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Published in:
European Respiratory Journal

DOI:
10.1183/13993003.01899-2015

Publication date:
2016

Document Version
Peer reviewed version

Title

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Authors

Stefano Aliberti MD[1], Sara Lonni MD[1], Simone Dore PhD[2], Melissa J. McDonnell MD[3], Pieter C. Goeminne MD[4,5], Katerina Dimakou MD[6], Thomas C Fardon MD[7], Robert Rutherford MD[3], Alberto Pesci MD[1], Marcos I. Restrepo MD[8], Giovanni Sotgiu MD, PhD[2], James D. Chalmers MD, PhD[7]

Affiliations

[1]Health Science Department, University of Milan Bicocca, AO San Gerardo, Via Pergolesi 33, Monza, Italy

[2]Clinical Epidemiology and Medical Statistics Unit, Department of Biomedical Sciences, University of Sassari - Research, Medical Education and Professional Development Unit, AOU Sassari, Sassari, Italy

[3] Department of Respiratory Medicine, Galway University Hospitals, Newcastle Road, Galway, Ireland

[4] University Hospital Gasthuisberg, Respiratory Medicine, Herestraat 49, B-3000 Leuven, Belgium

[5] Department of Respiratory Medicine, UZ Leuven, Herestraat 49, B-3000 Leuven, Belgium

[6] 5th Pulmonary Department, "Sotiria" Chest Hospital, Athens, Greece

[7] Tayside Respiratory Research Group, University of Dundee, Dundee, DD1 9SY, UK

[8] Division of Pulmonary Diseases & Critical Care, South Texas Veterans Health Care System and University of Texas Health Science Center at San Antonio Texas, USA

Corresponding author

Stefano Aliberti, MD, Health Science Department, University of Milan Bicocca, Respiratory Unit, AO San Gerardo, Via Pergolesi 33, 20052, Monza, Italy; e-mail: stefano.aliberti@unimib.it; tel: +390392339284; cell: +393394171538; fax: +390392339437

Take Home message

Chronic infection with *Pseudomonas* or other bacteria and daily sputum identify 4 clinical phenotypes in bronchiectasis

ABSTRACT

Bronchiectasis is a heterogeneous disease. This study aimed at identifying discrete groups of patients with different clinical and biological characteristics, and long-term outcomes.

This was a secondary analysis of five European databases of prospectively enrolled adult outpatients with bronchiectasis. Principal component and cluster analysis were performed using demographics, comorbidities, clinical, radiological, functional and microbiological variables collected during stable state. Exacerbations, hospitalizations and mortality during a 3-year follow-up were recorded. Clusters were externally validated in an independent cohort of patients with bronchiectasis, investigating also inflammatory markers in sputum.

Among 1,145 patients (median age: 66 years, 40% males), four clusters were identified driven by the evidence of chronic infection with *P. aeruginosa* or other pathogens and daily sputum: "*Pseudomonas*" (16%), "*Other chronic infection*" (24%), "*Daily sputum*" (33%) and "*Dry bronchiectasis*" (27%). Patients in the four clusters showed significant differences in terms of quality of life, exacerbations, hospitalizations and mortality during follow up. In the validation cohort, neutrophil elastase, myeloperoxidase and interleukin-1β levels in sputum were significantly different among the clusters.

Identification of four clinical phenotypes in bronchiectasis could favour focused treatments and interventions in further interventional studies designed to alter the natural history of the disease.

INTRODUCTION

Bronchiectasis is a chronic airway disease characterized by irreversibly damaged and dilated airways leading to recurrent episodes of bronchial sepsis. This results in poor mucus clearance and a vicious cycle of persistent bacterial colonization, airway obstruction, inflammation, and progressive tissue destruction [1].

The population of patients with bronchiectasis is extremely heterogeneous representing a group of disorders with a wide range of causes and varying clinical, radiological and microbiological features [2]. There are no licensed therapies for bronchiectasis and limited evidence even for widely used treatments such as physiotherapy and long-term macrolide treatment. Recently, a therapeutic approach based on the severity of the disease has been suggested [3]. A step towards a more individualized management of bronchiectasis patients would be the identification of distinct clinical phenotypes using a multidimensional approach that includes parameters available in daily clinical practice.

The majority of therapeutic approaches in bronchiectasis is aimed at the management of chronic bacterial infections, with short and long-term antibiotic courses [3]. It is not known, however, if those patients with chronic bacterial colonization or colonization with *Pseudomonas aeruginosa* present different clinical characteristics to those patients without. The clinical heterogeneity that characterizes patients with bronchiectasis might reflect different patho-physiological mechanisms, which could be considered potential targets for therapeutic interventions. Cluster analysis or unsupervised machine learning techniques have been successfully used to identify biological and clinical subgroups or "phenotypes" in other respiratory disorders such as asthma and COPD [4].

In light of the heterogeneity that characterizes patients with bronchiectasis and the need for better directing current management and targeting future treatments, we hypothesized that among these patients there would be discrete groups of subjects with different clinical and biological characteristics.

