.13–0.26] | 0.33[0.08–0.83] | 0.29[0.21–0.44] | 0.30[0.25–0.41] | 0.33[0.16–0.43] | 0.41[0.13–0.50] | 0.26[0.14–0.47] | 0.25[0.01–0.50] |
| *Expiratory phase* | | | | | | | | |
| Total | 3.29[2.06–4.50] | 2.88[2.30–6.22] | 3.52[2.38–4.47] | 4.30[2.36–6.94] | 3.69[2.73–4.96] | 4.11[2.25–4.94] | 3.27[2.60–5.41] | 5.08[2.21–6.25] |
| Coarse | 2.84[1.70–4.41] | 2.51[2.03–5.47] | 3.44[2.05–4.19] | 3.88[2.31–6.61] | 3.36[2.57–4.44] | 3.91[2.14–4.55] | 3.22[2.60–5.41] | 5.02[2.10–5.92] |
| Fine | 0.26[0.09–0.44] | 0.33[0.26–0.66] | 0.16[0.03–0.33] | 0.33[0.04–0.43] | 0.30[0.16–0.50] | 0.16[0.11–0.25] | 0.12[0.05–0.32] | 0.33[0.09–0.42] |
| Number of Wheezes [ǂ] | | | | | | | | |
| *Inspiratory phase* | | | | | | | | |
| Total | 0.47[0.15–0.57] | 0.57[0.31–0.78] | 0.76[0.28–0.78] | 1.00[0.64–1.33] | 0.31[0.19–0.71] | 0.62[0.52–0.99] | 0.31[0.22–0.38] | 0.36[0.23–0.40] |
| Monophonic | 0.32[0.11–0.57] | 0.50[0.23–0.61] | 0.59[0.24–0.63] | 0.71[0.55–0.98] | 0.21[0.19–0.64] | 0.57[0.32–0.74] | 0.23[0.14–0.31] | 0.28[0.23–0.33] |
| Polyphonic | 0.04[0.00–0.20] | 0.09[0.07–0.16] | 0.13[0.04–0.26] | 0.14[0.04–0.39] | 0.07[0.05–0.24] | 0.19[0.02–0.25] | 0.07[0.47–0.14] | 0.07[0.03–0.07] |
| Occupation rate (%) | 9.3[5.4–13.5] | 9.2[7.3–13.3] | 16.0[6.1–19.9] | 16.8[8.7–32.6] | 8.0[3.7–13.1] | 12.9[8.0–19.9] | 7.2[4.2–8.9] | 5.3[3.5–8.2] |
| *Expiratory phase* | | | | | | | | |
| Total | 0.57[0.43–0.75] | 0.83[0.26–1.58] | 0.93[0.54–1.48] | 1.58[1.02–1.78] | 0.64[0.45–0.93] | 0.84[0.53–0.91] | 0.43[0.28–0.57] | 0.57[0.28–0.78] |
| Monophonic | 0.50[0.33–0.57] | 0.74[0.23–0.93] | 0.59[0.50–1.38] | 0.95[0.74–1.27] | 0.50[0.40–0.78] | 0.75[0.42–0.80] | 0.28[0.24–0.50] | 0.43[0.24–0.64] |
| Polyphonic | 0.09[0.04–0.24] | 0.21[0.06–0.55] | 0.12[0.05–0.33] | 0.36[0.28–0.52] | 0.11-[0.04–0.14] | 0.11[0.04–0.16] | 0.14[0.03–0.15] | 0.14[0.11–0.22] |
| Occupation rate (%) | 6.52[4.43–7.69] | 8.30[4.52–11.67 | 13.1[6.5–24.6] | 14.6[9.0–23.7] | 6.8[5.1–8.3] | 11.1[7.7–11.6] | 4.8[3.2–11.0] | 5.9[3.5–8.5] |

Data are presented as median and [interquartile range] [†] Analysis without trachea point. [ǂ] Analysis across all anatomical points.

**Table 3.** Descriptive characteristics of adventitious respiratory sounds stratified by each one of the chest locations recorded.

| | Anterior regions | | Lateral regions | | Posterior regions | | Trachea | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Number of Crackles[†] | | | | | | | | |
| *Inspiratory phase* | | | | | | | | |
| Total | 1.50[1.00–2.66] | 2.00[1.06–2.45] | 1.73[1.05–2.33] | 2.00[1.50–2.66] | 1.66[1.05–2.31] | 2.00[1.06–2.68] | - | - |
| Coarse | 1.33[1.00–2.00] | 1.50[1.00–2.00] | 1.50[1.00–2.00] | 1.55[1.00–2.00] | 1.33[0.75–2.18] | 1.55[1.00–2.00] | - | - |
| Fine | 0.25[0.00–0.5] | 0.22[0.00–0.50] | 0.22[0.00–0.50] | 0.25[0.00–2.00] | 0.22[0.00–0.50] | 0.10[0.00–0.50] | - | - |
| Expiratory phase | | | | | | | | |
| Total | 3.70[2.00–4.66] | 3.66[1.76–5.50] | 3.00[2.42–5.33] | 4.33[2.33–5.91] | 3.00[1.80–4.25] | 3.83[2.50–5.00] | - | - |
| Coarse | 3.58[1.66–4.50] | 3.00[1.66–5.00] | 2.87[2.27–4.66] | 4.16[2.05–5.92] | 2.87[1.60–4.18] | 3.29[2.12–4.87] | - | - |
| Fine | 0.25[0.00–0.50] | 0.10[0.00–0.50] | 0.20[0.00–0.40] | 0.00[0.00–0.50] | 0.00[0.00–0.25] | 0.00[0.00–0.46] | - | - |
| Number of Wheezes[ŦŦ] | | | | | | | | |
| *Inspiratory phase* | | | | | | | | |
| Total | 0.33[0.21–0.80] | 0.50[0.17–1.00] | 0.25[0.00–0.57] | 0.50[0.00–1.00] | 0.33[0.00–0.60] | 0.50[0.00–1.19] | 0.33[0.11–0.50] | 0.42[0.15–0.85] |
| Monophonic | 0.33[0.00–0.66] | 0.33[0.00–1.00] | 0.20[0.00–0.47] | 0.33[0.00–1.00] | 0.20[0.00–0.40] | 0.29[0.00–0.66] | 0.25[0.00–0.50] | 0.36[0.00–0.54] |
| Polyphonic | 0.00[0.00–0.31] | 0.00[0.00–0.33] | 0.00[0.00–0.20] | 0.00[0.00–0.00] | 0.00[0.00–0.24] | 0.00[0.00–0.33] | 0.00[0.00–0.00] | 0.00[0.00–0.54] |
| Occupation rate (%) | 9.7[4.7–17.8] | 10.8[3.8–20.6] | 5.7[0.0–1.1] | 7.4[0.0–16.7] | 8.5[0.0–15.2] | 7.4[0.0–22.6] | 7.0[3.3–10.7] | 6.5[3.3–11.7] |
| Expiratory phase | | | | | | | | |
| Total | 0.66[0.00–1.33] | 0.67[0.50–1.33] | 0.60[0.00–1.15] | 0.71[0.05–2.00] | 0.40[0.00–0.79] | 0.66[0.25–1.33] | 0.50[0.31–1.00] | 1.00[0.47–1.50] |
| Monophonic | 0.55[0.21–1.00] | 0.50[0.27–1.00] | 0.50[0.00–1.00] | 0.58[0.00–1.62] | 0.31[0.00–0.66] | 0.50[0.21–1.00] | 0.33[0.25–0.64] | 0.50[0.20–1.00] |
| Polyphonic | 0.00[0.00–0.33] | 0.00[0.00–0.47] | 0.00[0.00–0.20] | 0.00[0.00–0.31] | 0.00[0.00–0.00] | 0.00[0.00–0.25] | 0.00[0.0–0.25] | 0.26[0.00–0.62] |
| Occupation rate (%) | 8.2-[3.9–14.7] | 8.3[3.1–16.0] | 6.7[0.0–13.1] | 6.8[0.6–13.7] | 5.2[0.0–9.9] | 6.4[2.9–12.4] | 7.4[3.4–10.6] | 10.6[6.6–13.8] |

Data are presented as median and [interquartile range]. [†] Analysis without trachea point. [ŦŦ] Analysis across all anatomical points.

an increase in the amount of inspiratory coarse crackles while the remaining three did not show any change. Six participants showed an increase in expiratory coarse crackles after slow-expiratory ACTs. A heterogeneous direction of change was observed for fine crackles (Supplementary Material, Figure A).

### Wheezes findings

The total number of wheezes and monophonic wheezes increased after intervention in all sessions, while no changes were observed for polyphonic wheezes (Table 2). Similarly, increases in the wheeze occupation rate were observed after intervention, mainly during expiration. The increase in the number of inspiratory wheezes were similar across all chest regions; however expiratory wheezes and wheeze occupation rate increased mainly at the trachea (Table 3).

Considering participants' individual results, after the airway clearance session, the number of monophonic wheezes increased in six participants during inspiration, and in four participants during expiration. Most participants also showed an increase of polyphonic wheezes after treatment (i.e. five during inspiratory phase, and four during expiratory phase) (Supplementary material, Figure A).

### Correlation between ARS and sputum expectorated

The number of expiratory coarse crackles and inspiratory monophonic wheezes were the computerized ARS parameters which experienced the major changes after the intervention (Table 4), and thus were chosen for the correlation analysis.

A moderate positive correlation was observed between the increase of expiratory coarse crackles and the sputum quantity ratio (r = 0.56), whereas changes in inspiratory monophonic wheezes presented a negative and small correlation with the sputum quantity ratio (r = −0.18) (Figure 2). Thus, expiratory coarse crackles seem to be the most appropriate primary outcome measure.

### Sample size estimation for future trials

Crackles and wheezes have shown high inter-subject variability in bronchiectasis and other respiratory disease (Jácome and Marques, 2015; Marques, Bruton, and Barney, 2009). Therefore, a randomized crossover trial might be the study design most appropriate (Mills et al, 2009) to assess the short-term effects of ACTs using computerized ARS as an outcome measure. The mean (SD) of the difference in response to slow-expiratory ACTs by the same participant in this study was 0.58 (1.23) for expiratory coarse crackles. Based on this assumption, an alpha risk of 0.05 with 80% power, in a two-sided test, it is estimated that a sample size of 38 participants will be required in future crossover trials. Considering a common drop-out rate of 20%, the final sample size required for future studies would be 46 participants.

## Discussion

According to the authors' best knowledge, this is the first study to determine feasibility of computerized ARS to slow-expiratory ACTs as an outcome measure in a small sample of stable patients with bronchiectasis. The main findings were: 1) computerized ARS presented acceptable feasibility in terms of completion rate, missing data, safety, cost, and the time taken to complete the ARS registration. However, environment noise negatively influenced the quality of data extracted from four patients and is potentially the main barrier of the assessment procedure; 2) the number of expiratory coarse crackle and inspiratory monophonic wheezes were the ARS parameters that experienced the major changes after slow-expiratory ACTs; and 3) differences in expiratory coarse crackles correlated positively and moderately with the sputum quantity ratio collected during sessions.

