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Lavage *versus* serum measurements of lysozyme, angiotensin converting enzyme and other inflammatory markers in pulmonary sarcoidosis

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ABSTRACT: The aim of this study was to explore whether amounts of angiotensin converting enzyme (ACE) and lysozyme produced within the lungs correlate more closely than serum levels of these enzymes, or other inflammatory markers, with chest radiographic profusion scores, lung function and therapy response in patients with pulmonary sarcoidosis. We have studied 25 patients, and levels in bronchoalveolar lavage (BAL) were used to determine "local" enzyme production by reference to serum and lavage albumin. Before treatment, serum lysozyme levels were elevated in more patients (80%) than serum ACE levels (40%). They also gave the best overall correlation with clinical measurements prior to treatment and falls in serum lysozyme closely paralleled improvement in lung function (transfer factor for carbon monoxide (D_{LCO})) on therapy. The only other markers showing significant correlations with disease severity were lavage neutrophil counts per ml and "local" ACE measurements prior to treatment. The value of pre-treatment levels of the different inflammatory markers in predicting response to corticosteroid therapy was explored and the only significant finding was that BAL lymphocyte percentages and numbers·ml^{-1} were initially higher in patients with lower post-treatment chest X-ray scores ($p<0.01$ and $p<0.05$, respectively). We conclude that serum lysozyme levels appear to be a more useful marker of overall disease activity in sarcoidosis than measurements of other inflammatory markers. However, BAL lymphocyte counts were the best predictive marker of radiographic response to corticosteroids.

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Numerous attempts have been made to assess disease "activity" in pulmonary sarcoidosis in order to select patients at risk of irreversible lung damage for early treatment. In the search for a prognostic test, bronchoalveolar lavage (BAL) lymphocyte counts, gallium^{67} uptake, serum angiotensin converting enzyme (serum ACE) and serum lysozyme levels have received most attention. Serum ACE [1–9] and serum lysozyme [10–16] are elevated in variable proportions of sarcoid patients but, since sarcoidosis is a multisystem disease, the origin of these enzymes is still a matter of debate. Both have been detected in alveolar macrophages [17–22] as well as in granuloma epithelioid cells [23, 24]. Thus, it seemed reasonable to postulate that levels of these enzymes in BAL fluid might reflect the inflammatory activity in the lungs more closely than serum levels.

The aim of this study was, therefore, to determine whether the levels of locally produced ACE and lysozyme measured in BAL fluid correlate better than serum levels with radiological and physiological findings in patients with pulmonary sarcoidosis. In addition, we wished to determine whether pre-treatment measurements of these enzymes can identify patients prone to develop irreversible changes not fully responsive to corticosteroid therapy. We also took the opportunity to compare the findings with measurements of other inflammatory markers, in particular BAL cell counts and albumin levels in this disease. A study group of patients with pulmonary parenchymal shadows was chosen because these patients are believed to be at the highest risk of developing irreversible lung damage [25].

Patients and methods

Patients

Twenty five patients with histologically proven sarcoidosis were studied. All had radiographic evidence of lung involvement at first lavage (15 at radiographic Stage II and 10 at Stage III) and were being considered for treatment because of persistent parenchymal shadows. There were 9 males and 16 females, with a mean age of 36±9 yrs (range 25–58 yrs). Fourteen were nonsmokers, 9 ex-smokers and 2 current smokers. The duration of disease prior to treatment, assessed on the basis of symptoms or radiographic changes, was a median 14 months, (range 1–146 months). The patients underwent BAL at the time of pre-treatment clinical assessment and were then treated with prednisolone according to a standard protocol [26]. This included 40 mg of prednisolone daily for 1–2 months, followed by gradual tapering to 15 mg daily over the subsequent two months. Further tapering was guided by the clinical course assessed at regular follow-up checks. Serum and BAL studies were repeated after a median period of 13 months (range 3–49 months). All patients gave their written informed consent to BAL and the studies were approved by the Ethical Committee of the National Heart and Chest Hospitals.

Bronchoalveolar lavage

BAL and BAL cell counts were performed using the standard procedure as previously reported [27]. Briefly, BAL was performed in the lateral segment of the right lower lobe during fibreoptic bronchoscopy by instillation of a standard volume of 240 ml of prewarmed buffered saline subject to clinical constraints.

