

Adenosine Deaminase in Progressive Systemic Sclerosis

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The aim of the present study was to confirm the increase of adenosine deaminase (ADA) activity in progressive systemic sclerosis (PSS), described by Sasaki & Nakajima, and to compare plasma ADA activity of patients in different stages of the disease. Enzyme activity was measured with a colorimetric assay.

The 48 patients were subdivided into 3 groups: subgroup 1 ($n=10$), disease still limited to the skin; subgroup 2 ($n=21$), involvement of the skin and oesophagus; and subgroup 3 ($n=17$), involvement of the skin and multiple internal organs.

ADA levels were highest in subgroup 3. However, the difference with respect to subgroup 2 did not reach statistical significance. Subgroup 1 was different from controls and subgroups 2 and 3 ($p<0.001$).

Our results confirm that ADA activity is increased in PSS, and that this finding is observed even in the early stages of the disease process. We speculate that the increase in ADA, a well-known marker of T-cell activation, might be an indicator of disease activity in PSS, in the beginning as well as during phases of exacerbation in later stages of the disease. **Key words:** ADA; PSS; T-cell activation; disease activity.

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Progressive systemic sclerosis (PSS) is a connective tissue disease of unknown etiology and pathogenesis, leading to fibrosis of skin and internal organs. It is characterized by alteration of the microvasculature and collagen deposition. T lymphocytes in cooperation with monocytes have been considered as major agents in the induction of the disease. These cells have complex interactions between each other as well as with fibroblasts, mast cells, endothelial cells and platelets. These interactions are not yet fully understood (1).

Recently an increase in adenosine deaminase (ADA; EC 3.5.4.4) activity has been found in the sera of patients with PSS (2). Since it is generally believed that increased enzyme activity in serum is in large part due to enzyme release from T-cells, these authors consider their finding as support for the implication of T-cells in the pathogenesis of PSS. ADA is a ubiquitous enzyme involved in purine metabolism, where it catalyses the irreversible deamination of adenosine or deoxyadenosine to inosine or deoxyinosine (3). It is an important enzyme in T-lymphocytes, since it plays a role in the maturation of this cell type (4). For this reason it has been extensively studied in leukemias (5, 6). An increased ADA activity has also been reported for diseases such as tuberculosis (7, 8), toxoplasmosis (9), salmonellosis (10), sarcoidosis (11), viral hepatitis (12) and HIV infection (13). The observation of increased ADA activity

is therefore not specific for PSS. However, as indicated by our results in a series of 48 patients, measurement of ADA activity may be of interest in the early stages and in the exacerbation phases of the disease.

MATERIAL AND METHODS

Patients

We examined 48 patients with PSS, 46 women and 2 men, with a mean age of 48 years, range 21-69. The plasma samples were obtained from 29 patients in France and 19 patients in Portugal. The patients fulfilled the standard criteria for PSS of the American Rheumatism Association (14). For each patient a severity score was established according to Hughes et al. (15). In the present study we subdivided the patients into 3 subgroups: subgroup 1 ($n=10$), patients having only skin involvement; subgroup 2 ($n=21$), patients having skin plus oesophagus involvement; and subgroup 3 ($n=17$), patients having skin, oesophagus as well as involvement of lung ($n=17$), heart ($n=4$) and kidney ($n=1$).

Reference plasma samples were obtained from healthy donors of similar age and sex (29 from France and 19 from Portugal). Patients and controls gave their consent to participate in the study.

Plasma ADA activity

Plasma samples obtained from patients and controls were kept at -20°C until measurement. The delay between sample collection and measurement of enzyme activity did not exceed one month.

ADA was determined with a colorimetric assay, described by Giusti & Galanti (16). In the presence of ADA, ammonia is produced from

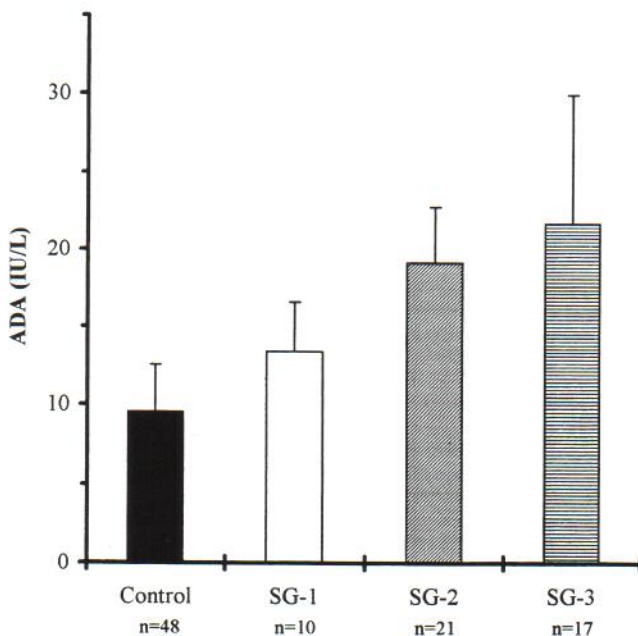


Fig. 1. Mean values \pm SD for ADA activity in plasma of 48 PSS patients. Comparison of subgroups: control/SG-1, $p<0.001$; SG-1/SG-2, $p<0.001$; SG-2/SG-3, NS; SG = subgroup; NS = not significant.

