Sputum neutrophil elastase in bronchiectasis: a Southern European cohort study

To the Editor:

Bronchiectasis is a chronic respiratory disease with neutrophilic airway inflammation playing a prominent role in its pathophysiology [1]. The inflammatory process depends on the release of neutrophil elastase and subsequent formation of neutrophil extracellular traps to facilitate the neutralisation of pathogens. An excessive release of neutrophil elastase can lead to several damaging lung effects, including mucus gland stimulation, increase in sputum production, impairment in ciliary beat frequency and extracellular matrix and airway epithelia destruction. The activity of neutrophil elastase (aNE) has been evaluated previously in sputum samples of a Scottish cohort of bronchiectasis patients [2]. The authors demonstrated that increased levels of aNE in sputum are associated with disease severity and poor clinical outcomes. This experience identified neutrophil elastase as one of the most promising biomarkers in bronchiectasis and, subsequently, a point-of-care assay for aNE was validated [3].

An external validation of the Scottish data is needed in view of the heterogeneity of bronchiectasis across Europe, especially in terms of microbiology, with chronic *Pseudomonas aeruginosa* infection being more prevalent in Southern *versus* Northern Europe [4] *P. aeruginosa* represents the major player in enhancing neutrophilic airway inflammation and leading to worse clinical outcomes [5–7]. In order to define the activity of neutrophil elastase in sputum of bronchiectasis patients in Southern Europe and its association with disease severity and other clinical characteristics, we designed a multicentric, prospective, observational study in two bronchiectasis referral centres in Italy and Spain.

Consecutive adults (aged ⩾18 years) with radiologically (at least one lobe involvement on chest computed tomography) and clinically (daily sputum production) significant bronchiectasis were enrolled during clinical stability (⩾1 month from the last exacerbation and antibiotic course) at the bronchiectasis programmes of the Policlinico Hospital in Milan, Italy and the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain, between March 2017 and March 2019. Patients with either cystic fibrosis or pulmonary fibrosis with secondary traction bronchiectasis were excluded. The study was approved by the local institutional review boards and all subjects provided written informed consent to participate. Sputum samples were collected during stable state, prepared by 8× dilution in PBS followed by centrifugation, and aNE levels were assessed using ProteaseTag Active Neutrophil Elastase Immunoassay (Proaxis, Belfast, UK) as per manufacturer's instructions [8]. Disease severity was evaluated according to both the Bronchiectasis Severity Index (BSI) and the E-FACED (forced expiratory volume in 1 s, age, chronic colonisation by *P. aeruginosa*, radiological extension and dyspnoea, plus exacerbations) score [9, 10]. The Quality of Life Bronchiectasis Questionnaire (QoL-B) was collected as a patient-reported outcome [11]. All bacteriology was performed on spontaneous sputum samples as per standard operating procedures. Chronic infection was defined by the isolation of potentially pathogenic bacteria in sputum culture on two or more occasions ⩾3 months apart over a 1-year period [12]. Three groups of patients were identified *a priori* based on the median and tertile concentrations of active neutrophil elastase on sputum: "low aNE" group including patients with aNE 0–6 $\mu g \cdot mL^{-1}$, "medium aNE" group including patients with aNE 7–20 $\mu g \cdot mL^{-1}$ and "high aNE" group including patients with aNE >20 $\mu g \cdot mL^{-1}$. Qualitative and quantitative variables were summarised with frequencies and medians (interquartile range (IQR)), respectively. Differences between groups were assessed with Chi-squared or Fisher exact tests for qualitative variables and with t-test or Mann–Whitney test for quantitative parametric and nonparametric

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TABLE 1 Disease severity and clinical characteristics across the three study groups