ACE, lysozyme and albumin measurements

ACE, lysozyme and albumin were determined in serum and in cell-free supernatant of the recovered BAL fluid. ACE was measured using the inhibitor binding assay reported by OKSANEN *et al.* [28]. Briefly, serum (10 µl) or BAL fluid (100 µl) was incubated with ^{125}I labelled ACE inhibitor at pH 7 at 37°C for two hours in a non-equilibrated system. Inhibitor bound to ACE was separated by adsorption to coated charcoal (dilution with serum 1:5 and with BAL fluid 1:20). The radioactivity remaining in the supernatant was counted, and the ACE value was calculated from a standard curve. The results were expressed as $kU \cdot l^{-1}$. Lysozyme was analysed by a radioimmunoassay method modified for the measurement of low lysozyme levels [29]. Purified lysozyme, isolated from the urine of a patient who had monocytic leukaemia, was iodinated by the chloramine-T method and used as a tracer in the assay which was based on the principle of competitive binding. The tracer was mixed with the test samples or with noniodinated preparations of purified lysozyme, which were used as the standards for the assay. Antiserum to human lysozyme (Dakopatts a/s, Copenhagen, Denmark) was added, the complexes formed were separated using polyethyleneglycol, and the amount of bound radioactivity determined. The lower limit of detection with this method is $0.004\ mg \cdot l^{-1}$. Albumin was analysed by a commercial albumin radioimmunoassay. The amount of enzyme (ACE or lysozyme) produced locally in the lungs, rather than derived by diffusion from serum, was calculated using the formula:

$$\text{local E} = \text{lavage E} - \frac{\text{serum E} \times \text{lavage albumin}}{\text{serum albumin}}$$

where E=ACE or lysozyme.

The formula derives from two assumptions:
1. Lavage E is the sum of E produced locally (local E) and the proportion diffused from the serum (diffused E). Therefore, local E=lavage E - diffused E.
2. Albumin is not produced locally, and so all lavage albumin has reached the alveolar space by diffusion from the blood stream. The ratio of diffused E:lavage albumin is assumed to be equal to the ratio of serum E: serum albumin.

$$\text{Therefore, diffused E} = \frac{\text{serum E} \times \text{lavage albumin}}{\text{serum albumin}}$$

$$\text{Thus, local E} = \text{lavage E} - \frac{\text{serum E} \times \text{lavage albumin}}{\text{serum albumin}}$$

Chest radiographs and lung function

Radiographs were graded using a modified Union Internationale Contre le Cancer (UICC)/International Labour Office (ILO) scheme [30]. A profusion score of 0–3 was allocated to 3 zones of each lung establishing a 9 point scale of abnormality for each lung separately and an 18 point scale overall [31].

Standard lung function tests including forced vital capacity (FVC), total lung capacity (TLC) and measurements of transfer factor for carbon monoxide (DLCO) were performed. Results were expressed as percentage of the mean of the predicted values for an age and height matched standard population [32].

Radiographic profusion scores and lung function measurements were re-evaluated at regular intervals and, for the purpose of this study, correlations with laboratory measurements were undertaken with the clinical measurements obtained at the time of repeat lavage, a median 13 months (range 3–49 months), and at a standard time point three years (median 36 months, range 34–39 months) after commencement of therapy.

Before treatment, all patients had parenchymal shadows, 16 had a reduction in DLCO (80% of mean predicted value) and 5 had a reduction in FVC and TLC. At the time of the follow-up lavage (*i.e.* after a median 13 months), radiographic profusion scores had deteriorated in one, improved in 17 and decreased to zero in 7. DLCO improved notably (≥10%) in 17 but

15 of the 25 still retained values below 80% of the mean predicted value. FVC and TLC showed a notable improvement (≥10%) in 8 and 9 patients, respectively. Group data analysis using the Wilcoxon matched-pairs test showed a significant overall improvement of chest radiographic profusion scores ($p<0.01$), FVC ($p<0.02$) and DLCO ($p<0.01$) but not of TLC. At a further clinical reassessment 3 yrs after the commencement of treatment, TLC had also improved significantly ($p<0.05$) compared to the pre-treatment assessment.

