

## Angiotensin-Converting Enzyme in Bronchoalveolar Lavage Fluid in Sarcoidosis

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**Abstract.** This is the first Australian study of angiotensin converting enzyme (ACE) in bronchoalveolar lavage fluid in 51 patients with sarcoidosis. The aim was: 1) to establish the range of lavage ACE in healthy smokers and non-smoking patients with sarcoidosis. 2) to evaluate the clinical usefulness of lavage ACE. Seventeen control subjects and 51 sarcoid patients all underwent bronchoalveolar lavage, the latter also having <sup>67</sup>Gallium scan, spirometry and carbon monoxide uptake. Eighteen patients had all tests repeated six months later. Lavage ACE was significantly higher in sarcoid non-smokers than control non-smokers ( $p < 0.05$ ). In the 51 sarcoid patients, lavage ACE/albumin ratios were 10-fold higher than serum ACE/albumin ratios ( $p < 0.0001$ ). In sarcoid patients with raised intrathoracic <sup>67</sup>Gallium uptake, lavage ACE was significantly higher than those patients with normal uptake ( $p < 0.05$ ). Expressing lavage ACE as ACE/albumin ratios reduced the statistical significance of correlations with other parameters, eg, lavage % lymphocytes, and lavage IgG. Lavage ACE levels changed concordantly with lung function <sup>67</sup>Gallium scan and lavage lymphocytes, albumin and IgG. However, the wide distribution of lavage ACE in control and sarcoid subjects and the influence of smoking history severely limits its clinical application.

**Key Words.** Angiotensin-converting enzyme. Bronchoalveolar lavage fluid.

### Introduction

There have been no studies in Australia of lavage fluid angiotensin converting enzyme (ACE) in sarcoidosis and its relationship with other indices of disease activity, lung function or clinical aspects. Additionally, most of the overseas studies have expressed the results of lavage fluid protein levels not as absolute concentrations but as protein/albumin ratios to reduce the variability inherent in the technique [1, 2, 3, 4]. However, as the albumin concentration in lavage supernatant is itself usually elevated in active interstitial lung disease [1, 2, 3, 5, 6] changes in protein/albumin ratios can also be misleading.

Lavage fluid ACE is known to be increased in healthy smokers, compared with non-smokers, probably because activated alveolar macrophages [7, 8, 9] are increased in smokers [10]. One report of the effect of smoking on lavage fluid ACE levels in sarcoidosis showed no difference between smokers and non-smokers [5]. A significant correlation was found between lavage fluid ACE levels and lavage fluid lymphocyte concentrations in the sarcoid non-smokers.

The aims of this study were to establish the range of lavage ACE fluid in healthy subjects (smokers and non-smokers) and in patients with active sarcoidosis and to evaluate the usefulness of lavage ACE measurement as a test of disease activity in the lungs.

## Subjects and Methods

### 1. Subjects

Seventeen control subjects, fourteen males and three females, age range 23–64 years ( $46 \pm 14$ ) underwent bronchoalveolar lavage. All were patients being investigated for suspected haemoptysis and/or non-productive cough who had normal chest radiographs and bronchoscopic findings. In none was any disease of the lung parenchyma suspected or later identified. There were six smokers and eleven non-smokers, of whom four had never smoked and the other seven had not smoked for at least two years (mean  $\pm$  S.D.,  $13 \pm 6$  years).

Fifty-one sarcoid patients, 29 males and 22 females, age range 18–69 years ( $39 \pm 2$ ) all had the following investigations – bronchoalveolar lavage, serum ACE, and respiratory function tests. Thirty-five entered the study at the time of diagnosis and the remainder had been diagnosed  $49 \pm 8$  months (mean  $\pm$  S.E.M.) before, and were being re-assessed because of suspected active disease. None were taking corticosteroids at the time of investigation and only one had been treated with corticosteroids in the past. Six patients had normal chest radiographs, 13 had bilateral hilar lymphadenopathy (B.H.L.), 20 had B.H.L. and pulmonary infiltrates, and 12 had pulmonary infiltrates alone. There were 13 smokers and 38 non-smokers. Smokers were defined as those smoking at the time of the study and non-smokers as those who had never smoked or not for the previous two years.  $^{67}$ Gallium scan was performed on 45/51 patients, and in six was not done because of suspected pregnancy. All patients had transbronchial biopsies done at initial assessment which revealed non-caseating granulomata with no pathogens isolated histologically or on culture. Investigations were completed within a fortnight in each case.