Based on our findings, computerized ARS seem to be a feasible outcome measure for use in clinical practice and future studies in patients with bronchiectasis. Nevertheless, achieving an optimal background noise level (below 60 dB) (Rossi et al, 2000) within a hospital environment appears to be a barrier for ARS recording. For practical purpose, it is recommended to choose a room with less transient noise with appointments schedule during quieter times. The only additional equipment required (electronic stethoscope) and its cost may be acceptable for clinical practice and future research with low funding.

Globally, the mean number of expiratory crackles after slow-expiratory ACTs increased and this pattern was presented in six out of the seven participants involved in this study. Oliveira, Pinho. and Marques (2015) observed similar results after one single session of physiotherapy with slightly lower ES (pre 2.64 ± 1.68 *vs*. post 3.22 ± 1.99, ES = 0.31) in obstructive patients with lower respiratory tract infection. These findings might suggest that the direction of crackles change is toward an increase after ACTs sessions. However, our findings contrast with those reported by Marques, Bruton, Barney, and Hall (2012), who suggested that the mean number of crackles is not able to change after one session of ACTs in patients with bronchiectasis.

**Table 4.** Changes in adventitious respiratory sound after slow-expiratory airway clearance techniques.

| OUTCOME | PRE Mean (SD) | POST Mean (SD) | MEAN DIFFERENCE (95%CI) | PRE Median [IQR] | POST Median [IQR] | MEDIAN DIFFERENCE (95% CI) | ES (95% CI) |
|---|---|---|---|---|---|---|---|
| Number of crackles[†] | | | | | | | |
| *Inspiratory Phase* | | | | | | | |
| Total | 1.90 (0.78) | 1.99 (0.63) | 0.09 [−0.18–0.37] | 1.71 [1.29–2.59] | 1.96 [1.43–2.30] | 0.18 [−0.14–0.36] | 0.24 [−0.15–0.62] |
| Coarse | 1.57 (0.68) | 1.64 (0.55) | 0.07 [−0.17–0.31] | 1.49 [1.13–1.87] | 1.57 [1.33–1.99] | 0.15 [−0.12–0.27] | 0.26 [−0.13–0.65] |
| Fine | 0.25 (0.16) | 0.33 (0.26) | 0.08 [−0.02–0.18] | 0.27 [0.08–0.36] | 0.33 [0.12–0.42] | 0.04 [−0.05–0.14] | 0.22 [−0.17–0.61] |
| *Expiratory Phase* | | | | | | | |
| Total | 3.66 (1.59) | 4.31 (2.03) | 0.65 [0.13–1.17] | 3.38 [2.66–4.56] | 4.14 [2.31–5.74] | 0.63 [0.10–1.21] | 0.40 [0.01–0.79] |
| Coarse | 3.41 (1.52) | 3.98 (1.88) | 0.58 [0.10–1.05] | 3.21 [2.57–4.44] | 3.90 [2.27–5.35] | 0.55 [0.08–1.05] | 0.40 [0.01–0.79] |
| Fine | 0.33 (0.25) | 0.35 (0.25) | 0.02 [−0.09–0.13] | 0.26 [0.15–0.43] | 0.33 [0.15–0.49] | 0.05 [−0.02–0.16] | 0.15 [−0.24–0.54] |
| Number of wheezes[ŦŦ] | | | | | | | |
| *Inspiratory Phase* | | | | | | | |
| Total | 0.47 (0.31) | 0.66 (0.37) | 0.19 [0.06–0.31] | 0.37 [0.23–0.70] | 0.59 [0.32–0.97] | 0.17 [0.06–0.31] | 0.51 [0.11–0.90] |
| Monophonic | 0.35 (0.24) | 0.50 (0.28) | 0.15 [0.06–0.23] | 0.26 [0.19–0.59] | 0.46 [0.27–0.68] | 0.14 [0.05–0.22] | 0.61 [0.22–1.00] |
| Polyphonic | 0.12 (0.11) | 0.15 (0.13) | 0.04 [−0.02–0.10] | 0.07 [0.05–0.20] | 0.10 [0.07–0.24] | 0.03 [−0.02–0.09] | 0.18 [−0.21–0.57] |
| Occupation Rate (%) | 11.2 (8.0) | 13.0 (9.3) | 1.8 [−1.6–5.2] | 8.8 [5.4–15.4] | 9.3 [7.4–18.2] | −0.17 [−2.64–2.13] | 0.22 [−0.17–0.61] |
| *Expiratory Phase* | | | | | | | |
| Total | 0.70 (0.39) | 0.96 (0.62) | 0.25 [0.08–0.43] | 0.57 [0.43–0.93] | 0.84 [0.50–1.51] | 0.25 [0.04–0.44] | 0.45 [0.06–0.84] |
| Monophonic | 0.57 (0.34) | 0.72 (0.48) | 0.15 [0.02–0.28] | 0.50 [0.30–0.74] | 0.74 [0.41–0.92] | 0.14 [0.02–0.27] | 0.37 [−0.02–0.76] |
| Polyphonic | 0.13 (0.10) | 0.23 (0.20) | 0.10 [0.03–0.17] | 0.12 [0.05–0.18] | 0.15 [0.07–0.36] | 0.08 [0.02–0.16] | 0.52 [0.13–0.91] |
| Occupation Rate (%) | 9.5 (7.1) | 10.6 (8.1) | 1.0 [−1.5–3.4] | 6.9 [4.8–11.6] | 8.8 [6.1–11.7] | 0.91 [−0.66–2.96] | 0.20 [−0.19–0.59] |

[†] Analysis without trachea point. [ŦŦ] Analysis across all anatomical points.

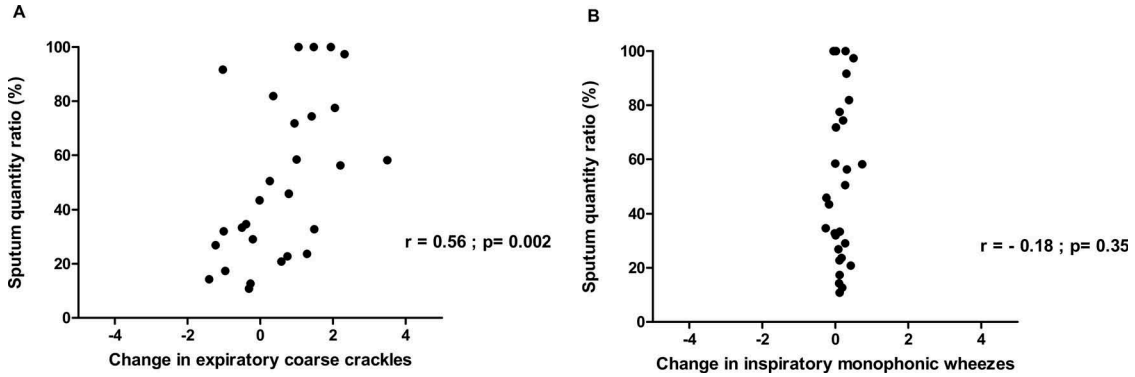


**Figure 2.** (a) Relationship between changes in the mean number of coarse crackles during expiratory phase and the sputum quantity ratio (%); (b) relationship between changes in the mean number monophonic wheezes during inspiratory phase and the sputum quantity ratio (%). Sputum quantity ratio (%) represented the sputum expectorated during the physiotherapy session/ 24-h overall sputum expectoration.

Although the target population included in both studies was similar and presented a comparable pre-intervention number of crackles (4.14 ± 2.31 *vs.* 5.55 ± 2.19 in our study), the ACT performed were different (i.e. active cycle of breathing technique *vs* ELTGOL/AD in our study). Also, the time period of the session was shorter for Marques, Bruton, Barney, and Hall (2012) study (average of 24 minutes *vs.* 40 minutes in our study) and the data was based only on a single session (*vs.* repeated measured in our study) which may justify the differences found.

It is known that slow-expiratory ACTs enhance mucus clearance from small/medium to larger airways (Button and Button, 2013). The motion of intraluminal mucus to larger airways produces a major airflow in small/medium airways and this process may allow a sudden reopening of abnormally closed airways, which in turn might generate an increased number of crackles (Oliveira, Pinho, and Marques, 2015). In our study, most changes occurred in the number of expiratory coarse crackles which were also correlated with the sputum quantity ratio, whereas inspiratory coarse crackles and fine crackles remained almost unchanged presenting a heterogeneous direction of change among participants (i.e. some participants presented increases and other presented decreases).

It is believed that obstructive diseases are associated with early inspiratory coarse crackles, and thus the present data are consistent with the concept that inspiratory coarse crackles depend mainly on the pathophysiology of the surrounding tissue (Piirilä and Sovijärvi, 1995), whereas expiratory coarse crackles seem to be able to respond to short-term effects of ACTs in stable patients with bronchiectasis. Therefore, for a future RCT in patients with bronchiectasis, expiratory coarse crackles might be the most appropriate primary endpoint.

Similar to crackles, the mean number of wheezes also increased after sessions. Inspiratory monophonic wheezes was the parameter that changed the most after the treatment, increasing in six participants; however poor correlation with the sputum quantity ratio collected during intervention was found. Otherwise, the occupation rate of wheezes presented a slightly change after treatment, suggesting that despite the increased wheezes, the level of obstruction remained almost unchanged. The higher number of wheezes after the session, specifically observed at the trachea, could be associated with the number of forced expiratory maneuvers (cough) performed. The relationship between wheezes and forced expiratory maneuvers has already been shown in patients with asthma and COPD (Fiz et al, 2002); however no studies have been performed in patients with bronchiectasis. It is possible that the same mechanism may be observed in this population. Nevertheless, as the numbers of cough maneuvers were not registered and computerized ARS were recorded at the end of the session, definite conclusions cannot be drawn.

Previous data on the behavior of wheezes after physiotherapy interventions in adults are limited to a pre/post study conducted by Oliveira, Pinho, and Marques (2015) in patients with lower respiratory tract infections. These patients performed a protocol composed of breathing techniques to enhance sputum expectoration (20–25 min), exercises to increase pulmonary volumes (15 min) and education (15 min). Considering all chest locations, no differences in the mean number of wheezes and wheeze occupation rate after the intervention were found in the subgroup of patients with obstructive diseases (Oliveira, Pinho, and Marques, 2015). These different results may be related with the higher inspiratory volumes associated with the exercises performed after the ACTs, which helped reverse the

airway collapse related to cough maneuvers, or due to the different timing of computerized ARS recordings (after the physiotherapy session vs immediately after the ACTs in this study).

A sample of 46 participants would be required for future crossover trials using expiratory coarse crackles as the primary outcome measure. Assuming that the rate of recruitment in previous crossover trials evaluating short-term effects ACTs in bronchiectasis was around 65% (Herrero-Cortina et al, 2016; Paneroni et al, 2011), at least 71 eligible patients would need to be invited to take part in a future study.

### *Limitations and future work*

The results of this feasibility study should be interpreted with caution particularly due to the small sample size included. However, the study was designed to maximize the accuracy of the findings as repeated measures were performed in four non-consecutive physiotherapy sessions.

Equipment to standardize airflows and volumes were not acquired and this may have affected the results on crackles and wheezes parameters. However, this study focused on analyzing the feasibility of an outcome measure to be easily applied in clinical practice (Marques, Bruton, Barney, and Hall, 2012). Despite the chest locations were recorded individually with only one stethoscope, the time burden was low and generally well tolerated. Future trials might be included two recordings for each one chest location to improve the results accuracy.