Statistical methods

Non-parametric tests were used, unless otherwise stated, since most of the data did not show a normal distribution. Changes before and after treatment were tested using the Wilcoxon matched-pairs signed-ranks test.

Correlations between quantitative measurements were tested using the Spearman rank correlation coefficient. Differences between groups of unrelated samples were compared using the Mann-Whitney U test. A paired t-test was used to compare lavage fluid input volumes, which were normally distributed. P values ≤0.05 were accepted as significant. Trends at $p\leq0.1$ are also shown in the tables for information but are rarely referred to in the text.

Results

Lavage compared with serum albumin, ACE and lysozyme measurements

Albumin. Albumin levels were measured, as well as ACE and lysozyme, in the serum and lavage samples of the 25 patients with sarcoidosis to provide an indicator of lung permeability. As shown in figure 1, the serum albumin levels were similar both before and after treatment of the patients with corticosteroids for a median 13 months. Lavage albumin levels were on average 320-fold lower than the serum levels before treatment (median 0.12, range 0.023–2.13 $g\cdot l^{-1}$ compared with median 38.4, range 30.3–47.2 $g\cdot l^{-1}$, respectively) and, by contrast with the serum levels, they fell to even lower levels after treatment (median 0.073, range 0.011–0.225 $g\cdot l^{-1}$), indicating a reduction in lung permeability ($p<0.01$ compared with pre-treatment lavage values; Wilcoxon matched-pairs signed-ranks test). The falls were not explained by variability in the lavage fluid input volumes, which did not differ significantly in the 25 patients before and after the median 13 month treatment (mean 266 ml±75 SD compared with mean 247 ml±40 SD, respectively; paired t-test).