	Sputum neutrophil elastase level			p-value
	Low 0–6 μg·mL^{-1}	Medium 7–20 μg·mL^{-1}	High >20 μg·mL^{-1}	
Subjects	91	77	98	
Male	19 (27.9)	19 (25.0)	21 (25.3)	0.91
Age years	65 (51–75)	63 (53–73)	64 (54–74)	0.83
Current/former smoker	32 (47.1)	36 (47.4)	32 (38.6)	0.45
BMI kg·m^{-2}	23.2 (20–26)	22 (18.9–25.0)	21 (19.4–24.5)	0.15
Disease severity				
BSI	6 (4–9)	7 (4–10)	8 (5–12)	0.009[¶]
Mild	28 (31.1)	23 (29.1)	17 (17.9)	0.09
Moderate	39 (43.3)	28 (35.4)	35 (36.8)	0.52
Severe	23 (25.6)	28 (35.4)	43 (45.3)	0.02[+]
E-FACED	2 (1–3)	2 (1–3)	3 (1–4)	0.0006[§]
Mild	73 (84.9)	55 (76.4)	59 (64.8)	0.008[f]
Moderate	12 (14.0)	12 (16.7)	25 (27.5)	
Severe	1 (1.2)	5 (6.9)	7 (7.7)	
mMRC 3–4	6 (6.6)	8 (10.4)	14 (14.4)	0.22
Comorbidity				
Cardiovascular diseases	23 (33.8)	20 (26.3)	27 (32.5)	0.57
Chronic renal failure	2 (2.9)	3 (4.0)	2 (2.4)	0.89
Diabetes	4 (5.9)	8 (10.5)	2 (2.4)	0.11
History of pneumonia	41 (60.3)	48 (63.2)	48 (57.8)	0.79
History of tuberculosis infection	5 (7.4)	8 (10.5)	7 (8.4)	0.79
Rheumatoid arthritis	1 (1.7)	1 (1.5)	1 (1.3)	1.0
History of other CTD	3 (5.2)	0 (0.0)	1 (1.3)	0.11
History of IBD	2 (2.9)	2 (2.6)	0 (0.0)	0.33
History of autoimmune disease	0 (0.0)	2 (3.1)	0 (0.0)	0.19
Primary ciliary dyskinesia	5 (8.6)	6 (9.2)	10 (13.0)	0.66
Asthma	10 (14.7)	12 (15.8)	10 (12.1)	0.79
COPD[#]	6 (8.8)	7 (9.2)	7 (8.4)	0.99
Rhinosinusitis	24 (35.3)	25 (32.9)	29 (34.9)	0.95
Gastro-oesophageal reflux disease	26 (44.8)	26 (40.0)	35 (45.5)	0.79
Primary immunodeficiency	7 (12.1)	10 (15.4)	13 (16.9)	0.74
Secondary immunodeficiency	2 (3.5)	4 (6.2)	1 (1.3)	0.34
Clinical status				
Sputum colour				
Mucoid	12 (27.3)	9 (16.7)	6 (9.5)	0.16
Mucopurulent	16 (36.4)	25 (46.3)	27 (42.9)	
Purulent/severe purulent	16 (36.4)	20 (37.0)	30 (47.6)	
Sputum volume	6 (5–20)	15 (5–50)	25 (7–75)	<0.0001[##]
Exacerbation previous year	2 (1–3)	2 (1–3)	2 (1–3)	0.77
Patients with ⩾2 exacerbations in previous year	49 (54.4)	47 (60.3)	58 (60.4)	0.65
>1 hospitalisation in previous year	15 (16.7)	12 (15.2)	23 (24.0)	0.27
Quality of life				
QoLB physical	59.8±25.1	61.1±25.3	52.8±27.1	0.31
QoLB role	66.7 (50–80)	73.3 (46.7–86.7)	66.7 (40–80)	0.43
QoLB vitality	53.1±19.9	51.2±23.5	49.8±19.7	0.79
QoLB emotion	70.9 (58.3–87.5)	75 (66.7–91.7)	75 (50.0–91.7)	0.83
QoLB social	68.9±23.0	59.7±26.6	54.7±26.6	0.05
QoLB treatment burden	66.7 (55.6–77.8)	66.7 (55.6–77.8)	66.7 (44.4–77.8)	0.86
QoLB health	41.7 (20.9–58.3)	41.7 (25–50)	33.3 (16.7–50.0)	0.33
QoLB respiration	74.1 (66.7–81.5)	70.4 (59.3–77.8)	66.7 (51.9–74.1)	0.04[¶¶]
Standard microbiology				
Chronic infection	26 (30.2)	34 (44.7)	66 (71.0)	<0.0001[++]
Chronic infection *P. aeruginosa*	12 (14.0)	21 (27.6)	44 (47.3)	<0.0001[§§]
Chronic infection other bacteria	16 (18.6)	21 (27.6)	25 (26.9)	0.31
FEV_1 L	1.9 (1.5–2.5)	1.9 (1.4–2.4)	1.7 (1.2–2.1)	0.02[ff]
FEV_1 %	80.2±24.4	74.0±20.0	73.0±27.1	0.10
FEV_1 <50% predicted	8 (8.9)	9 (12.0)	22 (23.2)	0.02[###]
FEV_1 <35% predicted	2 (2.2)	3 (4.0)	9 (9.5)	0.09

Data are presented as n, n (%), median (interquartile range) or mean±SD, unless otherwise stated. BMI: body mass index; BSI: Bronchiectasis Severity Index; E-FACED: forced expiratory volume in 1 s (FEV_1), age, chronic colonisation by *Pseudomonas aeruginosa*, radiological extension and dyspnoea, plus exacerbations; mMRC: modified Medical Research Council dyspnoea score; CTD: connective tissue disease; IBD: inflammatory bowel disease; QoLB: Quality of Life Bronchiectasis Questionnaire. [#]: defined as fixed ratio of post-bronchodilator FEV_1/forced vital capacity <0.7 in addition to FEV_1 <80% predicted in a patient with a smoking history of >10 pack-years; [¶]: low *versus* high p=0.004; [+]: low *versus* high p=0.005; [§]: low *versus* high p=0.0003, medium *versus* high p=0.01; [f]: low *versus* high p=0.002; [##]: low *versus* medium p=0.03, low *versus* high p<0.0001, medium *versus* high p=0.04; [¶¶]: low *versus* high p=0.02; [++]: low *versus* high p<0.0001, medium *versus* high p=0.0005; [§§]: low *versus* medium p=0.03, low *versus* high p<0.0001, medium *versus* high p=0.009; [ff]: low *versus* high p=0.01; [###]: low *versus* high p=0.008.

variables, respectively. Active neutrophil elastase was correlated with continuous variables with the Spearman correlation. A two-tailed p-value was considered statistically significant when <0.05.

