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Azathioprine in the treatment of systemic lupus erythematosus. A three-year prospective study

A.J.G. SWAAK*, ***, L.W. STATIUS VAN EPS*,
L.A. AARDEN**, T.E.W. FELTKAMP**

**Department of Internal Medicine, Slotervaart Hospital, Amsterdam; **Department of Autoimmune Disease of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology of the University of Amsterdam, Amsterdam; ***Department of Rheumatology, Dr. Daniel den Hoed Kliniek, Rotterdam, The Netherlands.*

SUMMARY In a prospective study, the effect of azathioprine on the clinical course and the anti-dsDNA profile was evaluated in 17 patients with systemic lupus erythematosus (SLE). During this prospective longitudinal study, exacerbations were never observed. Three periods of continuous anti-dsDNA increases with a doubling time (T2) shorter than 10 weeks were noted. Both the clinical symptoms and the anti-dsDNA levels improved after the administration of azathioprine. These results necessitate a careful double-blind trial for the use of this drug to prevent SLE exacerbations.

Key words: Azathioprine, Systemic Lupus Erythematosus, Anti-dsDNA

INTRODUCTION

The literature on the treatment of systemic lupus erythematosus (SLE) illustrates the difficulties in judging the value of immunosuppressive treatment. The sub-acute remitting and relapsing course of the disease makes it nearly impossible to investigate the effect of immunosuppressive drugs in a small number of patients; till now, studies of large groups of SLE patients are lacking.

The only valid conclusion, based on a review of the literature (1), is the statement by Decker et al. (2) that the use of either azathioprine or cyclophosphamide in the treatment of SLE should be considered investigationally. Our retro- and later prospective studies (3, 4, 5) on the relationship between anti-dsDNA levels and disease activity showed that all observed clinical exacerbations of SLE were preceded by a continuous increase of the anti-dsDNA levels. All patients showing a period of continuous increase of the anti-dsDNA levels with a doubling time (T2) shorter than 10 weeks developed an exacerbation (13 of the 18 exacerbations were even characterized by a T2 shorter than 5 weeks) (5). Four other patients with an increase of the anti-dsDNA

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Correspondence to:
A.J.G. Swaak,
Dr. Daniel den Hoed Kliniek,
Groene Hilledijk 301,
3075 EA Rotterdam, The Netherlands

level showed no exacerbation. In these 4 patients the T2 was longer than 10 weeks.

The possibility of predicting exacerbations by following the anti-dsDNA course raised the question whether this could be used to prevent exacerbations by the administration of immunosuppressives during the period of increasing anti-dsDNA levels. It is possible that the deregulation of the immune response (i.e., shortage of T-suppressor activities and/or increase of T-helper functions) is maximal and, therefore, more sensitive for immunosuppressive drugs during such a stage of the disease. To support the need of such a study, we described 4 remarkable case reports in this study.

METHODS

Determinations of anti-dsDNA

Anti-dsDNA was determined with the Farr assay, modified as described by Aarden (6). As antigen we used ^{3}H-labelled circular bacteriophage (PM_2) DNA with a molecular weight of 5.9×10^6. This ^{3}H-labelled PM_2 DNA was purified in accordance with the method suggested by Espejo and Canelo (7) and found to be free of ssDNA contamination. Up to 50 ul serum were incubated with 100 ug ^{3}H-labelled dsPM_2 DNA. The total amount of antibodies was expressed in Units/ml. One unit is defined as the amount of anti-dsDNA that binds 30% of 100 ng PM_2 DNA under standard conditions.

Selection of patients

After the completion of our prospective longitudinal study (5) on the precise relation between anti-dsDNA levels and the occurrence of exacerbations, from the end of 1977 till the end of 1980, 17 SLE patients meeting the preliminary ARA criteria for the diagnosis SLE (8) were selected. All 17 patients described in this paper participated in the investigations after having been fully informed about our studies. The aim of our study was to treat the patients with azathioprine when the anti-dsDNA levels increased.

According to the following criteria, an increase is said to occur when a) the anti-dsDNA level rises with a doubling time shorter than 6 weeks over two consecutive periods of 3 to 6 weeks, with an anti-dsDNA starting point of at least 10 units/ml, or when b) there was an eightfold or greater increase within 6 weeks. Clinical activity was divided into minor and major symptoms, as described earlier (3, 4, 5, 9).

