Original Article

trd Tuberculosis & Respiratory Diseases

Increased Neutrophil Elastase in Affected Lobes of Bronchiectasis and Correlation of Its Levels between Sputum and Bronchial Lavage Fluid

https://doi.org/10.4046/trd.2024.0078
ISSN: 1738-3536(Print)/ 2005-6184(Online)
Tuberc Respir Dis, Published online Jan. 14, 2025

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Received May. 14, 2024
Revised Oct. 8, 2024
Accepted Jan. 13, 2025
Published online Jan. 14, 2025

Abstract

Background: Neutrophil elastase (NE) has been proposed as a potential biomarker for evaluating the severity and prognosis of bronchiectasis. This study aimed to compare bronchial lavage quantification of NE levels and activities with those of sputum.
Methods: A cross-sectional study was conducted in which 24 Vietnamese adults with bronchiectasis were enrolled from June 2023 to August 2023. All participants underwent bronchoscopy to collect bronchial lavage fluid (BLF) from two bronchial locations: one in the region with the greatest bronchial dilatation and one in the normal bronchi or in patients with all lobes affected, the least abnormal lobe (abnormal BLF [ABLF] and normal BLF [NBLF], respectively). Spontaneously expectorated sputum was also collected.
Results: Out of 24 cases, the prevalence of mild, moderate and severe bronchiectasis was 14/24 (58.4%), 5/24 (20.8%), and 5/24 (20.8%), respectively. NE concentration and activity were significantly higher in sputum and ABLF than in NBLF ($p<0.001$). Sputum and ABLF were highly correlated ($r=0.841$, $p<0.001$) with no significant difference in NE activity between sputum and ABLF. Higher levels of NE activity were seen in more severe bronchiectasis than in mild bronchiectasis in all samples but were only statistically significant for NE activity in sputum ($r=0.418$, $p=0.042$).
Conclusion: NE activity and concentration are elevated in areas of the lung most affected by bronchiectasis. Sputum is a valid surrogate of pulmonary NE levels, as they correlate strongly with ABLF and confirm in a Vietnamese population the relationship between NE activity and disease severity.

Keywords: Bronchial Lavage Fluid; Bronchiectasis; Bronchoscopy; Neutrophil Elastase Activity; Neutrophil Elastase Concentration; Sputum

Introduction

Bronchiectasis is an abnormal and usually permanent dilation of the bronchi associated with varying degrees of airflow obstruction and impaired clearances of mucus and bacteria from the airway[1]. Bronchiectasis

subsequently leads to the recurrence and persistence of respiratory infection, inflammation, and other airway damage[2]. Progressive damages can result in lung dysfunction, respiratory failure, and even death. The pathophysiology of bronchiectasis is poorly understood because of the complexity of underlying etiologies, influential factors, and the lack of experimental models[3]. In particular, bronchiectasis may occur as a consequence of severe infection, immune dysregulation, and genetic disorders[4].

Several studies have reported an increased prevalence of bronchiectasis, foreseeing a significant burden on the healthcare system for managing affected cases[5,6]. Several promising treatments for bronchiectasis have been reported to improve airway clearance, which is a crucial approach in reducing mucus obstruction and exacerbation, and to prevent respiratory infections[7]. It has been suggested that personalized medicine based on the endotype and phenotype of a patient is an effective strategy for treatment advances[8]. Therefore, the identification of treatable traits and novel biomarkers of bronchiectasis is urgently needed to better classify high-risk individuals and enhance patient care.

Airway neutrophilic inflammation is an important pathophysiological hallmark of bronchiectasis. In normal conditions, neutrophils play a major role in the immune response to inflammation triggered by different agents. However, neutrophil hyperactivation can lead to the disruption of host cells and tissues due to exceeded secretions of cytokines, chemokines and proteases, such as neutrophil elastase (NE), cathepsin G, and proteinase 3[3]. Among these mediators, NE is a 218-amino acid serine protease mainly found in the granules of neutrophils. In bronchiectasis patients, an excess of NE driven by the extensive recruitment of neutrophils damages the airway barrier, provokes mucus production, slows down the ciliary beating, and subsequently worsens disease conditions[2].