MATERIALS AND METHODS

Study population

This was a secondary analysis of five databases of prospectively enrolled outpatients with bronchiectasis referred to the bronchiectasis clinics of university teaching hospitals in Monza (Italy), Dundee (UK), Leuven (Belgium), Athens (Greece) and Galway (Ireland). Consecutive patients aged ≥18 years with a diagnosis of bronchiectasis on high-resolution computed tomography (HRCT) scan in stable state were enrolled. Patients with cystic fibrosis or traction bronchiectasis due to pulmonary fibrosis were excluded. A further exclusion criterion for the Athens cohort was either admission to the hospital or use of antibiotics in the prior four weeks and for the Leuven cohort was the presence of active cancer. Collection of selected variables was approved at each individual center by the local ethical committee or institutional review board.

Data collection

At the time of clinical assessment, all patients underwent the same comprehensive diagnostic work-up in each site as suggested by the 2010 British Thoracic Society (BTS) guidelines [1]. Demographics, comorbidities, disease severity, etiology of bronchiectasis, respiratory symptoms, sputum evaluation, radiological, functional and laboratory findings on stable state, quality of life, long-term treatments and outcomes (including exacerbations, hospitalizations and mortality) during a three-year follow-up were uniformly recorded in each database.

The Charlson comorbidity index was used to assess comorbidities; this is a sum score of 19 weighted diseases with higher scores denoting increasing burden of comorbidities [5]. The severity of bronchiectasis was evaluated according to both the Bronchiectasis Severity Index (BSI) and FACED score (evaluating FEV_1, Age, Ahronic infection with Pseudomonas, radiological Extension and Dyspnea) [6,7]. Radiological severity of bronchiectasis was assessed using a modified Reiff score, which rates the number of involved lobes (with the lingula considered to be a separate lobe) and the

degree of dilatation (range: 1-18) [8]. Patients completed the St. George's Respiratory Questionnaire (SGRQ) as a measure of quality of life [9]. All bacteriology was performed on spontaneous sputum samples as previously described [10]. Murray-Washington criteria for sputum quality was used in all cases, with all samples having less than 10 squamous cells and more than 25 leukocytes per low-power microscope field. Chronic infection was defined by the isolation of potentially pathogenic bacteria in sputum culture on two or more occasions, at least 3 months apart over a 1-year period [11]. The predominant pathogen was the organism grown most frequently over the study period. Patients who were unable to provide sputum samples due to absence of a productive cough were classified as not having chronic infection for the purposes of analysis.

Study outcomes

Exacerbations. An exacerbation of bronchiectasis was defined as a clinical diagnosis of exacerbation for which antibiotics were prescribed in the presence of at least one (and usually more than one) of the following symptoms: increasing cough, increasing sputum volume, worsening sputum purulence, worsening dyspnea, increased fatigue/malaise, fever, and haemoptysis [1]. *Hospitalization for severe exacerbations.* Severe exacerbations were defined according to the BTS guidelines, and unscheduled hospitalizations or emergency department visits for severe bronchiectasis exacerbations or complications were recorded from patient histories and verified using an administrative database that records all regional hospital admissions [1]. *Mortality*. All-cause mortality for up to 3 years was evaluated.

Statistical analysis

An electronic form was used to collect epidemiological, demographic, clinical, and follow-up variables in the clinical centers participating in the project. Qualitative and quantitative variables were summarized using relative frequencies (percentages) and medians (interquartile ranges, IQR), when appropriate. A chi-squared test and Kruskall-Wallis test were performed to evaluate qualitative and

quantitative variables, respectively. A p-value of <0.05 was used to consider a difference statistically significant. A Spearman correlation analysis was performed between all the collected variables; a *rho* >0.3 was used to select variables to be included in the principal component analysis (PCA). A cut-off eigenvalue of 0.7 was adopted to choose the components, and a factor loading of 0.4 was considered to identify the most important variables to be selected in the cluster analysis [12]. After hierarchical analysis a dendrogram was prepared to visually assess the distribution of the clusters related to the recruited cohort. The number of clusters was decided on the basis of a 20 Gower dissimilarity value. A descriptive and an inferential analysis of the collected variables in between the selected clusters was carried out to detect any statistical differences [13]. All the statistical analyses were performed with the statistical software STATA®13 (StataCorp, College Station, TX, USA).

Validation cohort

In order to validate the primary cluster analysis, we recruited an independent population of patients with HRCT confirmed bronchiectasis at Ninewells Hospital, Dundee, UK during 2014. We hypothesized that the clinical clusters identified in the primary study would show differences in neutrophil mediated inflammation. The validation study was conducted as a case control study, with a consecutive cohort of 30 patients recruited in each of the 4 identified phenotype arms (identified by their primary clustering characteristic), see online supplement. Spontaneous sputum samples were obtained and ultracentrifuged at 50,000G for 90mins to obtain supernatant for inflammatory marker measurement as previously described [10]. Clinical evaluation was conducted as described above.

RESULTS

Study population

A total of 1,145 patients were enrolled in the five centers (median [IQR] age: 66 [56-74] years; 40% males): 286 patients in Dundee, 280 in Galway, 230 in Monza, 190 in Leuven, and 159 in Athens. Demographics, clinical, functional and radiological status, microbiology, severity of the disease, and long-term antibiotic treatment of the entire study population are presented in Table 1. A detailed overview of the five cohorts is reported in Table A in the online supplementary material. PCA and cluster analysis, which are reported in the online supplementary material, allowed the identification of four clusters.

