Universitat Autònoma de Barcelona

Colonización bronquial por Pseudomonas aeruginosa en pacientes con bronquiectasias: Nuevos métodos diagnósticos

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Tesis para optar al grado de Doctor presentada por

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Barcelona 2017

Foto de la portada: Versión coloreada de una imagen de *Pseudomonas aeruginosa* obtenida mediante microscopía electrónica de barrido.

Cortesía de Janice Haney Carr (Centers for Disease Control and Prevention's Public Health Image Library).

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AUTORIZACIÓN DEL DIRECTOR Y TUTOR DE TESIS

El Dr. Oriol Sibila Vidal, médico adjunto del Servicio de Neumología del Hospital de la Santa Creu i Sant Pau, y el Dr. Vicente Plaza Moral, Profesor Titular del Departamento de Medicina de la Universitat Autònoma de Barcelona y Jefe del Servicio de Neumología del Hospital de la Santa Creu i Sant Pau,

CERTIFICAN:

Que la Tesis Doctoral que lleva por título **"Colonización bronquial por *Pseudomonas aeruginosa* en pacientes con bronquiectasias: Nuevos métodos diagnósticos"**, presentada por Guillermo Rafael Suárez Cuartín para optar al grado de Doctor en Medicina, ha sido realizada bajo su dirección. Una vez finalizada alcanza los requisitos formales y científicos para proceder a su presentación en público y a su evaluación por el tribunal correspondiente.

Y para que quede constancia a los efectos oportunos, firman la presente:

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Barcelona, 2017

A mi familia

AGRADECIMIENTOS

Agradezco al Dr. Oriol Sibila por dirigir esta tesis y por darme la oportunidad de iniciar este camino bajo su guía. Poder trabajar con alguien a quien admiro y que más allá de ser un gran neumólogo es también un gran amigo, es una suerte increíble. Al Dr. Vicente Plaza por su ayuda como tutor de esta tesis y Jefe del Servicio de Neumología donde he iniciado mi carrera profesional.

Al Dr. Alfons Torrego, un excelente tutor durante mi formación y luego un mejor compañero y amigo. Al Dr. Diego Castillo por estar siempre presente y por sus palabras y consejos oportunos.

To Dr. James D. Chalmers, thank you for your kind help with this project. You are a role model as a scientist and as a human being.

A Sílvia Barril, Ana Rodrigo y Ana Muñoz por compartir tantas risas y penurias como residentes y como becarios, pero sobre todo por ser parte de mi familia extendida. A Helena, Patricia, Laura y todos los “R” mayores y menores con los que he compartido grandes momentos tanto dentro como fuera del hospital.

A todos los adjuntos del Servicio de Neumología del Hospital Sant Pau que han participado en mi formación y que han sido un ejemplo en mis primeros pasos como especialista. También al equipo de enfermería, auxiliar y administrativo del servicio, por ser grandes profesionales con quienes es un placer trabajar. Me enorgullece decir que llevaré conmigo un poco de todos ustedes.

Al equipo del Laboratorio de Inmunología y del Servicio de Microbiología por su paciencia y excelente labor. A todos los pacientes que han participado en este proyecto. Sin ustedes, esta tesis no habría sido posible.

A mi madre por su fuerza, entrega y amor infinito. A mi familia, mi soporte y el motor que me mueve. A Guillermo y Alejandro Cuartín, por enseñarme la inquietud por la ciencia y por ser los faros que me guían. A César, Andrea, Víctor, Elena, Santi, Mercedes, Adri y Abdel, mi familia lejos de casa.

A Enrique, mi apoyo incondicional y mi hogar donde quiera que vaya.

A todos, muchas gracias.

PRESENTACIÓN

La presente Tesis Doctoral es el resultado de una serie de estudios que pertenecen a una misma línea de investigación, dirigida a conocer nuevos métodos diagnósticos de colonización bacteriana de la vía aérea, específicamente por *Pseudomonas aeruginosa*, en pacientes con bronquiectasias. Los resultados de estos estudios aportan información relevante y novedosa en esta área de trabajo, y podrían potencialmente ayudar en el manejo clínico de los pacientes con bronquiectasias.

El proyecto de tesis se llevó a cabo bajo la supervisión del Director de tesis, el Dr. Oriol Sibila Vidal, y bajo la tutoría del Dr. Vicente Plaza Moral, Jefe del Servicio de Neumología del Hospital de la Santa Creu i Sant Pau de Barcelona.

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LISTADO DE ABREVIATURAS

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ABPA	Aspergilosis broncopulmonar alérgica
AUROC	*Area under the Receiver Operating Characteristic Curve*
BSI	*Bronchiectasis Severity Index*
COV	Compuestos orgánicos volátiles
DE	Desviación estándar
ELISA	*Enzyme-Linked Immunosorbent Assay*
E-nose	Nariz electrónica
EPOC	Enfermedad pulmonar obstructiva crónica
FEV_1	Volumen espiratorio forzado en un Segundo
FQ	Fibrosis quística
FVC	Capacidad vital forzada
IC	Intervalo de confianza
ICS	*Inhaled corticosteroids*
IgG	Inmunoglobulina G
IMC	Índice de masa corporal
LABA	*Long acting beta-agonists*
LAMA	*Long acting muscarinic antagonists*
MPO	Mieloperoxidasa
MPP	Microorganismos potencialmente patógenos
MUC	Mucina
PA	*Pseudomonas aeruginosa*

PCA *Principal Component Analysis*

ROC *Receiver Operating Characteristics*

SGRQ *Saint George's Respiratory Questionnaire*

TACAR Tomografía axial computarizada de alta resolución

RESUMEN

RESUMEN

Las bronquiectasias representan una condición caracterizada por la presencia de dilataciones bronquiales irreversibles, que predisponen al desarrollo de infecciones respiratorias recurrentes y a la colonización bronquial por microorganismos potencialmente patógenos (MPP), entre los cuales la *Pseudomonas aeruginosa* (PA) es uno de los más frecuentes. La colonización bronquial por PA se asocia a un peor pronóstico de la enfermedad, a un rápido deterioro funcional pulmonar y a una mayor tasa de mortalidad. Por este motivo, es de gran importancia identificar a los pacientes con colonización por PA. Sin embargo, el diagnóstico se basa en cultivos microbiológicos que requieren tiempo y personal entrenado, entre otras limitaciones.

En este proyecto de Tesis, se llevaron a cabo dos estudios dirigidos a la valoración de nuevos métodos diagnósticos para la identificación de la colonización bronquial por PA como los niveles séricos de inmunoglobulina G (IgG) específica anti-PA y el análisis de los patrones de compuestos orgánicos volátiles (COV) en el aire exhalado mediante la nariz electrónica (e-nose).

Para ello fueron incluidos pacientes con bronquiectasias clínicamente estables, que fueron catalogados como no colonizados o colonizados por PA u otros MPP según la microbiología del esputo. Se evaluó la eficacia diagnóstica de ambas pruebas para identificar a los pacientes con bronquiectasias colonizados por PA. En ambos estudios se observó una elevada precisión de validación cruzada, así como una alta sensibilidad, especificidad y valores predictivos positivos y negativos para identificar a los pacientes con bronquiectasias y colonización bronquial por PA.

En conclusión, la medición de los títulos de anticuerpos IgG específicos anti-PA y la identificación de patrones de COV en el aire exhalado con la e-nose son dos métodos que muestran resultados prometedores para detectar la colonización bronquial bacteriana, específicamente por la PA, en pacientes con bronquiectasias.

SUMMARY

Bronchiectasis is a respiratory condition characterized by the presence of irreversible bronchial dilatations, which predispose to the development of recurrent respiratory infections and bronchial colonization by potentially pathogenic microorganisms (PPM), among which *Pseudomonas aeruginosa* (PA) is one of the most frequent. Bronchial colonization by PA is associated with a worse prognosis of the disease, a rapid pulmonary functional decline and a higher mortality rate. Therefore, it is highly important to identify patients with airway PA colonization. However, its diagnosis is based on microbiological cultures that require time and trained personnel, among other limitations.

In this Thesis project, two studies were conducted to evaluate new diagnostic methods for identifying PA bronchial colonization such as serum levels of anti-PA specific immunoglobulin G (IgG) and the analysis of volatile organic compound (VOC) in the exhaled air with the electronic nose (e-nose).

Patients with clinically stable bronchiectasis were included, and they were classified as non-colonized or colonized by PA or other PPM according to sputum microbiology. The diagnostic efficacy of both tests to identify bronchiectasis patients with PA airway colonization was evaluated. In both studies, a high cross-validation accuracy was observed, as well as a high sensitivity, specificity and positive and negative predictive values to identify patients with bronchiectasis and PA airway colonization.

In conclusion, the measurement of specific anti-PA IgG antibody titres and the identification of VOC patterns in exhaled air with the e-nose are two methods that show promising results to detect bacterial bronchial colonization, specifically by PA, in bronchiectasis patients.

1.INTRODUCCIÓN

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1.1 Generalidades de las bronquiectasias

Las bronquiectasias no asociadas a fibrosis quística (FQ) -de ahora en adelante llamadas bronquiectasias- se definen como dilataciones irreversibles de los bronquios con alteración del epitelio pulmonar que impiden el correcto aclaramiento mucociliar (1, 2).

Clínicamente se manifiesta en gran parte de los pacientes con tos persistente, broncorrea, disnea, infecciones respiratorias recurrentes, y en algunos casos, hemoptisis (1). Dado que se trata de una condición muy heterogénea, las formas de presentación clínica varían ampliamente según la extensión de la afectación de las bronquiectasias y de acuerdo a las características y comorbilidades de cada paciente. Sin embargo, son las infecciones respiratorias recurrentes o exacerbaciones, una de las causas más importantes de morbilidad y mortalidad en estos pacientes (3, 4).

Estudios recientes han observado un aumento importante en la prevalencia de las bronquiectasias en Europa y Estados Unidos, con especial predominio en mujeres y en pacientes de edad avanzada (5–7). Existe además una elevada carga económica anual que aumenta en relación con la gravedad de la enfermedad y con el número de exacerbaciones infecciosas (8).

Las bronquiectasias pueden ser consecuencia de múltiples y muy diferentes etiologías. Entre las causas más frecuentes se encuentran las infecciones respiratorias graves (neumonías, abscesos pulmonares, tuberculosis pulmonar), las inmunodeficiencias (innatas o adquiridas), las enfermedades del

tejido conectivo, la enfermedad pulmonar obstructiva crónica (EPOC) entre otras; aunque en una proporción importante de los casos, no se puede identificar una etiología definida (9–13).

El proceso fisiopatológico que condiciona al desarrollo de bronquiectasias es poco comprendido debido a su alta complejidad y a la ausencia de modelos experimentales adecuados dirigidos a su estudio (4). La hipótesis más aceptada actualmente es la del círculo vicioso de Cole (14). Este modelo propone que tras una determinada lesión de la vía aérea, se produce una reacción inflamatoria que a su vez genera un daño tisular en un sujeto predispuesto. La alteración que se produce en el epitelio bronquial dificulta el correcto aclaramiento mucociliar, lo cual perpetúa el proceso de infección e inflamación de la vía aérea, continuando así el ciclo **(Figura 1)**.

Actualmente se conoce que existe un proceso inflamatorio de predominio neutrofílico en la vía aérea de los pacientes con bronquiectasias, incluso en ausencia de infección bacteriana (15, 16). No obstante, la regulación de este proceso inflamatorio es disfuncional, y esto unido a la alteración del aclaramiento mucociliar, favorece la colonización bacteriana bronquial (17).

1.2 Colonización bacteriana bronquial

El proceso inflamatorio persistente en el epitelio bronquial de los pacientes con bronquiectasias y el daño tisular que ocurre como consecuencia, favorecen la presencia de microorganismos potencialmente patógenos (MPP) en sus vías aéreas (1). La colonización por MPP se define como la persistencia de una o más poblaciones bacterianas en el tracto respiratorio, sin que esto implique

necesariamente un cambio en la situación clínica basal del paciente (1, 2); y puede ser intermitente o crónica, según el aislamiento persistente o no de una población bacteriana en los cultivos de muestras de la vía aérea (1, 2, 18).

La colonización por MPP es una de las principales causas de morbilidad de los pacientes con bronquiectasias. Diversos estudios han demostrado que los pacientes con colonización de la vía aérea por MPP tienen una peor evolución clínica y formas más graves de la enfermedad en comparación con sujetos no colonizados (3, 19). Además de la repercusión que tiene en la calidad de vida y en la evolución de la enfermedad, los pacientes con colonización por MPP requieren un mayor uso de recursos sanitarios, en gran medida por ingresos hospitalarios frecuentes, lo cual aumenta el impacto económico que tienen las bronquiectasias (8).

Entre los MPP más frecuentemente asociados a la colonización de la vía aérea de los pacientes con bronquiectasias se encuentran la *Pseudomonas aeruginosa* (PA) y el *Haemophilus influenzae*, si bien se ha evidenciado un número considerable de pacientes colonizados por otros MPP como *Escherichia coli*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, entre otros (3, 20–27). La **Tabla 1** resume la distribución de los MPP colonizadores más frecuentes en las bronquiectasias.

1.3 *Pseudomonas aeruginosa*

Es un bacilo Gram-negativo extracelular, aerobio facultativo y ubicuo que afecta de forma oportunista al ser humano, causando principalmente infecciones agudas y crónicas a pacientes con compromiso de la respuesta

inmune y/o con condiciones predisponentes como, entre otros casos, enfermedades pulmonares crónicas (28–30). La *Pseudomonas aeruginosa* se puede encontrar bajo dos formas de crecimiento, de tipo planctónico o formando biopelículas (31, 32). Ambas formas tienen mecanismos de patogenicidad y virulencia potentes, aunque diferentes. La forma planctónica posee diversos apéndices que median su virulencia (lipopolisacáridos, exotoxinas y exoenzimas, percepción de quórum o autoinducción) y motilidad (pili, flagelos). Por otra parte, las bacterias que forman biopelículas disminuyen su actividad metabólica, haciéndose más resistentes a la acción de diversas familias de antibióticos y a la propia actividad del sistema inmune (28, 32, 33). La **Figura 2** muestra la presencia de bacilos Gram-negativos compatibles con PA en una muestra de esputo.

En sujetos sanos es poco frecuente observar infecciones por PA. Sin embargo, individuos con compromiso del sistema inmune o alteraciones en los mecanismos de aclaramiento suelen ser los más afectados por esta bacteria (32). En patologías respiratorias crónicas como la Enfermedad Pulmonar Obstructiva Crónica (EPOC) se ha observado una incidencia de infecciones por PA en hasta un 15% de los pacientes (34, 35). Por otra parte, en la FQ la prevalencia aumenta con la edad, alcanzando más de un 80% en pacientes adultos (36–38).

En pacientes con bronquiectasias, la PA es uno de los PPM más frecuentemente relacionados con colonización bronquial (20, 25). La prevalencia de colonización por PA en estos pacientes varía ampliamente según la población estudiada, pudiendo alcanzar entre un 8 y un 32% (3, 18,

20–25, 39–42), aunque un meta-análisis realizado por Finch et al. estimó una tasa de colonización bronquial por PA de 21,4% (43).

Las características propias de la PA y su capacidad de colonizar las vías aéreas de los pacientes con bronquiectasias, determinan el impacto que tiene esta bacteria sobre la patogenia de la enfermedad. La colonización por PA puede además jugar un papel importante en la desregulación del proceso inflamatorio ya presente en la vía aérea de individuos con bronquiectasias. Se ha demostrado que los pacientes colonizados por PA presentan niveles más elevados de marcadores de actividad neutrofílica, citoquinas, quimioquinas y mucinas en la vía aérea, en comparación con sujetos no colonizados o colonizados por otros MPP (44, 45). Clínicamente, la colonización bronquial por PA en bronquiectasias se asocia a un mayor número de exacerbaciones infecciosas, peor calidad de vida y a un peor pronóstico de la enfermedad (19, 43, 46). Además, un estudio realizado por Martínez-García et al. demostró que la colonización por PA es un factor asociado de forma independiente a un rápido deterioro de los valores de función pulmonar, en concreto del volumen espiratorio forzado en 1 segundo (FEV_1) (46). Estudios más recientes han desarrollado herramientas para estratificar la gravedad de las bronquiectasias, como lo son el índice de gravedad de bronquiectasias o *Bronchiectasis Severity Index* (BSI) y la escala FACED (3, 41). En los estudios de validación de ambas escalas uno de los marcadores de gravedad fue la presencia de colonización por PA, puesto que los pacientes colonizados tenían una mayor tasa de mortalidad (3, 41). En la **Tabla 2** se resumen los estudios más relevantes que han analizado el impacto de la colonización bronquial por PA en pacientes con bronquiectasias.

Figura 1. Hipótesis del círculo vicioso de Cole.

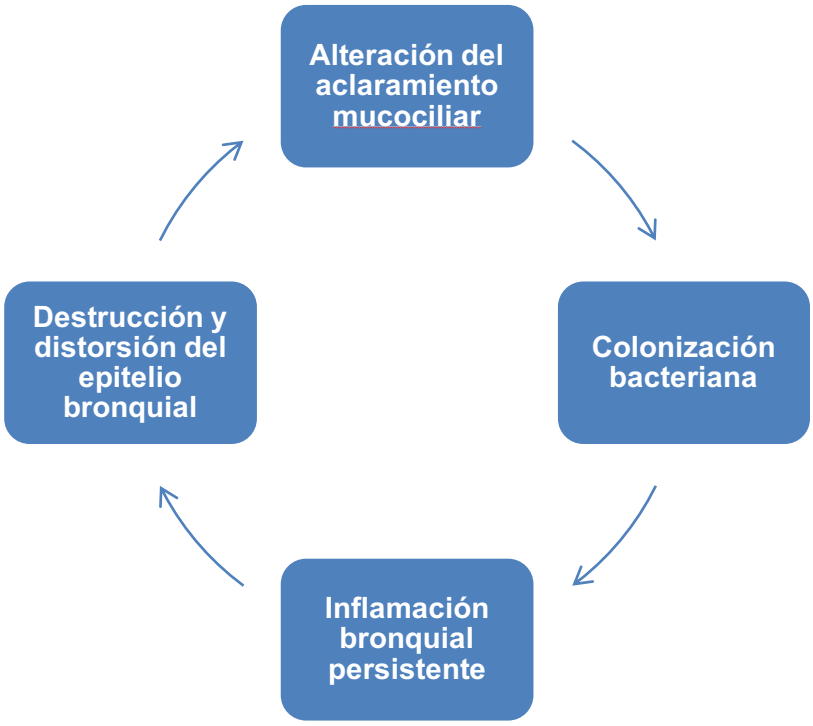
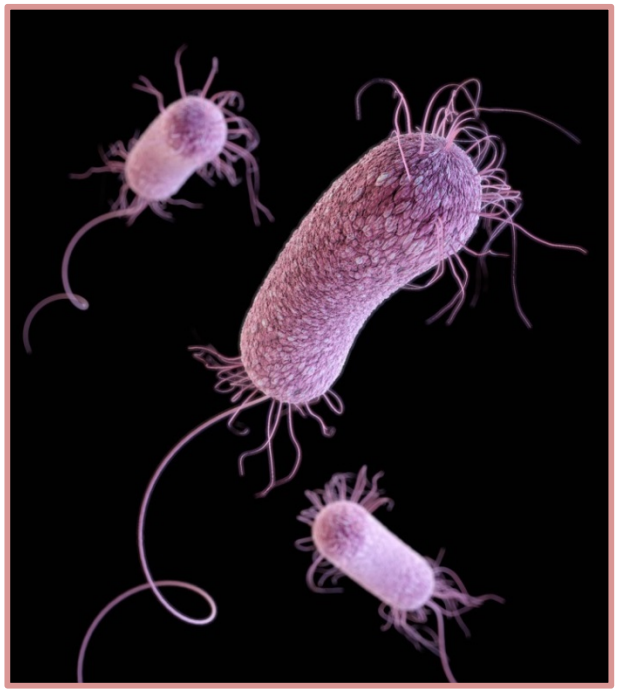


Figura 2. Ilustración tridimensional de *Pseudomonas aeruginosa* basada en imágenes de microscopía electrónica de barrido.



Cortesía de James Archer (Centers for Disease Control and Prevention's Public Health Image Library).

Tabla 1. Distribución de la colonización bronquial por microorganismos potencialmente patógenos (MPP) más frecuentes en los estudios de pacientes con bronquiectasias en fase de estabilidad clínica.

Primer autor / Año de publicación	País	N	Objetivo del estudio	Distribución de MPP
King et al., 2007 (20)	Australia	89	Determinar el perfil microbiológico de pacientes con bronquiectasias	Al inicio del estudio: *Haemophilus influenzae* 47% *Pseudomonas aeruginosa* 12% *Moraxella catarrhalis* 8% *Streptococcus pneumoniae* 7% *Staphylococcus aureus* 3% Otros MPP 2% Sin MPP 21% Seguimiento a 5 años: *Haemophilus influenzae* 40% *Pseudomonas aeruginosa* 18% *Moraxella catarrhalis* 7% *Streptococcus pneumoniae* 4% *Staphylococcus aureus* 3% Otros MPP 2% Sin MPP 26%
Martínez-García et al., 2007 (46)	España	76	Identificar factores modificables y no modificables asociados al empeoramiento de la función pulmonar	*Pseudomonas aeruginosa* 20% *Haemophilus influenzae* 18% No colonizados 42%
Rogers et al., 2013 (23)	Australia	41	Evaluar la relación entre la microbiota y la gravedad de las bronquiectasias	Según cultivo de esputo: *Haemophilus influenzae* 29% *Pseudomonas aeruginosa* 27% Flora habitual 44%
Chalmers et al., 2014 (3)	Reino Unido	608	Desarrollar un índice de gravedad para pacientes con bronquiectasias	*Haemophilus influenzae* 29% *Pseudomonas aeruginosa* 12% *Moraxella catarrhalis* 10% *Staphylococcus aureus* 8% *Streptococcus pneumoniae* 6% Otros MPP Gram-negativos 7% No colonizados 28%
Martínez-García et al., 2014 (41)	España	819	Desarrollar un índice de gravedad para pacientes con bronquiectasias	*Pseudomonas aeruginosa* 32% *Haemophilus influenzae* 15% *Staphylococcus aureus* 5%
Rogers et al., 2014 (42)	Australia	107	Estratificar a los pacientes con	Según cultivo de esputo:

			bronquiectasias según el taxón predominante en su microbiota	*Haemophilus influenzae* 22% *Pseudomonas aeruginosa* 30% Otros MPP 4% Sin MPP 44% <u>Según la dominancia en la secuenciación del ARNr 16s</u>: *Haemophilus influenzae* 35% *Pseudomonas aeruginosa* 27% *Veillonella spp.* 10% *Prevotella spp.* 9% Otras especies 19%
Aliberti et al., 2016 (19)	Italia Irlanda Bélgica Grecia Reino Unido E.E.U.U.	1145	Identificar grupos de pacientes con características clínicas y biológicas similares, y evaluar su evolución	*Pseudomonas aeruginosa* 16% Otros MPP 24%
McDonnell et al., 2016 (27)	Italia Reino Unido Irlanda Bélgica	986	Investigar las comorbilidades de pacientes con bronquiectasias y establecer su valor pronóstico	*Pseudomonas aeruginosa* 12% Otros MPP 23%

N: número de pacientes incluidos; MPP: Microorganismos potencialmente patógenos.

Tabla 2. Estudios dirigidos a estudiar el impacto de la colonización por *Pseudomonas aeruginosa* en pacientes con bronquiectasias.

Primer autor / Año de publicación	País	N	Objetivo del estudio	Porcentaje de colonización por PA	Resultados
Evans et al., 1996 (39)	Reino Unido	49	Estudiar el efecto de la infección por PA en la función pulmonar	Colonización crónica: 24% No colonizados: 76%	El deterioro de la función pulmonar es más rápido en pacientes con colonización crónica por PA
Davies et al., 2006 (25)	Reino Unido	163	Evaluar el impacto de la PA sobre el FEV_1	Colonización crónica: 9% Aislamiento intermitente: 50% No colonizados: 41%	La colonización por PA ocurre en pacientes con peor función pulmonar, pero no se asocia con un deterioro más rápido
Martínez-García et al., 2007 (46)	España	76	Identificar factores modificables y no modificables asociados al empeoramiento de la función pulmonar	Colonización crónica: 20%	La colonización crónica por PA, las exacerbaciones graves y la inflamación sistémica están asociados con una progresión rápida de la enfermedad
Loebinger et al., 2009 (21)	Reino Unido	91	Investigar factores que influyen en la mortalidad en bronquiectasias	Colonización crónica: 22% No colonizados: 78%	La mortalidad se asocia con un peor función pulmonar, peor intercambio de gases y con infección crónica por PA
Hester et al., 2012 (22)	Reino Unido	117	Estudiar la fatiga en pacientes con bronquiectasias	Colonización crónica: 10% Aislamiento puntual: 50% Sin aislamiento: 40%	La infección por PA se asocia a peor función pulmonar y a mayor disnea, pero no se asocia con la fatiga
Rogers et al., 2013 (23)	Australia	41	Evaluar la relación entre la microbiota y la gravedad de las bronquiectasias	Según cultivo de esputo: 27%	Las características de la microbiota de las vías aéreas inferiores se relacionan con marcadores clínicos de gravedad de la enfermedad
Chalmers et al., 2014 (3)	Reino Unido	608	Desarrollar un índice de gravedad para pacientes con bronquiectasias	Colonización crónica: 12%	La mortalidad es significativamente mayor en pacientes con colonización crónica por PA
Goeminne et al., 2014	Bélgica	245	Evaluar la mortalidad en	Colonización	Aumento del riesgo de mortalidad al

(24)			pacientes con diagnóstico reciente de bronquiectasias	crónica: 8% Aislamiento intermitente: 4%	aumentar la edad y el número de lóbulos afectados, así como la presencia de EPOC concomitante
Martínez-García et al., 2014 (41)	España	819	Desarrollar un índice de gravedad para pacientes con bronquiectasias	Colonización crónica: 32%	La colonización crónica por PA es un factor predictor de mortalidad a 5 años
Rogers et al., 2014 (42)	Australia	107	Estratificar a los pacientes con bronquiectasias según el taxón predominante en su microbiota	Según cultivo de esputo: 30% Según dominancia en la secuenciación del ARNr 16s: 27%	La predominancia de PA en la microbiota es un predictor de mayor frecuencia de exacerbaciones
McDonnell et al., 2015 (18)	Reino Unido	155	Valorar el perfil microbiológico longitudinalmente y relacionarlo con marcadores clínicos de la enfermedad	Colonización crónica: 30%	La tasa de exacerbaciones que requieres hospitalización es mayor en pacientes con colonización por PA. El aislamiento de PA multirresistente es infrecuente.
Aliberti et al., 2016 (19)	Italia Irlanda Bélgica Grecia Reino Unido E.E.U.U.	1145	Identificar grupos de pacientes con características clínicas y biológicas similares, y evaluar su evolución	Colonización crónica: 16%	Los pacientes con colonización crónica por PA tienen una frecuencia más elevada de exacerbaciones e ingresos hospitalarios, y una mayor tasa de mortalidad a 3 años.
McDonnell et al., 2016 (27)	Italia Reino Unido Irlanda Bélgica	986	Investigar las comorbilidades de pacientes con bronquiectasias y establecer su valor pronóstico	Colonización crónica: 12%	El BACI complementa al BSI en la valoración y predicción de mortalidad y de los marcadores de la enfermedad

N: número de pacientes incluidos; PA: *Pseudomonas aeruginosa*; FEV_1: Volumen espiratorio forzado en un segundo; MPP: Microorganismos potencialmente patógenos; EPOC: Enfermedad pulmonar obstructiva crónica; BACI: Bronchiectasis Aetiology Comorbidity Index; BSI: Bronchiectasis Severity Index.

1.4 Diagnóstico de colonización bronquial por PA

El análisis del esputo espontáneo es uno de los procedimientos más ampliamente utilizados para valorar el estado microbiológico de los pacientes con bronquiectasias y se compone principalmente de la tinción de Gram y el cultivo. Este análisis microbiológico, a pesar de ser de indudable utilidad en la práctica clínica, presenta una serie de limitaciones relevantes. Algunos pacientes tienen dificultad para producir una muestra de esputo de buena calidad y existe una alta tasa de contaminación de la muestra con flora de la vía aérea superior, lo cual puede disminuir la fiabilidad de la prueba (47, 48). Por otra parte, su procesamiento y análisis toman tiempo y requieren de un personal capacitado (47). La **Tabla 3** describe las principales limitaciones del estudio microbiológico actual del esputo.

Tabla 3. Principales limitaciones del estudio microbiológico del esputo mediante tinción de Gram y cultivo
• Dificultad para obtener muestras de esputo de buena calidad • Probabilidad de contaminación de la muestra con flora de la vía aérea superior • Necesidad de personal capacitado para el procesamiento y análisis de las muestras • Requiere tiempo (>24h) para obtener resultados • Algunos microorganismos necesitan tinciones y medios de cultivo específicos para poder ser identificados • Sensibilidad diagnóstica limitada

Además, otro de los problemas añadidos es la utilización de diferentes definiciones de colonización bronquial por PA. En bronquiectasias, la definición más comúnmente usada es la presencia de dos cultivos de esputo positivos para PA con al menos 3 meses de separación a lo largo de un año (43). Sin embargo, en FQ la definición de colonización crónica es más estricta puesto

que requiere la realización de cultivos de esputo cada 3 meses, de los cuales al menos el 50% deben positivos para PA (49, 50).

Actualmente no se realiza un seguimiento microbiológico con cultivos de esputo rutinarios como parte de la práctica clínica habitual en pacientes con bronquiectasias a nivel europeo. En auditorías recientes se ha podido observar que solo el 62% y 27% de pacientes con bronquiectasias en el Reino Unido e Italia respectivamente, tenían al menos un cultivo de esputo anual (51, 52). Esto dificulta la identificación de pacientes con colonización bronquial en la práctica clínica.

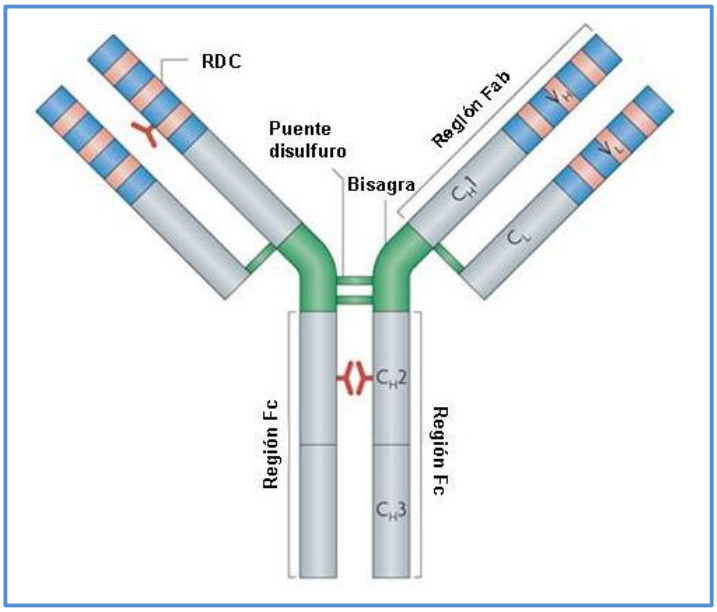
En los últimos años se han propuesto nuevos métodos diagnósticos para detectar la presencia de colonización bacteriana bronquial en pacientes con bronquiectasias, dado el impacto que tiene sobre la evolución y manejo de la enfermedad. En esta línea, se ha planteado que la determinación de los niveles de anticuerpos específicos contra la PA y el estudio de compuestos presentes en el aire exhalado mediante nuevos dispositivos como la nariz electrónica, pueden jugar un papel importante en el diagnóstico de colonización bronquial por PA en sujetos con bronquiectasias.

1.5 Inmunoglobulina G

La inmunoglobulina G (IgG) es el anticuerpo más abundante en el suero de los humanos y se compone de 4 subclases (IgG1–IgG4) (53). La estructura de una molécula de IgG se representa en la **Figura 3**. La IgG forma parte de la inmunidad adaptativa y la memoria inmunológica frente a procesos infecciosos y su función es principalmente pro-inflamatoria, aunque estudios más recientes

indican que puede ejercer una actividad anti-inflamatoria en determinadas situaciones (53–56). Estos anticuerpos activan de forma eficiente diferentes vías efectoras a mediante su fragmento constante o fragmento Fc. Los componentes de la inmunidad innata son capaces de reconocer los fragmentos Fc, lo cual activa sus funciones efectoras (55). Asimismo, la IgG puede activar también la vía del complemento, generando más mediadores pro-inflamatorios (54). Dadas las características de este anticuerpo, actualmente se utiliza tanto para el diagnóstico de enfermedades, como también para su tratamiento (57). En vista de la estrecha relación que guarda con la activación de la cascada inflamatoria frente a procesos infecciosos y de su largo tiempo de vida media, se ha propuesto a la IgG como un probable marcador eficiente de infección crónica en diversas entidades clínicas como la FQ (58).

Figura 3. Estructura de una molécula de inmunoglobulina G.



CDR: Región determinante de complementariedad. Adaptado de Beck A, et al. Strategies and challenges for the next generation of therapeutic antibodies (57)

Diversos estudios han demostrado la utilidad de la IgG específica anti-PA para diagnosticar el estado de infección crónica por esta bacteria en pacientes con FQ (58, 59). La ventaja que podría aportar esta prueba es su capacidad de realizar el diagnóstico del estado microbiológico en un momento puntual, sin necesidad de un seguimiento longitudinal con múltiples muestras de esputo en pacientes con FQ. Para esta patología ya existen varios test serológicos dirigidos a determinar los niveles de IgG anti-PA en suero mediante las técnicas de *Enzyme-Linked Immunosorbent Assay* (ELISA) y Contrainmunoelectroforesis (CIE), las cuales han probado tener una alta sensibilidad y especificidad para detectar infección bronquial crónica por PA (59–67). Los métodos más ampliamente usados se basan en la medición de la respuesta inmune contra una mezcla de 64 antígenos de los 17 serotipos más frecuentes de PA, o contra factores de virulencia específicos producidos por la PA como la Exotoxina A, la elastasa y la proteasa alcalina (58).

