*Thorax* 1982;37:936–940

# Serum beta-2-microglobulin and angiotensin-converting enzyme activity in sarcoidosis

RW PARRISH, JD WILLIAMS, BH DAVIES

*From Llandough Hospital, Penarth, South Glamorgan*

ABSTRACT Angiotensin-converting enzyme activity in sarcoidosis is regarded both as a diagnostic feature and as an index of disease activity. Increased activity of this enzyme is thought to parallel macrophage and epithelioid cell activity. Beta-2-microglobulin, a low-molecular-weight protein associated with the histocompatibility antigens, is thought to reflect activation of immunocompetent cells, particularly lymphocytes. In 132 patients with known sarcoidosis no close association was found to exist between the results of the two assays (r = 0·53). Angiotensin-converting enzyme activity was raised in 33% and $\beta_2$-microglobulin concentrations in 63% of patients with sarcoidosis. When analysed prospectively, the results of the two assays showed no correlation in 29 patients over periods of up to 19 months. Stage, duration of disease, and corticosteroid treatment showed no significant effect on levels of either angiotensin-converting enzyme or $\beta_2$-microglobulin. The disparity between indices of macrophage and lymphocyte activation requires further study in sarcoidosis.

Detection of activity in sarcoidosis by conventional criteria such as those based on chest radiography or physiological studies is difficult. Cytological analysis of bronchoalveolar lavage fluid may be more informative,[1] but is impractical for repetitive studies. There is a need for a simple test which would reflect the activity of immunocompetent cells concerned in the pulmonary immune effector response.

In 1974 Lieberman reported increased activity of serum angiotensin-converting enzyme in 13 patients with sarcoidosis who were not receiving corticosteroids.[2] He noted that raised levels fell towards normal with corticosteroid treatment, and confirmed these findings in a larger study.[3]

Beta-2-microglobulin is a low-molecular-weight protein which seems to be associated with several membrane proteins. It is present in very low concentrations normally, in a wide variety of body fluids. Mornex[4] reported increased concentrations in sarcoidosis—in all patients with the acute disease or in relapse and also in most patients with quiescent disease. In two of his patients raised concentrations of $\beta_2$-microglobulin fell towards normal with increased corticosteroid dose.

The purpose of the present study was to study serum levels of angiotensin-converting enzyme and $\beta_2$-microglobulin in patients with sarcoidosis and to correlate any relationship between the results of the two assays.

Address for reprint requests: Dr Brian H Davies, Llandough Hospital, Penarth, South Glamorgan.

## Methods

We studied 132 patients with sarcoidosis; 100 patients had angiotensin-converting enzyme assays performed, and all had $\beta_2$-microglobulin assays. The age range was 20–73 years (mean 40·7). There were 23 controls for the angiotensin-converting enzyme and 76 for $\beta_2$-microglobulin, their ages ranging from 20 to 78 years (mean 49). All patients and all controls had normal renal function and none was diabetic. Patients were divided into acute and chronic disease groups, chronic disease being defined as evidence of clinical, radiographic, or physiological sarcoidosis for more than one year. Radiographic staging of pulmonary disease activity was performed according to international criteria.[5] Cases of sarcoidosis with a normal chest radiograph are defined as stage 0. Stage 1 comprises patients with bilateral hilar lymphadenopathy with otherwise normal lung fields. Stage 2 patients have pulmonary infiltration in addition to bilateral hilar lymphadenopathy and stage 3 is the late stage of pulmonary infiltration without lymphadenopathy.

Serum angiotensin-converting enzyme activity was assayed with hippuryl-L-histidyl-L-leucine as a

substrate and orthophthaldialdehyde for fluorometric analysis, the results being expressed in nmol/min/ml.[6] Beta-2-microglobulin was assayed with a standard commercial solid-phase radioimmunoassay (Pharmacia Ltd) and the results are expressed in mg/litre.

Results were analysed using the Student *t* test.

## Results

The serum levels of angiotensin-converting enzyme in controls and patients with sarcoidosis, acute and chronic, with and without corticosteroid treatment, are shown in figure 1. There are significant differences between the population means for controls and those with acute sarcoidosis ($p < 0{\cdot}001$) and between controls and those with chronic sarcoidosis ($p < 0{\cdot}01$). We found no significant difference in angiotensin-converting enzyme activity between the acute and chronic sarcoidosis groups. Angiotensin-converting enzyme activity in patients treated with corticosteroids and in those not so treated did not differ significantly. Classification of patients by radiographic stage did not show significant differences in angiotensin-converting enzyme activity (fig 2).