All patients were followed at the Department of Internal Medicine of the Slotervaart Hospital by A.J.G. Swaak and L.W. Statius van Eps. Serum samples of these patients were obtained at intervals of at least 3 to 4 weeks for routine clinical-biochemical determinations (ESR, haemoglobulin concentration, amounts of leukocytes and thrombocytes, creatinine, serum transaminase), and for anti-dsDNA. Urine analyses were also carried out at the same time.

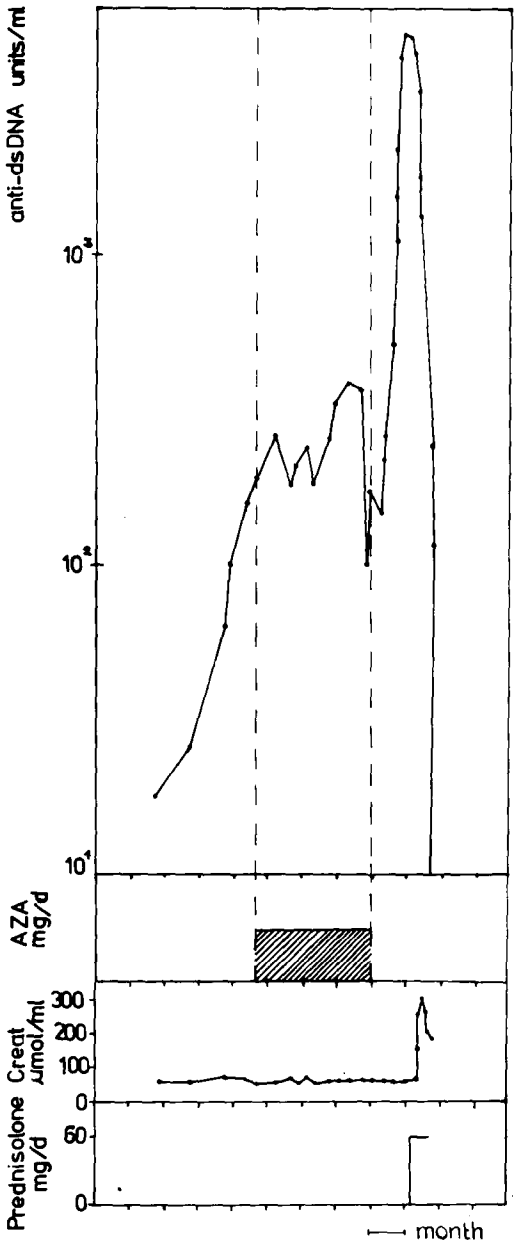
Clinical screening of patients at 6 to 8 weeks intervals was routinely performed by either one of us. When the patients had more complaints of disease, the frequency of clinical and serological investigation was increased to, if possible, weekly intervals.

RESULTS

In 1977, during our prospective study (3), a remarkable observation was made in the clinical course of a 33-year old female patient with SLE. Since 1964, when she was hospitalized with a nephritis, she was known to be suffering from SLE. After that period, she was frequently readmitted to hospital (1967, 1970, 1973, 1976) with exacerbations of her disease (mostly with haematological abnormalities and pleuropericarditis). At the end of 1976, she complained of increasing pain in the joints, subfebrile temperature and fatigue. During this period, an increase of the anti-dsDNA was observed (Fig. 1). Because of these two phenomena (minor

symptoms and a continuous anti-dsDNA increase), the decision to start therapy with azathioprine (2 mg/kg/day) was made. After a very short interval, the anti-dsDNA increase halted, and she also felt better. At the end of May 1977, azathioprine was stopped; within a few days, a very sharp increase of the anti-dsDNA level was observed which ended in an exacerbation.

This observation and the conclusion of our prospective studies (3, 4), that every continuous increase of anti-dsDNA with a T2 shorter than 10 weeks heralds an exacerbation, prompted us to evaluate the effect of azathioprine in the phase of continuously increasing anti-dsDNA levels.



Effect of azathioprine in the period of a well-defined increase of the anti-dsDNA levels

In Table I, the follow-up data and the clinical characteristics of the 17 patients with SLE are depicted. During the 3 years of observation, from the end of 1977 till the end of 1980, all patients had periods of minor symptoms mainly of the musculo-skeletal system, well-controlled with anti-inflammatory drugs, plaquenil or low doses of prednisolone (<20 mg//day). During this observation period, three episodes of defined anti-dsDNA increases could be investigated (figs. 2, 3, 4). In all the cases, the observation of our first patient was confirmed. Almost immediately after the start of azathioprine, the anti-dsDNA increase halted, followed by a slow decrease. In the other 14 patients the anti-dsDNA levels