Actualmente existe información muy limitada en cuanto a la utilidad de esta prueba en pacientes con bronquiectasias. En el año 2001, Caballero et al. observaron una correlación significativa entre los niveles de IgG anti-PA en suero mediante Western-blot y la frecuencia de aislamiento de PA en esputo de 56 pacientes con bronquiectasias, sugiriendo que podría diferenciarse entre diferentes estados de infección por esta bacteria (68). Estos resultados favorables, sin embargo, son difíciles de extrapolar debido al tamaño limitado de la muestra. En la **Tabla 4** se resumen los principales estudios dirigidos a valorar la utilidad de la IgG anti-PA para diagnosticar infección bronquial crónica por PA.

Tabla 4. Principales estudios dirigidos a determinar la utilidad diagnóstica de la inmunoglobulina G anti-*Pseudomonas aeruginosa* en la colonización bronquial crónica de pacientes con bronquiectasias asociadas y no asociadas a fibrosis quística.

Primer autor / Año de publicación	Condición	N	Objetivo del estudio	Método usado	Resultados
Høiby et al., 1977 (60)	FQ	133	Detección de anticuerpos anti-PA en suero	CIE	El incremento de niveles de precipitinas se asoció a un peor pronóstico de la enfermedad
Pedersen et al., 1987 (61)	FQ	243	Desarrollar un test ELISA para detectar anticuerpos anti-PA, comparando con CIE	ELISA CIE	La sensibilidad y especificidad de ELISA son similares a CIE, pero el procedimiento es más simple
Brett et al., 1988 (62)	FQ	33	Monitorizar los niveles de anticuerpos durante 3 años tras el primer aislamiento de PA	ELISA	-Los niveles de anticuerpos permanecían alterados hasta 24 meses tras el aislamiento de PA. -Estos disminuyeron después del tratamiento antibiótico
Caballero et al., 2001 (68)	BQ no FQ	56	Estudiar la utilidad de los anticuerpos anti-PA para identificar diferentes estados de infección por PA	Western blot	El estado de infección por PA se puede determinar mediante el número y tipo de bandas de proteínas de membrana
Kappler et al., 2006 (63)	FQ	183	Validación de un test ELISA comercializado para detectar anticuerpos anti-PA e infección por PA	ELISA	- La determinación regular de anticuerpos puede ser útil en pacientes con aislamiento negativo o intermitente de PA. - Un aumento en los títulos de anticuerpos puede ser indicativo de infección por PA
Tramper-Stranders et al., 2006 (64)	FQ	220	Evaluación de un test serológico para detección precoz de PA y de colonización crónica	ELISA	Los test serológicos específicos para PA son sensibles para la detección de colonización crónica, pero no para su detección precoz
Pressler et al., 2006 (65)	FQ	89	Analizar los factores de riesgo para desarrollar infección crónica por PA	ELISA	Los niveles elevados de IgG anti-PA pueden tener un significado pronóstico para el desarrollo de colonización por PA

Ratjen et al., 2007 (66)	FQ	375	Investigar la respuesta de anticuerpos ante antígenos de PA en una cohorte de pacientes	ELISA	-El estudio de anticuerpos anti-PA tiene una alta sensibilidad y especificidad para detectar la presencia de PA. -Puede ser de utilidad para monitorizar la respuesta al tratamiento
Pressler et al., 2009 (59)	FQ	791	Evaluar la eficacia de tres métodos serológicos diferentes para identificar diferentes estados de infección por PA	ELISA CIE	- Los diferentes métodos serológicos lograron diferenciar el estado de infección por PA con una alta sensibilidad. - Los títulos elevados de anticuerpos son un factor de riesgo para desarrollar infección crónica por PA
Anstead et al., 2013 (67)	FQ	303	Estudiar si la serología positiva para PA puede predecir el fallo de tratamiento, el tiempo hasta la siguiente exacerbación y el riesgo de aislamiento recurrente de PA después de la erradicación	ELISA	La positividad serológica no está relacionada con el fallo de tratamiento inicial ni con el tiempo entre exacerbaciones, pero si con el riesgo de aislamiento recurrente de PA tras la erradicación

N: número de pacientes incluidos; FQ: Fibrosis quística; PA: *Pseudomonas aeruginosa*; CIE: Contrainmunoelectroforesis; ELISA: *Enzyme-Linked ImmunoSorbent Assay*; S: Sensibilidad; E: Especificidad; VPP: Valor predictivo positivo; VPN: Valor predictivo negativo; BQ: Bronquiectasias.

En FQ el uso de la IgG anti-PA sigue siendo controvertido, en parte debido a que en estos pacientes, la infección crónica por PA se considera prácticamente inevitable a medida que el sujeto se hace mayor (37). Sin embargo, en sujetos con bronquiectasias la prevalencia de la infección bronquial crónica por PA es más baja, lo cual dificulta su diagnóstico y

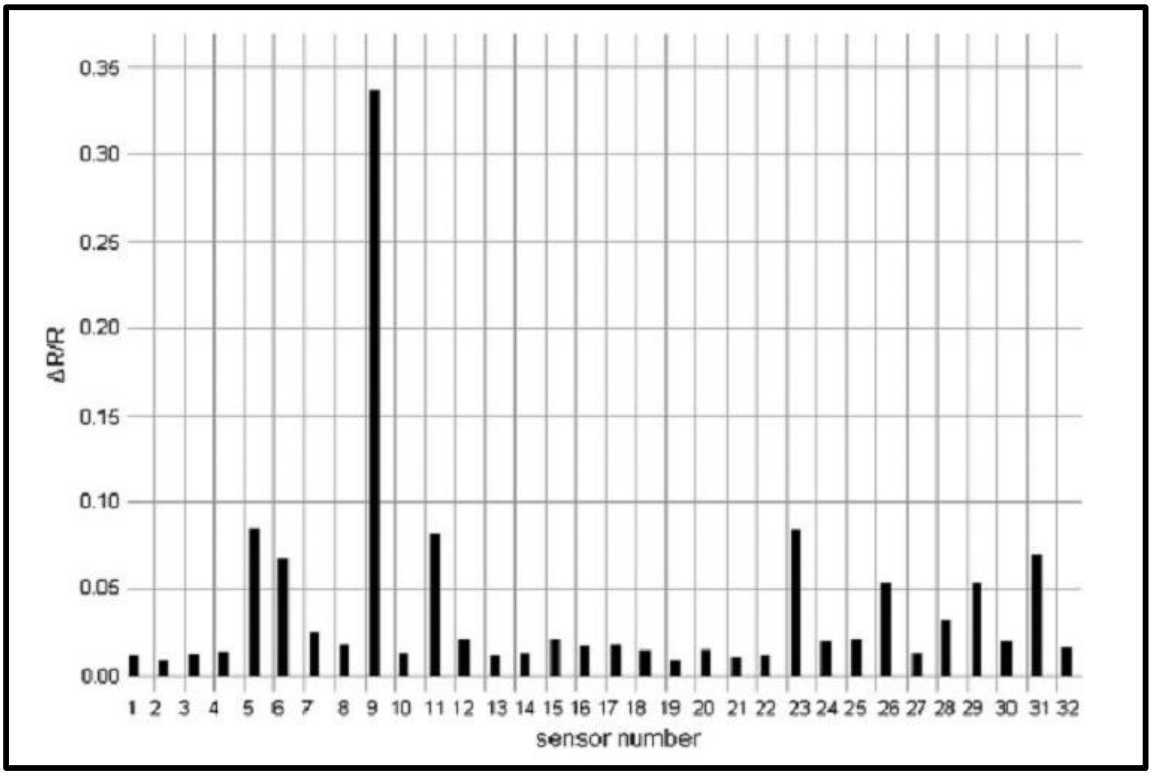
aumentaría la aplicabilidad diagnóstica de la determinación de IgG anti-PA en estos pacientes.

1.6 Nariz electrónica

La nariz electrónica (e-nose) es un dispositivo capaz de identificar compuestos orgánicos volátiles (COV) en el aire exhalado de forma no invasiva (69, 70). Existen varios modelos comerciales, siendo el Cyranose 320® (Smith Detections, Pasadena, E.E.U.U.) uno de los dispositivos más ampliamente usados **(Figura 4)**. Este equipo contiene una matriz de 32 nano-sensores que producen una serie de cambios en su resistencia eléctrica al ser expuestos a los COV. Dichos cambios de resistencia generan un patrón de COV o "imprenta olfatoria" característico para cada compuesto **(Figura 5)**.

Figura 4. Nariz electrónica Cyranose 320®

Figura 5. Patrón de Compuestos Orgánicos Volátiles o "imprenta olfatoria" obtenido mediante el dispositivo Cyranose 320®.



Se observan los 32 puntos obtenidos, uno por cada sensor del dispositivo. Adaptado de Dragonieri S, et al. An electronic nose in the discrimination of patients with asthma and controls (71).

Los COV presentes en el aire exhalado son producidos en distintas reacciones metabólicas o inflamatorias de la vía aérea (72, 73). De esta forma, la e-nose ha demostrado que posee un alto valor diagnóstico para identificar diversas patologías respiratorias como la EPOC (74), asma (71, 75, 76) y FQ (77), además de procesos neoplásicos pulmonares y pleurales (78–80) y neumonías (81). La **Tabla 5** resume los principales estudios que usan la e-nose en el diagnóstico de enfermedades pulmonares.

Por otra parte, diferentes estudios han objetivado que la e-nose puede detectar también COV procedentes del metabolismo bacteriano, identificando así la presencia de infecciones respiratorias. En EPOC, la e-nose ha diferenciado exitosamente las imprentas olfatorias de pacientes con colonización bronquial bacteriana de los no colonizados, tanto en fase de estabilidad clínica como durante exacerbaciones (82, 83). Algunos estudios han sugerido que ciertas bacterias como la PA puede producir COV específicos (84–86). Basándose en esto, se ha podido utilizar la e-nose para identificar la presencia de infección bronquial por PA tanto en pacientes con FQ como en frotis de bacterias obtenidas de cultivos *in vitro* (87, 88). En el estudio realizado por Lai et al. se logró identificar y diferenciar también de forma eficiente los COV generados por otras bacterias patógenas de la vía aérea como *Staphylococcus aureus*, *Streptococcus pneumoniae* y *Haemophilus influenzae*, además de la *Pseudomonas aeruginosa* (88). Los principales estudios que relacionan la e-nose con el diagnóstico de infecciones respiratorias se pueden observar en la **Tabla 5**.

Actualmente la información disponible sobre la utilidad de la e-nose en pacientes con bronquiectasias, y más aún, sobre su potencial papel en la identificación de la colonización bronquial por PA en estos pacientes, es muy limitada.

Tabla 5. Estudios más relevantes dirigidos a identificar la utilidad diagnóstica de la nariz electrónica en las patologías respiratorias.

Primer autor / Año de publicación	Patología	N	Objetivo del estudio	Resultados
Lai et al., 2002 (88)	Infecciones de la vía aérea superior (in vitro)	-	Evaluar la habilidad de la e-nose para detectar la presencia de bacterias en escobillones de cultivos bacterianos	La e-nose puede detectar la presencia de bacterias y distinguir entre diferentes especies (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, y *Pseudomonas aeruginosa*)
Hockstein et al., 2004 (81)	Neumonía	25	Valorar la utilidad de la e-nose para detectar neumonía asociada al ventilador en pacientes conectados a ventilación mecánica invasiva	Existe una buena correlación entre los patrones detectados por la e-nose y los hallazgos radiológicos en pacientes con neumonía asociada al ventilador
Machado et al., 2005 (78)	Neoplasia pulmonar	76	Valorar la capacidad diagnóstica de la e-nose para diferenciar pacientes con carcinoma broncogénico de controles sanos	La e-nose tiene una sensibilidad y especificidad elevadas para detectar patrones COV de pacientes con carcinoma broncogénico
Dragonieri et al., 2007 (71)	Asma	30	Discriminar pacientes sujetos sanos de asmáticos con diferentes grados de gravedad	-La e-nose tiene un elevado valor diagnóstico para discriminar entre asmáticos graves y controles sanos. -Menor eficacia para discriminar asmáticos leves y moderados
Fens et al., 2009 (74)	Asma EPOC	90	Evaluar la eficacia diagnóstica de la e-nose en pacientes asmáticos, con EPOC y controles sanos fumadores y no fumadores	-Los patrones de aire exhalado de pacientes asmáticos, EPOC y controles sanos se distinguen con una precisión elevada. -Los perfiles de aire exhalado de pacientes EPOC y fumadores asintomáticos se solapan parcialmente
Dragonieri et al., 2009 (80)	Neoplasia pulmonar EPOC	30	Discriminar los patrones de COV de pacientes con carcinoma pulmonar (no-célula pequeña) de los de pacientes con EPOC y controles sanos	La e-nose puede discriminar con precisión entre el patrón de COV de pacientes con cáncer de pulmón de los de pacientes con EPOC y controles sanos
Dragonieri et al., 2012 (79)	Mesotelioma maligno	39	Evaluar la capacidad diagnóstica de la e-nose en pacientes con mesotelioma pleural maligno	Los patrones de COV de pacientes con mesotelioma maligno se diferencian de los de controles sanos con y sin exposición al asbesto, con una alta precisión

van der Schee et al., 2013 (75)	Asma	25	-Comparar la capacidad de la e-nose con la FeNO y el recuento de eosinófilos en esputo para diferenciar asmáticos de controles. -Valorar su capacidad de predicción de respuesta a la terapia corticoidea.	-La e-nose tiene una elevada eficacia para diferenciar asmáticos de controles sanos, similar a la de la FeNO y el recuento de eosinófilos en esputo. -La capacidad de predicción de respuesta a la terapia corticoidea es mayor con la e-nose que con la FeNO y el recuento de eosinófilos en esputo.
Paff et al., 2013 (77)	FQ DCP	73	Determinar los perfiles de COV de pacientes con FQ y DCP	-Los perfiles de COV de los pacientes con FQ y DCP son significativamente diferentes de los de controles sanos. -Los pacientes con y sin exacerbaciones tienen patrones de COV significativamente diferentes.
Sibila et al., 2014 (82)	EPOC	50	Evaluar la precisión de la e-nose para diferenciar pacientes EPOC estables con y sin colonización bronquial bacteriana	Los patrones de COV de pacientes EPOC en fase de estabilidad clínica con y sin colonización bronquial son significativamente diferentes entre sí, y en comparación con aquellos de controles sanos
Joensen et al., 2014 (87)	FQ PCD	106	-Investigar las diferencias entre los patrones de COV de pacientes con y sin infección bronquial crónica. -Evaluar la influencia de bacterias Gram negativas en los perfiles de COV de pacientes con infección bronquial crónica	El uso de la e-nose permite diferenciar pacientes con y sin infección bronquial crónica por *Pseudomonas aeruginosa*
Shafiek et al., 2016 (83)	EPOC Neumonía	173	Estudiar la utilidad de la e-nose para discriminar entre patrones de COV de pacientes con EPOC estables y agudizados	Mediante la e-nose es posible diferenciar patrones de COV de pacientes EPOC estables y agudizados, especialmente si esta agudización se debe a una infección respiratoria bacteriana

N: número de pacientes incluidos; e-nose: nariz electrónica; EPOC: Enfermedad Pulmonar Obstructiva Crónica; FeNO: Fracción exhalada de óxido nítrico; COV: Compuestos orgánicos volátiles; FQ: Fibrosis quística; DCP: Discinesia ciliar primaria.

2.JUSTIFICACIÓN

2. JUSTIFICACIÓN DE LA TESIS

Las bronquiectasias representan una condición heterogénea que se manifiesta de formas muy diversas. Un porcentaje elevado de los pacientes con esta patología presentan una colonización de su vía aérea por microorganismos potencialmente patógenos (MPP), entre los cuales la *Pseudomonas aeruginosa* (PA) es uno de los más frecuentes. La colonización bronquial crónica por PA está claramente asociada a un aumento en el número de exacerbaciones infecciosas, con la consiguiente limitación de la calidad de vida y aumento en la tasa de mortalidad a mediano plazo. Debido a esto es de gran importancia realizar el diagnóstico de colonización bronquial por esta bacteria.

Actualmente el método de elección para diagnosticar el estado microbiológico de la vía aérea de los pacientes con bronquiectasias es el cultivo de esputo. Esta técnica posee una serie de limitaciones, por lo que realizar el diagnóstico microbiológico dependerá principalmente de la capacidad que tenga el paciente para producir una muestra de esputo adecuada y evitar la contaminación con flora de la vía aérea superior, de la facilidad que tenga el centro sanitario para contar con un personal capacitado para realizar el procesamiento y análisis de la muestra de esputo, así como de las características propias de la bacteria. Esto condiciona, por lo tanto, un tiempo de espera considerable entre la toma de la muestra y la obtención del resultado de la tinción y cultivo del mismo. Por estos motivos es necesario desarrollar métodos diagnósticos alternativos para identificar eficazmente la presencia de colonización bronquial por MPP.

La determinación de los niveles de anticuerpos IgG específicos anti-PA ha sido ampliamente estudiada en la fibrosis quística, demostrando un elevado valor diagnóstico para detectar la colonización bronquial crónica por PA en esta patología. De la misma manera, la nariz electrónica (e-nose) también ha demostrado un alto valor diagnóstico para discriminar entre sujetos sanos y pacientes con patologías respiratorias como el asma, EPOC, fibrosis quística y neoplasias, así como también en la identificación de determinadas especies bacterianas asociadas a infecciones respiratorias. Sin embargo, su utilidad en pacientes con bronquiectasias aún no ha sido estudiada. Tomando en cuenta esto, se ha postulado que la determinación de los niveles de IgG anti-PA y el estudio del aire exhalado mediante la e-nose pueden ser de gran valor para el diagnóstico de la colonización bronquial por PA en individuos con bronquiectasias.

3. HIPÓTESIS Y OBJETIVOS

3.1 HIPÓTESIS Y OBJETIVOS DEL ESTUDIO 1

"Anti-*Pseudomonas aeruginosa* IgG antibodies and chronic airway infection in bronchiectasis" (*Respir Med*. 2017 Jul;128:1-6).

Hipótesis:

La determinación de los niveles de IgG específica anti-PA en suero de pacientes con bronquiectasias puede ser de utilidad para identificar los casos con colonización bronquial crónica por PA.

Objetivo principal:

- Determinar la utilidad de la prueba de IgG anti-PA para identificar la presencia de colonización bronquial crónica por PA en pacientes con bronquiectasias.

Objetivos secundarios:

- Investigar la relación entre los niveles séricos de IgG anti-PA y la gravedad de las bronquiectasias, determinada por las puntuaciones del BSI y FACED, así como con el número de exacerbaciones.
- Valorar la relación entre los títulos de IgG anti-PA en suero y los niveles de marcadores inflamatorios neutrofílicos de la vía aérea como la elastasa y la mieloperoxidasa, en pacientes con bronquiectasias.
- Analizar la asociación entre los niveles en sangre de IgG anti-PA y la respuesta al tratamiento de erradicación anti-PA en pacientes con bronquiectasias, durante un período de seguimiento de 12 meses.

3.2 HIPÓTESIS Y OBJETIVOS DEL ESTUDIO 2

"Identification of Pseudomonas aeruginosa airway colonization by an electronic nose in Bronchiectasis" (Enviado a *Respirology*. Manuscrito en revision. ID: RES-17-236)

Hipótesis:

La e-nose puede discriminar con precisión entre los patrones de COV del aire exhalado de pacientes con bronquiectasias sin colonización bronquial crónica y sujetos colonizados por MPP, especialmente en aquellos con colonización por PA.

Objetivo principal:

- Explorar si una e-nose puede discriminar de forma fiable la colonización bacteriana de las vías respiratorias en pacientes clínicamente estables con bronquiectasias.

Objetivos secundarios:

- Estudiar la utilidad de la e-nose para identificar pacientes con bronquiectasias colonizados por MPP y no colonizados.
- Analizar la precisión diagnóstica de la e-nose para diferenciar los pacientes con bronquiectasias colonizados por PA de los no colonizados y con colonización bronquial por otros MPP.

4. MATERIALES Y MÉTODOS

4.1 MATERIALES Y MÉTODOS DEL ESTUDIO 1

4.1.1 Diseño del estudio y aspectos éticos

Se trata de un estudio prospectivo en el que fueron incluidos 408 pacientes con bronquiectasias clínicamente estables. El protocolo del estudio fue aprobado por el *East of Scotland Research Ethics Committee* (12/ES/0059), y todos los participantes dieron su consentimiento informado para participar.

4.1.2 Población del estudio

Los pacientes fueron reclutados de forma consecutiva de una consulta especializada en bronquiectasias del Hospital Ninewells en Dundee, Reino Unido, entre 2012-2015, y se les realizó un seguimiento clínico y microbiológico durante 12 meses. El diagnóstico de bronquiectasias se confirmó en todos los casos mediante la historia clínica (síntomas de tos con broncorrea y/o infecciones respiratorias recurrentes) y los hallazgos radiológicos de dilataciones bronquiales en la tomografía computarizada de tórax de alta resolución. Se excluyeron a todos los pacientes menores de 18 años de edad, a los que no otorgaron el consentimiento informado, con diagnóstico de FQ, aspergilosis broncopulmonar alérgica (ABPA) activa, enfermedad por micobacterias no tuberculosas activa, bronquiectasias de tracción por fibrosis pulmonar, y a aquellos sujetos con inmunodeficiencias o recibiendo tratamiento con reemplazo de inmunoglobulinas.

4.1.3 Evaluación clínica

Todos los pacientes incluidos se encontraban en fase de estabilidad clínica, definida por la ausencia de una exacerbación que requiriese tratamiento antibiótico o esteroideo sistémico en las 4 semanas previas a la inclusión. La calidad de vida fue evaluada mediante el *Saint George's Respiratory Questionnaire* (SGRQ), dado que este estudio inició antes de la disponibilidad del cuestionario específico para bronquiectasias *Quality of Life Bronchiectasis Questionnaire* (QOL-B). La etiología de las bronquiectasias fue determinada según las recomendaciones de la British Thoracic Society (1). La gravedad de la enfermedad se determinó mediante las escalas *Bronchiectasis Severity Index* (BSI) y FACED (3, 41).

4.1.4 Bacteriología

Se obtuvieron muestras de esputo espontáneo de todos los pacientes para análisis bacteriológico y medición de marcadores inflamatorios. La calidad del esputo se evaluó mediante los criterios de Murray-Washington (89). El estudio bacteriológico cuantitativo y cualitativo se llevó a cabo según protocolos pre-establecidos (44).

Los pacientes fueron clasificados en dos grupos según la presencia de aislamiento crónico previo de PA en esputo. La colonización crónica por PA fue definida como 2 o más muestras de esputo positivas con al menos 3 meses de separación entre ellas y/o fallo del tratamiento de erradicación de PA (9). El manejo clínico rutinario del centro donde se realizó el estudio consiste en analizar muestras de esputo en todas las visitas clínicas, con un objetivo de al

menos 3 muestras al año en pacientes con expectoración habitual. Para un análisis de sensibilidad posterior, evaluamos la definición de colonización crónica según Lee y colaboradores mediante los criterios de Leeds (90). Esta definición requería que los pacientes tuviesen un mínimo de 3 muestras de esputo analizadas en el último año, de las cuales al menos 50% debían ser positivas para PA.

4.1.5 Medición de los anticuerpos IgG específicos anti-PA

Se obtuvieron muestras de sangre de todos los pacientes incluidos, las cuales fueron procesadas para posteriormente realizar la titulación de los anticuerpos mediante un kit de ELISA validado y disponible comercialmente (Pseudomonas-CF-IgG ELISA kit. Statens Serum Institut, Denmark), siguiendo las instrucciones del fabricante (59, 61, 91). El valor usado como punto de corte para un resultado positivo fue de 2,96 unidades ELISA/10, según establecido por el fabricante.

4.1.6 Marcadores inflamatorios de la vía aérea

Las muestras de esputo fueron centrifugadas a 50.000g durante 90 minutos para obtener la fracción soluble. La actividad de la elastasa de neutrófilos y de la mieloperoxidasa (MPO) fueron determinadas mediante ensayo cromogénico, según descrito previamente (44).

4.1.7 Análisis estadístico

Los resultados se presentan como media y desviación estándar (DE) para los datos continuos paramétricos, y mediana y rango intercuartílico (RIQ) para los datos continuos no paramétricos. Los datos categóricos se presentan como frecuencias y porcentajes. Las variables fueron analizadas usando las pruebas “t” y ANOVA, mientras que las variables categóricas se analizaron mediante la prueba de X^2. Los niveles de marcadores inflamatorios y los niveles de IgG anti-PA se correlacionaron mediante regresión lineal. Se consideró significativo un p-valor menor de 0.05. El análisis estadístico se llevó a cabo mediante el software SPSS 22 para Windows (SPSS, Chicago, Illinois, EE.UU.) y GraphPad Prism Versión 6 (GraphPad Software Inc., San Diego, California, EE.UU.).

4.2 MATERIALES Y MÉTODOS DEL ESTUDIO 2

4.2.1 Diseño del estudio y aspectos éticos

Se trata de un estudio transversal que incluyó 73 pacientes con bronquiectasias clínicamente estables con y sin colonización bronquial bacteriana. El protocolo del estudio fue aprobado por el comité de ética institucional (IIBSP-BRO-2013-154) y los pacientes dieron su consentimiento informado para participar. Este estudio se registró en www.clinicaltrials.gov en abril de 2014. ClinicalTrials.gov ID: NCT02163642.

4.2.2 Población del estudio

Los pacientes fueron reclutados consecutivamente de una consulta especializada en el Hospital de la Santa Creu i Sant Pau de Barcelona entre junio de 2014 y mayo de 2016. El diagnóstico de bronquiectasias y su estudio etiológico se realizaron de acuerdo con las guías nacionales e internacionales (1, 2). Fueron excluidos del estudio los pacientes con edad inferior a 18 años; los sujetos que no pudieron dar su consentimiento informado y aquellos con otras patologías respiratorias como FQ, ABPA activa, enfermedad por micobacterias no tuberculosas activa y bronquiectasias de tracción por fibrosis pulmonar, así como pacientes recibiendo terapia de reemplazo de inmunoglobulinas o tratamiento inmunosupresor. El tamaño de la muestra se calculó como descrito en estudios previos (82, 83).

4.2.3 Evaluación clínica

Todos los sujetos fueron incluidos durante la fase de estabilidad clínica, definida por la ausencia de una exacerbación que requiriese tratamiento antibiótico o esteroideo sistémico en las 4 semanas previas a la inclusión. Se obtuvo una historia clínica detallada de todos los participantes, incluyendo datos demográficos, hábito tabáquico, comorbilidades relevantes, tratamiento actual y el número de exacerbaciones previas ambulatorias y hospitalarias. La gravedad de la enfermedad se evaluó mediante las escalas BSI y FACED (3, 41). La espirometría se realizó de acuerdo con las recomendaciones internacionales, utilizando los valores de referencia para la población mediterránea (92, 93).

4.2.4 Bacteriología

Se obtuvieron muestras de esputo espontáneo para análisis bacteriológico de todos los participantes, y fueron procesadas como descrito anteriormente (45). La calidad del esputo se evaluó usando los criterios de Murray-Washington (89). Los pacientes se clasificaron según el estudio bacteriológico del esputo en tres grupos: no colonizados, colonizados por PA y colonizados por otros MPP (*Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus* y otros bacilos gramnegativos).

4.2.5 Análisis del aire exhalado

Se obtuvieron muestras del aire exhalado de todos los participantes para evaluar los patrones de COV con la e-nose, como descrito anteriormente (76, 82). En resumen, se recogieron muestras del aire exhalado en bolsas Tedlar de 10 litros después de 3 minutos de respiración a volumen corriente a través de una válvula Hans-Rudolph con un depósito espiratorio de sílice expuesto al aire seco y un filtro inspiratorio. El dispositivo e-nose (Cyranose 320®, Smith Detections, CA, EE.UU.), un analizador químico de vapor con 32 matrices de sensores de nano-compuestos poliméricos orgánicos, era conectado posteriormente a la bolsa Tedlar durante 5 minutos. Esta exposición al aire exhalado generó un patrón de COV para cada sujeto.

Todos los participantes suspendieron sus tratamientos inhalados y ayunaron durante al menos 12 horas antes de la colección de las muestras.

4.2.6 Análisis estadístico

Los resultados se presentan como media y desviación estándar (DE) para datos paramétricos continuos, y mediana y rango intercuartílico (RIQ) para datos continuos no paramétricos. Los datos categóricos se presentan como frecuencias y porcentajes. Las variables fueron analizadas usando las pruebas "t" y ANOVA, mientras que las variables categóricas se analizaron mediante la prueba de X^2. El análisis estadístico se realizó utilizando el software SPSS 22 para Windows (SPSS, Illinois, EE.UU.). Se consideró significativo un valor de p inferior a 0,05.

Los perfiles del aire exhalado de todos los participantes fueron analizados utilizando una aplicación de reconocimiento de patrones construida

en el software MATLAB (v.R2012a) como descrito anteriormente (76, 82). En resumen, los datos brutos se redujeron a tres factores principales mediante el análisis de componentes principales (ACP). Estos factores se utilizaron para realizar un ANOVA univariante, seguido del método *post-hoc* de la diferencia mínima significativa. Los pacientes fueron clasificados luego en una división categórica utilizando un análisis discriminante canónico lineal, calculado como el que obtuvo el mayor porcentaje de sujetos correctamente clasificados. La función discriminante fue probada con todas las muestras de los sujetos menos una; y a continuación, se evaluaron las muestras restantes. Se repitió este proceso conocido como el método "*leave-one-out*" para todos los sujetos, construyendo así el porcentaje de pacientes correctamente clasificados, el cual definió el valor de precisión de la validación cruzada (74, 76, 82, 83). Se obtuvo una curva de características operativas del receptor (ROC) utilizando los resultados de la función discriminante. El área bajo la curva ROC se calculó mediante regresión logística múltiple.

5. RESULTADOS

5.1 RESULTADOS DEL ESTUDIO 1

5.1.1 Descripción de los pacientes

Cuatrocientos ocho pacientes con bronquiectasias clínicamente estables fueron incluidos en el estudio. De ellos, 247 (60,5%) eran mujeres y la media de edad fue 65,4 ± 12,7 años. Las etiologías de bronquiectasias más frecuentes fueron la idiopática (43,9%) y post-infecciosa (19,6%). La media del FEV_1 fue 70,7 ± 24,4 % del valor predicho, y la media de puntuación del BSI fue de 7,6 ± 4,7 puntos.

Sesenta (14,7%) pacientes cumplieron los criterios de colonización crónica por PA al inicio del estudio. La **Tabla 6** resume las características de los sujetos, agrupados según la presencia o no de colonización crónica por PA. Los pacientes colonizados tenían puntuaciones de gravedad significativamente mayores (mediana de puntuación del BSI 14,5 vs. 6 puntos, $p<0.001$; y la mediana de puntuación del FACED 5 vs 1 punto, $p<0.001$), más exacerbaciones previas (mediana 4 vs 1, $p<0.001$) y peor puntuación de disnea (mediana de la escala MRC 3 vs 2 puntos, $p<0.001$). Los pacientes con colonización bronquial crónica por PA tuvieron valores más bajos de FEV_1 (mediana de 72,5 vs 55,3 % del valor predicho, $p<0.001$) y peores puntuaciones de calidad de vida según el SGRQ (media 62,3 ± 21,1 vs 42,9 ± 21,9 puntos).

Tabla 6. Características demográficas y clínicas de los pacientes con bronquiectasias con y sin colonización bronquial crónica por *Pseudomonas aeruginosa*.

	No colonizados por PA (N=348)	**Colonización bronquial crónica por PA (N=60)**	***P*-valor**
Edad	67 (58 – 73,5)	70 (62 - 75)	0,06
Mujeres (n,%)	215 (61,8%)	32 (53,3%)	0,21
Tabaquismo (n,%) **Nunca fumadores** **Exfumadores** **Fumadores activos**	 213 (61,2%) 124 (35,6%) 11 (3,2%)	 40 (66,7%) 17 (28,3%) 3 (5%)	0,46
Escala MRC de disnea	2 (1 - 3)	3 (2,3 - 4)	*<0,001*
FEV_1 (% predicho)	72,5 (56,3 – 90,2)	55,3 (37,9 – 80,7)	*<0,001*
FVC (% predicho)	83,4 (70,9 – 99,1)	73,3 (59,4 - 92)	*0,003*
IMC (Kg/m^2)	25,2 (22,3 – 28,9)	25 (22,2 – 27,7)	0,31
Etiología (n,%) **Idiopáticas** **Post-infecciosas** **ABPA** **Asma** **EPOC** **Conectivopatías** **Inmunodeficiencias** **EII** **Otras**	 152 (43,7%) 71 (20,4%) 28 (8%) 15 (4,3%) 16 (4,6%) 22 (6,3%) 19 (5,5%) 8 (2,3%) 17 (4,9%)	 27 (45%) 9 (15%) 8 (13,3%) 0 (0) 3 (5%) 3 (5%) 2 (3,3%) 2 (3,3%) 6 (10%)	0,41
Exacerbaciones previas (n)	1 (0 - 2)	4 (3 - 7)	*<0,001*
Puntuación del BSI	6.0 (4 - 8)	14,5 (11,3 - 17)	*<0,001*
Puntuación FACED	1 (1 - 3)	5 (4 - 5)	*<0,001*
Puntuación del SGRQ media (±DE)	42,9 (21,9)	62,3 (21,1)	*<0,001*
Niveles de IgG anti-PA (unidades ELISA/10)	1,3 (0,6 – 3,1)	6,2 (4,6 – 10,2)	*<0,001*

Todos los datos se presentan como mediana (cuartiles 1-3) a menos que se indique lo contrario.

PA: *Pseudomonas aeruginosa*; FEV_1: Volumen espiratorio forzado en 1 segundo; FVC: Capacidad vital forzada; IMC: índice de masa corporal; ABPA: Aspergilosis broncopulmonar alérgica; EPOC: Enfermedad pulmonar obstructiva crónica; EII: Enfermedad inflamatoria intestinal; BSI: *Bronchiectasis Severity Index*; SGRQ: *Saint George's Respiratory Questionnaire*; IgG: Inmunoglobulina G.