Participants with lower probability to generate enough airflow (i.e. severe lung function impairment) were excluded from the present study to ensure greater quality of ARS recordings. Future studies evaluating the tolerability of ARS recordings in people with bronchiectasis and severe airflow obstruction should be conducted to test the feasibility of using this measure also in severe patients.

Further studies are required to explore if other parameters, such as normal respiratory sounds, i.e., intensity and frequency, are able to respond to slow-expiratory ACTs. It is also recommended to study the measurement validity and responsiveness of computerized ARS and the most appropriate time point to record the ARS after a session in patients with bronchiectasis. Finally, building on the findings of our study, future larger studies are needed to explore whether ARS are also an appropriate outcome to assess long-term efficacy of ACTs and for comparing the effects of different ACTs on ARS changes.

## Conclusion

These preliminary findings support the feasibility and potential use of computerized ARS as an objective and simple clinical outcome to assess the short-term effects of slow-expiratory ACTs in patients with bronchiectasis. The mean number of expiratory coarse crackles and monophonic inspiratory wheezes were the ARS parameters that appeared to change after an intervention. However, only changes in expiratory coarse crackles correlated with sputum quantity ratio, highlighting the usefulness of this parameter to assess the effects of slow-expiratory ACTs in patients with bronchiectasis.

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## Disclosure Statement

The authors declare no conflict of interest.

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**Table A** Wet sputum expectorated in response to slow-expiratory airway clearance techniques during airway clearance sessions and in the 24-h period after the intervention.

| Period of sputum collection | Sputum quantity (g) | Sputum quantity ratio (%)[†] |
|---|---|---|
| Physiotherapy session (40 min) | 5.9 [3.4-22.9] | 39.0 [23.8-76.7] |
| 24-h (excluding treatment)[₮] | 9.8 [5.4-12.9] | 61.0 [23.2-76.2] |
| 24-h (including treatment) | 17.1 [13.5-31.4] | 100 |

Data are presented as median [interquartile range]. [†] Sputum quantity ratio (%) represented the sputum expectorated during the physiotherapy session/ 24-h overall sputum expectoration x 100; [₮] Samples collected during 24 hours after the physiotherapy intervention without any intervention. The amount of sputum collected during the physiotherapy session and 24-h after the intervention were similar for both techniques (Herrero-Cortina et al, 2016)

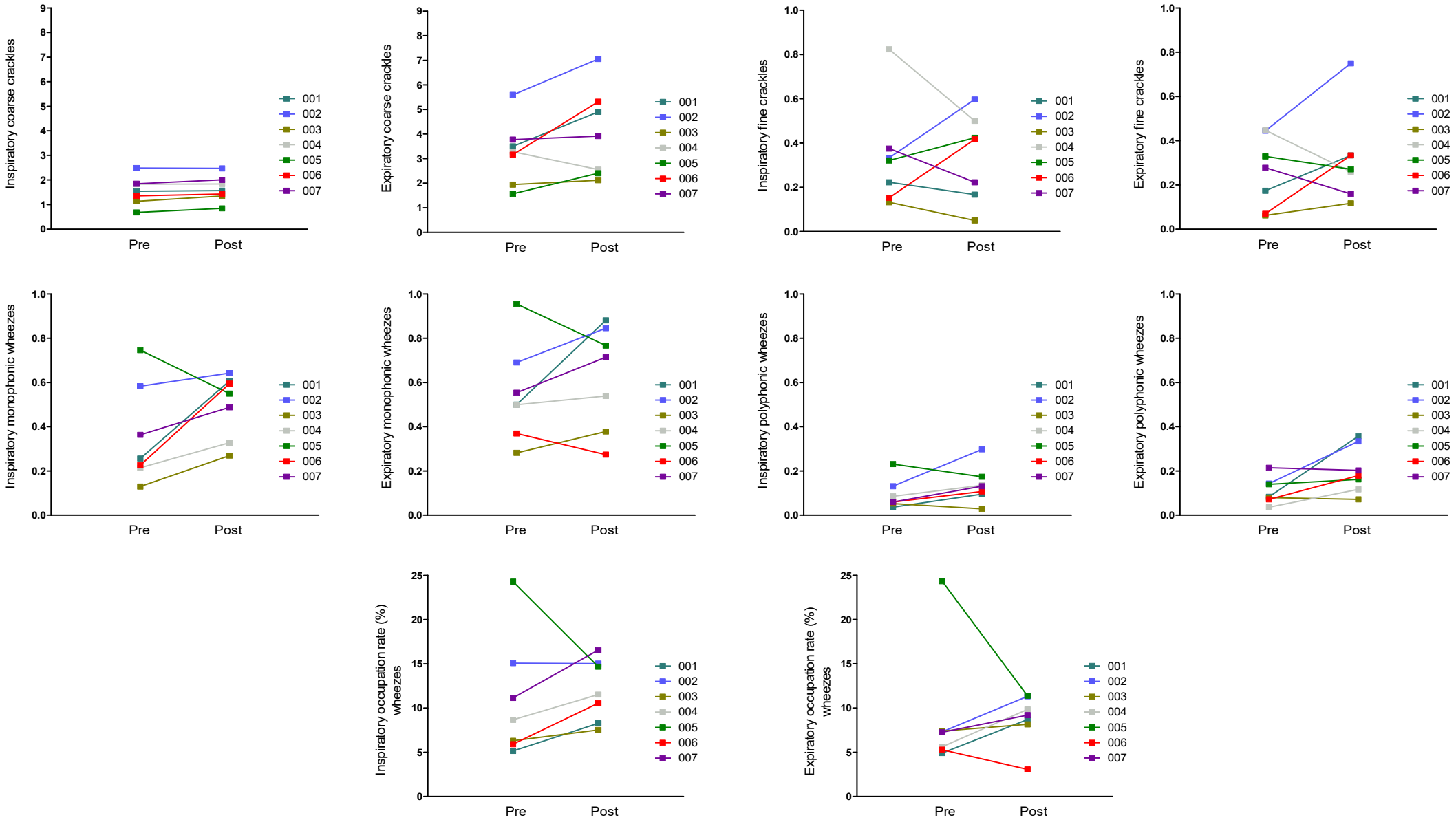


**Figure A** Patient profiles of changes in adventitious respiratory sound after slow-expiratory airway clearance techniques.

**NARRATIVE REVIEW**

# **Airway clearance techniques,** pulmonary rehabilitation and physical activity

**Herrero-Cortina B**, Lee AL, O'Neill B, Bradley J.

In: Chalmers JD, Polverino E, Aliberti S, eds. Bronchiectasis (ERS Monograph). Sheffield, European Respiratory Society, 2018; pp. 331–352.

Chapter 20

# Airway clearance techniques, pulmonary rehabilitation and physical activity

Beatriz Herrero-Cortina[1], Annemarie L. Lee[2,3], Brenda O'Neill[4] and Judy Bradley[5]

People with bronchiectasis are characterised by a combination of an impaired mucociliary clearance system with functional limitation and lower physical activity levels; hence, physiotherapy interventions should be a priority strategy for the management of this population. ACTs used regularly reduce the respiratory symptoms related to cough and, therefore, improve health-related quality of life (HRQoL). Short-term clinical benefits in people with bronchiectasis are observed for functional exercise capacity, symptoms and HRQoL after completing a pulmonary rehabilitation programme, but these improvements are not maintained long term. Consequently, interventions to increase physical activity and reduce sedentary time may be incorporated in pulmonary rehabilitation programmes and in the overall management of people with bronchiectasis. Furthermore, strategies to promote behavioural change and adherence are needed to ensure successful implementation of these interventions in clinical practice. Further research is needed to explore the effects of physiotherapy interventions during an acute exacerbation and continued beyond discharge, and their impact on disease severity.

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**Airway clearance techniques, pulmonary rehabilitation and interventions to promote physical activity are useful strategies to improve the quality of life and reduce the impact of daily respiratory symptoms in people with bronchiectasis** http://ow.ly/Yva330ksJkB

Chronic cough, sputum production, dyspnoea, fatigue, anxiety, depression and functional limitation are the main clinical manifestations reported for people with bronchiectasis. These symptoms and disorders tend to worsen during acute exacerbations,

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which have a negative impact on health-related quality of life (HRQoL). Physiotherapy interventions (ACTs, pulmonary rehabilitation and interventions to increase physical activity levels and reduce sedentary time) are useful nonpharmacological strategies to reduce symptoms and exacerbation frequency and improve HRQoL. This chapter focuses on: 1) understanding the beneficial effect of physiotherapy interventions in people with bronchiectasis; 2) providing validated tools of measurement to evaluate their effects; 3) identifying useful strategies to implement these interventions into clinical practice and tips to promote adherence to these physiotherapy interventions; and 4) reviewing the current evidence and giving suggestions for future research in these areas.

## Airway clearance techniques in bronchiectasis

### Why are airway clearance techniques beneficial in bronchiectasis?

A productive cough or difficulty in expectorating sputum is a clinical symptom that reflects the presence of an impaired mucociliary clearance system in people with bronchiectasis. Abnormalities in mucus production, ciliary function and in biophysical and surface mucus properties contribute directly to a decreased mucus clearance rate [1–3].

Although there are few data evaluating the function of airway surface liquid in bronchiectasis, the hypothesis that the dehydration of the mucus layer results in mucus transport impairment [2, 4, 5] may be extrapolated to people with bronchiectasis. First, neutrophil elastase activity plays an important role in the pathogenesis and progression of bronchiectasis [6]. The excess activity of neutrophil elastase within the inflamed airway decreases ciliary beating and stimulates mucin secretion [6, 7]. The MUC2 and MUC5B mucins seem to be the most predominant in bronchiectasis. Furthermore, higher airway mucin levels are also associated with disease severity [8], although further studies are needed to confirm these results. An excess of secreted mucin leads to mucus layer dehydration and generates an osmotic imbalance between the mucus layer and the periciliary liquid (PCL) region, which, ultimately, compresses the PCL and cilia system (figure 1) [9]. As result, the ciliary beat is slowed down and the mucus layer adhesion to the airway epithelial surface (termed “adhesivity”, a surface property of mucus) is facilitated, thereby reducing mucus

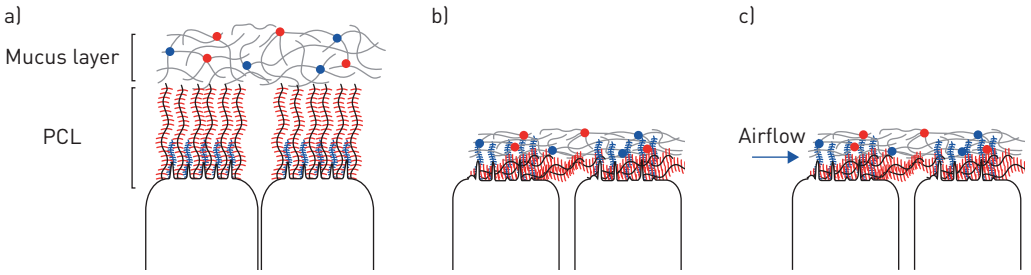


Figure 1. a) Representation of the airway surface layer, including mucus layer and periciliary liquid (PCL) layer, under normal conditions. b) Representation of the airway surface layer under dehydration conditions. An excess of mucin concentration leads to mucus layer dehydration and collapse of the PCL and ciliary system. As a result, the mucus transport is impaired and this produces mucus adhesion to the airway surface (adhesivity). c) Action of ACTs using expiratory airflow to enhance sputum removal. Reproduced and modified from [9] with permission.