Fig. 1: Relationship between the start of azathioprine, anti-dsDNA course and the disease activity (renal function). To illustrate the continuous increases of the anti-dsDNA levels, the values are expressed on a semi-log scale. On February 18, azathioprine (100 mg/day) was started due to the increases of the anti-dsDNA level and disease activity (arthralgia weight loss). Clinically, all signs of disease activity disappeared.
The decisions to stop the azathioprine was made (May 27, 1977) on clinical grounds (reappearance of the minor disease symptoms). Laboratory investigation showed increasing anemia and leukocytopenia. From June 24 onwards, signs of high temperature and arthritis developed. Urine analysis showed hyaliene cell cylinders. At this moment, treatment with prednisolone (60 mg/day) was started, however, a further deterioration of the renal function developed and the patient died.

remained stable; in none of these patients was it necessary to change treatment. At the end of this three-year prospective study none of the 17 patients had developed an exacerbation.

DISCUSSION

This open pilot study describes the effect of azathioprine on the anti-dsDNA course in 4 SLE patients. What was remarkable in this prospective longitudinal study over 3 years was that an exacerbation never occurred in the 17 patients with SLE. In the same period, 13 other patients with SLE were followed in collaboration with our department at the Department of Internal Medicine of the Free University Hospital (Amsterdam). These patients were treated on clinical grounds and irrespective of their anti-dsDNA profile. In this group of 13 patients, 3 developed an exacerbation (4). Each of the observed exacerbations was preceded by a continuous increase of the anti-dsDNA levels, fulfilling the criteria of the present study. The obtained results combined with our prospective longitudinal study (5) strongly support the possibility of preventing exacerbations by the administration of immunosuppressives during the period of increasing anti-dsDNA levels. One drawback to our results is that all patients were characterized as having anti-dsDNA antibodies (Farr assay). Recently, a patient with CNS involvement was described (10). In this patient anti-dsDNA antibodies could only be

Table I: The clinical characteristics and follow-up dates of the 17 patients with systemic lupus erytgematosus

					Year and kind of the last exacerbation		Follow-up data		
Patient no.	Date of birth	Sex	ARA criteria*	Onset of disease	year	clinical signs*	clinical signs*	anti-dsDNA levels units/ml	therapy
1	1917	♂	7, 8, 12, 14	1962	1977	14	7	< 10	7,5 mg P
2	1943	♀	1, 2, 5, 6, 8, 14	1969	1972	1, 2, 5	6	20-80	plaq.
3	1943	♀	1, 2, 3, 4, 7, 8, 12	1975	1975	1, 2, 3, 7, 8, 12	3, 6	10-100	–
4	1944	♀	1, 2, 3, 4, 7, 8, 10, 11, 14	1965	1977	10, 11	1, 7	< 20	5-7,5 mg P
5	1911	♂	3, 6, 7, 8, 12, 14	1974	1974	12, 14	7	< 10	–
6	1930	♀	1, 2, 3, 6, 7, 8, 9, 10, 11, 14	1973	1974	10, 11, 14	1, 2, 6	10-40	plaq.
7	1943	♀	3, 7, 8, 12	1970	1970	12, 14	7	< 20	–
8	1946	♀	1, 2, 3, 4, 7, 8, 9, 13, 14	1965	1977	13, 14	1, 2, 3	< 10	plaq.
9	1945	♀	7, 8, 12, 14	1977	1977	12	–	< 10	–
10	1902	♀	1, 2, 7, 8, 12	1974	1974	12	7	< 10	–
11	1923	♀	7, 8, 12, 14	1976	1976	12, 14	7	< 10	5 mg P
12	1951	♀	1, 2, 3, 7, 8, 12, 14	1972	1974	12	1, 2	10-20	plaq.
13	1950	♀	1, 2, 3, 4, 7, 8, 10, 11, 14	1968	1972	10, 11, 14	2, 7	< 10	7,5 mg P
14	1914	♀	1, 3, 7, 8 14	1960	1973	14	7	< 10	7,5 mg P
15	1937	♂	1, 3, 4, 6, 7, 8, 11, 14	1972	1972	11	2, 7	– *Figure 2* –	
16	1899	♀	1, 2, 3, 4, 6, 7, 8, 12, 14	1945	1977	12, 14	2, 7	– *Figure 3* –	
17	1931	♀	7, 8, 12, 14	1980	1980	12, 14	7	– *Figure 4* –	

