ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Original research article

Comparison of different sets of immunological tests to identify treatable immunodeficiencies in adult bronchiectasis patients

Stefano Aliberti, Francesco Amati, Andrea Gramegna, Barbara Vigone, Martina Oriano, Giovanni Sotgiu, Marco Mantero, Edoardo Simonetta, Laura Saderi, Anna Stainer, Serena Tammaro, Paola Marchisio, Eva Polverino, James D. Chalmers, Francesco Blasi

Please cite this article as: Aliberti S, Amati F, Gramegna A, *et al*. Comparison of different sets of immunological tests to identify treatable immunodeficiencies in adult bronchiectasis patients. *ERJ Open Res* 2021; in press (https://doi.org/10.1183/23120541.00388-2021).

Comparison of different sets of immunological tests to identify treatable immunodeficiencies in adult bronchiectasis patients

Authors

Stefano Aliberti MD[1,2*], Francesco Amati MD[1*], Andrea Gramegna MD[1,2], Barbara Vigone MD[3], Martina Oriano PhD[1,2], Giovanni Sotgiu MD, PhD[4], Marco Mantero MD[1,2], Edoardo Simonetta MD[1,2], Laura Saderi BSc[4], Anna Stainer MD[5], Serena Tammaro PT[1], Paola Marchisio MD[2,6], Eva Polverino MD[7], James D. Chalmers PhD[8], Francesco Blasi MD, PhD[1,2]

Affiliations

[1]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Respiratory Unit and Cystic Fibrosis Adult Center, Milan, Italy

[2]Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy

[3] Scleroderma Unit, Referral Center for Systemic Autoimmune Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy

[4] Clinical Epidemiology and Medical Statistics Unit, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy

[5] Respiratory Unit, San Gerardo Hospital, Department of Medicine and Surgery, University of Milan-Bicocca, Milan, Italy.

[6] Pediatric Highly Intensive Care Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[7] Pneumology Department, Hospital Universitari Vall d'Hebron, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain.

[8] College of Medicine, University of Dundee, Dundee, UK

*Shared first authorship

Corresponding author

Stefano Aliberti, MD, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Respiratory Unit and Cystic Fibrosis Adult Center, Milan, Italy; Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy

Take home message: A 4-fold increase in the diagnosis of immunodeficiencies was found in adults with bronchiectasis when IgG subclasses and lymphocyte subsets are added to the bundle of tests recommended by ERS guidelines

ABSTRACT

Background: Reported prevalence of immunodeficiencies in bronchiectasis patients is variable depending on the frequency and extent of immunological tests performed. ERS Guidelines recommend a *minimum* bundle of tests. Broadening the spectrum of immunological tests could increase the number of patients diagnosed with an immunodeficiency and those who could receive specific therapy. The primary objective of the present study was to assess the performance of different sets of immunological tests in diagnosing any, primary, secondary, or treatable immunodeficiencies in adults with bronchiectasis.

Methods: An observational, cross-sectional study was conducted at the Bronchiectasis Program of the Policlinico University Hospital in Milan, Italy, from September 2016 to June 2019. Adult outpatients with a clinical and radiological diagnosis of bronchiectasis underwent the same immunological screening during the first visit when clinically stable consisting of: complete blood count, IgA, IgG, IgM, IgG subclasses, total IgE, lymphocyte subsets, and HIV antibodies. The primary

endpoint was the prevalence of patients with any immunodeficiencies using five different sets of immunological tests.

Results: A total of 401 bronchiectasis patients underwent the immunological screening. A significantly different prevalence of bronchiectasis patients diagnosed with any, primary, or secondary immunodeficiencies was found across different bundles. 44.6% bronchiectasis patients had a diagnosis of immunodeficiency using when IgG subclasses and lymphocyte subset are added to the *minimum* bundle suggested by the guidelines.

Conclusion: A 4-fold increase in the diagnosis of immunodeficiencies can be found in adults with bronchiectasis when IgG subclasses and lymphocyte subsets are added to the bundle of tests recommended by guidelines.

Keywords: Bronchiectasis, immunodeficiency, treatable traits

INTRODUCTION

Bronchiectasis is a chronic respiratory disease characterized by abnormal dilations of the bronchi in the context of chronic symptoms (e.g., cough and daily sputum) and frequent respiratory infections [1]. International guidelines recommend an individualized work-up to detect treatable causes of bronchiectasis [2]. Immunodeficiency is one of the most prevalent etiologies of bronchiectasis, and specific treatments, including intravenous immunoglobulins, might improve patients' outcomes, including the frequency of severe respiratory infections such as pneumonia. [3,4].

Immunodeficiency encompasses a *spectrum* of multiple disorders, including innate and adaptive immune system defects, phagocytic, complement, syndromic disorders, as well as secondary immunodeficiencies [5]. From one hand, bronchiectasis is a very common pulmonary complication of common variable immunodeficiency (CVID) [6]. From the other hand, the reported prevalence of immunodeficiencies in bronchiectasis patients ranges from 1% to 9% and this variability might rely on the frequency and extent of immunological tests performed across different clinical centers [3,7-12]. A lack of standardized diagnostic testing panels for bronchiectasis exists with a marked variation in the performance of some diagnostic assays or variation in the use of reference intervals to define presence or absence of a disease. In terms of immunological work-up, guidelines on the management of bronchiectasis published by the European Respiratory Society (ERS) in 2017 recommend a *minimum* bundle of tests, including complete blood count, and total IgG, IgA, and IgM serum levels [2]. Broadening the spectrum of immunological tests could increase the number of patients diagnosed with an immunodeficiency and those who could receive specific therapy.

The objectives of the present study were: 1) to assess the performance of different sets of immunological tests in diagnosing any, primary, secondary, or treatable immunodeficiencies in

adults with bronchiectasis; 2) to evaluate the clinical and microbiological (including microbiome) characteristics of bronchiectasis in adults with immunodeficiencies.

MATERIALS AND METHODS

Study design and population

An observational, cross-sectional study was conducted at the Bronchiectasis Program of the Policlinico University Hospital in Milan, Italy, from September 2016 to June 2019. Adult (≥18 years of age) outpatients with a clinical (daily sputum production) and radiological (at least one lobe involvement on high-resolution CT scan) diagnosis of bronchiectasis underwent the same immunological screening during the first visit when clinically stable (defined as the absence of exacerbation and antibiotic exposure for 1 month). Patients with either cystic fibrosis (CF) or traction bronchiectasis due to pulmonary fibrosis were excluded. The study was approved by the local ethical committee, and all recruited subjects provided a written informed consent.

Data collection

Demographic, clinical, functional, radiological, and microbiological data were collected. At the time of enrolment and during their clinical stability patients were asked to provide a sputum sample to assess microbiome and inflammatory biomarkers. Complete methodology and results for airway microbiome and inflammation are reported in the online supplement. All patients underwent a systematic and standardized immunological screening consisting of: complete blood count (CBC), IgA, IgG, IgM, IgG subclasses, total IgE, lymphocyte subsets, and HIV antibodies (reference values of the tests are listed in the online supplement). Patients with at least one positive result of the immunological screening underwent a second evaluation after at least one month apart. In case of

a positivity at a second evaluation, patients were referred to a clinical immunologist (B.V.) for additional and individualized immunological tests including B and T cell typing (CD3, CD4, CD19, CD56, CD21low, Switched-memory B cells) and evaluation of immunological response to polysaccharide and proteic antigens (*Pneumococcus* and *Tetanus* vaccination). Bronchiectasis etiology was evaluated following the recommendations of the 2017 ERS guidelines and the etiological classification was based on the algorithm published by Araújo et al. [2,13].