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transport and enhancing mucus accumulation [2, 4]. Ciliary dysfunction can also be due to a genetic disorder of the cilia structure and function in people with bronchiectasis (PCD).

Secondly, the adhesivity seems to be the strongest dependent factor of cough clearance effectiveness [1, 3] when respiratory muscle strength is preserved. This is independent of mucus viscoelastic properties [10]. Greater adhesivity appears when the interfacial tension is high between the mucus layer and the airway epithelium and/or the mucus wettability is low [1, 11]. The limited data available suggest that cough clearance is impaired in bronchiectasis [12, 13], although it is still more effective when compared to other diseases (*e.g.* CF or bronchitis) [14]. Despite producing certain improvements on the sputum surface properties after inhalation of hydrator therapies in patients with bronchiectasis [12], the long-term clinical benefits of hyperosmolar solutions (such as mannitol or hypertonic saline) still remain unclear [15–17]. Cough clearance may be less impaired in bronchiectasis due to the lower sputum adhesivity found in this population [14], which may partly explain the variable beneficial effects found using hyperosmolar solutions in cases of bronchiectasis and other respiratory conditions.

Mechanical stress applied to the airways is a strategy to stimulate hydration of the mucus layer and, therefore, enhance airway clearance [18, 19]. During normal breathing, two physical stresses relevant for the regulation of normal airway surface hydration, the airflow and the trans-airway pressure gradient, are generated during both respiratory phases [18]. Previous studies have reported that fluid shear stress, compression/stretch and osmotic shock are the main physical mechanisms that stimulate airway surface hydration [18]. In addition, an *in vitro* flow model suggests two conditions that improve airway clearance [20–22]: 1) the peak expiratory flow rate should be greater than the peak inspiratory flow rate (by at least 10%) for mucus to move proximally; and 2) a peak expiratory flow rate of 30–60 $L \cdot min^{-1}$ is required to break the adhesive bonds generated between the mucus layer and the airway epithelial surface. Accordingly, ACTs based on generating greater mechanical stress on the airways compared to normal breathing and the achievement of both aforementioned conditions may play an important role in improving airway clearance in people with bronchiectasis.

So far, little attention has been paid to the impact of chronic productive cough as an independent prognostic factor in bronchiectasis, despite the fact that productive cough is associated with higher cough frequency [23] and poor HRQoL [24]. Furthermore, the duration of chronic productive cough is also associated with lower lung function and more exacerbations [25]. ACTs applied regularly in people with bronchiectasis may help increase the clearance of inflammatory markers in the airways, reduce the frequency of exacerbations and reduce the daily symptoms related to cough, thereby improving overall HRQoL.

## How to implement airway clearance techniques in clinical practice

ACTs should be taught to all people with bronchiectasis and chronic productive cough or inability to cough effectively, based on the recommendations of both international and national guidelines [26, 27]. Additionally, people with nonproductive cough who report an increase in cough frequency and sputum volume/consistency during a pulmonary exacerbation should also be trained (preferably when stable) in the most appropriate ACTs [28].

The main ACTs used in people with bronchiectasis [29] are described in table 1 and further information on the procedure for each one is available on specific multi-media web

Table 1. An overview of the main ACTs used in people with bronchiectasis

| Technique | Procedure [30, 31] | | Physiological basis to enhance sputum removal [19, 20] | Short-term clinical benefits | Long-term clinical benefits |
|---|---|---|---|---|---|
| | Positioning | Breathing | | | |
| **Active cycle of breathing technique** | Generally performed in sitting position; however, an alternative position (supine or side-lying) may also be used. | A combination of exercises including breathing control, thoracic expansions with breath-hold after inspiration and finished by forced expiratory technique (huff). | Thoracic expansion exercises with breath-hold generate a greater trans-airway pressure gradient (mechanical stress) than normal breathing. This mechanism also uses interdependence and collateral ventilation to allow the presence of air behind obstructed lung units. | Enhances sputum removal during treatment [32].<br>Slight improvement in lung function after treatment [32]. | NE |
| **Autogenic drainage** | Generally performed in sitting position (figure 2d); however, an alternative position (supine or side-lying) may also be used. | Commence breathing from lower lung volume levels in the expiratory reserve volume, through higher lung volume levels into the inspiratory reserve volume with the glottis opened and including a breath-hold after inspiratory phase.<br>The sputum is cleared by cough or forced expiratory technique.<br>Before starting autogenic drainage, patients should be taught how to exhale with glottis opened, either with or without a mouthpiece. | Stage 1, "Loosening phase": the cross-sectional area of the medial and peripheral airways is reduced (mechanical stress) and the airflow velocity increases in these areas. This is achieved by breathing repeatedly using low lung volumes in the expiratory reserve volume. The slow expirations with an open glottis avoid dynamic compression during manoeuvres and maintain the airway patency [34, 35].<br>Stages 2 and 3, "Collect and move up phase": breathing progressively with high lung volumes towards the | Increases sputum removal during treatment and reduces the sputum expectoration for the remainder of the day [36].<br>May improve ventilation homogeneity [37].<br>Reduces cough impact [36]. | NE |

Continued

https://doi.org/10.1183/2312508X.10017017

Table 1. Continued

| Technique | Procedure [30, 31] | | Physiological basis to enhance sputum removal [19, 20] | Short-term clinical benefits | Long-term clinical benefits |
|---|---|---|---|---|---|
| | Positioning | Breathing | | | |
| **Autogenic drainage (cont.)** | | | inspiratory reserve volume including a breath-hold generates a greater trans-airway pressure gradient (mechanical stress) and also allows the air to move behind the obstructed lung units *via* collateral ventilation. | | |
| **ELTGOL** | Performed in the lateral decubitus position with the affected lung in the dependent position. Both lateral decubitus positions should be recommended when both lungs are affected (figure 2a and c). | Slow expirations from functional residual capacity to the end of expiratory reserve volume with the glottis opened. The sputum is cleared by cough or forced expiratory technique. Before starting ELTGOL, patients should be taught how to exhale with glottis opened, either with or without a mouthpiece. | Placing the patient in the side-lying position, the airways in the dependent lung are stretched (mechanical stress) and, therefore, the airflow velocity increases in the medial and peripheral areas [35]. Slow exhalations with an open glottis from the functional residual capacity to the end of the expiratory reserve volume maintain the airways slightly narrowed without dynamic compression [34, 35]. | Increases sputum removal during treatment and reduces sputum expectoration for the remainder of the day [36]. Reduces pulmonary hyperinflation [38]. Reduces cough impact [36]. | Reduces the frequency of exacerbations [39]. Improves HRQoL [39]. Reduces cough impact [39]. Increases sputum removal [39]. |
| **Non-oscillating PEP** | Generally performed in sitting position; however, an alternative position (supine or side-lying) may also be used. | Slow inspirations with slightly greater volume than tidal volume followed by an end breath-hold. After this breath-hold, exhalation against a resistance occurs through the PEP device, using a | Active expiration against a mild expiratory resistance (10–25 $cmH_2O$) increases the expiratory phase time and generates a greater trans-airway pressure gradient (mechanical stress) compared to normal breathing. This mechanism | NE | NE |

Continued

Table 1. Continued

| Technique | Procedure [30, 31] | | Physiological basis to enhance sputum removal [19, 20] | Short-term clinical benefits | Long-term clinical benefits |
|---|---|---|---|---|---|
| | Positioning | Breathing | | | |
| **Non-oscillating PEP (cont.)** | | mouthpiece or a mask. The sputum is cleared by cough or forced expiratory technique. A manometer may be used for education purposes to ensure a correct expiratory pressure (10–25 $cmH_2O$). | also uses collateral ventilation to enable the presence of air behind obstructed lung units.<br>It may be useful to combine a PEP device with autogenic drainage or the ELTGOL technique in patients with higher airway resistance and/or lower elastic recoil pressure, moving the equal pressure point towards the cartilaginous airways [34]. | | |
| **Oscillating PEP** | Generally performed in sitting position; however, an alternative position (supine or side-lying) may also be used. | Slow inspirations with slightly greater volume than tidal volume followed by an end breath-hold. After this breath-hold, exhalation against a resistance occurs through the oscillating PEP device, using a mouthpiece or a mask (figure 2b). The sputum is cleared by cough or forced expiratory technique. | High frequency airflow oscillation (mechanical stress) during the expiratory phase improves the biophysical properties of the mucus (viscoelasticity) and stimulates the ciliary beat.<br>Active expiration against a mild expiratory resistance (10–25 $cmH_2O$) increases the expiratory phase time and generates a greater trans-airway pressure gradient (mechanical stress) than normal breathing. This mechanism also uses collateral ventilation | Enhances sputum removal during treatment in stable conditions and during a pulmonary exacerbation [40].<br>Reduces sputum viscosity [41].<br>Decreases sputum adhesivity [42, 43].<br>Increases cough transportability [42, 43].<br>Decreases airway inflammation [43].<br>Reduces airway resistance [44].<br>Slight improvement in lung function after treatment. | Improves HRQoL [45].<br>Reduces cough impact [45].<br>Improves exercise capacity [45].<br>Increases sputum removal [45]. |

Continued

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Table 1. Continued

| Technique | Procedure [30, 31] | | Physiological basis to enhance sputum removal [19, 20] | Short-term clinical benefits | Long-term clinical benefits |
|---|---|---|---|---|---|
| | Positioning | Breathing | | | |
| **Oscillating PEP (cont.)** | | | to allow the presence of air behind obstructed lung units. It may be useful to combine an oscillating PEP device with autogenic drainage or the ELTGOL technique in patients with higher airway resistance and/or lower elastic recoil pressure, moving the equal pressure point towards the cartilaginous airways [34]. The vibration effect on the properties of the mucus may also improve the effectiveness of autogenic drainage or ELTGOL when both techniques are used in combination. | | |

NE: not established; ELTGOL: slow expiration with the glottis opened in the lateral posture; HRQoL: health-related quality of life.

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resources (including images and videos) for people with bronchiectasis [30, 31]. No one ACT has been shown to be more beneficial than any other [46, 47]. Therefore, the selection of the ACTs should be targeted according to the patient's characteristics (biophysical sputum properties, breathlessness, tolerability, fatigue, preference and even economic resources) and it may be beneficial to schedule check-ups to ensure that patients are using ACTs correctly [48] or to re-evaluate their use and effectiveness [20]. People with bronchiectasis should be taught by a respiratory physiotherapist trained and with expertise in all possible ACTs [26]. In clinical practice, it is a recommendation that ACTs are applied after hyperosmolar treatment and before inhaled antibiotics. Previous studies performed in people with CF suggest that hypertonic saline inhalation during ACTs have similar clinical benefits to hypertonic saline inhalation before ACTs, with the benefit of saving time [49, 50]. Further research is needed in people with bronchiectasis.

