

Sputum neutrophil elastase and its relation to pediatric bronchiectasis severity: A cross-sectional study

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Abstract

Background and Aims: Sputum neutrophil elastase (NE) is a marker of neutrophilic airway inflammation in bronchiectasis. Yet, not much is known about its role in pediatric bronchiectasis severity. This study aimed to assess the sputum NE value as a biomarker of clinical and radiological severity in pediatric bronchiectasis.

Methods: This was a cross-sectional study assessing sputum NE in a total of 50 bronchiectasis patients under the age of 18 years—30 patients with cystic fibrosis (CF) and 20 patients with non-CF bronchiectasis were included. Bronchiectasis severity was assessed using Shwachman–Kulczycki (SK) score, CF-ABLE score, and CF risk of disease progression score, among CF patients, and bronchiectasis severity index (BSI) and FACED criteria among non-CF bronchiectasis patients, associations between sputum NE and bronchiectasis severity were assessed in both patient groups.

Results: Sputum NE was directly correlated with C-reactive protein ($r = 0.914$, $p < 0.001$), ($r = 0.786$, $p < 0.001$), frequency of exacerbations ($r = 0.852$, $p < 0.001$) ($r = 0.858$, $p < 0.001$), exacerbations severity ($r = 0.735$, $p = 0.002$), ($r = 0.907$, $p < 0.001$), and the number of hospital admissions ($r = 0.813$, $p < 0.001$), ($r = 0.612$, $p = 0.004$) in the last year among CF, and non-CF bronchiectasis patients, respectively. Additional linear correlations were found between sputum NE, CF risk of disease progression score ($p < 0.001$), CF-ABLE score ($p < 0.001$), and lower forced expiratory volume 1% of predicted ($p = 0.017$; $\rho = -0.8$) among CF patients. Moreover, sputum NE was positively correlated with the neutrophil count ($p = 0.018$), and BSI severity score ($p = 0.039$; $\rho = 0.465$) among non-CF bronchiectasis patients.

Conclusions: Sputum NE may be considered a good biomarker of bronchiectasis severity in both CF and non-CF bronchiectasis patients, as confirmed by the exacerbations rate, CF risk of disease progression, and BSI scores.

KEYWORDS

colonization, exacerbations, cystic fibrosis, non-CF bronchiectasis, neutrophil elastase, severity scores

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1 | INTRODUCTION

Bronchiectasis is defined as an abnormal and permanent dilation of the bronchi associated with daily cough, sputum production, and recurrent respiratory infections.¹ Bronchiectasis is classified into bronchiectasis secondary to cystic fibrosis (CF) and bronchiectasis not related to CF, which is called non-CF bronchiectasis.²

Regarding the pathophysiology of bronchiectasis, a fundamental role is played by both impaired mucus clearance and chronic bacterial infections leading to a serious neutrophilic activation by the release of chemotactic mediators.³ In this context, neutrophil elastase (NE) may have a determinant role in tissue damage and could be a predictor of long-term clinical outcomes, and a treatment target.⁴

Human NE is a proteolytic enzyme belonging to the chymotrypsin-like family of the serine-proteinases. NE is a 218 amino acid long protein stored in cytoplasmatic azurophilic granules which may be released during neutrophil degranulation.⁵ NE is a pro-inflammatory mediator which slows down ciliary beat rate, increases mucus secretion,⁶ and directly damage epithelium of the airways.⁷

There are no validated appropriate biomarkers of disease severity in pediatric bronchiectasis. For this reason, this study tested the hypothesis that elevated sputum NE concentrations may be implicated in increased bronchiectasis severity in pediatric patients with CF and non-CF bronchiectasis.

2 | SUBJECTS AND METHODS

2.1 | Study subjects

This cross-sectional study assessed sputum NE concentrations among 30 CF and 20 non-CF bronchiectasis children under the age of 18 years when clinically stable (defined as not receiving antibiotic treatment within the previous 4 weeks, excluding chronic usual medications; in the form of inhaled aminoglycoside antibiotics which were received by all the studied patients, in addition to pancreatic enzyme replacement therapy and multivitamins which were prescribed specifically for CF patients), during their routine visits to the pulmonology clinic, Children's Teaching Ain Shams University Hospital, in the period between the first January 2020 to the first July 2020.

The patients were enrolled in the study if they had (1) confirmed CF diagnosis based on documented diagnostic CF criteria⁸ or (2) a documented diagnosis of non-CF bronchiectasis by confirmed bronchiectasis findings using high-resolution computed tomographic (HRCT) lung scanning, and clinical symptoms consistent with bronchiectasis with a negative sweat test.⁹

The patients were omitted from the study if, (1) they had any hematological or immunological conditions affecting the neutrophil count or function, (2) there was a history of a recent exacerbation during the previous month preceding the study, or (3) the studied patients had any other systemic illness or comorbidities.