Previous research has indicated that NE is an important indicator of disease progression and severity[9,10]. In particular, a significant increase in NE activity was observed in the sputum of bronchiectasis patients with more severe symptoms, higher frequency of exacerbation, and declining lung function[11]. NE has also been proposed to be a potential diagnosis and prognosis biomarker, as well as a treatment target for bronchiectasis[12,13]. A new class of drugs, dipeptidyl peptidase-1/cathepsin C inhibitors, that prevent the activation of neutrophil serine proteases, including NE, is in development for bronchiectasis. Given the prominent roles of NE and the lack of a gold standard for measuring inflammation in bronchiectasis, further studies are required in different cohorts and sample types to support the clinical utility of NE. To date, the vast majority of studies investigating NE have used sputum. The validity of sputum is sometimes questioned because it may be a mix of secretions from different pulmonary areas and is at risk of upper airway contamination. Whether sputum accurately reflects the NE levels obtained using the gold standard of bronchoscopic sampling is unknown. Currently, nearly all studies on the levels and prognostic significance of NE have come from European countries. Asian patients with bronchiectasis show distinct clinical characteristics[14] and outcomes[15]. Therefore, we investigated the features of NE in Vietnamese patients with bronchiectasis and assessed whether NE could reflect the severity of bronchiectasis in our cohort.

Materials and Methods

We conducted a cross-sectional study, in which we enrolled 24 bronchiectasis patients consecutively from June 2023 to August 2023.

1. Patient recruitment

All patients (age ≥18 years) were screened at two endoscopy units of University Medical Center Ho Chi Minh City and Cho Ray's Hospital. Inclusion criteria were the following: (1) the patient was diagnosed with bronchiectasis based on the criteria of British Thoracic Society guidelines 2019[16]; (2) radiological features of bronchiectasis on chest computed tomography (CT) were confirmed by a radiologist and a pulmonologist (both with more than 5 years of experience calculated the modified Reiff score for each patient); (3) the patient's disease was stable at the time of sampling as indicated by the absence of symptoms of an exacerbation; and (4) the patient was given bronchoscopy for diagnosis and collecting bronchial lavage fluid (BLF). Exclusion criteria included traction bronchiectasis, active pulmonary tuberculosis, or failure to undertake bronchoscopy.

Data related to age, gender, comorbidities, clinical symptoms, past history of bronchiectasis exacerbation, and lung function test (forced expiratory volume in 1 second and forced vital capacity) were collected. Bronchiectasis severity index (BSI) was evaluated independently by two authors (Nguyen-Ho L and Le-Thuong V). Mild bronchiectasis was defined as a BSI score of 0–4, moderate bronchiectasis with a BSI score of 5–8, and severe bronchiectasis with a BSI score ≥9.

This study was approved by the ethics committee

of the University of Medicine and Pharmacy at Ho Chi Minh City (528/HDDD-DHYD). All participants provided written informed consent to participate in this study.

2. Flexible bronchoscopy, collection of sputum, and bronchial lavage fluid

Flexible bronchoscopy was undertaken according to protocols of University Medical Center Ho Chi Minh City and Cho Ray's Hospital. Briefly, patients were prepared and administered pre-medication following hospitals' protocols. A bronchoscope (Olympus Evis Exera III CLV-190, Olympus Medical, Center Valley, PA, USA) was put into tracheobronchial tree via nasal or oral routine under local anesthesia with lidocaine 2%. BLF from two bronchial locations, one in the region with the greatest bronchial dilatation (defined as abnormal BLF [ABLF]) and one at normal bronchi or in patients with all lobes affected, the least abnormal lobe (defined as normal BLF [NBLF]), were collected from all patients. The lobes to be targeted were based on a pre-procedure assessment of the chest CT. After washing the working channel with 5 to 10 mL NaCl 0.9%, the NBLF was collected, followed by the ABLF. The ABLF was sent to the laboratory to evaluate cellular differentiation, bacterial and fungal culture, and detection for mycobacterium tuberculosis (MTB) and nontuberculous mycobacteria (NTM). The multiplex real-time polymerase chain reaction was applied to directly detect MTB and NTM from specimens. An aliquot of 3 to 5 mL of ABLF was kept for NE measurement.

Sputum could be collected via spontaneous expectoration before or after bronchoscopy. All patients were monitored at the endoscopy unit post-procedure for 30 minutes and kept fasting for at least 2 hours post-procedure.

All specimens (NBLF, remaining ABLF, and sputum) were kept at 2°C to 8°C and delivered to the Center for Molecular Biomedicine for further experiments.