Definitions of primary and secondary immunodeficiencies

Primary immunodeficiency conditions were defined according to European Society for Immuno Deficiency (ESID) Diagnostic criteria [14]. Selective IgA deficiency was defined in the presence of undetectable serum level of IgA (when measured with nephelometry less than 0.07 g/L) and normal level of other immunoglobulins. CVID was defined in the presence of low total serum concentrations of immunoglobulin G (IgG, at least 2 SD below the mean for age), as well as low IgA with or without low IgM levels and low switched memory B cells (<70% of age-related normal value). Severe combined immunodeficiency was defined by the presence of at least two of the following T cell criteria fulfilled: low or absent CD3 or CD4 or CD8 T cells; reduced naive CD4 and/or CD8 T cells; elevated gamma-delta (g/d) T cells; reduced or absent proliferation to mitogen or T-cell receptor (TCR) stimulation. These criteria should be identified in the context of invasive bacterial, viral or fungal/opportunistic infections within the first year of life. Combined immunodeficiency was defined by the presence of at least two of the following T cell criteria fulfilled: reduced CD3 or CD4 or CD8 T cells (using age-related reference values); reduced naïve CD4 and/or CD8 T cells; elevated g/d T cells; reduced proliferation to mitogen or TCR stimulation. These criteria were identified in the context of: at least one severe infection (requiring hospitalization) and/or one manifestation of immune dysregulation (such as autoimmunity, inflammatory bowel diseases, severe eczema, lymphoproliferation, granuloma) and/or malignancy and/or affected family

member. DiGeorge Syndrome was defined by the presence of documented microdeletion 22q11 or 10p and recurrent or severe infections. Hyper-IgE syndrome was defined by the presence of IgE > 10 times the normal limit of age and pathologic susceptibility to infectious diseases and no evidence of T-cell or B-cell deficiency. IgG subclasses deficiency was defined by persistently low levels of one or more IgG subclasses and a normal total IgG, IgA and IgM serum levels and exclusion of T cell defect. Selective IgM deficiency was defined by low IgM plasma level and normal IgG and IgA plasma level and exclusion of T-cell defect. Unclassified antibody deficiency was defined by marked decrease of at least one of total IgG, IgG1, IgG2, IgG3, IgG4, IgA or IgM levels and no clinical signs of T-cell related disease and does not fit any of the other definitions (excluding unclassified immunodeficiencies). Unclassified immunodeficiency was defined by at least one numeric or functional abnormal finding upon immunological investigation and does not fit any of the other working definitions. A narrow definition of primary immunodeficiency conditions was consider excluding patients with both isolated IgM and Isolated IgG4 subclasses deficiency.

Secondary immunodeficiencies that could lead to hypogammaglobulinemia and/or lymphopenia included: AIDS; organ transplantation or graft-versus-host disease; splenectomy; bone marrow aplasia; haematological malignancies (lymphoma, leukemia, multiple myeloma); immunosuppressive agents (chemotherapy, long-term steroids, immunomodulatory agents and monoclonal antibodies). Other study definitions are reported in the online supplement.

Definitions of treatable immunodeficiencies

Candidates to treatment with immunoglobulin intravenous (IGIV) or subcutaneous immunoglobulins were patients suffering from either primary immunodeficiency syndromes with impaired antibody production or secondary immunodeficiency with proven specific antibody insufficiency or serum IgG level <4 g/L *plus* at least one of the following criteria: 1) ≥3 annual

exacerbations; 2) ≥1 systemic infection occurred during the previous year; 3) ≥1 hospitalization due to bacterial infection in the previous year; or 4) poor quality of life due to recurrent infections [15].

Study endpoints and comparison of five different bundles of immunological tests

The primary endpoint was the prevalence of patients with any immunodeficiencies using five different sets of immunological tests. Secondary endpoints were the prevalence of patients with primary or secondary immunodeficiencies using five different sets of immunological tests. The five sets were: S1: complete blood count, total IgG, IgA, and IgM serum levels (bundle suggested by the 2017 ERS Guidelines [2]); S2: S1 *plus* IgG subclasses; S3: S2 *plus* lymphocyte subset; S4: S3 *plus* total IgE; S5: S4 *plus* HIV testing.

Study groups

Three study groups were compared according to the results of the immunological tests and the etiology of bronchiectasis: primary immunodeficiency (Group A), secondary immunodeficiency (Group B), and idiopathic bronchiectasis (Group C).

Statistical analysis

Qualitative variables were summarized with absolute and relative (percentage) frequencies. Quantitative variables were summarized with means (standard deviations, SD) and medians (interquartile ranges, IQR) depending on their normal and non-normal distribution, respectively. Qualitative variables were compared with chi-squared and Fisher exact tests, when appropriate. Anova and Kruskall-Wallis were used to compare quantitative variables with a normal and non-normal distribution, respectively. Sidak correction was adopted for multiple comparisons. A two-tailed p-value less than 0.05 was considered statistically significant. Statistical analysis was conducted using STATA version 16 (StatsCorp, Texas, USA).

RESULTS

A total of 401 bronchiectasis patients [78% female; median (IQR) age: 62 (48-71) years] underwent the immunological screening. Patients' characteristics of the entire cohort are reported in Table 1.

Comparison of bundles of immunological tests

A significantly different prevalence of bronchiectasis patients diagnosed with any, primary, or secondary immunodeficiencies was found across different bundles (Table 2). A significantly higher prevalence of patients was diagnosed with treatable immunodeficiencies if S3 VS. S2 VS. S1 was chosen (16.7% VS. 9.2% VS. 3.7%, respectively, P: 0.00001). Isolated IgG subclasses deficiency, isolated IgM deficiency, unclassified antibody deficiency, CVID, unclassified immunodeficiency, as well as secondary immunodeficiency were diagnosed with S3 when both IgG subclasses and lymphocyte subsets were added to the minimum bundle of immunological tests. The addition of total IgE evaluation and HIV testing to S3 did not improve the detection of immunodeficiencies. If both isolated IgM and Isolated IgG4 subclasses deficiency were not considered as primary immunodeficiencies (narrow definitions), still a significantly higher prevalence of patients was diagnosed with any, primary and treatable immunodeficiencies if S3 VS. S2 VS. S1 was chosen (Any: 37.9% VS. 23.2% VS. 8.9%, P< 0.00001; Primary: 32.7% VS. 20.7% VS. 7.2%, P< 0.00001; Treatable: 13% VS. 9.2% VS. 3.7%, P: 0.00001). In terms of costs, the total cost per patient was 18.35 Euros for S1, 74.15 Euros for S2, 159.7 Euros for S3, 167.4 Euros for S4, and 176.2 Euros for S5.

Clinical and microbiological characteristics of bronchiectasis patients with immunodeficiencies

158 (39.4%) bronchiectasis patients had a diagnosis of primary and 21 (5.2%) of secondary immunodeficiency when S3 was adopted. The most frequent diagnosis among those with primary immunodeficiencies was isolated IgG subclasses deficiency (36, 9%), whereas 6 (1.5%) had unclassified antibody deficiency, and 108 (26.9%) unclassified immunodeficiency (Table 2).

Among the entire cohort, 67 (16.7%) patients with immunodeficiency met the pre-specified criteria for treatment with IGIV or subcutaneous immunoglobulins: 58 (14.5%) had primary immunodeficiency (including 36 patients with unclassified immunological deficiency, 17 with isolated IgG subclasses deficiency, 2 with CVID, 1 isolated with IgM deficiency, 1 with DiGeorge Syndrome, and 1 with unclassified antibody deficiency) and 9 (2.2%) secondary immunodeficiency (including 5 patients with hematological malignancy, 2 undergoing immunosuppressive drugs, and 2 who underwent transplant).

Bronchiectasis was idiopathic in 155 (38.7%) and caused by other etiologies in 67 (16.7%), including 26 post-infective, 8 primary ciliary dyskinesia, and 5 allergic bronchopulmonary aspergillosis. Patients with either primary and secondary immunodeficiency were older and with a higher rate of comorbidities (Table 3).

DISCUSSION

A 4-fold increase in the diagnosis of treatable immunodeficiencies can be found in adults with bronchiectasis (up to 17% of the patients) when IgG subclasses and lymphocyte subsets are added to the minimum bundle of immunological tests recommended by the ERS guidelines. In particular, S3 of immunological tests increases the diagnosis of isolated IgG subclasses deficiencies. If diagnosis of isolated IgM and isolated IgG4 subclasses deficiency were not considered, still 37.9% of the patients had any immunodeficiencies, 32.7% a primary immunodeficiency and 13% a treatable immunodeficiency. No differences in terms of disease severity, radiological impairment, lung function, clinical characteristics, and exacerbation frequencies are found between patients with immunodeficiencies and those with idiopathic bronchiectasis. Finally, patients with primary

immunodeficiency have a lower diversity of sputum microbiome in comparison with those with idiopathic bronchiectasis.

Isolated IgG subclasses deficiency is a heterogeneous group of disorders with a wide range of clinical manifestations in adults, and it is associated with an increased susceptibility to bacterial infection in general and in bronchiectasis patients [16]. International guidelines for the management of bronchiectasis do not recommend measuring IgG subclasses in all patients with bronchiectasis [2,17]. The clinical meaning of measuring IgG subclasses is still subject of debate: reduced levels of IgG subclasses have been demonstrated also in healthy subjects and are not necessarily associated with an increased risk of bacterial infection [18,19]. The prevalence of deficiency in one or more IgG subclasses in patients with bronchiectasis ranges from 6 to 48% and we reported this in 9% of our population [20-27]. We found a prevalence of 3.1% of IgG1, IgG2, or IgG3 deficiency in patients with bronchiectasis while previous experiences using stringent criteria to define this condition reported a prevalence less than 1% [22,23,25]. Among our patients, 5.9% of bronchiectasis patients were diagnosed with an isolated IgG4 subclasses deficiency although a reduced IgG4 concentration is not usually regarded as a marker of humoral immune deficiency [28]. An isolated IgG subclasses deficiency, in severe patients despite an optimized clinical management, might represent an important treatable trait in bronchiectasis [29]. Different experiences showed that correction of IgG subclass deficiency using intravenous or subcutaneous immunoglobulin replacement therapy resulted in a clinically meaningful reduction in bacterial chest infections [30,31]. However, specific randomized controlled trials (RCT) on bronchiectasis are needed.