Bronchoscopy, lung function tests,  $^{67}$ Gallium scan and serum ACE were repeated in 18 of 51 sarcoid patients who volunteered. This was done  $6 \pm 2$  months (mean  $\pm$  SD) after the initial assessment. There were 14 males and four females, age range 24–69 years (mean  $\pm$  SD,  $41 \pm 3$ ). Two patients had stage 0 radiographs, six had stage I, five had stage II and five had stage III. Four were non-smokers and none changed smoking habits between tests. Seven patients received oral prednisolone between initial and follow-up tests, all but two for respiratory indications. Corticosteroids were ceased at least a week before testing. Investigations were performed with the patients' informed consent and in accordance with the standards of each hospital's ethics committee.

### 2. Methods

#### Bronchoalveolar Lavage

Patients were given a premedication of oral diazepam and intramuscular atropine 30 minutes before the bronchoscopy. The nose and throat were sprayed with 4% lignocaine solution and the fiberoptic bronchoscope passed nasally with the patient supine. With the bronchoscope gently wedged in the right middle lobe or lingula, and with the lumen in view, five aliquots of 20 ml of sterile normal saline at  $37^\circ\text{C}$  were injected down the suction channel. As the saline was injected, the patient inhaled

slowly from residual volume to total lung capacity. Gentle suction ( $< -5$  cm  $\text{H}_2\text{O}$ ) was applied to recover each aliquot before the next aliquot was injected. Particular care was taken to avoid bleeding or bronchial collapse. Blood-stained lavage was excluded because of associated plasma protein contamination. The fluid recovered was collected in a siliconised glass jar and stored on ice before processing immediately afterwards.

#### Cellular Analysis of Lavage

The lavage fluid recovered was strained through two layers of sterile white surgical gauze into a plastic measuring cylinder to remove excess mucus and debris. After the lavage had been gently agitated, 0.2 ml aliquots were centrifuged onto albuminised glass slides in a Shandon–Elliott cytopsin (Runcorn, England) for three minutes at 1000 r.p.m. The slides were then stained with a Romanovsky stain and a differential cell count done on 1000 cells. Cell concentrations were determined with a haemocytometer.

#### Biochemical Analyses

The remaining lavage fluid was centrifuged in siliconised glass tubes for fifteen minutes at 500 g,  $10^\circ\text{C}$ , in a Mistral 4–L centrifuge. The supernatant was stored at  $-20^\circ\text{C}$  for later analyses. Unconcentrated supernatant was used for all assays. Albumin and IgG were assayed by laser nephelometry (Beckman Instruments, Fullerton, California). Serum albumin was measured by autoanalyser.

#### Angiotensin-Converting Enzyme Assay

Serum and lavage samples were transported on ice and stored at  $-20^\circ\text{C}$  until processing. ACE assay was by the spectrofluorometric method of Piquilloud et al. (1970) [11], employing the synthetic tripeptide substrate, L-Hip-His-Leu (Protein Research Laboratories, Japan). ACE activity was expressed as nmol/ml/min of His-Leu generated. The duration of incubation and volumes of lavage fluid and serum used were 2 hr/50  $\mu\text{l}$  and 15 min/10  $\mu\text{l}$  respectively. Validity of the assay was assessed by measuring the rate of generation of His-Leu with different volumes of lavage fluid from a control subject and two patients with sarcoidosis. The 95% confidence limits of serum ACE (mean  $\pm$  2 SD) for normal subjects was 14–41 nmol/ml/min for males and 16–34 nmol/ml/min for females.

To convert the results into the spectrophotometric units used previously [12], spectrofluorimetric results were adjusted by the following equation;

$$\text{ACE activity} = (0.634 \times) + 3.593 \text{ nmol/ml/min}$$

where  $x$  is the spectrofluorimetric value. This relationship was derived by submitting 50 samples to both assays and fitting the relationship between them by linear regression.

#### Respiratory Function Testing

Forced vital capacity (F.V.C.) was derived from flow volume measurements obtained using a heated pneumotachograph (Fleisch N° 4) and a Hewlett–Packard Pulmonary Calculator System 47804A (Palo Alto, Ca, USA). Carbon monoxide diffusion ( $D_{\text{LCO}}$ ) was obtained by the standard single breath technique using a Morgan Transfer Test Model B (Rainham,

Kent, UK). At each test, the patients' haemoglobin was measured and the carbon monoxide diffusion corrected to a haemoglobin concentration at 14.6 g/dl [6]. Predicted normal values were taken from Cotes (1979) [13].