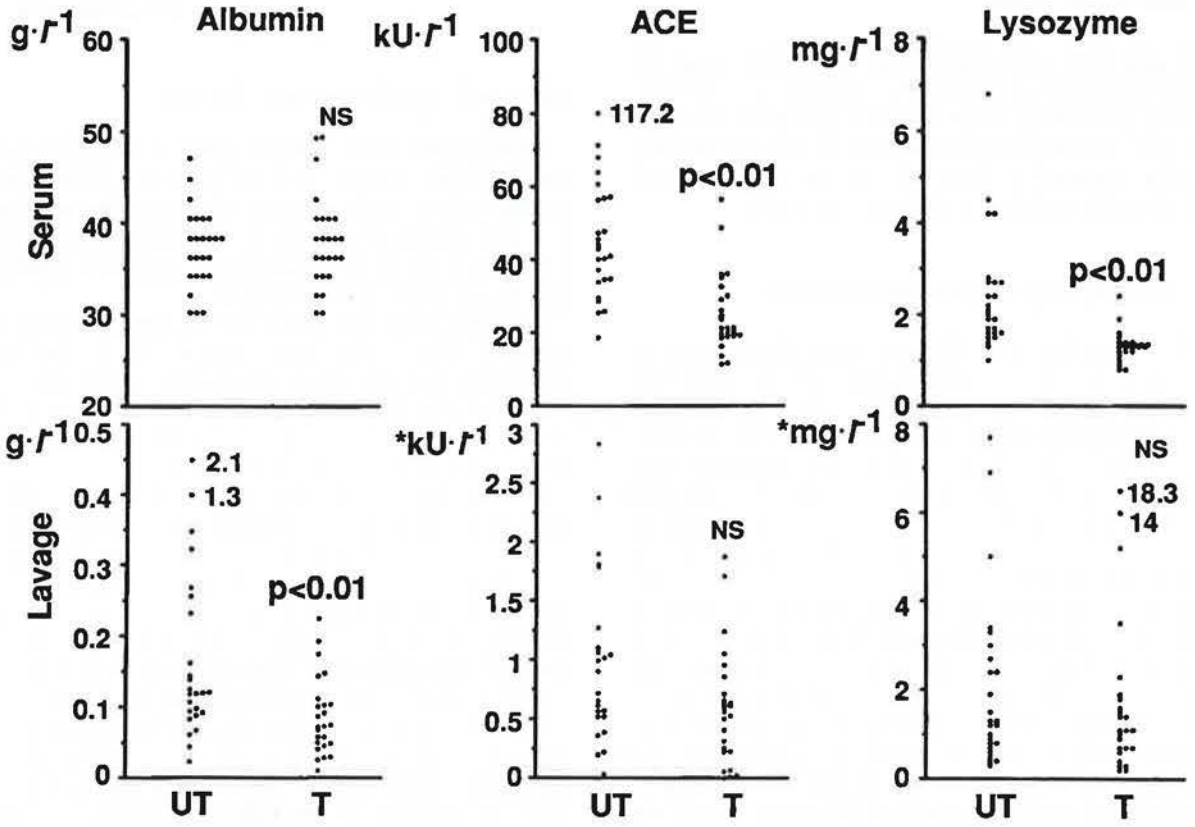

Fig. 1. – Levels of albumin, angiotensin converting enzyme (ACE) and lysozyme in serum and bronchoalveolar lavage fluid from 25 patients with pulmonary sarcoidosis. UT: untreated; T: after a median 13 months of corticosteroid treatment; *: corrected for diffusion from serum (*i.e.* "local" levels); NS: not significant.

ACE. Before treatment, serum ACE levels were increased in 10 (40%) of the 25 patients compared with the normal control range for the laboratory (20–45 kU·l^{-1}) [28], but after the median 13 months on steroids all but two patients had values within the normal range and the levels had fallen significantly for the group overall (median 42.95, range 18.77–117.24 kU·l^{-1} before treatment compared with median 21.48, range 11.46–56.49 kU·l^{-1} on treatment, $p<0.01$; Wilcoxon matched-pairs signed-ranks test; fig. 1). Lavage ACE levels (calculated using the formula set out in the Methods section to exclude diffusion from serum) were on average sixtyfold lower before treatment than in the serum (median 0.72, range 0–2.84 kU·l^{-1}) but unlike the serum values local levels did not fall significantly after treatment (median 0.56, range 0–1.87 kU·l^{-1}).

Lysozyme. More patients (20/25 *i.e.* 80%) had elevated levels of serum lysozyme (normal range 0.4–1.5 mg·l^{-1}) than elevated serum ACE (10/25 *i.e.* 40%) before treatment (fig. 1). After treatment, however, serum lysozyme levels were increased in only three patients and the fall in serum lysozyme levels was significant for the group overall (median 2.0, range 1–6.8 mg·l^{-1} before treatment compared with median 1.3, range 0.8–2.4 mg·l^{-1} after treatment, $p<0.01$; Wilcoxon matched-pairs signed-ranks test). Lavage lysozyme levels were almost as high as serum levels indicating that the levels of lysozyme *in vivo* in lung lining fluid are likely to be higher than serum levels. However, like lavage ACE measurements they showed no significant reduction following treatment (median 1.3, range 0.3–7.7 mg·l^{-1} before treatment compared with median 1.1, range 0.2–18.3 mg·l^{-1} after treatment).

BAL cell counts. Counts of cells in the lavage samples were also evaluated before and after treatment for a median 13 months and, like lavage ACE and lysozyme, they showed random changes after treatment rather than any consistent directional change for the group (table 1).

The relationship between the BAL cell counts and coincident measurements of serum and lavage ACE, lysozyme and albumin was also investigated. Before treatment, a significant correlation was observed between serum ACE and the numbers of macrophages·ml^{-1} in the lavage fluid ($r_s=0.34$, $p<0.05$, Spearman rank correlation), while lavage albumin levels correlated with the percentages and numbers·ml^{-1} of lymphocytes in the lavage fluid ($r_s=0.43$, $p<0.025$ and $r_s=0.34$, $p<0.05$, respectively). After a median 13 months treatment with corticosteroids, lavage albumin levels still showed a correlation with the numbers·ml^{-1} of lymphocytes ($r_s=0.55$, $p<0.005$) and also with the numbers of macrophages·ml^{-1} ($r_s=0.44$, $p<0.025$) and the total numbers of cells·ml^{-1} ($r_s=0.53$, $p<0.005$). There was also a correlation between the serum lysozyme levels and the numbers·ml^{-1} and percentages of BAL neutrophils after treatment ($r_s=0.58$, $p<0.005$ and $r_s=0.44$, $p<0.025$, respectively). No significant correlations were observed between local levels of lysozyme or ACE and BAL cell counts.