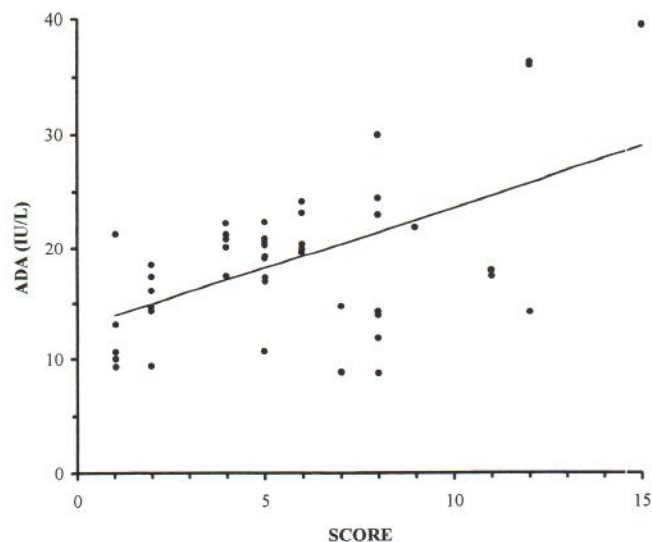


Fig. 2. Absence of correlation between ADA activity values in plasma and disease severity scores in 48 PSS patients (regression analysis: $y = 1.0707x + 12.8121$, $r = 0.2855$, NS).

adenosine and forms an intensively blue indophenol with sodium hypochlorite and phenol in alkaline solution. The reaction catalysed by ADA is stopped at the end of the incubation period by the addition of the phenol nitroprusside solution. The ammonia concentration is directly proportional to the absorbance of the indophenol, which is measured with a spectrophotometer Varian (Series 634) at a UV-wavelength of 620 nm. The reaction obtained with patients' plasma was compared to the standard ammonia, purchased from Boehringer, Mannheim Germany.

The other chemical reagents were purchased from Sigma, Paris.

Statistical analysis

All assays were performed in triplicate. The data were expressed as mean \pm standard deviation and analysed using Student's *t*-test. Linear regression analysis was used to determine correlation coefficients.

RESULTS

According to Giusti & Galanti (16), normal human plasma contains between 10 and 25 IU/ml. The values obtained by us with the 48 reference plasma samples yielded lower values (range 4–13, mean 9.53 ± 3.0 IU/ml). They did not follow a Gaussian distribution.

The results of the ADA assays performed with patients' plasma are summarized in Fig. 1. The plasma ADA activity was 18.65 ± 6.58 IU/l. The difference is statistically significant from controls ($p < 0.001$).

The highest ADA values were observed in subgroup 3, but the difference between subgroups 2 and 3 did not reach statistical significance. The increase observed for patients of subgroup 1 compared to control values and to mean values of subgroups 2 and 3 was statistically highly significant ($p < 0.001$).

The highest value observed in controls was 13 IU/ml. In subgroup 1, 50% of patients had higher values, while this was the case for 90% and 88%, respectively, for subgroups 2 and 3. Values higher than 30 IU/ml were found only in subgroup 3 (4 patients out of 17).

As may be seen in Fig. 2, there was no correlation between individual scores of severity and plasma ADA activity.

The duration of the disease for patients of the three subgroups was 6.6 ± 6.8 years for subgroup 1, 8.8 ± 5.0 for subgroup 2 and 9.4 ± 6.2 for subgroup 3. Again there was no correlation between ADA activity and duration of disease for individuals.

DISCUSSION

The present study confirms the findings of the previous report (2), indicating an increase in ADA activity for PSS patients. The kit used by the Japanese group yielded values of 12.55 ± 2.20 IU/l for the 46 controls studied. The method used by us indicated lower values (mean 9.53 ± 3.00 IU/l for 48 reference samples). Since the distribution of individual results in the control population did not follow a Gaussian distribution, we could not apply the statistical criteria used by the Japanese authors (mean values + 2 SD). However, according to Student's *t*-test, the differences between normal plasma samples and PSS samples were highly significant ($p < 0.001$). The mean values observed in patients were approximately the double of those observed in controls.