3. Sputum and bronchial lavage fluid processing

Sputum samples (selecting mucus plug and removing saliva) were divided into two parts and processed separately. A portion was spread onto a slide to fix and stain with hematoxylin and eosin to assess sputum quality (≤10 squamous epithelium and >25 leukocytes). The remaining part was treated with dithiothreitol (DTT) to lyse sputum and measure NE concentration and activity. Briefly, DTT (Thermo Fisher Scientific, Waltham, MA, USA) was suspended in 100 μL of nuclease-free water and gently mixed to completely dissolve (final concentration of 500 mM). Next, the entire 100 μL of freshly prepared DTT was added to 5 mL of cold sterile 0.01 M phosphate-buffered saline (pH 7.2) and mixed. The diluted DTT was mixed with an equal volume of sputum. The sample was incubated at 4°C with intermittent mixing until the sample was dissolved. Subsequently, the samples were centrifuged at 3,000 rpm for 10 minutes, and the supernatant was collected and stored at –80°C.

Table 1. Characteristics of 24 bronchiectasis patients

Characteristic	Total (n=24)
Age, yr	63 (51–75)
Female sex	18 (75.0)
BMI, kg/m^2	21.8 (19.6–23.6)
Symptoms	
Productive cough	16 (66.7)
Sputum volume, mL	8.0 (5.0–10.0)
Dry cough	8 (33.3)
Dyspnea	11 (45.8)
History of hemoptysis	8 (33.3)
History of exacerbation	6 (25.0)
Comorbidities	
Hypertension	8 (33.3)
Diabetes mellitus	5 (17.9)
COPD	2 (8.3)
Asthma	1 (4.2)
Complete blood count	
White blood cells, K/mm^3	6.9 (5.5–8.1)
Eosinophil, K/mm^3	0.2 (0.1–0.4)
Neutrophil-to-lymphocyte ratio	2.6 (1.4–4.0)
FEV1, %	72.0 (66.8–81.3)
FVC, %	75.5 (69.0–80.5)
Chest computed tomography features of bronchiectasis	
Cylindrical pattern	11 (45.8)
Cystic pattern	6 (25.0)
Varicose pattern	3 (12.5)
Combined pattern	4 (16.7)
Upper lobe	15 (62.5)
Middle lobe/lingula	15 (62.5)
Lower lobe	16 (66.7)
Bilateral abnormality	13 (54.2)
Modified Reiff score	3 (1–6)

Values are presented as median (interquartile range) or number (%).
BMI: body mass index; COPD: chronic obstructive pulmonary disease; FEV1: forced expiratory volume in 1 second; FVC: forced vital capacity.

In terms of BLF, about 2 to 5 mL for each NBLF and ABLF were centrifuged at 6,000 ×g for 5 minutes at 4°C to remove the cellular fraction. The cell-free supernatant was immediately aliquoted into 1.5 mL Eppendorf tubes and stored at −80°C until further analysis.