Mean serum concentrations of $\beta_2$-microglobulin for both acute and chronic sarcoidosis differ significantly from the control mean ($p < 0{\cdot}001$) (fig 3). As with angiotensin-converting enzyme levels, no significant differences emerged between acute and chronic groups or between those treated and those not treated with corticosteroids. We found a small difference, however, between $\beta_2$-microglobulin levels in nine stage 0 patients and 42 stage 1 patients ($p < 0{\cdot}05$). In 84 patients with all stages of sarcoidosis, who had blood taken on the same day for both angiotensin-converting enzyme and $\beta_2$-microglobulin assays, there was no significant correlation between the results of the two assays, either for the group as a whole (fig 4: $r = 0{\cdot}53$) or for patients classified according to stage of disease.

In sequential studies in 29 patients, using within-patient multiple-regression techniques, we found no significant association between levels of angiotensin-converting enzyme and $\beta_2$-microglobulin in individual patients over periods of up to 19 months after the onset of the disease.

## Discussion

Our data on serum angiotensin-converting enzyme activity confirm the previous findings that levels are increased in a proportion of patients with sarcoidosis. Our mean levels (± SE) for acute ($51{\cdot}2 \pm 3{\cdot}0$) and chronic ($47{\cdot}5 \pm 2{\cdot}6$) disease significantly exceed the control mean ($37{\cdot}5 \pm 1{\cdot}7$). We have not confirmed previous reports of significant differences between acute and chronic sarcoidosis,[7] but have confirmed the lack of association with radiographic stage.[8] The mean serum angiotensin-converting enzyme activity

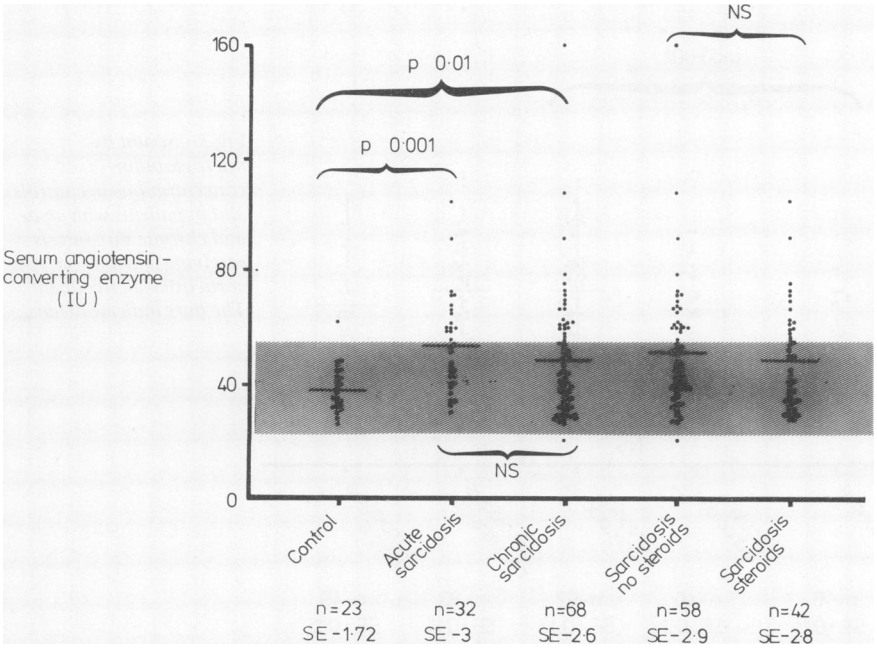


Fig 1 *Serum angiotensin-converting enzyme activity in controls and in patients with acute and chronic sarcoidosis receiving and not receiving corticosteroid treatment. The bars indicate means and the shaded area the control mean ± 2 SD.*