Cluster characteristics

The clinical features of the four clusters are shown in Table 2. 13 patients were allocated in none of the four clusters because of missing data and excluded by further analysis. The study population was almost equally distributed among the four clusters. Age, sex, body mass index (BMI) and Charlson comorbidity index were not significantly different among the four clusters. On the other hand, the presence and type of chronic infection were the major key factors defining two of the four clusters. Cluster 1 included 16% (n= 179) of the patients and all of them had chronic infection with *P. aeruginosa*. In comparison to all the other clusters, the patients belonging to this cluster presented the most severe disease, showing the worse radiological and the highest inflammatory patterns, the lowest functional status, the highest number of exacerbations and hospitalizations, and the worse quality of life at baseline. Based on these characteristics, Cluster 1 was labelled as "*Pseudomonas*".

There were two intermediate clusters in terms of disease severity (*i.e.*, Cluster 2 and 3), including patients with moderate levels of systemic inflammation and functional status, of whom 20% experienced at least one hospitalization per year.

Cluster 2 included 24% (n= 273) of the study population and was characterized by the presence of chronic infection with pathogens other than *P. aeruginosa*. Accordingly, this cluster was labelled as "*Other chronic infection*".

Cluster 3 was the largest cluster, including 33% (n= 373) of the study population. No patients within this cluster had chronic infection, but almost all of them had daily sputum and the majority of them were smokers or ex-smokers. Accordingly, this cluster was labelled as "*Daily sputum*".

Cluster 4 was composed by 27% (n= 307) of the patients who were the less severe ones, showing the lowest level of inflammatory biomarkers, the less severe radiological and functional impairment. None of these patients had chronic infection and none had daily sputum. Accordingly, this cluster was labelled as "*Dry bronchiectasis*".

The etiology of bronchiectasis was identified in 66% (n= 756) of the patients. Excluding idiopathic bronchiectasis, the first three most commonly defined etiologies were post-infective (290, 26%), COPD-related (120, 11%), and CTD-related (89, 8%), (Table 1). A detailed distribution of different etiologies among the four clusters is depicted in Table 3. No clinically and statistically significant differences in terms of bronchiectasis etiology were detected among the four clusters, but for post-infective and COPD-related bronchiectasis.

A significant difference in terms of long-term antibiotic treatment, including macrolide and inhaled antibiotic, was detected among the four clusters (Table 2). More than 50% of the patients in the "*Pseudomonas*", one-third of the patients in "*Other chronic infection* and "*Daily sputum*" clusters were exposed to macrolides. More than one-third of the patients in the "*Pseudomonas*" cluster were on inhaled antibiotic treatment, while few patients received this treatment in the other clusters.

Quality of life and clinical outcomes during follow up

Quality of life was evaluated in 389 (34%) patients, showing a median (IQR) SGRQ value of 39 (26-58). Significant differences in median values of SGRQ were detected across the four clusters with the worse quality of life for patients in the "*Pseudomonas*" cluster (Table 4).

Significant differences in terms of both exacerbations and hospitalizations due to exacerbations were detected along the four clusters during the one-year follow-up, with the higher rates detected in the "*Pseudomonas*" and "*Other chronic infection"* clusters. At one-year follow up no significant differences in mortality were detected among the different clusters; however, the "*Pseudomonas*" cluster showed a significant higher mortality rate during the three-year follow up period (Table 4).

Biological and clinical validity of the clusters

A total of 120 patients were included in a case-control study, using the major characteristics, namely the presence of *Pseudomonas aeruginosa*, chronic colonization with other pathogens, daily sputum production without colonization or the absence of these 3 characteristics, see Table 5. Patients in "*Pseudomonas*", "*Other Chronic infection*" and "*Daily sputum*" clusters were recruited consecutively. As sputum production was less frequent in the "*Dry bronchiectasis*" cluster, 66 patients were recruited in order to obtain 30 with sputum samples available for analysis. Higher levels of neutrophil elastase, myeloperoxidase and interleukin-1β were found in "*Pseudomonas*" and "*Other chronic infection*" clusters, while there were no differences in other cytokines, see Figure 1.

DISCUSSION

The major finding of our study is the evidence of microbiology, including chronic infections with *P. aeruginosa* or other pathogens, and the daily presence of sputum production as the major drivers to classify patients with bronchiectasis in four discrete groups. We have further demonstrated that these clusters represent clinical phenotypes because they exhibit differences in terms of not only inflammatory biomarkers on sputum but also quality of life and long-term clinical outcomes.

PCA and cluster analysis have been successfully used in recent years to classify patients with asthma and COPD in discrete groups according to similar combinations of disease characteristics [14-22]. No previous studies have used PCA and cluster analysis to identify clinical phenotypes in bronchiectasis. Previous literature defined disease severity through the identification of predictors of mortality and hospital admissions using multivariable analysis [6,7]. Although scores such as the BSI and the FACED might recognize which patient is globally severe, they do not help physicians in identifying which aspect of patient's management should be addressed firstly in daily clinical practice.

The present analysis, which does not include the derivation cohort of the BSI paper, sought clusters that were not necessarily related to outcomes, but rather patient characteristics providing important new information beyond the available scoring systems.

