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

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Conclusion A four-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.



Introduction

Bronchiectasis is a chronic respiratory disease characterised 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 individualised work-up to detect treatable causes of bronchiectasis [2]. Immunodeficiency is one of the most prevalent aetiologies of bronchiectasis. 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 and syndromic disorders, as well as secondary immunodeficiencies [5]. On one hand, bronchiectasis is a very common pulmonary complication of common variable immunodeficiency (CVID) [6]. On 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 centres [3, 7–12]. A lack of standardised 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 serum levels of IgG, IgA and IgM [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; and 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 (\geq 18 years of age) outpatients with a clinical (daily sputum production) and radiological (at least one lobe involved on a high-resolution computed tomography 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 or traction bronchiectasis due to pulmonary fibrosis were excluded. The study was approved by the local ethical committee and all recruited subjects provided 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 their microbiome and inflammatory biomarkers. The complete methodology and results for the airway microbiome and inflammation analyses are reported in the supplementary material. All patients underwent a systematic and standardised immunological screening consisting of: IgA, IgG, IgM and IgG subclasses; total IgE; lymphocyte subsets; and HIV antibodies (reference values for the tests are listed in the supplementary material). Patients with at least one positive result in the immunological screening underwent a second evaluation ≥ 1 month after the first. In cases of a positive at a second evaluation, patients were referred to a clinical immunological (B. Vigone) for additional and individualised immunological tests including B- and T-cell typing (CD3, CD4, CD19, CD56, CD21^{low} and switched memory B-cells), and evaluation of immunological response to polysaccharide and protein antigens (*Streptococcus pneumoniae* and tetanus vaccination). Bronchiectasis aetiology was evaluated following the recommendations of the 2017 ERS guidelines [2] and the aetiological classification was based on the algorithm published by ARAÚJO *et al.* [13].

Definitions of primary and secondary immunodeficiencies

Primary immunodeficiency conditions were defined according to European Society for Immunodeficiencies (ESID) diagnostic criteria [14]. Selective IgA deficiency was defined in the presence of undetectable serum levels of IgA (when measured by nephelometry, $<0.07 \text{ g} \cdot \text{L}^{-1}$) and normal levels of other immunoglobulins. CVID was defined in the presence of low total serum concentrations of IgG (≥ 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: low or absent CD3, CD4 or CD8 T-cells; reduced naïve CD4 and/or CD8 T-cells; elevated $\gamma\delta$ T-cells; and reduced or absent proliferation in response 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: reduced CD3, CD4 or CD8 T-cells (using age-related reference values); reduced naïve CD4 and/or CD8 T-cells; elevated γδT-cells; and reduced proliferation in response to mitogen or TCR stimulation. These criteria were identified in the context of at least one severe infection (requiring hospitalisation) and/or one manifestation of immune dysregulation (such as autoimmunity, inflammatory bowel diseases, severe eczema, lymphoproliferation or granuloma) and/or malignancy and/or an affected family member. DiGeorge Syndrome was defined by the presence of documented microdeletion at 22q11 or 10p and recurrent or severe infections. Hyper-IgE syndrome was defined by the presence of IgE >10 times the normal limit for age, pathological susceptibility to infectious diseases and no evidence of T- or B-cell deficiency. IgG subclass deficiency was defined by persistently low levels of one or more IgG subclass, normal total IgG, IgA and IgM serum levels, and exclusion of a T-cell defect. Selective IgM deficiency was defined by low IgM plasma level, normal IgG and IgA plasma levels, and exclusion of a T-cell defect. Unclassified antibody deficiency was defined by a marked decrease of at least one of total IgG, IgG₁, IgG₂, IgG₃, IgG₄, IgA or IgM levels, no clinical signs of T-cell related disease and not fitting 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 not fitting any of the other working definitions. A narrow definition of primary immunodeficiency conditions was consider excluding patients with both isolated IgM and isolated IgG₄ subclass deficiency.

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

Definitions of treatable immunodeficiencies

Candidates for treatment with intravenous 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 $<4 \text{ g} \cdot \text{L}^{-1}$ plus at least one of: 1) three or more exacerbations per year; 2) one or more systemic infection during the previous year; 3) one or more hospitalisation 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 as follows. S1: complete blood count, and total serum IgG, IgA and IgM levels (bundle suggested by the 2017 ERS Guidelines [2]); S2: S1 plus IgG subclasses; S3: S2 plus lymphocyte subsets; 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 aetiology of bronchiectasis: primary immunodeficiency (group A), secondary immunodeficiency (group B) and idiopathic bronchiectasis (group C).