4. Measurement of neutrophil elastase concentration and activity

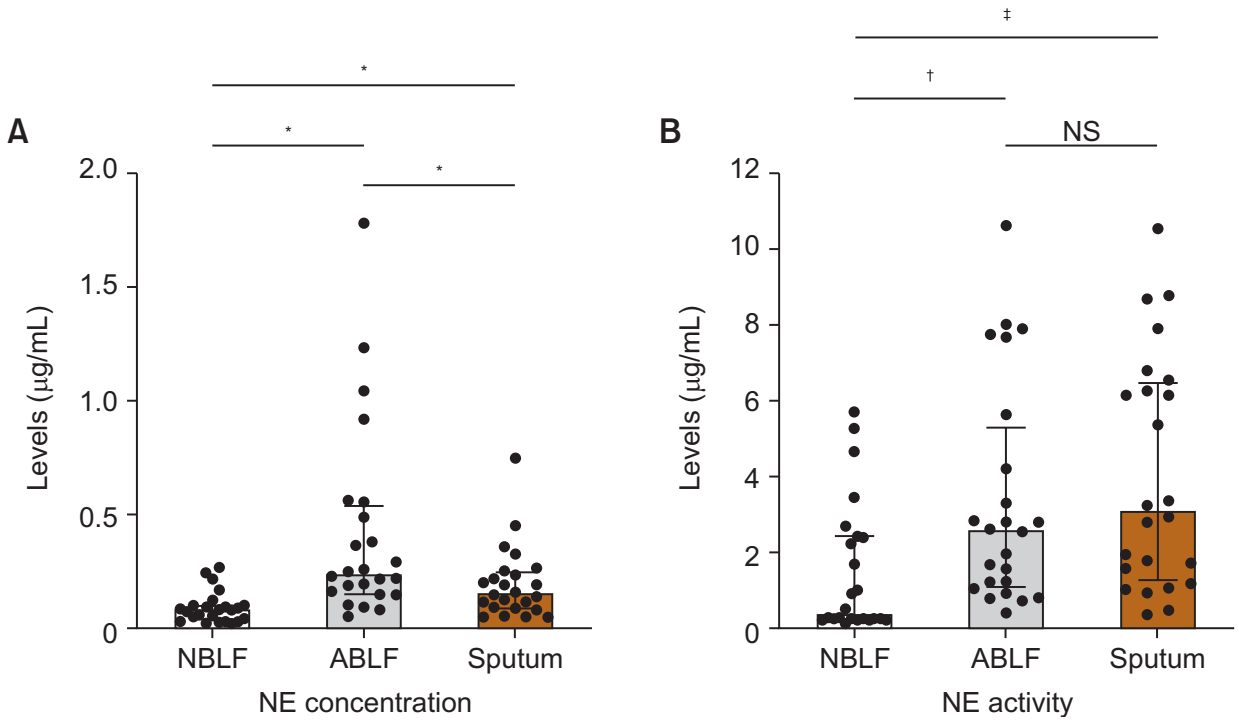
The NE concentration was examined using the Human PMN-Elastase enzyme-linked immunosorbent assay (ELISA) Kit (BMS269, Thermo Fisher Scientific) in accordance with the manufacturer's instructions. Briefly, anti-Human PMN-Elastase coating antibody was pre-coated in the wells of microplate. The samples, standards, or controls were then added into these wells, followed by adding horseradish peroxidase-conjugated anti-α 1-PI antibody. Subsequently, the substrate solution was added to the wells to produce color reaction, which was terminated by the addition of stop solution. The absorbance was measured at 450 nm.

The NE activity was examined using the Neutrophil Elastase Activity Assay Kit (Fluorometric, MAK246, Abcam, Cambridge, UK) in accordance with the manufacturer's instructions. The Neutrophil Elastase Activity Assay Kit utilized the ability of NE in samples to proteolytically cleave a synthetic substrate and release a fluorophore AFC (7-amino-4-trifluoromethylcoumarin). NE enzyme standard was prepared by reconstituting with 10 μL of NE dilution buffer to prepare 100 ng/L of stock solution. NE enzyme working solution (5 ng/L) was transferred into a series of wells in a 96-well plate to prepare 0, 5, 10, 15, 20, and 25 ng/well of NE standard. About 50 μL of patient BLF and sputum samples were added per well of the 96-well plate and adjusted the volume to 50 μL/well with NE assay buffer. A 50 μL of NE substrate mix was added into each well and mixed. The fluorescence results were measured in kinetic mode for 10 to 20 minutes at 37°C (λ_{ex}=380 nm/λ_{em}=500 nm). Delta relative fluorescence unit (ΔRFU) of the samples was compared to the NE standard curve to obtain corresponding NE amount. The activity of NE in the sample was calculated by dividing the NE amount to the sample volume.

5. Statistical analysis

We hypothesized that NE could be different between activity versus concentration, normal bronchi versus dilated bronchi, the severity of bronchiectasis (mild, moderate, and severe), and based on the type of specimens (sputum vs. BLF). The Shapiro-Wilk test was used to determine the normal distribution of continuous variables. The Wilcoxon signed-rank test for paired sam-

Figure 1. Comparison of neutrophil elastase in sputum and bronchial lavage fluid. (A) Neutrophil elastase concentration. (B) Neutrophil elastase activity. *p<0.01; †p<0.001; ‡p<0.0001. NBLF: normal bronchial lavage fluid; ABLF: abnormal bronchial lavage fluid; NE: neutrophil elastase; NS: non-significant.



ples was used to compare NE in NBLF and NE in ABLF and sputum. Spearman's correlation coefficient was used to test the association of NE activity in sputum and BLF, likewise to NE concentration, with the severity of bronchiectasis. Differences between groups were measured by the Mann-Whitney test. A two-sided p-value <0.05 was considered as statistical significance. Our study analyzed data by IBM SPSS version 22.0 (IBM Co., Armonk, NY, USA) which created the figure with the support of the PhotoScape software version 3.7 (www.photoscape.org) and the JASP software version 0.18.3.0 (www.jasp-stats.org).

