EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Research letter

Sputum neutrophil elastase in bronchiectasis: a Southern European cohort study

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Please cite this article as: Gramegna A, Aliberti S, Sibila O, *et al*. Sputum neutrophil elastase in bronchiectasis: a Southern European cohort study. *Eur Respir J* 2020; in press (https://doi.org/10.1183/13993003.01702-2020).

This manuscript has recently been accepted for publication in the *European Respiratory Journal*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.

Title

Sputum neutrophil elastase in bronchiectasis: a Southern European cohort study

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Keywords. Neutrophil elastase; Bronchiectasis; Pseudomonas aeruginosa

Take Home message. Activity of Neutrophil Elastase is a generalizable biomarker related to disease severity and clinical characteristics across different populations of patients with bronchiectasis.

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 (NE) and subsequent formation of neutrophil extracellular traps to facilitate the neutralization of pathogens. An excessive release of NE 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 NE (aNE) has been previously evaluated 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 NE as one of the most promising biomarkers in bronchiectasis and, subsquently, 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 been more prevalent in Southern vs. 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 NE 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 centers in Italy and Spain.

Consecutive adults (≥18 years) with radiologically (at least one lobe involvement on chest CT) and clinically (daily sputum production) significant bronchiectasis were enrolled during their clinical stability (at least one month from the last exacerbation and antibiotic course) at the Bronchiectasis Programs 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 IRBs and all subjects provided written informed consent to participate. Sputum samples were collected during stable state, prepared by 8x dilution in PBS followed by centrifugation and aNE levels were assessed by ProteaseTag® Active Neutrophil Elastase Immunoassay (Proaxis, Belfast, Northern Ireland, UK) as per manufacturer's instructions [8]. Disease severity was evaluated according to both the Bronchiectasis Severity Index (BSI) and the E-FACED score [9,10]. The Quality-of-Life Bronchiectasis Questionnaire (QoL-B) was collected as 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 at least 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 NE on sputum: "Low aNE" group including patients with aNE 0-6 ug/ml; "Medium aNE" group including patients with aNE 7-20 ug/ml; and "High aNE" group including patients with aNE >20 ug/ml. Qualitative and quantitative variables were summarized with frequencies and medians (interquartile ranges, IQR), respectively. Differences between groups were assessed with chi-squared or Fisher exact test for qualitative variables and with Student t test or Mann-Whitney for quantitative parametric and non-parametric variables, respectively. Active NE was correlated with continuous variables with the Spearman correlation. A two-tailed p-value was considered statistically significant when less than 0.05.

Among the 266 patients (female: 77.8%; median [IQR] age: 64 [53-73] years) enrolled, aNE evaluation was under the lower limit of detection in 2 patients. Among the entire study cohort, the median (IQR) aNE level was 12.8 (4.1-29.3) ug/ml. 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 levels [IQR] of aNE significantly increased across mild, moderate and severe BSI (8.7 [2.8-20.7] VS. 11.1 [4.7-29.3] VS. 18.2 [8.1-37.7], p=0.001) and E-FACED

(10.0 [3.6-24.4] VS. 21.4 [8.2-34.4] VS. 31.5 [11.4-42.3], p=0.004) risk classes. Median levels [IQR] of aNE were higher in patients with VS. without any chronic infection (22.4 [9.1-35.5] VS. 8.0 [2.8-17.3] ug/ml, P<0.0001), and chronic *P. aeruginosa* infection (25.1 [11.3-40.5] VS. 9.2 [3.1-22.7] ug/ml, 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, gender and comorbidities, see Table. Significant differences in terms of disease severity, chronic infection and lung function were found across the three study groups, see Table. Daily sputum volume significantly increased across the study groups, while quality of life, assessed through the QoL-B RD worsened, see Table. No differences in terms of exacerbations/hospitalizations 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-off of aNE 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 relation 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 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 generalizability 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 own combined population. The 20 ug/ml 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 also identified a 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 multicenter design of our experience strengths the generalizability of our findings. Next research should aim at confirming our findings in cohorts of bronchiectasis patients enrolled in other European and non-European countries to increase their generalizability and understand if aNE could be implemented as biomarker in future randomized 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.

Acknowledgements: none

Support statement: no finacial support for this study

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

Variables		Sputum neutrophil elastase level			p-value
Demographics		**Low (0-6 ug/ml) (n= 91)**	**Medium (7-20 ug/ml) (n= 77)**	**High (>20 ug/ml) (n= 98)**	
Male, n (%)		19 (27.9)	19 (25.0)	21 (25.3)	0.91
Median (IQR) age, years		65 (51-75)	63 (53-73)	64 (54-74)	0.83
Current/former smoker, n (%)		32 (47.1)	36 (47.4)	32 (38.6)	0.45
Median (IQR) BMI, kg/m^2		23.2 (20-26)	22 (18.9-25.0)	21 (19.4-24.5)	0.15
Disease severity					
Median (IQR) BSI		6 (4-9)	7 (4-10)	8 (5-12)	0.009(1)
BSI risk class, n (%)	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(2)
Median (IQR) E-FACED		2 (1-3)	2 (1-3)	3 (1-4)	0.0006(3)
E-FACED risk class, n (%)	Mild	73 (84.9)	55 (76.4)	59 (64.8)	0.008(4)
	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, n (%)		23 (33.8)	20 (26.3)	27 (32.5)	0.57
Chronic renal failure, n (%)		2 (2.9)	3 (4.0)	2 (2.4)	0.89
Diabetes, n (%)		4 (5.9)	8 (10.5)	2 (2.4)	0.11

