CORRESPONDENCE

Decrease in \(D_{L,CO}\) in systemic sclerosis correlates with acceleration of DTPA clearance

To the Editor:

We read with great interest the article by Koon et al. [1] in which the authors demonstrated that clearance of diethylene-triamine pentaacetate (DTPA) is preserved in systemic sclerosis (SSc) patients with pulmonary vascular disease and may be useful in distinguishing fibrosing alveolitis (FA) from vascular disease. This is of considerable value in interpreting decreases in total lung diffusing capacity for carbon monoxide \((D_{L,CO})\) in SSc. Indeed, \(D_{L,CO}\) in SSc can be decreased either through FA or as a result of microvascular involvement, and it is difficult to measure their respective influences. We have focused, in the past few years, on testing the potential of noninvasive methods of detecting early lung involvement in SSc using scintigraphic methods [2], high-resolution computed tomography (HRCT) of the lungs [3] and biological markers such as procollagen III [4] and procollagen I [5]. Using this approach and involving overall >80 patients, we also measured \(D_{L,CO}\) and found a correlation between changes in HRCT alterations and \(D_{L,CO}\) [3], suggesting a link between \(D_{L,CO}\) and parenchymal involvement \((i.e. FA)\) rather than between \(D_{L,CO}\) and microvascular injury. In order to test this hypothesis, we used DTPA scintigraphy, assuming the fact, now firmly established by Koon et al. [1] that acceleration in DTPA clearance in SSc patients is specifically related to FA.

Forty-five nonsmokers (one male, 44 female; aged 23–79 yrs, mean age 54 yrs) suffering from SSc, as defined according to the American Rheumatism Association criteria [6], with normal heart ultrasound findings were included in this prospective study. DTPA clearance was measured using the technique described by Koon et al. [1]. Pulmonary function tests, i.e. forced inspiratory and expiratory flow/volume curves and absolute lung volumes were measured using a constant volume plethysmograph (Sensor Medics 28000; Sensor Medics, USA). A 10-s single-breath \((D_{L,CO})\) was carried out (Morgan, UK). Total lung capacity (TLC) and \(D_{L,CO}\) were expressed as a percentage of normal predicted values for age and sex [7]. All patients underwent HRCT scanning. One-millimetre thick slices were performed from apex to base using a High Speed (GE Milwaukee, USA) at end-inspiration at intervals of 100 mm on a 512×512 matrix. An HRCT score was established using a score that has been validated in previous studies [3, 8]. Spearman’s correlation coefficient was used to assess the correlation between these quantitative parameters \((D_{L,CO}, TLC, HRCT\) score and DTPA clearance).

There was a correlation between decrease in \(D_{L,CO}\) and DTPA clearance \((r=0.52, p=0.0002)\). We confirmed the correlation between decrease in \(D_{L,CO}\) and HRCT score \((r=0.45, p=0.002)\) and also found a correlation between decrease in \(D_{L,CO}\) and TLC \((r=0.31, p=0.038)\).

We conclude that decrease in total lung diffusing capacity for carbon monoxide is related to fibrosing alveolitis in systemic sclerosis and that a decrease in total lung diffusing capacity for carbon monoxide obviates the need for follow-up by imaging parenchymal involvement using high-resolution computed tomography and treating it early.

E. Diot*, B. Giraud**, F. Maillot*, P. Diot***
UPRES-EA 2638 Epithélium Respiratoire et Inflammation, Service de MEDECINE Interne B*, Biostatistique** et Pneumologie***, CHU Bretonneau, 2 Boulevard Tonnellé, 37044 Tours Cedex, France. 33 247473882

References


From the authors:

We thank E. Diot and colleagues for their interest in our article. We read with interest their observation that total lung diffusing capacity for carbon monoxide \((D_{L,CO})\) correlates well with high-resolution computed tomography (HRCT) appearances. This confirms previous work from our group [1] and, in this regard, there is no doubt that the extent of diffuse lung disease in scleroderma, as assessed by HRCT, is best reflected in the gas transfer measurement. However, gas transfer is a measure of effective pulmonary vasculature and is therefore also an index of the microvascular impairment found in individuals with scleroderma who have this pure vascular form of lung disease without diffuse lung disease.
The very strong correlation between DLCO and diethylenetriamine pentaacetate (DTPA) clearance is of interest. Our previous DTPA work in fibrosing alveolitis [2] showed that individuals with a rapid DTPA clearance were more likely to show reduced gas transfer, but we did not look at direct correlations with gas transfer in that study.