Statistical analysis

Qualitative variables are presented as n (%). Quantitative variables are presented as mean±sD or median (interquartile range (IQR)) depending on their normal or non-normal distribution, respectively. Qualitative variables were compared with chi-squared and Fisher exact tests, when appropriate. ANOVA and Kruskall–Wallis tests 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 <0.05 was considered statistically significant. Statistical analysis was conducted using STATA version 16 (StataCorp, College Station, TX, USA).

Results

A total of 401 bronchiectasis patients (79.1% female; median (IQR) age 63 (50–71) years) underwent the immunological screening. Patients' characteristics of the entire cohort are reported in table 1.

Variables Study population (n=401) Demographics 317 (79.1%) Female sex 317 (79.1%) Age, years 63 (50–71) Body mass index, kg·m ⁻² 21.5 (19.5–24.0) Underweight 58 (14.6%) Former or current smoker 180 (44.9%) Comorbidities 180 (44.9%) GORD 180 (44.9%) Cardiovascular diseases 142 (35.4%) Systemic hypertension 93 (23.2%) Asthma 60 (15.0%) Osteoporosis 69 (17.2%) OCPP 32 (8.0%) Depression 34 (8.5%) Astima 60 (15.0%) Mistory of neoplastic disease 56 (14.0%) Diabetes 17 (4.2%) BACI score 0 (0–3) Functional evaluation 110 FEV, % pred 87 (71–101) FEV, % % pred, meanse 93 (52.7%) Chronic infection 120 (35.9%) Chronic infection 120 (35.9%) Chronic infection 120 (35.9%) Chronic infection		
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1 Demographics, disease severity, clinical, radiological, functional and microbiological characteristics of dv population

Data are presented as median (interquartile range) unless otherwise stated. GORD: gastro-oesophageal reflux disease; COPD: chronic obstructive pulmonary disease; BACI: before, after, control, impact; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; NTM-PD: nontuberculous mycobacterial pulmonary disease; LTOT: long-term oxygen therapy; BSI: bronchiectasis severity index; FACED: FEV₁, age, chronic colonisation, extension, dyspnoea.

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 *versus* S2 *versus* S1 was chosen (16.7% *versus* 9.2% *versus* 3.7%, respectively; p=0.00001). Isolated IgG subclass deficiency, isolated IgM deficiency, unclassified antibody deficiency, CVID, unclassified immunodeficiency and secondary

	\$1	S2	S3	S4	S5			
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 subclass deficiency	0	0	36 (9%)	36 (9%)	36 (9%)			
Isolated IgG ₁ subclasses deficiency	0	0	3 (0.8%)	3 (0.8%)	3 (0.8%)			
Isolated IgG ₂ subclasses deficiency	0	0	2 (0.5%)	2 (0.5%)	2 (0.5%)			
Isolated IgG ₃ subclasses deficiency	0	0	6 (1.5%)	6 (1.5%)	6 (1.5%)			
Isolated IgG ₄ subclasses deficiency	0	0	24 (5.9%)	24 (5.9%)	24 (5.9%)			
IgG ₁ and IgG ₃ subclasses deficiency	0	0	1 (0.2%)	1 (0.2%)	1 (0.2%)			
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%)			
CVID	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 aetiologies	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%)			

TABLE 2 Prevalence of bronchiectasis adults (n=401) with any, primary, secondary or treatable immunodeficiencies according to five different sets of immunological tests (S1–S5)

S1: complete blood count, and total IgG, IgA and IgM serum levels; S2: S1 plus IgG subclasses; S3: S2 plus lymphocyte subsets; S4: S3 plus total IgE; S5: S4 plus HIV testing. CVID: common variable immunodeficiency. #: including 26 post-infective, eight primary ciliary dyskinesia and five allergic bronchopulmonary aspergillosis.

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 subclass deficiencies were not considered as primary immunodeficiencies (narrow definitions), a significantly higher prevalence of patients was still diagnosed with any, primary or treatable immunodeficiencies if S3 *versus* S2 *versus* S1 was chosen (any: 37.9% *versus* 23.2% *versus* 8.9%, p<0.00001; primary: 32.7% *versus* 20.7% *versus* 7.2%, p<0.00001; treatable: 13% *versus* 9.2% *versus* 3.7%, p=0.00001). In terms of costs, the total cost per patient was EUR 18.35 for S1, EUR 74.15 for S2, EUR 159.70 for S3, EUR 167.40 for S4 and EUR 176.20 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 subclass deficiency (36, 9%), whereas six (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 intravenous or subcutaneous immunoglobulins: 58 (14.5%) had primary immunodeficiency (including 36 patients with unclassified immunological deficiency, 17 with isolated IgG subclasses deficiency, two with CVID, one with isolated with IgM deficiency, one with DiGeorge Syndrome and one with unclassified antibody deficiency) and nine (2.2%) secondary immunodeficiency (including five patients with haematological malignancy, two undergoing immunosuppressive drugs and two who underwent transplant).