5.1.2 Precisión de la prueba de IgG específica anti-PA

Los pacientes con colonización crónica por PA tuvieron mayores niveles basales de IgG anti-PA (mediana 6,2 vs 1,3 unidades, p<0.001). Se encontró un resultado positivo de IgG >2,96 unidades en 57 (95%) de pacientes que cumplían criterios de colonización bronquial crónica por PA, y en 89 (25,6%) de los sujetos que no cumplían dichos criterios. Los valores de sensibilidad, especificidad y valores predictivos positivo y negativo se muestran en la **Tabla 7**. El área bajo la curva ROC (AUROC) de la prueba fue de 0,87.

Entre todos los pacientes incluidos, 127 (31,1%) estaban colonizados por *Haemophilus influenzae*. Con el fin de estudiar el efecto de posibles reacciones cruzadas de anticuerpos inducidos por otros microorganismos Gram-negativos (59), se realizó un análisis de subgrupos excluyendo a estos pacientes. No hubo cambios en la sensibilidad de la prueba, pero la especificidad y los valores predictivos positivos aumentaron y el valor predictivo negativo y el área bajo la curva ROC disminuyeron ligeramente. La **Tabla 7** muestra estos valores de precisión de la prueba para el análisis de subgrupos.

Se investigaron las características de los 89 pacientes "falsos positivos" que tuvieron una prueba positiva de IgG anti-PA sin una colonización bronquial por PA conocida. De ellos, 33 (37,1%) estaban colonizados por *Haemophilus influenzae* y 10 (11,2%) tenían un cultivo de esputo positivo para PA en el año previo a la inclusión en el estudio, pero no cumplían con los criterios de colonización crónica. Estos pacientes no presentaron diferencias estadísticamente significativas con los sujetos "verdaderamente negativos" (n=259) en cuanto a la gravedad de las bronquiectasias, el número de

exacerbaciones previas, las puntuaciones del SGRQ, los valores de función pulmonar o los niveles de elastasa y MPO en el esputo. De los 10 sujetos con aislamiento de PA en el año previo al estudio, 6 cumplieron con los criterios de infección crónica por PA durante el período de seguimiento del estudio, lo que sugiere que pueden haber sido "verdaderos positivos".

Tabla 7. Sensibilidad, especificidad y valores predictivos positivos y negativos para la prueba de IgG anti-PA en todos los sujetos y excluyendo a los pacientes con colonización bronquial crónica por *Haemophilus influenzae.*

	Sensibilidad	**Especificidad**	**Valor predictivo positivo**	**Valor predictivo negativo**	**AUROC**
Todos los pacientes (n=408)	95%	74%	39%	99%	0,87
Excluyendo a pacientes con colonización crónica por Hi (n=281)	95%	75%	50%	98%	0,86

Hi: *Haemophilus influenzae*; AUROC: Área bajo la curva ROC.

Investigamos también la variación de los puntos de corte para determinar la positividad de las prueba de IgG anti-PA, ya que este fue diseñado para los pacientes con FQ. Reducir el punto de corte a 2 unidades no mejoró la sensibilidad, pero redujo la especificidad al 63%. Sólo se logró una sensibilidad del 100% con la reducción del punto de corte a 0,1 con una especificidad del 9%. Aumentar el punto de corte a 4 unidades (por ejemplo) aumentó la especificidad al 83% pero redujo la sensibilidad al 78,3%. Se

requirió un punto de corte por encima de 13 unidades para lograr una especificidad del 100%, a expensas de una sensibilidad muy baja.

El uso de una definición más rigurosa de colonización bronquial crónica por PA resultó en una mayor precisión de la prueba. 56/60 pacientes con PA tuvieron 3 o más muestras de esputo en el año anterior y 52 cumplieron con los criterios de Leeds de colonización crónica por PA. Todos estos pacientes tenían una prueba de IgG anti-PA positiva. De los 3 pacientes con una prueba de anticuerpos "falsa negativa", 2 no cumplieron con los criterios de Leeds. En un caso, el paciente tenía 6 cultivos de esputo en el año anterior con sólo 2 (aunque con más de 3 meses de diferencia) positivas para PA. En el segundo caso tuvo 2 cultivos de esputo positivos para PA a comienzos del año, pero se habían cultivado *Haemophilus influenzae* y *Moraxella catarrhalis* en muestras subsecuentes de esputo. El tercer paciente fue excluido debido a que sólo tenía 2 muestras de esputo en el año anterior. Así, aplicando el criterio de Leeds, la prueba de anticuerpos IgG anti-PA tenía una sensibilidad del 100% y una especificidad del 89% (n=293 pacientes con 3 o más muestras de esputo disponibles en los 12 meses anteriores).

5.1.3 Niveles de IgG anti-PA y gravedad de la enfermedad

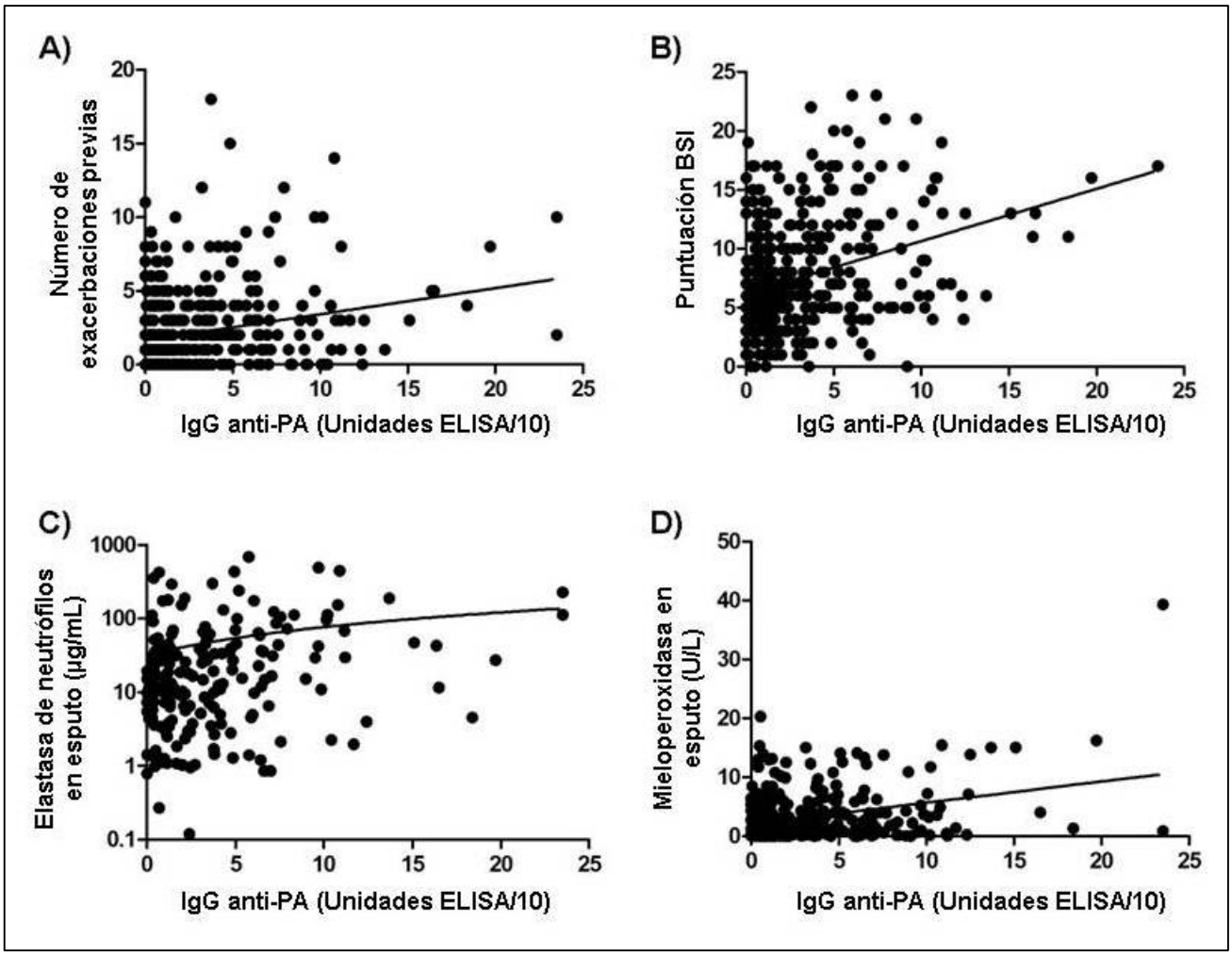
Hubo una correlación significativa entre los niveles de IgG anti-PA y el número de exacerbaciones previas (r=0,168, p<0,001), las puntuaciones del BSI y FACED (r=0,281, p<0,001 y r=0,278, p<0,001 respectivamente), la puntuación del SGRQ (r=0,152; p=0,006) y los niveles de elastasa (r=0,228; p<0,001) y MPO (r=0,168; p<0,001) en esputo, tal como se presenta en la

Figura 6. Tras excluir a los pacientes con colonización bronquial por PA conocida, no hubo correlaciones significativas con las puntuaciones del BSI (p=0,2), FACED (p=0,1) y SGRQ (p=0,5), o con cualquier otro parámetro.

5.1.4 Seguimiento de los pacientes

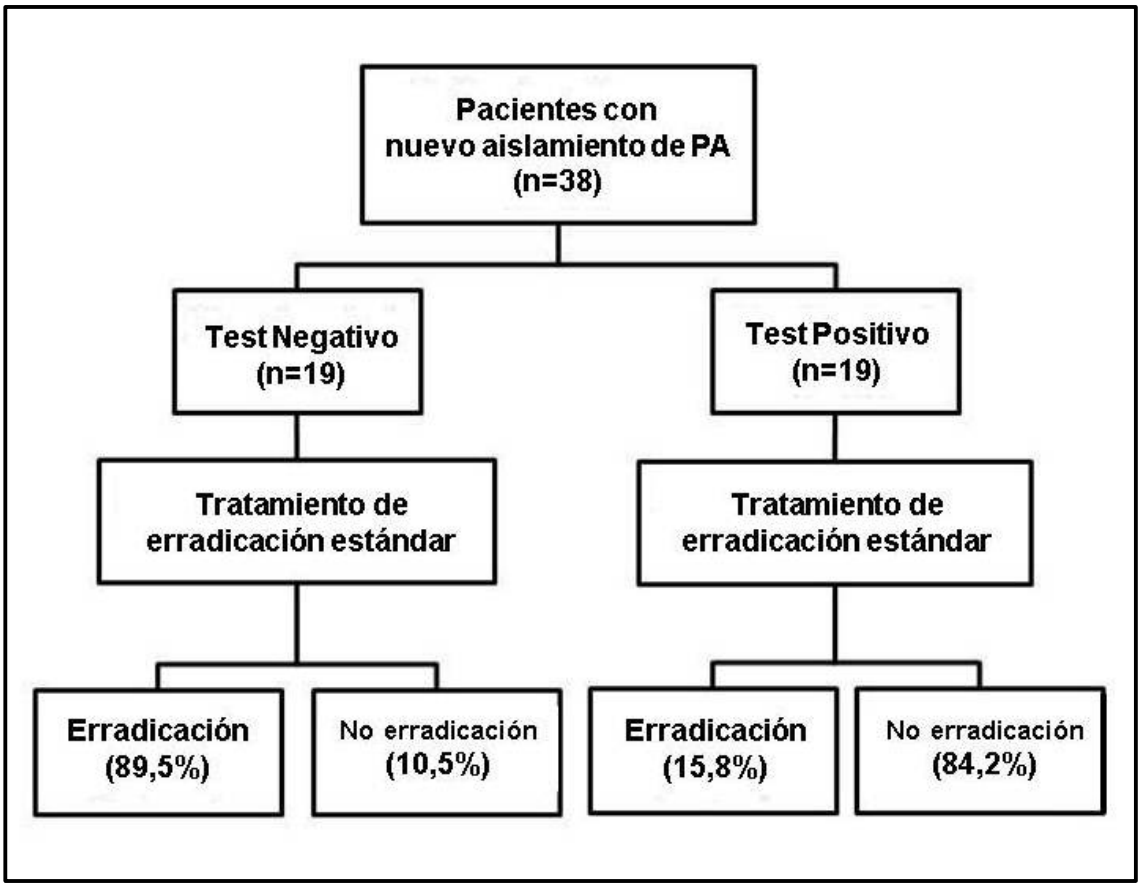
Durante el estudio, 38 pacientes tuvieron un nuevo aislamiento de PA en el esputo. De ellos, 19 (50%) tuvieron una prueba anti-PA IgG negativa en el momento del aislamiento. De acuerdo con el protocolo local, todos ellos se sometieron a un tratamiento de erradicación consistente en 2 semanas de ciprofloxacino, seguido de 2 semanas de antibióticos intravenosos y 3 meses de colistina nebulizada (1). La erradicación, definida como la ausencia de aislamiento de PA en el esputo después de 3 y 12 meses, se logró en 17 (89,5%) pacientes con una prueba de IgG negativa y en 3 (15,8%) con una prueba de anticuerpos positiva. La **Figura 7** resume la distribución de la eficacia del tratamiento de erradicación de PA en pacientes con prueba de IgG anti-PA positiva y negativa.

Figura 6. Correlación de los niveles de IgG anti-PA con el número de exacerbaciones previas (A), gravedad de la enfermedad (B) y marcadores inflamatorios del esputo (C, D).



PA: *Pseudomonas aeruginosa*, IgG: Inmunoglobulina G, BSI: Bronchiectasis severity index, MPO: Mieloperoxidasa.

Figura 7. Distribución de la eficacia del tratamiento de erradicación de *Pseudomonas aeruginosa* según el resultado de la prueba de IgG anti-PA.



PA: *Pseudomonas aeruginosa*; IgG: Inmunoglobulina G.

5.2 RESULTADOS DEL ESTUDIO 2

5.2.1 Descripción de los pacientes

Se incluyeron 73 pacientes con bronquiectasias clínicamente estables. De ellos, 47 (64%) eran mujeres y la mediana de edad fue de 69 años (RIQ 60-76,5 años). El FEV_1 medio fue de 65,9 ± 23,3% de lo predicho; la mediana de la puntuación BSI fue de 7 puntos (RIQ 6-11 puntos) y la mediana de la puntuación FACED fue de 2 puntos (RIQ 1-4 puntos). Las etiologías más frecuentes fueron post-infecciosas (47%) e idiopáticas (19%).

Cuarenta y un (56%) pacientes fueron clasificados como colonizados. Los MPP aislados con mayor frecuencia fueron *Pseudomonas aeruginosa* (n=27; 66%), *Haemophilus influenzae* (n=7; 17%), *Escherichia coli* (n=2; 5%) y *Streptococcus pneumoniae* (n=2; 5%). Otros MPP aislados fueron *Moraxella catarrhalis*, *Achromobacter xylosoxidans* y *Staphylococcus aureus* (n=1 cada uno, 2%).

Las características basales de los sujetos colonizados y no colonizados se resumen en la **Tabla 8**. Los pacientes con colonización bronquial bacteriana tuvieron valores de función pulmonar más bajos (FEV_1 medio 76,8 ± 22,5% frente a 57,6 ± 20,5%, p<0,001) y bronquiectasias más graves (puntuación mediana del BSI 6 vs. 10, p<0,001; y puntuación FACED mediana 2 vs. 3, p<0,001). Los pacientes colonizados fueron posteriormente clasificados en 2 subgrupos según el microorganismo aislado en el cultivo de esputo; 27 sujetos (66%) estaban colonizados por PA y 14 (34%) por otros MPP. Las características demográficas y clínicas de estos subgrupos se muestran en la **Tabla 9**. Los pacientes con colonización bronquial por PA se asociaron más

con la etiología post-infecciosa, tenían bronquiectasias más graves (puntuación BSI mediana 11 vs. 6, p=0,01 y puntuación FACED mediana 4 vs. 2, p=0,001), y además presentaban un mayor uso de beta-agonistas de acción prolongada (85% vs. 35%, p=0,001) y de corticosteroides inhalados (74% vs. 35%, p=0,01) en comparación con aquellos pacientes colonizados por otros MPP.

Tabla 8. Características demográficas y clínicas de pacientes con bronquiectasias con y sin colonización bronquial.

	No Colonizados (N=32)	**Colonizados (N=41)**	**p-valor**
Edad	69,5 (59-75,8)	68 (60,5-77,5)	0,726
Mujeres (n,%)	22 (68,8%)	25 (61%)	0,491
Tabaquismo (n,%) Nunca Exfumador Activo	 24 (75%) 7 (21,9%) 1 (3,1%)	 31 (75,6%) 10 (24,4%) 0	0,514
Comorbilidad cardiovascular (n,%)	5 (15,6%)	9 (22%)	0,496
Diabetes mellitus (n,%)	2 (6,3%)	4 (9,8%)	0,588
Escala MRC de disnea	2 (1-2)	2 (2-3)	*<0,001*
Etiología (n,%) Post infecciosa Conectivopatías Discinesia ciliar primaria Inmunodeficiencia ABPA inactiva EPOC Otras Idiopáticas	 16 (50%) 2 (6,3%) 2 (6,3%) 1 (3,1%) 0 2 (6,3%) 1 (3,1%) 8 (25%)	 18 (43,9%) 7 (17,1%) 2 (4,9%) 2 (4,9%) 3 (7,3%) 1 (2,4%) 2 (4,9%) 6 (14,6%)	0,521
FVC % predicho (media ± DE)	87,9 ± 20,6	74,7 ± 18,4	*0,005*
FEV_1 % predicho (media ±DE)	76,8 ± 22,5	57,6 ± 20,5	*<0,001*
Exacerbaciones previas	2 (1-3)	3 (2-4)	0,392
Puntuación del BSI	6 (4-8)	10 (6-13)	*<0,001*
Puntuación del FACED	2 (1-3)	3 (2-4)	*<0,001*
LABA (n,%)	18 (56,3%)	28 (68,3%)	0,290
LAMA (n,%)	8 (25%)	19 (46,3%)	0,061
ICS (n,%)	16 (50%)	25 (61%)	0,348
Macrólidos (n,%)	6 (18,8%)	10 (24,4%)	0,563

Los datos se presentan como mediana (cuartiles 1-3) a menos que se indique lo contrario. MRC: Medical Research Council; ABPA: Aspergilosis broncopulmonar alérgica; EPOC: Enfermedad pulmonar obstructiva crónica; FVC: Capacidad vital forzada; FEV_1: Volumen espiratorio forzado en 1 segundo; LABA: Beta-agonistas de acción prolongada; BSI: Bronchiectasis Severity Index; LAMA: Antagonistas de los receptores muscarínicos de acción prolongada; ICS: Corticosteroides inhalados.

Tabla 9. Características demográficas y clínicas de pacientes con bronquiectasias y colonización bronquial por *Pseudomonas aeruginosa* u otros microorganismos potencialmente patógenos.

	Colonizados por *PA* (N=27)	**Colonizados por otros MPP (N=14)**	**p-valor**
Edad	68 (63-77)	67 (58-78)	0,591
Mujeres (n,%)	18 (66,7%)	7 (50%)	0,300
Tabaquismo (n,%) Nunca Exfumador Activo	 19 (70,4%) 8 (29,6%) 0	 12 (85,7%) 2 (14,3%) 0	0,278
Escala MRC de disnea	3 (2-3)	2 (2-3)	0,166
Etiología (n,%) Post infecciosa Conectivopatías Discinesia ciliar primaria Inmunodeficiencia ABPA inactiva EPOC Otras Idiopáticas	 16 (59,3%) 2 (7,4%) 0 0 2 (7,4%) 1 (3,7%) 1 (3,7%) 5 (18,5%)	 2 (14,3%) 5 (35,7%) 2 (14,3%) 2 (14,3%) 1 (7,1%) 0 1 (7,1%) 1 (7,1%)	*0,013*
FVC % predicho (media±DE)	72,3 ± 19,1	79.4 ± 16,6	0,248
FEV_1 % predicho (media±DE)	54,9 ± 22,2	62.7 ± 16,2	0,251
Exacerbaciones previas	3 (2-4)	2,5 (1-3,3)	0,370
Puntuación del BSI	11 (9-14)	6 (5,8-12,5)	*0,016*
Puntuación del FACED	4 (2-5)	2 (1-3)	*0,001*
LABA (n,%)	23 (85,2%)	5 (35,7%)	*0,001*
LAMA (n,%)	14 (51,9%)	5 (35,7%)	0,326
ICS (n,%)	20 (74,1%)	5 (35,7%)	*0,017*
Macrólidos (n,%)	7 (25,9%)	3 (21,4%)	0,750

Todos los datos se presentan como mediana (cuartiles 1-3) a menos que se indique lo contrario. PA: *Pseudomonas aeruginosa*; MPP: Microorganismos potencialmente patógenos; MRC: Medical Research Council; ABPA: Aspergilosis broncopulmonar alérgica; EPOC: Enfermedad pulmonar obstructiva crónica; FVC: Capacidad vital forzada; FEV_1: Volumen espiratorio forzado en 1 segundo; LABA: Beta-agonistas de acción prolongada; BSI: Bronchiectasis Severity Index; LAMA: Antagonistas de los receptores muscarínicos de acción prolongada; ICS: Corticosteroides inhalados.

5.2.2 Patrones de Compuestos Volátiles Orgánicos

Los pacientes con bronquiectasias con y sin colonización bronquial bacteriana tuvieron patrones de COV significativamente diferentes **(Figura 8)**. La precisión de validación cruzada para detectar estos patrones con la nariz

electrónica fue del 72,1%, y el área bajo la curva ROC (AUROC) fue 0,75 (p=0,01) **(Tabla 10)**.

También se analizaron los sujetos con colonización bronquial bacteriana. Los patrones de COV en el aire exhalado de los sujetos colonizados por PA y los de pacientes colonizados por otros MPP fueron significativamente diferentes **(Figura 9)**, con una precisión de validación cruzada de 89,2% y el AUROC de 0,96 (p<0,001) **(Tabla 10)**.

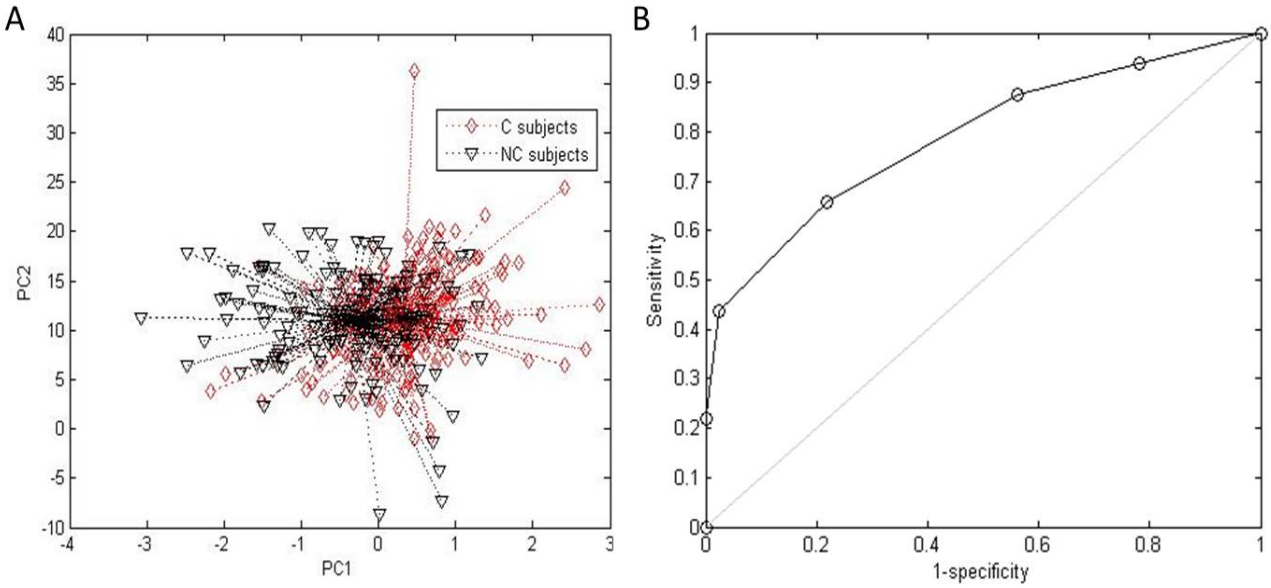
Además, los pacientes con colonización bronquial por PA se compararon luego con los sujetos no colonizados. Este análisis también mostró diferencias significativas en los patrones de COV en el aire exhalado **(Figura 10)**, con una precisión de validación cruzada de 72,7% y el AUROC de 0,82 (p=0,007) **(Tabla 10)**.

Tabla 10. Análisis de precisión de los patrones de compuestos volátiles orgánicos en el aire exhalado entre pacientes con bronquiectasias y colonización bronquial por *Pseudomonas aeruginosa*, colonización por otros microorganismos potencialmente patógenos y no colonizados.

	Colonizados vs. No colonizados	**Colonizados por PA vs. Colonizados por otros MPP**	**Colonizados por PA vs. No colonizados**
Precisión de validación cruzada	72,1%	89,2%	72,7%
Sensibilidad	0,84	0,92	0,83
Especificidad	0,58	0,85	0,65
AUROC	0,754	0,968	0,829
Valor predictivo positivo	0,70	0,92	0,65
Valor predictivo negativo	0,75	0,85	0,83
p-valor	*0,01*	*<0,001*	*0,007*

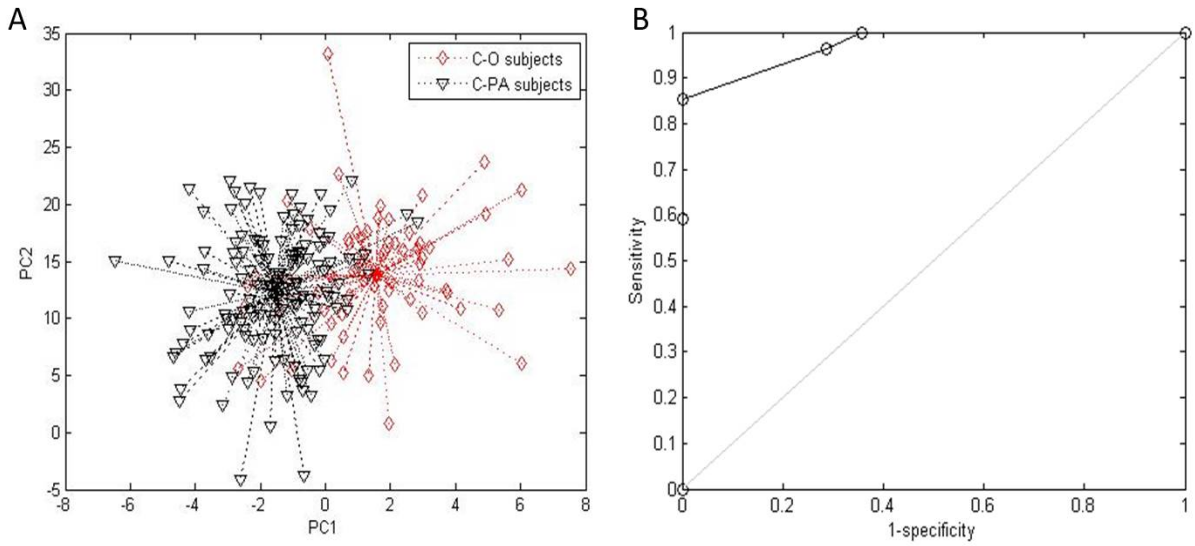
PA: *Pseudomonas aeruginosa*; MPP: Microorganismos potencialmente patógenos; AUROC: Área bajo la curva de características operativas del receptor.

Figura 8. Discriminación de patrones de imprenta olfatoria de pacientes colonizados versus no colonizados con la nariz electrónica



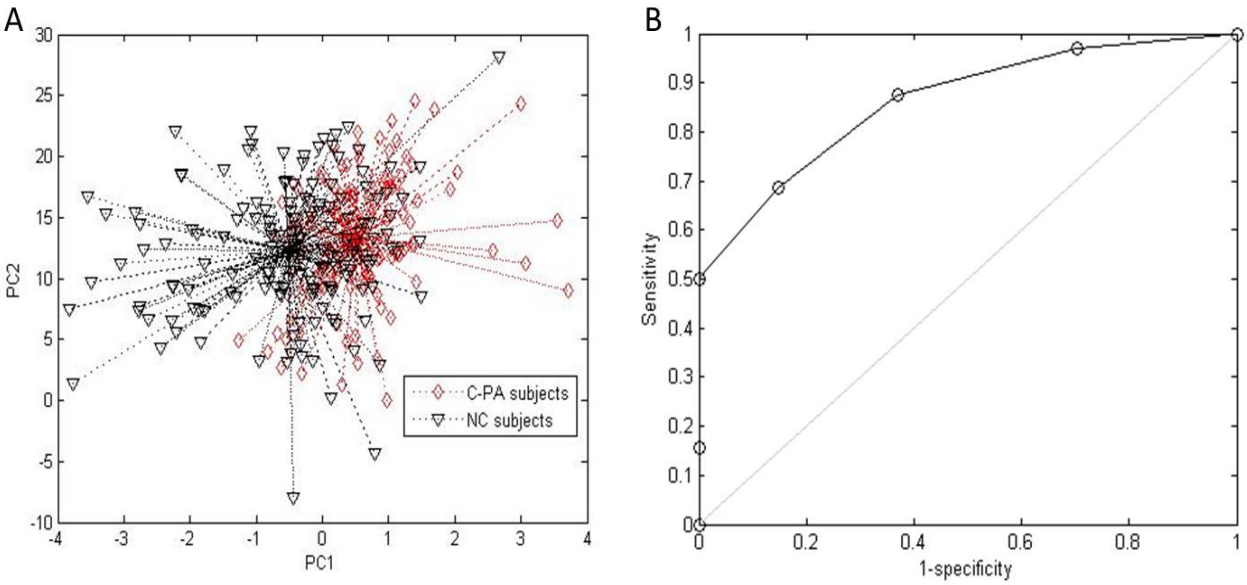
A: Gráfico bidimensional del análisis de los componentes principales (PC) que muestra la discriminación de los patrones de imprenta olfatoria. B: Área bajo la curva ROC (AUROC) de 0,75. C: Colonizados; NC: No colonizados.

Figura 9. Discriminación de patrones de imprenta olfatoria de pacientes colonizados por *Pseudomonas aeruginosa* versus colonizados por otros microorganismos potencialmente patógenos con la nariz electrónica.



A: Gráfico bidimensional del análisis de los componentes principales (PC) que muestra la discriminación de los patrones de imprenta olfatoria. B: Área bajo la curva de ROC (AUROC) de 0,96. C-PA: Colonización por *Pseudomonas aeruginosa*; C-O: Colonización por otros microorganismos potencialmente patógenos.

Figura 10. Discriminación de patrones de imprenta olfatoria de pacientes colonizados por *Pseudomonas aeruginosa* versus no colonizados con la nariz electrónica.



A: Gráfico bidimensional del análisis de los componentes principales (PC) que muestra la discriminación de los patrones de imprenta olfatoria. B: Área bajo la curva ROC (AUROC) de 0,82. C-PA: Colonizados por *Pseudomonas aeruginosa*; NC: No colonizados.

6. DISCUSIÓN

6. DISCUSIÓN GENERAL

La colonización bacteriana bronquial, y especialmente cuando se debe a la PA, tiene un impacto negativo muy significativo en la evolución clínica y funcional de los pacientes con bronquiectasias. Su identificación, por tanto, es fundamental, y los métodos usados actualmente presentan múltiples limitaciones para realizar un correcto diagnóstico microbiológico.

La presente tesis doctoral consta de dos estudios dirigidos a la valoración de nuevos métodos diagnósticos de colonización bronquial por PA que tienen un gran potencial para apoyar a las técnicas estandarizadas actuales. Estos métodos son la determinación de los niveles de anticuerpos IgG anti-PA en suero y el análisis de los COV en el aire exhalado. En ambos casos se ha podido demostrar un elevado valor diagnóstico para la detección de colonización bronquial por PA, y por ende, podrían ser de utilidad en el seguimiento clínico de pacientes con bronquiectasias.

6.1 DISCUSIÓN DEL ESTUDIO 1

Este estudio ha demostrado que los niveles séricos de IgG anti-PA tienen una alta precisión diagnóstica para la identificación de la colonización crónica de las vías respiratorias por PA en pacientes con bronquiectasias. Además, la prueba fue también un marcador de respuesta al tratamiento antibiótico de erradicación anti-PA, aunque este análisis está limitado por la ausencia de un grupo control sin tratamiento antibiótico.

La infección bronquial por PA desempeña un papel importante en la patogénesis de las bronquiectasias. Estudios anteriores han demostrado que los pacientes con bronquiectasias e infección por PA tienen mayores niveles de marcadores inflamatorios de neutrófilos de las vía aérea, así como de citocinas, quimiocinas y mucinas, en comparación con los sujetos colonizados por otros microorganismos (44, 45). Además, la infección crónica por PA está relacionada con exacerbaciones más frecuentes, peor calidad de vida, menor FEV_1 y una mayor tasa de mortalidad (39, 43, 46). En la cohorte de 408 pacientes incluidos en este estudio, alrededor de un 15% cumplía los criterios de colonización crónica por PA. Esto muestra similitudes con lo observado en el metaanálisis de Finch y colaboradores, donde fueron incluidos 3.683 pacientes distribuidos en 55 estudios, observando una prevalencia de PA global de un 21% aproximadamente (43). Además, los pacientes crónicamente infectados por PA tenían peores valores de función pulmonar y peores puntuaciones del cuestionario SGRQ, así como más exacerbaciones previas y en general, una mayor gravedad de las bronquiectasias. Por lo tanto, la identificación de la colonización crónica por PA es clínicamente importante en los pacientes con bronquiectasias.