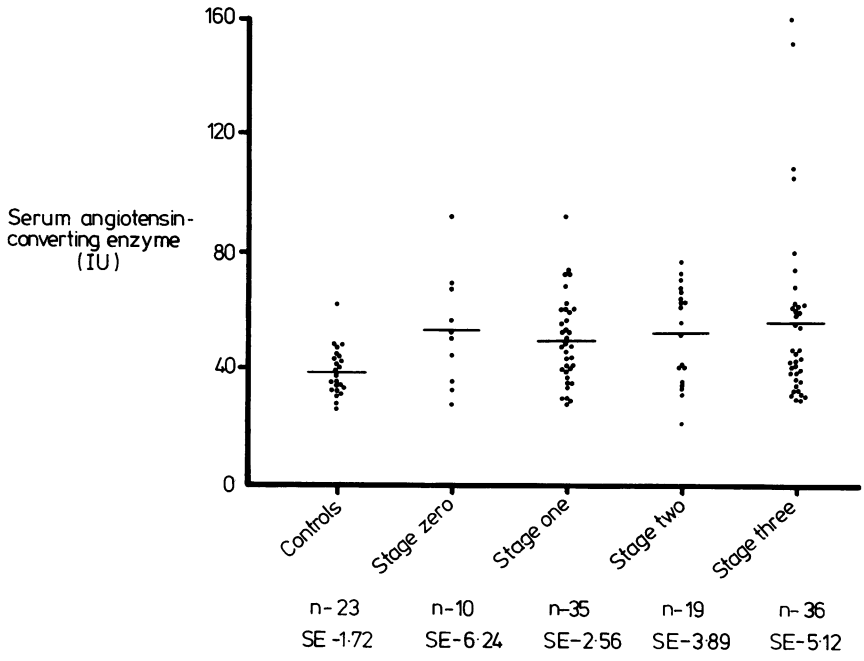


Fig 2 *Serum angiotensin-converting enzyme activity in controls and in patients with sarcoidosis classified by stage of disease. The bars indicate means.*

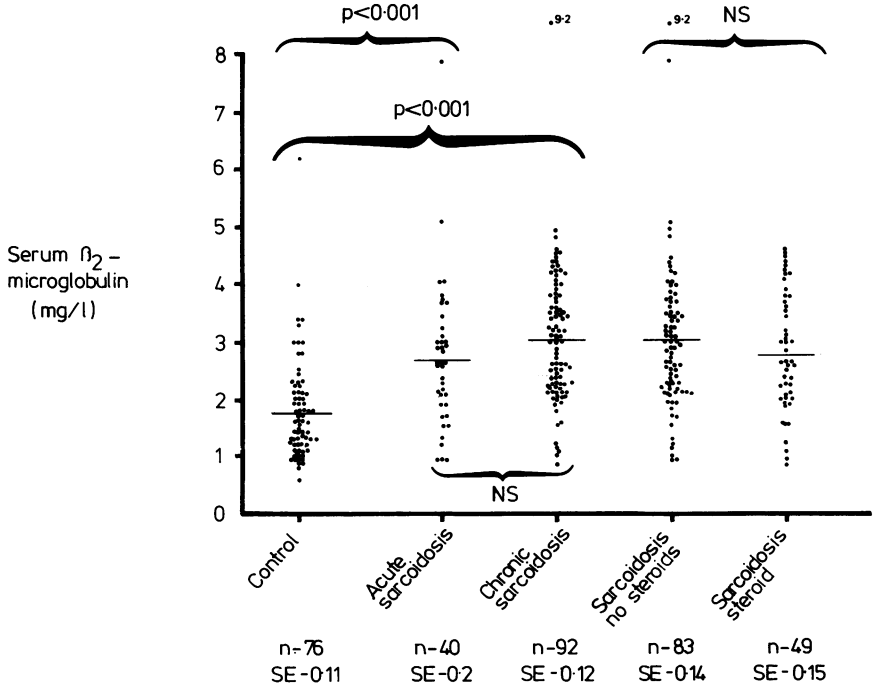


Fig 3 *Serum $\beta_2$-microglobulin concentrations in controls and in patients with acute and chronic sarcoidosis receiving and not receiving corticosteroid treatment. The bars indicate means.*

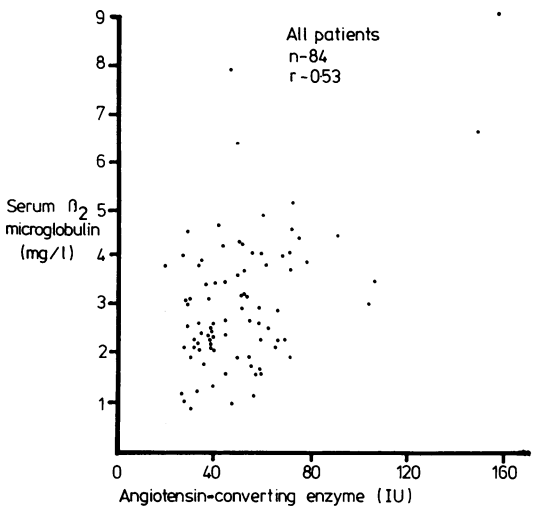


Fig 4 *Scatter diagram showing lack of correlation between levels of serum $\beta_2$-microglobulin and angiotensin-converting enzyme.*

in patients receiving corticosteroids did not differ significantly from that in non-treated patients. This is not what might have been anticipated in view of extensive evidence that corticosteroids depress raised levels of angiotensin-converting enzyme in sarcoidosis.

