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A 10-Year Matched-Pairs Study Comparing Azathioprine and no Immunosuppression in Multiple Sclerosis

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Summary. In a retrospective matched-pairs study the efficacy of azathioprine treatment was compared with no treatment over a period of at least 10 years. Of 277 patients with clinically definite multiple sclerosis seen during the years 1973 and 1974, 42 pairs were selected by similarity in disability score, sex, age and disease duration. Only 3 patients were lost to follow-up, and in 2 cases the initial diagnosis could not be confirmed. At the end of the 10-year period the number of wheelchair-bound, bedridden or deceased patients was double in the untreated as compared with the azathioprine-treated group but the number of non- or only minimally handicapped patients was similar in each group. The mean disability score was significantly lower in the treated group. Although caution is warranted as in every retrospective study because of insufficiently controlled confounding variables, these results support a positive but weak long-term effect of azathioprine.

Key words: Multiple sclerosis – Azathioprine treatment – Immunosuppressive – Long-term

Introduction

Based on evidence supporting an important role of autoimmunity in the pathogenesis of multiple sclerosis [26], numerous immunosuppressive and immunomodulating agents have been used in attempts to control the disease. Since the early 1960s azathioprine (Aza) has probably been the drug most frequently used for the long-term immunosuppressive treatment of multiple sclerosis.

The authors of several uncontrolled studies have claimed a lower frequency of relapse and a slowing down of disease progression in Aza-treated patients (for review see [5, 9, 17]).

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One open controlled study [20, 21] including 98 patients who were observed for 2 years showed a decrease in the progression of the neurological deficit in Aza-treated patients as compared with linoleic acid in the group with an intermittent-progressive course, i.e. patients with relapses and incomplete remissions, but not in those with complete remissions or a chronic progressive course.

A small double-blind study by Mertin et al. [18] did not show a significant difference but a tendency in favour of Aza as compared with placebo. It was followed by a large British and Dutch multicentre double-blind study including 354 patients, randomized either to placebo or to Aza and treated for at least 3 years. From the 2nd year of treatment onwards, Aza-treated patients had a lower disease progression as assessed by several scales, but the difference was only marginal [2].

It may be argued that a slowing down of disease progression, which may look small in a period of 2 or 3 years, can result in a very important difference in a disease with a mean duration of 30 years [13]. In order to evaluate the effects of Aza treatment over a period of 10 years, we have conducted this retrospective matched-pairs analysis.

Methods

Patient Selection. In a first step the charts of all 277 patients with a clinically definite diagnosis of multiple sclerosis [25] who were seen in the Department of Neurology of the University of Würzburg during the years 1973 and 1974 were reviewed. Only those patients who at this time fulfilled the criteria for being offered treatment with Aza (relapsing course with two or more exacerbations with or without conversion to secondary progression or primary chronic progressive course) [16] were included if they met the following conditions:

1. Well-documented neurological examination at the beginning of the observation period (not during an acute relapse)
2. Age less than 55 years at the beginning of the observation period

3. Residence within 150 km of the study centre (Würzburg)
4. Ambulatory at the beginning of the observation period; expanded disability status scale (EDSS) ≤ 6.5 [12]
5. Documented treatment with Aza for 2 or more years or no immunosuppressive treatment at all (short-term steroid treatment in acute relapse was not regarded as immunosuppressive in this context).

From these patients, we formed pairs with one Aza-treated and one untreated patient. The pairs were chosen to be comparable in respect to the following criteria: EDSS score: 0–2.5 or 3–4.5 or 5–6.5; sex; age (± 4 years); duration of disease.

Assessment. Patients were asked by letter to come to our clinic for re-examination. If after several attempts no re-examination in our clinic could be achieved, we tried to obtain information on the patient's actual clinical state by telephone calls to them and their doctors as well as by sending them questionnaires. Of patients who had died in the meantime, we obtained information from their general practitioners and from their death certificates. Re-examinations consisted of a quantified neurological examination (neurostatus) by two examiners as well as the EDSS [12] and the ambulation index [8, 10].

Compliance. The compliance of the Aza-treated patients was regarded as good if an intermitting leucopenia and/or elevated corpuscular volume of the erythrocytes (≥ 95 fl) could be verified and to be insufficient if the patient said at re-examination that he/she did not take the drug regularly.

Statistical Analysis. The main criterion for the statistical analysis was the EDSS score at the end of the follow-up. A *t*-test was used for paired samples where values were normally distributed and Wilcoxon's U-test for the analysis of not normally distributed values. Proportions were evaluated with the chi-square test. *P* values were based on a two-tailed test.