El estudio de los niveles de IgG anti-PA ha demostrado una alta precisión en el diagnóstico de infección crónica por PA en pacientes con FQ. Pressler y colaboradores realizaron un estudio dirigido a evaluar la utilidad de la medición de anticuerpos específicos contra PA en pacientes con FQ con diferentes estados de colonización por PA: 381 sin aislamientos previos de PA, 129 con colonización intermitente por PA y 281 con colonización bronquial crónica por PA (59). Para ello utilizaron tres métodos serológicos diferentes:

CIE, ELISA de Exotoxina A y ELISA Pseudomonas-CF-IgG, y todos ellos tuvieron una elevada sensibilidad y especificidad para la identificación de diferentes estados de colonización por PA. Específicamente, con el método usado en nuestro estudio (ELISA Pseudomonas-CF-IgG) se observó una sensibilidad del 97%, especificidad del 83% y valores predictivos positivo y negativo de 80% y 98% respectivamente para la identificación de diferentes estados de infección por PA (59). Sin embargo, en la actualidad todavía no se utiliza esta prueba de forma rutinaria en la práctica clínica. Esto probablemente esté relacionado, en parte, con la alta prevalencia de la colonización por PA y con el seguimiento microbiológico estrecho de los pacientes con FQ. Las guías clínicas actuales de la FQ recomiendan realizar cultivos de esputo al menos cada 3 meses (37, 50), haciendo que la determinación de estos anticuerpos sea dispensable en la mayoría de los casos.

Sin embargo, existen diferencias importantes entre FQ y bronquiectasias que podrían determinar una mayor utilidad de la determinación de los niveles de IgG anti-PA en esta última patología. Por un lado, en pacientes con bronquiectasias la prevalencia de la infección por PA es significativamente menor que en FQ (43). Por otra parte, auditorías recientes llevadas a cabo en Italia y el Reino Unido han observado que existe una gran variabilidad en el manejo microbiológico de estos pacientes (51, 52). En muchos centros se solicita solo un cultivo de esputo por año o no se realiza ningún tipo de monitorización microbiológica en absoluto (51, 52). Además, hasta el 27% de los pacientes con bronquiectasias no producen esputo diario, lo que puede dificultar el seguimiento microbiológico (19). Tomando en cuenta esto, la identificación de la colonización bronquial crónica por PA en pacientes con

bronquiectasias es probablemente más difícil que en FQ, y por tanto la prueba de IgG anti-PA podría potencialmente proporcionar una mayor ayuda clínica.

En bronquiectasias existen datos limitados sobre la utilidad de los anticuerpos IgG anti-PA. Caballero y colaboradores estudiaron a 56 pacientes con bronquiectasias y los clasificaron en tres grupos dependiendo de la ausencia o aislamiento crónico o intermitente de PA en el esputo. En dicho estudio se observó una correlación significativa entre los anticuerpos anti-PA detectados por Western blot y la frecuencia de aislamiento de PA en el esputo, lo que sugiere que los diferentes grados de infección por PA podrían ser identificados en sujetos con bronquiectasias (68). Sin embargo, estos resultados son difíciles de extrapolar para la práctica clínica habitual debido entre otras cosas al tamaño reducido de la muestra y a la complejidad de la técnica empleada en dicho trabajo. En nuestro estudio se utilizó un kit ELISA validado, mostrando un valor diagnóstico similar con una sensibilidad y especificidad de 95% y 74,4%, respectivamente.

Las implicaciones clínicas de nuestro estudio son varias. Aunque el escenario ideal es que los pacientes con bronquiectasias deben ser seguidos regularmente con cultivos de esputo al menos en cada visita según las guías británicas (1), esto no se realiza actualmente como parte de la práctica clínica en la mayoría de los centros (51, 52). Por lo tanto, tras el aislamiento de PA en una sola muestra de esputo, no es posible saber con seguridad si esta infección es crónica y potencialmente requiera tratamiento supresor para prevenir las exacerbaciones, o si es probable que sea transitoria y se erradicará con tratamiento antibiótico o espontáneamente. En esta situación, la medición de los anticuerpos IgG anti-PA puede tener un papel importante. Los

niveles elevados de IgG anti-PA pueden alertar al clínico sobre una alta probabilidad de colonización bronquial crónica y una baja probabilidad de éxito del tratamiento de erradicación. Por el contrario, los niveles bajos de IgG anti-PA alertarán al clínico de una alta probabilidad de erradicación de la bacteria con tratamiento antibiótico o espontáneamente. Sin embargo, claramente esta prueba no reemplaza la necesidad de un control microbiológico regular y debe utilizarse conjuntamente con la tinción de Gram y cultivo del esputo, ya que se demostró una elevada tasa de pruebas positivas por reactividad cruzada en los individuos con colonización bronquial crónica por *Haemophilus influenzae*. La importancia y utilidad de la determinación de los títulos de IgG anti-PA debe interpretarse en el contexto clínico de cada paciente, especialmente en aquellos casos con una prueba de anticuerpos positiva y un cultivo negativo de esputo.

Definir la colonización bronquial crónica por PA es crucial para identificar a los sujetos que deben ser tratados con terapias dirigidas contra la PA, como los antibióticos inhalados. Este estudio resalta la dificultad en la definición de la colonización bronquial crónica por PA en bronquiectasias. La definición más ampliamente utilizada se basa en el aislamiento de esta bacteria en al menos dos ocasiones con una separación mínima de 3 meses durante un año. En este estudio, la prueba de los anticuerpos IgG anti-PA tuvo una sensibilidad del 95% utilizando esta definición estandarizada de colonización bronquial. Sin embargo, utilizando una definición más rigurosa que requería 3 cultivos de esputo en un año de los cuales al menos el 50% fueran positivos para PA, el test de anticuerpos fue 100% sensible y más específico. Esto se ha demostrado claramente en ensayos clínicos aleatorizados. En un ensayo de

colistina nebulizada realizado por Haworth y colaboradores (94), requería que los pacientes tuvieran 2 muestras de esputo positivas para PA en los 12 meses previos a la inclusión, y un cultivo positivo para PA en el cribado. A pesar de este riguroso criterio de inclusión, sólo en 53/62 (85%) de los pacientes en el grupo placebo se reportaba crecimiento de PA en el cultivo de esputo de la visita final del estudio, indicando una alta tasa de "erradicación espontánea" o cultivos negativos intermitentes (94). En el grupo placebo de los ensayos clínicos de aztreonam y gentamicina se observaron resultados similares (95, 96). Nuestros datos sugieren que el uso de una prueba de anticuerpos IgG anti-PA puede aumentar la probabilidad de que los pacientes tengan realmente una colonización bronquial crónica por PA si se utiliza como criterio de inclusión para los ensayos clínicos de bronquiectasias.

El tratamiento de erradicación de la PA es complejo, aunque ampliamente practicado, tanto en la FQ como en las bronquiectasias. Se ha reportado una tasa de erradicación satisfactoria de la infección inicial por PA en el 70-80% de los pacientes con FQ (97–99) y en hasta el 22-55% de los sujetos con bronquiectasias (100–103). Sin embargo, a pesar de la erradicación inicial, sólo en el 34% de los pacientes con bronquiectasias colonizados por PA no se vuelve a aislar este microorganismo en los cultivos de esputo (18). En nuestro estudio, la erradicación de la PA a los 12 meses se logró sólo en el 15,8% de los pacientes con una prueba positiva de IgG anti-PA y en el 89,5% de los sujetos que tuvieron un test negativo. Esto sugiere que la determinación de los niveles de IgG anti-PA puede ayudar a predecir la respuesta al tratamiento de erradicación, aunque la decisión de realizar o no dicho tratamiento dependerá por supuesto del clínico y de las características de cada paciente.

Este estudio tiene algunas limitaciones. Aunque se incluyó un gran número de sujetos, se trata de un estudio realizado en un solo centro. Otra limitación es la ausencia de diagnósticos moleculares tales como la reacción en cadena de la polimerasa (PCR-PA) o la caracterización del microbioma, que pueden definir mejor la colonización bronquial por PA en comparación con el cultivo de esputo. Sin embargo, elegimos conscientemente no llevar a cabo el análisis molecular porque hasta la fecha, la importancia clínica de una PCR-PA positiva o la presencia de PA como parte del microbioma no está clara. El objetivo de este estudio es utilizar la medición de IgG anti-PA para añadir información clínica relevante a la proporcionada por el cultivo de esputo en la práctica clínica, ya que sigue siendo la prueba de referencia para la evaluación microbiológica de pacientes con bronquiectasias. Además, toda la información existente en la actualidad sobre la impacto de la colonización bronquial por PA en el pronóstico de la enfermedad se basa en el aislamiento repetido de dicha bacteria en cultivos de esputo; por lo tanto, el valor clínico y pronóstico de una prueba de PCR-PA positiva debería ser evaluado antes en un estudio separado. Otra limitación del estudio es que estos resultados no son aplicables a pacientes con inmunodeficiencias, ya que este subgrupo de pacientes fue excluido con el fin de evitar posibles interferencias en la medición de los anticuerpos.

6.2 DISCUSIÓN DEL ESTUDIO 2

El principal hallazgo de este estudio fue demostrar que la e-nose es una tecnología no invasiva capaz de identificar los patrones de COV en el aire exhalado de pacientes con bronquiectasias y colonización bronquial por PA, y diferenciarlos de los de pacientes colonizados por otros MPP y no colonizados. Además, permitió distinguir con precisión entre los patrones de COV de pacientes con colonización bronquial y no colonizados. Estos hallazgos sugieren que la e-nose podría ser una herramienta útil para identificar la colonización bronquial por PA en pacientes con bronquiectasias clínicamente estables.

La colonización bronquial por MPP es una de las causas de morbilidad más frecuentes e importantes en los pacientes con bronquiectasias. Diferentes estudios han observado que los pacientes con colonización bronquial bacteriana tienen una peor evolución clínica y mayor gravedad de la enfermedad que los pacientes no colonizados (3, 19, 41). Nuestro estudio mostró una prevalencia del 56% de pacientes colonizados durante la fase de estabilidad clínica y, en general, estos pacientes también tenían antecedentes de exacerbaciones más frecuentes, valores más bajos de FEV_1 y peores puntuaciones en las escalas de gravedad de la enfermedad. La identificación de la colonización bronquial bacteriana es muy importante no solo para conocer el pronóstico de la patología, sino también porque nos alerta para iniciar tratamientos específicos dirigidos a su control, como los antibióticos nebulizados.

La e-nose ha demostrado ser capaz de detectar la colonización bronquial en estudios previos realizados en pacientes con EPOC. Concretamente, en el estudio realizado por Sibila y colaboradores (82) se incluyeron 37 pacientes con EPOC clínicamente estables y 13 controles sanos, a los cuales se les realizó un estudio microbiológico mediante un cepillado bronquial protegido. Los autores objetivaron que la e-nose puede discriminar de forma precisa entre los patrones de COV de pacientes con EPOC y colonización bronquial bacteriana de aquellos con EPOC sin colonización bronquial y de los de controles sanos (sensibilidad del 82%, especificidad del 96%) (82). En el presente estudio, los pacientes con bronquiectasias y colonización bronquial también presentaron diferentes patrones de COV en comparación con los no colonizados, usando la misma tecnología de e-nose (sensibilidad del 84%, especificidad del 58%).

La PA es un patógeno frecuentemente asociado con la colonización de las vías respiratorias en las bronquiectasias (43). Se ha demostrado que los pacientes con bronquiectasias colonizados por PA presentan exacerbaciones más frecuentes, una disminución más pronunciada del FEV_1 y una mayor tasa de mortalidad que otros pacientes (3, 19, 41, 43, 46). Además, los pacientes con colonización bronquial por PA han demostrado tener mayores niveles de mucinas en el esputo y de citoquinas, quimiocinas y elastasa de neutrófilos en la vía aérea, en comparación con otros sujetos con bronquiectasias (44, 45), estando ésta también relacionada con peores resultados clínicos y gravedad de la enfermedad (104). En nuestro estudio, la PA fue el MPP más frecuentemente aislado en el 66% de los pacientes colonizados, y como se describió en estudios previos, estos sujetos presentaron peores valores de función pulmonar

y puntuaciones más altas en las escalas de gravedad. Por lo tanto, no sólo es importante identificar la colonización bronquial bacteriana, sino también discriminar la presencia de MPP específicos tales como la PA.

Diversos estudios han utilizado la tecnología e-nose para discriminar la colonización bronquial por diferentes MPP basándose en el hecho de que los COV pueden producirse como parte del metabolismo bacteriano (105, 106). Lai y colaboradores utilizaron con éxito la e-nose para discriminar patrones de COV de escobillones de muestras *in vitro* de patógenos bacterianos respiratorios comunes como *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus* y *Pseudomonas aeruginosa* de muestras de control (88). Asimismo, Shafiek y colaboradores (83) demostraron que los pacientes con EPOC expresaban perfiles de COV diferentes durante las exacerbaciones infecciosas dependiendo del microorganismo causante usando el mismo dispositivo de e-nose de nuestro estudio, especialmente cuando se comparó PA versus *Haemophilus influenzae* (aunque sólo se incluyeron 8 pacientes en esta comparación). En nuestro estudio, utilizando un mayor tamaño de muestra, la e-nose mostró una muy buena precisión para discriminar los patrones de COV de pacientes con colonización bronquial por diferentes microorganismos, especialmente al comparar PA con otros MPP (sensibilidad del 92%, especificidad del 85%). Para nuestro conocimiento, este es el primer estudio en pacientes con bronquiectasias estables cuyo objetivo es explorar la utilidad de la tecnología e-nose no sólo para discriminar sujetos colonizados de no colonizados, sino también para identificar la presencia de colonización por PA.

Este estudio tiene limitaciones como el tamaño de muestra relativamente pequeño y la participación de un solo centro; sin embargo hemos validado previamente la utilidad de la e-nose en una cohorte menor de pacientes EPOC (82) y varios estudios que utilizan el mismo dispositivo tienen tamaños de muestra más reducidos (71, 76, 77). Además, las características de los pacientes en nuestro centro son muy similares a los reportados en Europa, en términos de demografía, etiología y bacteriología. Esto fortalece en gran medida la validez externa de este estudio (12, 27). El uso del cultivo de esputo para la evaluación bacteriológica comprende otra limitación del estudio, debido a la dificultad de algunos pacientes para obtener una buena muestra de esputo y la posibilidad de contaminación de la muestra con flora de la vía aérea superior. Sin embargo, la calidad de todas las muestras incluidas se evaluó usando los criterios de Murray-Washington. Finalmente, no se utilizó cromatografía de gases o espectrometría de masas para estudiar la correspondencia molecular de los diferentes patrones de COV. Para ello podría ser de gran interés desarrollar un estudio destinado a identificar los diferentes compuestos que caracterizan a cada grupo de pacientes.

7. CONCLUSIONES

7. CONCLUSIONES

Los resultados obtenidos de los estudios realizados en el marco de esta Tesis Doctoral han permitido llegar a las siguientes conclusiones:

1. La medición de los títulos de anticuerpos IgG específicos contra *Pseudomonas aeruginosa* tiene una precisión muy alta para detectar la colonización bronquial crónica por este microorganismo en pacientes con bronquiectasias. Además, esta prueba puede ser un marcador de gravedad de la enfermedad y de predicción de respuesta al tratamiento, aunque la decisión de iniciar el tratamiento de erradicación anti-PA dependerá del clínico tratante y de las características de cada paciente.

2. La nariz electrónica es una tecnología no invasiva que muestra resultados prometedores en la identificación de patrones de Compuestos Orgánicos Volátiles en el aire exhalado relacionados con la colonización bronquial bacteriana en pacientes con bronquiectasias durante la fase de estabilidad clínica, especialmente en aquellos colonizados por *Pseudomonas aeruginosa*. Por lo tanto, la e-nose puede convertirse en una herramienta útil junto con la microbiología del esputo para optimizar el manejo adecuado de los pacientes con bronquiectasias.

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BIBLIOGRAFÍA

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9.ANEXOS

9. ANEXOS

9.1 Publicaciones y manuscritos originales incluidos en la Tesis Doctoral:

- Suarez-Cuartin G, Smith A, Abo-Leyah H, Rodrigo-Troyano A, Perea L, Vidal S, Plaza V, Fardon TC, Sibila O, Chalmers JD. “Anti-Pseudomonas aeruginosa IgG antibodies and chronic airway infection in bronchiectasis”. *Respir Med*. 2017;128:1-6.

- Suarez-Cuartin G, Giner J, Merino JL, Rodrigo-Troyano A, Feliu A, Perea L, Sanchez-Reus F, Castillo D, Plaza V, Chalmers JD, Sibila O. “Identification of Pseudomonas aeruginosa airway colonization by an electronic nose in Bronchiectasis” (Enviado a *Respirology*. Manuscrito en revision. ID: RES-17-236).

9.2 Publicaciones relacionadas

En relación con los trabajos que forman parte de la presente Tesis Doctoral, los siguientes estudios también han sido publicados:

- Sibila O, Suarez-Cuartin G, Rodrigo-Troyano A, Fardon TC, Finch S, Mateus EF, Garcia-Bellmunt L, Vidal S, Sanchez-Reus F, Restrepo MI, Chalmers JD. “Secreted mucins and airway bacterial colonization in non-CF bronchiectasis”. *Respirology*. 2015;20(7):1082-8.

- Suarez-Cuartin G, Chalmers JD, Sibila O. “Diagnostic challenges of bronchiectasis”. *Respir Med*. 2016;116:70-7.

- Chalmers JD, Moffitt KL, Suarez-Cuartin G, Sibila O, Finch S, Furrie E, Dicker A, Wrobel K, Elborn JS, Walker B, Martin SL, Marshall SE, Huang JT, Fardon TC. “Neutrophil Elastase Activity Is Associated with Exacerbations and Lung Function Decline in Bronchiectasis”. *Am J Respir Crit Care Med*. 2017 May 15;195(10):1384-1393.

Respiratory Medicine 128 (2017) 1–6

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Anti-*Pseudomonas aeruginosa* IgG antibodies and chronic airway infection in bronchiectasis

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ARTICLE INFO

Article history:
Received 17 October 2016
Received in revised form
3 April 2017
Accepted 1 May 2017
Available online 2 May 2017

Keywords:
Bronchiectasis
Pseudomonas aeruginosa
Antibody
Chronic infection

ABSTRACT

Background: Identification of chronic *Pseudomonas aeruginosa* (PA) infection is important in the management of bronchiectasis, but requires repeated sputum sampling. We hypothesized that serum anti-PA IgG antibodies could diagnose chronic PA infection at a single visit.
Methods: Clinically stable bronchiectasis patients were studied prospectively. Chronic PA infection was defined as 2 or more positive sputum samples at least 3 months apart and/or failure to clear PA following eradication treatment. Baseline serum anti-PA IgG was determined by a validated ELISA kit.
Results: A total of 408 patients were included. Sixty of them (14.7%) had chronic PA infection and had higher anti-PA IgG levels (median 6.2 vs. 1.3 units, $p < 0.001$). Antibody levels showed direct significant correlations with exacerbation frequency, the bronchiectasis severity index and sputum inflammatory markers. Fifty-seven patients with chronic PA infection had a positive test, giving 95% sensitivity, 74.4% specificity and AUROC of 0.87. During follow-up, 38 patients had a new PA isolation. Eradication at 12 months was achieved in 89.5% of subjects with a negative antibody test and 15.8% of patients with a positive test.
Conclusions: Anti-PA IgG test is highly accurate to detect chronic PA infection in bronchiectasis patients. In addition, it may be a marker of disease severity and treatment response.

1. Introduction

Bronchiectasis is a chronic lung disease characterized by irreversible dilation of the bronchi, leading to failure of mucociliary clearance and neutrophilic inflammation [1]. This condition predisposes patients to chronic respiratory bacterial infection, which perpetuates airway inflammation [2].

Pseudomonas aeruginosa (PA) is one of the most common organisms isolated in bronchiectasis patients [3,4]. PA infection can be chronic or intermittent, depending on the presence of persistent isolation of this microorganism in respiratory samples or not [5]. Chronically infected patients with PA have worse quality of life, increased exacerbations and poorer prognosis [6]. Therefore, monitoring sputum microbiology to identify PA infection status in bronchiectasis is essential in order to select the best treatment option for each individual [7].

Studies to date have used different definitions of chronic PA infection, with a recent systematic review identifying that 2 positive sputum cultures at least 3 months apart in 1 year is the most widely used definition in bronchiectasis [6]. Methods used in CF are more rigorous, requiring samples every 3 months and at least 50% of them being positive for PA [8,9]. Standard of care for bronchiectasis across Europe currently does not incorporate regular sampling of patients, and in recent audits only 62% of patients in the UK and 27% of bronchiectasis patients from Italy had a sputum sample sent even once per year [10,11]. This presents challenges in clinical practice to identify patients with chronic PA, and in clinical trials to identify a target population with chronic PA.

Serum IgG PA antibodies have been proposed to diagnose

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http://dx.doi.org/10.1016/j.rmed.2017.05.001
0954-6111/

Abbreviations list

PA	*Pseudomonas aeruginosa*
CF	Cystic fibrosis
SGRQ	St. George's Respiratory Questionnaire
BSI	Bronchiectasis severity index
MPO	Myeloperoxidase

chronic bronchial infection, being a solution that can potentially identify this status at a single time point without longitudinal sputum sampling. In CF, different serological tests are available and most of these have shown a high sensitivity and specificity to detect chronic PA bronchial infection [12]. However, their routine use remains controversial [13]. Whilst PA infection is considered almost inevitable in CF with a prevalence of over 90% in adults [14], the rate of PA colonisation in bronchiectasis patients is around 21% [6]. Thus, identification of PA infection status in this latter group can be more challenging.

We hypothesized that specific anti-PA IgG antibody determination may be useful for identifying chronic PA infection in bronchiectasis patients.

2. Methods

2.1. Study design and ethics

This is a prospective study that included 408 clinically stable bronchiectasis patients. The study protocol was approved by the East of Scotland Research Ethics Committee (12/ES/0059) and all participants gave written informed consent to participate.

2.2. Participants

Patients were consecutively recruited from a specialist clinic at Ninewells Hospital in Dundee, United Kingdom 2012–2015 and were followed-up for 12 months. The diagnosis of bronchiectasis was confirmed in all cases with compatible clinical history of cough with sputum production and/or recurrent respiratory infections and presence of bronchial dilatation on high-resolution chest computed tomography scan. Patients with age less than 18 years; unable to give informed consent; patients with CF; active allergic bronchopulmonary aspergillosis (ABPA); active non-tuberculous mycobacterial disease; a primary diagnosis of pulmonary fibrosis with traction bronchiectasis and patients with immunodeficiency or receiving immunoglobulin replacement therapy were excluded.

2.3. Clinical assessment

All patients were clinically stable as defined by the absence of an exacerbation that required antibiotic or steroid treatment within 4 weeks prior to inclusion. Quality of life was assessed using the St. George's Respiratory Questionnaire (SGRQ) as this study was initiated prior to the availability of the disease specific Quality of Life Bronchiectasis Questionnaire (QOL-B). The aetiology of bronchiectasis was assessed as recommended by the British Thoracic Society guidelines [1]. Bronchiectasis severity index (BSI) and FACED score were determined as previously described [15,16].

2.4. Bacteriology

Spontaneous sputum samples were obtained for bacteriology and inflammatory marker measurement. Qualitative and quantitative bacteriology determination was performed in all samples as described previously [7]. Quality of sputum was evaluated using the Murray-Washington criteria [17].

Patients were classified into two groups according to previous chronic isolation of PA in sputum. Chronic PA infection was defined as 2 or more positive sputum samples at least 3 months apart and/or failure to clear PA following eradication treatment [18]. Standard of care at the study centre is to send sputum at all clinical encounters with a target for a minimum of 3 sputum cultures per year in expectorating patients. In a sensitivity analysis we evaluated a definition described by Lee et al. [19], referred to as the Leeds criteria. This required patients to have at least 3 sputum samples in the previous 12 months and at least 50% of samples to be positive for PA.

2.5. Specific anti-PA IgG measurement

Blood samples were obtained from all patients and processed for later antibody analysis by a validated commercially available ELISA kit (Pseudomonas-CF-IgG ELISA Kit. Statens Serum Institut, Denmark) following the manufacturer instructions [12,20,21]. The cut-off value for a positive ELISA Unit/10 result was 2.96 as determined by the manufacturer.

2.6. Airway biomarkers

Sputum samples were centrifuged at 50,000*g* for 90 min to obtain the soluble fraction. Neutrophil elastase activity and myeloperoxidase (MPO) activity in sputum supernatants were measured by chromogenic assay as previously described [7].

2.7. Statistical analysis

Results are presented as mean and standard deviation (SD) for continuous parametric data, and median and interquartile range for continuous non-parametric data. Categorical data is presented as frequencies and percentages. Continuous variables were analysed using t and ANOVA tests, whereas categorical variables were analysed using X^2 tests. Biomarkers and Anti-PA IgG levels were correlated by linear regression. A p value of less than 0.05 was considered significant. Statistical analysis was performed using the SPSS 22 software for Windows (SPSS, Chicago, Illinois, USA) and GraphPad Prism Version 6 (GraphPad Software Inc., San Diego, California, USA).

3. Results

3.1. Patient description

Four hundred and eight patients with clinically stable bronchiectasis were included. Of them, 247 (60.5%) were female, and mean age was 65.4 ± 12.7 years. The most frequent bronchiectasis aetiologies were idiopathic (43.9%) and post-infective (19.6%). Mean FEV_1 was 70.7 ± 24.4% of predicted value, and mean BSI score was 7.6 ± 4.7 points.

Sixty (14.7%) patients met the criteria for chronic PA infection at baseline. Table 1 shows the characteristics of the subjects, grouped by whether they met the criteria for chronic PA airway infection or not. Patients with chronic PA infection had significant more severe bronchiectasis (BSI score median 14.5 vs. 6 points, $p < 0.001$; and FACED score median 5 vs. 1 point, $p < 0.001$), more prior exacerbations (median 4 vs. 1, $p < 0.001$), and worse MRC dyspnoea score (median 3 vs. 2 points, $p < 0.001$). Patients with chronic PA infection had lower FEV_1% of predicted (median 72.5 vs. 55.3, $p < 0.001$)

Table 1
Demographics and clinical characteristics of bronchiectasis patients with and without chronic airway infection of *Pseudomonas aeruginosa*.

	No chronic Pa infection (N = 348)	Chronic Pa infection (N = 60)	*P* value
Age	67 (58–73.5)	70 (62–75)	0.06
Female (n,%)	215 (61.8%)	32 (53.3%)	0.21
Smoking status (n,%)			
Never	213 (61.2%)	40 (66.7%)	0.46
Ex-smoker	124 (35.6%)	17 (28.3%)	
Current	11 (3.2%)	3 (5%)	
MRC dyspnea score	2 (1–3)	3 (2.3–4)	*<0.001*
FEV_1 (% predicted)	72.5 (56.3–90.2)	55.3 (37.9–80.7)	*<0.001*
FVC (% predicted)	83.4 (70.9–99.1)	73.3 (59.4–92)	*0.003*
BMI (Kg/m^2)	25.2 (22.3–28.9)	25 (22.2–27.7)	0.31
Aetiology (n,%)			
Idiopathic	152 (43.7%)	27 (45%)	0.41
Post-infective	71 (20.4%)	9 (15%)	
ABPA	28 (8%)	8 (13.3%)	
Asthma	15 (4.3%)	0 (0)	
COPD	16 (4.6%)	3 (5%)	
CTD	22 (6.3%)	3 (5%)	
Immunodeficiency	19 (5.5%)	2 (3.3%)	
IBD	8 (2.3%)	2 (3.3%)	
Others	17 (4.9%)	6 (10%)	
Prior exacerbations (n)	1 (0–2)	4 (3–7)	*<0.001*
BSI score	6.0 (4–8)	14.5 (11.3–17)	*<0.001*
FACED score	1 (1–3)	5 (4–5)	*<0.001*
SGRQ score mean (±SD)	42.9 (21.9)	62.31 (21.1)	*<0.001*
Anti-Pa IgG levels (ELISA unit/10)	1.3 (0.6–3.1)	6.2 (4.6–10.2)	*<0.001*

All data is presented in median (quartiles 1–3) unless otherwise indicated.
Pa: *Pseudomonas aeruginosa*; FEV_1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; BMI: Body mass index; ABPA: Allergic bronchopulmonary aspergillosis; COPD: Chronic obstructive pulmonary disease; CTD: Connective tissue disease; IBD: Inflammatory bowel disease; BSI: Bronchiectasis severity index; SGRQ: Saint George's respiratory questionnaire; IgG: Immunoglobulin G.

and worse SGRQ scores (mean 62.3 ± 21.1 vs. 42.9 ± 21.9 points).

3.2. Specific anti-PA IgG test accuracy

Patients with chronic PA infection had higher baseline anti-PA IgG levels (median 6.2 vs. 1.3 units, $p < 0.001$). A positive IgG result at >2.96 units was found in 57 (95%) patients meeting the definition of chronic PA infection, and 89 (25.6%) of the patients without chronic PA infection. Sensitivity, specificity, positive and negative predictive value are shown in Table 2. Area under the ROC curve (AUROC) of the test was 0.87.

Among all patients, 127 (31.1%) were chronically infected with *Haemophilus influenzae*. In order to study the effect of possible cross-reacting antibodies induced by other Gram-negative micro-organisms [12], a subgroup analysis was performed excluding these patients. There was no change in the sensitivity of the test, but specificity and positive predictive values increased and negative predictive value and AUROC slightly decreased. Table 2 shows these test accuracy values for the subgroup analysis.

We investigated the characteristics of the 89 "false positives" patients who had a positive anti-PA IgG test without known chronic PA infection. Of them, 33 (37.1%) were chronically infected with *H. influenzae*, and 10 (11.2%) had a positive sputum culture for PA in the year prior to enrolment but did not meet criteria for chronic infection. They did not present statistically significant differences with "true negative" patients (n = 259) regarding bronchiectasis severity, number of prior exacerbations, SGRQ scores, percentage of predicted spirometric values or sputum elastase and MPO levels. Of the 10 subjects with isolation of PA in the year prior to the study, 6 subsequently met the criteria for chronic PA infection during the study period, suggesting they may have been "true positives".

We investigated varying cut-offs of the PA IgG test, since the manufacturers cut-off was developed for CF patients. Lowering the cut-off to 2 units did not improve sensitivity, but reduced specificity to 63%. A sensitivity of 100% could only be achieved with lowering the cut-off to 0.1 with a specificity of 9%. Increasing the cut-off to 4 units (for example) increased the specificity to 83% but reduced sensitivity to 78.3%. A cut-off above 13 was required to achieve a specificity of 100%, at the expense of very low sensitivity.

Using a more rigorous definition of chronic PA infection resulted in an increased accuracy of the test. 56/60 patients with PA had 3 or more sputum samples in the previous year and 52 met the Leeds criteria for chronic PA infection. All of these patients had a positive anti-PA IgG test. Of the 3 patients with a "false negative" antibody test, 2 did not meet the Leeds criteria. In one case the patient had 6 sputum cultures in the previous year with only 2 (nevertheless >3 months apart) showing PA infection. In the second case they had 2 positive sputum cultures for PA early in the year, but had grown *H. influenzae* and *Moraxella catarrhalis* in subsequent sputum samples. The third patient was excluded due to only having 2

Table 2
Sensitivity, specificity and positive and negative predictive values for the Anti-PA IgG test in all subjects and excluding patients with chronic *Haemophilus influenzae* airway infection.

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	AUROC
All patients (n = 408)	95%	74.4%	39%	98.9%	0.87
Excluding patients with chronic Hi infection (n = 281)	95%	74.7%	50.4%	98.2%	0.86

Hi: *Haemophilus influenzae*; AUROC: Area under ROC curve.

sputum samples in the previous year. Thus applying the Leeds criteria, anti-PA IgG antibody had a 100% sensitivity and specificity of 89% (n = 293 patients with 3 or more sputum samples available in the previous 12 months).

3.3. Anti-PA IgG levels and severity of disease

There was a significant correlation between anti-PA IgG levels and the number of prior exacerbations ($r = 0.168$; $p < 0.001$), BSI and FACED scores ($r = 0.281$; $p < 0.001$ and $r = 0.278$; $p < 0.001$ respectively), the SGRQ ($r = 0.152$; $p = 0.006$), and sputum neutrophil elastase ($r = 0.228$; $p < 0.001$) and MPO ($r = 0.168$; $p < 0.001$), as presented in Fig. 1. After excluding patients with known PA infection, there were no significant correlations with BSI ($p = 0.2$), FACED ($p = 0.1$), SGRQ ($p = 0.5$) or any other parameters.

3.4. Patient follow-up

During the study, 38 patients had a new isolation of PA in sputum. Of them, 19 (50%) had a negative anti-PA IgG test at isolation. According to local protocol, all of them underwent eradication treatment consisting of 2 weeks of ciprofloxacin, followed by 2 weeks of intravenous antibiotics and 3 months of nebulized colistin [1]. Eradication, defined as absence of PA isolation in sputum after 3 and 12 months, was achieved in 17 (89.5%) patients with a negative IgG test and 3 (15.8%) patients with a positive antibody test. Fig. 2 summarizes the distribution of PA eradication treatment efficacy in patients with positive and negative anti-PA IgG test.

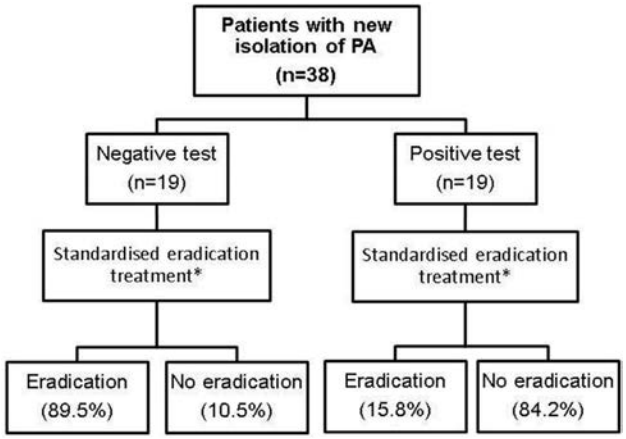

Fig. 2. Distribution of *Pseudomonas aeruginosa* eradication treatment efficacy in patients with positive and negative anti-PA IgG test.
(PA: *Pseudomonas aeruginosa*; IgG: Immunoglobulin G.
*Standardised eradication treatment: 2 weeks of ciprofloxacin, followed by 2 weeks of intravenous antibiotics and 3 months of nebulized colistin).