Relationship with clinical findings

Disease severity. To determine whether the serum and lavage ACE and lysozyme levels, or levels of other inflammatory markers, reflected the extent of clinical impairment, their correlations with coincident measurements of lung function and radiographic profusion scores were investigated (table 2). Before treatment, patients with higher serum lysozyme levels had significantly higher radiographic profusion scores ($p<0.025$) and lower lung function measurements of FVC ($p<0.05$) and DLCO ($p<0.025$; Spearman rank correlation). Lavage neutrophil counts (per ml) were also higher in patients with higher radiographic profusion scores ($p<0.05$) and lower FVC ($p<0.05$). The only other significant correlations were between higher lavage ACE levels and lower DLCO ($p<0.025$) and between higher lavage albumin and lower TLC ($p<0.05$). No significant

Table 1. – Percentage counts and numbers·ml^{-1} of cells in lavage samples of the 25 patients with sarcoidosis before and after a median 13 months of corticosteroid therapy

	Before therapy		On steroids		
	Median	Range	Median	Range	p*
Percentage counts					
Macrophages	70.7	28.8–89.2	72.0	23.4–93.6	NS
Lymphocytes	19.9	4.8–66.4	21.1	5.4–67.4	NS
Neutrophils	3.3	0.4–32.7	5.7	0.7–20.3	NS
Cell counts·ml×10^4					
Total cells	26.0	5.0–90.0	20.0	2.0–101.0	NS
Macrophages	15.2	2.9–75.7	14.7	1.5–64.1	NS
Lymphocytes	5.2	0.6–31.9	4.5	0.4–44.8	NS
Neutrophils	1.2	0.1–15.1	1.0	0.03–15.0	NS

*: Wilcoxon matched-pairs test; NS: not significant.

Table 2. – Correlations of serum and lavage markers with clinical findings before therapy in the 25 patients with sarcoidosis

	CXR score	FVC	TLC	DLCO
Serum ACE	NS	NS	NS	NS
Lavage ACE*	NS	NS	NS	r_s=-0.40 p<0.025
Serum lysozyme	r_s=0.42 p<0.025	r_s=-0.38 p<0.05	r_s=-0.32 p<0.10	r_s=-0.40 p<0.025
Lavage lysozyme*	NS	NS	NS	NS
Serum albumin	NS	NS	NS	NS
Lavage albumin	NS	NS	r_s=-0.38 p<0.05	r_s=-0.30 p<0.10
BAL cell counts				
Total cells·ml^{-1}	r_s=0.30 p<0.10	NS	NS	NS
Macrophages·ml^{-1}	NS	NS	NS	NS
Lymphocytes·ml^{-1}	NS	NS	NS	r_s=-0.33 p<0.10
Lymphocytes %	NS	NS	NS	r_s=-0.29 p<0.10
Neutrophils·ml^{-1}	r_s=0.38 p<0.05	r_s=-0.36 p<0.05	NS	NS
Neutrophils %	NS	r_s=-0.29 p<0.10	NS	NS

*: Corrected for diffusion from serum (*i.e.* "local" levels); r_s: Spearman rank correlation coefficient; CXR: chest X-ray; FVC: forced vital capacity; TLC: total lung capacity; DLCO: transfer factor for carbon monoxide; BAL: bronchoalveolar lavage; ACE: angiotension converting enzyme.

correlations were observed after a median 13 months of treatment.

Longitudinal changes. We next explored whether the longitudinal changes in any of the inflammatory markers reflected the parallel changes in clinical measurements in individual patients. This was done by determining whether the extent of change in each inflammatory marker (pre-treatment level minus level at re-assessment on steroids) showed any significant correlation with the extent of change in each clinical measurement over the same period; table 3. In the case of lung function, change was calculated as post-treatment minus pre-treatment values in order to achieve positive values rather than negative values for the purpose of correlations. The closest correlation was between the extent of fall in serum lysozyme and the extent of improvement in DLCO (p<0.005, Spearman rank correlation). There were also significant correlations between the extent of fall in serum ACE and lavage ACE and the extent of improvement in DLCO (p<0.05 and p<0.01, respectively). The decrease of lavage albumin levels reflected the improvement in TLC (p<0.025). The extent of fall in counts of total cells·ml^{-1} macrophages·ml^{-1} and lymphocyte percentages in lavage showed a relationship with the extent of improvement in radiographic profusion scores (p<0.05, table 3) but, otherwise, changes in lavage cell counts showed no correlations with clinical changes.