The presence of chronic infection with *Pseudomonas* seems to define by itself a specific clinical phenotype of patients with bronchiectasis who share a more relevant inflammatory status, a more severe disease, worse clinical, functional, and radiological characteristics, and worse quality of life and long-term outcomes, in line with previous literature [23]. Two thirds of the patients in the *Pseudomonas* phenotype were on long-term antibiotic treatment, with half of the patients receiving a macrolide. From a clinical perspective, it is reasonable to assume that patients belonging to this phenotype deserve almost all of the currently available treatment options, including mucus clearance techniques, bronchodilators, inhaled or oral long-term antibiotics, long-term oxygen therapy, embolization and other measurements to control haemoptysis, and vaccinations to prevent

exacerbations. However, this clinical phenotype characterized only 16% of the bronchiectasis patients in our cohort, prompting the need to better evaluate characteristics of other phenotypes in order to individualize and optimize management.

Patients with chronic infections other than *Pseudomonas* recognize a distinct clinical phenotype accounting for 24% of the patients with bronchiectasis. A recent study demonstrated worse outcomes for patients with a chronic infection due to pathogens other than *Pseudomonas* compared to patients without a chronic infection, but to a lesser extent than those infected with *Pseudomonas* [6]. Our findings related to the *Other chronic infection* phenotype, showing intermediate rates of exacerbations, hospitalizations, and mortality in between the *Pseudomonas* and the *Dry bronchiectasis* phenotypes, confirming these data. More than one third of the patients in the *Other chronic infection* phenotype received long-term antibiotic treatment, especially macrolide, with only 5.5% exposed to inhaled antibiotics. There is a paucity of data in literature concerning the evaluation of inhaled antibiotics specifically developed for chronic infections with bacteria other than *Pseudomonas*, although the percentage of these patients in the bronchiectasis population is not negligible and they do suffer from adverse outcomes.

The largest clinical phenotype in our cohort, the *Daily sputum* phenotype, was composed by patients who were active smokers, with daily sputum production and without chronic infections. These patients had similar level of disease severity and systemic inflammation, with a similar history of exacerbations, clinical outcomes and chronic antibiotic use in comparison with patients belonging to the *Other chronic infection* phenotype. These patients would deserve high treatment intensity, although the use of long-term antibiotics cannot be guided by microbiological data obtained by standard methods. However, it would be conceivable that, although with standard method we were not able to identify a chronic infection, new techniques, such as 16S rRNA gene pyrosequencing, could allow the microbiological classification of those patients, broadening potential therapeutic options [24].

Patients in the *Dry bronchiectasis* phenotype showed the lowest systemic inflammation level, disease severity, and clinical impact of bronchiectasis, with the best functional status and without any chronic infections. Very few data have been reported in the literature regarding non-clinically significant bronchiectasis and certainly further clinical and translational research is needed to identify early therapeutic targets in order to prevent disease progression.

When we validated our findings in an independent cohort of patients with bronchiectasis, we found that this classification based on the predominant feature of each cluster (chronic infection with *Pseudomonas* or other bacteria, daily sputum or the absence of daily sputum) is clinically valid with clear differences in lung function, symptoms, and disease severity between groups consistent with the primary analysis. In addition, we found higher levels of neutrophil elastase, myeloperoxidase and interleukin-1β in "*Pseudomonas*" and "*Other chronic infection"* clusters, while there were no differences in other cytokines. This is very much consistent with previous published data showing higher levels of neutrophil inflammatory markers in patients with bacterial colonization and higher bacterial loads [10]. This previous literature, together with the present findings, confirms that the bacterial colonization status is the most useful clinical parameter to sub-classify patients.

This study is the first one to apply cluster analysis principles for the identification of clinical phenotypes in bronchiectasis. It represents one of the largest cohorts of patients with bronchiectasis published so far in literature, including patients from five European countries with a three-year follow-up. The sample size and the multi-center design increase the inference of our findings. One of the strengths of the current analysis is the identification of different phenotypes in bronchiectasis through variables that are usually collected during daily clinical practice. Finally, the presence of differences in sputum biomarkers and quality of life in a validation cohort strengthens the validity of these phenotypes.

One of the limitations of the present study is that, although we merged multiple cohorts obtained in different settings across Europe to ensure representation of different subgroups of patients, all centers

were tertiary care ones enrolling a selected population of patients. Further studies should also include cohorts of in/outpatients recruited in both secondary and primary care settings and also collect other important comorbidities not assessed by the Charlson index, such as depression or anxiety. Furthermore, we did not collect the exact date of death and thus we were not able to perform a survival analysis on our cohort.

Cluster analysis, using clinical, microbiological, functional, and radiological variables identified four clinical phenotypes easily detected according to the presence of chronic infection with *P. aeruginosa* or other pathogens and daily sputum. Patients belonging to these clinical phenotypes show distinctly different patterns of sputum biomarkers, quality of life, and outcomes. Identification of clinical phenotypes showing similar biological profiles and prognosis could favour a focused treatment as well as test interventions in further randomized controlled trials designed to alter the natural history of the disease.

