

Online supplement

A point of care neutrophil elastase activity assay identifies bronchiectasis severity, airway infection and risk of exacerbation

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Supplementary methods

The supplementary video shows the performance of two NEATstik[®] assays, one positive and one negative. In the positive test it is possible to see both the control and test bands appear after adding the sample. In the negative test it is only possible to see the control band appear.

Supplementary results

To establish the reliability of the grading of the NEATstik[®] method two observers independently determine the Grade from 0-10. This was correlated as shown in Figure E1. Agreement within 1 grade was seen in 150/185 samples tested (81%), with a smaller number of outliers and a maximum disagreement of 5 grades in 3 cases. The results of the two observers were highly correlated as shown in Figure E1A (correlation 0.93, $p < 0.0001$). The Bland Altman plot is shown in figure E1B. The relationship between sputum neutrophil elastase evaluated using the Immunoassay method (y-axis) and NEATstik[®] elastase assessment is shown in figure E1C, Spearman correlation $r = 0.73, p < 0.0001$.

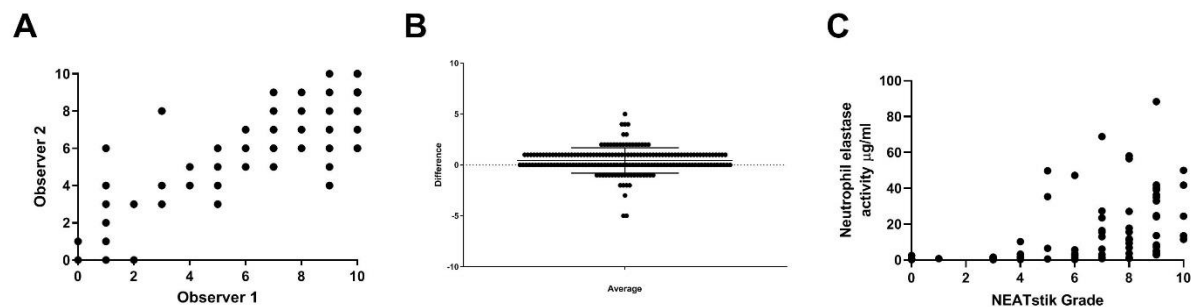


Figure E1. Reproducibility of NEATstik® grades between two observers (A), Bland Altman plot of the difference between two observers evaluating samples using the NEATstik® (B) and the relationship between neutrophil elastase activity determined using the activity based immunoassay and NEATstik® (C).

Figure E2 shows the relationship between positive NEATstik® results and clinical parameters. The correlations between NEATstik® grades and outcomes are shown in the main manuscript. There was a statistically significantly higher BSI score and lower FEV1 in patients with positive NEATstik® (figure E2). There was a strong statistically significant relationship between positive NEATstik® test and the presence of airway infection.

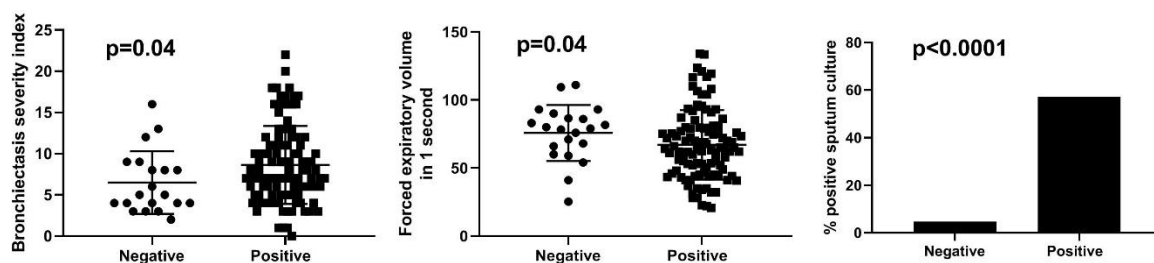


Figure E2. Relationship between positive NEATstik® and clinical parameters.

The predictive value of NE for exacerbations was consistent across all strata of the bronchiectasis severity index and prior exacerbation history (figure E3 below).

Interestingly while hazard ratios suggested a that patients with a high NEATstik® grade were at higher risk of exacerbation across the spectrum, the highest hazard ratios were in patients with 0-1 exacerbations per year at baseline suggesting NE activity may be most useful in identifying otherwise “low risk” patients who are at risk of deterioration (table E1).