#### <sup>67</sup>Gallium Scanning

A <sup>67</sup>Gallium citrate scan (110 MBq intravenously) preceded the bronchoscopy by about one week. Whole body anterior and posterior studies, including selected views as indicated, were performed at 24 and 72 hours. Quantitative counts in regions of interest were performed in the lungs, liver and mid-thigh with a Toshiba GCA-402 gamma camera. Lung-liver, lung-thigh and liver-thigh ratios were thus determined. The latter were used to supplement the visual assessment of <sup>67</sup>Gallium in the lung parenchyma. <sup>67</sup>Gallium activity in the lung parenchyma was judged either normal or increased by nuclear physicians unaware of the results of the bronchoalveolar lavage or respiratory function tests.

#### Statistical Methods

Comparisons of data fitting a normal distribution, eg, age, volume of fluid recovered, percent lymphocytes, were assessed by two-tailed, non-paired t-test [14]. For non-parametric data, eg, lavage protein concentrations and albumin ratios, the Mann-Whitney U-test was used [15]. For analysis of serial data, the logistic regression to test for trend was used [16] as well as one-way analysis of variance [17]. Pearson's correlation co-efficient was employed for parametric data and for non-parametric data, Spearman's rank correlation co-efficient [15]. A Hewlett-Packard-85 computer (Palo Alto, Ca) with a "general statistics pac" programme (Pennsylvania State University, Penn) was used for calculations. Results were expressed as mean  $\pm$  SE unless otherwise indicated. Tests were regarded as significant if  $p < 0.05$ .

## Results

### Lavage and Serum ACE

The generation of His-Leu from the tripeptide substrate was linear with time of incubation and with volume of lavage fluid, for both control ( $r = 0.98, 0.99$  respectively) and sarcoid lavage fluid ( $r = 1.0, 0.97$  respectively).

### Control and Sarcoid Subjects

There were no significant differences between control and sarcoid subjects in age, volume of lavage recovered and total cell concentrations. The absolute concentrations of lavage

albumin and IgG were significantly higher in sarcoid subjects but lavage fluid ACE activity was not significantly different between sarcoid and control groups (Table I). Serum ACE was significantly higher in the sarcoid patients (Table I).

### Smoking

In the control group, there were no significant differences in age, volume of lavage fluid recovered, total cell concentration or concentrations of lavage fluid ACE, albumin or IgG between smokers and non-smokers. Sarcoid non-smokers had significantly higher lavage fluid IgG, albumin and % lymphocytes than sarcoid smokers. Their ages, volume of lavage fluid recovered, lavage fluid cell concentrations and serum ACE were very similar (Table II). Lavage fluid ACE was higher in sarcoid non-smokers but not significantly so. In both these sarcoid groups, the ratio of lavage fluid ACE/albumin was approximately 10-fold that of serum (Table II). Although there was no significant difference in lavage fluid ACE in sarcoid patients and the control group as a whole, there was a significant difference in lavage fluid ACE in the non-smokers between these two groups (sarcoid non-smokers  $1.18 \pm 0.18$ , control non-smokers  $0.56 \pm 0.12$  nmol/ml/min,  $p < 0.05$ ).

Table I  
BAL Fluid Cell Counts and Protein Levels

	CONTROL SUBJECTS	SARCOID SUBJECTS	P
Subjects, n	17	51	N.S.
Age in years	46 $\pm$ 4	39 $\pm$ 2	N.S.
% vol recovered	55 $\pm$ 13	56 $\pm$ 13	N.S.
Total cell conc ( $\times 10^3$ /ml)	3.10 $\pm$ 0.65	3.40 $\pm$ 0.32	N.S.
% macrophages	92.5 $\pm$ 0.9	78.1 $\pm$ 2.2	< 0.001
% lymphocytes	5.2 $\pm$ 0.8	18.6 $\pm$ 1.9	< 0.001
Lavage fluid ACE	0.71 $\pm$ 0.20	1.05 $\pm$ 0.14	N.S.
Lavage fluid albumin (g/l)	0.039 $\pm$ 0.006	0.099 $\pm$ 0.025	< 0.05
Lavage fluid IgG (g/l)	0.016 $\pm$ 0.009	0.054 $\pm$ 0.017	< 0.005
Serum ACE (nmol/ml/min)	33.6 $\pm$ 2.0	53.5 $\pm$ 3.2	< 0.001

Lavage ACE/albumin was significantly higher than serum ACE/albumin by at least 10-fold in both smokers and non-smoking patients with sarcoidosis (lavage, median = 13.7; serum, median = 1.2,  $n = 51$ ,  $p < 0.0001$ ).