Results

1. Characteristics of bronchiectasis patients

We evaluated NE (concentration and activity) in sputum and BLF from 24 bronchiectasis patients. The majority of cases (22/24, 91.67%) had after-bronchoscopy sputum because the patients in our study had dry

Figure 2. The correlation between neutrophil elastase (NE) levels in sputum and bronchial lavage fluid (BAL) was demonstrated as follows: the correlation between NE concentration in sputum and that in either (A) normal BAL or (B) abnormal BAL; the correlation between NE activity in sputum and that in either (C) normal BAL or (D) abnormal BAL. Abnormal bronchial lavage fluid was collected from the region with the greatest bronchial dilatation and normal bronchial lavage fluid was collected from the region with the normal bronchi or in patients with all affected lobes of bronchiectasis, the least abnormal lobe was selected.

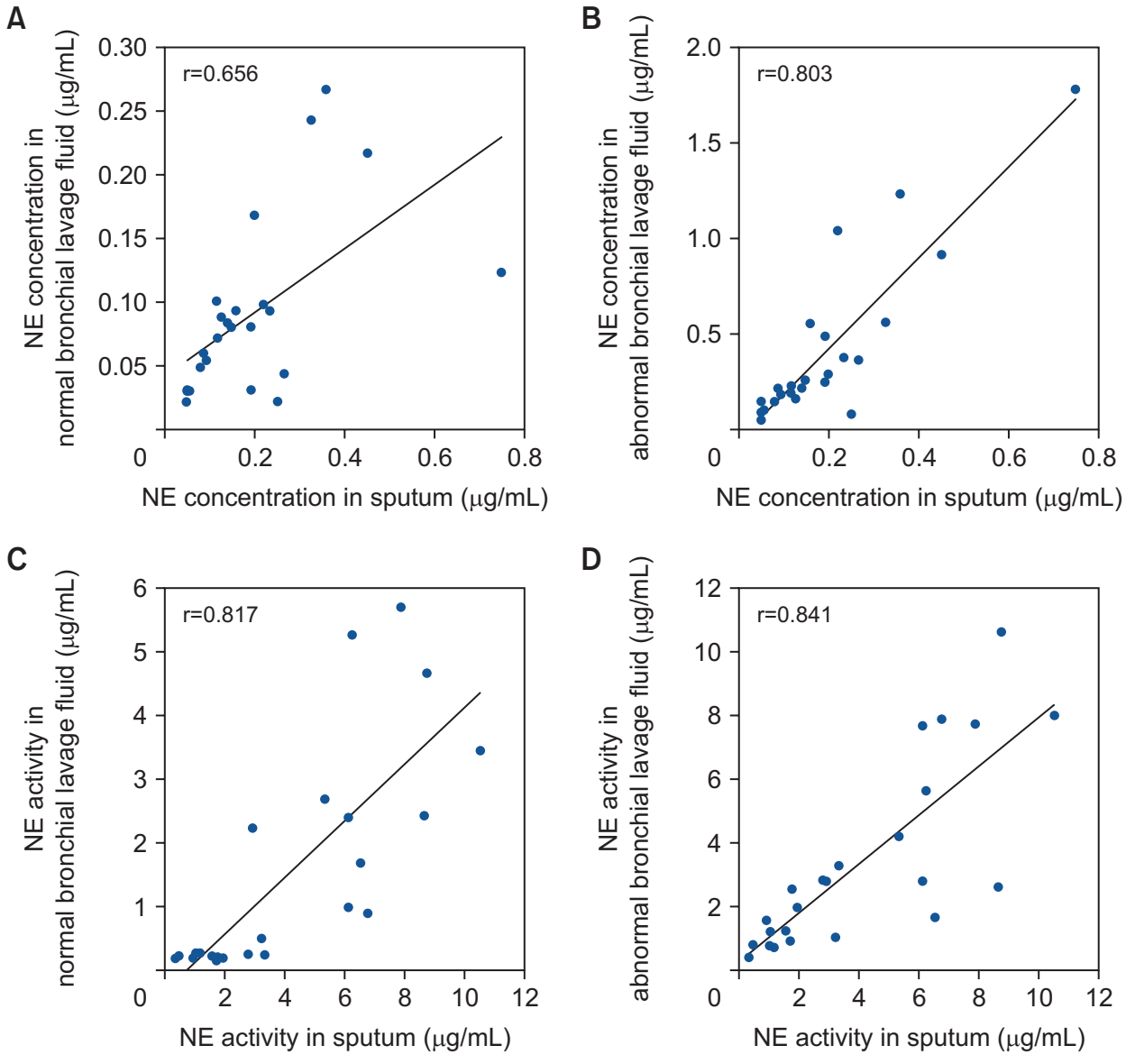


Table 2. Neutrophil elastase and leukocyte differentiation in bronchial lavage fluid

	Neutrophil in ABLF <50% (n=7)	Neutrophil in ABLF ≥50% (n=12)	p-value
NE concentration in ABLF, μg/mL	0.19 (0.15–0.25)	0.29 (0.11–0.54)	0.773*
NE activity in ABLF, μg/mL	1.96 (0.80–2.79)	3.06 (1.32–7.17)	0.227*

Values are presented as median (interquartile range).
*Mann-Whitney U test.
ABLF: abnormal bronchial lavage fluid; NE: neutrophil elastase.

cough during the screening time. Their demographic and clinical characteristics are presented in Table 1. Three-quarters of the population were female, with a median age of 63 years. The patients manifested productive cough (66.7%) with a medium sputum volume (8ml), dyspnea (45.8%), and dry cough (33.3%). The etiologies of bronchiectasis included 10 cases related to NTM infection, six cases with post-tuberculosis, one case with bronchial foreign body, one case with second immunodeficiency (Kahler's disease), and six idiopathic cases. The neutrophil-to-lymphocyte ratio (NLR) was 2.6 (interquartile range [IQR], 1.4 to 4.0) and showed a significant association with the severity of bronchiectasis (r=0.658, p=0.001).