2.2 | Ethical considerations

The study was approved by the Research Ethical Committee, faculty of medicine, Ain Shams University, Children's Hospital. Informed consents were obtained from the parents and the patients older than 8 years before the inclusion in the study.

2.3 | Data collection

At enrollment, from each patient, a detailed history was undertaken including respiratory symptoms such as type of cough, expectoration, dyspnea, respiratory distress, hemoptysis, and cyanosis with a special emphasis on symptoms suggestive of pulmonary exacerbations over the last 12 months. Socioeconomic status in all participants was also evaluated using El-Gilani score.¹⁰

The 50 studied bronchiectasis patients were also assessed by clinical parameters including weight, height, body mass index (BMI) percentile. Chest auscultation and pulse oximetry were also performed.

Furthermore, the studied bronchiectasis patients were subjected to chronic infection status assessment, and pulmonary function tests (PFTs) measurements using standardized spirometry for cooperative patients older than 6 years according to European Respiratory Society (ERS) guidelines.¹¹ Moreover, serum laboratory inflammatory parameters such as complete blood count and C-reactive protein (CRP) as well as sputum NE were also recorded. The severity of bronchiectasis was assessed using forced expiratory volume in 1 s (FEV1), and bronchiectasis severity scores which included bronchiectasis severity index (BSI),¹² and FACED score¹³ which enclosed (FEV1, age, chronic colonization, extension of lobes, dyspnea), which were performed to assess non-CF bronchiectasis patients, while Shwachman-Kulczycki (SK) score,¹⁴ CF risk of disease progression (CF RD-Pro) score,¹⁵ and the CF-ABLE score¹⁶ were used to evaluate the disease severity among CF patients. The radiological severity of bronchiectasis was evaluated using the radiological findings measured by assessing the most recent chest X ray and HRCT of the chest performed in the last six months using Bhalla score¹⁷ in both groups of patients.

2.4 | Study tools

The SK score¹⁴ was used to monitor the severity of CF patients, which included four domains, general activity, physical examination, nutrition, and radiological findings. The scores of the four domains were summed to obtain the final score, from which the condition of the patient was categorized as excellent (86–100), good (71–85), average (56–70), poor (41–55), or severe (≤ 40).¹⁵

In addition, CF RD-Pro score¹⁵ was used to identify the high-risk CF patients liable for disease progression, with a risk of 10% or more fall in FEV1% of predicted. The CF RD-Pro score included the number of *Staphylococcus aureus* infections and the BMI with consideration to

age and sex. Patients with CF RD-Pro scores (higher or equal to 2 points) were considered at high risk for disease advancement.

Furthermore, the CF-ABLE score¹⁶ was used to assess disease severity among CF patients, it is a simple clinical score that used clinical parameters (age, body mass index, lung function as FEV1% predicted, and number of exacerbations) to score in a scale from 0 to 7. Patients presenting with a score higher than five points were associated with higher disease severity and poor outcomes.

A pulmonary exacerbation was defined based on Fuchs criteria¹⁸ for the CF patients, and British Thoracic Society (BTS) bronchiectasis recommendations¹⁹ for non-CF bronchiectasis patients. The frequency and severity of acute exacerbations in the last year were assessed in the studied patients by at least two pediatric pulmonologists during the examination and from the hospital records.

Chronic infection was defined as isolation of a pathogenic organism in sputum when clinically stable on at least two occasions 3 months apart in the previous 12 months.²⁰

2.5 | Quantitative sputum NE assay

2.5.1 | Sputum sampling and processing

Adequate sputum samples as previously reported,²¹ either by expectoration (5 CF, 8 non-CF bronchiectasis patients), sputum induction (17 CF, 6 non-CF bronchiectasis patients), or bronchoalveolar lavage (BAL) samples (8 CF, 6 non-CF bronchiectasis patients) were collected. More detailed description of the procedure of sputum induction based on the previous literature,^{22,23} has been presented in the supplementary data.

The sputum and bronchial lavage fluid samples were centrifuged (at 2000–3000 revolutions per minute) for approximately 20 min, and the supernatants were carefully collected. When sediments occurred during storage, centrifugation would be performed again.

2.6 | Measurement

NE concentration was measured in the sputum supernatants using Human Neutrophil Elastase ELISA Kit (Shanghai Korain Biotech Co. Ltd.) in accordance with the manufacturer's instructions.^{24,25} The lowest detection limit is (0.251 ng/ml).