## Tips to improve adherence to airway clearance techniques

The low rate of adherence to ACTs is worrisome in people with bronchiectasis [51], especially when they report no change (or little change) in their daily respiratory symptoms and they fail to believe in the need for ACTs as a part of their chronic treatment [52]. Therefore, an ACT training programme should include education on the possible clinical benefits of using ACTs regularly. Strategies to improve ACTs adherence are shown in figure 2 [33, 48] and may include: 1) offering possibilities to reduce the ACT treatment burden; 2) teaching at least two independent ACTs, avoiding monotonous treatment and improving self-confidence; 3) regular reminders to encourage patients to follow their self-management strategy; 4) empowering patients with knowledge regarding the clinical benefits achieved after regular treatment using ACTs (amount and colour of sputum expectorated [53], lower number of hospitalisations and exacerbations, decreased need for extra medication, greater HRQoL); and 5) promoting the use of technology for providing bronchiectasis-specific information [30, 31] and granting patients the opportunity to interact online with other people with bronchiectasis for social support.

## What is the current evidence for airway clearance techniques?

The level of recommendation for ACTs is still weak and with a low quality of evidence in people with bronchiectasis [26]. The lack of long-term studies and methodological issues are the main underlying factors [54]. Table 1 summarises the main short- and long-term benefits of ACTs in this target population. Recently, Muñoz *et al.* [39] found that the ELTGOL technique (slow expiration with the glottis opened in the lateral posture) performed twice daily over 1 year increases sputum removal compared to upper-limb stretching exercises (primary end-point) in people with bronchiectasis. This study also found that regular performance of the ELTGOL technique improves HRQoL, reduces exacerbation frequency and improves cough impact. In addition, the length of time to the first exacerbation tended to be longer for the ELTGOL group, although this finding was not statistically significant. These results are consistent with the previous findings by Murray *et al.* [45], who found that the use of an oscillatory PEP device used twice a day improved the HRQoL and perceived cough severity, enhancing sputum removal and improving exercise capacity in people with bronchiectasis.

Overall, the short-term studies performed to date demonstrate that all the ACTs enhance sputum removal to a similar degree in cases of bronchiectasis [26]. A

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Figure 2. Some strategies to improve ACT adherence. a and b) A patient with bronchiectasis is performing two independent ACTs combined with an oscillating PEP device at home. He assesses the efficacy of the session using a volumetric container and a sputum colour chart validated in bronchiectasis [33]. c and d) Regular appointments at a hospital/institution are encouraged to assist/educate in the procedure of ACTs. Images used with permission from the patients.

randomised crossover trial has shown that both the ELTGOL and autogenic drainage techniques are able to concentrate the sputum removal during the treatment, reducing the need to expectorate during the remainder of the day and improving the cough impact [36]. Biophysical and surface mucus properties seem to be improved after the use of oscillating PEP devices in bronchiectasis. Tambascio and co-workers. [42, 43] found that a flutter device used over 4 weeks improves cough clearance, reduces sputum adhesivity (contact angle) and may reduce airway inflammation. Further research is needed to better understand the mechanism of action of ACTs on the biophysical level and regarding the properties of the airway mucus surface. Finally, the role of ACTs during an acute exacerbation in people with bronchiectasis is still unknown. ACTs seem to be safe and well tolerated during exacerbations in patients with bronchiectasis [40, 55]. Specifically, oscillating PEP devices slightly enhanced greater sputum expectoration than other techniques [40]. However, it should be a research priority in the upcoming years to explore whether ACTs have an impact on

the rate of symptom recovery, length of hospital stay and time to next hospital admission in people with an acute exacerbation of bronchiectasis.

### Future research on airway clearance techniques

We suggest that future research on the use of ACTs in bronchiectasis should focus on the following questions. When along the disease trajectory should the use of ACTs be investigated? How should ACTs be personalised based on patients' severity and preferences? What are the most effective strategies to improve adherence to ACTs and maintain their long-term benefits in bronchiectasis? Are all ACTs able to reduce the number of exacerbations in patients with bronchiectasis? Are ACTs during acute exacerbation effective in patients with bronchiectasis? Does the timing of hyperosmolar solutions have an impact on effectiveness of ACTs? Could exercise be used instead of ACTs to enhance sputum removal in selected patients with bronchiectasis?

## Pulmonary rehabilitation in bronchiectasis

### Why is pulmonary rehabilitation beneficial in bronchiectasis?

In people with bronchiectasis, peripheral muscle dysfunction has been reported, with evidence of quadriceps femoris weakness and biceps brachii weakness and a corresponding reduction in endurance compared to age-matched, healthy control subjects [56, 57]. Accompanying these changes are increased levels of fatigue and dyspnoea [58, 59]. Despite limited study, it is possible that alterations noted in COPD, including muscle atrophy, mitochondrial dysfunction, change in fibre type and poor oxidative capacity [59], also affect those with bronchiectasis. This reduction in peripheral muscle strength and endurance is a key contributor to the reduced functional exercise capacity noted in this population, with lower distances recorded in field walking tests compared to healthy control subjects [56, 58, 60]. A reduction in peak oxygen consumption during a maximal exercise test in bronchiectasis illustrates a decrease in maximal exercise capacity [56, 61]. In addition, those with bronchiectasis are less active, with lower proportions of physical activity undertaken each day [56, 60, 62].

Respiratory dysfunction, with inspiratory and expiratory muscle weakness, is also evident in bronchiectasis [63–65]. These changes, together with the combination of impaired mucociliary clearance, bronchial inflammation and irreversible dilatation, are linked to decreased expiratory flow, which further contributes to reduced exercise tolerance [66]. This combination of peripheral and respiratory muscle impairment, a predisposition to acute exacerbations, dyspnoea, fatigue and higher levels of anxiety and depression [67, 68] all contribute to poorer HRQoL [24, 69, 70].

Pulmonary rehabilitation is a comprehensive intervention for people with chronic lung diseases, with therapies including exercise training, education and behaviour change [71, 72]. National and international guidelines for the management of bronchiectasis accept the referral and support the role of pulmonary rehabilitation for those experiencing functional limitations [26–28, 73], regardless of an individual's disease severity according to lung function or HRCT findings. Contributing factors to these functional limitations are peripheral muscle weakness, symptoms of dyspnoea, fatigue and respiratory dysfunction. Most people typically undertake pulmonary rehabilitation when clinically stable. Although there is less evidence for the effects of pulmonary rehabilitation either during or

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# Discussion

This discussion focusses on providing a comprehensive evaluation as to which airway clearance therapeutic approach showed the greatest short-term benefits in adults with clinically stable bronchiectasis and an in-deep analysis of the potential use and clinical interpretation of wet sputum weight and computerised ARS as outcome measures to assess the short-term effects of airway clearance interventions.

This general discussion is a complement to the discussion sections of each manuscript included in this thesis[193, 194, 257, 258], with the aim of providing a broader and more integrated interpretation of the findings.

## Interpretation of the findings and clinical implications

Comparing short-term findings among studies in the airway clearance field is complex because the utilised methods are heterogenic (time frame of intervention and outcome measures)[46, 109] and it is unclear how to correctly interpret the changes observed from some outcome measures, such as sputum weight or computerised ARS[192].

Although wet sputum weight is considered to be a controversial outcome measure, it is widely used to assess airway clearance therapies. Traditionally, it is believed that a greater amount of sputum means a more successful airway clearance treatment[114]. However, it remains unclear which direction of change (increase or decrease) corresponds with clinical improvements in

bronchiectasis[192]. The findings observed in this thesis have shed light on this question: the most short-term efficacious airway clearance therapeutic approach in outpatients with bronchiectasis appears to be the one that increases the sputum amounts during the treatment period and reduces the need to expectorate (an approximate reduction of 6 grams or 17%) for the rest of the day[193, 194, 258]. This finding is consistent with patient´s perception because it has been reported that patients use airway clearance interventions prior to going out as a strategy to reduce the need to expectorate and, thus, avoid embarrassing situations related to sputum[100, 101]. Therefore, for a correct interpretation of the short-term effects of airway clearance interventions in clinical practice and future research, it is suggested that clinicians focus on the change of sputum expectoration after intervention and not only during the intervention period itself.

Nevertheless, it is difficult to ascertain whether people with bronchiectasis in stable state respond to an airway clearance intervention if only a single measure is performed using sputum weight as an outcome measure. Considering that the agreement intervals of 24-hour wet sputum weight were not sufficiently narrow, particularly for high levels of expectorated sputum weight (≥ 15 grams)[258], we recommend the use of repetitive measurements. Moreover, the ability to detect differences among groups using 24-hour sputum weight is limited due to the high variability we observed[258]; thus, intrasubject comparisons are more appropriated. In summary, our findings suggest using repetitive measurements to improve the accuracy of sputum weight results and only compare results on the same subject (intrasubject comparison), as performed in clinical practice or crossover designs.

Computerised ARS may be a potential alternative to sputum weight as an outcome measure to assess short-term effects of airway clearance interventions in clinical practice. Indeed, it showed

acceptable feasibility in people with bronchiectasis[257]. The use of validated algorithms allows one to objectively identify the type of ARS and their parameters; thus, the information obtained does not depend on the assessor's interpretation[245, 246]. The findings in this thesis suggest that expiratory coarse crackles and inspiratory monophonic wheezes are the ARS parameters that exhibited the greatest changes after a short-term ACT intervention in people with bronchiectasis[257]. Intriguingly, only the increase in expiratory coarse crackles correlated with the sputum quantity ratio obtained during sessions. In fact, an increase in the number of monophonic wheezes after airway clearance interventions may be associated with excessive airway dynamic collapse during sessions (cough manoeuvres). Therefore, physiotherapists should consider these parameters in clinical practice to immediately identify the benefits/adverse events of these interventions.

The international guidelines for the management of people with bronchiectasis agree that ACTs represent first-line treatment in people with daily sputum-related symptoms[46-48]. However, there is a lack of consensus on which ACTs should be taught to people with bronchiectasis. Because no one ACT has been shown to be more beneficial than any other[109, 114], they should (particularly manual techniques) be targeted according to patients´ characteristics and preferences rather than the physiotherapists' skills or countries' preferences.

Slow-expiratory manual techniques (i.e., autogenic drainage and ELTGOL) have been poorly explored in people with bronchiectasis (Table 3). Thus, we considered it appropriate to first conduct a short-term trial to compare and analyse in depth the benefits of these techniques. The degree of patients´ autonomy in performing the ACTs (total autonomy, assistance required or device-dependent) may play an important role on long-term adherence, and so we compared three slow-expiratory techniques with different levels of autonomy (autogenic drainage, ELTGOL

and TPEP) in adults with stable bronchiectasis. Patients´ perception is another substantial factor that influences long-term ACT adherence. Thus, the crossover design is the most appropriate for obtaining patients´ feedback because participants experience all treatment arms[153].

Our findings suggest that autogenic drainage (self-administered) and the ELTGOL technique (assistance required) have a similar sputum clearance effects during sessions and 24 hours post-intervention[193]. Indeed, both techniques are based on the same mechanism of action: an increase in the airflow velocity during the expiratory phase due to a reduction of the cross-sectional area of the medial airways (Table 2). Participants have to repeat slow and long exhalations with an open glottis, avoid dynamic compression during manoeuvres and maintain airway patency[110]. Therefore, a mechanical stress is generated in the airways and the peak expiratory flow increase in comparison with normal breathing, and this mechanism is an optimal strategy to enhance sputum clearance from a physiological point of view[103, 104].