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

(table 3). Comparison of sputum microbiome characteristics in terms of alpha diversity and inflammatory biomarker levels between idiopathic and primary immunodeficiency groups is reported in supplementary figures S1 and S2.

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	122 (77.2%)	13 (61.9%)	129 (83,2%)	0.06
Age, years	65 (52–73)	70 (65–73)	62 (48–70)	0.009#
Body mass index, kg·m ⁻²	21.1 (19.2–24.0)	23.5 (21.9–25.5)	21.4 (19.7–24.0)	0.08
Underweight	27 (17.2%)	2 (10.0%)	20 (13.0%)	0.48
Former or current smoker	63 (39.9%)	13 (61.9%)	77 (49.7%)	0.07
Comorbidities		(,	(,.,	
GORD	71 (44.9%)	11 (52.4%)	74 (47.7%)	0.76
Rhinosinusitis	58 (36.7%)	7 (33.3%)	45 (29.0%)	0.35
Cardiovascular diseases	66 (41.8%)	12 (57.1%)	43 (27.7%)	0.004 [¶]
Systemic hypertension	43 (27.2%)	8 (38.1%)	29 (18.7%)	0.06
Asthma	24 (15.2%)	1 (4.8%)	29 (18.7%)	0.25
Osteoporosis	24 (15.2%)	10 (47.6%)	26 (16.8%)	0.001^{+}
COPD	16 (10.1%)	2 (9.5%)	9 (5.8%)	0.32
Depression	17 (10.8%)	3 (14.3%)	10 (6.5%)	0.24
Anxiety	11 (7.0%)	2 (9.5%)	11 (7.1%)	0.84
History of neoplastic disease	23 (14.6%)	12 (57.1%)	13 (8.4%)	<0.0001 [§]
Diabetes	6 (3.8%)	7 (33.3%)	3 (1.9%)	<0.0001 ^f
Functional				
FEV ₁ , % pred	85.9±24.9	84.6±27.2	85.6±20.8	0.97
FEV ₁ <50% pred	13 (8.8%)	2 (10.0%)	9 (6.2%)	0.62
FVC, % pred	98.5±23.2	97.5±25.1	97.3±18.6	0.89
Microbiological				
Chronic infection	49 (36.6%)	5 (29.4%)	46 (35.4%)	0.84
Chronic Pseudomonas aeruginosa	31 (23.1%)	3 (17.7%)	30 (23.1%)	0.95
infection				
Chronic Haemophilus influenzae infection	7 (5.3%)	3 (17.7%)	8 (6.2%)	0.14
NTM-PD	18 (13.4%)	3 (17.7%)	11 (8.5%)	0.29
Clinical				
Exacerbations	2 (1–3)	1 (0–3)	2 (1–3)	0.97
≥3 exacerbations in the previous year	51 (32.3%)	8 (38.1%)	49 (31.6%)	0.84
LTOT	11 (7.0%)	2 (9.5%)	4 (2.6%)	0.09
Daily sputum	104 (65.8%)	15 (71.4%)	107 (69.0%)	0.77
Sputum volume, mL	7 (4.5–20.0)	5.5 (3–50)	6 (5–20)	0.93
Chronic macrolide therapy	16 (10.1)	1 (4.8)	11 (7.1)	0.62
Chronic inhaled antibiotic therapy	8 (5.1)	1 (4.8)	10 (6.5)	0.86
Radiological				
Reiff score	4 (2–6)	4 (2–6)	4 (3–6)	0.99
Number of lobes involved	3 (2–4)	4 (2–6)	4 (2–5)	0.59
Disease severity				
BSI score	6 (4–9)	6 (3.0–8.5)	6 (3–8)	0.11
FACED score	2 (1–3)	2 (2–3)	2 (1–3)	0.06
BACI score	0 (0–3)	6 (3–10)	0 (0–3)	0.0001##

Data are presented as median (interquartile range) or mean±sD, unless otherwise stated. GORD: gastro-oesophageal reflux disease; COPD: chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; FVC: forced vital capacity; NTM-PD: nontuberculous mycobacterial pulmonary disease; LTOT: long-term oxygen therapy; BSI: bronchiectasis severity index; FACED: FEV₁, age, chronic colonisation, extension, dyspnoea; BACI: before, after, control, impact. [#]; Group B *versus* Group C, p=0.008. [¶]: Group A *versus* Group C, p=0.0001; [§]: Group A *versus* Group C, p=0.0001; ^f: Group A *versus* Group C, p<0.0001; Group B *versus* Group C, p<0.0001; ^f: Group A *versus* Group C, p<0.0001; Group B *versus* Group C, p<0.0001; ^f: Group A *versus* Group C, p<0.0001; Group B *versus* Group C, p<0.0001; Group B *versus* Group C, p<0.0001.