However, the conclusion that a decrease in total lung diffusing capacity for carbon monoxide is related only to fibrosing alveolitis is not correct because we have shown in our previous studies, including that of Ko et al. [3], that pure pulmonary vascular disease in scleroderma, in the absence of fibrosing alveolitis, is associated with a reduced total lung diffusing capacity for carbon monoxide. There is, therefore, still a role for follow-up high-resolution computed tomography measurements in individuals whose lung function changes are equivocal and when clearer information is required about a change in the parenchymal disease. Nevertheless, we do not routinely use high resolution computed tomography as a follow-up index because of the radiation burden but rather restrict its use to those patients in whom there is doubt about change in extent. We agree completely that early treatment of the parenchymal complication of systemic sclerosis is crucial and at present are co-ordinating the first European prospective double-blind placebo controlled study of the efficacy of treatment in fibrosing alveolitis of scleroderma.

R.M. du Bois
Royal Brompton & Harefield NHS Trust, Sydney Street, London SW3 6NP, UK.

References

Human lung volumes and the mechanisms that set them

To the Editor:

In a recent paper, Leith and Brown [1] reviewed the "definitions of human lung volumes and the mechanisms that set them in the context of pulmonary function testing".

Discussing the definition of restriction, they quoted the 1975 American College of Chest Physicians (ACCP)-American Thoracic society (ATS) joint committee [2]: "Restrictive Pattern (restrictive ventilatory defect): Reduction of vital capacity not explainable by airways obstruction". The authors further emphasized that "some find this definition unsatisfactory and substitute the criterion that there must be a reduction in [total lung capacity] TLC before a 'restrictive pattern' is said to exist". Among the "some" are the ATS and the European Respiratory Society who published their statements in 1975. The statement of the ATS [3], in 1991, was: "a restrictive ventilatory defect is characterized physiologically by a reduction in TLC. One may infer the presence of a restrictive ventilatory defect when [vital capacity] VC is reduced and [forced expiratory volume in one second] FEV1/ [forced vital capacity] FVC is normal or increased . . . . If there is a contradiction between VC and TLC in defining restriction the classification should be based on TLC."

The definition of the European Respiratory Society [4] in 1993, was: "A restrictive ventilatory defect is best described on the basis of a reduced TLC rather than from vital capacity measurements. The vital capacity, i.e. the volume change between [residual volume] RV and TLC, may be diminished by both restrictive and obstructive ventilatory defects; in the latter case it is due to an increase in residual volume due to (premature) airways closure (gas trapping) and airflow limitation at low lung volumes, leading to incomplete lung emptying. However, in small airways disease the RV is increased with no change in TLC: accordingly the VC is reduced (with a proportionate decrease in FEV1). Hence, the vital capacity alone is of little use in discriminating between restrictive, obstructive and mixed ventilatory defects".

So definitions are here with us and I see no reason to go back to older ones when newer accepted definitions are available. To suggest VC as a criterion to define a restrictive defect would lead to overestimation of restrictive defects and conversely to underestimation of obstructive ones. In a recent paper on consecutive adult Caucasian patients who had undergone both spirometry and lung volume measurements, Aaron et al. [5] reported that, in patients with a low FVC and normal (or above normal) FEV1/FVC, only 153 out of 264 (58%) had a true restrictive syndrome, i.e. a decreased TLC. The others (111, 42%) had a normal TLC.

D. Stanescu
Service De Pneumologie, Universite Catholique De Louvain, Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, 1200 Bruxelles.

References

From the authors:

We thank D. Stanescu for his comments and for his good summary of current definitions of restriction. We cited an old definition not to advocate it but rather to introduce the related physiological and practical problems.