Intriguingly, device-dependent TPEP was not as efficacious as the other techniques during sessions[193]. Participants had to repeat expirations against a expiratory resistance[110]. Thus, the mechanical stress created in the airways by TPEP is an oscillation during the expiratory phase in combination with a greater transairway pressure gradient that allows the expiratory phase to be prolonged while keeping the airways open[110]. Consequently, the reduction of the cross-sectional airway area may act faster than oscillating or resistance (pressure) during expiratory phase on enhancing sputum expectoration in clinically stable people with bronchiectasis. Although TPEP achieved lower amounts of sputum during sessions compared to autogenic drainage and ELTGOL, the three techniques were equally efficacious in reducing the need to expectorate for the rest of day after intervention[193]. Surprisingly, the total amount of sputum collected (sessions + 24-hour post-session) was not greater compared to baseline assessment

(24 hours without airway clearance intervention) for any technique. This finding supports the hypothesis that the short-term goal of a once daily ACT session may not be to increase the total daily amount of sputum expectorated, but to achieve the highest level of expectoration during a session and thus, reduce the need to expectorate the rest of the day in people with stable bronchiectasis and a moderate level of expectoration (≥ 15 grams).

However, this finding contrasts those recently reported by Muñoz et al.[120], who suggested that twice daily ELTGOL sessions increase the 24-hour sputum volume compared to baseline (without any ACT intervention). The target population (adults with stable bronchiectasis, with approximately 20 mL sputum volume over 24 hours at baseline) and the intervention performed (ETLGOL technique) were similar in both studies but not the daily frequency of ACTs. This finding should be interpreted with caution for several reasons. It was based on a single measurement, used a parametrical test for a non-normally distributed data, utilised sputum volume rather than sputum weight and the person who reported the sputum data (physiotherapist or patient) was not well described. While twice daily ACT sessions apparently promoted greater expectoration, it is still unknown whether this benefit in sputum quantity is also extrapolated to other long-term clinical outcomes. Nevertheless, treatment burden is the main barrier for ACT adherence in people with bronchiectasis. Thus, we must achieve an ideal balance to guarantee benefits at long term using these techniques in our target population.

Autogenic drainage and ELTGOL demonstrated similar effects in bronchiectasis, but autogenic drainage was selected by most of participants (49%), possibly due to the fact that this technique was performed independently and reinforces personal satisfaction[193]. Moreover, the side-laying position required by the ELTGOL technique, although well tolerated, may be uncomfortable for some people (i.e., older, overweight) in comparison to the back-laying/sitting

position using with autogenic drainage technique. These factors are important determinants for adherence and should be considered when designing future long-term trials.

The clinical characteristics that a patient with bronchiectasis must meet to be a good candidate for hyperosmolar agents remain unclear. However, the latest BTS guidelines reinforce the use of this treatment only after reviewing that ACTs treatment is optimal in patients with frequent exacerbations (≥ 3) or with a significant deterioration in symptoms[47]. The findings in this thesis support the combined use (once a day) of hyperosmolar agents and ACTs to achieve greater expectoration during the airway clearance sessions and greater reduction in the need to expectorate after intervention in bronchiectasis[194]. These benefits may improve the sputum-related symptoms and their impact on social life.

Notably, while there was a trend for lower expectoration after a combined session (using HA + HS + autogenic drainage) when compared to a single session of autogenic drainage in people with bronchiectasis, there was no significant difference in sputum weight for any assessment point (session, 24-hour post-intervention and the overall period; supplementary material in Study 3) despite the 24-hour baseline sputum was slightly higher for the single session group[258]. Although this finding should be properly tested, it indicates that before substantially increasing the treatment burden to the patient, research must verify whether patients obtain substantial additional benefits using combined therapy. Moreover, people with bronchiectasis reported that the therapeutic equipment needed to inhale mucoactive treatments may be an important barrier for long-term adherence[46].

In clinical practice, hyperosmolar agents are commonly administered twice daily based on efficacy and safety in cystic fibrosis. The lower clinical impact of these treatments observed in bronchiectasis[164, 170] suggests that the mucus clearance impairment may be different between diseases. Indeed, the mucin concentration seems to be lower in bronchiectasis[83] and cough clearance appears to be less impaired due to the lower sputum adhesivity found in this population[90], which may reflect a lower level of ASL dehydration. The efficacy of HS may depends on the level of ASL dehydration; thus, HS treatment would likely benefit people with greater ASL dehydration[82]. Therefore, the concentration of HS solution (6-7 %) and daily doses (twice a day) selected for cystic fibrosis may not be fully appropriate for people with bronchiectasis. Similar long-term benefits were previously reported for IS and HS solutions in bronchiectasis[164]. Our findings suggest that although hyperosmolar solutions are more efficacious during the inhalation period, the 24-hour sputum expectorated after a combined session was similar independent of the solution inhaled[194].

Lower rates of HS tolerability and the presence of minor adverse events during inhalation are more common in elderly people and those with a major lung function impairment[173]. These factors limit the long-term adherence to these treatments in people with bronchiectasis; however, those patients are likely to present higher ASL dehydration and, therefore, greater benefits are expected with the use of hyperosmolar solutions[82]. The HA + HS treatment may represent a potential solution because it presents a better safety profile (minor adverse events reported) compared to HS solution alone while increasing sputum weight during the inhalation period to a similar extent. This benefit may be the reason why it was chosen as the preferred inhaled solution by most of participants[194]. Safety, tolerability and preference are key points to maintain long-term airway clearance treatment, and these characteristics should be considered when a hyperosmolar solution is prescribed in people with bronchiectasis.

The reduction of the need to expectorate after airway clearance interventions (single or combined sessions) is reflected in the improvement in the LCQ score[193, 194]. This improvement was similar among the compared interventions. However, after only a 1-week wash-out period, the LCQ score decreased back to the baseline level in both studies. Therefore, regular use of airway clearance interventions is essential to maintain the benefits in reducing cough severity in bronchiectasis, as previously reported in long-term trials[120, 126, 164].

# Future lines of research

Although the findings of this thesis have provided important key points in the short-term airway clearance management of people with bronchiectasis in stable state, further research is needed to better understand the mucociliary clearance impairment in bronchiectasis and identify how to transfer the knowledge acquired from this thesis to long-term airway clearance management using appropriate outcome measures. I am currently exploring some of these strategic research lines in parallel to the present thesis, an endeavour that reflects a clear thematic continuity in my research.

***Research line 1: airway clearance management***

- ✓ What are the ASL characteristics in people with clinically stable bronchiectasis with mild/moderate disease severity? Is ASL dehydration related to any clinical characteristics or disease prognosis?
- ✓ In people with stable bronchiectasis, does the regular use of the autogenic drainage technique generate long-term clinical benefits? #
- ✓ In adults with stable bronchiectasis and a lower basal expectoration level (< 10 grams per 24 hours), does the regular use of ACT generate long-term clinical benefits? #
- ✓ In people with stable bronchiectasis, is one ACTs session per day enough to impact the clinical status in the long term? #
- ✓ What are the most efficacious concentrations and doses of hyperosmolar agents in people with stable bronchiectasis?
- ✓ In individuals with clinically stable bronchiectasis, does a simultaneous intervention of hyperosmolar solutions and ACTs provide similar benefits and safety profile compared to a traditional combined session (ACT after hyperosmolar inhalation) but save time?

- ✓ In adults with clinically stable bronchiectasis, does once daily use of combined sessions (hyperosmolar agents + ACT) generate similar benefits to twice daily hyperosmolar solution sessions?[¥]
- ✓ In people with clinically stable bronchiectasis, what are the most effective strategies to improve airway clearance treatment adherence and maintain long-term benefits?[#]
- ✓ What barriers and enablers are related to long-term airway clearance adherence in people with stable bronchiectasis?
- ✓ Could exercise be used instead of airway clearance treatments to enhance sputum removal in selected patients with bronchiectasis in stable condition?
- ✓ When during the disease trajectory should the use of airway clearance interventions be investigated?
- ✓ What clinical factors are related to a greater response to airway clearance management in adults with stable bronchiectasis?

***Research line 2: outcome measures to assess airway clearance management***

- ✓ In people with clinically stable bronchiectasis,
    - Are the computerised ARS a valid outcome measure?*
    - Are the computerised ARS reliable over time?*
    - Do the computerised ARS respond to long-term airway clearance management?*
    - What are the MIDs/MCIDs of computerised ARS after a treatment?*
- ✓ In people with stable bronchiectasis, when is the best time to use the computerised ARS to assess the immediate airway clearance effects?
- ✓ In people with clinically stable bronchiectasis, what tools are more appropriate to analyse the impact of airway clearance management in social life?
- ✓ In people with stable bronchiectasis, could the use of cough monitors validate the MID of 24-hour sputum weight after airway clearance interventions?

- ✓ In people with clinically stable bronchiectasis, what are the most appropriate outcome measures (cough frequency, sputum percentage solids) to assess short-term effects of airway clearance management?

[#] These questions are being investigated in an ongoing study entitled "*Long-term efficacy of a home-based airway clearance programme to improve cough severity in people with bronchiectasis: a randomised controlled trial (NTC02324855)"* that I am leading.

[¥] This question will be investigated in a new project entitled *"Is it enough once combined session (HS + ACTs) to generate long-term clinical benefits in people with bronchiectasis?"* that I will co-lead.

*These questions are being investigated in an ongoing study entitled "*Psychometric properties of computerised respiratory sounds in people with bronchiectasis"* that I am leading.

# Strengths and limitations

The thematic unity of this thesis is its main strength and there is also a clear line of continuity for the future research. However, there are other strengths that are worth mentioning. First, the use of repetitive measures to assess the short-term effects of airway clearance interventions, the MID estimation of 24-hour sputum weight after interventions and the feasibility of computerised ARS improve the accuracy of the findings[151]. Second, the randomised crossover design selected for both trials allowed intra-subject comparisons, which are more appropriate for the primary outcome chosen (wet sputum weight) due to its high variability as well as knowing the patients´ preferences[153]. Third, the prospective collection of sputum samples at distinct time points (baseline, sessions and 24-hour post intervention) facilitated the identification of the immediate and the long-lasting effects of interventions[154]. Although the sputum weight is considered to be a controversial outcome measure, it is appropriate for analysing short-term effects[192], as it was evaluated in the present thesis. Furthermore, the election to express the amount of sputum in weight rather than volume and the continuous reminder to participants to correctly collect sputum samples (avoiding confounding factors such as salivary contamination or inadvertently swallowed secretions) contributed to more accurate results. Finally, the physiotherapists in charge of the interventions in both trials had lengthy experience with airway clearance in patients with bronchiectasis, a factor that ensured the interventions were performed correctly.

The main limitations of this thesis should also be noted and require additional discussion. Blinding is unequivocally more difficult to guarantee in trials of non-pharmacological interventions, as in the present thesis. In fact, despite trying to blind participants in the

hyperosmolar solutions trial, they clearly identified which solution was the saltiest[194]. Participants could not be blinded because all treatment options were active treatment arms. Thus, the use of sham interventions was not possible. Moreover, it was decided not to use quinine sulphate as a blinding agent for hyperosmolar solutions because it is unknown whether it may influence the HA + HS properties[194]. However, participants were blinded to the study hypothesis, and most of them were naïve to the interventions. This design slightly reduced the risk of performance bias and detection bias. Additionally, data were coded before starting the statistical analysis to ensure blinding even if the analysis was performed by the same investigator who executed the intervention.