While the previous report (2) did not take into account the stages of the disease, we compared the three subgroups and found significantly higher values in patients with internal organ involvement (subgroups 2 and 3) compared to scleroderma limited to the skin (subgroup 1). However, the difference between the patients with oesophagus involvement only (subgroup 2) and those in whom lung, kidney and/or heart were also involved (subgroup 3) did not reach statistical significance because of the important individual variation. Similarly, no positive correlations were observed, if the patients were subdivided according to the severity scores proposed by Hughes et al. (15). Both classifications are based on clinical and morphological findings, such as fibrosis, but do not take into account the infiltration of tissues by T-cells or the presence of biological markers of T-cell activation such as cytokine production (17, 18). Among patients with similar severity scores, certain have high, others low ADA activity.

If ADA activity reflects T-cell activation, as suggested by others (2), one may speculate whether high ADA activity indicates an exacerbation of the disease process in later stages of the disease. We have no data to confirm this hypothesis. Only long term follow-up of larger groups of patients can answer the question whether the ADA assay indeed reflects disease activity. It could then be helpful for therapeutic decisions.

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REFERENCES

1. Perez MI, Kohn SR. Systemic sclerosis. *J Am Acad Dermatol* 1993; 28: 525–547.
2. Sasaki T, Nakajima H. Serum adenosine deaminase activity in systemic sclerosis and related disorders. *J Am Acad Dermatol* 1992; 27: 411–414.

3. Van der Weyden MB, Kelley WN. Human adenosine deaminase. Distribution and properties. *J Biol Chem* 1976; 251: 5448-5456.
4. Adams A, Harkness RA. Adenosine deaminase activity in thymus and other human tissues. *Clin Exp Immunol* 1976; 26: 647-649.
5. Tritsch GL, Minowada J. Differences in purine metabolizing enzyme activities in human leukemia T-cell, B-cell, and null cell lines. *J Natl Cancer Inst* 1978; 60: 1301-1304.
6. Smyth JF, Poplack DG, Holiman BJ, Leventhal BG, Yarbro G. Correlation of adenosine deaminase activity with cell surface markers in acute lymphoblastic leukemia. *J Clin Invest* 1979; 62: 710-712.
7. Ocana I, Martinez-Vazquez JM, Segura RM, Fernandez-De-Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluids, test for diagnosis of tuberculous pleural effusion. *Chest* 1983; 84: 51-53.
8. Burnat P, Le Brumant-Payen C, Perrier F, Renaudeau C, Yvert JP. Adénosine déaminase du liquide pleural: intérêt de sa détermination dans le diagnostic de la tuberculose. *Médecine et Armée* 1993; 21: 431-433.
9. Jaqueti J, Martinez-Hernandez D, Hernandez-Garcia R, Navarro-Gallar F. Adenosine deaminase increased in serum in toxoplasmosis (Letter). *Clin Chem* 1991; 37: 2021.
10. Galanti B, Nardiello S, Russo M, Fiorentino F. Increased lymphocyte adenosine deaminase in typhoid fever. *Scand J Infect Dis* 1981; 13: 47-50.
11. Taylor A. Value of measuring adenosine deaminase in serum of sarcoidosis patients. *Clin Chem* 1996; 32: 2225.
12. Ungerer JPJ, Oosthuizen HM, Bissbort SH, Vermaak WJH. Serum adenosine deaminase: isoenzymes and diagnostic application. *Clin Chem* 1992; 38: 1322-1326.
13. Niedzwicki JG, Mayer KH, Abushanab E, Abernethy DR. Plasma adenosine deaminase 2 is a marker for human immunodeficiency virus-1 seroconversion. *Am J Hematol* 1991; 37: 152-155.
14. Masi AT, Rodnan GP, Medsger TA Jr, Altman RD, D'Angelo WA, Fries JF, et al. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980; 23: 581-590.
15. Hughes P, Gelsthorpe K, Doughty RW, Rowell NR, Rosenthal FD, Sneddon IB. The association of HLA-B8 with visceral disease in systemic sclerosis. *Clin Exp Immunol* 1978; 31: 351-356.
16. Giusti G, Galanti B. Colorimetric method. In: Bergmeyer HU, ed. *Methods of enzymatic analysis*. 3rd edn. Weinheim: Verlag Chemie, 1984: 315-323.
17. Kahaleh MB, LeRoy EC. Interleukin-2 in scleroderma: correlation of serum level with extent of skin involvement and disease duration. *Ann Intern Med* 1989; 110: 446-450.
18. Postlethwaite AE. Early immune events in scleroderma. *Rheum Dis Clin North Am* 1990; 16: 125-139.