Stable inflammatory phenotype in smokers and smokers with COPD

O. Holz1, S. Roepecke2, G. Lauer3, N. Krug1, P. Ernst3, G. Lahu2, J.M. Hohlfeld1

1Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany; 2Nycomed, a Takeda company, Konstanz, Germany; 3GeneData AG, Basel, Switzerland

Introduction

COPD is a chronic inflammatory disease and the inflammatory processes or the extent of inflammation should be related to disease activity, exacerbation rate and prognosis. Sputum neutrophils, however, showed no clear relationship to exacerbation rate (Singh, 2010). But there appears to be a COPD patient phenotype that is more susceptible to exacerbations, with stable exacerbation rates that are related to the white blood cell count [Hurst 2006]. Single parameters of airway inflammation may not be sufficient to characterise a phenotype. It was therefore the aim to find and define an inflammatory phenotype that is based on multiple sputum markers, is stable and shows a better reproducibility compared to these individual markers.

Methods

24 COPD patients (GOLD II) and 23 age and gender matched healthy controls (all current smokers with ≥ 10PY) were included. Blood, bronchial biopsies, BAL, and induced sputum were collected on 2 occasions within 6 weeks. Differential cell counts and a broad panel of proteins were analyzed. Sputum supernatant was analysed by ELISA and Luminex. The intra-class correlation coefficients (ICC) were derived from one-way ANOVA tables as the ratio of variance among subjects to total variance based on the 2 visits. To obtain the inflammatory phenotype, we first transformed each selected marker and assigned a score of 1 (lowest values, first quartile of the distribution) to 4 (highest values, fourth quartile). Then we computed the mean value of the resulting scores for different combinations of markers separately for each visit.

The list of markers analysed in induced sputum is presented in the 2 following tables. These show the differences between groups for the first visit. The levels of a1-AT, HSA, and MMP3 were lower in smokers with COPD.

Results

The top four most reproducible sputum inflammatory markers (A1AT, IL6, MMP7, HSA, see table below) and the percentage of sputum neutrophils were combined to define an inflammatory phenotype (IP) independently for both visits.

The IP showed a better reproducibility (r=0.70; p<0.001) between visits as compared to the percentage of sputum neutrophils and correlated significantly with BAL (r=0.55, p<0.001) but not with serum calprotectin levels (r=0.25). Correlations with other serum markers were weak, being best for WBC count (r=0.5, p=0.002). No relationship to biopsy neutrophils was found.

Conclusion

Using a combination of neutrophil cell counts and fluid phase inflammatory mediators we defined an inflammatory phenotype that was shown to be reproducible and more robust than sputum neutrophils alone. The approach to use a combination of inflammatory markers could be helpful in studies that try to relate persistent airway inflammation to exacerbation rates, to long term changes in disease severity and prognosis, especially in the field of COPD research and for ongoing large cohort trials.

Acknowledgements

The authors would like to thank all volunteer subjects and patients for their participation, and the staff of the Clinical Airway Research Unit and the team of the Nycomed biomarker group for conduction the study, for excellent technical assistance and their contributions to the study logistics.

Contact

Fraunhofer ITEM, Hannover, Germany
olaf.holz@item.fraunhofer.de