Another limitation may be that the power of both trials was only calculated based on the primary outcome (sputum weight during airway clearance sessions). Thus, the possibility of a type II error may make it difficult to clearly identify differences among treatments after 24-hour intervention. Moreover, the amount of sputum collected over the 24-hour period after interventions depended on patient compliance. Nevertheless, some strategies were implemented to reduce this impact: patients were continuously encouraged to collect the sputum samples correctly, and the 24-hour sputum containers were weighed immediately after the assessment period[193, 194, 258]. Indeed, there were no differences among the consecutive days using the same treatment during this period, a fact that reflects that the patients´ collaboration was similar over the study period.

The high drop-out rate observed in the hyperosmolar solutions study may be considered another limitation. However, the sample size was appropriate for the primary outcome, and the primary reason for withdrawal was related to the time burden perceived by participants and not for the interventions themselves[194].

Finally, although the acquisition of computerised ARS did not follow all the high-quality recommended standards (airflow was not monitored and each chest location was recorded only once) and could negatively affect the accurately of the findings[277, 278], the study was focused on analysing the feasibility of an outcome measure that could be easily applied in clinical practice[257].

# Conclusions

Overall, a deeper understanding of which short-term airway clearance therapeutic approach is most efficacious in people with stable bronchiectasis and how to interpret its clinical benefits are the main conclusions of this doctoral thesis. Specifically, the following conclusions can be drawn from this thesis:

1. Autogenic drainage and ELTGOL techniques induce greater sputum expectoration than TPEP during sessions in people with stable bronchiectasis. However, the significant reduction in the need to expectorate for the rest of the day compared to baseline (without intervention) is similar for the three techniques, data that reflect the comparable improvement in the impact of cough observed for all techniques. Participants prefer the autogenic drainage technique, possibly due to the fact that it is performed independently and without the need to use any device.

2. The HA + HS solution is as efficacious as HS solution and greater than IS in improving sputum expectoration during sessions but with a better safety profile than HS in people with clinically stable bronchiectasis. Thus, it is selected as the preferred solution. Moreover, the additional performance of autogenic drainage technique after hypertonic solutions achieve a greater sputum weight during sessions and a substantial reduction in expectoration for the rest of the day.

3. The wet sputum weight is an acceptable and reliable measure over a 24-hour period, but the level of agreement is slightly wide, particularly for higher expectoration levels. Therefore, multiple measurements are recommended to increase the accuracy of the

findings. Moreover, a reduction of at least 6.4 grams in the amount of sputum expectorated during the 24 hours following an airway clearance intervention—or a relative change of approximately -17% from baseline—is required to achieve a MID in patients with stable bronchiectasis.

4. Computerised ARS is a feasible outcome measure for assessing the short-term effects of ACTs in people with clinically stable bronchiectasis. Expiratory coarse crackles appear to be the most appropriate primary outcome for use in future studies, as their changes after an intervention present a moderate positive correlation with the sputum quantity ratio collected during sessions.

# Conclusiones

De forma global, las principales conclusiones de la presente tesis doctoral ayudan a comprender que enfoque terapéutico es más efectivo en personas con bronquiectasias clínicamente estables y cómo interpretar correctamente los beneficios clínicos obtenidos con estas intervenciones. De forma específica, se pueden extraer las siguientes conclusiones:

1. Las técnicas de drenaje autógeno y ELTGOL favorecen en mayor grado la expectoración durante las sesiones que la técnica TPEP en personas con bronquiectasias en periodo de estabilidad clínica. Sin embargo, las tres técnicas consiguen una reducción significativa en la necesidad de expectorar a lo largo del día en comparación con la medición basal (sin intervención) y una mejora semejante en el impacto de la tos. Los participantes prefieren la técnica drenaje autógeno, posiblemente por el hecho de poder realizarla de forma independiente y sin necesidad de utilizar ningún dispositivo.

2. La solución de suero hipertónico + ácido hialurónico (SH+AH) es tan efectiva como la solución de SH y superior a la solución de suero salino (SI) facilitando la expectoración; sin embargo, presenta un mejor perfil de seguridad que el SH en personas con bronquiectasias estables clínicamente y por esto, fue la técnica preferida por los participantes. Además, con la realización de técnicas de DS (drenaje autógeno) después de la inhalación de las soluciones hipertónicas se consigue obtener una mayor cantidad de expectoración durante las sesiones y una reducción más importante en la necesidad de expectorar durante el resto del día.

3. El peso húmedo de la cantidad de esputo expectorada durante 24 horas presenta una fiabilidad aceptable; sin embargo, su nivel de concordancia es ligeramente amplio, especialmente para niveles de expectoración elevados. Por esta razón, se recomienda la realización de múltiples medidas para incrementar la precisión de los resultados cuando se utiliza esta variable. Además, se estimó que una reducción de al menos 6.4 g. en la cantidad de esputo expectorada durante las 24 horas posteriores a una intervención de DS, o un cambio relativo de alrededor del -17% con respecto al nivel basal, es la cantidad necesaria para alcanzar una DIM en pacientes con bronquiectasias.

4. El uso de los ruidos respiratorios adventicios analizados de forma computacional es una herramienta viable/factible para analizar los efectos a corto plazo de las técnicas de DS en personas con bronquiectasias; así mismo, parece que el número de crujidos espiratorios graves es la variable primaria más apropiada para utilizar en futuros estudios, ya que los cambios observados tras una intervención se correlacionan de forma positiva y moderada con el ratio de expectoración obtenido durante las sesiones de DS en bronquiectasias.

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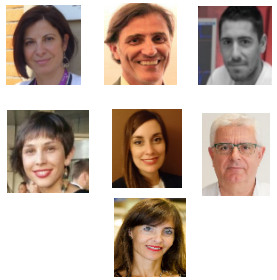
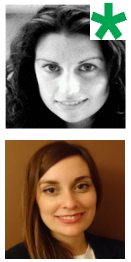
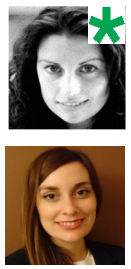
# Appendix

## 1. Appendix 1

The contribution of the PhD student to the present thesis is summarise in the Table 9

**Table A.** The contribution of researchers in the present thesis

| Studies | Research project draft | Funding | Ethical Committee and Registration | Fieldwork | Data analysis | Manuscript draft | Critical review |
|---|---|---|---|---|---|---|---|
| **Study 1** |  |  |  |  |  |  |  |
| **Study 2** |  |  |  |  |  |  |  |
| **Study 3** |  | |  |  |  |  |  |

**Study 4**

**Narrative review**

* PhD candidate – Beatriz Herrero Cortina. Anonymous picture in the Study 4, Gómez-Trullén E; Anonymous pictures in the narrative review, O´Neill B, Bradley J and Lee AL.

## 2. Appendix 2

### 2.1 List of manuscript co-authored by the PhD candidate

- San Miguel Pagola M, Reychler G, Cebrià I Iranzo MA, Gómez-Romero M, Díaz-Gutiérrez F, **Herrero-Cortina B**. Impact of hypertonic saline nebulization combined with oscillatory positive expiratory pressure on sputum expectoration and related symptoms in cystic fibrosis: a randomized crossover trial. Physiotherapy. 2019. Recently accepted. Impact Factor (JCR,2018) 2.534. Quartil (JCR):1

- Reychler G, San Miguel-Pagola M, Aubriot AS, **Herrero-Cortina B**, Lecocq V, Hesse G, Jamar F. Targeted Lung Deposition From Nebulisation is not improved in the lateral decubitus position in healthy volunteers. Respir Care. 2019 Sep. DOI: 10.4187/respcare.06978. Impact Factor (JCR, 2018) 1.736. Quartil (JCR): 4

- Alcaraz-Serrano V, Fernández-Barat L, Scioscia G, Llorens-Llacuna J, Gimeno-Santos E, **Herrero-Cortina B**, Vàzquez N, Puig de la Bellacasa J, Gabarrús A, Amaro-Rodriguez R, Menéndez R, Torres A. Mucoid Pseudomonas aeruginosa alters sputum viscoelasticity in patients with non-cystic fibrosis bronchiectasis. Respir Med. 2019 Jun 11;154:40-46. DOI: 10.1016/j.rmed.2019.06.012. Impact Factor (JCR, 2018) 3.237. Quartil (JCR): Q2

### 2.2 List of funded research projects during the PhD period

- **Name of the project:** Reference Equations for PIM, PEM and sniff test in Spanish Population. **Role:** Co-investigator. **Founded by:** SEPAR (Spanish Society of Pulmonology) in 2018.

- **Name of the project:** Is the mucus concentration a new biomarker for the management of people with bronchiectasis? **Role:** Co-investigator. **Founded by:** SADAR (Aragon Society of Pulmonology) in 2018.

- **Name of the project:** Comparative assessment of lung consolidation diagnosis using lung ultrasound and computerized respiratory sounds. **Role:** Co-investigator. **Founded by:** NEUMOCAN (Canarias Society of Pulmonology) in 2017.

- **Name of the project:** Assisted airway clearance techniques in patients with acute exacerbation of bronchiectasis requiring intravenous antibiotic treatment: a randomized controlled trial. **Role:** Principal Investigator. **Founded by:** CAI-Estancias de Investigación in 2016

### 2.3 List of funded research projects leading by the PhD candidate (not related to the present thesis) during the thesis period

- **Name of the project:** Long-term airway clearance therapy in non-cystic fibrosis bronchiectasis: a randomised controlled trial. **Role:** Principal Investigator. **Founded by:** SEPAR (Spanish Society of Pulmonology) in 2011.

- **Name of the project:** Resistive inspiratory manoeuvre as airway clearance technique in cystic fibrosis. **Role:** Principal Investigator. **Founded by:** Spanish Cystic Fibrosis Foundation in 2011.

### 2.4. List of communications accepted to national or international conferences

- **Herrero-Cortina B,** San Miguel-Pagola M, Francín-Gallego M, Sáez-Pérez JA, Polverino E. Long-term efficacy of a home-based airway clearance programme to improve cough severity in people with bronchiectasis. Oral presentation at: European Respiratory Congress; 2019 Sep 30; Madrid, España
- Sáez-Pérez J, **Herrero-Cortina B,** Alcaraz-Serrano V, Francín-Gallego M, San Miguel-Pagola M, Hernández-Secorún M, Gimeno-Santos E, Torres A, Arbillaga-Etxarri A. Heart rate recovery after 6 min walk test in people with bronchiectasis: a cross-sectional study. Poster presented at: European Respiratory Congress; 2019 Sep 30; Madrid, España
- **Herrero-Cortina B,** San Miguel-Pagola M, Francín-Gallego M, Sáez-Pérez JA, Polverino E. Efectividad a largo plazo de un programa domiciliario de drenaje de secreciones en

pacientes con bronquiectasias: ensayo clínico aleatorizado. Poster presented at: 52 Congreso Nacional SEPAR; 2019 Jun 14- 6; Santiago de Compostela, España.