2.7 | Statistical analysis

All statistical calculations were done using computer programs; Statistical Package for the Social Science (IBM SPSS) version 23. Data were analyzed in the form of mean \pm standard deviation (\pm SD), and range for quantitative data, or the number of cases and percentages for qualitative data. Kolmogorov–Smirnov test was used to determine the normality of data. In addition, the χ^2 test was used to determine the differences between groups with qualitative data, and the

independent *t* test was used to determine the difference between groups with quantitative data and parametric distribution, while, Mann–Whitney test was used to compare between two groups regarding quantitative data with the nonparametric distribution. One-way analysis of variance test was used to compare between more than two groups regarding quantitative data with parametric distribution, while Kruskal–Wallis test was used to compare between more than two groups regarding quantitative data with nonparametric distribution. *p* Values less than 0.05 was considered statistically significant and *p* less than 0.01 was considered highly significant.

Correlations between different variables (demographic data, anthropometric measurements, inflammatory markers, PFTs, sputum microbiology, clinical, radiological scores and sputum NE) were performed using Spearman correlation coefficients.

Receiver operating characteristic curve (ROC) and area under the curve (AUC) were applied to identify the sputum NE cutoff score that best discriminated between different grades of CF, and non-CF bronchiectasis severity. The AUC was calculated, with a value of 1.0 representing 100% sensitivity and specificity.

Univariate and multivariate regression analyses were used to assess predictors of elevated sputum NE concentrations.

Sample size was calculated using PASS 11.0 which achieved 81% power to detect a difference in NE of -0.49000 between the null hypothesis correlation of 0.00000 and the alternative hypothesis correlation of 0.49000 between NE and severity of lung disease using a two-sided hypothesis test with a significance level of 0.05000 .

3 | RESULTS

3.1 | Characteristics of the studied patients

The present study was conducted on 50 bronchiectasis patients under the age of 18 years during times of clinical stability. The subjects were classified into two groups: group (A); CF patients ($n = 30$), group (B); non-CF bronchiectasis patients ($n = 20$). Demographics, anthropometry, parameters suggestive of bronchiectasis severity, and sputum NE levels were obtained from the studied groups.

3.2 | Demographic data

The studied subjects' mean age was (5.1 ± 3.6) in CF patients, (8.8 ± 3.8) in non-CF bronchiectasis which was ranged from (1–15) years, males were presented among (53.3%, 60.0%) of CF and non-CF bronchiectasis patients respectively. Non-CF bronchiectasis etiology was postinfectious in (12/20) 60%, immunodeficiency in (4/20) 20%, and idiopathic in (4/20) 20% of the patients (Supporting Information: Table S1). Chronic pseudomonas infection represented (66.7%, 10%) of the airway pathogens in both CF and non-CF

bronchiectasis patients. The main demographic and clinical characteristics are reported in (Table 1).

As regards the Bhalla score, (40%, 53.3%) had mild score and (60%, 46.7%) had moderate score, among CF and non-CF bronchiectasis, respectively. Concerning the disease severity scores, the median CF RD-Pro score, and CF-ABLE score were 1 (0–1), 2 (2–3.5) among CF patients, respectively, while the median BSI score, and FACED score were 9.5 (8–12), 0 (0–1) among non-CF bronchiectasis patients (data not shown).

3.3 | Sputum NE levels among the studied groups

Regarding sputum NE, the median level was 33 ng/ml, with interquartile range (IQR) of (8–140 ng/ml) among CF patients, while, it was 27.5 ng/ml, with IQR of (6–72 ng/ml) among non-CF bronchiectasis patients ($p = 0.27$), as shown in Figure 1.

According to bronchiectasis severity subgroups using CF RD-Pro score, and BSI among CF and non-CF bronchiectasis patients, median

sputum NE (IQR) was significantly higher among the severe groups (126 ng/ml [64–140], 67 ng/ml [51.5–72]) than among the moderate (30 ng/ml [24–32], 24 ng/ml [21.25–28.75]) and mild (16 ng/ml [8–25], 11 ng/ml [6–16]) groups ($p < 0.001$, < 0.001), among CF and non-CF bronchiectasis patients, respectively, as presented in Figure 2.

3.4 | Sputum NE and bronchiectasis severity

Sputum NE concentrations, showed statistical linear correlations with several parameters of disease severity including inflammatory markers as C-reactive protein ($r = 0.854$, $p < 0.001$), frequency of exacerbations ($r = 0.624$, $p < 0.001$), number of hospital admissions ($r = 0.523$, $p < 0.001$), and number of ICU admissions in the last year ($r = 0.515$, $p < 0.001$) among all the studied bronchiectasis patients (Figure 3).