Prognostic value of pre-treatment ACE and lysozyme measurements

Relationship with post-treatment clinical measurements. The pre-treatment serum and lavage ACE, lysozyme and albumin measurements did not correlate with measurements of lung function or radiographic profusion scores assessed after a standard follow-up time of three years from the start of corticosteroid therapy (median 36 months, range 34–39 months). However, the percentage counts and numbers·ml^{-1} of lymphocytes in the lavage fluid before treatment were significantly higher in patients with lower radiographic profusion scores at three years (r_s=-0.48, p<0.01 and r_s=-0.35, p<0.05, respectively; Spearman rank correlation). There was also a correlation between the total cell count·ml^{-1} prior to treatment and higher TLC measurements at three years (r_s=0.36, p<0.05).

Prediction of radiographic or lung function "normalization". To address the important clinical question whether pre-treatment inflammatory markers can predict which patients will normalize completely on treatment with corticosteroids and which will be left with some degree of irreversible impairment, we sub-divided the patients according to: a) whether their chest radiographs had cleared completely and remained clear over the three year follow-up (n=12) compared with those who had residual shadows or who suffered radiographic relapse over the same period (n=13); b) whether the DLCO had normalized (≥80% mean predicted values) over the three year follow-up period (n=11) compared with those who were left with residual DLCO impairment (<80% mean predicted) (n=14). The only differences observed were that pre-treatment local ACE levels and percentages of lavage lymphocytes were slightly higher in the patients who achieved maintained radiographic clearing than in those with residual shadows (p<0.1 for both comparisons; Mann-Whitney U test). The FVC and TLC could not be compared in this way since all but three patients had normalized in these measurements after treatment.

Table 3. – Correlations of extent of decrease in inflammatory markers with the extent of clinical improvement

	Extent of improvement at 13 months			
	CXR score	FVC	TLC	DLCO
Serum ACE	NS	NS	NS	r_s=0.37 p<0.05
Lavage ACE*	NS	r_s=0.29 p<0.10	r_s=0.29 p<0.10	r_s=0.47 p<0.01
Serum lysozyme	r_s=0.32 p<0.10	NS	NS	r_s=0.53 p<0.005
Lavage lysozyme*	NS	NS	NS	NS
Serum albumin	NS	NS	NS	r_s=-0.27 p<0.10
Lavage albumin	NS	NS	r_s=0.40 p<0.025	r_s=-0.30 p<0.10
BAL cell counts				
Total cells·ml^{-1}	r_s=0.35 p<0.05	NS	NS	NS
Macrophages·ml^{-1}	r_s=0.36 p<0.05	NS	NS	NS
Lymphocytes·ml^{-1}	r_s=0.30 p<0.10	NS	NS	NS
Lymphocytes %	r_s=0.35 p<0.05	NS	NS	NS
Neutrophils·ml^{-1}	NS	NS	NS	NS
Neutrophils %	NS	NS	NS	NS

For abbreviations see legend to table 2.

Discussion

Serum levels of ACE and lysozyme have been used in the diagnosis and follow-up of sarcoidosis for many years. It has been reported that serial measurements can help distinguish between steroid-responsive and nonresponsive individuals [3, 5, 11, 33, 34] and that rising serum ACE levels can predict radiographic relapses [5, 35]. However, the pre-treatment levels do not appear to have great prognostic value [16]. In view of the evidence that ACE [19–22, 24] and lysozyme [18, 23] can be produced in the lungs, we wondered whether the "local" levels of these enzymes might be better markers of activity and prognosis than the serum levels in patients with pulmonary sarcoidosis.

Rat models show that local production of ACE and alveolar-capillary membrane permeability for proteins can be altered independently from each other in different forms of lung parenchymal injury [36]. Concentration of soluble substances in BAL fluid are difficult to interpret because of the unknown dilution factor and the unknown serum-derived proportion of the substance under investigation. Albumin is considered a good reference substance to correct for alveolar-capillary permeability, because it is produced exclusively outside the lungs and passes into the alveolar space by passive transudation from the serum [37]. In our patients with pulmonary sarcoidosis lavage albumin levels were higher in untreated than in treated patients indicating that there is a disease-associated leakage of the alveolar capillary membrane [7] which is reversible after steroid treatment [38]. We found an inverse correlation between lavage albumin and TLC before treatment, and between the extent of fall in lavage albumin and the amount of improvement in TLC, indicating that the factors responsible for both alveolar-capillary leakage and decrease in TLC, such as inflammation and oedema of the alveolar walls, may be corrected by steroid therapy.