Notably, up to 27% of the patients might have unclassified immunodeficiency according to ESID definitions. The clinical significance of unclassified immunodeficiency is still a matter of debate, especially in asymptomatic patients [14]. However, in our cohort unclassified immunodeficiency is

associated with the presence of clinically significant bronchiectasis. A large proportion of patients with unclassified immunodeficiency was characterized by alteration in both lymphocyte and immunoglobulin production. Thus, even if lymphocyte were not amenable to target therapy, immunoglobulin therapy represents a valuable therapy in this subgroup of patients. Several other specialist immunological tests might be required in a small proportion of patients with bronchiectasis to reach a diagnosis and classify primary iimmunodeficiency or evaluate its severity, such as mannose binding lectin genotype and function [32,33].

Finally, no patients' characteristics seem help physicians identify those with immunodeficiencies and, thus, individualize the immunological workup. Patients enrolled in our cohort belong to a tertiary care center and different findings could be described when patients are recruited from primary or secondary care centers where a minimum bundle of immunological tests is adopted. We could speculate that patients with severe bronchiectasis and several comorbidities attending a tertiary care center could be the ideal candidate for a comprehensive immunological screening. The study limitations are related to its monocentric nature, run in a tertiary care setting which could hinder the reproducibility of the findings. Different prevalence of immunodeficiencies across different settings could be found [3]. Thus, our epidemiological analysis needs an external, international validation. Another limitation is the missing follow-up to evaluate outcomes of patients with immunodeficiencies, including those who underwent treatment. This limitation negatively impacts on our ability to evaluate the cost-effectiveness of different bundles, although we could suggest a possible indirect advantage. A comprehensive economic analysis is needed to demonstrate the cost-effectiveness of early diagnosis, and eventually of a replacement therapy, of immunodeficiencies. Finally, third level functional studies concerning B and T cell such as proliferation to mitogen or T cell stimulation were not performed in our center.

The evaluation of a large cohort of adult bronchiectasis patients who underwent the same comprehensive immunological work-up is a strength of our manuscript. Furthermore, the classification of immunodeficiencies we used in our study followed the latest ESID criteria and the classification of bronchiectasis etiology also followed a standardized algorithm recently published.

CONCLUSION

In conclusion we found a significantly different prevalence of adult bronchiectasis patients diagnosed with any, primary, or secondary immunodeficiencies across different bundles. We demonstrated that a 4-fold increase in the diagnosis of treatable immunodeficiencies can be found in adults with bronchiectasis when IgG subclasses and lymphocyte subsets are added to the minimum bundle of immunological tests recommended by guidelines. However, no patients' characteristics seem help physicians identify those with immunodeficiencies and, thus, individualize the immunological workup. Thus, we recommended the following bundle of immunological tests in adult patients with bronchiectasis: CBC, IgA, IgG, IgM, IgG subclasses and lymphocyte subsets (including CD4+ T cells, CD8+ T cells, B cells and Natural Killer cells). Further studies are needed in external cohort of bronchiectasis patients to validate our result. RCTs are needed to verify if immunoglobulin replacement in bronchiectasis patients with treatable immunodeficiencies might improve relevant outcomes.

Author's contribution: Conception and design, S.A. and F.A. ; Formal Analysis S.A., M.O., G.S., L.S. and F.A. ; Investigation, S.A. , M.O. and F.A.; Resources, F.B., S.A.; Data Curation, M.O., F.A., A.G., S.A., G.S., L.S. ; Writing – Original Draft Preparation, S.A., F.A., M.O.; Writing – Review & Editing, A.G., B.V., G.S., M.M., E.S., L.S., A.S., S.T., P.M., E.P. , J.D.C., F.B.; Supervision, F.B., and S.A. ; Project Administration, S.A.; Funding Acquisition, F.B. and S.A.

Conflict of interest: F.A., A.G., B.V., M.O., G.S., E.S., L.S., A.S., S.T. and P.M. report no conflict of interest.

M.M. declares honoraria for educational events from GlaxoSmithKline and Boehringer Ingelheim outside the present study. J.D.C. reports grants and personal fees from Astrazeneca, grants and personal fees from Boehringer Ingelheim, personal fees from Chiesi, grants and personal fees from Glaxosmithkline, grants from Gilead Sciences, grants and personal fees from Insmed, personal fees from Novartis, personal fees from Zambon, outside the submitted work. F.B. reports grants and personal fees from astrazeneca, grants from bayer, grants and personal fees from chiesi, grants and personal fees from gsk, personal fees from guidotti, personal fees from grifols, grants and personal fees from insmed, personal fees from menarini, personal fees from mundipharma, personal fees from novartis, grants and personal fees from pfizer, personal fees from zambon, outside the submitted work. S.A. reports personal fees from Bayer Healthcare, personal fees from Grifols, personal fees from AstraZeneca, personal fees from Zambon, grants and personal fees from Chiesi, grants and personal fees from INSMED, personal fees from GlaxoSmithKline, personal fees from Menarini, personal fees from ZetaCube Srl, grants from Fisher & Paykel, outside the submitted work. A.D.S. re-ports grants, speakers fees and travel support from AstraZeneca, Bayer, Chiesi, Forest labs, GSK, Insmed, Pfizer, Medimmune, Novartis and Zambon outside the submitted work.

Support statement: This work was supported by Takeda Pharmaceutical Company (ID 9891332)

REFERENCES

1. Chalmers JD, Aliberti S, Blasi F. Management of bronchiectasis in adults. Eur Respir J. 2015 May;45(5):1446-62.
2. Polverino E, Goeminne PC, McDonnell MJ, Aliberti S, Marshall SE, Loebinger MR, Murris M, Cantón R, Torres A, Dimakou K, De Soyza A, Hill AT, Haworth CS, Vendrell M, Ringshausen FC, Subotic D, Wilson R, Vilaró J, Stallberg B, Welte T, Rohde G, Blasi F, Elborn S, Almagro M, Timothy A, Ruddy T, Tonia T, Rigau D, Chalmers JD. Eur European Respiratory Society guidelines for the management of adult bronchiectasis. Respir J. 2017 Sep 9;50(3):1700629.
3. Lonni S, Chalmers JD, Goeminne PC, McDonnell MJ, Dimakou K, De Soyza A, Polverino E, Van de Kerkhove C, Rutherford R, Davison J, Rosales E, Pesci A, Restrepo MI, Torres A, Aliberti S. Etiology of Non-Cystic Fibrosis Bronchiectasis in Adults and Its Correlation to Disease Severity. Ann Am Thorac Soc. 2015 Dec;12(12):1764-70.
4. de Gracia J, Vendrell M, Alvarez A, Pallisa E, Rodrigo MJ, de la Rosa D, Mata F, Andreu J, Morell F. Immunoglobulin therapy to control lung damage in patients with common variable immunodeficiency. Int Immunopharmacol. 2004 Jun;4(6):745-53.
5. Wood P, Stanworth S, Burton J, et al. Recognition, clinical diagnosis and management of patients with primary antibody deficiencies: a systematic review. Clin Exp Immunol 2007; 149:410.
6. Touw CM, van de Ven AA, de Jong PA, Terheggen-Lagro S, Beek E, Sanders EA, van Montfrans JM. Detection of pulmonary complications in common variable immunodeficiency. Pediatr Allergy Immunol. 2010 Aug;21(5):793-805.