4. Discussion

This study has shown that serum anti-PA IgG levels have a high diagnostic accuracy for identification of chronic PA airway infection in bronchiectasis patients. Importantly; the test was also a marker of treatment response, identifying patients likely to clear PA infection following eradication treatment, although this analysis is

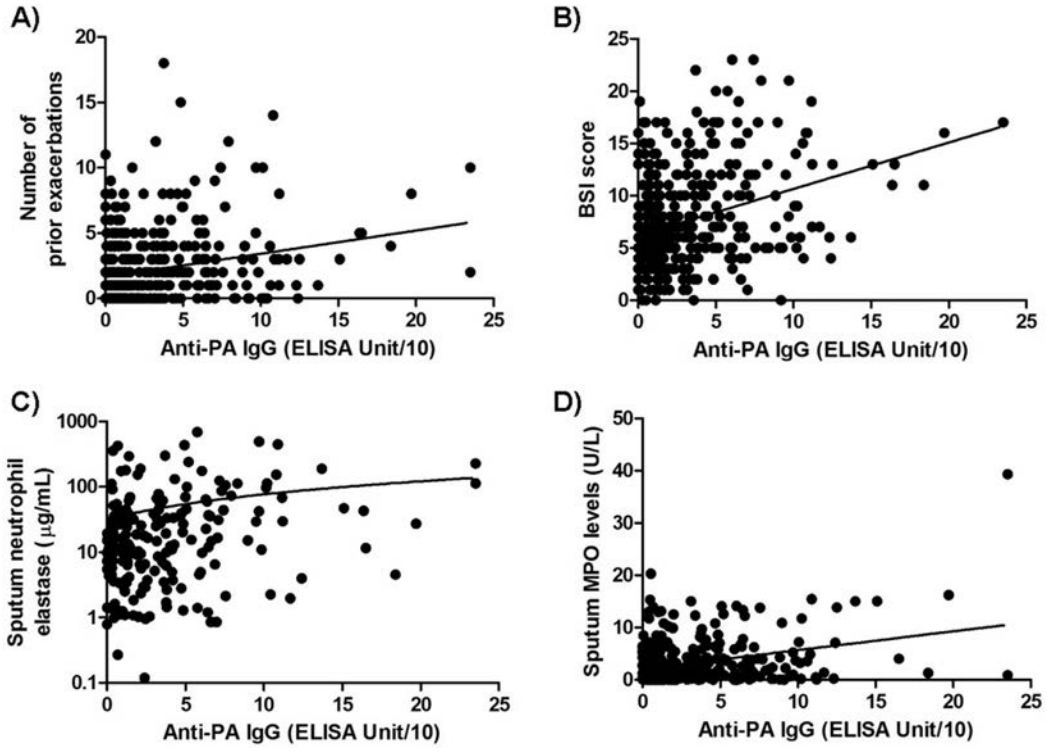

Fig. 1. Correlation of anti-PA IgG levels with the number of prior exacerbations per year (A), disease severity (B) and sputum inflammatory markers (C,D).
(PA: *Pseudomonas aeruginosa*; IgG: Immunoglobulin G; BSI: Bronchiectasis severity index; MPO: Myeloperoxidase).

limited by the absence of a control group without antibiotic treatment.

PA infection plays an important role in the pathogenesis of bronchiectasis. Previous studies have demonstrated that patients with bronchiectasis and PA infection have higher levels of airway neutrophil biomarkers, cytokines, chemokines and mucins compared to subjects colonised with other microorganisms [7,22]. Furthermore, chronic PA infection is related to more frequent exacerbations, worse quality of life, lower FEV_1 and a higher mortality rate [6,23,24]. Our cohort had 14.7% of patients with chronic PA infection, showing similarities with these previous studies. In addition, chronically PA infected patients had worse lung function values and SGRQ scores, as well as more prior exacerbations and more severe bronchiectasis. Thus, identification of chronic PA infection is clinically important in patients with bronchiectasis.

Anti-PA IgG test has shown a high accuracy diagnosing chronic PA infection in patients with CF. Pressler et al. demonstrated a sensitivity of 96% and specificity of 83% for identification of different PA infection status using this test [12]. However, this test is still not widely used in clinical practice. This is probably related in part to the high prevalence of PA infection and to the close microbiologic monitoring of CF patients. Current CF guidelines recommend performing sputum cultures at least every 3 months [9,14], making the antibody test dispensable in most cases. On the other hand, the prevalence of PA infection in bronchiectasis is significantly lower [6] and there is a high variability in the microbiologic management of these patients, as many centres perform only one sputum culture per year or no sputum monitoring at all [10,11]. Also, up to 27% of bronchiectasis patients do not produce daily sputum, which can make the microbiologic follow-up more difficult [25]. These differences suggest that the anti-PA IgG test could potentially have a more important role in discriminating chronic PA infection in bronchiectasis patients. Limited data is available regarding the utility of anti-PA IgG antibodies in bronchiectasis. Caballero et al. studied 56 bronchiectasis patients and classified them into three groups depending on the absence or the chronic or intermittent isolation of PA in sputum. They found a significant correlation between anti-PA antibodies detected by Western blot and the frequency of PA isolation in sputum, suggesting that different degrees of PA infection could be identified in bronchiectasis [26]. Nevertheless, these positive results are difficult to extrapolate. Our study used a validated ELISA kit, showing a similar diagnostic value with a sensitivity and specificity of 95% and 74.4%, respectively.

The clinical implications of our study are as follows. Although the ideal scenario is that patients with bronchiectasis should be regularly followed-up with a minimum of 2–3 sputum samples per year, this is not currently performed as part of clinical practice across most of Europe, North America and the rest of the world. Thus, upon isolation of PA from a single sputum sample, it is not clear if this infection is likely to be chronic and potentially requiring suppressive treatment to prevent exacerbations, or is likely to be transient and will be cleared with antibiotic treatment or spontaneously. In this situation, measurement of anti-PA IgG antibodies may have a role. A high anti-PA IgG will alert the clinician to a high likelihood of chronic infection and a low likelihood of success of eradication treatment. Conversely, a low anti-PA IgG will alert the clinician to a high likelihood of clearance either with antibiotic treatment or spontaneously. Nevertheless, this test clearly does not replace the need for regular sputum monitoring and must be used in conjunction with sputum positivity for PA, as we demonstrated cross-reactivity and high rate of positive tests accompanying infection with *H. influenzae*. The significance and utility of the anti-PA IgG test needs to be interpreted in the clinical context of each patient, especially in those cases with a positive antibody test and negative sputum culture.

Defining chronic PA infection is crucial to identifying populations that should be treated with antibiotics directed against PA, such as inhaled antibiotics. Our study highlights the difficulty in defining chronic PA infection in bronchiectasis. The most widely used definition incorporates the isolation of the organism on two occasions at least 3 months apart over 1 year. In this study PA IgG antibody test had a 95% sensitivity using this “gold standard” to define colonisation. Nevertheless using a more rigorous definition requiring 3 positive cultures at least 50% of which were positive for PA, the antibody test was 100% sensitive and was more specific. This has been clearly demonstrated in randomized trials. In a trial of nebulized colistin, Haworth and colleagues required patients to have 2 positive sputum samples for PA in the previous 12 months for entry, and a positive PA culture at screening. Despite this rigorous entry criteria, only 53/62 (85%) of patients in the placebo group were growing PA at the final study visit, indicating a high rate of “spontaneous clearance” or intermittent negative cultures even with the use of a rigorous definition [27]. Similar results were observed in the placebo arm of aztreonam and gentamicin trials [28,29]. Our data suggests that using a PA IgG antibody test may increase the likelihood that patients have chronic PA infection if used as an inclusion criterion for bronchiectasis trials.

Eradication treatment for PA is challenging, though widely practised, in both CF and bronchiectasis. Initial PA infection is reported to be successfully eradicated in 70–80% of CF patients [30–32] and in up to 22–55% of bronchiectasis subjects [33–36]. However, only 34% of bronchiectasis patients colonised with PA cease to grow this microorganism in their sputum [5]. In our study, PA eradication at 12 months was achieved only in 15.8% of patients with a positive anti-PA IgG test and in 89.5% of subjects who had a negative test. This suggests that the antibody test is not sufficiently sensitive or specific to guide treatment decisions but predicts treatment response. It may be appropriate to test this first in a prospective trial.

This study has some limitations. Although a large number of subjects were included, it is a single centre study. Another limitation is the absence of molecular diagnostics such as PA PCR or microbiome characterisation which may better define PA colonisation compared to culture. Nevertheless we consciously chose not to conduct molecular analysis because to date, the clinical significance of a positive PA PCR or the presence of PA as part of the microbiome is not clear. The aim of this study is to use the anti-PA IgG test to provide relevant clinical information to sputum culture in clinical practice, since it still is the standard of care for microbiologic assessment in bronchiectasis patients. Furthermore, all of our existing data on the prognostic implications of PA infection is based on repeated isolation in cultures and therefore PA PCR testing should first be evaluated for its clinical and prognostic value in a separate study. Another limitation of the study is that these results are not applicable to patients with immunodeficiency, as this subgroup of patients was excluded in order to avoid potential complications in the antibody assessment.

5. Conclusions

In summary, the anti-PA IgG test has a very high accuracy to detect chronic PA infection in bronchiectasis patients. This test is not sufficiently sensitive or specific to guide the decision to eradicate PA but may be a marker of severity of disease and treatment response.

Funding sources

The research was supported by Tenovus Scotland, Sociedad

Española de Neumología (SEPAR), Fundació Catalana de Pneumologia (FUCAP), Societat Catalana de Pneumologia (SOCAP) and EMBARC (A European Respiratory Society Clinical Research Collaboration).

Conflicts of interest

Dr. Chalmers has received research grants in the field of bronchiectasis from Bayer Healthcare, Aradigm Corporation and Chiesi on behalf of the European Bronchiectasis Network (EMBARC). Rest of authors have no conflicts of interest.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.rmed.2017.05.001.

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Respirology

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Journal:	*Respirology*
Manuscript ID	RES-17-236
Manuscript Type:	Original Article
Date Submitted by the Author:	03-May-2017
Complete List of Authors:	Suarez-Cuartin, Guillermo; Hospital de la Santa Creu i Sant Pau, Respiratory Medicine; Institut d'Ivestigació Biomédica Sant Pau (IIB) Giner, Jordi; Hospital de la Santa Creu i Sant Pau, Respiratory Medicine Merino, Jose; Universitat de les Illes Balears, Electronic Systems Group Rodrigo-Troyano, Ana; Hospital de la Santa Creu i Sant Pau, Respiratory Medicine Feliu, Anna; Hospital de la Santa Creu i Sant Pau, Respiratory Medicine Perea, Lidia; Institut d'Ivestigació Biomédica Sant Pau (IIB), Experimental Immunology Sanchez-Reus, Ferran; Hospital de la Santa Creu i Sant Pau, Microbiology Department Castillo, Diego; Hospital de la Santa Creu i Sant Pau, Respiratory Medicine Plaza, Vicente; Hospital de la Santa Creu i Sant Pau, Respiratory Medicine Chalmers, James; University of Dundee, Scottish Centre for Respiratory Research Sibila, Oriol; Hospital de la Santa Creu i Sant Pau, Respiratory Medicine
Subject Category – Select up to 3 subject categories that best match your manuscript and list them in order of preference.:	Bronchiectasis, Respiratory Infections
Keywords - Select up to 5 keywords:	Bronchiectasis, Respiratory Infections (non-tuberculous)

Manuscript word count: 2136 Abstract word count: 210

Identification of *Pseudomonas aeruginosa* airway colonization by an electronic nose in Bronchiectasis

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All authors declare to have no conflicts of interest regarding the elaboration of this manuscript.

Summary at a Glance

This study aims to determine the utility of the electronic nose to detect airway colonization in clinically stable bronchiectasis patients. The electronic nose showed accurate breath-print pattern discrimination of subjects with *Pseudomonas aeruginosa* airway colonization from bronchiectasis patients non-colonized or colonized by other potentially pathogenic microorganisms.

Abstract

Background: Airway colonization by Potentially Pathogenic Microorganisms (PPM) in bronchiectasis is associated with worse clinical outcomes. The electronic nose is a non-invasive technology capable of distinguishing volatile organic compounds (VOC) breath-prints in exhaled breath. We aim to explore if an electronic nose can reliably discriminate airway bacterial colonization in patients with bronchiectasis.

Methods: Seventy-three clinically stable bronchiectasis patients were consecutively included in a cross-sectional study. The presence of PPM in the airways was determined using sputum culture. At the same time, exhaled breath was collected in Tedlar bags and VOC breath-prints were detected by the commercially available electronic nose Cyranose 320®.

Results: Forty-one patients with bronchiectasis (56%) were colonized with PPM. *Pseudomonas aeruginosa* (n=27, 66%) was the most common PPM, followed by *Haemophilus influenzae* (n=7, 17%). VOC breath-prints from colonized and non-colonized patients were significantly different (accuracy of 72%, AUROC 0.75, $p<0.001$). VOC breath-prints from *Pseudomonas aeruginosa* colonized patients were significantly different from those of patients colonized with other PPM (accuracy of 89%, AUROC 0.97, $p<0.001$) and non-colonized patients (accuracy 73%, AUROC 0.83, $p=0.007$).

Conclusions: An electronic nose can accurately identify VOC breath-prints of clinically stable bronchiectasis patients with airway bacterial colonization, especially in those with *Pseudomonas aeruginosa.*

This study was registered in www.clinicaltrials.gov on April 2014. ClinicalTrials.gov ID: NCT02163642.

Keywords: Bronchiectasis; Electronic nose; Pseudomonas aeruginosa; Volatile organic compounds.

Short title: *P. aeruginosa* & e-nose in Bronchiectasis

Abbreviations list

BSI: Bronchiectasis severity index

CF: Cystic fibrosis

E-nose: Electronic nose

PPM: Potentially Pathogenic Microorganisms

VOC: Volatile Organic Compounds

Introduction

Bronchiectasis is a chronic respiratory condition characterized by irreversible dilation of the bronchi and chronic airway inflammation.[1] Recent studies have observed an increased prevalence of bronchiectasis across Europe and the United States,[2–4] and a high annual economic burden that increases with disease severity and with the number of exacerbations.[5]

Airway colonization by potentially pathogenic microorganisms (PPM) is an important cause of morbidity in bronchiectasis patients, and *Pseudomonas aeruginosa* is one of the most frequently isolated pathogens. The presence of *P. aeruginosa* airway colonization is associated with more frequent exacerbations and a higher mortality rate compared to patients without *P. aeruginosa* infection.[6,7] Therefore, microbiological assessment determined by sputum culture analysis is one of the key factors in the characterization of bronchiectasis patients. However, sputum culture has limitations such as time delay for results and the difficulty to obtain proper sputum samples.[8,9] Thus, sputum analysis is not used routinely as a standard of care in many hospitals.[10,11] In these cases, other techniques for microbiological characterization may be helpful.

The electronic nose (e-nose) is a non-invasive diagnostic device that contains an array of electronic chemical sensors capable of identifying volatile organic compounds (VOC) breath-prints.[12,13] The e-nose has demonstrated a good diagnostic value in identifying different airway respiratory diseases such as COPD,[14] asthma[15] and cystic fibrosis (CF)[16]. Furthermore, several studies have shown that the e-nose is also able to detect respiratory infections. In COPD, the e-nose has successfully distinguished patients with and without

airway bacterial infection during clinical stability[17] and acute exacerbations[18]. Some studies have suggested that specific bacteria such as *P. aeruginosa*, may produce concrete VOC.[19–21] Thus, the e-nose has been able to identify the presence of *P. aeruginosa* infection both in CF patients[22] and in swabs of bacteria obtained from *in vitro* cultures.[23] However, data regarding the use of the e-nose in bronchiectasis, and its potential role identifying *P. aeruginosa* is scarce.

We hypothesized that the e-nose could accurately discriminate VOC breath-prints from bronchiectasis patients with and without airway bacterial colonization, especially in those with *P. aeruginosa*. Therefore, the aim of this study is to explore if an electronic nose can reliably discriminate airway bacterial colonization in clinically stable patients with bronchiectasis.

Methods

Study design and Ethics

This is a cross-sectional study that included clinically stable bronchiectasis patients with and without airway bacterial colonization. The study protocol was approved by the institutional ethics committee (IIBSP-BRO-2013-154) and patients gave their informed consent. This study was registered in www.clinicaltrials.gov on April 2014. ClinicalTrials.gov ID: NCT02163642.

Study population

Patients were consecutively recruited from a specialist clinic at the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain, between June 2014 and May 2016. **Figure 1** shows the study approach for patient enrolment. The

diagnosis of bronchiectasis and its etiological assessment were established according to national and international guidelines.[1,24] Patients with age less than 18 years; unable to give informed consent or with other respiratory diseases such as CF, active allergic bronchopulmonary aspergillosis, active non-tuberculous mycobacterial infection or pulmonary fibrosis with traction bronchiectasis, as well as patients receiving immunoglobulin replacement therapy or chronic systemic corticosteroid treatment were excluded. Sample size was calculated as described in previous studies[17,18].

Clinical and functional assessment

All subjects were included during clinical stability, defined as the absence of an exacerbation requiring antibiotic or systemic corticosteroid treatment within the previous 30 days of inclusion. A detailed clinical history was obtained from all participants, including demographic data, smoking status, relevant comorbid conditions, current treatment and the number of previous outpatient and hospitalized exacerbations. Severity of disease was assessed with the Bronchiectasis Severity Index (BSI) and FACED scores.[25,26] Spirometry was performed according to international recommendations,[27] using the reference values for Mediterranean population.[28]

Bacteriology

Spontaneous sputum samples for bacteriology were obtained from all participants on inclusion, and were processed as we described previously.[29] Quality of sputum was evaluated using the Murray-Washington criteria.[30] Patients were classified according to sputum bacteriology assessment into

three groups: non-colonized, colonized by *P. aeruginosa* and colonized by other PPM (*Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus* and other Gram-negative bacilli).

Exhaled breath analysis

Breath samples were obtained from all participants to assess VOC profiles with the e-nose as we described previously.[17,31] In summary, exhaled breath samples were collected in 10 litres Tedlar bags after 3 minutes of tidal breathing through a Hans-Rudolph valve with an expiratory silica reservoir exposed to dry air and an inspiratory filter. The e-nose device (Cyranose 320®; Smith Detections, CA, USA), a chemical vapour analyser with 32 organic polymeric Nano-composite sensor arrays, was then connected to the Tedlar bag for 5 minutes. The exposure to exhaled breath generated a breath-print VOC profile for each subject.

All participants stopped their inhaled medications and fasted for at least 12 hours before the breath sampling.

Data analysis

Results are presented as mean and standard deviation (SD) for continuous parametric data, and median and interquartile range (IQR) for continuous non-parametric data. Categorical data is presented as frequencies and percentages. Statistical analysis was performed using the SPSS 22 software for Windows (SPSS; Illinois, USA). A p value of less than 0.05 was considered significant.

Breath-print data from all participants was analysed using a pattern-recognition application built in the MATLAB software (v.R2012a) as we described previously.[17,31] In short, raw data was reduced to three principal factors by principal component analysis (PCA). These PCA factors were used to perform a univariate ANOVA, followed by *post-hoc* least significant difference test. Patients were then classified into a categorical division using a linear canonical discriminant analysis, calculated as the one that obtained the better percentage of correctly classified subjects. The discriminant function was trained with all minus one subject samples. Then, the remaining samples were tested. This process known as the "leave-one-out" method was repeated for all subjects, thus building the percentage of correctly classified patients which defined cross-validation accuracy values.[14,17,18,31] A Receiver Operating Characteristics (ROC) was obtained using the discriminant function results. The area under the ROC curve was calculated with multiple logistic regression.

Results

Patient description

Seventy-three clinically stable bronchiectasis patients were included. Of them, 47 (64%) were female and median age was 69 years (IQR 60-76.5 years). Mean FEV_1 was 65.9 ± 23.3% of predicted; median BSI score was 7 points (IQR 6-11 points) and median FACED score was 2 points (IQR 1-4 points). The most frequent aetiologies were post-infective (47%) and idiopathic (19%).

Forty-one (56%) patients were classified as colonized. The most frequent isolated PPM were *P. aeruginosa* (n=27; 66%), *Haemophilus influenzae* (n=7;

17%), *Escherichia coli* (n=2; 5%) and *Streptococcus pneumoniae* (n=2; 5%). Other isolated PPM included *Moraxella catarrhalis*, *Achromobacter xylosoxidans* and *Staphylococcus aureus* (n=1 each, 2%).

Baseline characteristics of colonized and non-colonized subjects are summarized in **Table 1**. Patients with airway colonization had lower lung function values (mean FEV1 76.8 ± 22.5 vs. 57.6 ± 20.5, $p<0.001$) and more severe bronchiectasis (BSI score median 6 vs. 10, $p<0.001$; and FACED score median 2 vs. 3, $p<0.001$). Colonized patients were subsequently classified into 2 subgroups according to the isolated microorganism in sputum culture; 27 subjects (66%) were colonized with *P. aeruginosa*, and 14 (34%) with other PPM. Demographic and clinical characteristics of these subgroups are showed in **Table 2**. Patients with *P. aeruginosa* airway colonization were more associated with post-infective aetiology, had more severe bronchiectasis (BSI score median 11 vs. 6, $p=0.01$; and FACED score median 4 vs. 2, $p=0.001$), and had a higher use of long-acting beta agonists (85% vs. 35%, $p=0.001$) and inhaled corticosteroids (74% vs. 35%, $p=0.01$) compared to those patients colonized with other PPM.

Breath-print analysis

Bronchiectasis patients with and without airway bacterial colonization had significantly different breath profiles (**Figure 2**). Cross-validation accuracy was 72.1%, and Area under ROC curve (AUROC) was 0.75 ($p=0.01$) (**Table 3)**.

Subjects with airway bacterial colonization were then analysed. VOC breath-print profiles from subjects colonized by *P. aeruginosa* and by other PPM

were marked different (**Figure 3**), with a cross-validation accuracy of 89.2% and AUROC of 0.96 (p<0.001) (**Table 3**).

In addition, patients with *P. aeruginosa* airway colonization were compared with non-colonized subjects. This analysis also showed significant differences in breath-print profiles (**Figure 4**), with a cross-validation accuracy of 72.7% and AUROC of 0.82 (p=0.007) (**Table 3**).

Discussion

This study showed that an electronic nose is a non-invasive technology capable of identifying VOC breath-prints from bronchiectasis patients with and without airway bacterial colonization. Moreover, it can accurately distinguish breath-prints of bronchiectasis subjects with *P. aeruginosa* airway colonization from those colonized with other PPM and non-colonized. These findings suggest that the e-nose could be a useful tool to identify airway bacterial colonization in clinically stable bronchiectasis patients.

Airway bacterial colonization by PPM is one of the most frequent and important causes of morbidity in bronchiectasis patients. Different studies have observed that patients with airway bacterial colonization, either by *P. aeruginosa* or other PPM, have worse clinical outcomes and more severity of disease than non-colonized patients.[9,25] Our study showed a 56% prevalence of colonized patients during clinical stability, and overall these patients also had a history of more frequent exacerbations, lower FEV_1 values and worse disease severity scores. Identifying airway bacterial colonization is highly important because of its prognostic significance and also because most therapies for bronchiectasis target airway infection, such as inhaled antibiotics. We

previously demonstrated an accurate discrimination of COPD patients with and without airway bacterial colonization using the e-nose (sensitivity of 82%; specificity of 96%).[17] In this study, bronchiectasis patients with airway colonization also had different VOC breath-prints compared to non-colonized ones using the same e-nose technology (sensitivity of 84%; specificity of 58%).

P. aeruginosa is a pathogen frequently associated with airway colonization in bronchiectasis.[7] It has been demonstrated that bronchiectasis patients colonized by *P. aeruginosa* have more frequent exacerbations, a steeper decline in FEV_1 and a higher mortality rate than other patients.[6,7,9,25,26] In addition, patients with airway *P. aeruginosa* colonization have shown higher levels of sputum mucins and airway cytokines, chemokines and neutrophil elastase when compared to other bronchiectasis subjects,[29,32] with this latter being also related to worse clinical outcomes and disease severity.[33] In our study, *P. aeruginosa* was the most frequently isolated PPM in 66% of the colonized patients, and as described in previous studies, these subjects had worse lung function values and higher severity scores. Therefore, it is important not only to identify airway bacterial colonization but also to discriminate the presence of specific PPM such as *P. aeruginosa*.

Several studies have used the e-nose technology to discriminate between airway colonization by different PPM, based on the fact that VOC may be produced as a part of bacterial metabolism.[34,35] Lai et al. successfully used the e-nose on swabs from *in vitro* samples to discriminate VOC patterns of common respiratory bacterial pathogens such as *H. influenzae*, *S. pneumoniae*, *S. aureus* and *P. aeruginosa* from control samples.[23] Using the same e-nose as in our study, Shafiek et al.[18] demonstrated that COPD patients expressed

different VOC profiles during infectious exacerbations depending on the causative bacteria, especially when comparing *P. aeruginosa* versus *H. influenzae*, although only 8 patients were included in this comparison. In our study, using a higher sample size, the e-nose showed a high accuracy to discriminate VOC breath-prints between airway colonization by different microorganisms, especially when comparing *P. aeruginosa* with other PPM (sensitivity of 92%, specificity of 85%). To our knowledge, this is the first study in stable bronchiectasis patients aimed to explore the utility of the e-nose technology not only for discriminating colonized from non-colonized subjects, but also for identifying the presence of *P. aeruginosa* airway colonization.

This study has limitations as the relatively small sample size obtained from a single centre; however we have previously validated the utility of the e-nose in a smaller COPD cohort[17] and several studies using the same device have lower sample sizes.[15,16,31] In addition, the characteristics of patients in our centre are very similar to those reported from across Europe in terms of demographics, aetiology and bacteriology. This greatly strengthens the external validity of this study.[36,37] The use of sputum analysis for bacteriology assessment comprehends another limitation of the study, due to the difficulty of some patients to obtain a good sputum sample and the possibility of ample contamination with bacteria from the upper respiratory tract. Nevertheless, the quality of all samples included was assessed using the Murray-Washington criteria as mentioned above. Finally, we did not use gas chromatography or mass spectrometry to study the molecular correspondence of the different VOC patterns. A study aimed to identify the different compounds that characterize each group would be of great interest.

In conclusion, the electronic nose is a non-invasive technology that shows promising results in the identification of VOC breath-prints related to airway bacterial colonization in bronchiectasis patients during clinical stability, especially in those colonized by *P. aeruginosa*. Therefore, it may become a useful tool alongside sputum microbiology to improve the proper management of bronchiectasis patients.

Acknowledgments

All authors have critically revised and given their final approval of the manuscript.

This project was supported by: Sociedad Española de Neumología y Cirugía Torácica (SEPAR), Societat Catalana de Pneumologia (SOCAP) and Fundación AstraZeneca (Programa de Fomento de los jóvenes científicos españoles).

Role of sponsors: The sponsors had no role in the design of the study, acquisition, analysis and interpretation of the data, or in the preparation of the manuscript.

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Table 1. Demographics and clinical characteristics of bronchiectasis patients with and without airway colonization.

	Non Colonized (N=32)	**Colonized (N=41)**	**p**
Age (median (IQR)	69.5 (59-75.8)	68 (60.5-77.5)	0.726
Female gender (n,%)	22 (68.8%)	25 (61%)	0.491
Smoking status (n,%) Never Former Current	 24 (75%) 7 (21.9%) 1 (3.1%)	 31 (75.6%) 10 (24.4%) 0	0.514
Cardiovascular disease (n,%)	5 (15.6%)	9 (22%)	0.496
Diabetes mellitus (n,%)	2 (6.3%)	4 (9.8%)	0.588
MRC dyspnoea score (median (IQR)	2 (1-2)	2 (2-3)	*<0.001*
Aetiology (n,%) Post infective Connective tissue disease Primary ciliary dyskinesia Immunodeficiency Inactive ABPA COPD Others Idiopathic	 16 (50%) 2 (6.3%) 2 (6.3%) 1 (3.1%) 0 2 (6.3%) 1 (3.1%) 8 (25%)	 18 (43.9%) 7 (17.1%) 2 (4.9%) 2 (4.9%) 3 (7.3%) 1 (2.4%) 2 (4.9%) 6 (14.6%)	0.521
FVC % of predicted (mean ± SD)	87.9 ± 20.6	74.7 ± 18.4	*0.005*
FEV_1 % of predicted (mean ± SD)	76.8 ± 22.5	57.6 ± 20.5	*<0.001*
Number of exacerbations in the previous year (median (IQR)	2 (1-3)	3 (2-4)	0.392
Bronchiectasis severity index score (median (IQR)	6 (4-8)	10 (6-13)	*<0.001*
FACED score (median (IQR)	2 (1-3)	3 (2-4)	*<0.001*
LABA use (n,%)	18 (56.3%)	28 (68.3%)	0.290
LAMA use (n,%)	8 (25%)	19 (46.3%)	0.061
ICS use (n,%)	16 (50%)	25 (61%)	0.348
Chronic macrolides (n,%)	6 (18.8%)	10 (24.4%)	0.563

All data is presented in median (quartiles 1-3) unless otherwise indicated.
MRC: Medical Research Council; ABPA: Allergic bronchopulmonary aspergillosis; COPD: Chronic obstructive pulmonary disease; FVC: Forced vital capacity; FEV_1: Forced expiratory volume in 1 second; SABA: Short-acting beta agonists; LABA: Long-acting beta agonists; LAMA: Long-acting muscarinic receptor antagonists; ICS: Inhaled corticosteroids.

Table 2. Demographics and clinical characteristics of bronchiectasis patients with airway colonization by *Pseudomonas aeruginosa* and other potentially pathogenic bacteria.

	Colonized with *P. aeruginosa* (N=27)	**Colonized with other PPB (N=14)**	**p**
Age (median (IQR)	68 (63-77)	67 (58-78)	0.591
Female gender (n,%)	18 (66.7%)	7 (50%)	0.300
Smoking status (n,%) Never Former Current	 19 (70.4%) 8 (29.6%) 0	 12 (85.7%) 2 (14.3%) 0	0.278
MRC dyspnoea score (median (IQR)	3 (2-3)	2 (2-3)	0.166
Aetiology (n,%) Post infective Connective tissue disease Primary ciliary dyskinesia Immunodeficiency Inactive ABPA COPD Others Idiopathic	 16 (59.3%) 2 (7.4%) 0 0 2 (7.4%) 1 (3.7%) 1 (3.7%) 5 (18.5%)	 2 (14.3%) 5 (35.7%) 2 (14.3%) 2 (14.3%) 1 (7.1%) 0 1 (7.1%) 1 (7.1%)	*0.013*
FVC % of predicted (mean ± SD)	72.3 ± 19.1	79.4 ± 16.6	0.248
FEV_1 % of predicted (mean ± SD)	54.9 ± 22.2	62.7 ± 16.2	0.251
Number of exacerbations in the previous year (median (IQR)	3 (2-4)	2.5 (1-3.3)	0.370
Bronchiectasis severity index score (median (IQR)	11 (9-14)	6 (5.8-12.5)	*0.016*
FACED score (median (IQR)	4 (2-5)	2 (1-3)	*0.001*
LABA use (n,%)	23 (85.2%)	5 (35.7%)	*0.001*
LAMA use (n,%)	14 (51.9%)	5 (35.7%)	0.326
ICS use (n,%)	20 (74.1%)	5 (35.7%)	*0.017*
Chronic macrolides (n,%)	7 (25.9%)	3 (21.4%)	0.750

All data is presented in median (quartiles 1-3) unless otherwise indicated.
PPB: Potentially pathogenic bacteria; MRC: Medical Research Council; ABPA: Allergic bronchopulmonary aspergillosis; COPD: Chronic obstructive pulmonary disease; FVC: Forced vital capacity; FEV_1: Forced expiratory volume in 1 second; SABA: Short-acting beta agonists; LABA: Long-acting beta agonists; LAMA: Long-acting muscarinic receptor antagonists; ICS: Inhaled corticosteroids.

Table 3. Receiver operating characteristics analyses of breath-prints between bronchiectasis patients with Pseudomonas aeruginosa colonization, other PPB colonization and non-colonized.

	Colonized vs. non-colonized bronchiectasis patients	***P. aeruginosa* colonized vs. other PPB colonized bronchiectasis patients**	***P. aeruginosa* colonized vs. non-colonized bronchiectasis patients**
Cross-validation accuracy	72.1%	89.2%	72.7%
Sensitivity	0.84	0.92	0.83
Specificity	0.58	0.85	0.65
AUROC	0.754	0.968	0.829
Positive predictive value	0.70	0.92	0.65
Negative predictive value	0.75	0.85	0.83
p value	0.01	<0.001	0.007

PPB: Potentially pathogenic bacteria; AUROC: Area under receiver operating characteristics.

Figure Legends

Figure 1. Flow diagram of study approach for patient enrolment

Figure 2. Electronic nose discrimination of colonized vs. non-colonized bronchiectasis patients.

A: Two-dimensional principal component (PC) analyses plot showing the breath-print discrimination.
B: Area Under Roc Curve (AUROC) of 0.75

C: Colonized; NC: Non-colonized.

Figure 3. Electronic nose discrimination of airway colonization with *Pseudomonas aeruginosa* vs. airway colonization with other potentially pathogenic bacteria in bronchiectasis patients.

A: Two-dimensional principal component (PC) analyses plot showing the breath-print discrimination.
B: Area Under Roc Curve (AUROC) of 0.96

C-PA: Colonization with Pseudomonas aeruginosa; C-O: Colonization with other potentially pathogenic microorganisms.

Figure 4. Electronic nose discrimination of bronchiectasis patients with *Pseudomonas aeruginosa* airway colonization vs. non-colonized subjects.

A: Two-dimensional principal component (PC) analyses plot showing the breath-print discrimination.
B: Area Under Roc Curve (AUROC) of 0.82.

C-PA: Colonization with Pseudomonas aeruginosa; NC: Non-colonized.