Among the 266 patients (female 77.8%; median (IQR) age 64 (53–73) years), aNE evaluation was under the lower limit of detection in two patients. Among the entire study cohort, the median (IQR) aNE level was 12.8 (4.1–29.3) $\mu g \cdot mL^{-1}$. Levels of aNE in sputum correlated with bronchiectasis severity, evaluated through both the BSI (r=0.23, p=0.0002) and the E-FACED (r=0.26, p<0.0001) scores. Median (IQR) levels of aNE increased significantly across mild, moderate and severe BSI (8.7 (2.8–20.7) *versus* 11.1 (4.7–29.3) *versus* 18.2 (8.1–37.7), respectively, p=0.001) and E-FACED (10.0 (3.6–24.4) *versus* 21.4 (8.2–34.4) *versus* 31.5 (11.4–42.3), respectively, p=0.004) risk classes. Median (IQR) levels of aNE were higher in patients with *versus* without any chronic infection (22.4 (9.1–35.5) *versus* 8.0 (2.8–17.3) $\mu g \cdot mL^{-1}$, respectively, p<0.0001), and chronic *P. aeruginosa* infection (25.1 (11.3–40.5) *versus* 9.2 (3.1–22.7) $\mu g \cdot mL^{-1}$, p<0.0001). The QoL-B respiratory domain inversely correlated with increasing aNE concentrations in sputum (r=−0.25, p=0.009). Low, medium and high aNE groups included 91 (34%), 77 (29%) and 98 (37%) patients, respectively. The three study groups did not differ in term of age, sex and comorbidities (table 1). Significant differences in terms of disease severity, chronic infection and lung function were found across the three study groups (table 1). Daily sputum volume significantly increased across the study groups, while quality of life, assessed through the QoL-B respiratory domain worsened (table 1). No differences in terms of exacerbations/hospitalisations in the previous year were detected among the three study groups.

With the present experience, we confirmed the correlation of sputum aNE with disease severity, lung function, chronic infection (especially with *Pseudomonas*) and quality of life of bronchiectasis patients in two Southern European cohorts. Furthermore, we identified different cut-offs of aNE values which were able to identify different bronchiectasis populations in terms of disease severity and clinical characteristics. This study succeeded to validate previous findings about the relationship between aNE and disease severity and clinical characteristics in a large population of bronchiectasis patients from two major Southern European countries. Different findings from the present experience should be highlighted. Firstly, the large majority of our patients showed aNE values above the lower limit of detection. This could reflect the disease severity and prevalence of chronic *P. aeruginosa* infection we found in our population. Indeed, we could speculate that the higher prevalence of *P. aeruginosa* in our cohort is the driver of different aNE levels in sputum, as previously reported in the literature [13]. Secondly, aNE showed a good performance in correlating with disease severity and clinical characteristics in two Southern European cohorts, in addition to what already demonstrated in a large population of patients from Scotland [4]. This highlights the generalisability of aNE as a biomarker across different populations of bronchiectasis patients. Thirdly, the cut-offs of aNE proposed in the present study derives from the *a priori* analysis of our combined population. The 20 $\mu g \cdot mL^{-1}$ cut-off is consistent with previously published data, and might help in identifying the most severe patients and those who could be more likely the candidates and responders to different interventions [4]. In addition, we identified low and medium aNE groups, which might support physicians in better stratifying their patients and follow their clinical course and treatment response. This could be the case for new neutrophil protease inhibitors which have been tested as modulators of neutrophilic inflammation in bronchiectasis. Notably, in a recent phase 2 trial a selective inhibitor of enzyme dipeptidyl peptidase 1 significantly reduced time to first exacerbation in this population (clinicaltrial.gov ID NCT03218917).

Our study suffers of different limitations, including its cross-sectional design and the lack of the evaluation of the predictive value of aNE on long-term clinical outcomes in bronchiectasis. However, the multicentre design of our experience strengthens the generalisability of our findings. Future research should aim at confirming our findings in cohorts of bronchiectasis patients enrolled in other European and non-European countries to increase their generalisability and understand if aNE could be implemented as biomarker in future randomised clinical trials. Finally, future studies should further explore our findings by the comparison of patients with pure bronchiectasis *versus* patients with both bronchiectasis and obstructive lung disease.

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