7. Habesoglu MA, Ugurlu AO, Eyuboglu FO. Clinical, radiologic, and functional evaluation of 304 patients with bronchiectasis. Ann Thorac Med. 2011 Jul;6(3):131-6.
8. Dimakou K, Triantafillidou C, Toumbis M, Tsikritsaki K, Malagari K, Bakakos P. Non CF-bronchiectasis: Aetiologic approach, clinical, radiological, microbiological and functional profile in 277 patients. Respir Med. 2016 Jul;116:1-7.
9. Buscot M, Pottier H, Marquette CH, Leroy S. Phenotyping Adults with Non-Cystic Fibrosis Bronchiectasis: A 10-Year Cohort Study in a French Regional University Hospital Center. Respiration. 2016;92(1):1-8.
10. Olveira C, Padilla A, Martínez-García MÁ, de la Rosa D, Girón RM, Vendrell M, Máiz L, Borderías L, Polverino E, Martínez-Moragón E, Rajas O, Casas F, Cordovilla R, de Gracia J.Etiology of Bronchiectasis in a Cohort of 2047 Patients. An Analysis of the Spanish Historical Bronchiectasis Registry. Arch Bronconeumol. 2017 Jul;53(7):366-374.
11. Goussault H, Salvator H, Catherinot E, Chabi ML, Tcherakian C, Chabrol A, Didier M, Rivaud E, Fischer A, Suarez F, Hermine O, Lanternier F, Lortholary O, Mahlaoui N, Devillier P, Couderc LJ. Primary immunodeficiency-related bronchiectasis in adults: comparison with bronchiectasis of other etiologies in a French reference center. Respir Res. 2019 Dec 4;20(1):275.
12. Aksamit TR, O'Donnell AE, Barker A, Olivier KN, Winthrop KL, Daniels MLA, Johnson M, Eden E, Griffith D, Knowles M, Metersky M, Salathe M, Thomashow B, Tino G, Turino G, Carretta B, Daley CL; Bronchiectasis Research Registry Consortium. Adult Patients With Bronchiectasis: A First Look at the US BronchiectasisResearch Registry. .Chest. 2017 May;151(5):982-992.
13. Araújo D, Shteinberg M, Aliberti S, Goeminne PC, Hill AT, Fardon T, Obradovic D, Dimakou K, Polverino E, De Soyza A, McDonnell MJ, Chalmers JD. Standardised classification of the

aetiology of bronchiectasis using an objective algorithm. Eur Respir J. 2017 Dec 14;50(6):1701289.

14. https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria
15. Jolles S, Chapel H, Litzman J. When to initiate immunoglobulin replacement therapy (IGRT) in antibody deficiency: a practical approach. Clin Exp Immunol. 2017 Jun;188(3):333-341.
16. Khokar A, Gupta S. Clinical and Immunological Features of 78 Adult Patients with Primary Selective IgG Subclass Deficiencies. Arch Immunol Ther Exp (Warsz). 2019 Oct;67(5):325-334.
17. Hill AT, Sullivan AL, Chalmers JD, De Soyza A, Elborn SJ, Floto AR, Grillo L, Gruffydd-Jones K, Harvey A, Haworth CS, Hiscocks E, Hurst JR, Johnson C, Kelleher PW, Bedi P, Payne K, Saleh H, Screaton NJ, Smith M, Tunney M, Whitters D, Wilson R, Loebinger MR. British Thoracic Society Guideline for bronchiectasis in adults. Thorax. 2019 Jan;74(Suppl 1):1-69.
18. Jefferis R, Kumararatne DS. Selective IgG subclass deficiency: quantification and clinical relevance. Clinical and experimental immunology 1990;81(3):357-67
19. King PT, Hutchinson P, Holmes PW, et al. Assessing immune function in adult bronchiectasis. Clinical & Experimental Immunology 2006;144(3):440-6
20. Pasteur MC, Helliwell SM, Houghton SJ, et al. An investigation into causative factors in patients with bronchiectasis. American Journal of Respiratory & Critical Care Medicine 2000;162(4 Pt 1):1277-84
21. De Gracia J, Rodrigo MJ, Morell F, Vendrell M, Miravitlles M, Cruz MJ, Codina R, Bofill JM. IgG subclass deficiencies associated with bronchiectasis. Am J Respir Crit Care Med. 1996 Feb;153(2):650-5

22. Parker AR, Skold M, Ramsden DB, Ocejo-Vinyals JG, López-Hoyos M, Harding S. The Clinical Utility of Measuring IgG Subclass Immunoglobulins During Immunological Investigation for Suspected Primary Antibody Deficiencies. Lab Med. 2017 Nov 8;48(4):314-325
23. Anwar GA, McDonnell MJ, Worthy SA, et al. Phenotyping adults with non-cystic fibrosis bronchiectasis: a prospective observational cohort study. Respiratory medicine 2013;107(7):1001-7
24. De Gracia J, Rodrigo MJ, Morell F, et al. IgG subclass deficiencies associated with bronchiectasis. American journal of respiratory and critical care medicine 1996;153(2):6505
25. Hill SL, Mitchell JL, Burnett D, et al. IgG subclasses in the serum and sputum from patients with bronchiectasis. Thorax 1998;53(6):463-8
26. King PT, Holdsworth SR, Freezer NJ, Villanueva E, Holmes PW. Characterisation of the onset and presenting clinical features of adult bronchiectasis. Respir Med. 2006 Dec;100(12):2183-9
27. Goussault H, Salvator H, Catherinot E, Chabi ML, Tcherakian C, Chabrol A, Didier M, Rivaud E, Fischer A, Suarez F, Hermine O, Lanternier F, Lortholary O, Mahlaoui N, Devillier P, Couderc LJ. Primary immunodeficiency-related bronchiectasis in adults: comparison with bronchiectasis of other etiologies in a French reference center. Respir Res. 2019 Dec 4;20(1):275.
28. Bonilla FA, Khan DA, Ballas ZK, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. The Journal of allergy and clinical immunology 2015;136(5):1186-205

29. Abdou NI, Greenwell CA, Mehta R, Narra M, Hester JD, Halsey JF. Efficacy of intravenous gammaglobulin for immunoglobulin G subclass and/or antibody deficiency in adults. Int Arch Allergy Immunol. 2009;149(3):267-74.
30. Olinder-Nielsen AM, Granert C, Forsberg P, et al. Immunoglobulin prophylaxis in 350 adults with IgG subclass deficiency and recurrent respiratory tract infections: a longterm follow-up. Scandinavian journal of infectious diseases 2007;39(1):44-50
31. Abrahamian F, Agrawal S, Gupta S. Immunological and clinical profile of adult patients with selective immunoglobulin subclass deficiency: response to intravenous immunoglobulin therapy. Clinical and experimental immunology 2010;159(3):344-50
32. Litzman J, Freiberger T, Grimbacher B, et al. Mannose-binding lectin gene polymorphic variants predispose to the development of bronchopulmonary complications but have no influence on other clinical and laboratory symptoms or signs of common variable immunodeficiency. Clinical and experimental immunology 2008;153(3):324-30
33. Macfarlane JG, Jary H, Hester KL, et al. Low serum mannose-binding lectin level is not associated with disease severity in non-cystic fibrosis bronchiectasis. Innate immunity 2012;18(6):787-92

TABLES

Table 1. Demographics, disease severity, clinical, radiological, functional and microbiological characteristics of the study population.

Variables	Study Population (N=401)
Demographics	
Female sex, n (%)	317 (79.1)
Age, median (IQR)	63 (50-71)
Body mass index, median (IQR)	21.5 (19.5-24.0)
Underweight, n (%)	58 (14.6)
Former or current smoker, n (%)	180 (44.9)
Comorbidities	
GERD, n (%)	180 (44.9)
Rhinosinusitis, n (%)	138 (34.4)
Cardiovascular diseases, n (%)	142 (35.4)
Systemic hypertension, n (%)	93 (23.2)
Asthma, n (%)	60 (15.0)
Osteoporosis, n (%)	69 (17.2)

COPD, n (%)	32 (8.0)
Depression, n (%)	34 (8.5)
Anxiety, n (%)	27 (6.7)
History of neoplastic disease, n (%)	56 (14.0)
Diabetes, n (%)	17 (4.2)
BACI score median (IQR)	0 (0-3)
Functional evaluation	
FEV1, median (IQR)	87 (71-101)
FEV1 <50%, n (%)	30 (7.9)
FVC, mean (SD)	97.5 (21.7)
Microbiology	
Chronic infection, n (%)	120 (35.9)
Chronic *P. aeruginosa* infection, n (%)	76 (22.7)
H. influenzae chronic infection, n (%)	20 (6.0)
NTM-PD, n (%)	41 (12.2)
Clinical status	
Exacerbations, median (IQR)	2 (1-3)

Exacerbations ≥3 previous year, n (%)	132 (32.9)
LTOT, n (%)	19 (4.7)
Daily sputum, n (%)	267 (66.6)
Sputum volume, median (IQR)	6 (4-20)
Chronic treatment	
Chronic macrolide therapy, n (%)	40 (10.0)
Chronic antibiotic inhaled therapy, n (%)	21 (5.2)
Radiology	
Reiff score, median (IQR)	4 (2-6)
Number of lobes involved, median (IQR)	3 (2-5)
Disease severity	
BSI score median (IQR)	6 (3-9)
BSI moderate-severe, n (%)	244 (63.9)
BSI severe, n (%)	98 (25.7)
FACED score median (IQR)	2 (1-3)
FACED moderate-severe, n (%)	141 (36.0)