In sarcoid patients there was a positive correlation between lavage fluid ACE and % lavage fluid lymphocytes only in the non-smokers, whereas lavage fluid IgG correlated significantly with lavage fluid lymphocyte concentration in both smokers and non-smokers with sarcoidosis (Table III). When these lavage fluid proteins were expressed as albumin ratios, the above correlations were not significant (Table III).

**Table II**  
BAL Fluid Cell Counts and Protein Levels of Sarcoid Patients Grouped According to Smoking History

	SMOKERS	NON-SMOKERS	P
Subjects, n	13	38	
Age in years	37 ± 4	39 ± 2	N.S.
% vol recovered	57 ± 2	54 ± 4	N.S.
Total cell conc (x 10 <sup>7</sup> /ml)	4.0 ± 0.8	3.2 ± 0.3	N.S.
% lymphocytes	11.5 ± 3.5	21.0 ± 2.0	< 0.01
Lavage fluid ACE (nmol/ml/min)	0.69 ± 0.17	1.18 ± 0.18	N.S.
Lavage fluid albumin (g/l)	0.046 ± 0.012	0.114 ± 0.34	< 0.05
Lavage fluid IgG (g/l)	0.024 ± 0.010	0.066 ± 0.023	< 0.05
Serum ACE (nmol/ml/min)	64.4 ± 8.6	49.8 ± 2.9	N.S.

**Table III**  
Initial Correlations in Sarcoid Patients

	NON-SMOKERS (N = 38)	SMOKERS (N = 13)	TOTAL (N = 51)
Lavage fluid % lymphocytes vs BALF-ACE	0.504*	-0.193	0.435*
Lavage fluid % lymphocytes vs BALF-ACE/albumin	0.109	-0.197	0.071
Lavage fluid lymphocyte concentration # vs BALF-ACE	0.385*	-0.137	0.333*
Lavage fluid lymphocyte concentration # vs BALF-ACE/albumin	0.109	-0.298	0.207
Lavage fluid lymphocyte concentration * vs BALF-IgG	0.400*	0.700*	0.505**
Lavage fluid lymphocyte concentration * vs BALF-IgG/albumin	0.119	0.151	0.030

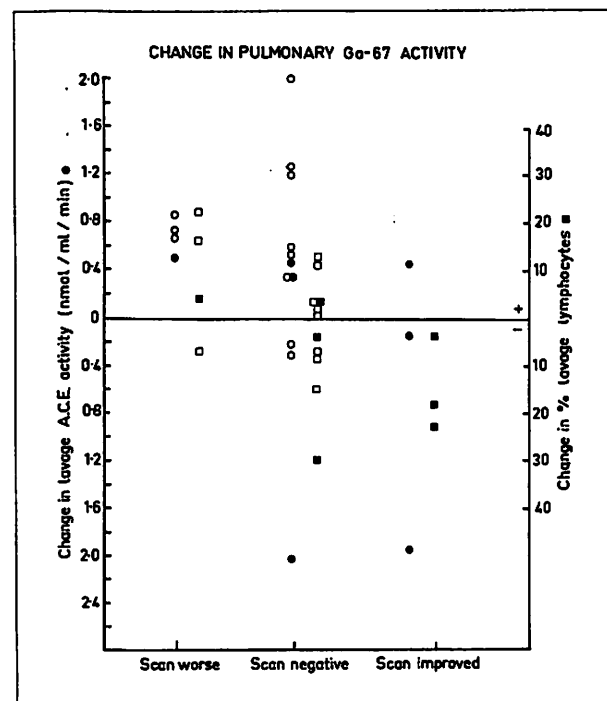
# Pearson correlation co-efficient; \* Spearman's rank correlation co-efficient; \*  $p < .01$ ; \*\*  $p < .001$

### <sup>67</sup>Gallium Scan

There were 35 patients with positive <sup>67</sup>Gallium uptake in the thorax; 12 with hilar node uptake, 13 with lung parenchymal uptake and 10 with both. Serum ACE was not significantly different in any of these groups. However, the patients with positive scans had significantly higher

**Table IV**  
Serum and BAL ACE, and BAL Lymphocytes and Proteins in Sarcoid Patients Grouped According to <sup>67</sup>Ga Result