2. Neutrophil elastase in sputum and bronchial lavage fluid

The NE concentration and activity were significantly higher in sputum and ABLF than in NBLF (Figure 1), but no significant difference was observed between NE activity in sputum and ABLF (p=0.18). Significant correlations of either NE concentration or NE activity were found in sputum and ABLF, and even the NE concentration/activity significant in sputum was correlated with that in NBLF (Figure 2) (p<0.05 for all).

Leukocyte differentiation in ABLF was conducted in 19 cases. Although there was an increasing trend of NE levels in ABLF for patients with a prominent neutrophil component (%neutrophil ≥50%), no statistically significant differences were observed (Table 2).

Table 3. Results of abnormal bronchial lavage fluid

Features	No. of cases (total=24)
Cellular analysis	
Neutrophil (≥50%)	13
Eosinophil (≥3%)	2
Bacterial culture	
Pseudomonas aeruginosa	2
Pseudomonas peptida	1
Staphylococcus aureus	2
Klebsiella pneumoniae	2
Haemophilus influenzae	1
Escherichia coli	1
Acinetobacter baumannii	2
Fungal culture	
Candida spp.	2
Aspergillus niger	1
Multiplex real-time polymerase chain reaction assay for nontuberculous mycobacteria	
Mycobacterium avium complex	2
Mycobacterium abcessus complex	2
Mycobacterium chelonae	2
Mycobacterium szulgai	2
Mycobacterium tilburgii	2
Mycobacterium xenopi	1

3. Neutrophil elastase with the microbiological profile of bronchial lavage fluid

The results of the BLF analysis are presented in Table 3. All cases had bacterial cultures for ABLF, 22 cases were evaluated for NTM infection, and 17 cases received fungal cultures of BLF. There was no correlation between the positive bacterial culture or the positive NTM detection and the increased NE activity or NE concentration in both sputum and BLF, except for a higher NE concentration in sputum of NTM-positive patients (0.24 [IQR, 0.14 to 0.36]) than that of NTM-negative patients (0.10 [IQR, 0.05 to 0.19]; p=0.009).

