Development Of A Novel Proteasetag™ Activity Immunoassay For The Specific Measurement Of Neutrophil Elastase In Clinical Samples From Patients With Respiratory Disease

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Rationale Neutrophil elastase (NE), a biomarker of infection and inflammation, correlates with the severity of several respiratory diseases. Its detection and quantification in biological samples is, however, confounded by a lack of reliable and robust methodologies. Standard assays using chromogenic or fluorogenic peptide-based substrates, are non-specific when used with complex clinical samples containing multiple host and bacterial enzymes which hydrolyse the substrate resulting in an over-estimation of the target protease. Standard ELISA systems measure total protease levels, which can be a mixture of latent, active and protease-inhibitor complexes. In contrast, our novel ProteaseTag™ Active NE Immunoassay couples an activity dependent ProteaseTag™ with a specific antibody step, resulting in the selective and specific determination of **active** NE only. This study was designed to clinically validate ProteaseTag™ Active NE Immunoassay for quantifying NE in sputum and Bronchoalveolar Lavage (BAL) from chronic respiratory disease patients.

Methods Matched sputum sol samples (n=20) were collected from COPD patients during stable and exacerbation phases whilst the adult CF sputum came from patients hospitalised for acute exacerbation. Pediatric BAL samples were collected through the Study of Host Immunity and Early Lung Disease in CF (SHIELD CF) (Table 1). Samples were analysed for NE activity using both ProteaseTag™ Active NE (ProAxsis Ltd) and a fluorogenic substrate-based assay (Suc-AAPV-AMC; Sigma). Results were analysed to explore possible correlations in NE activity between the two assays and with a range of clinical parameters, where available.

Table 1: Patient Demographics

	Patient Number	Gender	Age
COPD	10	6M, 4F	73.0 ± 6.0 years
Adult CF	45	22M, 23F	29.6 ± 11.0 years
Pediatric CF	28	56M, 39F	5.4 ± 2.6 years

Results A highly significant correlation was found between the two assays in all samples ($r=0.89$; $p<0.0001$). In the COPD population both assays detected elevated levels of NE in the majority of patients (n=7) during an exacerbation (mean=217.2 µg/ml ±296.6) compared to their stable phase (mean=92.37 µg/ml ±259.8). Within adult CF, NE activity measured by ProteaseTag™ Active NE correlated appropriately with clinical parameters: inversely with FEV_1 ($p = 0.036$) and positively with CRP ($p = 0.035$), neutrophils and total white cell counts ($p < 0.001$). **No correlations with any of the clinical parameters were observed when NE was measured using the standard fluorogenic substrate.**

Conclusion NE is an established biomarker of inflammation but its measurement has been hampered by the lack of a robust and simple to use assay. ProteaseTag™ Active NE Immunoassay has been shown to be superior to currently available assays. It specifically measures only **active** NE, is quick and easy to use (< 3 hours) and has no dependency on a kinetic readout.

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