	<sup>67</sup> Ga-NEGATIVE (N = 10)	<sup>67</sup> Ga-POSITIVE (N = 35)	P
Serum ACE (nmol/ml/min)	54.7 ± 9.2	55.7 ± 3.6	N.S.
% Lavage fluid lymphocytes	13.2 ± 2.0	21.2 ± 2.6	< 0.05
Lavage fluid ACE (nmol/ml/min)	0.48 ± 0.14	1.21 ± 0.19	< 0.05
Lavage fluid albumin (g/l)	0.086 ± 0.022	0.111 ± 0.036	N.S.
Lavage fluid IgG (g/l)	0.030 ± 0.010	0.065 ± 0.025	N.S.



**Fig. 1a.** Changes in BAL fluid ACE activity—(circles), and in % lymphocytes—(squares) in patients whose <sup>67</sup>Gallium scan worsened ( $n = 4$ ), remained negative ( $n = 11$ ) or improved ( $n = 3$ ). Corticosteroids between tests (closed symbols), no corticosteroids (open symbols). One-way analysis of variance showed significant differences in these parameters in the groups whose scans worsened and improved ( $p < 0.05$ ).

lavage fluid ACE and % lymphocytes than those with negative scans ( $p < 0.05$ , Table IV).

In the repeat study, the subjects could be grouped as follows according to the changes observed in their  $^{67}\text{Ga}$  scans; lung parenchymal uptake increased (4 patients "scan worse"), decreased (3 "scan improved") or still negative (11 "scan negative") (Fig. 1a, b, c). Corticosteroids were prescribed between scans in one patient whose scan increased, in the three whose scan decreased and in 3/11 whose scan remained negative. The indication in the first two groups was functional and symptomatic deterioration of pulmonary sarcoidosis. In the third group the indications for corticosteroids were for extra-thoracic disease in all but one patients.

Serum ACE fell in all patients over the study period with the exception of 3/4 patients whose scans had increased  $^{67}\text{Ga}$  lung uptake and who were not taking corticosteroids. Their serum ACE continued to rise during the study.

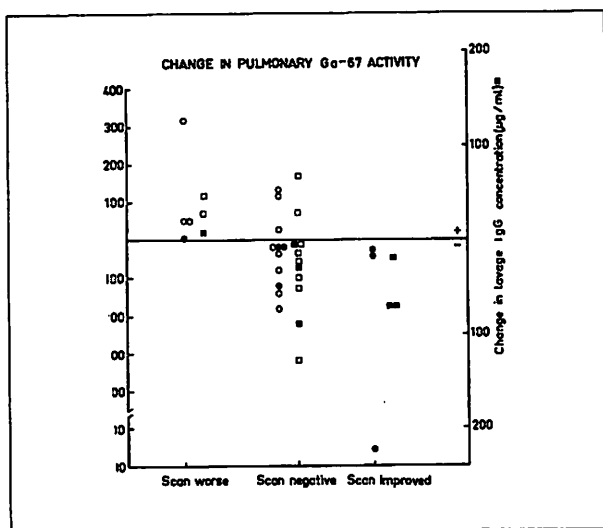


Fig. 1b. Changes in concentration of lavage fluid albumin—(circles), lavage IgG—(triangles) in patients whose  $^{67}\text{Ga}$  scan worsened, remained negative or improved. Corticosteroids (closed), no corticosteroids (open symbols). For change in albumin concentration, there was a significant difference between "scan worse" and "scan negative" groups and also between all groups by one-way analysis of variance ( $p < 0.05$ ). For IgG concentration, for all groups by one-way analysis of variance, ( $p < 0.05$ ), "scan worse/scan negative" ( $p < 0.05$ ), "scan worse/scan improved" ( $p = 0.06$ ).

The volume of lavage recovered in the initial and repeat study was very similar ( $56 \pm 3$  ml,  $55 \pm 3$  ml respectively). Lavage fluid ACE, albumin, IgG and % lymphocytes in all three groups changed concordantly (Fig. 1a, b). There was a rise in all these parameters in the three patients whose lung  $^{67}\text{Ga}$  uptake increased with a corresponding fall in pulmonary function (Fig. 1c). In the group whose scans remained negative, 9/11 had a fall in lavage fluid IgG and albumin with more variable changes occurring in lavage fluid ACE. Lung function remained stable in this group. In the group whose lung  $^{67}\text{Ga}$  uptake decreased, there was a fall in lavage fluid ACE, albumin IgG concentrations with an improvement in lung function.