4. Neutrophil elastase and the radiological extent and severity of bronchiectasis

We documented 14 cases (58.4%) with mild bronchiectasis, five cases (20.8%) with moderate bronchiectasis, and five cases (20.8%) with severe bronchiectasis. Table 4 presents the NE concentration and activity in sputum and BLF according to the severity of bronchi-

Table 4. Neutrophil elastase and severity of bronchiectasis according to BSI

Variable	Total (n=24)	Mild bronchiectasis (n=14)	Moderate-severe bronchiectasis (n=10)	r-value*
BSI	4.0 (3.0–8.0)	≤4	≥5	NA
NE concentration in NBLF, μg/mL	0.08 (0.03–0.10)	0.09 (0.03–0.13)	0.07 (0.04–0.09)	–0.233
NE concentration in ABLF, μg/mL	0.24 (0.15– 0.54)	0.23 (0.15–0.65)	0.30 (0.15–0.51)	–0.176
NE concentration in sputum, μg/mL	0.15 (0.09–0.25)	0.17 (0.11 - 0.27)	0.14 (0.07–0.24)	–0.389
NE activity in NBLF, μg/mL	0.38 (0.22–2.42)	0.25 (0.19 -2.34)	1.09 (0.24–2.68)	0.340
NE activity in ABLF, μg/mL	2.58 (1.08–5.28)	1.76 (0.89–4.56)	2.72 (1.50–7.77)	0.264
NE activity in sputum, μg/mL	3.09 (1.27–6.47)	1.83 (0.99–6.17)	4.74 (2.53–8.70)	0.418†

Values are presented as median (interquartile range).
*r: coefficient of Spearman's correlation between neutrophil elastase and BSI. †Statistical significance with p-value <0.05.
BSI: bronchiectasis severity index; NA: not available; NE: neutrophil elastase; NBLF: normal bronchial lavage fluid; ABLF: abnormal bronchial lavage fluid.

ectasis. Although higher levels of NE activity were seen in more severe bronchiectasis than in mild bronchiectasis in all samples, only NE activity in sputum showed a significant correlation (r=0.418, p=0.042).

Regarding the radiological extent of bronchiectasis, 21 cases with mild bronchiectasis (Reiff score ≤6) and three cases with moderate bronchiectasis (7≤ Reiff score ≤12) were found. There was no correlation between NE levels (concentration and activity in sputum and BLF) and the radiological extent of bronchiectasis. Moreover, no correlation was observed between NLR and NE levels in sputum and BLF.

Discussion

Many studies on NE in respiratory specimens of European bronchiectasis patients (adult and children) have shown its association with the severity of bronchiectasis, decline in lung function, the risk of bronchiectasis exacerbation and chronic bacterial infection in the airways[9-11,17]. Data on Asian bronchiectasis patients remain scarce, with the exception of a study on NE in soluble sputum from 10 Chinese bronchiectasis patients[18] and a recent study on NE in sputum from 46 Turkish bronchiectasis patients[13]. To the best of our knowledge, this study is the first to evaluate NE in both sputum and BLF in Vietnamese bronchiectasis patients. Our study also showed a significant association of NE activity in sputum with the severity of bronchiectasis. This is an important contribution to generalize NE as a biomarker for the global bronchiectasis population. High NE is associated with airway mucus hypersecretion and airway hyperinflammation, one of the essential features of the pathophysiological cycle of bronchiectasis.

NE is particularly important as a marker in bronchiectasis, as a novel therapeutic approach targeting NE and other serine proteases is in clinical development. Dipeptidyl peptidase-1 inhibitors block the activation of NE in the bone marrow, resulting in reduced sputum levels of NE and prolongation of the time to first exacerbation[17]. Studies have suggested that the etiology, clinical characteristics and outcomes of patients with bronchiectasis are different in Asia compared with those in Europe or North America[14,15,19]. Thus, our results provide confidence that NE is a potential treatable trait, specifically in Vietnamese bronchiectasis patients.

Our study showed that NE (concentration and activity) in sputum and ABLF was higher than that in NBLF. This confirmed the abnormal increase in NE in Vietnamese bronchiectasis patients and showed that the levels of NE could be different between dissimilar bronchial locations. Angrill et al.[20] described the hyperinflammatory status in the bronchioalveolar lavage fluid of bronchiectasis patients which was higher than the systemic inflammatory response and the poor correlation between the level of cytokines such as interleukin 1β (IL-1β), IL-6, IL-10, and tumor necrosis factor-α, in serum and the bronchioalveolar lavage fluid. Although the severity of bronchiectasis was not evaluated, this study implied that local inflammation could exist in bronchiectasis patients, and it was re-authenticated through our measurement of NE in BLF. Excluding the mild bronchiectasis cases, our study still showed a higher NE in ABLF than in NBLF.