Table 2. Prevalence of bronchiectasis adults (N=401) with any, primary, secondary and treatable immunodeficiencies according to five different sets of immunological tests (see footnotes)

	Set #1	Set #2	Set #3	Set #4	Set #5
Any Immunodeficiency	36 (8.9)	93 (23.2)	179 (44.6)	179 (44.6)	179 (44.6)
Primary Immunodeficiency	29 (7.2)	83 (20.7)	158 (39.4)	158 (39.4)	158 (39.4)
Isolated IgG subclasses deficiency	0	0	36 (9)	36 (9)	36 (9)
• Isolated IgG1 subclasses deficiency	0	0	3 (0.8)	3 (0.8)	3 (0.8)
• Isolated IgG2 subclasses deficiency	0	0	2 (0.5)	2 (0.5)	2 (0.5)
• Isolated IgG3 subclasses deficiency	0	0	6 (1.5)	6 (1.5)	6 (1.5)
• Isolated IgG4 subclasses deficiency	0	0	24 (5.9)	24 (5.9)	24 (5.9)
• IgG1 and IgG3 subclasses deficiency	0	0	1 (.3)	1 (.3)	1 (.3)
Isolated IgM deficiency	0	0	3 (0.7)	3 (0.7)	3 (0.7)
Unclassified antibody deficiency	0	0	6 (1.5)	6 (1.5)	6 (1.5)
Common variable immunodeficiency	0	0	2 (0.5)	2 (0.5)	2 (0.5)

Severe combined immunodeficiency	0	0	0	0	0
Combined immunodeficiency	0	0	0	0	0
Hyper-IgE syndrome	0	0	0	0	0
Isolated IgA deficiency	2 (0.5)	2 (0.5)	2 (0.5)	2 (0.5)	2 (0.5)
DiGeorge Syndrome	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)
Unclassified immunodeficiency	26 (6.5)	80 (20)	108 (26.9)	108 (26.9)	108 (26.9)
Secondary immunodeficiency	7 (1.7)	10 (2.5)	21 (5.2)	21 (5.2)	21 (5.2)
Immunosuppressive drugs	1 (0.2)	3 (0.7)	11 (2.7)	11 (2.7)	11 (2.7)
• Steroids	1 (0.2)	3 (0.7)	8 (2)	8 (2)	8(2)
• Biologics	0 (0)	0 (0)	2 (0.5)	2 (0.5)	2 (0.5)
• Antiproliferative agents	0 (0)	0 (0)	1 (0.2)	1 (0.2)	1 (0.2)
Haematological malignancy	6 (1.5)	6 (1.5)	7 (1.7)	7 (1.7)	7 (1.7)
Transplant	0	1 (0.2)	3 (0.7)	3 (0.7)	3 (0.7)
Other etiologies	113 (28.2)	113 (28.2)	67 (16.7)	67 (16.7)	67 (16.7)
Idiopathic bronchiectasis	252 (62.8)	195 (48.6)	155 (38.7)	155 (38.7)	155 (38.7)
Treatable immunodeficiencies	15 (3.7)	37 (9.2)	67 (16.7)	67 (16.7)	67 (16.7)

Footnotes: Data are presented as n (%); [§]including 26 post-infective, 8 primary ciliary dyskinesia, 5 allergic bronchopulmonary aspergillosis. Set #1: complete blood count, total IgG, IgA, and IgM

serum levels; Set #2: complete blood count, total IgG, IgA, and IgM serum levels, IgG subclasses; Set #3: complete blood count, total IgG, IgA, and IgM serum levels, IgG subclasses; Lymphocyte subset; Set #4: complete blood count, total IgG, IgA, and IgM serum levels, IgG subclasses; Lymphocyte subset, total IgE; Set #5: complete blood count, total IgG, IgA, and IgM serum levels, IgG subclasses; Lymphocyte subset, total IgE, HIV.

Table 3. Demographic, clinical, functional, radiological, and microbiological characteristics of the three study groups: patients with primary immunodeficiency (Group A), patients with secondary immunodeficiency (Group B) and patients with idiopathic bronchiectasis (Group C)

Variables	**Group A (n= 158)**	**Group B (n= 21)**	**Group C (n= 155)**	**p-value**
Demographics				
Female sex, n (%)	122 (77.2)	13 (61.9)	129 (83.2)	0.06
Age, median (IQR)	65 (52-73)	70 (65-73)	62 (48-70)	0.009[1]
BMI, median (IQR)	21.1 (19.2-24.0)	23.5 (21.9-25.5)	21.4 (19.7-24.0)	0.08

Underweight, n (%)	27 (17.2)	2 (10.0)	20 (13.0)	0.48
Former or current smoker, n (%)	63 (39.9)	13 (61.9)	77 (49.7)	0.07
Comorbidities				
GERD, n (%)	71 (44.9)	11 (52.4)	74 (47.7)	0.76
Rhinosinusitis, n (%)	58 (36.7)	7 (33.3)	45 (29.0)	0.35
Cardiovascular diseases, n (%)	66 (41.8)	12 (57.1)	43 (27.7)	0.004[3]
Systemic hypertension, n (%)	43 (27.2)	8 (38.1)	29 (18.7)	0.06
Asthma, n (%)	24 (15.2)	1 (4.8)	29 (18.7)	0.25
Osteoporosis, n (%)	24 (15.2)	10 (47.6)	26 (16.8)	0.001[4]
COPD, n (%)	16 (10.1)	2 (9.5)	9 (5.8)	0.32
Depression, n (%)	17 (10.8)	3 (14.3)	10 (6.5)	0.24
Anxiety, n (%)	11 (7.0)	2 (9.5)	11 (7.1)	0.84
History of neoplastic disease, n (%)	23 (14.6)	12 (57.1)	13 (8.4)	<0.0001[5]
Diabetes, n (%)	6 (3.8)	7 (33.3)	3 (1.9)	<0.0001[6]
Functional				
FEV1, mean (SD)	85.9 (24.9)	84.6 (27.2)	85.6 (20.8)	0.97
FEV1 <50%, n (%)	13 (8.8)	2 (10.0)	9 (6.2)	0.62
FVC, mean (SD)	98.5 (23.2)	97.5 (25.1)	97.3 (18.6)	0.89
Microbiological				
Chronic infection, n (%)	49 (36.6)	5 (29.4)	46 (35.4)	0.84
P. aeruginosa chronic	31 (23.1)	3 (17.7)	30 (23.1)	0.95

infection, n (%)				
H. influenzae chronic infection, n (%)	7 (5.3)	3 (17.7)	8 (6.2)	0.14
NTM-PD, n (%)	18 (13.4)	3 (17.7)	11 (8.5)	0.29
Clinical				
Exacerbations, median (IQR)	2 (1-3)	1 (0-3)	2 (1-3)	0.97
Exacerbations ≥3 previous year, n (%)	51 (32.3)	8 (38.1)	49 (31.6)	0.84
LTOT, n (%)	11 (7.0)	2 (9.5)	4 (2.6)	0.09
Daily sputum, n (%)	104 (65.8)	15 (71.4)	107 (69.0)	0.77
Sputum volume, median (IQR)	7 (4.5-20.0)	5.5 (3-50)	6 (5-20)	0.93
Chronic macrolide therapy, n (%)	16 (10.1)	1 (4.8)	11 (7.1)	0.62
Chronic antibiotic inhaled therapy, n (%)	8 (5.1)	1 (4.8)	10 (6.5)	0.86
Radiological				
Reiff score, median (IQR)	4 (2-6)	4 (2-6)	4 (3-6)	0.99
Number of lobes involved, median (IQR)	3 (2-4)	4 (2-6)	4 (2-5)	0.59
Disease severity				
BSI score median (IQR)	6 (4-9)	6 (3.0-8.5)	6 (3-8)	0.11
FACED score median (IQR)	2 (1-3)	2 (2-3)	2 (1-3)	0.06
BACI score median (IQR)	0 (0-3)	6 (3-10)	0 (0-3)	0.0001[8]

Footnotes: [1] Group B VS. Group C p-value: 0.008

[2] *Group A VS. Group C p-value: 0.04; Group B VS. Group C p-value: 0.01*

[3] *Group A VS. Group C p-value: 0.009; Group B VS. Group C p-value: 0.006*

[4] *Group A VS. Group B p-value: 0.0004; Group B VS. Group C p-value: 0.001*

[5] *Group A VS. Group C p-value <0.0001; Group B VS. Group C p-value <0.0001*

[6] *Group A VS. Group C p-value <0.0001; Group B VS. Group C p-value <0.0001*

[7] *Group A VS. Group C p-value: 0.001; Group B VS. Group C p-value: 0.04*

[8] *Group A VS. Group B p-value: <0.0001; Group B VS. Group C p-value: <0.0001*

ONLINE SUPPLEMENT

MATERIALS AND METHODS

Reference values for immunological tests

Reference values of the following tests: white blood cells (4800-10800/mm^3), IgA (70-400 mg/dL), IgG (700-1600 mg/dL), IgM (40-230 mg/dL), IgG1 (3.150-8.550 g/L), IgG2 (0.640-4.950 g/L), IgG3 (0.230-1.096 g/L), IgG4 (0.110-1.570 g/L), total IgE (0-100 kUA/dL), CD4+ T cells (300-1400cells/µL), CD8+ T cells (200-900cells/µL), B cells (100-500cells/µL), Natural Killer cells (90-600cells/µL); Serum electrophoresis was defined as "positive" in presence of a monoclonal component; HIV test was ELISA antibodies HIV1/2 assay, if positive, a confirm with western-blot test was required.