Specifically, among CF patients, additional correlations were observed between sputum NE and older age ($r = 0.364$, $p = 0.048$),

TABLE 1 The baseline characteristics of the studied groups

Characteristics	CF patients (n = 30)	Non-CF bronchiectasis patients (n = 20)	Test value	p Value
Age (year) (mean ± SD)	5.1 ± 3.6	8.8 ± 3.8	14.166 ^a	
Range	1.3–15	1–15		<0.001*
Gender, n (%)				
Male	16 (53.3%)	12(60.0%)	0.216 ^b	0.642
Female	14 (46.7%)	8(40.0%)		
Socioeconomic status, N (%)				
Low	18(60%)	16(80%)	2.206 ^b	0.137
Middle	12(40%)	4 (20%)		
Consanguinity, N (%)	16(53.3%)	8(40.0%)	0.855 ^b	0.355
BMI (kg/m ²)				
Median (IQR)	14.16 ± 2.30	14.95 ± 2.76	1.319 ^a	0.263
Range	(10.9–18.5)	(11.7–19.8)		
FEV1% of predicted (N = 36), (mean ± SD)	70 ± 21.3	70 ± 27.9	3.064 ^a	0.547
Radiology				
Cystic bronchiectasis	10(33.3%)	10 (50%)	10.357 ^b	0.350
Chronic infection				
Pseudomonas	20 (66.7%)	2 (10.0%)	29.230 ^b	<0.001*
MRSA	8 (26.6%)	10 (50.0%)	29.230 ^b	0.092
Median exacerbations in the last year	2 (1–4)	2.5 (1–3)		
Range	1–6	1–4	0.624 ^a	0.775
Bhalla score (mean ± SD)	16.70 ± 4.32	16.33 ± 3.46	0.055 ^a	0.816

Abbreviations: CF, cystic fibrosis; IQR, interquartile range; MRSA: methicillin-resistant staph aureus.

* $p < 0.01$.

^at Independent sample t test.

^b χ^2 test.

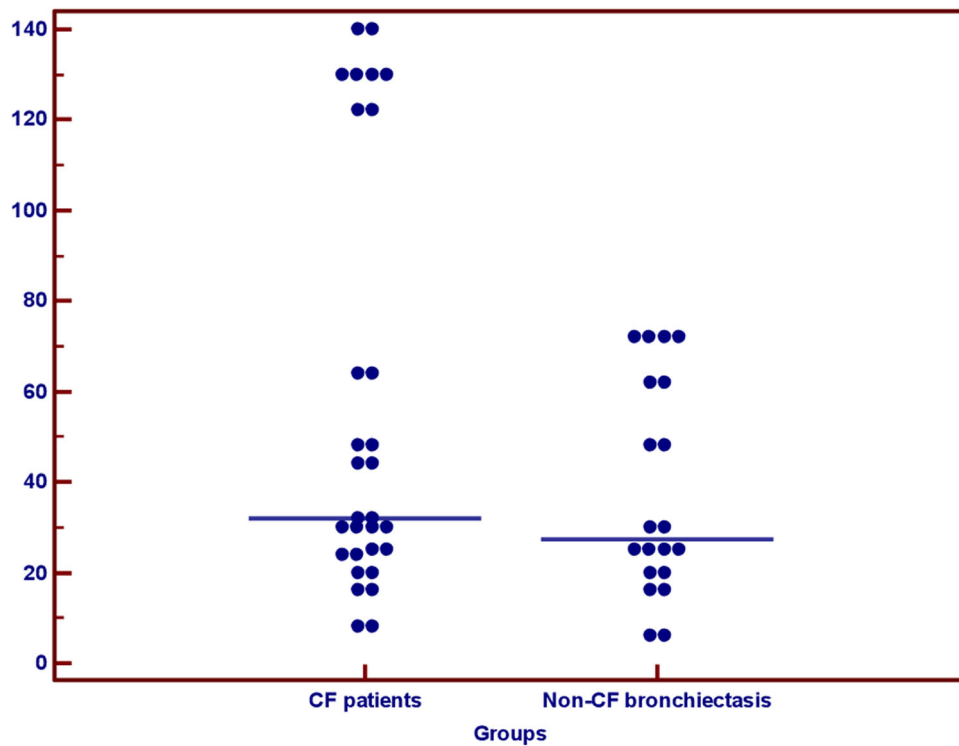
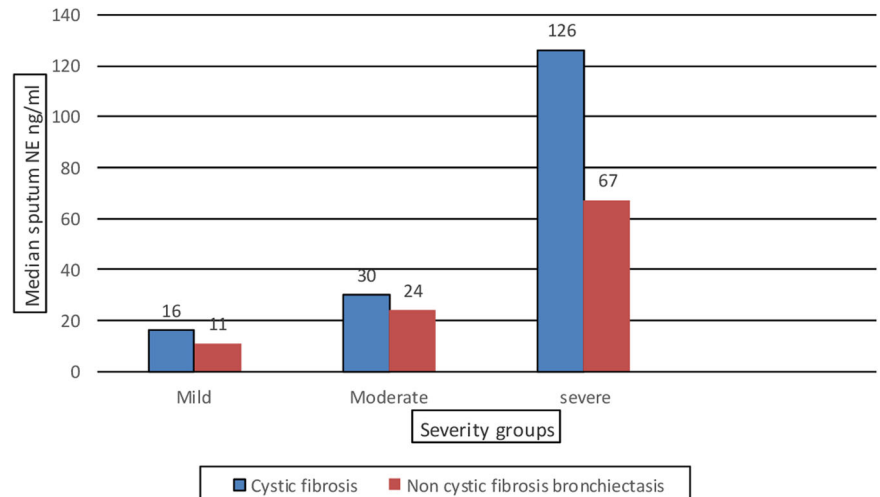