Discussion

A four-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 subclass deficiencies. If diagnosis of isolated IgM and isolated IgG4 subclass deficiencies were not considered, 37.9% of the patients still 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 or exacerbation frequencies were found between patients with immunodeficiencies and those with idiopathic bronchiectasis.

Isolated IgG subclass 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 to debate: reduced levels of IgG subclasses have been demonstrated 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% [11, 20–25] and we reported this in 9% of our population. We found a prevalence of 3.1% of IgG_1 , IgG_2 or IgG_3 deficiency in patients with bronchiectasis while previous experiences using stringent criteria to define this condition reported a prevalence <1% [22–24]. Among our patients, 5.9% of bronchiectasis patients were diagnosed with an isolated IgG₄ subclass deficiency although a reduced IgG₄ concentration is not usually regarded as a marker of humoral immune deficiency [26]. An isolated IgG subclass deficiency in severe patients despite optimised clinical management might represent an important treatable trait in bronchiectasis [27]. Different experiences have shown that correction of IgG subclass deficiency using intravenous or subcutaneous immunoglobulin replacement therapy resulted in a clinically meaningful reduction in bacterial chest infections [28, 29]. However, specific randomised controlled trials (RCTs) 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 was associated with the presence of clinically significant bronchiectasis. A large proportion of patients with unclassified immunodeficiency were characterised by alteration in both lymphocyte and immunoglobulin production. Therefore, even if lymphocytes 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 immunodeficiency or evaluate its severity, such as mannose-binding lectin genotype and function [30, 31].

Finally, no patients' characteristics seemed to help physicians identify those with immunodeficiencies and, thus, individualise the immunological workup. Patients enrolled in our cohort belong to a tertiary care centre and different findings could be described when patients are recruited from primary or secondary care centres where a minimum bundle of immunological tests is adopted. We could speculate that patients with severe bronchiectasis and several comorbidities attending a tertiary care centre could be the ideal candidate for comprehensive immunological screening. The study limitations are related to its monocentric nature: it being conducted in a tertiary care setting could have hindered the reproducibility of the findings. Different prevalence of immunodeficiencies across different settings could be found [3]. Therefore, 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-cells, such as proliferation to mitogen or TCR stimulation, were not performed in our centre.

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 aetiology also followed a standardised algorithm recently published.

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 four-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 seemed help physicians identify those with immunodeficiencies and, thus, individualise the immunological workup. Therefore, we recommend the following bundle of immunological tests in adult patients with bronchiectasis: complete blood count; IgA, IgG, IgM and IgG subclasses; and lymphocyte subsets (including CD4 and 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.

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Conflict of interest: S. Aliberti reports grants or contracts from Insmed, Chiesi and Fisher & Paykel; royalties or licences from McGraw Hill; consulting fees from Insmed, Zambon, AstraZeneca, CSL Behring GmbH, Grifols, Fondazione Charta, Boehringer Ingelheim, Chiesi, Zcube Srl and Menarini; and payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or educational events from GlaxoSmithKline, and for participation on a Data Safety Monitoring Board or Advisory Board for Insmed and AstraZeneca, all outside the submitted work. M. Mantero declares honoraria for educational events from GlaxoSmithKline and Boehringer Ingelheim, outside the present study. A. Stainer reports grants, speaker fees and travel support from AstraZeneca, Bayer, Chiesi, Forest Laboratories, GlaxoSmithKline, Insmed, Pfizer, Medimmune, Novartis and Zambon, outside the submitted work. E. Polverino reports grants or contracts from Grifols; consulting fees from Insmed, Chiesi and Zambon; payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or educational events from Insmed, GlaxoSmithKline, Teva, Boehringer Ingelheim, Chiesi and Zambon; support for attending meetings from Insmed and Teva, and for participation on a Data Safety Monitoring Board or Advisory Board from Insmed and Chiesi, all outside the submitted work. J.D. Chalmers reports grants and personal fees from AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline and Insmed, grants from Gilead Sciences, and personal fees from Chiesi, Novartis and Zambon, outside the submitted work. F. Blasi reports grants and personal fees from AstraZeneca, Chiesi, GlaxoSmithKline, Pfizer and Insmed; grants from Bayer; and personal fees from Guidotti, Grifols, Menarini, Mundipharma, Novartis and Zambon, outside the submitted work. F. Amati, A. Gramegna, B. Vigone, M. Oriano, G. Sotgiu, E. Simonetta, L. Saderi, S. Tammaro and P. Marchisio report no conflict of interest.

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