The characteristics of bronchiectasis patients in our study were similar to those of previous research, ex-

cept for the high rate of NTM-induced bronchiectasis and post-tuberculosis bronchiectasis. It is noteworthy that Vietnam is an endemic country of tuberculosis. NTM infection in bronchiectasis patients should be considered in similar areas. All 24 bronchiectasis patients conducted bronchoscopy to collect BLF and exclude the causes of local bronchial obstruction (tumor or foreign body) with no documented post-bronchoscopy complications. There are only a few bronchoscopy studies on adult bronchiectasis patients, such as Park et al.'s[21] study evaluating bronchoscopy in bronchiectasis patients with hemoptysis or McGarvey et al.'s[22] study on bronchoalveolar lavage fluid in cystic fibrosis patients, which found that bronchoscopy was not safe. Conversely, bronchoscopy has been examined frequently in bronchiectasis children, particularly in the age range of 6 to 17[23].

Thus far, NE in BLF has rarely been evaluated in bronchiectasis patients, with the exception of Angrill et al.'s study[20]. Our study found a significant difference between NE concentration in sputum and ABLF but not for NE activity. McGarvey et al.[22] mentioned that the dilution of NaCl 0.9% when collecting BLF and sputum processing could contribute to this difference. Moreover, our study showed a strong correlation between NE (concentration and activity) in sputum and ABLF. Despite the limitations of its invasive nature, the evaluation of NE in BLF in bronchiectasis patients who have dry cough (another option using induced sputum) provides more information related to the inflammatory status of mild bronchiectasis. This is also important information for future studies on NE in bronchiectasis patients because it suggests that sputum, despite its potential limitations, provides a valid and representative estimation of NE levels in areas of the lung affected by bronchiectasis, thus supporting the ongoing use of sputum in clinical trials and prognostic studies.

NE concentration is often associated with extracellular free NE or the antiprotease-NE complex[24], while NE activity depends not only on free NE but also on other factors such as NE on the surface of neutrophils, polyanions, and the interaction with endogenous protease inhibitors[18]. Therefore, NE activity can more precisely describe the hyperinflammatory status of airways, consequently being better in correlating with the pathogenic abnormality in bronchiectasis patients. Our study also showed a significant correlation between NE activity in sputum and the severity of bronchiectasis but not for NE concentration. Although higher levels of NE activity were seen in the ABLFs of patients with more severe bronchiectasis, no statistically significant correlation was found.

This study has several limitations. The sample size was small due to the feasibility of enrolling patients in bronchoscopy, and this study was conducted at two centers in Vietnam. These impaired the generalization of our results. The effect of lidocaine on the result of NE measurement was not evaluated. Moreover, the use of BLF instead of bronchoalveolar lavage cannot avoid potential contamination from secretions from other bronchial trees and lobes. Previous research has shown that NE activity is associated with long-term outcomes in bronchiectasis patients, but this was not observed in our study. Thus, further follow-up studies with larger sample sizes are necessary.

In conclusion, this study is the first to evaluate NE in specimens of Vietnamese bronchiectasis patients. NE activity and concentration are elevated in areas of the lung most affected by bronchiectasis. We show that sputum is a valid substitute for pulmonary NE levels because they are strongly correlated with ABLF and confirm the relationship between NE activity and disease severity in a Vietnamese population.

Authors' Contributions

Conceptualization: Nguyen-Ho L, Trinh HKT, Le-Thuong V, Vu DM, Tran-Van N, Chalmers JD. Methodology: Nguyen-Ho L, Trinh HKT, Vu DM, Chalmers JD. Formal analysis: Nguyen-Ho L, Le KM, Vo VTN. Data curation: Nguyen-Ho L, Le KM, Vo VTN. Investigation: Nguyen-Ho L, Le-Thuong V, Le KM, Vo VTN, Tran-Van N. Writing - original draft preparation: all authors. Writing - review and editing: all authors. Approval of final manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Funding

This research was funded by the University of Medicine and Pharmacy at Ho Chi Minh City under contract number 112/2021/HĐ-ĐHYD, dated 30/09/2021.

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