FIGURE 1 Median sputum neutrophil elastase levels (ng/ml) among cystic fibrosis and noncystic fibrosis bronchiectasis

FIGURE 2 Sputum neutrophil elastase levels (ng/ml) in cystic fibrosis and noncystic fibrosis bronchiectasis severity subgroups



more severe pulmonary exacerbations ($r = 0.735$, $p = 0.002$), higher pancreatic insufficiency ($r = 0.696$, $p < 0.001$), chronic pseudomonas aeruginosa infection ($r = 0.635$, $p = 0.008$), CF RD-Pro score ($r = 0.667$, $p < 0.001$), CF-ABLE score ($r = 0.707$, $p < 0.001$), and lower FEV1% of predicted ($r = -0.800$, $p = 0.017$), as demonstrated in Supporting Information: Table S2.

Moreover, strong correlations were demonstrated between sputum NE, age ($r = 0.66$, $p = 0.002$), neutrophil count ($r = 0.521$, $p = 0.018$), severity of pulmonary exacerbations ($r = 0.907$, $p < 0.001$), and BSI ($r = 0.465$, $p = 0.039$) among non-CF bronchiectasis patients, as presented in Supporting Information: Table S3.

Furthermore, the best fitting multiple linear regression tests for the sputum NE, displayed that elevated CRP and pulmonary exacerbation frequency ($p \leq 0.001$, 0.011) were the best independent predictors for NE concentrations among CF patients, while BSI ($p = 0.014$) was the best independent predictor for NE activity among non-CF bronchiectasis patients as demonstrated in Tables 2 and 3.

Finally, ROC analysis of sputum NE was accurate in determining the disease severity of the studied CF and non-CF bronchiectasis patients with a cutoff value of more than 44 and 30 ng/ml with (AUC = 1.000), among CF and non-CF bronchiectasis patients respectively as illustrated in Figure 4a,b.