Our data for $\beta_2$-microglobulin show that in both acute ($2{\cdot}7 \pm 0{\cdot}2$) and chronic ($3{\cdot}05 \pm 0{\cdot}12$) disease the population means significantly exceed the control population mean ($1{\cdot}75 \pm 0{\cdot}11$). This elevation may be a marker of the known pulmonary lymphocyte activation in sarcoidosis. As with angiotensin-converting enzyme, serum $\beta_2$-microglobulin concentrations did not vary significantly with duration of disease or with corticosteroid treatment. In view of macrophage and lymphocyte activation in sarcoidosis we expected that there would be a correlation between indices of activation of these two types of immunological effector cells.

It is currently accepted that estimation of serum angiotensin-converting enzyme in sarcoidosis has an important supportive role in the diagnosis and assessment of the disease.[7] It has been shown to be increased in some patients with sarcoidosis. Levels tend to be higher in acute, disseminated disease, especially when corticosteroid treatment has not been given—as many as 70% of these patients having raised levels.[9] Nearly all reports suggest that high levels of angiotensin-converting enzyme fall towards normal with corticosteroid treatment. Lieberman suggested that a fall in angiotensin-converting enzyme activity in response to corticosteroid treatment was a good prognostic sign, and that serial estimations could be of value in titrating an appropriate maintenance dose.[3]

Silverstein pointed out that angiotensin-converting enzyme activity in the lymph nodes of patients with sarcoidosis was almost always increased and was diagnostically significant.[10] Immunofluorescence techniques have shown that the enzyme is present in most epithelioid and giant cells of sarcoid granulomas and it has been suggested that this is the site of synthesis.[10]

In 1968 Berggård and Bearn isolated $\beta_2$-microglobulin from the urine of patients with renal tubular disorders.[11] In view of its low molecular weight (11 800) unbound $\beta_2$-microglobulin passes freely into the glomerular filtrate. Normally reabsorption by the proximal convoluted tubule is almost complete, and the globulin is catabolised within renal tissue.

Cunningham *et al* determined the entire amino-acid sequence of $\beta_2$-microglobulin and noted similarities with the $CH_3$ region of the IgG molecule.[12] Beta-2-microglobulin, however, does not cross-react with the immunoglobulins or their subunits.

In man $\beta_2$-microglobulin seems to be produced by all cells except mature erythrocytes and the trophoblastic layer of the placenta.[13] It is suggested that HLA antigens are composed of two distinct polypeptide chains—a heavy chain, MW 44 000, which carries the alloantigenic determinants, and a light chain, $\beta_2$-microglobulin, in non-covalent association. This seems to be shed from cells at a constant rate in health. Nilsson[14] showed that human lymphocytes possess on their surface $3\text{–}5 \times 10^5$ molecules of $\beta_2$-microglobulin, but less than $10^5$ molecules of HLA antigen. The amount of transplantation antigens varies with cell type, but cells of the immune system are particularly rich in these antigens. Synthesis of $\beta_2$-microglobulin by PHA stimulated and normal lymphocytes was demonstrated by Bernier and Fanger[15] and several workers have described increased concentrations of $\beta_2$-microglobulin in patients with various lymphoproliferative disorders. High concentrations in the presence of normal renal function are thought to indicate lymphocyte activation. In view of its widespread distribution, serum levels of $\beta_2$-microglobulin are raised in states associated with high cell turnover, as in the fetus[16]; in pregnancy[17]; and in a wide variety of neoplastic,[18 19] inflammatory, and immunological diseases.[20] Because of the many possible causes of a raised concentration of $\beta_2$-microglobulin, it cannot be expected to have discriminating diagnostic value. It is as a marker of lymphocyte activation that levels may parallel the intense lymphocytic infiltrate known to occur in pulmonary sarcoidosis. Beta-2-microglobulin levels in normal individuals are known

to rise with age,[18] but the effect of age on angiotensin-converting enzyme activity is not clear.[21 22]

Our findings suggest that $\beta_2$-microglobulin concentrations do not adequately reflect the activity of sarcoidosis and a more sensitive marker in the systemic circulation is required for appropriate study of this disease.

We are grateful to Drs B Spragg and R Fifield for their co-operation and to Mrs J Holmes for typing the manuscript.

## References


[1] Stanislas-Leguern G, Marsac J, Arnoux A, Lecossier D. Serum angiotensin-converting enzyme and broncho-alveolar lavage in sarcoidosis. *Lancet* 1979;i:723.