D.E. Leith, R. Brown
5025 Lakewood Drive, Manhasset, KS 66503-8406, USA.
Defensins: where and how do they work against micro-organisms on human airways?

To the Editor

We appreciated the fine review article by Hiemstra et al. [1] concerning defensins and pulmonary epithelium. Defensins are broad spectrum antimicrobial peptide products of neutrophils (α-defensins) [2, 3] and epithelia β-defensins [4, 5]. Since airway infection with Pseudomonas aeruginosa and neutrophilic inflammation are the major cause of lung disease in cystic fibrosis (CF), bacterial killing by human β-defensins (HBD) predominantly produced by airway epithelium and in the airway surface liquid (ASL) may be of clinical/pathological importance in both healthy subjects and CF patients. Extensive evidence suggests that epithelial tissue provides the first line of defence between foreign organisms and the environment. Disruption of this barrier leads to bacterial invasion and subsequent inflammation. The first direct evidence for the expression of defensin peptides in the oral mucosa was the identification of a novel epithelial β-defensin peptide in the mammalian tongue [6]. It was shown to be up-regulated in inflammation, suggesting that it participates in host defence. There is now evidence indicating that normal airway epithelial cells and tissues express two β-defensins, human β-defensins (HBD)-1 and HBD-2 [4, 5]. It has also been reported that both HBDs are detected in bronchoalveolar lavage (BAL) fluid [7, 8]. However, the antimicrobial activities of both HBD-1 and HBD-2 were known to be inhibited by NaCl.

The key issue about the antimicrobial action of β-defensins is the composition and osmolality of ASL. Wine [9] has perceptively summarized the two current hypotheses regarding the pathogenesis of CF airway disease in reaction to the composition of ASL and defensins. In healthy subjects, ASL has been reported to be hypotonic, however elevated ASL Na+ and Cl− concentrations in airways in CF patients have been reported by several investigators in both in vivo and in vitro studies [10, 11]. Smith et al. [12] demonstrated that normal airways reabsorb salt in greater quantities than water from the ASL, thus producing the sufficiently low NaCl concentrations needed (≤50 mM NaCl) to activate defensins, but that salt is poorly absorbed in CF airways, resulting in excessively salty ASL that disrupts the bacterial killing activities of HBDs. This is the "hypertonic (high salt) ASL in CF airways" hypothesis. However, the second hypothesis, "low, but isotonic volume of ASL in CF airways", has recently been considered to link defects in cystic fibrosis transmembrane conductance regulator (CFTR)-mediated ion transport with CF airway disease [13]. Matsur and Bouchier [13] have demonstrated that the airways absorbs salt/water isotonically to ad-just the volume/height of the ASL, components to maintain efficient mucus clearance. They suggested that airway epithelia are too water permeable to maintain hypotonic ASL. Several investigators have also reported that the ASL is isotonic rather than hypotonic in both healthy subjects and child and adult patients with CF [14–16]. The "low volume of ASL in CF airways" hypothesis may support one of the earliest hypotheses to explain CF lung disease, the "thick mucous" hypothesis [17, 18]. If it is true, ASL cannot produce a sufficiently low NaCl concentration to activate HBDs in vivo. Although the HBDs are mainly produced by airway epithelia, how and where do the HBDs work on airways? There are a number of questions still outstanding.

Firstly, do the β-defensins work in ASL in human airways? The "low salt in normal airways/high salt in CF airways" theory [10–12] strongly supports the idea that HBDs derived from the epithelium act directly against micro-organisms in the relatively hypotonic ASL of non-CF subjects, but not in CF patients. However, the "isotonic ASL in normals as well as CF airways" theory [13–16] contradicts the idea of the effective action of HBDs on the bacteria in lungs in both CF and non-CF airways.

Secondly, do the other components of ASL and the mucous inhibit or augment the action of HDBs on human airways? Since ASL is not composed of salt-water alone, (there are proteases, lysosomes, and another ions/anions)? It has been reported that ASL from healthy subjects has markedly higher K+ concentrations than plasma [11]. In addition, α-defensins are known to increase the interleukin (IL)-8 messenger ribonucleic acid (mRNA) of epithelial cells [3].