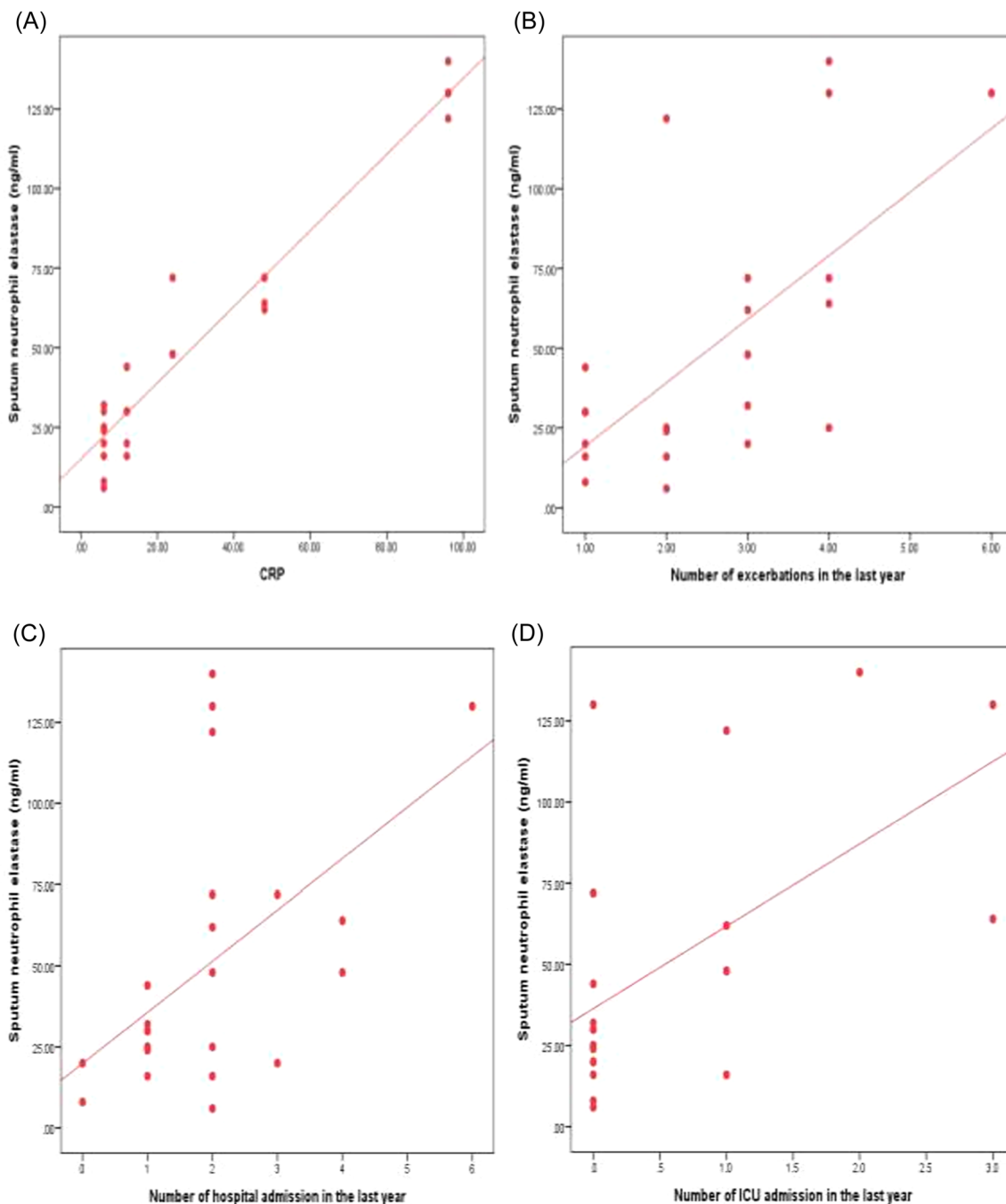


FIGURE 3 Correlations between sputum neutrophil elastase in all the studied bronchiectasis patients with C-reactive protein (A), exacerbations frequency (B), hospital admissions (C), and intensive care unit admissions in the last year (D)

4 | DISCUSSION

To our knowledge, this is one of the fewest studies that addressed the sputum NE measurements among pediatric CF and non-CF bronchiectasis patients and examined its role in bronchiectasis severity. We found that sputum NE might be a good biomarker of bronchiectasis severity as confirmed by its correlation with several disease severity parameters as a number of hospital admissions, pulmonary exacerbations frequency, severity, and bronchiectasis

severity scores. This means that sputum NE may be considered an excellent indicator of disease severity suggesting more aggressive management.

The present study showed that younger age (5.1 ± 3.6) was significantly observed among CF patients (Table 1), which may be due to earlier presentation and diagnosis among CF patients.

In our analysis, according to CF RD-Pro score, and BSI (43.3%, 40.0%), of the studied patients, were classified as moderate, and (10%, 60.0%) were classified as severe among CF and non-CF

TABLE 2 Multiple linear regression analysis using enter method for the factors associated with elevated sputum neutrophil elastase among cystic fibrosis group

Parameter	Regression coefficients	Standard error	β	t	p value
(Constant)	11.707	8.225		1.423	0.172
Age	0.404	0.439	0.032	0.92	0.37
Body mass index	0.673	0.709	0.034	0.948	0.356
C-reactive protein	1.195	0.058	1.007	20.53	<0.001**
Bronchiectasis severity index	0.95	0.544	0.056	1.745	0.098
Number of hospital admissions	0.224	4.873	0.003	0.046	0.964
Number of intensive care unit admissions	1.052	2.927	0.018	0.359	0.724
Frequency of pulmonary exacerbations in the last year	-25.683	9.121	0.274	2.816	0.011*
Pulmonary exacerbation severity	-4.107	3.227	0.067	1.273	0.219

* $p < 0.05$.

** $p < 0.01$.

TABLE 3 Multiple linear regression analysis using enter method for the factors associated with elevated sputum neutrophil elastase among non-CF bronchiectasis group

Parameter	Regression coefficients	Standard error	β	t	p value
(Constant)	-21.857	20.685		-1.057	0.313
Age	2.953	1.725	0.494	1.711	0.115
Body mass index	-2.674	1.638	-0.323	-1.632	0.131
Number of hospital admission in the last year	-19.353	11.834	-0.388	-1.635	0.13
Number of intensive care unit admission in the last year	24.09	15.702	0.422	1.534	0.153
Pulmonary exacerbation severity	8.147	4.774	0.296	1.707	0.116
Bronchiectasis severity index	2.689	0.918	0.943	2.929	0.014*

Abbreviation: CF, cystic fibrosis.

* $p < 0.05$.

bronchiectasis, respectively (data not shown), indicating a population of moderate to severe bronchiectasis severity.

The current study showed that the higher levels of sputum NE levels were significantly observed among the severe groups of both CF and non-CF bronchiectasis patients (Figure 2) indicating a higher inflammatory response with more neutrophilic inflammation associated with bronchiectasis severity.