Official Journal of the Asian Pacific Society of Respirology
Respirology 20

ORIGINAL ARTICLE

Secreted mucins and airway bacterial colonization in non-CF bronchiectasis

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ABSTRACT

Background and objective: **Secreted mucins play a key role in antibacterial defence in the airway, but have not previously been characterized in non-cystic fibrosis (CF) bronchiectasis patients. We aim to investigate the relationship between secreted mucins levels and the presence of bacterial colonization due to potentially pathogenic microorganisms (PPM) in the airways of stable bronchiectasis patients.**

Methods: **Clinically stable bronchiectasis patients were studied prospectively at two centres. Patients with other pulmonary conditions were excluded. Spontaneous sputum was subject to bacterial culture, and secreted mucins (MUC2, MUC5AC and MUC5B) were measured in sputum supernatants by ELISA.**

Results: **A total of 50 patients were included. PPM were identified from sputum samples in 30 (60%), with *Pseudomonas aeruginosa* (*n* = 10) and *Haemophilus influenzae* (*n* = 10) as the most common PPM. There were no baseline differences among airway colonized and non-colonized patients. Patients with airways colonized by PPM presented higher levels of airway MUC2. No differences in MUC5AC levels were found among groups, whereas MUC5B levels were undetectable. Patients with *P. aeruginosa* colonization expressed the highest levels of MUC2. High levels of MUC2 and MUC5AC are also correlated with disease severity using the Bronchiectasis Severity Index.**

Conclusions: **Airway MUC2 levels were higher in bronchiectasis patients colonized with PPM compared with those without airway colonization, especially in patients with *P. aeruginosa*. These findings suggest that airway-secreted mucins levels may play a role in the pathogenesis of airway infection in non-CF bronchiectasis.**

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Received 22 December 2014; invited to revise: 20 February 2015; revised 17 April 2015; accepted 23 April 2015 (Associate Editor: Yuanlin Song).

SUMMARY AT A GLANCE

Secreted mucins are important for airway defence. However, their role in airway bacterial colonization in non-CF bronchietasis has not been studied previously. Our study demonstrates that airway MUC2 levels are higher in patients with non-CF bronchiectasis and airway bacterial colonization, especially in those with severe disease and *Pseudomonas aeruginosa.*

Key words: bronchiectasis, bronchial colonization, lung defense mechanism, mucin.

Abbreviations: BMI, body mass index; BSI, Bronchiectasis Severity Index; BTS, British Thoracic Society; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; CT, computed tomography; CTD, connective tissue disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; IL-1b, Interleukin-1b; PPB, potentially pathogenic bacteria; PPM, pathogenic microorganisms; SD, standard deviation; SEM, standard error of the mean; SEPAR, Spanish Respiratory Society.

INTRODUCTION

Non-cystic fibrosis (non-CF) bronchiectasis (hereafter referred to as bronchiectasis) is an inflammatory lung disease characterized by permanent dilatation of the bronchi.[1] It produces neutrophilic airway inflammation, impairment of pulmonary host defence and recurrent bronchial infection.[2,3] More than 50% of patients are chronically infected with bacteria.[4] This airway colonization leads to damage the bronchial wall, promoting more airways inflammation and bacterial infection, leading to a vicious cycle.[5]

The pathogenesis of chronic airway infection in bronchiectasis is poorly understood. Mucus is a protective coating secreted in the healthy airways, composed of water, salt and proteins. The correct balance

doi: 10.1111/resp.12595

of these components is essential for the protective function of the mucus layer.[6] Mucins are the major macromolecular component of the mucus gel in health.[7] They are glycoproteins responsible for the protective and clearance properties of the mucus. Several mucins have been described in the lower respiratory tract from healthy individuals,[8–10] although MUC5AC, MUC5B and MUC2 are the major secreted mucins detected in sputum.[11] In CF bronchiectasis, some studies showed that the concentration of secreted mucins is decreased compared with that in normal subjects.[12,13] In addition, during a CF exacerbation, concentrations of mucins increase, suggesting that those molecules are important in the response to an infectious or inflammatory stimulus in these patients.[14] Recent experimental studies confirmed the crucial role of secreted mucins for airway defence.[15] However, no data regarding the role of secreted mucins and its relationship with airway bacterial infection in bronchiectasis are available.

We postulated that bacterial colonization and disease severity in bronchiectasis would be associated with increased levels of secreted mucins. Therefore, we measured mucin levels in patients with bronchiectasis with and without airway bacterial colonization.

METHODS

Study design and ethics

This is a prospective, multicentre, cross-sectional study that included clinically stable bronchiectasis patients with and without airway bacterial colonization ($n = 30$ and $n = 20$, respectively). The study protocol was approved by the institutional review board at both institutions (IIBSP-BRO-2013154 and 12/ES/0059), and all subjects gave signed informed consent.

Participants

Patients were recruited from two regional specialist bronchiectasis clinics at the Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) and Ninewells Hospital (Dundee, UK).

Bronchiectasis was defined as presence of bronchial dilatation on high-resolution computed tomography (CT) scanning with compatible clinical history of cough with sputum production and/or recurrent respiratory infections.

Patients with CF, primary immunodeficiency (e.g., common variable immunodeficiency), active malignant disease, active allergic bronchopulmonary aspergillosis, interstitial lung disease, active mycobacteria disease, current smoking (within 1 year), human immunodeficiency virus infection, oral corticosteroid treatment or current chronic liver disease were excluded. For the purposes of this analysis, patients treated with long-term macrolide treatment were excluded.

Clinical assessments

At the time of clinical assessment, all patients were clinically stable as defined by the absence of an exacerbation that required antibiotic or steroid treatment within 30 days prior to inclusion. Demographic data, level of current symptoms, number of exacerbations in the previous year, time from last exacerbation, relevant co-morbid conditions and current treatments were recorded at inclusion using standardized questionnaires. All patients underwent spirometry (forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) with the highest of three technically satisfactory measurements recorded). The underlying aetiology of bronchiectasis was determined after testing recommended by the Spanish Respiratory Society (SEPAR) and British Thoracic Society (BTS) guidelines.[16,17]

The Bronchiectasis Severity Index (BSI), a validated composite severity tool, was calculated as previously described.[18]

Bacteriology

Spontaneous early-morning sputum samples were collected. Samples containing less than 10 squamous cells and more than 25 leukocytes per low-power microscope field were considered acceptable. Sputum was separated from saliva, and the sample split for bacteriology and assessment mucins levels. Samples were processed for qualitative and quantitative bacteriology as previously described.[19]

Specific microorganisms were identified according to standard laboratory methods and classified as potentially pathogenic microorganisms (PPM) (*Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, other enteric Gram-negative bacilli and *Staphylococcus aureus*) or non-PPM (*Streptococcus viridans*, *Candida* spp, *Corynebacterium* spp and coagulasse-negative staphylococci) for analysis.[20]

Mucins measurement

Fresh sputum was ultracentrifuged at $5000 \times g$ for 10 min at 10°C. The sol phase was removed and frozen at −70°C.[19] Proteases inhibitors (Calbiochem, San Diego, CA, USA) were added in equal volumes to the sputum sample during thawing.

MUC2, MUC5AC and MUC5B were measured by a validated commercially available ELISA kits (USCN Life Science Inc., Wuhan, China) following the manufacturer's instructions. The limit of detection was 1.56 ng/mL for MUC2, 78 pg/mL for MUC5AC and 0.625 ng/mL for MUC5B.

Airway biomarkers

Neutrophil elastase activity and myeloperoxidase activity in sputum supernatants were measured by chromogenic assay as previously described.[19] Interleukin-1b (IL-1b) was measured by ELISA (R&D Systems, Abingdon, UK).

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 software program (SPSS Inc, Chicago, IL, USA). Results are presented as mean, standard deviation

(SD), standard error of the mean (SEM), frequency or percentage, as required. Continuous variables were analyzed using *t* and analysis of variance tests, whereas categorical variables were analyzed using χ^2 tests. Biomarkers and bacterial load were correlated by linear regression. Non-parametric tests were used when necessary. A *P* value of less than 0.05 was considered significant.

RESULTS

A total of 50 patients with stable bronchiectasis were included in the study. The majority of patients had idiopathic or post-infective bronchiectasis (Table 1) and the mean BSI score was 10, indicating a population with relatively severe bronchiectasis. Sputum cultures were positive for PPM in 30 (60%) and negative in 20 (40%) patients; the former were considered colonized and the latter non-colonized.

P. aeruginosa and *H. influenzae* were both isolated in sputum culture in 10 patients each (33.3%), *Escherichia coli* in 3 patients (10%), *M. catarrhalis* and *S. aureus* in 2 patients each (6.7%) and *S. pneumoniae*, *P. mirabilis* and *Stenotrophomas maltophila* in 1 patient each (3.3%).

Patient characteristics

Table 1 shows the characteristics of the subjects, grouped by whether they had airway bacterial colonization. There were no statistical significant differences in sex, age, lung function tests, aetiology of bronchiectasis, prior medications used or pre-existing co-morbid conditions among groups.

Secreted mucin levels

MUC2 was the secreted mucin with highest expression in the airways in patients with bronchiectasis, with a mean (SD) of 62.3 (±49.6) ng/mL. MUC5AC levels were lower, with a mean (SD) of 0.53 (±0.86) ng/mL. MUC5B levels were below the level of detection of the assay in the sputum of 48 patients (96%).

When comparing airway colonized versus non-colonized patients, MUC2 levels were higher in those patients with airway bacterial colonization (76.8 ± 53.1 vs 40.6 ± 34.9 ng/mL, $P = 0.01$). No differences in MUC5AC levels were found (Fig. 1).

In order to assess the influence of *P. aeruginosa* colonization, we performed an additional analysis including only patients with sputum culture positive for *P. aeurginosa* ($n = 10$). There were no differences in the baseline characteristics among patients colonized by *P. aeruginosa* compared with those colonized by other PPM in this small subgroup ($n = 20$) (Table 2).

Patients colonized by *P. aeruginosa* expressed a trend to higher MUC2 (87.2 ± 60.7 vs 71.6 ± 49.7 ng/mL, $P = 0.09$) and MUC5AC levels (687.2 ± 333.6 vs 466.7 ± 196.2 pg/mL, $P = 0.4$) compared with those patients colonized by other PPM. When patients

Table 1 Patient demographics, clinical characteristics and prior treatments among colonized and non-colonized bronchiectasis patients

	Colonized ($n = 30$)	Non-colonized ($n = 20$)	*P* value
Age (mean ± SD)	63.1 (12.4)	67.4 (11.4)	0.9
Male	15 (50.0)	7 (35.0)	0.2
Smoking status			
Never	19 (63.3)	14 (70.0)	0.6
Ex-smoker	11 (36.6)	6 (30.0)	
Current	0 (0.0)	0 (0.0)	
Chronic cardiac disease	4 (13.3)	5 (25.0)	0.2
Diabetes mellitus	3 (10.0)	0 (0.0)	0.1
Stroke	3 (10.0)	1 (4.0)	0.5
Inhaled bronchodilators	17 (56.7)	9 (45.0)	0.4
Inhaled corticosteroids	12 (40.0)	7 (35.0)	0.7
FEV_1 (% pred) (mean ± SD)	64.7 (24.9)	77.3 (27.3)	0.7
FVC (% pred), (mean ± SD)	76.5 (20.0)	82.7 (23.8)	0.5
Ratio (%) (mean ± SD)	67.6 (18.3)	70.6 (13.5)	0.1
BMI (kg/m^2) (mean ± SD)	25.7 (6.8)	23.3 (3.8)	0.1
Aetiology			
Idiopathic	16 (53.3)	12 (60.0)	0.1
Post-infective	8 (26.6)	6 (30.0)	
CTD	4 (13.3)	2 (10.0)	
Others	2 (6.6)	0 (0.0)	
Exacerbations previous 12 months			
0	5 (16.7)	8 (40.0)	0.1
1	4 (13.3)	3 (15.0)	
2 or more	21 (70.0)	9 (45.0)	
BSI (mean ± SD)	12.3 (4.5)	7.5 (4.3)	0.002

Data are presented as *n*(%) unless otherwise indicated.

BMI, body mass index; BSI, Bronchiectasis Severity Index; CTD, connective tissue disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; SD, standard deviation.

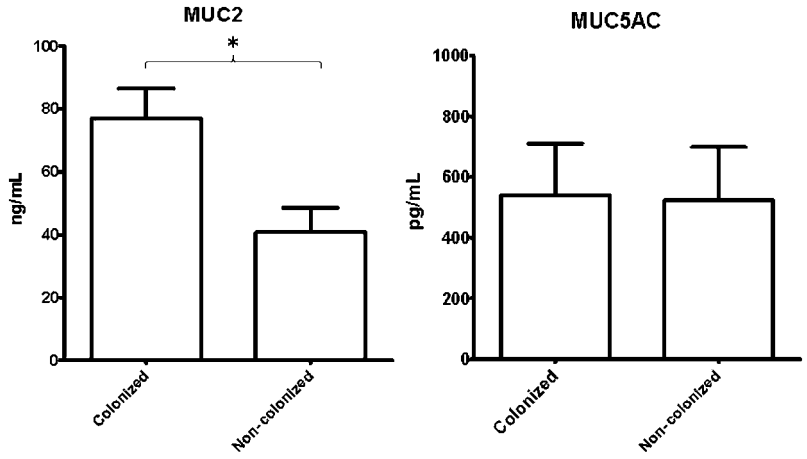

Figure 1 MUC2 and MUC5AC sputum levels in patients with bronchiectasis colonized and non-colonized by potentially pathogenic bacteria. Data are presented as median ± standard error of the mean. $*P < 0.05$.

Table 2 Patient demographics, clinical characteristics and prior treatments among colonized by *P. aeruginosa* and colonized by other PPM

	Colonized by *P. aeruginosa* ($n = 10$)	Colonized by other PPM ($n = 20$)	*P* value
Age (mean ± SD)	63.4 (10.1)	62.9 (13.6)	0.9
Male	4 (40.0)	11 (55.0)	0.4
Smoking status			
Never	8 (80.0)	11 (55.0)	0.1
Ex-smoker	2 (20.0)	9 (45.0)	
Current	0 (0.0)	0 (0.0)	
Chronic cardiac disease	1 (10.0)	3 (15.0)	0.7
Diabetes mellitus	1 (10.0)	2 (10.0)	1.0
Stroke	0 (0.0)	3 (15.0)	0.1
Inhaled bronchodilators	5 (50.0)	9 (45.0)	0.6
Inhaled corticosteroids	12 (40.0)	7 (35.0)	0.7
FEV_1 (% pred) (mean ± SD)	64.0 (24.3)	65.1 (23.5)	0.9
FVC (% pred), (mean ± SD)	81.8(24.3)	73.9(17.7)	0.3
Ratio (%) (mean ± SD)	62.3(20.7)	70.3 (16.9)	0.2
BMI (kg/m^2) (mean ± SD)	25.0 (7.7)	26.1 (5.8)	0.6
Aetiology			
Idiopathic	5 (50.0)	11 (55.0)	0.3
Post-infective	4 (40.0)	4 (20.0)	
CTD	0 (0.0)	4 (20.0)	
Others	1 (10.0)	1 (050)	
Exacerbations previous 12 months			
0	2 (20.0)	3 (15.0)	0.8
1	1 (10.0)	3 (15.0)	
2 or more	7 (70.0)	14 (70.0)	
BSI (mean ± SD)	15 (4.6)	11.3 (4.2)	0.08

Data are presented as n(%) unless otherwise indicated.

BMI, body mass index; BSI, Bronchiectasis Severity Index; CTD, connective tissue disease, FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PPM, pathogenic microorganisms; SD, standard deviation.

colonized by *P. aeruginosa* were compared with those colonized by other PPM and patients without airway bacterial colonization, the highest levels of MUC2 were observed in those bronchiectasis patients colonized with *P. aeruginosa* ($P = 0.02$) (Fig. 2).

The mean bacterial load in airway colonized patients was 9×10^7 cfu/g. In addition to being associated with the presence of airway bacteria, there was a correlation between levels of MUC2 and airway bacterial load ($r^2 = 0.16$, $P = 0.009$; Fig. 3a). In addition, there was a significant correlation between MUC5AC levels and airway bacterial load using linear regression ($r^2 = 0.15$, $P = 0.02$).

Secreted mucins and severity of disease

There was a significant correlation between both MUC2 and MUC5AC with the BSI score. The relationship between BSI and MUC2 levels are shown in Fig. 3b ($r^2 = 0.18$, $P = 0.006$). The strength of correlation between BSI and MUC5AC was weaker, but still statistically significant ($r^2 = 0.14$, $P = 0.02$).

There were no significant differences in MUC2 or MUC5AC levels between ex-smokers and never smokers, and no correlation between prior exacerbation frequency and secreted mucin levels.

Secreted mucins and neutrophil biomarkers

MUC2 was significantly correlated with neutrophil elastase activity ($r^2 = 0.18$, $P = 0.007$; Fig. 3c) and myeloperoxidase activity ($r^2 = 0.13$, $P = 0.02$), but not with cytokines including IL-1b ($r^2 = 0.06$, $P = 0.1$). Relationships between MUC5AC and these markers were all not statistically significant.

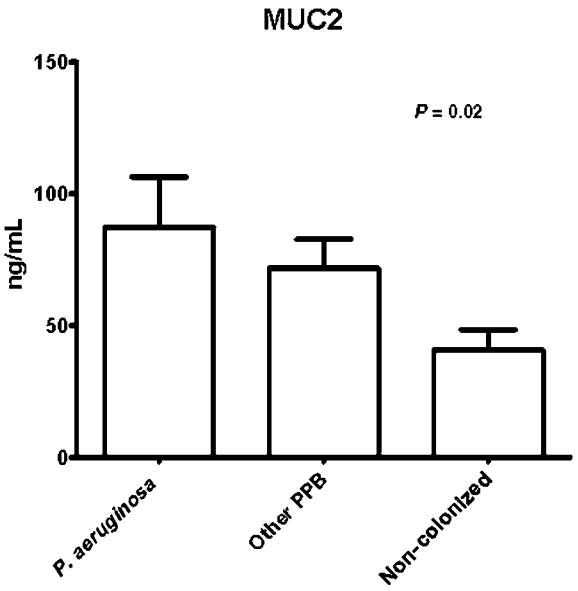

Figure 2 MUC2 sputum levels in patients with bronchiectasis colonized by *P. aeurginosa*, colonized by other potentially pathogenic bacteria (PPB) and non-colonized. Data are presented as median ± standard error of the mean. *P* value (analysis of variance test) = 0.02.

DISCUSSION

This study has demonstrated that MUC2 levels in the sputum were higher in those patients with bronchiectasis and airway bacterial colonization, especially in those colonized by *P. aeruginosa*. No differences in MUC5AC levels were found among groups, whereas MUC5B levels were undetectable in the majority of the patients. This is partly in contrast to previous studies of mucins in chronic obstructive pulmonary disease (COPD) and asthma where MUC5AC and MUC5B were the major ones and may suggest a specific role for MUC2 in the response to bacterial colonization in bronchiectasis. As has recently been emphasized, bronchiectasis has a different pathogenesis and different inflammatory profile to asthma and COPD, and so our findings emphasize the importance of conducting specific research into the pathogenesis of bronchiectasis rather than extrapolating from other airway diseases. In addition, airway mucin levels were associated with markers of disease severity in bronchiectasis, and with the BSI indicated that high levels of secreted mucins, both MUC2 and MUC5AC, are associated with more severe disease. Whether mucins are causative in disease progression or simply markers of disease severity requires further study.

Secreted mucins are proteins produced by respiratory epithelial cells.[21] They are essential for the correct airway mucus gel formation.[21–23] Patients with bronchiectasis have extensive mucus plugging in their airways, chronic cough and sputum production.[1] Although it has been speculated that mucus hypersecretion is crucial in the pathogenesis of bronchiectasis, there are few data evaluating mucus properties in these patients. In our study, MUC2 was the predominant secreted mucin in the sputum from bronchiectasis patients. Previous studies in patients with asthma and COPD showed higher levels of sputum MUC5AC and MUC5B rather than MUC2.[11,24] We also found MUC5AC expression in the sputum of bronchiectasis patients, but MUC5B were not detectable in most of the patients (only in 2 out of 50).

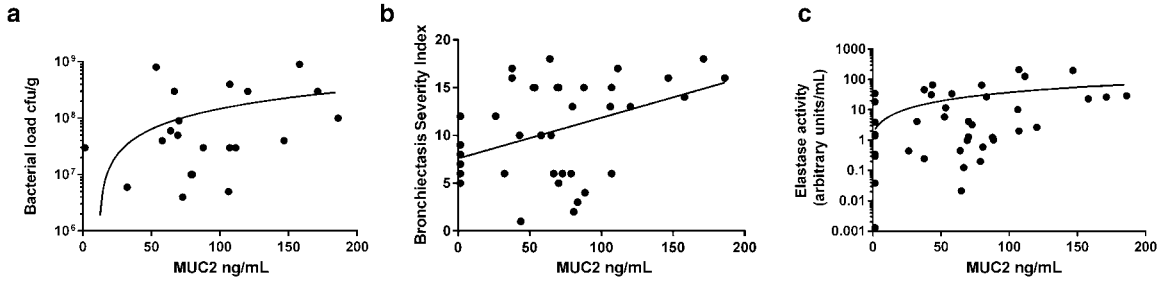

Figure 3 (a) Relationship between MUC2 sputum levels and airway bacterial load; (b) MUC2 sputum levels of the Bronchiectasis Severity Index; (c) MUC2 sputum levels and neutrophil elastase levels.

Kirkham *et al*. proved that MUC5B was the major mucin in the gel phase of sputum in COPD patients as compared with smokers without airway obstruction, whereas MUC5AC was the major one.[24] In CF bronchiectasis, Henke *et al*. demonstrated that MUC5AC and MUC5B levels were decreased in CF airway secretions compared with subjects without lung disease, with a decline of 93% and 70% respectively.[13] These findings suggested that the expression of secreted mucins is highly variable among different chronic airway respiratory diseases.

Airway bacterial colonization plays an important role in the pathogenesis and prognosis of bronchiectasis. The vicious cycle hypothesis argues that chronic bacterial infection perpetuates airway inflammation.[25] This inflammation leads to airway structural damage and further impairment of local host defence, leading to increase bacterial load.[2,26] In addition, recent clinical studies have demonstrated that patients with bronchiectasis and airway colonization by PPM had a greater risk of exacerbation, hospitalization and mortality,[18] especially those colonized by *P. aeruginosa*.[18,27] Mechanisms underlying why some patients are colonized are not well known.[1] Mucins have been postulated as natural antimicrobial agents.[10] In the gastrointestinal tract, mucins have demonstrated an antibiotic function against *Helicobacter pylori*,[28] but no data are available regarding the relationship between mucins expression and airway infection. In our study, we found that patients colonized by PPM expressed higher levels of MUC2 than those without airway bacterial colonization. Furthermore, the highest MUC2 values were detected in patients colonized by *P. aeruginosa*. In CF bronchiectasis, Henke *et al*.[14] showed that mucins increased in airways secretions during pulmonary exacerbations, especially MUC5AC with an increase of 908% compared with periods of stable disease. It suggested that the capacity to secrete mucin in response to an exogenous stimulus (e.g. infection) is preserved in CF airways. Our study supports the view that MUC5AC is upregulated in the context of bacterial infection, as we found a significant correlation between MUC5AC and bacterial load as well as severity of disease according to the BSI. Future studies should evaluate mucin levels during exacerbations.

Regulation of mucin secretion might be a key factor to understand these findings. In patients with inflammatory airways disease, mucus secretion is stimulated by several inflammatory stimuli such as cytokines,[29] proteases including neutrophil elastase[30] and *P. aeruginosa* proteases.[31] All of these factors are common in patients with bronchiectasis, especially in those colonized by PPM,[19] and may stimulate mucin secretion. Fujisawa *et al*. demonstrated that MUC5B expression is stimulated by inflammatory cytokines in a time- and dose-dependent manner.[32] Using human bronchial epithelial cells, these authors showed that inflammatory cytokines stimulates mucin secretion, but the persistence of inflammatory stimulus over time or the presence of excessive inflammation (both features present in most of patients with bronchiectasis) inhibits mucin induction.[32] Further studies are needed to interpret this complex pathway that may contribute to better understand the role of secreted mucins as natural antimicrobial agents in the airways of patients with bronchiectasis.

Our study has limitations. First, sample size is small, although it is larger than previous studies that evaluated mucin levels in patients with CF,[13,14] asthma[11] and COPD.[11,24] Second, sputum samples may reflect large-airway rather than small-airway phenomena. However, expectorated sputum, if collected and processed in a standardized manner as was done in this study, has been used to obtain important information about the airway features in bronchiectasies.[19,33] And third, a control group is not included, and it would be helpful to obtain normal levels of the secreted mucins studied.

In conclusion, we found that airway-secreted mucin patterns in patients with bronchiectasis are different from that of other chronic respiratory diseases, whereas MUC2 levels are increased in those patients with airway bacterial colonization. High levels of MUC2 and MUC5AC are associated with disease severity in bronchiectasis. This justifies a larger, definitive investigation of the role of mucins in airway defence in bronchiectasis.

Acknowledgements

The project described was supported by Sociedad Española de Neumología (SEPAR), Fundació Catalana de Pneumologia (FUCAP) and Tenovus Scotland. Dr Restrepo's time is partially protected by Award Number K23HL096054 from the National Heart, Lung, and Blood Institute.

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Respiratory Medicine 116 (2016) 70–77

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Review article

Diagnostic challenges of bronchiectasis

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ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form
18 April 2016
Accepted 16 May 2016
Available online 17 May 2016

ABSTRACT

Bronchiectasis is a condition of increasing incidence and prevalence around the world. Many different diseases have been associated with bronchiectasis, and their treatment can differ widely. Recent guidelines have helped to approach aetiological diagnosis but it is still a complex process. Identifying the cause of the bronchiectasis may determine a change in the treatment of a large group of subjects. That is one of the main reasons why the aetiological diagnosis is crucial in the proper management of bronchiectasis patients.

Postinfectious bronchiectasis is the most frequent entity among different studies, but a high percentage of cases still remain without a clear aetiology. Bronchiectasis related to allergic bronchopulmonary aspergillosis (ABPA), immunodeficiencies with antibody production deficiency, primary ciliary dyskinesia, cystic fibrosis and alpha-1-antitrypsin deficiency, among others, require a specific management that may improve quality of life and prognosis in a large group of individuals.

Therefore, the aim of this article is to review the main bronchiectasis related diseases and to simplify the aetiological diagnosis, in order to improve the management of bronchiectasis patients, especially in those where a specific treatment is available.

Contents

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http://dx.doi.org/10.1016/j.rmed.2016.05.014
0954-6111/

1. Introduction

Non-cystic fibrosis (CF) Bronchiectasis (henceforth referred as bronchiectasis) is a progressive disease characterized by a permanent dilatation of bronchi, retention of mucus and ciliary clearance impairment. These changes are a result of very diverse pulmonary or systemic conditions, which can influence the course of the disease. Therefore, aetiological investigation is one of the key aspects in the management of patients with bronchiectasis [1,2].

Causes of bronchiectasis are many and varied, making the aetiological diagnosis process difficult. The most common causes are previous lung infections such as pneumonia or pulmonary tuberculosis, primary and secondary immunodeficiencies, CF, abnormal ciliary function, allergic bronchopulmonary aspergillosis (ABPA) and connective tissue diseases. Bronchiectasis has also been associated with other chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) [3]. In addition, recent studies focusing on the aetiology of bronchiectasis have revealed a high percentage of patients with no identifiable cause despite undergoing extensive studies for this purpose that are considered idiopathic [4–7].

The treatment of diseases associated with bronchiectasis can differ widely among them. Some bronchiectasis aetiologies can determine a change in patient management such as initiating a specific treatment, closer follow-up, genetic testing for relatives or modifying risk factors [6,7]. Therefore, it is important to identify the aetiological cause of the bronchiectasis for a proper management. Current guidelines recommendations for the study of the aetiology of bronchiectasis include an extensive laboratory and instrumental workup, as well as lung function tests and microbiological evaluation [1,2]. The aim of this article is to review the main diseases associated with bronchiectasis in order to simplify the process of the aetiological diagnosis and therefore to help improving patient management when a treatable or modifiable cause is identified.

2. Aetiologies

The causes most frequently identified in patients with bronchiectasis are summarized in Table 1. The entities that have been associated with the development of bronchiectasis are numerous, and their prevalence varies depending on the population studied. Most studies directed to characterising patients with bronchiectasis to date have focused on specific populations in the UK, which can make it difficult to extrapolate the results to other countries and outside of highly specialized centres [4–6]. Recently, Lonni and colleagues conducted an analysis of 1258 patients from seven cohorts in different countries included in the European Bronchiectasis Registry (EMBARC) in the study directed to the most extensive identification of aetiologies of bronchiectasis to date [7]. This study has shown that the cause of bronchiectasis was identified in approximately 60% of individuals. Among these, the most frequent were postinfectious (20%), COPD-related bronchiectasis (15%), connective tissue disease-related (10%), immunodeficiencies (5.8%) and asthma-related bronchiectasis (3.3%) [7]. Table 2 summarizes the distribution of aetiologies most frequently identified in recent studies.

2.1. Postinfectious

This is the most frequently identified aetiology in most studies with a prevalence of 10–32% [4–8]. This variability may be due to a higher prevalence of postinfectious aetiology in the most disadvantaged communities [9]. Infections that have been associated with the presence of bronchiectasis are bacterial or viral pneumonia, pulmonary tuberculosis and childhood infections such as measles and whooping cough [10–12].

Identifying this aetiology is often complicated because there may be biases in the data collection. Sometimes patients cannot recall previous infections or their severity, making it difficult to establish a temporal relationship between these infections and bronchiectasis. Shoemark et al. observed an important delay in the diagnostic of bronchiectasis. While the average age of onset of symptoms in these patients was 7 years, the average age for the diagnostic study of bronchiectasis was 49 years [5]. Therefore, in patients for whom the connection between the antecedent infection and the onset of symptoms of bronchiectasis is unclear, a more comprehensive aetiological study is recommended [2].

The incidence of bronchiectasis caused by non-tuberculosis mycobacterial (NTM) infections is a controversial point [11]. Although the presence of bronchiectasis predisposes colonization by opportunistic microorganisms such as NTM, it is also suggested that they play a role in the development of these bronchial disorders. Fujita et al. retrospectively evaluated resected lung specimens from 9 patients infected with *Mycobacterium avium* complex [13]. In all the cases they identified destruction of bronchial cartilage and of the smooth muscle layer as well as bronchial mucosa ulcerations and the presence of airway granulomas. Given these histopathological changes, the authors concluded that NTM infection could be a cause rather than a consequence of the appearance of bronchiectasis [13]. It has been suggested that certain host factors may increase the risk to develop pulmonary NTM infection [14]. Kartalija et al. studied 103 patients with NTM infection and found that they were taller, had a significantly lower body mass index and body fat, and a higher prevalence of scoliosis and pectus excavatum than the 101 uninfected control subjects. Also, abnormal serum leptin and adiponectin levels were measured in NTM infected patients, resulting in suppressed blood IFN-γ and IL-10 levels [15]. Patients with *Mycobacterium avium* infection classically are middle aged and elderly female patients with middle lobe bronchiectasis, who may have little cough (the so called, “Lady Windemere” syndrome), although disease associated with NTM should be considered in all patients.

2.2. Immunodeficiencies

Immunodeficiency is defined as the partial or complete failure to conduct an effective immune response to an infectious agent. It is classified as primary (or congenital) and secondary (or acquired). It comprises a heterogeneous group of conditions that can occur in both childhood and adulthood. In patients with primary or secondary immunodeficiencies, bronchiectasis may be the result of a state of persistent systemic and airway inflammation due to recurrent infectious episodes [16,17]. A study from Hurst et al. observed greater airway and systemic inflammation in patients with primary antibody deficiency compared to immunocompetent bronchiectasis controls. The severity of this systemic inflammatory response correlated with the rate of progression of lung disease and also to airway inflammation [17].

The presence of an immune deficiency in patients with bronchiectasis varies from 2 to 18% depending on the population studied [6,18]. The deficiency in the function or production of one or more kinds of immunoglobulines is the most common and clinically important manifestation of primary immunodeficiencies. Common variable immunodeficiency, X-linked agammaglobulinemia or immunoglobulin A deficiency are therefore common causes of bronchiectasis [4,19]. Other secondary immunodeficiencies related to bronchiectasis are infection by the human immunodeficiency virus, immunosuppressive therapy or chemotherapy, and patients with haematological malignancies.

There is often a history of recurrent respiratory (pneumonias,

Table 1
Aetiologies of bronchiectasis.

Postinfectious
Necrotising pneumonia
Tuberculosis and non-tuberculosis mycobacterium
Viruses (adenovirus, measles and other childhood infections)
Immunodeficiencies
* *Primary:* antibody deficiency, combined immunodeficiency, neutrophil dysfunction, Wiskott-Aldrich syndrome, among others
* *Secondary:* HIV infection, haematological malignancies, chemotherapy, transplant
Hypersensitivity
Allergic bronchopulmonary aspergillosis
Associated with lung diseases
Asthma
COPD
Swyer-James Syndrome
Diseases associated with connective tissue
Rheumatoid Arthritis
Sjögren Syndrome
Other: Ankylosing spondylitis systemic sclerosis, systemic lupus erythematosus, ankylosing spondylitis, relapsing polychondritis, sarcoidosis, Marfan syndrome and Ehlers-Danlos syndrome
Alteration of the mucociliary escalator
Cystic fibrosis
Primary ciliary dyskinesia
Young's syndrome
Inflammatory bowel disease
Ulcerative colitis
Crohn's disease
Inflammatory pneumonitis
Aspiration and gastroesophageal reflux
Toxic inhalation (drugs, gases, etc.)
Congenital defects of the airway
Tracheobronchomegaly (Mounier-Kuhn syndrome)
Cartilage defects (Williams-Campbell syndrome)
Pulmonary sequestration
Tracheobronchomalacia
Bronchial Obstruction
* *Intrinsic:* scar stenosis, broncholithiasis, foreign body, tumour
* *Extrinsic:* lymphadenopathy, tumour, aneurysm
Others
Alpha 1 antitrypsin deficiency
Yellow nail syndrome
Diffuse panbronchiolitis
Idiopathic or unknown aetiology

HIV: human immunodeficiency virus; COPD, chronic obstructive pulmonary disease.

Table 2
Distribution of the aetiologies of bronchiectasis in recent studies.