P = prednisone
plaq. = plaquenil
* Clinical signs refer to the ARA criteria (8)
1 Butterfly rash
2 Discoid lupus
3 Raunaud's phenomenon
4 Alopecia
5 Photosensitivity
6 Oral or nasopharyngeal ulceration
7 Arthritis without deformity
8 LE cells
9 False-positive STS
10 Profuse proteinuria
11 Cellular casts
12 Pleuritus and/or pericarditis
13 Psychosis and/or convulsions
14 Haemolytic anaemia and/or leukopenia, thrombocytopenia

detected in the PEG assay. Anti-dsDNA antibodies measured with the Farr assay are of high avidity; low avidity anti-dsDNA is not detected by this method. With the PEG essay both high and low avidity anti-dsDNA can be detected (10). These facts showed that our 17 patients were selected on having high avidity anti-dsDNA antibodies. However, so far, an exacerbation has never observed in an SLE patient with high avidity anti-dsDNA antibodies (Farr assay) not showing a continuous anti-dsDNA increase.

Till now prospective systematic longitudinal studies showing the relation between the anti-dsDNA level and disease activity are scarce and suffer from inadequate quantitation of anti-dsDNA and infrequent serum sampling. This might be the reason why our results (3, 5) have still not been confirmed by others. All 4 observed patients treated with azathioprine, showed a slope of the increase in the anti-dsDNA levels followed by a slow decrease immediately after the start of azathioprine. This decrease in the anti-dsDNA levels was much slower than found during a period of an exacerbation, in which $T\frac{1}{2}$ of the anti-dsDNA levels of less than 2 weeks is observed (5).

The patient depicted in Fig. 1 afforded two important observations; 1) the negative

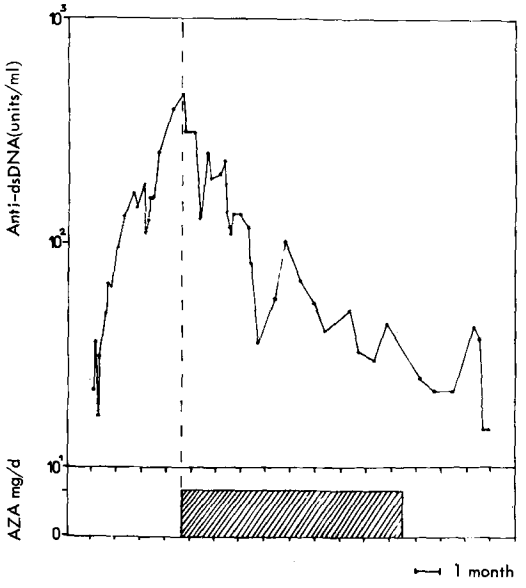


Fig. 2: Relationship between the anti-dsDNA course and the start of azathioprine in patient no. 15.
All clinical and biochemical parameters remained constant till September 1977. From this point, anti-dsDNA increased from 10 units/ml. At that moment, a T2 was reached of 3 to 4 weeks and the decision was made to start treatment with azathioprine (150 mg/day). At this point, the anti-dsDNA level decreased and no major symptoms developed in the 30 weeks following the beginning of the treatment with azathioprine.

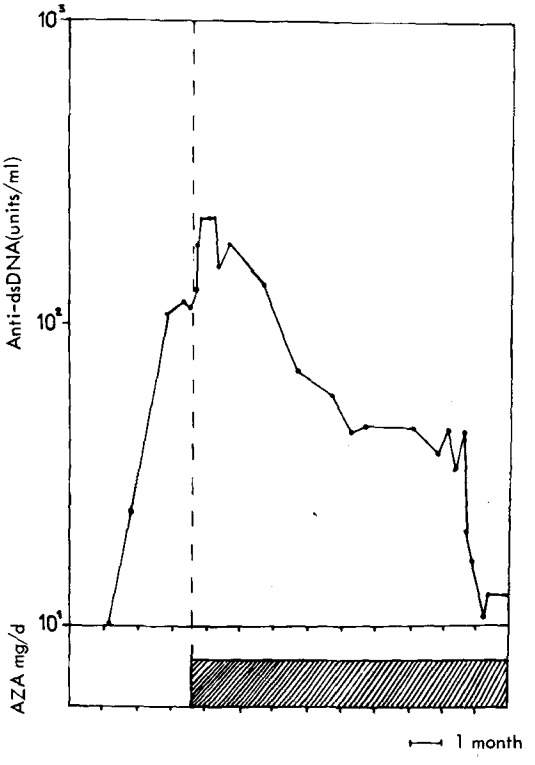


Fig. 3: Relationship between the anti-dsDNA course and the start of azathioprine in patient no. 16. In the beginning of January 1978 the patient developed periods of arthritis. The anti-dsDNA levels remained low till March. On March 31, the anti-dsDNA value was 24 units/ml; on April 28, 108 units/ml and a further increase in value to 125 units/ml appeared on May 19. At this moment, according to the defined continuous increase of the anti-dsDNA, azathioprine therapy (100 mg/day) was started. During the first 4 weeks, following the start of azathioprine, the anti-dsDNA increase continued, then slowly decreased. Clinically, all symptoms of fatigue and arthritis improved.