ACKNOWLEDGEMENTS

This study was supported by the European Multicentre Bronchiectasis Audit and Research Collaboration (EMBARC; www.bronchiectasis.eu) and the BRONCH-UK Consortium. EMBARC is an European Respiratory Society Clinical Research Collaboration and has received funding from the European Respiratory Society, Bayer HealthCare and Aradigm Corporation. James D Chalmers acknowledges fellowship support from the Medical Research Council and the Wellcome Trust. Melissa J McDonnell acknowledges fellowship support from the European Respiratory Society/European Lung Foundation and Health Research Board, Ireland. Dr. Restrepo time is partially protected by Award Number K23HL096054 from the National Heart, Lung, and Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, And Blood Institute or the National Institutes of Health. The funding agencies had no role in the preparation, review, or approval of the manuscript. The views

expressed in this article are those of the author and do not necessarily represent the views of the Department of Veterans Affairs.

REFERENCES

1. Pasteur MC, Bilton D, Hill AT; British Thoracic Society Bronchiectasis non-CF Guideline Group. British Thoracic Society guideline for non-CF bronchiectasis. Thorax 2010; 65 Suppl 1: i1-58.
2. Barker AF. Bronchiectasis. N Engl J Med 2002; 346: 1383–1393.
3. Chalmers JD, Aliberti S, Blasi F. Management of bronchiectasis in adults. Eur Respir J 2015; 45: 1446-1462.
4. Han MK, Agusti A, Calverley PM, Celli BR, Criner G, Curtis JL, Fabbri LM, Goldin JG, Jones PW, Macnee W, Make BJ, Rabe KF, Rennard SI, Sciurba FC, Silverman EK, Vestbo J, Washko GR, Wouters EF, Martinez FJ. Chronic obstructive pulmonary disease phenotypes: the future of COPD. Am J Respir Crit Care Med 2010; 182: 598–604.
5. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987; 40: 373–383.
6. Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, Poppelwell L, Salih W, Pesci A, Dupont LJ, Fardon TC, De Soyza A, Hill AT. The bronchiectasis severity index. An international derivation and validation study. Am J Respir Crit Care Med 2014; 189: 576-585.
7. Martínez-García MÁ, de Gracia J, Vendrell Relat M, Girón RM, Máiz Carro L, de la Rosa Carrillo D, Olveira C. Multidimensional approach to non-cystic fibrosis bronchiectasis: the FACED score. Eur Respir J 2014; 43: 1357-1367.
8. Reiff DB, Wells AU, Carr DH, Cole PJ, Hansell DM. CT findings in bronchiectasis: limited

value in distinguishing between idiopathic and specific types. AJR Am J Roentgenol 1995; 165: 261–267.

9. Wilson CB, Jones PW, O'Leary CJ, Cole PJ, Wilson R. Validation of the St. George's Respiratory Questionnaire in bronchiectasis. Am J Respir Crit Care Med 1997; 156: 536–541.
10. Chalmers JD, Smith MP, McHugh BJ, Doherty C, Govan JR, Hill AT. Short- and long-term antibiotic treatment reduces airway and systemic inflammation in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med 2012; 186: 657–665.
11. Pasteur MC, Helliwell SM, Houghton SJ, Webb SC, Foweraker JE, Coulden RA, Flower CD, Bilton D, Keogan MT. An investigation into causative factors in patients with bronchiectasis. Am J Respir Crit Care Med 2000; 162: 1277–1284.
12. Jolliffe IT. Discarding Variables in a Principal Component Analysis. II: Real Data Journal of the Royal Statistical Society. Series C (Applied Statistics) 1973; 22: 21-31
13. Bartholomew DJ, Steele F, Galbraith J, Moustaki I. Analysis of Multivariate Social Science Data, II Edition by Chapman and Hall/CRC, June 4, 2008.
14. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med 2008; 178: 218-224.
15. Everitt B, Landau S, Leese M, Stah lD. Cluster Analysis. 5th Edition editor. Hoboken NJ: John Wiley and Sons; 2011, Burgel PR, Paillasseur JL, Caillaud D, Tillie-Leblond I, Chanez P, Escamilla R, Court-Fortune I, Perez T, Carré P, Roche N, Initiatives BPCO Scientific Committee. Clinical COPD phenotypes: a novel approach using principal component and cluster analyses. Eur Respir J 2010; 36: 531-539.
16. Vanfleteren LE, Spruit MA, Groenen M, Gaffron S, van Empel VP, Bruijnzeel PL, Rutten EP, Op't Roodt J, Wouters EF, Franssen FM. Clusters of comorbidities based on validated objective measurements and systemic inflammation in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2013; 187: 728-735.