ACE is normally produced in pulmonary capillary endothelial cells. It converts the serum decapeptide angiotensin I into angiotensin II, which is a potent vasoconstrictor and plays a significant role in the short-term regulation of systemic blood pressure [39]. Normal blood monocytes can also be induced to produce ACE *in vitro* by activated T-lymphocytes, probably *via* an unidentified soluble factor [40, 41]. It is speculated that a similar mechanism is responsible for the induction of ACE synthesis in sarcoid alveolar macrophages [41]. The correlation between serum ACE levels and the numbers of macrophages·ml^{-1} in the lavage fluids of our patients with pulmonary sarcoidosis prior to treatment is consistent with this proposal. The elevations in ACE levels in BAL fluid of sarcoid patients [7, 22, 38, 42] suggest that ACE is not only synthesized but is also secreted into the lower respiratory tract. It seems reasonable to assume [8] that ACE may then act on its natural substrate angiotensin I to cause local vasoconstriction. In support of the view that an increase in local ACE production could affect the perfusion of the alveolar walls causing a decrease in DLCO, we observed an inverse correlation between local ACE and DLCO before treatment, and the improvement in DLCO after corticosteroid therapy was parallelled by a decrease in local ACE.

Serum levels of ACE were elevated in only 40% of our patients before treatment, although this percentage can vary in different groups of patients [2, 5]. By contrast with our findings for local ACE serum ACE levels did not correlate with change in lung function on treatment supporting the view [42] that levels of ACE produced in the lungs may be a better indicator of inflammatory activity than ACE levels in serum.

Lysozyme is a cationic enzyme [1,4-beta-N-acetylmuramidase) with bactericidal activity, cleaving beta 1-4 glycosidic bonds in cell walls of certain bacteria. It is normally present in granules of monocytes, macrophages and polymorphonuclear leucocytes and is constantly

released [43] into various body fluids, *e.g.* saliva, tears and airway secretions. It is also present in normal bronchoalveolar lavage fluid [44] but, to our knowledge, this is the first report of lavage levels in patients with sarcoidosis. We observed that the local lysozyme concentrations were in the same order as those in serum, thus, when the dilution effect of lavage is taken into account, levels *in vivo* in lung lining fluid must be far in excess of serum levels. We could not demonstrate any correlations between local lysozyme and lavage cell counts, clinical findings or prognosis; and it seems likely that high physiological concentrations of lysozyme in the BAL fluids may have masked any additional local production linked with the inflammatory disease process.

Lysozyme is not normally found in high concentrations in serum and, in contrast with the lavage findings, the elevated serum lysozyme levels in patients with pulmonary sarcoidosis appear to be a very reliable marker of overall inflammatory activity. Eighty percent of the patients had elevated serum levels before treatment and higher levels were associated with more extensive pulmonary involvement as determined both by radiographic profusion scores and lung function measurements. The decrease of serum lysozyme after treatment also closely reflected the improvement in DLCO. It is thought that the serum lysozyme probably originates mainly from epithelioid cells, macrophages and giant cells in granulomas within involved tissues [18] where it is produced in excess and released either by secretion or due to cell turnover [45]. Our observation of a correlation between serum lysozyme levels and percentages of BAL neutrophils after treatment also indicates that some of the circulating lysozyme in patients with pulmonary sarcoidosis may relate to the neutrophilic component of the inflammatory response. Diffusion of lysozyme from sites of inflammation into the blood stream may be facilitated by its low molecular weight (14,500) [29]. This could explain why serum lysozyme was elevated in a greater proportion of the patients in our study group than serum ACE [13, 14] which has a molecular weight about tenfold higher (150,000) [28].