	Pasteur et al. (n = 150)	King et al. (n = 103)	Shoemark et al. (n = 165)	Anwar et al. (n = 189)	Lonni et al. (n = 1258)
Mean age (SD)	52.7 (15.2)	56 (14)	49 (16)	66.1 (11.5)	67 (58–75)[a]
Gender (% M/F)	38/62	37/63	35/65	49/51	40/60
Idiopathic (%)	53	74	26	43	40
Postinfectious (%)	29	10	32	24	20
Immunodeficiencies (%)	8	9	7	2	6
ABPA (%)	7	4	8	4	5
Connective tissue diseases (%)	3	2	2	5	10
COPD (%)	–	–	–	12	15
Asthma (%)	–	–	–	3	3
Inflammatory intestinal disease (%)	1	–	3	2	2
Cystic Fibrosis (%)	3	0	1	<1	0
Ciliary dysfunction (%)	2	1	10	1	2
AAT Deficiency (%)	0	0	0	1	<1
Aspiration/GER (%)	4	0	1	1	<1
Panbronchiolitis (%)	<1	0	2	0	0
Young's Syndrome (%)	3	1	3	<1	0
Yellow nail Syndrome (%)	–	–	2	–	<1
Congenital defect of the airway (%)	<1	0	–	–	<1
Pink's disease (%)	<1	–	–	<1	<1
Other (%)	–	–	Mycobacteria Infection: 2	–	Bronchial obstruction: <1

SD: standard deviation; ABPA: allergic bronchopulmonary aspergillosis; COPD, chronic obstructive pulmonary disease; AAT: Alpha-1 antitrypsin; gastro-oesophageal reflux (GER).

[a] Data presented as median (interquartile range).

sinusitis) and non-respiratory (otitis, meningitis, diarrhea) infections, but in many cases impaired immunity can be identified in apparently healthy subjects, which may produce a delay in diagnosis in these patients [20]. A history of frequent non-respiratory infections can give a clue to the presence of an underlying immunodeficiency. Treatment with immunoglobulin has demonstrated an improvement in lung function in patients with hypogammaglobulinemia [21]. Therefore, identifying these patients and initiating early specific treatment is essential in cases that require it to avoid the appearance of bronchiectasis and to slow down the progression of the disease.

2.3. Chronic obstructive pulmonary disease (COPD)

The presence of bronchiectasis associated with COPD is the aetiology that has generated most interest and controversy in recent years. It is due to the high prevalence of patients with COPD and to the undertaking of more routine chest CT in these patients [22]. The prevalence of this bronchiectasis-COPD association differs depending on whether the studied population is a bronchiectasis series or a COPD series. Recent studies have found that about 12–15% of patients with bronchiectasis have a diagnosis of associated COPD [6,7]. However, it is believed that this association may be even greater when looking at COPD series. In a meta-analysis performed by Ni et al. including six observational studies with 881 COPD patients, the mean prevalence of bronchiectasis was 54.3% [23]. A study in the UK showed that 29% of patients with COPD followed in primary care had morphological alterations of airway potentially classifiable as bronchiectasis [24]. A recent population study on 18793 patients diagnosed with bronchiectasis also in the UK during 2004–2013 has shown that 36% of individuals had a diagnosis of COPD [25]. The prevalence of smoking history is also different depending on the studied population. In bronchiectasis series, 17–36% of patients were smokers or ex-smokers [5–8,18,26], while in COPD studies almost all patients included had a smoking history, since this is one of the main causes related to this disease.

The factors associated with the presence of bronchiectasis in COPD are severe airflow obstruction, isolation of potentially pathogenic microorganisms and at least one hospital admission due to an exacerbation of COPD in the last year [27]. In addition, the association of these diseases has shown a worse prognosis. Goeminne et al. observed that in patients with bronchiectasis and COPD mortality was almost three times higher than in patients with bronchiectasis without COPD (55% vs. 20%) [28]. Moreover, in patients with COPD and bronchiectasis on high resolution chest tomography, an increase in the number of exacerbations and hospitalizations as well as five-year mortality has been detected [29–31]. Martinez-Garcia and colleagues studied 201 patients diagnosed with COPD, of whom 57% had associated bronchiectasis. They noted that the coexistence of these entities is associated with a high risk of mortality from all causes in the group of patients with moderate to severe COPD [30].

These smaller studies may have overestimated the prevalence of bronchiectasis in COPD, as larger studies suggest a lower prevalence. In the ECLIPSE study (N = 2161), an international multicentre COPD cohort, bronchiectasis was reported in only 2% of males with GOLD II COPD (<1% of females), increasing to 9% of females and 7% of males in very severe COPD (GOLD IV) [32].

Whether the diagnosis is bronchiectasis with fixed airflow obstruction or COPD with suggestive anatomical abnormalities of bronchiectasis, this “overlap syndrome” has a significant impact on the management of both diseases and requires more studies to help understand its natural history and therefore to optimize the treatment given to date.

2.4. Asthma

The relationship between bronchiectasis and bronchial asthma is not clearly defined. Anwar et al. and Lonni et al. observed a prevalence of bronchiectasis associated with bronchial asthma of about 3% [6,7]. Nevertheless, studies aimed at the characterisation of radiological alterations of asthma have found bronchiectasis on chest high-resolution computed tomography (HRCT) in 17–35% of asthma patients [33–36]. These alterations have been associated more frequently with cases of non-allergic asthma and more severe forms of the disease [34,35]. However, a study performed by Menzies et al. also found a 2.01 increased hazard ratio of bronchiectasis among asthma patients with sensitization to *Aspergillus fumigatus* not meeting the diagnostic criteria for ABPA [36].

Unlike other aetiologies, morphological alterations of patients with asthma may affect all lung lobes, and both proximal and distal zones [37]. Finally, in a recent population study in the UK, the diagnosis of asthma was again found to be associated with a large number of patients with bronchiectasis, namely 42.5% [25]. It is difficult to define in which cases is bronchiectasis secondary to asthma and not the primary disease. Therefore, further studies are needed to better characterise this relationship and determine the impact on the prognosis of both diseases.

2.5. Allergic bronchopulmonary aspergillosis (ABPA)

This is a lung disease that occurs as a result of a hypersensitivity reaction to bronchial colonization by *Aspergillus fumigatus* [38]. The diagnosis of ABPA is performed using clinical and immunological criteria which are summarized in Table 3 [38]. The percentage of bronchiectasis associated with ABPA varies depending on the population analysed, from 1% in a study conducted in the US [18] to 7–8% in studies in the United Kingdom [5–7]. In some cases, it is difficult to make the diagnosis of ABPA because the result of serology tests such as total IgE and IgE specific to *Aspergillus fumigatus* can be similar to that observed in bronchial asthma [39]. Moreover, it is possible that the patient is in a stable phase at the time of the study and that structural damage occurred years before and that specific tests may give results that are normal or near normal [2]. In these cases, the identification of central bronchiectasis along with predominant impact on the upper lung lobes in chest HRCT can support the diagnosis of ABPA [40]. Chronic isolation of *Staphylococcus aureus* in bronchiectasis patients could also suggest ABPA as an aetiology, as this association has been reported in a previous study from Shah et al. [41]. Its routine screening is recommended, since the identification of this disease implies specific management [2].

2.6. Connective tissue diseases

Bronchiectasis have been associated with multiple systemic diseases and connective tissue disease is thought to be the cause in up to 10–16% of patients in studies in Europe and the US [7,18]. Among the more notable are connective tissue diseases such as rheumatoid arthritis (RA) and Sjögren’s syndrome, although bronchiectasis has been identified in patients with systemic sclerosis, systemic lupus erythematosus, ankylosing spondylitis, relapsing polychondritis, Marfan syndrome and Ehlers Danlos syndrome [42]. The association of bronchiectasis with RA is the most studied among these systemic diseases. An incidence of bronchiectasis of approximately 5% in patients with RA and respiratory symptoms has been reported, which is higher than the incidence of pulmonary fibrosis in those patients [43]. Like other pleuropulmonary manifestations of RA, bronchiectasis precedes articular manifestations in a large number of patients. This

Table 3
Criteria used by the American Academy of Allergy, Asthma, and Immunology (AAAAI) for the diagnosis of allergic bronchopulmonary aspergillosis.

ABPA diagnostic criteria
Minimum criteria
● Asthma or CF with impaired lung function
● Immediate skin reaction to the *Aspergillus* antigen
● Total serum IgE of 1000 ng/ml (416 iu/ml) or greater
● High levels of specific IgG and IgE for *Aspergillus* in serum
● Pulmonary infiltrates on chest radiograph
Additional criteria
● Peripheral blood eosinophilia
● Presence of precipitating antibodies for *Aspergillus* in serum
● Central bronchiectasis
● Isolation of *Aspergillus* in mucus plugs
Classified as ABPA-CB or ABPA-S (seropositive) according to the presence or absence of central bronchiectasis, respectively.

CF: Cystic fibrosis; Ig: Immunoglobulin; ABPA: allergic bronchopulmonary aspergillosis.

supports the hypothesis that chronic bronchial infection could be one of the triggers of RA [44]. Remy-Jardin et al. observed that approximately 30% of patients with RA who underwent chest HRCT had bronchiectasis. Although this finding was more frequent in patients with respiratory symptoms, about 8% of patients were asymptomatic [45]. A recent study by Perry et al. found that patients with RA and bronchiectasis present higher activity and severity of the disease and higher levels of anti-citullinated peptide antibodies when compared with patients with RA only [46].

Other collagenopathies have been much less studied. Studies aimed at characterizing the radiological changes of the lung by chest HRCT in patients with systemic sclerosis and systemic lupus erythematosus identified bronchiectasis in 59% and 21% respectively [47,48]. More studies are needed to elucidate the pathogenesis of the association between bronchiectasis and these diseases, as well as to determine the clinical impact that these bronchial abnormalities have on the course of the different systemic diseases.

It has been reported that connective tissue disease and in particular RA associated bronchiectasis is associated with a poorer prognosis, requiring more intensive monitoring. Whether this reflects the nature of the disease or the impact of the immunosuppressive drugs frequently used to treat RA is unclear [49].

2.7. Inflammatory bowel disease (IBD)

Bronchiectasis are the most common pulmonary manifestation of IBD [50]. They have been associated with IBD in approximately 1–3% depending on the population studied [4–7,18]. Among these, ulcerative colitis has the most clearly established relationship, but an association has also been suggested with Crohn's disease [42]. The most common form of presentation is the appearance of coughing with chronic bronchorrhea in patients with IBD, many after being colectomised [51]. One of the proposed theories suggests that this is because inflammatory mediators change from the resected intestine to the lung due to their common embryological origin [52]. In some cases, treatment with inhaled and oral glucocorticosteroids has been effective, including their instillation via bronchoalveolar lavage [42], but there is insufficient evidence to prescribe this treatment routinely [1,2].

2.8. Ciliary dysfunction

Primary ciliary dyskinesia (PCD) is a rare aetiology characterized by early onset. It is a hereditary transmitted disease and about 30 associated genes have been isolated, which can determine the heterogeneity and severity of the presentation between individuals [53,54]. It usually appears in childhood in the form of neonatal respiratory distress syndrome, chronic cough and/or chronic nasal congestion in more than 80% of cases [54]. Other frequent findings include chronic rhinosinusitis, dextrocardia, chronic otitis media, hearing loss, anosmia, infertility and diffuse bronchiectasis.

In screening patients with suspected PCD, the saccharin test has many limitations on the undergoing and interpretation of results, so new techniques have been studied to improve the diagnosis of this condition [53]. Nasal nitric oxide (nNO) levels are usually low in PCD patients (around 10–20% of normal values) [53,55]. Therefore, its use as a diagnostic test has grown as more specialized centres have standardized protocols and availability for this technique [53]. Measuring nNO levels has been proven useful in the diagnosis of PCD in adults and children over 5 years of age, but since the technique requires patients to perform trained velum closure or to exhale against resistance (in order to limit contamination with air from lower airways), reproducibility is limited in younger children [55]. However, low nNO levels can also be found in CF, acute upper airways infections, sinusitis and nasal polyposis, so it should not be used as single diagnostic test [56]. The gold standard for diagnosis is electron microscopy, although it is recommended that the workup be conducted combining various tests such as the frequency and pattern of ciliary beating and the determination of nNO to support the diagnosis [57]. Currently there is no specific treatment for PCD, and few data is available to support strong recommendations on the management of patients with this disease. Nevertheless, the European Respiratory Society task force recommends that patients with PCD should be evaluated and closely followed in centres specializing in this disease by a multidisciplinary team, including education in airway clearance techniques, based on CF guidelines [58].

2.9. Alpha-1-antitrypsin (AAT) deficiency

The association between AAT deficiency and bronchiectasis remains controversial. The prevalence of bronchiectasis in patients with AAT deficiency varies greatly between studies, probably because other aetiologies were not studied in many of them [59,60]. Screening of AAT deficiency is not recommended in the study of bronchiectasis, except in cases in which emphysema on chest HRCT is identified, particularly in a panlobular or basal distribution [2]. Although specific treatment for AAT deficiency is available and it is likely to improve the natural history of bronchiectasis, more evidence is needed to prove its benefit on these patients.

2.10. Gastrointestinal aspiration

The aspiration of gastrointestinal contents have been reported

as the cause of bronchiectasis in some studies [4–7,18]. Although few studies exist which aim to analyse this relationship, Lee and colleagues found a prevalence of gastroesophageal reflux (GER) in 40% of patients with bronchiectasis [61]. In the same vein, a recent study by McDonnell et al. observed a high prevalence of hiatus hernia and GER symptoms in individuals with bronchiectasis in stable phase [62]. The presence of GER has been also associated with greater severity of bronchiectasis and a worse outcome of the disease [7,62]. Whether GER is a common cause of bronchiectasis or not it seems reasonable to treat GER where it is identified.

2.11. Cystic fibrosis

Although the majority of cystic fibrosis is diagnosed by neonatal screening or presents during childhood, we continue to identify some cases of CF presenting with apparently idiopathic bronchiectasis during adulthood. Features suggesting the need to exclude CF will include early age at presentation, the presence of non-respiratory features such as malabsorption or infertility, the early presence of *Pseudomonas aeruginosa, Burkholderia cepacia* or *Staphylococcus aureus* and the presence of an upper lobe distribution on CT. Whether heterozygosity for CFTR mutations contribute to the development of bronchiectasis is unclear. Investigation is with sweat test and genetic screening.

2.12. Idiopathic causes

This category encompasses all patients with bronchiectasis in whom it was not possible to identify a cause despite a full aetiological study. The prevalence of this group varies greatly depending

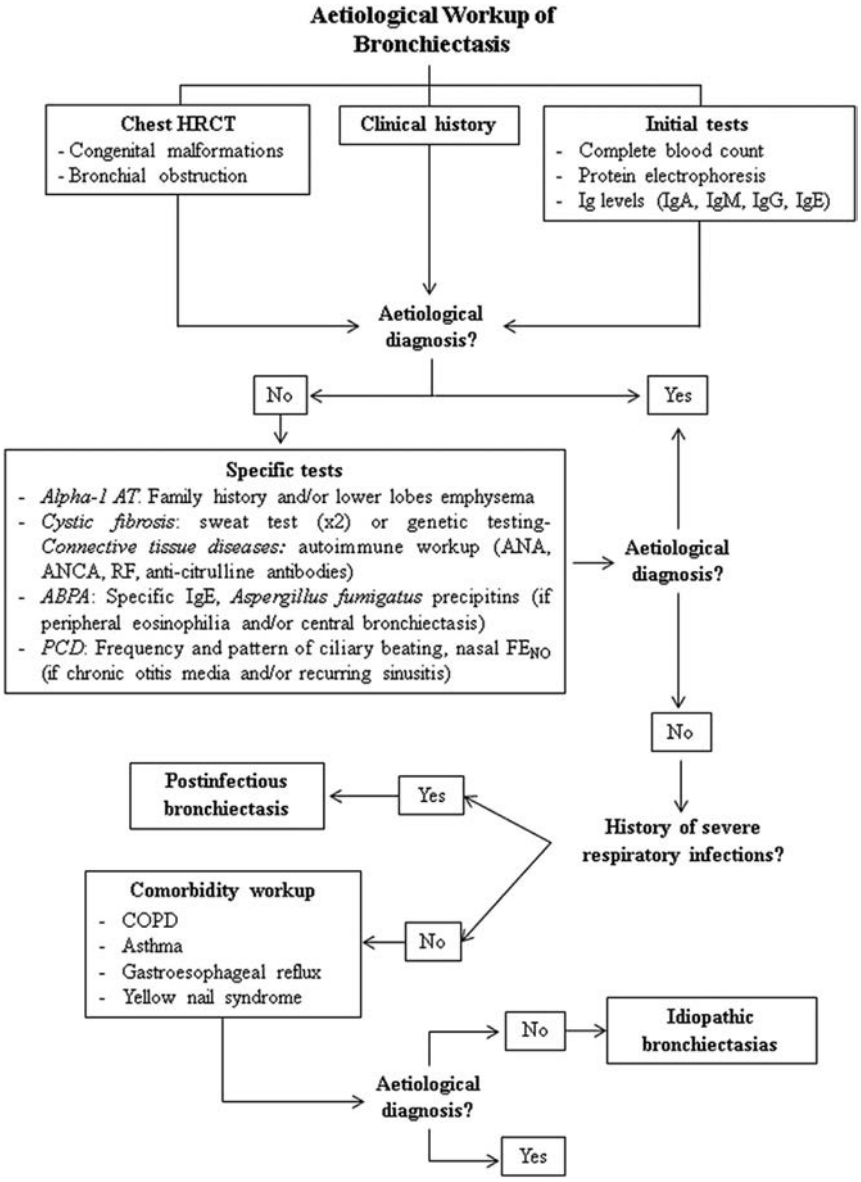

Fig. 1. Proposed algorithm for establishing the aetiologic diagnosis of bronchiectasis. HRCT: high-resolution computed tomography; Ig: Immunoglobulin; Alpha-1-AT: Alpha-1 antitrypsin; ANA: antinuclear antibodies; ANCA: anti neutrophil cytoplasmic antibodies; RF: rheumatoid factor; ABPA: allergic bronchopulmonary aspergillosis; PCD: Primary ciliary dyskinesia; $FE_{NO:}$ Fraction of exhaled nitric oxide, COPD, Chronic obstructive pulmonary disease.

on the population and the rigour of the diagnostic study, and lies between 26 and 74% [6]. A sub-analysis by Shoemark et al. reported an older mean age at diagnosis and predominant involvement of lower lobes in individuals with idiopathic bronchiectasis compared with patients with post infectious bronchiectasis, who had a mean age at diagnosis of 7 years and more diffuse lung involvement [5]. Lonni and colleagues also noted that the group of patients without a definite cause had more mild to moderate bronchiectasis compared to the other subjects [7]. Despite these findings, it is still a very heterogeneous group and more studies are needed to understand and identify the causative mechanisms of bronchiectasis in these patients.

3. Approaches to aetiological diagnosis

Bronchiectasis may be related to many diseases and many of them may require specific treatment. Identifying the cause of this bronchial disease may determine patient management or the need for genetic testing of individuals and their relatives in 7–13% of cases [6,7]. Therefore, it is recommended to try determining the diagnosis of the cause of bronchiectasis in all cases where possible.

Some aetiologies must be ruled out in all patients with bronchiectasis due to clinical implications in the management and prognosis. These include immunodeficiencies with antibody production deficiency, ABPA, primary ciliary dyskinesia, gastroesophageal reflux disease, mycobacterial infection, alpha-1 antitrypsin deficiency and CF [1]. Fig. 1 summarizes the proposed algorithm for addressing the aetiologic diagnosis [1,2].

In summary, all bronchiectasis patients should undergo a detailed medical background assessment and at least a basic study of immunoglobulin levels. If no immunodeficiency is detected, specific tests must be performed according to clinical history. The diagnosis of postinfectious bronchiectasis is made when other aetiologies have been ruled out and a history of severe respiratory infections exists. Finally, idiopathic bronchiectasis can be diagnosed only after a negative thorough aetiological study and no other bronchiectasis-related comorbidities are identified.

4. Conclusions

Identifying the aetiology of bronchiectasis is challenging, but important as early identification of treatable underlying disorders can lead to a change in management. We advocate a systematic approach to investigation for every patient with careful exclusion of each of the underlying conditions listed above. Continued improvements in the investigation and management of bronchiectasis will lead to better long term outcomes.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.rmed.2016.05.014.

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ORIGINAL ARTICLE

Neutrophil Elastase Activity Is Associated with Exacerbations and Lung Function Decline in Bronchiectasis

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Abstract

Rationale: Sputum neutrophil elastase and serum desmosine, which is a linked marker of endogenous elastin degradation, are possible biomarkers of disease severity and progression in bronchiectasis. This study aimed to determine the association of elastase activity and desmosine with exacerbations and lung function decline in bronchiectasis.

Methods: This was a single-center prospective cohort study using the TAYBRIDGE (Tayside Bronchiectasis Registry Integrating Datasets, Genomics, and Enrolment into Clinical Trials) registry in Dundee, UK. A total of 433 patients with high-resolution computed tomography–confirmed bronchiectasis provided blood samples for desmosine measurement, and 381 provided sputum for baseline elastase activity measurements using an activity-based immunosassay and fluorometric substrate assay. Candidate biomarkers were tested for their relationship with cross-sectional markers of disease severity, and with future exacerbations, mortality and lung function decline over 3 years.

Measurement and Main Results: Elastase activity in sputum was associated with the bronchiectasis severity index ($r = 0.49$; $P < 0.0001$) and was also correlated with the Medical Research Council dyspnea score ($r = 0.34$; $P < 0.0001$), FEV_1% predicted ($r = -0.33$; $P < 0.0001$), and the radiological extent of bronchiectasis ($r = 0.29$; $P < 0.0001$). During a 3-year follow-up, elevated sputum elastase activity was associated with a higher frequency of exacerbations ($P < 0.0001$) but was not independently associated with mortality. Sputum elastase activity was independently associated with FEV_1 decline (β coefficient, −0.139; $P = 0.001$). Elastase showed good discrimination for severe exacerbations with an area under the curve of 0.75 (95% confidence interval [CI], 0.72–0.79) and all-cause mortality (area under the curve, 0.70; 95% CI, 0.67–0.73). Sputum elastase activity increased at exacerbations ($P = 0.001$) and was responsive to treatment with antibiotics. Desmosine was correlated with sputum elastase ($r = 0.42$; $P < 0.0001$) and was associated with risk of severe exacerbations (hazard ratio 2.7; 95% CI, 1.42–5.29; $P = 0.003$) but not lung function decline.

Conclusions: Sputum neutrophil elastase activity is a biomarker of disease severity and future risk in adults with bronchiectasis.

Keywords: bronchiectasis; neutrophils; inflammation; biomarker; exacerbations

(Received in original form May 20, 2016; accepted in final form December 1, 2016)

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Supported by the Wellcome Trust (Postdoctoral Fellowship for Clinician Scientists to J.D.C.) and Tenovus Scotland. Additional support from the European Bronchiectasis Network (EMBARC), a European Respiratory Society Clinical Research Collaboration. EMBARC has received financial support from Bayer Healthcare and Aradigm Corporation. K.L.M. was supported by a grant to S.L.M., J.S.E., and B.W. from Cystic Fibrosis Foundation Therapeutics Inc. (MARTINXX130).

Author Contributions: Conception and design: J.D.C., E.F., S.E.M., and T.C.F. Recruited patients: J.D.C., S.F., S.E.M., and T.C.F. Performed experiments and sample processing: J.D.C., K.L.M., G.S.-C., O.S., S.F., E.F., A.D., K.W., S.L.M., and J.T.-J.H. Analysis and interpretation: J.D.C., K.L.M., G.S.-C., O.S., J.S.E., B.W., S.L.M., S.E.M., J.T.-J.H., and T.C.F. Drafting the manuscript: J.D.C., J.S.E., S.E.M., J.T.-J.H., and T.C.F. All authors participated in writing and revising the article before submission.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 195, Iss 10, pp 1384–1393, May 15, 2017

Originally Published in Press as DOI: 10.1164/rccm.201605-1027OC on December 2, 2016
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: There are no validated biomarkers of disease severity and progression in bronchiectasis. Studies in cystic fibrosis (CF) and pilot studies in non-CF bronchiectasis suggest that neutrophil elastase is associated with more severe disease and airway bacterial infection. We prospectively tested the hypothesis that exacerbations and lung function decline are associated with increased sputum neutrophil elastase activity and the related circulating biomarker desmosine.

What This Study Adds to the Field: Neutrophil elastase was associated with clinical and radiological extent of disease and with lung function. During follow-up, elevated levels of sputum neutrophil elastase activity identified patients at higher risk of exacerbations and severe exacerbations who required hospital admission over 3 years. Sputum elastase activity was also independently associated with lung function decline. Increased circulating desmosine was also associated with a higher risk of severe exacerbations. Because few clinical parameters have been shown to be associated with bronchiectasis outcomes, sputum neutrophil elastase and circulating desmosine may be useful adjuncts to clinical assessment or to patient evaluation in clinical trials.

Bronchiectasis is characterized by permanent bronchial dilatation associated with chronic neutrophilic airway inflammation (1). The pathogenesis of bronchiectasis is poorly understood, but activated neutrophils are believed to be a key component of the "vicious cycle" of lung damage (2). Neutrophil elastase (NE) is a 29-kD serine protease stored in azurophilic granules that may be released during degranulation, neutrophil extracellular trap formation, or cell death (3–8). NE is proinflammatory, slows ciliary beat frequency, and stimulates mucus secretion (9, 10). It is found in high concentrations in the sputum of patients with neutrophilic lung diseases, including bronchiectasis, chronic obstructive pulmonary disease, and cystic fibrosis (CF) (5–7). It is believed that unopposed action of NE directly contributes to the pathogenesis and progression of these diseases.

NE activity is inhibited by antiproteases, including secretory leukoproteinase inhibitor produced by bronchial epithelium and by serum-derived alpha-1 antitrypsin (11). In addition, the presence of high concentrations of DNA released during neutrophil extracellular trap formation inhibits elastase activity, both directly and indirectly by modulating the response to NE inhibitors (12). Epithelial-derived factors (e.g., syndecan-1) also complex with elastase in the airway and reduce the inhibitory capacity of alpha-1 antitrypsin (7, 12).

Thus, the activity of NE within the inflamed airway is usually controlled by a range of inhibitors. However, in bronchiectasis, release of NE overwhelms the antiproteinase defense, which leads to detectable levels of NE proteolytic activity in sputum and bronchoalveolar lavage (13–15). This can be measured readily using assays that detect cleavage of chromogenic or fluorogenic peptide-based substrates in sputum, or downstream by measuring the endogenous degradation of mature elastin through the quantification of the unique covalent cross-linking amino acids desmosine and isodesmosine in serum and/or plasma (circulating desmosine [cDES]) (16, 17).

There are no widely accepted biomarkers of disease progression in bronchiectasis, but evidence is accumulating that sputum NE activity correlates with disease severity. In a study of 30 patients, Tsang and colleagues showed that NE activity correlated strongly with 24-hour sputum volume, extent of bronchiectasis, and FEV_1 (13). In 385 patients with bronchiectasis from the UK, NE activity was correlated with airway bacterial load, the presence of *Pseudomonas aeruginosa*, and the extent of radiological bronchiectasis (14). No previous study has investigated the association of sputum NE activity or cDES with clinically relevant outcomes in bronchiectasis during long-term follow-up. In this study, we prospectively tested the hypothesis that elevated sputum NE activity or the related biomarker cDES is associated with increased frequency of exacerbations and lung function decline.

Methods

This study was conducted and is reported according to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (18). Patients were consecutively recruited to a prospective observational study (TAYBRIDGE [Tayside Bronchiectasis Registry Integrating Datasets, Genomics, and Enrolment into Clinical Trials] registry) at Ninewells Hospital, Dundee, UK 2012–2015. The study was approved by the East of Scotland Research Ethics committee (12/ES/0059), and all patients gave written informed consent. Inclusion criteria were age $\geq$18 years, high-resolution computed tomography–confirmed bronchiectasis, and clinical symptoms consistent with bronchiectasis. Exclusion criteria were inability to give informed consent, active nontuberculous mycobacterial infection, active allergic bronchopulmonary aspergillosis, active tuberculosis, active malignancy, CF, or pulmonary fibrosis with secondary traction bronchiectasis.

For inclusion in the present analysis, patients were asked to provide serum and sputum samples at the same baseline visit when clinically stable (defined as no antibiotic treatment within the preceding 4 weeks, excluding prophylactic oral or inhaled antibiotics).

Clinical Assessment

Full details of the clinical assessments are shown in the online supplement. The underlying cause of bronchiectasis was determined by standardized testing according to British Thoracic Society recommendations (19). The bronchiectasis severity index (BSI) was calculated as described (20). Quality of life was evaluated using the St. George's Respiratory Questionnaire (SGRQ) (21). Chronic infection was defined as the isolation of pathogens on at least 2 occasions 3 months apart during the preceding 12 months (22). Spirometry was performed according to American Thoracic Society/European Respiratory Society guidelines (23). The severity of radiological bronchiectasis was evaluated using the Reiff score (24). Exacerbations were defined according to British Thoracic Society recommendations, and severe exacerbations were defined as those requiring hospital admission (19).

Sputum Sampling and Processing

Spontaneous sputum samples were split for microbiology and inflammatory marker measurement. For measurement of inflammatory markers, including NE, spontaneous sputum was ultracentrifuged at 50,000 × *g* for 90 minutes, and the soluble fraction carefully removed (14).

Methods of Measurement of NE Activity and Other Inflammatory Markers

Because previous studies have used several different methods of NE quantification, we simultaneously evaluated three methods in this study; two assays for sputum NE activity and one for cDES measurement.

Active NE was measured using an activity-based immunoassay (ProteaseTag Active NE Immunoassay referred to as the ABI-NE assay) (ProAxsis Ltd, Belfast, UK) in accordance with the manufacturer's instructions (25, 26) and a fluorogenic substrate-based kinetic assay (referred to as kinetic-NE assay). The kinetic-NE assay used the substrate *N*-methoxysuccinyl-Ala-Ala-Pro-Val-7-amido-4-methylcoumarin (Sigma-Aldrich, St. Louis, MO).

Sputum samples were assayed at dilutions ranging from 5× to 2,000×, and assays that remained below the lowest limit of detection (0.016 μg/ml) at 5× dilution were recorded as zero for the purposes of analysis.

Measurement of serum and sputum inflammatory markers (C-X-C ligand 8 [CXCL8], IL-1β, tumor necrosis factor-α [TNF-α], and extracellular newly identified receptor for advanced glycation end-product–binding protein [EN-RAGE]) were performed using commercially available ELISA. Before use, kits were validated for use in sputum according to the methods described by Woolhouse and colleagues (27).

Serum Desmosine Measurement

cDES was measured in serum using a validated liquid chromatography–mass spectrometry/mass spectrometry method as previously described (16, 17).

Exacerbation Study

Patients (n = 26) included in the main study who visited a hospital for a severe exacerbation of bronchiectasis were enrolled in a substudy of changes in NE during exacerbations (19). Spontaneous sputum samples were collected on day 1 before commencement of antibiotics and after treatment at day 14. Patients received standardized treatment for 14 days based on their previous sputum microbiology (19). These 26 patients subsequently had additional sampling 6 months post-exacerbation to determine dynamics of NE.

Statistical Analysis

Mean and SD were used to display continuous normally distributed data with median and interquartile range (IQR) for continuous nonnormally distributed data, and frequencies and percentages for categorical data. The association of biomarkers with linear variables was performed using Spearman's correlation, whereas between group differences were evaluated by analysis of variance or Kruskal-Walis test. Frequency of exacerbations and severe exacerbations were evaluated using Poisson regression adjusted for duration of follow-up. Time to event data (time to first exacerbation, first hospital admission, and death) were analyzed using Kaplan-Meier survival analysis and Cox proportional hazard regression for multivariable analyses. Discrimination for mortality and severe exacerbations at 3 years was analyzed using the area under a receiver-operating characteristic curve (AUC). Analysis of FEV_1 decline over 3 years was performed using multiple linear regression with appropriateness of the linear regression modeling evaluated by examining the distribution of residuals. In some analyses, patients were split into three groups based on low, intermediate, and high elastase levels, with cutoffs selected using Youden's index. Patients with missing data were excluded from analysis of the specific test as outlined in the following. No imputation methods were used. Sample size was empirically based on previous studies with equivalent lengths of follow-up (14).

Results

Patient Cohort

The study included 433 patients, of whom 381 patients were able to provide a sputum sample sufficient for measurement of NE activity. The flow of patients through the study is shown in the STROBE flowchart (Figure 1).

Characteristics of the included patients are shown in Table 1. The median (IQR) age was 67 (58–74) years; 60.7% of patients were women, and 45% of patients had idiopathic bronchiectasis. The median exacerbation frequency was 1 per year (IQR, 0–3). The median BSI score was 6, which indicated a population with moderate to severe bronchiectasis (range, 0–24).

There were no significant differences between patients who were able and unable to produce sputum. A total of 42 patients were receiving long-term inhaled antibiotics, and 129 were receiving long-term oral antibiotic treatments at baseline.

Sputum NE Activity Is Associated with Disease Severity

The ABI-NE assay detected activity above the lower limit of detection in 249 patients (65.4%), whereas the kinetic-NE assay detected active NE in 204 (53.5%) samples. The two assay methods were highly correlated (*see* Figure E1 in the online supplement).

Sputum NE activity as measured by the ABI-NE assay showed a univariate association with cross-sectional markers of disease severity, including the Medical Research Council dyspnea score ($r = 0.34$; $P < 0.0001$), SGRQ score ($r = 0.28$; $P < 0.0001$), absolute FEV_1 ($r = -0.31$; $P < 0.0001$), FEV_1% predicted ($r = -0.33$; $P < 0.0001$), Reiff score ($r = 0.29$; $P < 0.0001$), and the BSI ($r = 0.49$; $P < 0.0001$). Similar results were obtained with the kinetic-NE assay (Figure 2 and *see* Figure E2).

Both ABI-NE and kinetic-NE assays were correlated with sputum myeloperoxidase activity ($P < 0.0001$), but there was no correlation with sputum CXCL8, IL-1β, TNF-α, or EN-RAGE (data not shown).

There was a relationship between NE activity and airway bacterial load (at bacterial load $>10^7$ cfu/ml) (Figures 3A and 3B). Patients chronically infected with *P. aeruginosa,* Enterobacteriaceae, and *Haemophilus influenzae* had increased levels of NE using both assays ($P < 0.0001$) compared with patients without chronic bacterial infection.

Sputum NE Activity and Longitudinal Clinical Outcomes

In the sputum producing cohort, the mortality rate was 8.7% and 25.5% of patients who had hospital admissions for severe exacerbations during follow-up. The median frequency of exacerbations was 1 per patient per year (IQR, 0–3).