History of Pneumonia, n (%)		41 (60.3)	48 (63.2)	48 (57.8)	0.79
History of Tuberculosis infection, n (%)		5 (7.4)	8 (10.5)	7 (8.4)	0.79
Rheumatoid arthritis, n (%)		1 (1.7)	1 (1.5)	1 (1.3)	1.0
History of other CTD, n (%)		3 (5.2)	0 (0.0)	1 (1.3)	0.11
History of IBD, n (%)		2 (2.9)	2 (2.6)	0 (0.0)	0.33
History of autoimmune disease, n (%)		0 (0.0)	2 (3.1)	0 (0.0)	0.19
Primary ciliary dyskinesia, n (%)		5 (8.6)	6 (9.2)	10 (13.0)	0.66
Asthma, n (%)		10 (14.7)	12 (15.8)	10 (12.1)	0.79
COPD, n (%)*		6 (8.8)	7 (9.2)	7 (8.4)	0.99
Rhinosinusitis, n (%)		24 (35.3)	25 (32.9)	29 (34.9)	0.95
Gastro-oesophageal reflux disease, n (%)		26 (44.8)	26 (40.0)	35 (45.5)	0.79
Primary immunodeficiency, n (%)		7 (12.1)	10 (15.4)	13 (16.9)	0.74
Secondary immunodeficiency, n (%)		2 (3.5)	4 (6.2)	1 (1.3)	0.34
Clinical status					
Sputum colour, n (%)	Mucoid	12 (27.3)	9 (16.7)	6 (9.5)	0.16
	Mucupurulent	16 (36.4)	25 (46.3)	27 (42.9)	
	Purulent/Severe purulent	16 (36.4)	20 (37.0)	30 (47.6)	
Median (IQR) sputum volume		6 (5-20)	15 (5-50)	25 (7-75)	<0.0001 (5)
Median (IQR) exacerbation previous year		2 (1-3)	2 (1-3)	2 (1-3)	0.77
Patients with 2+ exacerbations/previous year		49 (54.4)	47 (60.3)	58 (60.4)	0.65
>1 hospitalization previous year		15 (16.7)	12 (15.2)	23 (24.0)	0.27
Quality of Life					
Mean (SD) QoLB questionnaire-Physical		59.8 (25.1)	61.1 (25.3)	52.8 (27.1)	0.31
Median (IQR) QoLB questionnaire-Role		66.7 (50-80)	73.3 (46.7-86.7)	66.7(40-80)	0.43
Mean (SD) QoLB questionnaire-Vitality		53.1 (19.9)	51.2 (23.5)	49.8 (19.7)	0.79
Median (IQR) QoLB questionnaire-Emotion		70.9 (58.3-87.5)	75 (66.7-91.7)	75 (50.0-91.7)	0.83
Mean (SD) QoLB questionnaire-Social		68.9 (23.0)	59.7 (26.6)	54.7 (26.6)	0.05

Median (IQR) QoLB questionnaire-Treatment burden	66.7 (55.6-77.8)	66.7 (55.6-77.8)	66.7 (44.4-77.8)	0.86
Median (IQR) QoLB questionnaire-Health	41.7 (20.9-58.3)	41.7 (25-50)	33.3 (16.7-50.0)	0.33
Median (IQR) QoLB questionnaire-Respiration	74.1 (66.7-81.5)	70.4 (59.3-77.8)	66.7 (51.9-74.1)	0.04[6]
Standard Microbiology				
Chronic infection, n (%)	26 (30.2)	34 (44.7)	66 (71.0)	<0.0001[7]
Chronic infection *P. aeruginosa*, n (%)	12 (14.0)	21 (27.6)	44 (47.3)	<0.0001[8]
Chronic infection other bacteria, n (%)	16 (18.6)	21 (27.6)	25 (26.9)	0.31
Median (IQR) FEV1, L	1.9 (1.5-2.5)	1.9 (1.4-2.4)	1.7 (1.2-2.1)	0.02[9]
Mean (SD) FEV1, %	80.2 (24.4)	74.0 (20.0)	73.0 (27.1)	0.10
FEV1 <50%predict., n (%)	8 (8.9)	9 (12.0)	22 (23.2)	0.02[10]
FEV1 <35%predict., n (%)	2 (2.2)	3 (4.0)	9 (9.5)	0.09

*COPD defined as fixed ratio of post-bronchodilator FEV1/FVC less than 0.7 in addition to FEV1 <80% predicted in a patient with a smoking history of more than 10 pack-years.

1. Low VS. High p-value= 0.004
2. Low VS. High p-value= 0.005
3. Low VS. High p–value= 0.0003; Medium VS. High p-value= 0.01
4. Low VS. High p-value= 0.002
5. Low VS. Medium p-value= 0.03; Low VS. High p–value <0.0001; Medium VS. High p-value= 0.04
6. Low VS. High p–value= 0.02
7. Low VS. High p-value <0.0001; Medium VS. High p-value= 0.0005
8. Low VS. Medium p–value= 0.03; Low VS. High p-value <0.0001; Medium VS. High p-value= 0.009
9. Low VS. High p–value= 0.01
10. Low VS. High p–value= 0.008