Thirdly, although the natural bacterial killing activity of HBD is upregulated by inflammatory stimuli including bacteria, fungi, and lipopolysaccharides (LPS), it is not easy to maintain airway cells in culture without antibiotics, e.g. penicillin and tobramycin. The antimicrobial activity of HBDs may be not potent enough to protect airway cells in a culture dish against the contaminated bacteria.

Finally, the problem is that ASL exists as a very thin layer of fluid, making the collection of a sufficient volume for reliable analysis difficult [18, 19]. After many false starts, investigators are now coming closer to understanding the fundamental pathogenesis of CF [9]. The further knowledge about the difference and/or similarity, in the regulation of depth and composition of ASL between CF patients and healthy subjects may be the key to the real understanding of the function of defensins as well as CF airway disease pathogenesis [18, 19].

S. Teramoto, Y. Ouchi
Dept of Geriatric Medicine, Tokyo University Hospital, 7-3-1 Hongo Bunkyo-ku Tokyo 113-8655 Japan. Fax: 81 358006530.

References
The questions raised by S. Teramoto and Y. Ouchi in their letter are interesting. Indeed, based on the "low volume hypothesis", it would seem unlikely that β-defensins are active in ASL under normal conditions due to the isotonic nature of this fluid. Little is known about the effect of various components present in ASL on the activity of antimicrobial peptides. Therefore studies aimed at exploring the activity of antimicrobial peptides in their "natural environment" are needed. What is the impact of this new information on airway epithelial cell culture? While it is true that it appears to be difficult to maintain airway epithelial cells in culture without antibiotics, this does not necessarily imply that the antimicrobial peptides secreted by the cells in culture are not active. This observation is probably explained by the fact that these cells are cultured in isotonic culture medium. Because of the salt content of the medium, it does not seem likely that antimicrobial peptides display optimal activity against microorganisms in this medium. This also partially explains the results of a recent study [6], in which neutrophil defensins were found to increase the adherence of *Haemophilus influenzae* to cultured bronchial epithelial cells, in the absence of antimicrobial activity against the bacteria. The composition of the culture medium used in this study formed a reasonable explanation for the lack of antibacterial activity of the neutrophil defensins in these experiments. These results suggest that in conditions of high salt, as predicted in CF, neutrophil defensins do not kill but rather enhance the adherence of selected microorganisms.

In summary, the "high salt hypothesis" for the inactivation of antimicrobial peptides is an appealing addition to the various explanations that have been proposed to explain the increased susceptibility to bacterial infection in CF. It has led to rapid progress in research into endogenous antimicrobial peptides, and ultimately may aid in the development of new therapies.

**From the authors:**

We would like to thank S. Teramoto and Y. Ouchi for their comments in response to our review on neutrophil serine proteinases and defensins in the *European Respiratory Journal* [1]. Our review was focused on the effects of neutrophil serine proteinases and defensins on pulmonary epithelium, rather than addressing the recently developed new insights into the role of defensins in increased susceptibility to bacterial infection in cystic fibrosis (CF). Indeed, studies by Smith et al. [2] and Goldman et al. [3] have indicated that the activity of antimicrobial peptides produced by epithelial cells is possibly decreased in the airway surface liquid (ASL) of patients with CF as a result of an increased salt concentration in this fluid [4]. These studies are in line with the "high salt hypothesis". Although they have received much attention and led to new research initiatives in the field, it has to be noted that the results were obtained using cultured epithelial cells (including the elegant bronchial xenograft model). Therefore, their relevance to the in *vivo* situation remains to be shown. Studies in which the Na and Cl concentrations of ASL sampled from the airways of patients with CF is measured may provide important information. However, such studies have sometimes provided conflicting results, which may in part be related to the fragility of the bronchial mucosa especially in inflamed areas. Improved sampling techniques for the collection of ASL from the airways may provide more consistent data on this matter. Much of the attention on the inactivation of antimicrobial peptides in ASL in CF is focused on β-defensins. In addition, the activity of other cationic antimicrobial peptides and proteins, including that of secretory leukocyte proteinase inhibitor (SLPI); [5], is decreased under conditions of increased ionic strength.

**References**