These results are consistent with the results of previously published studies,^{21,26} which detected elevated NE concentrations in the sputum or BAL fluid in CF lung disease. Also, an old study conducted by Khan and colleagues²⁷ reported that higher level of NE has been found in BAL of infants with CF.

Similarly, a previous study conducted by Chalmers and colleagues²⁰ on 317 non-CF bronchiectasis patients, which showed elevated sputum NE concentrations among the studied bronchiectasis patients.

The previous findings highlight the crucial role of sputum NE in the pathogenesis of pediatric bronchiectasis.

The current study also revealed strong associations between elevated sputum NE concentrations and disease severity regarding the frequency of exacerbations, hospitalizations, and pancreatic insufficiency among non-CF bronchiectasis and CF patients ($p < 0.001$ for all) (Figure 3 and Supporting Information: Table S2).

In addition, sputum NE concentrations were significantly correlated with older age in both groups of patients ($p = 0.048$, 0.002) (Supporting Information: Tables S2 and S3), which may be attributed to the progression of inflammatory process with age. These findings declared a significant correlation between elevated sputum NE, and increasing bronchiectasis severity, highlighting the importance of its measurement during bronchiectasis assessment, and in monitoring the disease progression.

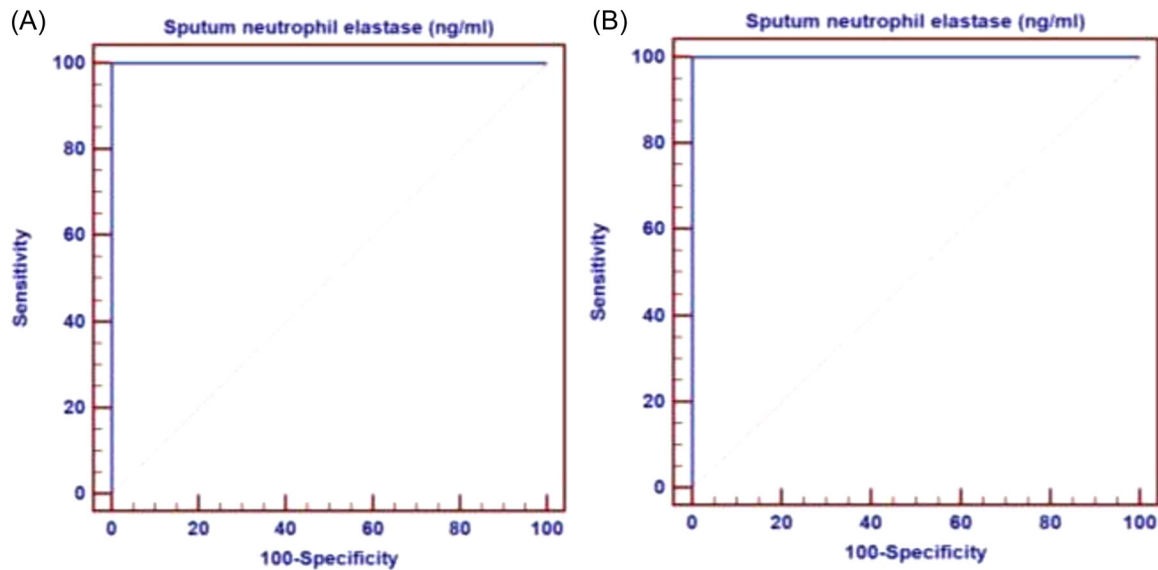


FIGURE 4 The best receiver operating characteristic curve cut-offs of sputum neutrophil elastase (ng/ml) for discriminating severe from mild to moderate disease among cystic fibrosis (CF) (A) and non-CF bronchiectasis patients (B)

Our results go parallel with a recent study conducted by Sly et al.²⁸ who showed a correlation between NE activity in BAL from CF children and the early development of bronchiectasis.

Similar to our results, Tsang et al.²⁹ showed in a study of 30 bronchiectasis patients in Hong Kong that NE activity correlated strongly with 24 h sputum volume, and extent of bronchiectasis. Also, Chalmers et al.³⁰ in a study conducted on 385 patients with bronchiectasis from the United Kingdom, found that NE activity correlated with airway bacterial load, and the extent of radiological bronchiectasis.

Moreover, previous studies have revealed an association between proteases and lung function parameters,³¹ illness severity scores,³² and radiological scores,³³ in children with bronchiectasis.

Destruction of elastin, basement membrane collagen and proteoglycans by proteases were implicated in bronchiectasis progression.²⁰ Consequently, our results found that the CF patients with elevated sputum NE tend to have higher CF RD-Pro score ($p < 0.001$), CF-ABLE score ($p < 0.001$), chronic pseudomonas infection ($p = 0.008$), and lower lung function ($p = 0.017$) (Supporting Information: Table S2).

