PostScript

LETTERS TO THE EDITOR

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Microvascular hyperpermeability in COPD airways

Chronic obstructive pulmonary disease (COPD) is characterised by an abnormal inflammatory response of the lungs. An increase in the albumin concentration in the sputum of COPD patients has previously been reported.[1] This may suggest that the airway microvascular permeability is increased in COPD airways because the albumin comes from the vasculature via endothelial contraction at post-capillary venule lesions. However, measurement of sputum samples has some limitations such as contamination by saliva. We have measured the albumin concentration of the airway lumen in patients with COPD using a new direct technique for collecting airway epithelial lining fluid.[2]

Eighteen untreated patients with peripheral type lung cancer undergoing a bronchoscopic examination for the diagnosis were recruited to the study. Approval was obtained from the Wakayama Medical University ethics committee and the patients gave their written informed consent. The mean (SE) age of the patients was 70.4 (2.0) years. Eight patients were current smokers, seven ex-smokers, and three non-smokers. Five of the subjects did not have COPD, four were at risk (stage 0), six had moderate COPD (stage II), and three had severe COPD (stage III) according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of the severity of COPD.[3] Epithelial lining fluid was collected using a microsampling probe under bronchoscopy at the main or intermediate bronchus on the tumour absent side. The albumin concentration in the extracted ELF was measured and normalised by the values in the serum.

The normalised airway albumin values showed a strong correlation with the forced expiratory volume in 1 second % predicted (%FEV_1) values ($r = -0.727$, $p = 0.0006$; fig 1). There was no significant difference in the airway albumin values according to smoking status (non-smokers: mean (SE) 1.21 (0.29)%, ex-smokers: 1.23 (0.28)%, current smokers: 1.14 (0.28)%) or age. These data suggest that an increase in airway microvascular permeability may be involved in the inflammatory and subsequent obstructive process of COPD.

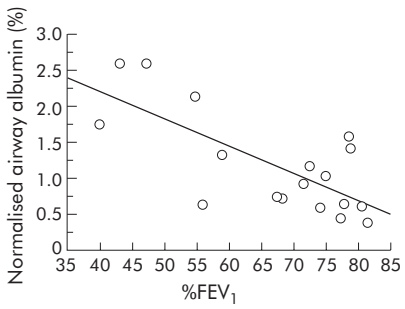

Figure 1 Relationship between normalised airway albumin and forced expiratory volume in 1 second % predicted (%FEV_1). Normalised airway albumin values were calculated as values of epithelial lining fluid/values of serum.

The precise mechanism of the microvascular hyperpermeability observed in COPD has not been well characterised. We have recently reported that oxidative and nitrosative stress is exaggerated in COPD airways.[4 5] Reactive oxygen/nitrogen species such as superoxide anion and peroxynitrite may participate in the microvascular hyperpermeability of COPD airways.

At present some airway/pulmonary cells (including epithelial cells, neutrophils, and macrophages) are considered therapeutic targets for future COPD treatment. In addition to these cells, the airway microvasculature may also be a target in the treatment of COPD. Furthermore, airway albumin values may be a good marker for the efficacy of COPD treatment.

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Assessing the validity of genetic association studies

We read with interest your approved guidance on the key issues which should be considered in preparing a genetic association study to be acceptable for publication in *Thorax*.[1 2] While we agree with several points in this guidance, other points we consider to be exaggerated or, at best, controversial. We note that, in the eight genetic association studies published in *Thorax* since 2004, some of them do not conform to this guidance with regard to population size, number of polymorphisms studied, and their functionality. This is seen clearly in the latest published association study by Yarden and colleagues[3] who examined four polymorphisms in the *TNFα* gene in patients with cystic fibrosis. Three of the studied polymorphisms were without functional information; no assessment of linkage disequilibrium, haplotype analysis or correction for multiple comparisons had been performed; and the population size—even after pooling the two different ethnic groups—showed that the study was underpowered.

With regard to the population size required in your guidance, the numbers in table 1 are too high (regardless of the typing error that caused the cases required for minor allele frequencies of 0.2 and 0.4 to be reversed). The reason for this is the unusual use of 90% power instead of the widely applied 80%. In fact, 80% power is the default for the online genetic power calculator you yourself provided in your editorial. Using this default of 80%, much smaller numbers of cases could be obtained and considered as having enough power. For example, with the relative risk set at 2, only 130 or 170 cases are required when the ''minor allele frequency'' is 0.4 and 0.2, respectively. We therefore think that your assumption that a study of 150 asthmatics and 150 controls is unlikely to be adequately powered needs some modification (such as adding to it if the minor allele frequency is less than 0.3).

As far as the functionality of a polymorphism is concerned, we agree that studying known functional polymorphisms rather than random polymorphisms in the gene of interest is advantageous in terms of detecting true disease associated variants. However, restricting genetic association studies to functional polymorphisms may lead to important polymorphisms being missed because the functional effects of many polymorphisms are difficult to assess, either as a result of technical problems (such as intronic, coding synonymous, or polymorphisms that are far upstream or downstream from the studied gene) or because of an absence of the full knowledge of the gene function and how it might be influenced by the polymorphism.

With regard to population stratification, there is no doubt that a study population that contains ethnically or geographically unmatched subjects may lead to spurious results, and we do not think any researcher would undertake an association study based on such a population. However, your assumption that even an apparently homogenous population may show substratification and your request that study populations should