17. Burgel PR, Roche N, Paillasseur JL, Tillie-Leblond I, Chanez P, Escamilla R, Court-Fortune I, Perez T, Carré P, Caillaud D; INITIATIVES BPCO Scientific Committee. Clinical COPD phenotypes identified by cluster analysis: validation with mortality. Eur Respir J 2012; 40: 495-496.
18. Garcia-Aymerich J, Gómez FP, Benet M, Farrero E, Basagaña X, Gayete À, Paré C, Freixa X, Ferrer J, Ferrer A, Roca J, Gáldiz JB, Sauleda J, Monsó E, Gea J, Barberà JA, Agustí À, Antó JM; PAC-COPD Study Group. Identification and prospective validation of clinically relevant chronic obstructive pulmonary disease (COPD) subtypes. Thorax 2011; 66: 430-437.
19. Cho MH, Washko GR, Hoffmann TJ, Criner GJ, Hoffman EA, Martinez FJ, Laird N, Reilly JJ, Silverman EK. Cluster analysis in severe emphysema subjects using phenotype and genotype data: an exploratory investigation. Respir Res 2010; 11: 30.
20. Burgel PR, Paillasseur JL, Peene B, Dusser D, Roche N, Coolen J, Troosters T, Decramer M, Janssens W. Two distinct chronic obstructive pulmonary disease (COPD) phenotypes are associated with high risk of mortality. PloS One 2012; 7: e51048.
21. Castaldi PJ, Dy J, Ross J, Chang Y, Washko GR, Curran-Everett D, Williams A, Lynch DA, Make BJ, Crapo JD, Bowler RP, Regan EA, Hokanson JE, Kinney GL, Han MK, Soler X, Ramsdell JW, Barr RG, Foreman M, van Beek E, Casaburi R, Criner GJ, Lutz SM, Rennard SI, Santorico S, Sciurba FC, DeMeo DL, Hersh CP, Silverman EK, Cho MH. Cluster analysis in the COPDGene study identifies subtypes of smokers with distinct patterns of airway disease and emphysema. Thorax 2014; 69: 415-422.
22. Rennard SI, Locantore N, Delafont B, Tal-Singer R, Silverman EK, Vestbo J, Miller BE, Bakke P, Celli B, Calverley PM, Coxson H, Crim C, Edwards LD, Lomas DA, MacNee W, Wouters EF, Yates JC, Coca I, Agustí A; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints. Identification of five chronic obstructive pulmonary disease subgroups with different prognoses in the ECLIPSE cohort using cluster analysis. Ann Am Thorac Soc 2015;12: 303-312.

23. Finch S, McDonnell MJ, Abo-Leyah H, Aliberti S, Chalmers JD. A Comprehensive Analysis of the Impact of Pseudomonas aeruginosa Colonisation on Prognosis in Adult Bronchiectasis. Ann Am Thorac Soc 2015 Sep 10.
24. Tunney MM, Einarsson GG, Wei L, Drain M, Klem ER, Cardwell C, Ennis M, Boucher RC, Wolfgang MC, Elborn JS. Lung microbiota and bacterial abundance in patients with bronchiectasis when clinically stable and during exacerbation. Am J Respir Crit Care Med 2013; 187: 1118-1126.

FIGURE LEGEND

Figure 1. Sputum markers of inflammation among different clusters in the validation cohort.

Footnotes. 1: *Pseudomonas*; 2: *Other chronic infection*; 3: *Daily sputum*; 4: *Dry bronchiectasis*. MPO: myeloperoxidase; TNF: tumor necrosis factor

TABLES

Table 1. Patient characteristics of the entire study population

Variables		
Study cohort	n. (%)	1,145
Demographics and comorbidities		
Age, years	median (IQR)	66 (56-74)
Male	n. (%)	455 (40)
Body Mass Index	median (IQR)	25 (22-28)
Smokers / Ex-smokers	n. (%)	437 (38)
Charlson Comorbidities Index>1	n(%)	373 (33)
Etiology of bronchiectasis		
Idiopathic	n. (%)	373 (34)
Post-infective	n. (%)	290 (26)
COPD	n. (%)	120 (11)
Connective tissue disease	n. (%)	89 (8)
Immunodeficiency	n. (%)	56 (5)
ABPA	n. (%)	54 (4.9)
Asthma	n. (%)	35 (3.2)
Inflammatory bowel disease	n. (%)	24 (2.2)
Ciliary dysfunction	n. (%)	20 (1.8)
Aspiration	n. (%)	14 (1.3)
$Alpha_1$-antitrypsin deficiency	n. (%)	10 (0.9)
Congenital	n. (%)	5 (0.5)
Other	n. (%)	20 (1.8)
Severity of the disease		
BSI score	median (IQR)	6 (4-11)

FACED score	median (IQR)	2 (1-3)
Radiological Status		
Reiff score	median (IQR)	4 (2-6)
Clinical status		
Daily cough	n(%)	899 (79)
Daily sputum	n(%)	744 (65)
Prior history of haemoptysis	n(%)	206 (18)
MRC breathlessness scale	median (IQR)	2 (1-3)
Long-term oxygen therapy	n(%)	86 (7.5)
Exacerbations in the previous year	median (IQR)	2 (1-3)
At least one hospitalization in the previous year	n(%)	307 (27)
Functional Status		
FEV_1, % predicted	median (IQR)	75 (55-95)
Microbiology		
Chronic infection with at least one pathogen*	n. (%)	455 (40)
H. influenzae	n. (%)	185 (16)
P. aeruginosa	n. (%)	180 (15)
Methicillin-sensitive *S. aureus*	n. (%)	61 (5.3)
M. catarrhalis	n. (%)	51 (4.5)
S. pneumoniae	n. (%)	47 (4.1)
Enterobacteriaceae	n. (%)	29 (2.5)
Methicillin-resistant *S. aureus*	n. (%)	21 (1.8)
E. coli	n. (%)	14 (1.2)
K. pneumoniae	n. (%)	13 (1.1)
Non-tuberculous mycobacteria	n. (%)	8 (0.7)

Other	n. (%)	31 (2.7)
Laboratory findings		
C-reactive protein, mg/L	median (IQR)	6 (4-8)
Long-term antibiotic treatment		
Either macrolide or inhaled antibiotics	n. (%)	388 (34)
Macrolide	n. (%)	358 (31.3)
Inhaled antibiotic treatment	n. (%)	90 (7.9)
Both macrolide and inhaled antibiotics	n. (%)	60 (5.2)

n: number; IQR: interquartile range 25-75; BSI: Bronchiectasis Severity Index; MRC: medical research council; FEV_1: forced expiratory volume in the first second. *150 patients had more than one pathogen as cause of chronic infection. Among those, 56 patients had *P. aeruginosa plus* another bacteria.