Our data are in consistence with those seen in the previous studies^{21,27,34} including CF patients, which demonstrated a clear association between NE and FEV1, where the last concluded that NE was the strongest predictor of lung function decline over 3 years.

Moreover, the current study revealed that BSI was the best independent predictor for NE concentrations among non-CF bronchiectasis ($p = 0.014$) (Table 3), which coincided with previous studies,^{3,29,35} which reported that sputum NE directly correlated with disease severity as assessed by the BSI score. In addition, Guan et al. and Taylor et al.^{36,37} found that sputum NE correlated with the radiological severity and the BSI score among 86 and 102 non-CF bronchiectasis patients, respectively.

On the same hand, previous findings in a large cohort,¹² demonstrated a clear association between elastase activity and several markers of disease severity such as breathlessness and FEV1. Also, there was a strong association between NE level and the multidimensional BSI.

The current study declared that sputum NE has been associated with increased bronchiectasis severity, higher risk of pulmonary exacerbations, hospital admissions, bacterial colonization, and lower FEV1. For this reason, it might be used as an accurate inflammatory marker in stable state bronchiectasis not only among CF but also among non-CF bronchiectasis patients.

Although sputum NE has been implicated in the progression of bronchiectasis. However, these data are missing regarding the cutoff value of sputum NE which can predict disease severity. To the best of our knowledge, this is the first pediatric study that has determined the cutoff value of nanograms per milliliter sputum NE associated with severe bronchiectasis disease in both groups of bronchiectasis patients.

Based on our results, ROC analysis showed that the best cut of point of sputum NE to differentiate severe from mild to moderate disease was found to be >44 ng/ml with a sensitivity of 100%, a specificity of 100.0% and an AUC of 100% among CF patients while the best cutoff point among non-CF bronchiectasis patients was found to be >30 ng/ml with a sensitivity of 100.0%, specificity of 100% and an AUC of 100.0% (Figure 4).

Compared to our results, Chalmers et al.²⁰ demonstrated that the bronchiectasis patients with sputum NE levels >20 $\mu\text{g/ml}$ (high NE) had the highest rate of pulmonary exacerbations frequency compared to those with levels below 0.016 $\mu\text{g/ml}$ (low NE).

This study tried to highlight the role of sputum NE concentrations as a potential biomarker of disease severity among CF patients and non-CF bronchiectasis patients. This suggests that sputum NE

may be a useful marker that may early detect bronchiectasis patients with more severe disease who may be in need for extra care, close follow up, and more intensive management, aiming to reduce the disease morbidity and thus saving the healthcare resources.

5 | STUDY LIMITATIONS

The current study has some limitations. First, the sample size was relatively small. Second, the design was cross-sectional and further longitudinal studies are needed to demonstrate the clinical utility of this biomarker as an adjunct to an optimal clinical assessment of bronchiectasis patients. Finally, different methods were used to collect sputum samples among the studied patients which may be attributed to the prevalence of the younger age group, particularly among CF patients, who were not able to expectorate, however, this difference did not affect the levels of sputum NE among the studied patients. In spite of the previous limitations, it is important to note that this study is one of the fewest studies that examined the association between sputum NE concentrations, and the disease severity among CF and non-CF bronchiectasis pediatric patients.

6 | CONCLUSION

Sputum NE level is associated with increased risk of exacerbations, specifically severe ones, higher rates of hospitalizations, and worse severity scores among CF, and non-CF bronchiectasis patients. Therefore, NE may be a marker of disease progression in bronchiectasis. Future studies in large international multicenter registries are needed to confirm the diagnostic accuracy of this novel biomarker and to evaluate its use as a feasible treatment target in pediatric bronchiectasis.

AUTHOR CONTRIBUTIONS

Conceptualization: Eman Fouda and Heba A. Ali. **Writing—review and editing, software:** Heba A. Ali. **Writing—original draft:** Heba A. Ali and Mona Salem. **Supervision and validation:** Eman Fouda and Heba A. Ali. **Investigation and methodology:** Mona Salem and Marwa Abdelwahad. **Formal analysis:** Heba A. Ali and Marwa Abdelwahad. **Data curation:** Heba H. Radwan. All authors have read and approved the final version of the manuscript. The corresponding author had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

TRANSPARENCY STATEMENT

Dr Heba Ali affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

The authors confirm that the relevant data supporting the findings of this study are included in the article and/or its supplementary information files.

ETHICS STATEMENT

The study was approved by the Research Ethical Committee, Faculty of Medicine, Ain Shams University, Children's Hospital. Informed consents were obtained from the parents and the patients older than eight years before the inclusion in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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