The possibility that lavage cell populations may have prognostic value in sarcoidosis has been investigated for many years, with a clear emphasis on lymphocytes and lymphocyte subsets [46–48]. Initial data suggested that higher lymphocyte counts may identify patients who are prone to functional deterioration [47]. However, a more recent study has concluded that patients who have higher percentages of lymphocytes (≥28%) before therapy tend to draw a greater benefit from steroid treatment [48]. Similar results have been obtained in the present study. The reason why patients with higher BAL lymphocyte counts achieve a greater degree of radiographic clearing is not understood. The lymphocytes in the lungs of patients with pulmonary sarcoidosis appear to be activated [46] and lymphokine mediators are thought to play a key role in promoting the granulomatous response in sarcoidosis by attracting monocytes to the sites of disease, retaining these cells at involved sites by inhibiting their migration, then activating the monocytes inducing the focalized granulomatous reactions. However, certain lymphokines are multifunctional and interferon-gamma, one of the lymphokines produced in increased amounts in sarcoidosis, can activate macrophages [49, 50], but can also down-regulate fibroblast proliferation [51] and collagen synthesis [52]. In a recent study [53], we have observed that sarcoid patients with higher serum levels of interferon-gamma are less likely to have irreversible parenchymal shadows suggestive of fibrosis. This raises the question whether certain lymphokines may promote the granulomatous reactions on the one hand, yet may also play a role in regulating the extent of the irreversible fibrogenic component of the tissue response.

In conclusion, we have investigated a panel of inflammatory markers in serum and BAL fluid of patients with pulmonary sarcoidosis and determined their correlation with clinical impairment and response to steroid therapy. Serum lysozyme and, to a lesser degree, local ACE and lavage albumin reflected the clinical course more closely than other markers. However, pre-treatment measurements of these markers did not "predict" the clinical outcome at three years. In this respect, BAL lymphocyte counts were superior. This suggests that secretory products, such as lysozyme derived from macrophages and other phagocytic cells, may reflect overall inflammatory activity in pulmonary sarcoidosis, while certain components of the lymphocyte response may have an influence in limiting the degree of residual impairment.

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Valeur comparative des mesures du lysozyme, de l'enzyme de conversion de l'angiotensine, et d'autres marqueurs inflammatoires, dans le lavage alvéolaire vs *le sérum, chez les patients atteints de sarcoïdose pulmonaire. C. Prior, R. Barbee, P.M. Evans, P.J. Townsend, Z.S. Primett F. Fyhrquist, C. Grönhagen-Riska, P.L. Haslam.*

RÉSUMÉ: Le but de cette étude est d'explorer dans quelle mesure les quantités d'ACE et de lysozyme produites dans les poumons sont en corrélation plus étroite que les niveaux sériques de ces enzymes, ou d'autres marqueurs inflammatoires, avec les scores de profusion lésionnelle au cliché thoracique, la fonction pulmonaire, et la réponse thérapeutique des patients atteints de sarcoïdose pulmonaire. Nous avons étudié 25 patients, et les niveaux du lavage bronchoalvéolaire ont été utilisés pour déterminer la production "locale" d'enzymes, en référence avec le sérum et l'albumine du lavage. Avant traitement, les taux de lysozyme sérique étaient élevés chez plus de patients (80%) que les taux sériques d'ACE (40%). Ils s'avèrent également présenter la meilleure corrélation globale avec les mesures cliniques avant le traitement; les chutes du lysozyme sérique sont en parallélisme étroit avec l'amélioration de la fonction pulmonaire (DLCO) ou après traitement. Les seuls autres marqueurs montrant des corrélations significatives avec la gravité de la maladie étaient les décomptes de neutrophiles par ml dans le lavage, ainsi que les mesures "locales" de l'ACE avant le traitement. La valeur des niveaux pré-thérapeutiques des différents marqueurs inflammatoires pour prédire la réponse à la thérapeutique par stéroïdes a été explorée. La seule observation significative était que les pourcentages de lymphocytes du BAL et les nombres par ml s'avèrent initialement plus élevés chez les patients dont les scores radiologiques post-traitement sont les plus bas ($p<0.01$ et $p<0.05$, respectivement). Nous concluons que les taux sériques de lysozyme s'avèrent des marqueurs plus utiles de l'activité globale de la maladie dans la sarcoïdose, que ne le sont les autres marqueurs inflammatoires. Toutefois, les décomptes des lymphocytes du BAL sont les marqueurs de prédiction les meilleurs pour une réponse radiologique aux corticostéroïdes.
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