Table 2. Baseline characteristics in the four identified clusters

Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Overall p-value

		"*Pseudomonas*"	"*Other chronic infection*"	"*Daily sputum*"	"*Dry bronchiectasis*"	
Total n. (%)		179 (100)	273 (100)	373 (100)	307 (100)	
Center	Dundee, UK	44 (25)	128 (47)	90 (24)	24 (68)	<0.0001
	Leuven, BE	16 (8.9)	19 (7.0)	66 (18)	89 (29)	
	Monza, IT	23 (13)	24 (8.8)	87 (23)	96 (31)	
	Galway, IR	39 (22)	78 (29)	74 (20)	89 (29)	
	Athens, GR	57 (32)	24 (8.8)	56 (15)	9 (2.9)	
Demographics and comorbidities						
Age, years	median (IQR)	67 (56-75)	65 (56-73)	67 (57-74)	66 (55-74)	0.52
Male	n.(%)	81 (45)	112 (41)	148 (40)	109 (36)	0.19
Body Mass Index	median (IQR)	25 (21-27)	25 (22-28)	25 (22-28)	25 (21-28)	0.47
Smoker or ex-smokers	n.(%)	56 (31)	90 (33)	165 (44)	121 (39)	0.005
Charlson Comorbidities Index>1	n.(%)	53 (30)	101 (37)	113 (30)	106 (35)	0.20
Severity of the disease						
BSI score	median (IQR)	14 (11-17)	7 (5-10)	6 (3-9)	5 (3-7)	0.0001
FACED score	median (IQR)	4 (2-5)	2 (1-3)	2 (0.5-3)	1 (0-3)	<0.001
Radiological status						
Reiff score	median (IQR)	6 (4-9)	4 (2-6)	3 (2-6)	3 (2-6)	0.0001
Clinical status						
Daily cough	n.(%)	170 (95)	241 (88)	322 (86)	154 (50)	<0.0001
Daily sputum	n.(%)	166 (93)	204 (75)	362 (97)	0 (0)	<0.0001
Prior history of haemoptysis	n.(%)	42 (24)	36 (13)	80 (22)	43 (14)	0.002
MRC breathlessness scale	median (IQR)	3 (2-5)	2 (1-3)	2 (1-3)	1 (1-2)	0.0001

Long-term oxygen therapy	n.(%)	34 (19)	14 (5.1)	36 (9.7)	0 (0)	<0.0001
Exacerbations in the previous year	median (IQR)	3 (2-4)	2 (1-3)	2 (1-3)	2 (1-3)	0.0001
At least one hospitalization in the previous year	n.(%)	109 (61)	63 (23)	90 (24)	36 (12)	<0.0001
Functional Status						
FEV_1 (% predicted)	median (IQR)	59 (46-78)	71 (55-93)	77 (57-95)	84 (68-101)	0.0001
Microbiology						
Chronic infection with *P. aeruginosa*	n.(%)	179 (100)	0 (0)	0 (0)	0 (0)	<0.0001
Chronic infection with other pathogens	n.(%)	0 (0)	273 (100)	0 (0)	0 (0)	<0.0001
Laboratory findings						
C-reactive protein, mg/L	median (IQR)	10.7 (4.0-36.0)	5.0 (3.7-9.0)	4.5 (2.0-7.7)	3.0 (1.2-7.2)	0.0001
Long-term antibiotic treatment						
Either macrolide or inhaled antibiotics	n.(%)	120 (67)	105 (39)	122 (33)	38 (12)	<0.0001
Macrolide	n.(%)	97 (54)	103 (38)	119 (32)	37 (12)	<0.0001
Inhaled antibiotics	n.(%)	64 (36)	15 (5.5)	7 (1.9)	2 (0.7)	<0.0001
Both macrolide and inhaled	n.(%)	41 (23)	13 (4.8)	4 (1.1)	1 (0.3)	<0.0001

n: number; IQR: interquartile range 25-75; BSI: Bronchiectasis Severity Index; MRC: medical research council; FEV_1: forced expiratory volume in the first second.