Because the ABI-NE assay was more sensitive and had stronger correlations with the most clinical outcomes for clarity, we

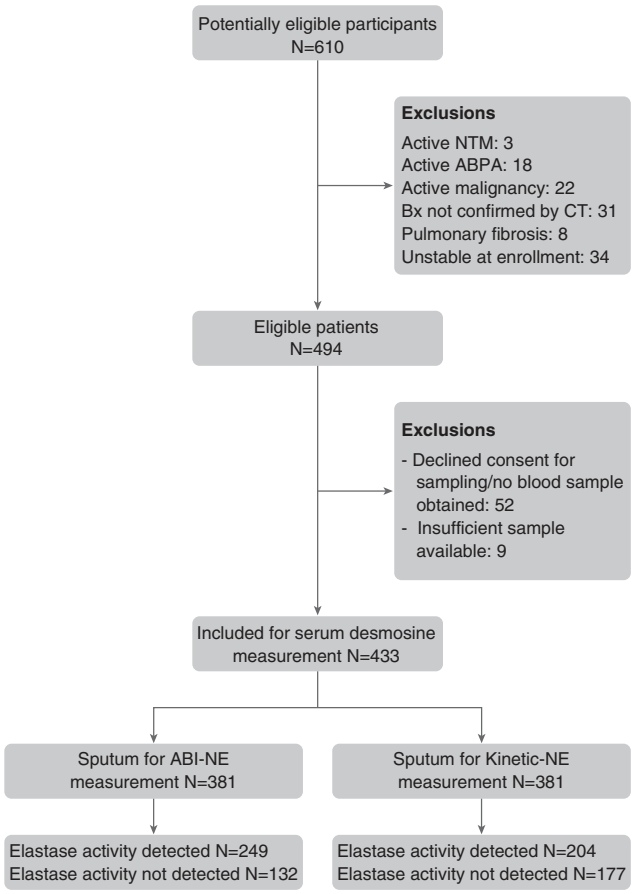

Figure 1. Strengthening the Reporting of Observational Studies in Epidemiology flowchart of study inclusion and exclusions. Patients with elastase levels below the lower limit of detection ("not detected") were included in the analysis with values treated as zero. ABI-NE = activity-based immunoassay for neutrophil elastase; ABPA = allergic bronchopulmonary aspergillosis, Bx = bronchiectasis, CT = computed tomography; NTM = nontuberculous mycobacteria.

only presented the results for the ABI-NE assay here. Using receiver-operating characteristic analysis, ABI-NE activity was associated with hospital admissions during follow-up (AUC, 0.75; 95% CI, 0.72–0.79) and mortality (AUC, 0.70; 95% CI, 0.67–0.73). Entering NE activity as a continuous variable, after multivariable adjustment, including the BSI, a 1 μg/ml increase in NE activity was independently associated with a 0.5% increased risk of hospital admission (hazard ratio [HR], 1.005; 95% confidence interval [CI], 1.002–1.008; $P < 0.0001$). No independent relationship with mortality was identified. Additional models are shown in Table E1 in the online supplement.

Using receiver-operating characteristic analysis, we determined candidate clinically meaningful cutoff values of sputum NE. A total of 132 patients had values below the lower limit of detection (<0.016 μg/ml [low NE]), 143 patients had values between 0.016 and 20 μg/ml (intermediate NE), and106 had values >20 μg/ml (high NE).

Comparing the frequency of exacerbation between the three ABI-NE cutoffs using Poisson regression, patients with the highest elastase values (>20 μg/ml) had a rate ratio (RR) of 3.18 (95% CI, 2.65–3.18; $P < 0.0001$), and patients with intermediate NE values had a RR of 1.61 (95% CI, 1.39–1.86; $P < 0.0001$) compared with the low elastase (reference) group, which indicated that high sputum NE activity was associated with a greatly increased frequency of future exacerbations. For severe exacerbations, the corresponding values were RR 1.69 (95% CI, 0.90–3.17; $P = 0.1$) for intermediate NE values and 4.73 (95% CI, 2.67–8.33; $P < 0.0001$) for the highest NE group (Figure 4A). Consistent with this, elevated NE activity was associated with a shorter time to next exacerbation ($P < 0.0001$) (Figure 4B), shorter time to next severe exacerbation ($P < 0.0001$) (Figure 4C), and increased all-cause mortality (Figure 4D) ($P < 0.0001$). An analysis of exacerbation frequency according to different severity groups using the BSI is shown in Table E2. Prediction statistics are shown in Table E3.

FEV_1 decline over 3 years was normally distributed. The mean FEV_1 decline was 48.2 ml/yr (SD 83.7). Across the defined three elastase groups, mean FEV_1 decline was 35.6 ml (SD 81.1) for NE activity <0.016 μg/ml, 49.5 ml (SD 92.5) for intermediate elastase levels, and 56.4 ml (SD 67.4) for those with NE >20 μg/ml. On univariate regression, there was a weak but statistically significant relationship between NE activity and FEV_1 decline ($P = 0.004$). After adjustment for BSI, sex, and baseline FEV_1, increasing NE-ABI elastase was associated with more rapid lung function decline (β coefficient, −0.139; $P = 0.001$; model fit $r = 0.7$).

Serum Desmosine Is Associated with Age and Disease Severity

cDES was most strongly correlated with age ($r = 0.48$; $P < 0.0001$ (Figure 5A). Additional univariate correlations were observed between cDES and Medical Research Council dyspnea score ($r = 0.32$; $P < 0.0001$), SGRQ ($r = 0.40$; $P < 0.0001$), absolute FEV_1 ($r = -0.39$; $P < 0.0001$), and Reiff score ($r = 0.15$; $P = 0.002$). cDES was also significantly higher in patients who had *P. aeruginosa* ($P < 0.0001$). There was an association between cDES and BSI ($r = 0.46$; $P < 0.0001$). Correlations were demonstrated with sputum NE activity (*see* Figure E1). Removing the outliers at >1 ng/ml showed similar correlations with markers of disease severity as described in the online supplement.

In the total cohort, mortality was 9.5% and 22.6% of patients admitted to hospital for severe exacerbations. Median exacerbation frequency was 1 per patient per year (IQR, 0–3).

In analysis of longitudinal clinical outcomes, there was no relationship between cDES and FEV_1 decline over 3 years ($P = 0.1$), but there was a strong relationship between cDES and severe exacerbations (HR, 6.0; 95% CI, 3.61–10.0;

Table 1. Baseline Characteristics of the Cohort

Baseline Characteristics	Full Cohort	Patients Providing Sputum
N	433	381
Age, yr	67 (58–74)	67 (58–74)
Sex, % female	263 (60.7)	225 (59.1)
Body mass index	25.0 (22.3–28.5)	25.1 (22.2–28.6)
Smoking status, never/ex/current	266 (61)/151 (34.9)/16 (3.7)	239 (62.7)/131 (34.4)/11 (2.9)
MRC dyspnea score	2 (1–3)	2 (1–3)
FEV_1, L	1.58 (1.10–2.20)	1.58 (1.10–2.23)
FEV_1% predicted	71.9 (50.0–91.0)	71.4 (49.4–90.9)
FVC, L	2.45 (1.84–3.21)	2.41 (1.85–3.19)
FVC, % predicted	83.9 (68.4–99.5)	83.2 (67.7–98.7)
Etiology of bronchiectasis		
Idiopathic	195 (45.0)	169 (44.4)
Postinfective	84 (19.4)	78 (20.5)
Previous ABPA	37 (8.5)	34 (8.9)
Asthma	15 (3.5)	14 (3.7)
COPD	22 (5.1)	19 (5.0)
Rheumatoid arthritis	21 (4.8)	17 (4.4)
Connective tissue disease	6 (1.4)	4 (1.0)
Inflammatory bowel disease	11 (2.5)	11 (2.9)
Primary immunodeficiency	18 (4.2)	17 (4.5)
Previous NTM infection	7 (1.6)	4 (1.0)
Primary ciliary dyskinesia	4 (0.9)	3 (0.8)
Alpha-1 antitrypsin deficiency	2 (0.5)	1 (0.3)
Others	11 (2.5)	10 (2.6)
Exacerbations per year	1 (0–3)	1 (0–3)
Previous hospitalization for severe exacerbations	107 (24.7)	101 (26.5)
St. George's Respiratory Questionnaire total score	44.3 (24.6–62.7)	46.1 (27.3–63.2)
Bronchiectatic on CT	3 (2–4)	3 (2–4)
Reiff score	3 (2–6)	3 (2–6)
Chronic colonization*	236 (54.5)	213 (55.9)
H. influenzae	129 (29.8)	116 (30.4)
P. aeruginosa	63 (14.5)	60 (15.7)
Moraxella catarrhalis	51 (11.8)	49 (12.9)
Streptococcus pneumoniae	25 (5.8)	23 (6.0)
Streptococcus aureus	34 (7.9)	30 (7.9)
Enterobacteriaceae	39 (9.0)	39 (10.2)
Bronchiectasis severity index	6 (4–10)	6 (4–11)
Mild	126 (29.1)	108 (28.3)
Moderate	170 (39.3)	144 (37.8)
Severe	137 (31.6)	129 (33.9)

Definition of abbreviations: ABPA = allergic bronchopulmonary aspergillosis; COPD = chronic obstructive pulmonary disease; CT = computed tomography; MRC = Medical Research Council; NTM = nontuberculous mycobacteria.
Data are presented as median (interquartile range) or n (%).
*Defined as isolation of a pathogenic microorganism in sputum when clinically stable on two occasions at least 3 months apart in a 12-month period.

$P < 0.0001$), which persisted after adjustment for BSI (HR, 2.7; 95% CI, 1.42–5.29; $P = 0.003$). There was no significant association between cDES and moderate exacerbations ($P = 0.2$), but after combining moderate and severe exacerbations, a statistically significant association more than 0.4 ng/ml was observed (RR, 1.96; 95% CI, 1.61–2.39; $P < 0.0001$).

There was similar association between cDES and all-cause mortality (HR, 2.60; 95% CI 1.24–5.45; $P = 0.01$), but this relationship was not statistically significant after adjustment for BSI (HR, 1.15; 95% CI, 0.45–2.91; $P = 0.8$). Additional models are shown in Table E2. The AUC values for biomarkers compared with individual recognized predictors of outcome in bronchiectasis is shown in Table E4.

A sensitivity analysis conducted in patients taking long-term antibiotics demonstrated that sputum NE and cDES had similar associations with long-term outcomes compared with the overall cohort (Table E5).

Changes in NE at Exacerbation and after Antibiotic Therapy

To determine whether sputum NE was responsive to treatment, we studied 26 patients during an acute exacerbation that required intravenous antibiotic therapy. Characteristics of the included patients compared with the overall population are shown in Table E6.

Median ABI-NE levels were 0.39 μg/ml (IQR, 0–23.5) at baseline, 57.0 μg/ml (IQR, 3.3–145 μg/ml) at onset of exacerbation, 0 μg/ml (IQR, 0–25.8) after 14 days of antibiotic therapy, and 1.3 μg/ml (IQR, 0–29.9) at the second stable measurement 6 months later (Figure 6). Although NE activity was generally higher at exacerbations than at baseline ($P = 0.0002$) and at recovery ($P < 0.0001$), the assay did not discriminate between exacerbation and disease quiescence because of the high baseline activity in some individuals. The ABI-NE assay level >50 μg/ml was associated with a sensitivity of 57.7% and specificity of 92.3%. An increase from baseline was present at exacerbation in 20 of 26 patients at exacerbation.

Remarkably, even with this small sample size, failure to return to NE baseline levels after completion of antibiotics was associated with a shorter time to the next exacerbation (HR, 2.92; 95% CI, 1.16–7.38; $P = 0.02$). Data on the correlation between elastase measurements at two stable visits more than 6 months apart are shown in Figure E3.

Discussion

This study indicated a role for sputum NE activity as a biomarker of disease severity and disease progression in bronchiectasis, while also providing the first data on the linked biomarker cDES. NE activity in sputum was independently associated with risk of exacerbations, severe exacerbations, and lung function decline, even after adjustment for underlying severity of the disease. This suggested that NE is a useful marker that might identify patients at future risk. Elastase is dynamic and responded to

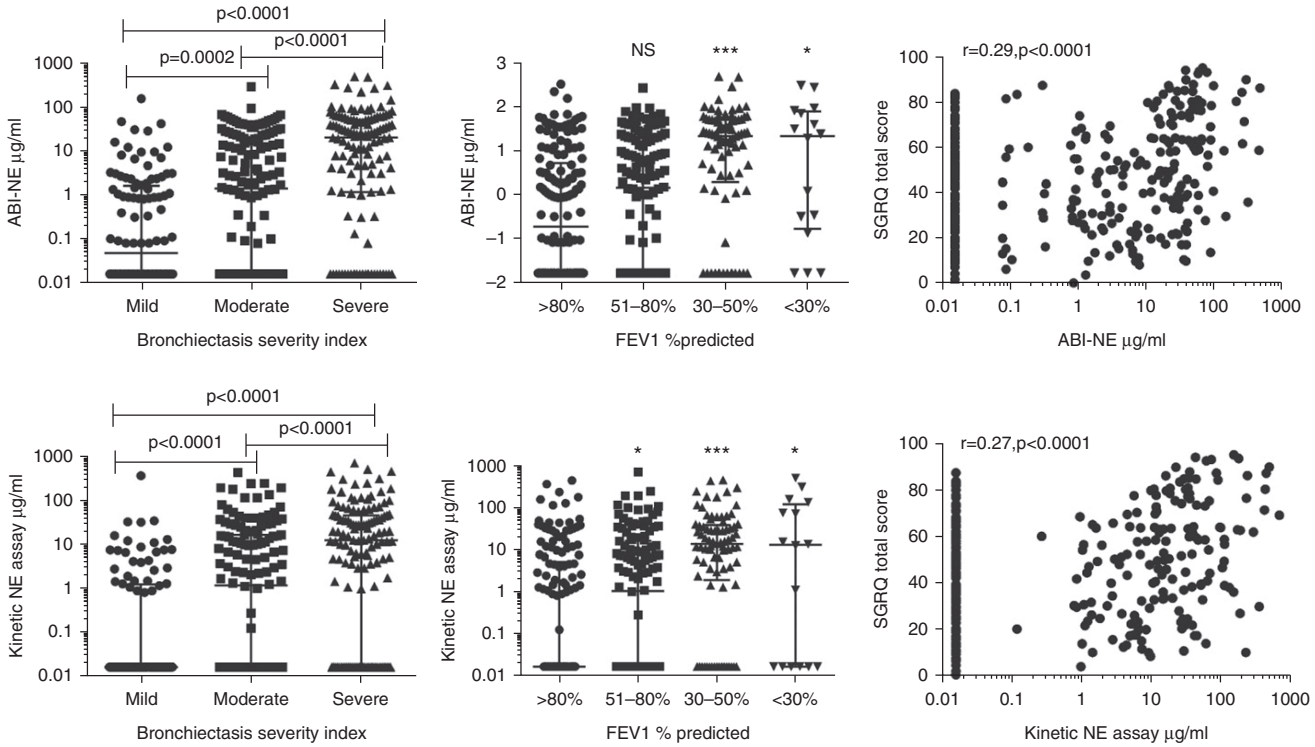

Figure 2. Association between neutrophil elastase (NE) and severity of disease. The activity-based immunoassay (ABI)-NE assay (*upper panels*) and kinetic NE assay (*lower panels*) are significantly different between bronchiectasis severity index and FEV_1% predicted groups and correlate with the St. George's Respiratory Questionnaire (SGRQ). *$P < 0.05$ compared with >80% predicted FEV_1; ***$P < 0.0001$ compared with >80% FEV_1% predicted. Across-group comparisons for FEV_1, $P < 0.0001$ by Kruskal-Walis test. Bronchiectasis severity index and FEV_1 cutoffs were chosen as those used in Reference 20. The median and interquartile range are shown. NS = no significant difference compared with FEV_1 >80% predicted.

treatment, and we showed that a failure to improve elastase with treatment predicted a shorter time to the next exacerbation. To the best of our knowledge, NE activity is the first biomarker to be associated with this range of clinically relevant outcomes in bronchiectasis. This confirmed and extended previous observations in diverse bronchiectasis populations in Hong Kong, Belgium, and the UK (13–15).

Tsang and colleagues previously showed that 24-hour NE output was correlated with 24-hour sputum volume, radiological severity of bronchiectasis, and FEV_1 (13). NE is not the only airway protease found in the bronchiectasis lung, but Goeminne and colleagues, in a study of 63 patients, showed that NE accounted for 82% of the total gelatinolytic activity of sputum, making a greater contribution than matrix metalloproteinases (15). Goeminne and colleagues also showed a statistically significant association between NE and FEV_1% predicted, which was not seen for matrix metalloproteinase-9 (MMP-9) (15). In the largest previous study on 385 patients with bronchiectasis, sputum NE activity measured using a kinetic assay was found to be associated with bacterial load, *P. aeruginosa* infection, radiological severity, and lung function (14). However, previous studies used a variety of different assays and a limited number of bronchiectasis severity indexes without longitudinal follow-up.

It is essential that candidate biomarkers undergo independent validation because markers typically perform better in their "discovery" or derivation cohort than in subsequent independent cohorts (28). Our study therefore validated these previous findings in a large cohort, because we demonstrated a clear association between elastase activity and a variety of markers of disease severity, including breathlessness, quality of life, and FEV_1. There was a

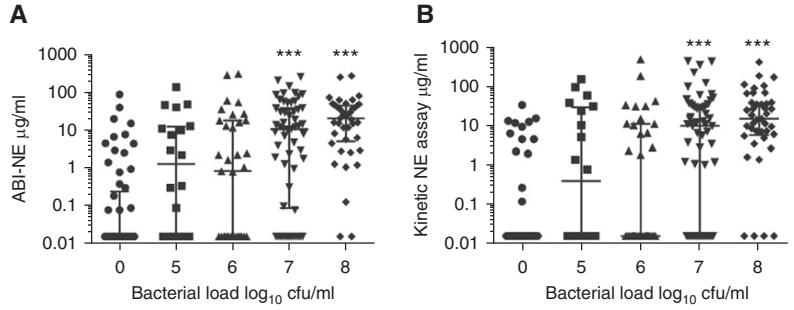

Figure 3. Association between neutrophil elastase (NE) and sputum bacterial load. (*A*) Data for the activity-based immunoassay (ABI)-NE assay. (*B*) Data for the kinetic assay. ***$P < 0.0001$ compared with no organism isolated. The median and interquartile range are shown.

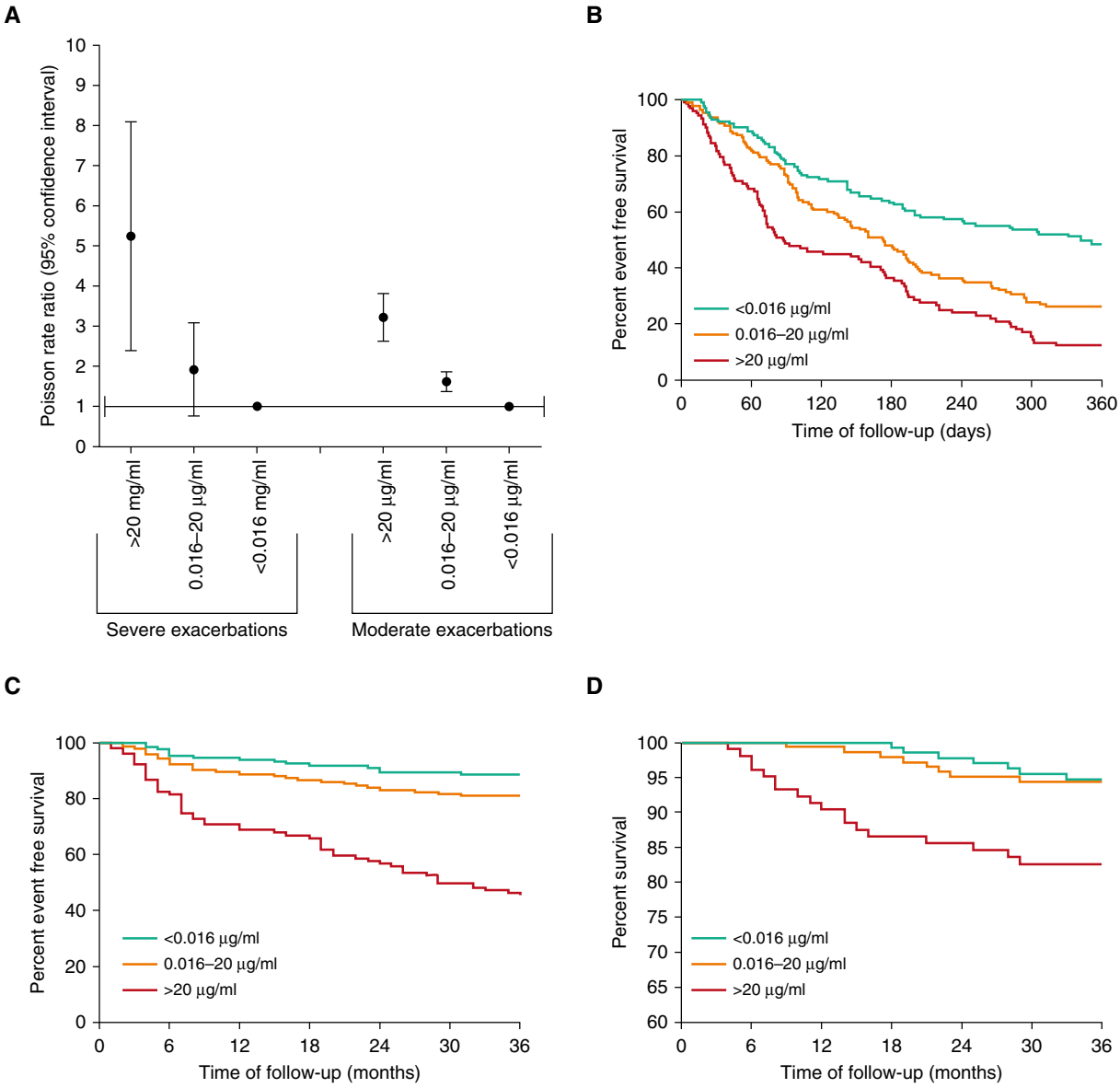

Figure 4. Association between neutrophil elastase and longitudinal clinical outcomes. (*A*) Rate ratio from Poisson regression; elastase levels >0.016 µg/ml are associated with significantly increased risk of moderate exacerbations ($P < 0.0001$), and levels >20 µg/ml are associated with increased severe exacerbations ($P < 0.0001$). (*B*) Time to next exacerbation. Elevated neutrophil elastase is associated with shorter time to next exacerbation ($P < 0.0001$ by log rank test). (*C*) Time to next hospitalization for severe exacerbation over 36 months ($P < 0.0001$ by log rank test). (*D*) All-cause mortality over 3 years ($P < 0.0001$ by log rank test; comparison between <0.016 and 0.016–20 µg/ml, not significant). Less than 0.016 µg/ml, n = 132; 0.016 to 20 µg/ml, n = 143; >20 µg/ml, n = 106.

strong relationship between elastase activity and the multidimensional BSI (20).

We observed strong relationships between NE activity and bacterial load. NE activity was also highest in patients with *P. aeruginosa* infection, and this was consistent with previous studies in bronchiectasis that showed that bacteria, and *P. aeruginosa* in particular, were the key drivers of airway neutrophilic inflammation (22), and that that *P. aeruginosa* infection represented a distinct clinical phenotype associated with earlier mortality, more frequent exacerbations, and worse quality of life (14, 22, 29, 30).

Although a biomarker that identifies patients with more severe disease is of interest, it is most important to find biomarkers that can identify patients at highest risk of future exacerbations and disease progression. Our study showed that NE activity was independently associated with lung function decline over 3 years, and identified elastase as the first biomarker associated with disease progression in bronchiectasis. In addition, elastase was independently associated with future exacerbations. Although patients with the highest elastase levels were at an increased risk of early death, this association was not

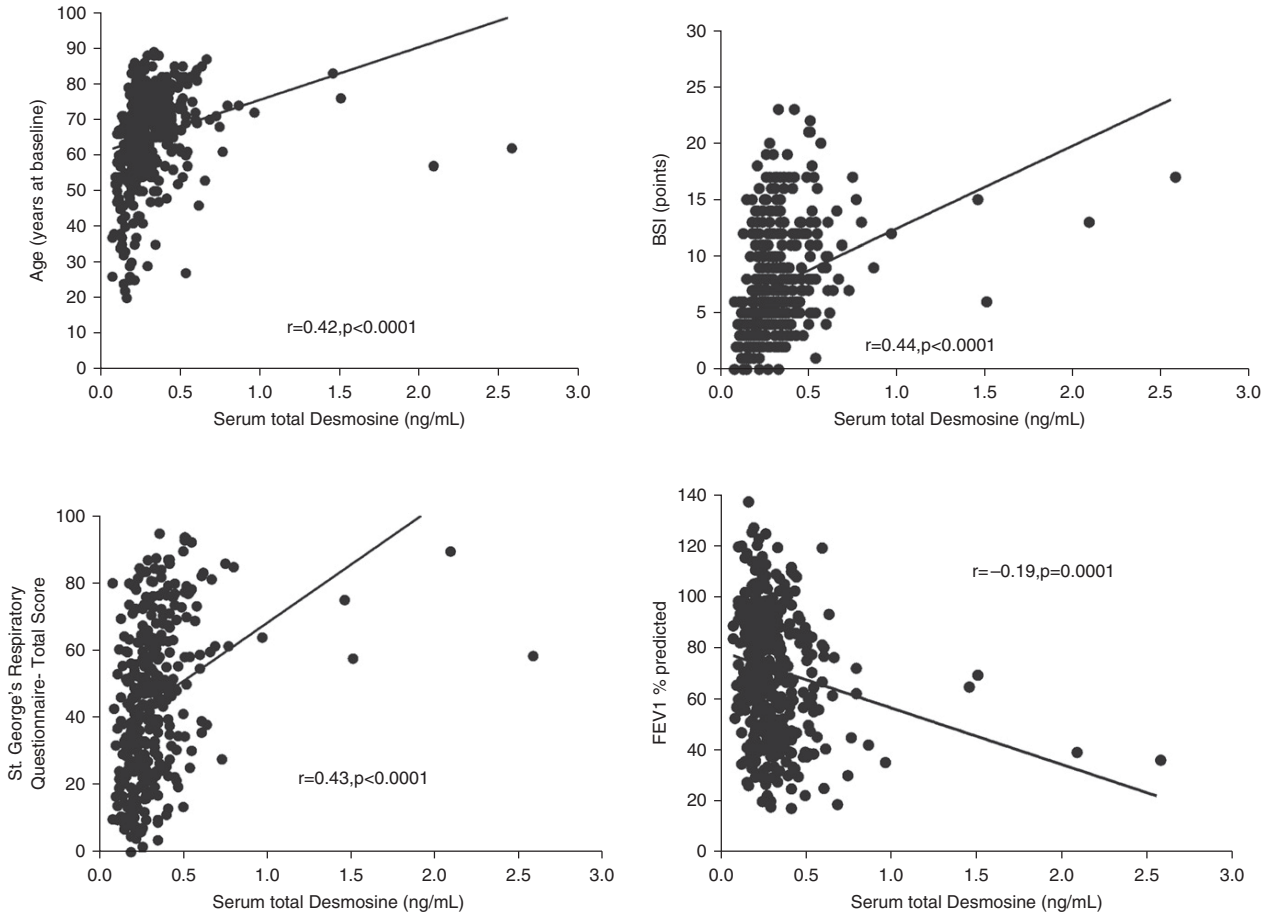

Figure 5. Spearman rank correlation analysis of the relationship between serum total desmosine and age, bronchiectasis severity index (BSI), St. George's Respiratory Questionnaire, and FEV_1% predicted.

independent of disease severity using the BSI, and only the highest levels of elastase were associated with increased mortality, which indicated that airway inflammation itself was not likely to be the primary driver of mortality in this population.

Our data were consistent with those seen in CF, in which NE has been shown to be a key biomarker (31–33). In a pooled analysis of 4 multicenter studies, Mayer-Hamblett and colleagues showed a clear relationship between elastase and FEV_1 (31). Sagel and colleagues extended these observations and demonstrated that elastase was the strongest predictor of lung function decline over 3 years (32). Sly and colleagues also demonstrated that elastase activity present in bronchoalveolar lavage was the strongest predictor of the early development of bronchiectasis in infants with CF (33).

Bronchiectasis has been a neglected disease and so, in contrast, biomarker studies are in their infancy. Markers identified in bronchiectasis include sputum MMP-8 and MMP-9, which were shown in a Chinese cohort to correlate with radiological severity, FEV_1, and the BSI (34). These findings were extended by Taylor and colleagues who showed that MMP-8 and MMP-9 were higher with *P. aeruginosa* or *H. influenzae* colonization and inversely associated with lung function (34, 35). These studies included 102 and 86 patients, respectively, and included only 1 year of follow-up; therefore, further large validation studies are required.

Inconsistent results were seen with cytokines such as CXCL8, TNF-α, and IL-1β in previous studies; in the present study, these were not significantly associated with clinical outcomes (14, 36).

No blood biomarkers have been studied in detail in bronchiectasis, which makes the identification of cDES as a potential marker of severity of significant interest. The results here are similar to those recently described in a large cohort with chronic obstructive pulmonary disease, in which cDES was associated with age and quality of life (17).

There was overwhelming evidence that elastase is involved in the pathophysiology of bronchiectasis. Destruction of elastin, basement membrane collagen, and proteoglycans by proteases contributes to disease progression and might explain the relationship between elastase and FEV_1 decline observed in this study (37). NE induces neutrophil dysfunction through multiple mechanisms, including cleavage of FcγRIIIb, and has also been shown to cleave complement receptor 1 in patients with CF (3, 38). NE can also cleave the opsonin iC3b from the surface of pathogens, leading to opsonin/receptor mismatch (39), although Vandivier and colleagues showed that elastase cleaved phosphatidylserine, which prevented the phagocytosis and clearance of apoptotic cells (40). Therefore, not surprisingly,

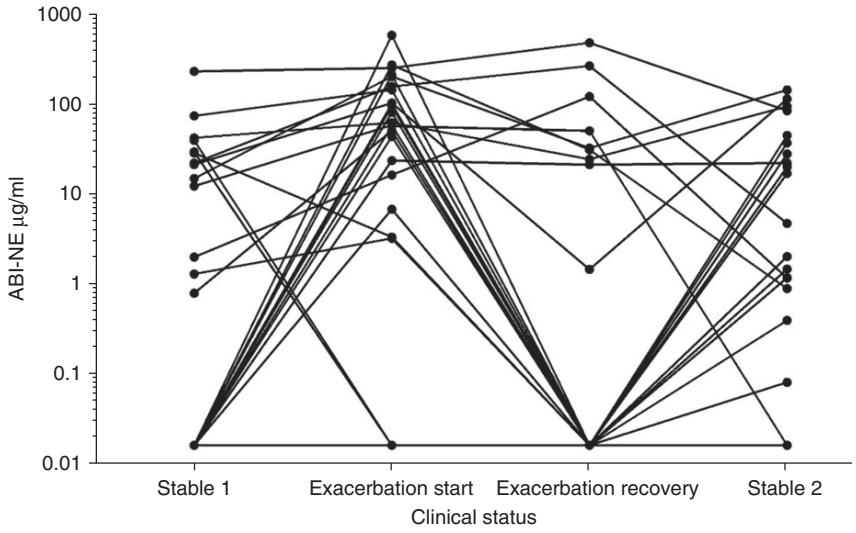

Figure 6. Changes in sputum neutrophil elastase (NE) activity at exacerbation and recovery. ABI = activity-based immunoassay.

therapeutic manipulation of elastase has been proposed in bronchiectasis. In a proof of concept study, Stockley and colleagues tested an oral NE inhibitor for 4 weeks in 38 patients with bronchiectasis (41). Although the primary outcome of a reduction in sputum neutrophils was not achieved, the study showed a clinically important and significant improvement in FEV_1 of 100 ml versus placebo, and a more than 4-point improvement in the SGRQ, which did not reach statistical significance (41).

Although NE activity appears able to stratify patients as high and low risk of disease progression, we cannot currently recommend management decisions based on elastase measurement. The next step would be implementation of such a strategy in a controlled clinical trial. Sputum biomarkers are not currently in routine use, and implementation would be greatly enhanced by the availability of a point-of-care device that could make the assay more rapid and accessible.

Because we and others have shown that elastase is responsive to change, and that changes in elastase correlate with clinical outcomes, measurement of NE might be particularly useful in clinical trials, where it could be used as an early "signal searching" or "early efficacy" endpoint for new antibiotics or anti-inflammatory therapies (14, 42). Such endpoints are essential to identify candidates in smaller clinical trials before embarking on large definitive phase 3 studies. It has been suggested that the absence of such an early response endpoint has contributed to the failure of a number of large phase 3 programs to reach their primary endpoints (43). NE should be further evaluated for this purpose.

There are a wide range of commercially available NE assays, and our data only demonstrated the validity of the ABI-NE, kinetic NE, and cDES assays in bronchiectasis. Additional studies with alternative assays, including those that quantify total elastase rather than elastase activity and urinary desmosine, may not give the same results. This was a single-center study, although the external validity of our results were strengthened by the similarity of the characteristics of these patients with other cohorts across Europe and the previous validation of findings from our center across multiple European centers (20, 44). We did not obtain multiple elastase measurements over time, except in a subset, and it will be important in the future to determine if repeated measurement of elastase could provide improved predictive accuracy. The cutoffs that we proposed here for intermediate and high levels of NE were not independently validated and should be tested in future cohort studies. The use of expectorated sputum for NE measurement might introduce sampling bias because only patients able to produce sputum could be included.

Conclusions

Sputum NE activity is associated with the future risk of exacerbations, including severe exacerbations and lung function decline in bronchiectasis. Elastase is therefore a marker of disease progression in bronchiectasis that may complement clinical assessment of multidimensional clinical scoring systems. NE levels reflect clinical status, and its response is associated with future risk of exacerbations. Future interventional studies should therefore evaluate whether elastase reduction can be used as a surrogate of efficacy in clinical trials. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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