effect of azathioprine on the anti-dsDNA increase and, 2) the relationship between the withdrawal of azathioprine and the rapidly developing exacerbation. That discontinuation of azathioprine might be related to a high incidence of exacerbation has been described previously (11, 12). Both observations indicate that azathioprine may have a beneficial effect on the course of the disease.

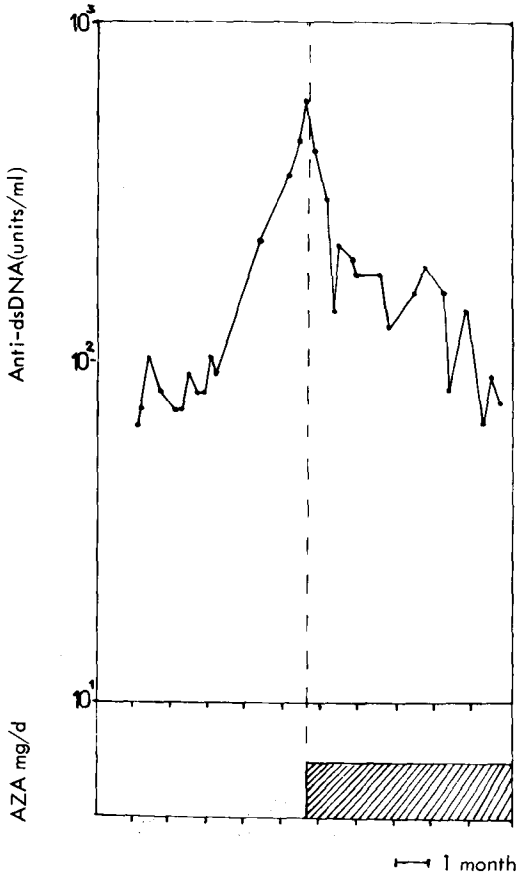


Fig. 4: Relation between the anti-dsDNA course and the start of azathioprine in patient no. 17. The anti-dsDNA level remained stable till the end of July 1980. Then an increase was noted from 100 to 250 units/ml (August 1, September 7, respectively). On October 1, the anti-dsDNA level amounted to 350 units/ml, and on the 17th of October 600 units/ml. At this moment, a continuous anti-dsDNA increase was reached with a T2 of 4 weeks over two periods of 4 weeks and azathioprine therapy (100 mg/day) was started. During the period of increasing anti-dsDNA, the patient had clinically increasing complaints of arthralgia with signs of arthritis and temperature. After the start of azathioprine therapy, she began to feel well within 2 weeks. All other parameters remained stable.

Till now, cytotoxic drugs are in widespread use in the management of systemic lupus erythematosus. The largest experience relates to the therapy with cyclophosphamide and azathioprine. Attempts to treat patients with SLE with immunosuppressive drugs have had varying successes, and the efficacy and specific indications for this treatment are still controversial.

In animal experiments, e.g. the immune-complex nephritis of the NZB mouse, both drugs are effective in suppressing the course of the disease (13, 14, 15). Also in patients with SLE, these drugs are reported to be beneficial when employed in combination with prednisolone (16, 17, 18, 19, 20, 21). Nearly all studies, evaluating the effect of cytotoxic drugs, have several drawbacks: a) The therapy with the cytotoxic drug is usually started when the patient is at a severe stage in his oscillating disease course; therefore, it is unlikely that an overall improvement will be obtained (16, 17). b) Several studies described the effect of cytotoxic therapy on the change of laboratory values rather than on the course of the disease (19). c) A third pitfall is that nearly all studies are characterized by a too short follow-up period, limited to small number of patients, non-prospective, uncontrolled and with differences in criteria used to define the disease activity.

In contrast to the mentioned beneficial effects of cytotoxic drugs (16, 17, 18, 19, 20, 21), other studies concluded that cytotoxic drugs in combination with prednisolone, compared with prednisolone alone, were of no value (22, 23, 24).

Our study also concerns a small number of patients, uncontrolled. Therefore, it prompted us to start a double-blind controlled clinical trial of the effect of azathioprine on the course of SLE.

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