Sputum evaluation of patients with immunodeficiencies

At the time of enrolment and during their clinical stability patients were asked to provide a sputum sample to assess microbiome and inflammatory biomarkers. Patients with active non-tuberculous mycobacterial pulmonary disease were excluded from sputum analysis. Sputum sample were split in two aliquots: the first one was used for DNA extraction and subsequently for microbiome and molecular biology (see below) analysis, whereas the second one was used to produce sputum supernatant for the evaluation of inflammatory biomarkers.

Microbiome. DNA extraction along with sequencing of bacterial 16S rRNA gene amplicons (V3-V4 region) was performed according to previously published studies [12,13]. V3-V4 16S rRNA genes were amplified using the 16S metagenomic sequencing library preparation protocol (Illumina, San Diego, CA, USA).

Bioinformatic analysis. Bioinformatic analysis was carried out as previously described [14]. Briefly, Quantitative Insights into Microbial Ecology (QIIME2) pipeline was used to analyze 16s sequences reads. ASV frequency table was used to report relative abundance of each type of bacteria. Shannon diversity index, Pielou' Evenness and richness were adopted to evaluate alpha diversity (within sample diversity). The DESeq2 (v3.5, R Bioconductor) package was used to evaluate differences between groups of 16S rRNA gene sequencing data through Differential gene expression analysis based on the negative binomial distribution [15]. Principal coordinates analysis (PCoA) was used to represent beta diversity data, specifically using Bray-Curtis dissimilarity. Data were analyzed at genus levels. Sequence data are available from the NCBI Sequence Read Archive (accession number to the bioproject PRJNA664034).

Molecular biology. A Real-time PCR for the detection and quantification of *Pseudomonas aeruginosa, Haemophilus influenzae, Staphylococcus aureus* and *Streptococcus pneumoniae* was performed as previously described [14]. *Inflammatory biomarkers.* Mucus plug was diluted 8X in PBS, vortexed and centrifuged at 4°C for 15 minutes at 3,000g. Supernatants were stored at -80°C. ProteaseTag® Active Neutrophil Elastase Immunoassay (Proaxis, Belfast, UK) was used to assess active neutrophil elastase (aNE) [16]. Similarly, TNFa, IL1b, IL10, and IL6 were tested using commercially available ELISA assay (Cusabio Technology LLC, Houston, USA). Further details on DNA extraction and molecular analysis are reported in the online supplement.

DNA extraction was performed using Roche High Pure PCR Template Preparation Kit (Hoffmann – La Roche. Basilea. Switzerland) with a pre-treatment with dithiothreitol (DTT; Sputafluid, Biolife Italiana Srl, Italy) and enzymatic lysis with 3.6 mg/ml lysozyme and 0.18 mg/ml lysostaphin (Sigma-Aldrich. Saint Louis. Missouri, USA). Control samples and mock microbial DNA were similarly treated. V3-V4 16S rRNA gene were amplified from DNA extracts using the 16S metagenomic sequencing library preparation protocol (Illumina, San Diego, CA, USA) using suggested primers. Amplicons were

barcoded using Nextera XT library prep kit (Illumina, San Diego, CA, USA) as per manufacturer's instructions. PCR products were visualized using Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA) and, then, cleaned using AMPure XP magnetic bead-based purification (Beckman Coulter, Brea, CA, USA) [1, 2].Sample libraries were quantified using Qubit and, then, pooled in an equimolar mode. Finally, the pool was sequenced on MiSeq (Illumina, San Diego, CA, USA) sequencing platform, using a 2 300 cycle V3 kit and following standard Illumina sequencing protocols.

Real time PCR for bacterial detection

Bacterial DNA was amplified and quantified using a real-time PCR assay with TaqMan probes for P. aeruginosa (gyrB gene), Staphylococcus aureus (nuc gene), Streptococcus pneumoniae (lytA gene), and H. influenzae (fucK gene) using AB7900HT Fast Real-Time PCR System (Applied Biosystems), with primers and probes previously published [3].

Quantitative PCR amplification was performed in 20-µL reaction mixture containing 2x QuantiFast Multiplex PCR Master Mix (Qiagen), primers and probes and 2 µL of DNA extracted from sputum samples. Moreover, a real-time PCR targeting human RNaseP gene was used to detect PCR inhibition or extraction failure. Amplification was performed with the following parameters: 95°C for 5 minutes, followed by 45 cycles of 95°C for 45 seconds and 60°C for 1 minute. To quantify the amount of bacterial DNA in each sample standard curves were prepared using quantitative genomic DNA from S. pneumoniae (ATCC® 700669DQTM), P. aeruginosa (ATCC® 47085DQTM), S. aureus (ATCC® 29213DQTM), and H. influenzae (ATCC® 51907DQTM). These were quantified with Qubit and Quant-iT dsDNA Assay Kit High Sensitivity and, then, diluted at 1ng/µL; subsequently, standard curve was prepared by 10-fold dilution from 1ng/µL to 1*10-7 ng/µL. Samples and controls were tested in triplicate and samples were assumed to be positive if Ct is <38. Target genes (gyrB, nuc, lytA and fucK) were in a single copy in the genome and the number of measured copies was assumed to be

equivalent to the bacterial load [4]. The number of genome copies were calculated based on 7.22 fg, 3.01 fg, 2.28 fg, and 1.95 fg of DNA per P. aeruginosa, S. aureus, S. Pneumoniae, and H. influenzae genomes, respectively. Quantification of bacteria found in each sample was based on standard curves generate by plotting the Ct values against known genome copies. Conversion of genome copies/reaction to genome copies/mL was based on a 2µL input per reaction derived from 100 µL of eluate extracted from 200 µL of treated specimen.

Briefly, Quantitative Insights into Microbial Ecology (QIIME2) pipeline was used to analyse 16s sequences reads. Sequences underwent de-multiplexing and quality filtering with default parameters. After demultiplexing, sequences were matched through the DADA2 pipeline generating amplicon sequence variants (ASV) and a frequency table [5, 6]. Quality control step filtered out non-microbiota sequences with a 97% homology to a Greengenes database. A classifier with 99% homology to the V3-V4 region was trained with remaining sequences assigned with taxonomy based on 99% homology [7]. No OTU was removed from the analysis.

ASV frequency table was used to report relative abundance of each type of bacteria. Shannon diversity index, Pielou' Evenness and richness were utilized to evaluate alpha diversity (within sample diversity). The DESeq2 (v3.5, R Bioconductor) package was used to evaluate differences between groups of 16S rRNA gene sequencing data through Differential gene expression analysis based on the negative binomial distribution [8]. PCoA was used to represent beta diversity data, specifically using Bray-Curtis dissimilarity. Data were analysed at genus levels.

Sequence data are available from the NCBI Sequence Read Archive (accession number to the bioproject PRJNA664034).

Other study definitions

The severity of bronchiectasis was evaluated according to both the Bronchiectasis Severity Index [9] and the FACED score [10]. 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) [11]. Bronchiectasis etiology was evaluated following the recommendations of the 2017 European Respiratory Society guidelines and the etiological classification was based on the algorithm published by Araújo et al. [12, 13]. Chronic bacterial infection was defined as the presence of 2 cultures positive for pathogenic bacteria at least 3 months apart over 12 months [9]. Chronic *P. aeruginosa* infection was defined as the presence of 2 cultures positive for *P. aeruginosa* at least 3 months apart over 12 months [14]. Patients completed the Quality of Life-Bronchiectasis (QOL-B) questionnaire as a measure of quality of life [15]. All bacteriology was performed on spontaneous sputum samples during stable state as previously described [Chalmers Am J Respir Crit Care Med 2012]. Murray-Washington criteria for sputum quality were adopted, with all samples having <10 squamous cells and m>25 leukocytes per low-power microscope field. Exacerbation of bronchiectasis was defined according to Hill et al. [16]

RESULTS

Sputum analysis

Microbiome and inflammatory biomarkers analyses were performed in 48 patients with primary immunodeficiency and 49 with idiopathic bronchiectasis. Patients with secondary immunodeficiency were excluded due to the small sample size.