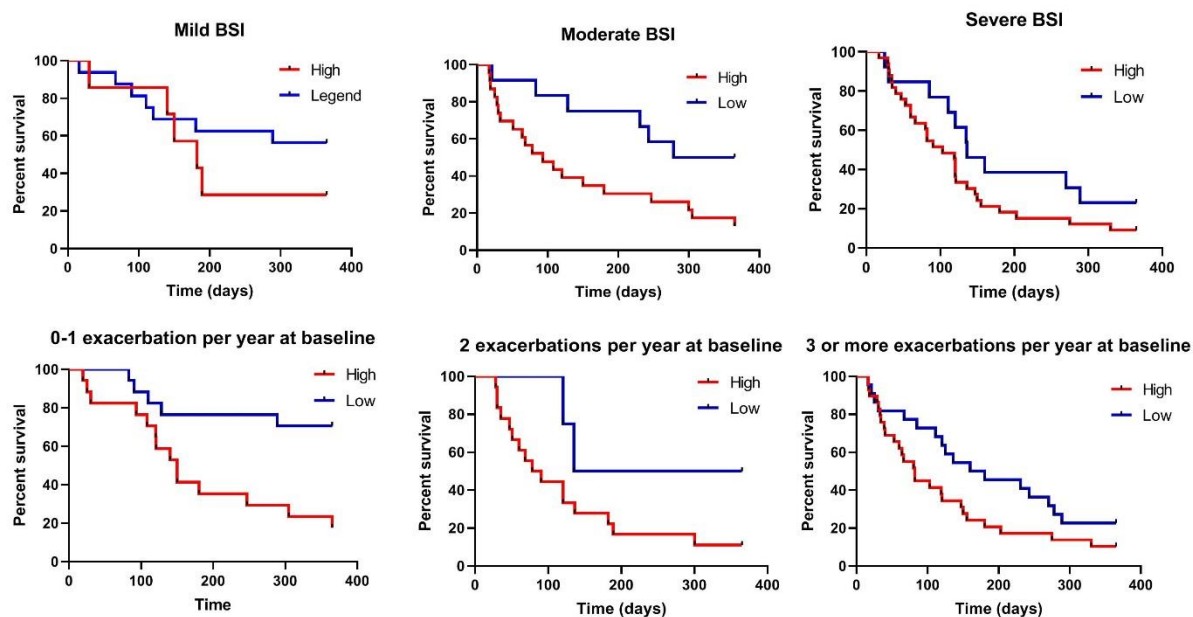


Figure E3. Kaplan meier survival curves for time to first exacerbation in subgroups based on the bronchiectasis severity index and baseline exacerbation history.

Patient subgroup	Hazard ratio (95% CI)	p-value
Bronchiectasis severity index		
Mild	1.75 95% CI 0.50-6.11	0.5
Moderate	2.81 95% CI 1.30-6.09	0.02
Severe	1.71 95% CI 0.89-3.25	0.1
Exacerbation history*		
0-1	3.98 (1.61-9.98)	0.009
2	3.03 (1.08-8.53)	0.1
3 or more	1.72 (0.95-3.20)	0.07

Table E1. Hazard ratios and corresponding 95% Confidence intervals for subgroups of time to first exacerbation for NEATstik® test >6. . *exacerbation history refers to the year prior to baseline.

Patient characteristics	Median (IQR) or N (%)
Age	67 (58-74)
Female sex (%)	39 (78%)
Bronchiectasis severity index	
Mild	8 (16%)
Moderate	18 (36%)
Severe	24 (48%)
Median BSI	8 (5-13)
Aetiology	
Idiopathic	31 (62%)
Immunodeficiency	6 (12%)
PCD	4 (8%)
Others	9 (18%)
Bronchiectasis background	
Modified MRC dyspnoea scale	1 (0-2)
Sputum volume per day (ml)	20 (6-50)
Exacerbations per year	2 (1-4)
Hospitalized in the previous year	16 (32%)
Chronic infection	25 (50%)
<i>P. aeruginosa</i> chronic infection	19 (38%)
Chronic infection other than PA	7 (14%)
FEV1 % predicted	79 (58-93)
Smoking	
Never	29 (58%)
Ex	20 (40%)
Current	1 (2%)
Treatment	
Chronic macrolide	4 (8%)

Chronic inhaled antibiotic	4 (8%)
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Table E2. Characteristics of the independent validation cohort.

Severity of disease and neutrophil elastase test results

The results for the correlation between the NEATSTIK grade and the bronchiectasis severity index is shown in the main manuscript. In the Dundee and Barcelona cohorts the correlation between NEATSTIK grade and the FACED and E-FACED scores were weak and not statistically significant. Similar relationships were observed in the Milan cohort, see Table E3 below. There was also no statistically significant relationship between NEATSTIK results and radiological severity using the Reiff score ($R=0.12$ 95% CI -0.17 to 0.39, $p=0.4$).

Score and cohort	R value	P-value
Dundee and Barcelona		
-FACED	-0.18 (-0.39 to 0.06)	0.14
-E-FACED	-0.19 (-0.41 to 0.04)	0.11
Milan		
-FACED	0.11 (-0.18 to 0.38)	0.46
-E-FACED	0.08 (-0.20 to 0.35)	0.57

Table E3. Alternative scoring systems and their relationship with NEATSTIK grade.