Table 3. Etiology of bronchiectasis in the four clusters

Variables	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Overall p-value**
	"*Pseudomonas*"	**"*Other chronic infection*"**	**"*Daily sputum*"**	**"*Dry bronchiectasis*"**	
Total n. (%)	179 (100)	273 (100)	373 (100)	307 (100)	
Idiopathic	46 (26)	86 (33)	131 (36)	110 (36)	0.09
Post-infective	63 (36)	54 (21)	96 (26)	77 (25)	0.004
COPD	21 (12)	29 (11)	50 (14)	20 (6.6)	0.03
Connective tissue disease	10 (5.6)	26 (9.8)	26 (7.1)	27 (8.9)	0.377
Immunodeficiency	11 (6.2)	17 (6.4)	14 (3.8)	14 (4.6)	0.436
ABPA	10 (5.6)	20 (7.6)	12 (3.3)	12 (3.9)	0.083
Asthma	2 (1.1)	10 (3.8)	8 (2.2)	15 (4.9)	0.071
Inflammatory bowel disease	3 (1.7)	6 (2.3)	12 (3.3)	3 (1)	0.233
Ciliary dysfunction	7 (4)	6 (2.3)	5 (1.4)	2 (0.7)	0.055
Aspiration	2 (1.1)	6 (1.9)	3 (0.8)	3 (1)	0.419
$Alpha_1$-antitrypsin deficiency	0 (0)	1 (0.4)	3 (0.8)	6 (2)	0.091
Congenital	0 (0)	2 (0.8)	3 (0.8)	0 (0)	0.284
Other	2 (1.1)	1 (0.4)	2 (0.5)	15 (4.9)	<0.001

Table 4. Quality of life and longitudinal outcomes for each identified cluster

Variable		Cluster 1	Cluster 2	Cluster 3	Cluster 4	Overall p-value
		"*Pseudomonas*"	"*Other chronic infection*"	"*Daily sputum*"	"*Dry bronchiectasis*"	
Total n. (%)		179 (100)	273 (100)	373 (100)	307 (100)	
Quality of life						
SGRQ	median (IQR)	58 (34-72)	43 (27-61)	39 (27-55)	29 (12-40)	<0.001
Outcomes						
Exacerbations during one-year follow-up	median (IQR)	2 (1-3)	2 (1-2)	1 (0-2)	1 (0-2)	0.0001
At least one hospitalization during one-year follow-up	n(%)	67 (42)	41 (16)	56 (16)	42 (14)	<0.0001
Mortality during one-year follow-up	n(%)	9 (5.1)	4 (1.5)	13 (3.6)	14 (4.9)	0.12
Mortality during three-year follow-up	n(%)	26 (17)	19 (7.6)	24 (8.2)	23 (11)	0.02

SGRQ: St. George's Respiratory Questionnaire.

Table 5. Patients' characteristics and quality of life in the validation cohort according to the four clusters.

Variables		Cluster 1	Cluster 2	Cluster 3	Cluster 4	Overall p-value
		"*Pseudomonas*"	"*Other chronic infection*"	"*Daily sputum*"	"*Dry bronchiectasis*"	
Demographics and comorbidities						
Age, years	median (IQR)	70 (62-72)	66 (57-74)	65 (55-72)	63 (55-75)	0.7
Male	n.(%)	14 (46.6%)	10 (33.3%)	13 (43.3%)	11 (36.7%)	0.7
Body Mass Index	median (IQR)	24 (21-26)	26 (22-30)	25 (24-30)	26 (22-30)	0.8
Smoker or ex-smokers	n.(%)	9 (30%)	8 (27%)	9 (30%)	4 (13.3%)	0.4
Charlson Comorbidities Index>1	n.(%)	11 (37%)	10 (33%)	8 (27%)	12 (40%)	0.7
Severity of the disease						
BSI score	median (IQR)	12 (9-15)	8 (5-11)	6 (3-9)	6 (4-8)	0.0001
Radiological status						
Reiff score	median (IQR)	6 (3-10)	3 (2-6)	2 (2-5)	3 (2-4)	<0.0001
Clinical status						
Daily cough	n.(%)	30 (100)	28 (93)	30 (100)	19 (64)	<0.0001
Daily sputum	n.(%)	29 (97)	27 (90)	30 (100)	0 (0)	<0.0001
Prior history of haemoptysis	n.(%)	8 (27)	4 (13)	3 (10)	0 (0)	0.02
MRC breathlessness scale	median (IQR)	3 (2-5)	3 (2-4)	1 (1-2)	1 (1-2)	<0.0001
Exacerbations in the previous year	median (IQR)	2 (1-5)	2 (0-2)	1 (0-1)	1 (0-2)	<0.0001

At least one hospitalization in the previous year	n.(%)	20 (66.7)	15 (50)	5 (16.6)	3 (10.0)	<0.0001
Functional Status						
FEV_1 (% predicted)	median (IQR)	51 (35-76)	61 (49-81)	83 (65-97)	93 (59-99)	<0.0001
Microbiology						
Chronic infection with *P. aeruginosa*	n.(%)	30 (100)	0	0	0	<0.0001
Chronic infection with other pathogens	n.(%)	0	30 (100)	0	0	<0.0001
Quality of life						
SGRQ	median (IQR)	58.3 (38.6-70.3)	44.3 (31.8-51.9)	33.3 (25.6-37.1)	36.6 (20-49.0)	<0.0001
Leicester cough questionnaire	median (IQR)	10 (9-16)	13 (10-17)	16 (14-19)	13.2 (11.0-19.0)	0.004

n: number; IQR: interquartile range 25-75; BSI: Bronchiectasis Severity Index; MRC: medical research council; FEV_1: forced expiratory volume in the first second; SGRQ: St. George's Respiratory Questionnaire.