Microbiome. 210 sequences were found in negative control, filtered out after quality control step. In terms of within-sample diversity, patients with primary immunodeficiency had lower median (IQR) values of Shannon [primary immunodeficiency 4.1 (2.3-5.3) VS. idiopathic 4.9 (3.6-5.8); P:

0.029], Evenness [0.7 (0.5-0.8) VS. 0.8 (0.6-0.9); P: 0.053] and Richness [47.5 (21.0-75.2) VS. 70.0 (51.0-96.0); P: 0.010] in comparison with those with idiopathic bronchiectasis (Figure 1a,b,c and Table 3). Compositional results of the primary immunodeficiency and idiopathic bronchiectasis groups are reported in (Figure 1d). The PCoA based on Bray-Curtis dissimilarity through PERMANOVA analysis did not show any statistical significance (p=0.265) between the two groups (Figure 1e).

Bacterial burden. The rate of *P. aeruginosa, H. influenzae*, and *S. pneumoniae* infection was not different in patients with primary immunodeficiency patients and in those with idiopathic bronchiectasis. *S. aureus* infection was significantly less frequent in primary immunodeficiency patients [26 (53.1%) VS. 38 (39.2%), P: 0.005) in comparison with those with idiopathic bronchiectasis (Table 4).

Inflammatory biomarkers. aNE levels were not different between primary immunodeficiency and idiopathic bronchiectasis groups. Furthermore, levels of inflammatory mediators (i.e., TNFα, IL1β, IL-6 and IL10) were not different when patients with primary immunodeficiency were compared with those with idiopathic bronchiectasis (Figure 2 and Table 4).

Table 4. Microbiome, molecular biology and airway inflammation in sputum of patients with primary immunodeficiency (Group A, N=48) *versus* patients with idiopathic bronchiectasis (Group C; N=49)

Variables	Group A (N=48)	Group C (N=49)	p-value
Sputum microbiome α diversity			
Median (IQR) Shannon	4.1 (2.3, 5.3)	4.9 (3.6, 5.8)	0.029
Median (IQR) Richness	47.5 (21.0, 75.2)	70.0 (51.0, 96.0)	0.01
Median (IQR) Evenness	0.7 (0.5, 0.8)	0.8 (0.6, 0.9)	0.053
Molecular detection of respiratory pathogens			
P. aeruginosa detection	28 (58.3%)	20 (40.8%)	0.084
P. aeruginosa genome copies/mL, log	7.6 (6.9, 8.1)	7.5 (6.8, 8.3)	0.95
H. influenzae detection	12 (25.0%)	15 (30.6%)	0.537
H. influenzae genome copies/mL, log	7.6 (5.3, 9.0)	5.1 (4.3, 7.8)	0.107
S. aureus detection	12 (25.0%)	26 (53.1%)	0.005
S. aureus genome copies/mL, log	4.5 (3.3, 7.7)	4.3 (3.6, 6.5)	0.925

S. pneumoniae detection	18 (37.5%)	20 (40.8%)	0.738
S. pneumoniae genome copies/mL, log	3.5 (3.0, 4.7)	3.5 (3.3, 4.6)	0.792
Inflammatory biomarkers			
Median (IQR) active neutrophil elastase, ng/mL	17.0 (5.9, 32.3)	14.4 (5.4, 31.9)	0.489
Median (IQR) TNFα, pg/mL	929.9 (542.1, 1148.5)	1145.8 (699.7, 1314.5)	0.163
Median (IQR) IL6, pg/mL	176.2 (79.0, 271.9)	144.1 (68.9, 239.0)	0.522
Median (IQR) IL1β, pg/mL	360990.2 (271474.7, 552694.3)	353607.4 (197558.8, 486900.3)	0.64
Median (IQR) IL10, pg/mL	31590.9 (13441.6, 55344.1)	19689.9 (10046.4, 37742.2)	0.456
Median (IQR) active neutrophil elastase, ng/mL	17.0 (5.9, 32.3)	14.4 (5.4, 31.9)	0.489

FIGURES

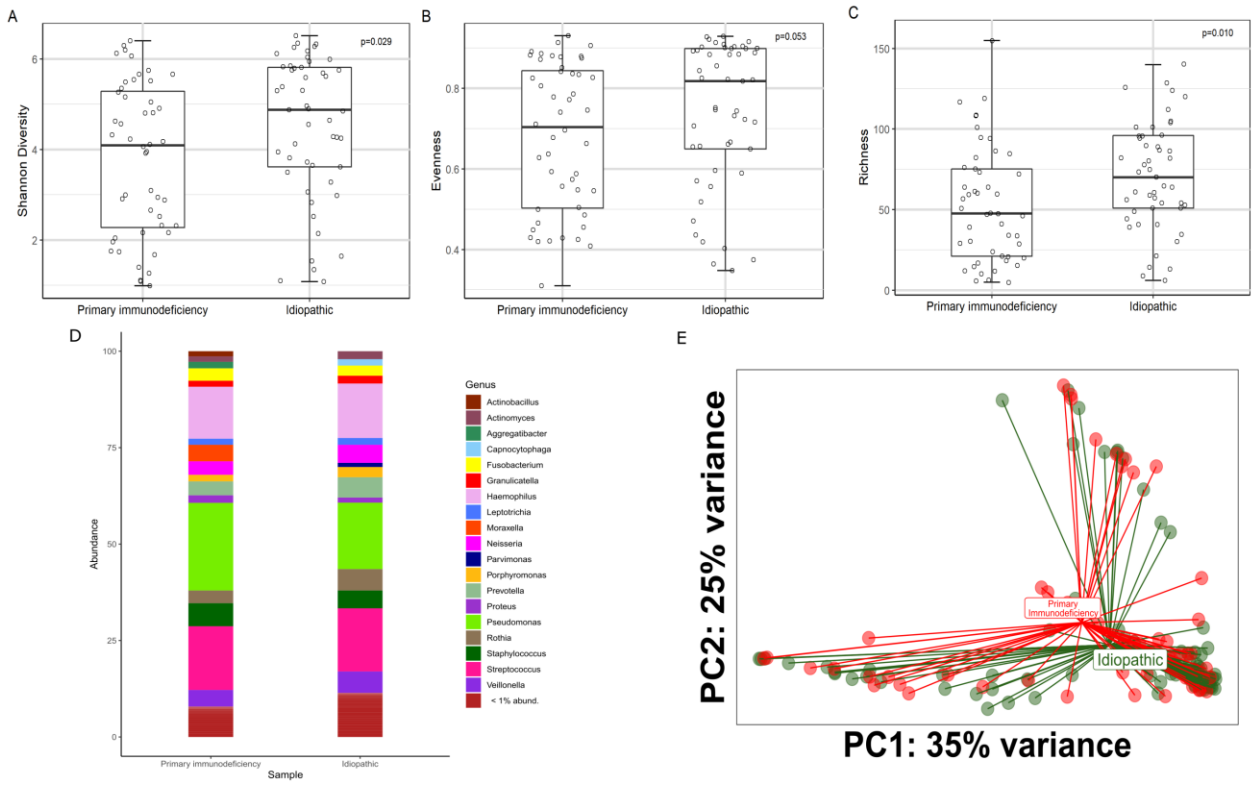

Figure 1: Comparison of alpha diversity levels in terms of a) Shannon diversity, b) Evenness, c) Richness across primary immunodeficiency and idiopathic groups; d) genera Barplots in idiopathic and primary immunodeficiency groups; e) PCoA according to Bray-Curtis dissimilarity in idiopathic and primary immunodeficiency groups