[2] Lieberman J. A new confirmatory test for sarcoidosis. Serum angiotensin converting enzyme: effect of steroids and chronic lung disease. *Am Rev Respir Dis* 1974;**109**:743.

[3] Lieberman J, Nosal A, Schlessner A, Sastre-Foken A. Serum angiotensin-converting enzyme for diagnosis and therapeutic evaluation of sarcoidosis. *Am Rev Respir Dis* 1979;**120**:329–35.

[4] Mornex JF, Revillard JP, Vincent C, Deteix P, Brune J. Elevated serum $\beta_2$-microglobulin levels and Clq-binding immune complexes in sarcoidosis. *Biomedicine* 1979;**31**:210–3.

[5] James DG, Neville E, Siltzbach LE, *et al.* A worldwide review of sarcoidosis. *Ann N Y Acad Sci* 1976;**278**: 321–34.

[6] Friedland J, Silverstein E. A sensitive fluorometric assay for serum angiotensin converting enzyme. *Am J Clin Pathol* 1976;**66**:416–24.

[7] Yotsumoto H, Ryujin Y, Hiraga Y, Mikami R. Clinical aspects of S-ACE in sarcoidosis. In: Mikami R, ed. *Proceedings of Nova symposium on sarcoidosis and other granulomatous diseases.* Tokyo: University of Tokyo Press, 1981.

[8] Studdy P, Bird R, James DG, Sherlock S. Serum angiotensin-converting enzyme (SACE) in sarcoidosis and other granulomatous disorders. *Lancet* 1978;ii: 1331–4.

[9] Lieberman J. Applications and limitations of the serum angiotensin-converting enzyme (ACE) assay in sarcoidosis. In: Mikami R, ed. *Proceedings of Nova symposium on sarcoidosis and other granulomatous disorders.* Tokyo: University of Tokyo Press, 1981.

[10] Silverstein E, Friedland J, Pertschuk LP. Angiotensin-converting enzyme (ACE) in sarcoidosis. In: Mikami R, ed. *Proceedings of Nova symposium on sarcoidosis and other granulomatous disorders.* Tokyo: University of Tokyo Press, 1981.

[11] Berggård I, Bearn AG. Isolation and properties of low molecular weight $\beta_2$-microglobulin occurring in human biological fluids. *J Biol Chem* 1968;**243**:4095–103.

[12] Cunningham BA, Wang JL, Berggård I, Peterson PA. A complete amino acid sequence of $\beta$2-microglobulin. *Biochemistry* 1973;**12**:4811–22.

[13] Faulk WP, Temple A. Distribution of $\beta_2$-microglobulin and HLA in chorionic villi of human placentae. *Nature* 1976;**262**:799–802.

[14] Nilsson K, Evrin PE, Welsh I. Production of $\beta_2$-microglobulin by normal and malignant human cell lines and peripheral lymphocytes. *Transplantation Rev* 1974;**21**:53–84.

[15] Bernier GM, Fanger MW. Synthesis of $\beta$2-microglobulin in stimulated lymphocytes. *J Immunol* 1972;**109**:407–9.

[16] Kithier K. Cejka J. Belamaric J. $\beta$2-microglobulin. Occurrence in fetal life and malignancy. *Clin Chim Acta* 1974;**52**:293–9.

[17] Jonasson LE, Evrin PE, Wibell L. Content of $\beta$2-microglobulin and albumin in human amniotic fluid. *Acta Obstet Gynecol Scand* 1974;**53**:49–58.

[18] Teasdale C, Mander AM, Fifield R, Keyser JW, Newcombe RG, Hughes LE. Serum $\beta$2-microglobulin in controls and cancer patients. *Clin Chim Acta* 1977; **78**:135–43.

[19] Takagi K, Itoh Y, Enomoto H, Koyamaishi Y, Maeda K, Kawai T. A comparative study of serum $\alpha_1$-microglobulin and $\beta_2$-microglobulin levels in cancerous and other diseases. *Clin Chim Acta* 1980;**108**:277–83.

[20] Talal N. Grey HM. Zvaifler N. Michaiski J. Daniels T. Elevated salivary and synovial fluid $\beta$2-microglobulin in Sjögren's syndrome and rheumatoid arthritis. *Science*

[21] Ashutosh K, Keighley JFH. Diagnostic value of serum angiotensin-converting enzyme activity in lung diseases. *Thorax* 1976;**31**:552–7.

[22] Khoury F, Teasdale PR, Smith L, Jones OG, Carter JR. Angiotensin-converting enzyme in sarcoidosis: a British study. *Br J Dis Chest* 1979;**73**:382–8.