- Alcaraz-Serrano V, Fernández-Barat L, Scioscia G, Llorens-Llacuna J, Gimeno-Santos E, **Herrero-Cortina B**, Amaro R, Torres A. Pseudomona aeruginosa mucoide, una bacteria que afecta a las propiedades visco-elásticas del esputo y a la clínica de los pacientes. Poster presented at: 52 Congreso Nacional SEPAR; 2019 Jun 14- 6; Santiago de Compostela, España.
- **Herrero-Cortina B**, Membrado O, San Miguel-Pagola M, Francín-Gallego, Saez-Perez JA, Polverino E. Effects of a home programme of airway clearance techniques on adventitious respiratory sounds in patients with bronchiectasis: a randomised controlled trial. Poster-discussion presented at: European Respiratory Congress; 2018 Sep 15-19; Paris, France.
- Walker P, **Herrero-Cortina B**, Spinou A, Dimakou K, Blasi F, Ringshausen F, De Souza A, Vendrell M, Goeminne P, Boersma W, Haworth C, Murris M, Hill AT, Loebinger R, Menendez R, Torres A, Welte T, Wilson R, Elborn JS, Aliberti S, Chalmers JD, Polverino E. Variability in access and referral to pulmonary rehabilitation in European bronchiectasis patients enrolled in the EMBARC registry. Oral presentation presented at: European Respiratory Congress; 2018 Sep 15-19; Paris, France.
- Tobajas-Sagaste I, Francín-Gallego M, San Miguel-Pagola M, Sáez-Perez JA, **Herrero-Cortina B**. Is there a relationship between adventitious respiratory sound and disease severity in patients with bronchiectasis? A cross-sectional study. Poster presented at: European Respiratory Congress; 2018 Sep 15-19; Paris, France.
- Alcaraz-Serrano V, Llorens-LLacuna J, Gimeno-Santos E, Gabarrus A, **Herrero-Cortina B**, Rosales-Mayor E, Fernández-Barat L, Polverino E, Amaro-Rodriguez R, Torres-Marti A. Does pseudomona aeruginosa infection alter sputum viscoelastic properties in bronchiectasis patients? Poster presented at: European Respiratory Congress; 2018 Sep 15-19; Paris, France.
- **Herrero-Cortina B**, Membrado O, San Miguel-Pagola M, Francín-Gallego, Saez-Perez JA, Polverino E. ¿Un programa domiciliario de drenaje de secreciones modifica las características de los ruidos adventicios en pacientes con bronquiectasias? Poster presented at: 51 Congreso Nacional SEPAR; 2018 May 31- 3; Mallorca, España.
- Sáez-Perez JA, San Miguel-Pagola M, Francín-Gallego M, **Herrero-Cortina B**. Identificar determinantes relacionados con la frecuencia cardiaca de recuperación en el primer minuto tras una prueba submáxima en pacientes con bronquiectasias: estudio transversal. Poster presented at: 51 Congreso Nacional SEPAR; 2018 May 31- 3; Mallorca, España.

- Francín-Gallego M, Tobajas-Sagaste I, San Miguel-Pagola M, Sáez-Perez JA, **Herrero-Cortina B**. Correlación entre los ruidos pulmonares adventicios y el pronóstico de la enfermedad en pacientes con bronquiectasias: estudio transversal. Poster presented at: 51 Congreso Nacional SEPAR; 2018 May 31- 3; Mallorca, España.
- Alcaraz-Serrano V, Llorens-LLacuna J, Gimeno-Santos E, Rosales-Mayor E, **Herrero-Cortina B**, Fernández-Barat L, Polverino E, Amaro R, Torres-Martí A. En pacientes con bronquiectasias, ¿la presencia de *Pseudomona aeruginosa* en el esputo altera sus propiedades reológicas? Poster presented at: 51 Congreso Nacional SEPAR; 2018 May 31-3; Mallorca, España.
- **Herrero-Cortina B**, Aliberti S, Blasi F, Vendrell M, Elborn S, Loebinger M, Menendez R, Torres A, Ringshausen F, Welte T, Wilson R, Chalmers J, Polverino E. Chest physiotherapy in European patients with bronchiectasis: Data from EMBARC registry. Poster presented at: European Respiratory Society Congress; 2017 Sep 9-13; Milan, Italia.
- **Herrero-Cortina B**, Aliberti S, Blasi F, Vendrell M, Elborn S, Loebinger M, Menendez R, Torres A, Ringshausen F, Welte T, Wilson R, Chalmers J, Polverino E. Chest physiotherapy in European patients with bronchiectasis: Data from EMBARC registry. Poster presented at: 2nd World Bronchiectasis Conference; 2017 Jul 6-8; Milan, Italia.
- **Herrero-Cortina B**, Membrado O, San Miguel-Pagola M, Francín-Gallego, Saez-Perez JA, Polverino E. Effects of a home programme of airway clearance techniques on adventitious respiratory sounds in patients with bronchiectasis: a pilot study. Poster presented at: 2nd World Bronchiectasis Conference; 2017 Jul 6-8; Milan, Italia.
- Alcaraz V, **Herrero-Cortina B**, Vilaró J, Rosales E, Torres A, Polverino E. Effects of hypertonic saline on sputum clearance in patients with bronchiectasis. Poster presentation at: American Thoracic Society Congress; 2017 May 19-24; Washington DC, EEUU.
- **Herrero-Cortina B**, San Miguel M, Fernández A, Rios A, Del Corral T, Sanchez M.D.M, Gomez C, Vilaró J. Resistive Inspiratory Manoeuvres as airway clearance technique in patients with cystic fibrosis: a randomised crossover trial. Poster presented at: European Respiratory Society Congress; 2016 Sep 3-7; London, United Kingdom.
- San Miguel M**,** **Herrero-Cortina B**, Cebria MA, Gómez M, Diaz F, Reychler G. Short term effects of hypertonic saline nebulization combined with oscillatory positive expiratory pressure in cystic fibrosis: randomised crossover trial. Poster presented at: European Respiratory Society Congress; 2016 Sep 3-7; London, United Kingdom.

- Alcaraz V, **Herrero-Cortina B**, Vilaró J, Rosales E, Torres A, Polverino E. Effects of hypertonic saline on sputum clearance in patients with bronchiectasis. Oral presentation at: European Respiratory Society Congress; 2016 Sep 3-7; London, United Kingdom.
- Alcaraz V, **Herrero-Cortina B**, Vilaró J, Rosales E, Torres A, Polverino E. Effects of hypertonic saline on sputum clearance in patients with bronchiectasis Poster presented at: 1st World Bronchiectasis Congress; 2016 Jul 7-9; Hannover, Germany.
- **Herrero-Cortina B**, San Miguel M, Fernández A, Rios A, Del Corral T, Sanchez M.D.M, Gomez C, Vilaró J. Maniobras inspiratorias contra resistencia como técnica de drenaje bronquial en fibrosis quistica: ensayo aleatorizado y cruzado. Modalidad Poster-discusión: 49 Congreso Nacional SEPAR; 2016 Jun 10; Granada, España.
- San Miguel M**,** **Herrero-Cortina B**, Cebria MA, Gómez M, Diaz F, Reychler G. Efectividad del suero hipertónico inhalado con un sistema de presión espiratoria positiva oscilante en pacientes con fibrosis quística: ensayo aleatorizado y cruzado Modalidad Poster-discusión: 49 Congreso Nacional SEPAR; 2016 Jun 10; Granada, España.
- Francín M, San Miguel M, **Herrero-Cortina B**. Influencia de la posición corporal en la computarización de los ruidos pulmonares en pacientes con Fibrosis Quística. Modalidad Poster-discusión: 49 Congreso Nacional SEPAR; 2016 Jun 10; Granada, España.
- Alcaraz V, **Herrero-Cortina B**, Vilaró J, Rosales E, Torres A, Polverino E. Efectos del suero hipertónico sobre el drenaje bronquial en pacientes con bronquiectasias: ensayo clínico aleatorizado, cruzado y doble ciego. Poster-discusión: 49 Congreso Nacional SEPAR; 2016 Jun 10; Granada, España.
- **Herrero-Cortina B**, San Miguel M, Fernández A, Rios A, Del Corral T, Sanchez M.D.M, Gomez C, Vilaró J. Resistive inspiratory manoeuvre as airway clearance technique in patients with cystic fibrosis: a randomised crossover trial. Presentación oral: 39th European Cystic Fibrosis Conference; 2016 Jun 9; Basel, Switzerland.
- **Herrero-Cortina B**, San Miguel M, Cebria MA, Gómez M, Diaz F, Reychler G. Short term effects of hypertonic saline nebulization combined with oscillatory positive expiratory pressure in cystic fibrosis: randomised crossover trial. Presentación oral: 39th European Cystic Fibrosis Conference; 2016 Jun 9; Basel, Switzerland.
- **Herrero-Cortina B**, Vilaró J, Marti D, Torres A, San Miguel M, Alcaraz V, Polverino E. Wet sputum as an objective outcome in a randomized crossover trial in nCF-BE: reliability and responsiveness. Poster exposition presented at: European Respiratory Society Congress; 2014 Sep 6-10; Munich, German.

- **Herrero-Cortina B**, Polverino E, San Miguel M, Alcaraz V, Marti D, Vilaró J, Torres A. Cuantificación del esputo como variable objetiva en un ensayo clínico cruzado en BQ-noFQ: fiabilidad y sensibilidad al cambio. Oral presentation at: XLVII Congreso nacional SEPAR; 2014 Jun 9; Bilbao, Spain.

## 2.5. Prizes awarded to PhD candidate during the thesis period

- **Young Scientific** Sponsorship from the European Respiratory Society for the European Respiratory Congress, Madrid, 2019.

- European Cystic Fibrosis Society **Travel Grant** for the 39th European Cystic Fibrosis Conference in Basel, Switzerland, 2016.

- **Best communication** in physiotherapy section. XLVII Congreso Nacional SEPAR (Spanish Society of Pulmonology); 2014 Jun; Bilbao, Spain.

## 3. Appendix 3

Letter from Dean R Hess, Managing Editor of Respiratory Care Journal

Decision on Manuscript ID RC-07175.R1 entitled *"Reliability and minimal important difference of sputum weight in people with bronchiectasis"*

Accepted

Date 15-10-2019

# Respiratory Care - Decision on Manuscript ID RC-07175.R1

1 message

**Respiratory Care** <onbehalfof@manuscriptcentral.com> 15 October 2019 at 11:22
Reply-To: dhess@aarc.org
To: Beafisiorespi@gmail.com
Cc: sara.moore@aarc.org, branson@aarc.org, gail.drescher@aarc.org

15-Oct-2019

Dear Miss Herrero-Cortina:

Your manuscript entitled "Reliability and minimal important difference of sputum weight in people with bronchiectasis" has been evaluated by 2 external consultants, whose comments are included at the foot of this letter. In accordance with their assessment and recommendations, I am pleased to accept it for publication in Respiratory Care in its current form.

Your paper will be scheduled for the next available issue of the Journal. We try to publish papers as soon as possible after they are accepted, but in some cases we experience a backlog of 6 to 9 months between acceptance and publication. You will receive page proof prior to final publication.

In the meantime, your paper will be uploaded as an Epub (paper in press) and will appear in PubMed. It usually takes several months for papers to move through production to Epub.

Please note that by this email notifying you that we have accepted your paper, copyright is automatically transferred to Daedalus Enterprises.

Thank you for submitting this work to the Journal.

Dean R Hess PhD RRT FAARC
Managing Editor
RESPIRATORY CARE

Reviewer(s)' Comments to Author:
Reviewer: 1

Comments to the Author
You have appropriately addressed my concerns and those of the other reviewers. There are a number of grammatical and punctuation errors that need to be corrected.

Reviewer: 2

Comments to the Author
Thank you to the authors for addressing all the comments made. The manuscript now reads better and is more transparent to readers. I look forward to seeing this work published.


**Universidad** Zaragoza