Although there was a significant negative correlation between the change in serum ACE and change in  $\text{D}_L\text{CO}$  % predicted ( $r = -0.553$ ,  $n = 16$ ,  $p < 0.05$ ), the correlation between change in  $\text{D}_L\text{CO}$  % predicted and lavage fluid ACE was not significant ( $r = 0.267$ ,  $n = 18$ ). There was a signi-

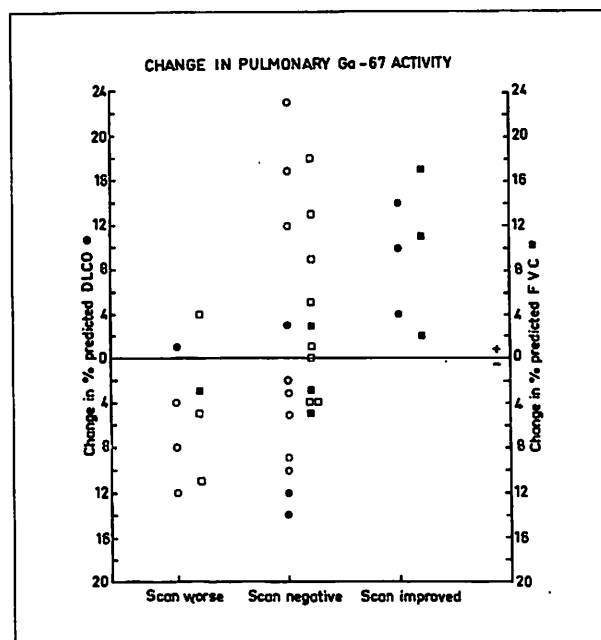


Fig. 1c. Changes in  $\text{D}_L\text{CO}$  % predicted—(circles), F.V.C. % predicted—(squares). Corticosteroids (closed), no corticosteroids (open symbols). By one-way analysis of variance for both change in  $\text{D}_L\text{CO}$  and F.V.C. in groups "scan worse/scan improved" ( $p < 0.05$ ).

ficant correlation between the change in lavage fluid IgG and  $D_LCO$  % predicted ( $r = 0.575$ ,  $n = 18$ ,  $p < 0.01$ ).

## Discussion

This study first established a "normal" range of lavage fluid ACE, as well as albumin and IgG, by a method using unconcentrated proteins which were expressed as absolute values rather than as ratios of albumin. Unfortunately, the normal ranges of lavage fluid ACE, albumin and IgG in control and sarcoid patients were not normally distributed and had large standard deviations. Lavage ACE did not differ between sarcoid and control groups. This would ultimately limit the use in clinical practice of lavage ACE as a pointer to the diagnosis in sarcoidosis.

There was no significant difference in lavage fluid ACE in the control smokers and non-smokers. However, the significantly higher lavage fluid ACE in sarcoid non-smokers than control non-smokers, suggests that a knowledge of smoking history is essential for any subsequent studies of lavage fluid ACE. Our study confirms the findings of one study [5] which found that the ratio of ACE/albumin in lavage fluid is 10-fold that of serum, both in smokers and non-smokers. This suggests that ACE found in lavage fluid is due to active production in the lungs rather than diffusion into the lung from the capillaries. Alveolar macrophages stimulated by adherent T-lymphocytes as well as epithelioid cells are likely to be responsible for this [18, 19].

The significantly higher levels of lavage fluid ACE in sarcoid patients with increased  $^{67}Ga$  activity in the chest is further evidence for the local production of lavage ACE in the lung in sarcoidosis. However, serum ACE did not differ between thoracic  $^{67}Ga$  uptake positive and negative patients. This is not unexpected as serum ACE is the product of the granuloma activity in the whole body.

The changes in lavage ACE and other lavage parameters concordantly with the  $^{67}Ga$

scan findings and lung function alteration (*Fig. 1a, b, c*) suggest that they may reflect disease activity in the patient group as a whole. There was considerable overlap, however, between lavage ACE values in those whose scans worsened or improved and those in whom the scan remained negative. This and the fact that lung function tests and gallium scanning also reflect changes in disease activity and are less invasive than bronchoscopy with lavage, suggest that lavage ACE may be of little clinical usefulness in the detection of changes in disease severity in the individual patient. Lavage fluid ACE measurements cannot therefore be recommended for routine clinical use, either for the diagnosis or for following the activity of sarcoidosis in the individual patient.

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