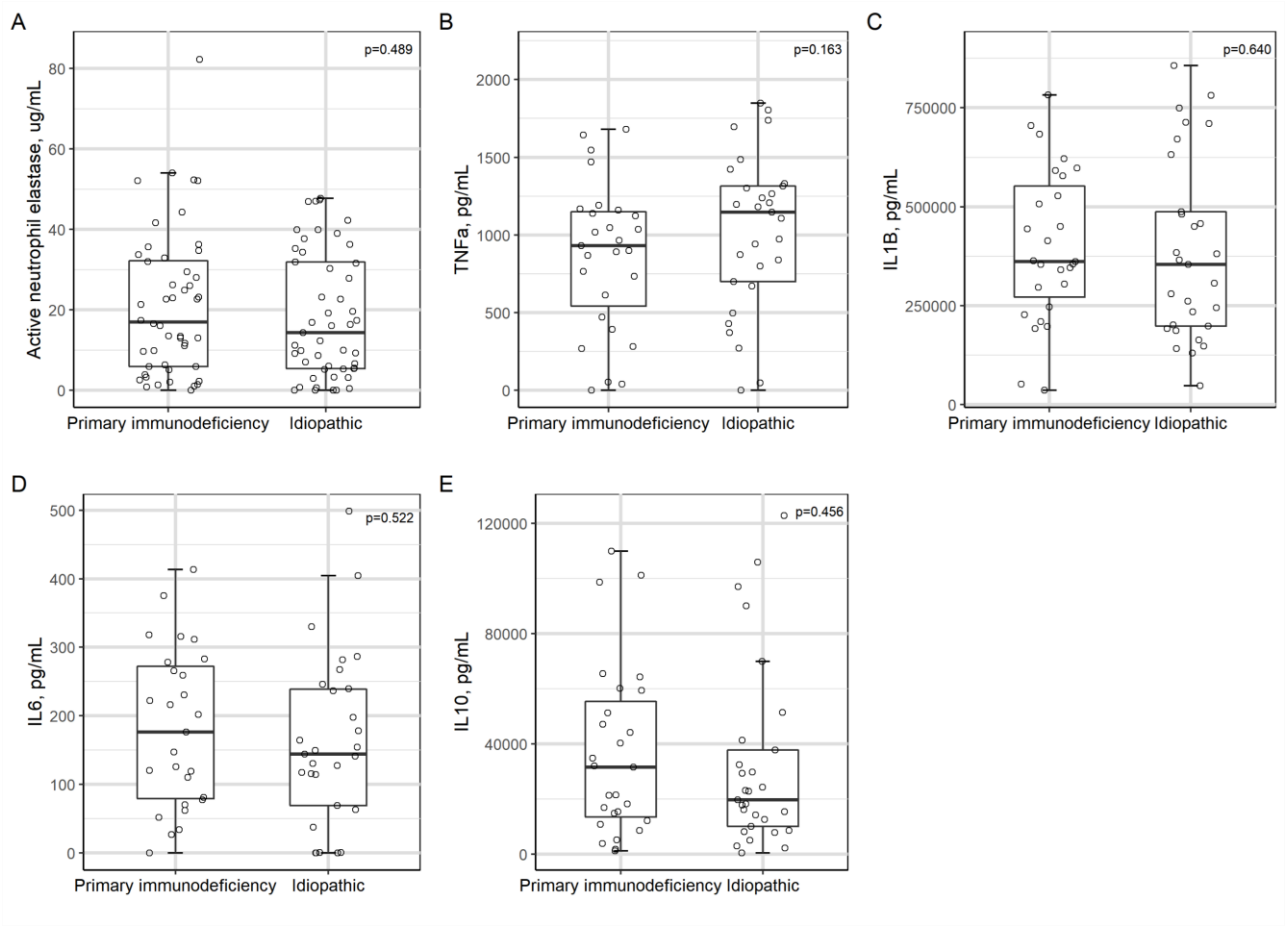

Figure 2: Comparison of inflammatory biomarkers levels in terms of A) Active neutrophil elastase B) TNFa, C) IL1B, D) IL6 and E) IL10 across primary immunodeficiency and idiopathic groups

References

1. Terranova L, Oriano M, Teri A, Ruggiero L, Tafuro C, Marchisio P, Gramegna A, Contarini M, Franceschi E, Sottotetti S, Cariani L, Bevivino A, Chalmers J, Aliberti S, Blasi F. How to Process Sputum Samples and Extract Bacterial DNA for Microbiota Analysis. *Int. J. Mol. Sci.* 2018; 19: 3256.

2. Oriano M, Terranova L, Teri A, Sottotetti S, Ruggiero L, Tafuro C, Marchisio P, Gramegna A, Amati F, Nava F, Franceschi E, Cariani L, Blasi F, Aliberti S. Comparison of different conditions for DNA extraction in sputum - a pilot study. *Multidiscip. Respir. Med.* Multidisciplinary Respiratory Medicine; 2019; : 1–8.

3. Gadsby NJ, McHugh MP, Russell CD, Mark H, Conway Morris A, Laurenson IF, Hill AT, Templeton KE. Development of two real-time multiplex PCR assays for the detection and quantification of eight key bacterial pathogens in lower respiratory tract infections. *Clin. Microbiol. Infect.* Elsevier; 2015; 21: 788.e1-788.e13.

4. Yang S, Lin S, Khalil A, Gaydos C, Nuemberger E, Juan G, Hardick J, Bartlett JG, Auwaerter PG, Rothman RE. Quantitative PCR assay using sputum samples for rapid diagnosis of pneumococcal pneumonia in adult emergency department patients. *J. Clin. Microbiol.* [Internet] American Society for Microbiology; 2005; 43: 3221–3226Available from: https://www.ncbi.nlm.nih.gov/pubmed/16000439.

5. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope E, Da Silva R, Dorrestein PC, Douglas GM,

Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, et al. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ Prepr.* [Internet] 2018; 6: e27295v2Available from: https://doi.org/10.7287/peerj.preprints.27295v2.

6. Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, A AJ. Dada2. *Nat Methods* 2016; 13: 581–583.

7. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* [Internet] 2012; 6: 1621–1624Available from: https://doi.org/10.1038/ismej.2012.8.

8. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* [Internet] 2014/12/05. BioMed Central; 2014; 15: 550Available from: https://www.ncbi.nlm.nih.gov/pubmed/25516281.

9. 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.

10. Martínez-García MA, De Gracia J, Relat MV, Girón RM, Carro LM, De La Rosa Carrillo D, Olveira C. Multidimensional approach to non-cystic fibrosis bronchiectasis: The FACED score. *Eur. Respir. J.* 2014; 43: 1357–1367.

11. Reiff DB, Wells AU, Carr DH, Cole PJ, Hansell DM. CT findings in bronchiectasis: limited value in distinguishing between idiopathic and specific types. *Am. J. Roentgenol.* [Internet] American Roentgen Ray Society; 1995; 165: 261–267Available from:

https://doi.org/10.2214/ajr.165.2.7618537.

12. Polverino E, Goeminne PC, McDonnell MJ, Aliberti S, Marshall SE, Loebinger MR, Murris M, Cantón R, Torres A, Dimakou K, De Soyza A, Hill AT, Haworth CS, Vendrell M, Ringshausen FC, Subotic D, Wilson R, Vilaró J, Stallberg B, Welte T, Rohde G, Blasi F, Elborn S, Almagro M, Timothy A, Ruddy T, Tonia T, Rigau D, Chalmers JD. European Respiratory Society guidelines for the management of adult bronchiectasis. *Eur. Respir. J.* [Internet] 2017; 50Available from: http://dx.doi.org/10.1183/13993003.00629-2017.

13. Araújo D, Shteinberg M, Aliberti S, Goeminne PC, Hill AT, Fardon T, Obradovic D, Dimakou K, Polverino E, De Soyza A, McDonnell MJ, Chalmers JD. Standardised classification of the aetiology of bronchiectasis using an objective algorithm. *Eur. Respir. J.* [Internet] 2017; 50: 1701289Available from: http://erj.ersjournals.com/content/50/6/1701289.abstract.

14. 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.* [Internet] 2015; 12: 1602–1611Available from: http://www.ncbi.nlm.nih.gov/pubmed/26356317.

15. Quittner A, O'Donnell A, Salathe M, Lewis S, Li X, Montgomery A, O'Riordan T, Barker A. Quality of Life Questionnaire-Bronchiectasis: Final psychometric analyses and determination of minimal important difference scores. *Thorax* 2014; 70.

16. Hill AT, Haworth CS, Aliberti S, Barker A, Blasi F, Boersma W, Chalmers JD, De Soyza A, Dimakou K, Elborn JS, Feldman C, Flume P, Goeminne PC, Loebinger MR, Menendez R, Morgan L, Murris M, Polverino E, Quittner A, Ringshausen FC, Tino G, Torres A, Vendrell M, Welte T, Wilson R, Wong C, O'Donnell A, Aksamit T. Pulmonary exacerbation in adults with bronchiectasis: a consensus definition for clinical research. *Eur. Respir. J.* [Internet] 2017; 49Available from: http://dx.doi.org/10.1183/13993003.00051-2017.