Results

Patients

According to the criteria outlined above, 42 pairs of patients were selected. From these 84 patients we were able to re-examine 51 patients, 21 of them without, 30 with Aza treatment. This included 17 pairs in which both partners have been re-examined. From 28 other patients (17 without and 11 on Aza treatment), we could obtain enough data on their actual state to evaluate the EDSS score. Twelve patients had died in the meantime. In 3 cases we only knew that the patients were still alive, all of them without immunosuppressive treatment. Two other patients had to be excluded from the study. One had died of a cervical spinal tumour, which was obviously the reason for the misdiagnosis in our clinic. In a second case we found that the diagnostic criteria had not been fulfilled.

These 5 patients were not considered in our statistical analysis. At the end we had 37 pairs of which we could compare the EDSS at the beginning and the end of follow-up.

Duration and Compliance of Aza Treatment

The prescribed Aza dosage was 2–2.5 mg/kg body weight. It could be seen that the duration and the compliance of Aza treatment showed great differences during the 10-year follow-up. Two patients had only taken Aza before 1974, but because of the criteria fixed above they had to be taken into consideration in the Aza group. Two other patients had taken Aza less than 12 months and 4 patients not longer than 24 months after 1974. The mean duration of Aza treatment to January 1974 was 33 months [standard error of the mean (SEM) 3.0 months] and during the follow-up 40 months (SEM 6.4 months). Twenty-six patients had a good compliance; 10 of them took Aza for 5 and more years during the 10-year follow-up. For 12 patients not enough data were available to determine the compliance and in 3 patients the compliance was insufficient.

Comparison of the Groups

At the beginning of the 10-year period, both groups, the 37 Aza-treated and the corresponding 37 untreated patients, were comparable with regard to age and average disability measured by EDSS. Only the Aza patients' mean duration of the disease on the 1 January 1974 was somewhat, but not significantly, higher (see Table 1). The 17 pairs who could be re-examined thoroughly in our clinic were also very comparable (see Table 2). Significant differences could only be seen concerning the course of the disease up to the beginning of the 10-year follow-up [*P* (chi-square) = 0.01]. The course of the disease at the time of re-examination could only be evaluated for 51 patients who had been re-examined by us. In the Aza-treated group, there was a significant tendency to change from purely remittent to chronic progressive disease courses, whereas among the untreated patients the distribution of different disease courses remained quite stable. At the end of the follow-up, the difference in the distribution of disease courses was no longer significant [*P* (chi-square) > 0.05; see Table 3].

Comparison of the Disability at the End of the 10-Year Period

The mean EDSS increased in both groups: from 3.5 to 6 in patients without immunosuppression and from 3.4 to

Table 1. Comparison of the 37 pairs of treated and untreated patients for whom we could define an expanded disability status score (EDSS) at the end of the 10-year follow-up (mean ± SEM, *t*-test and U-test for linked random samples)

	Patients without immuno-suppression	Patients with Aza treatment	Probability for α-error
Sex (F/M)	22/15	22/15	
Age at entry (years)	36.9 ± 1.3	36.5 ± 1.3	$P(t) = 0.34$
Duration of the disease at entry (years)	7.6 ± 1.1	9.0 ± 1.0	$P(U) = 0.36$
EDSS at entry	3.5 ± 0.3	3.4 ± 0.2	$P(t) = 0.24$
EDS at the end of the follow-up	6.0 ± 0.5	4.9 ± 0.5	$P(t) = 0.028$

Aza, Azathioprine

Table 2. Comparison of the 17 pairs of patients who could both be re-examined at the end of the 10-year follow-up (mean ± SEM, *t*-test and U-test for linked random samples

	Patients without immuno-suppression	Patients with Aza treatment	Probability *P* for α-error
Sex (F/M)	9/8	9/8	
Age at entry (years)	33.9 ± 2.2	33.4 ± 2.1	$P(t) = 0.37$
Duration of the disease at entry (years)	5.9 ± 1.2	6.7 ± 1.4	$P(U) = 0.44$
EDSS at entry	2.8 ± 0.4	2.9 ± 0.3	$P(U) = 0.90$
EDSS at the end of the follow-up	4.1 ± 0.7	3.7 ± 0.5	$P(t) = 0.57$
Neurostatus score	89 ± 21	65 ± 11	$P(U) = 0.37$
Ambulation index at the end of the follow-up	3.4 ± 0.8	2.3 ± 0.4	$P(U) = 0.149$
Duration of Aza treatment before entry of the follow-up	–	24 ± 4.9	–
During the follow-up (months)	–	59 ± 9.5	–

Table 3. Distribution of different disease courses[a] in both groups at the beginning and at the end of the follow-up

	Course at entry					
	Relapsing		Relapsing-progressive		Chronic-progressive	
	Untreated	Aza-treated	Untreated	Aza-treated	Untreated	Aza-treated
Course 10 years later						
Relapsing	11	13	0	0	0	0
Relapsing-progressive	1	5	0	1	0	0
Chronic-progressive	0	8	3	2	6	1

[a] The relapsing-remitting category includes patients with complete remissions (EDSS < 2.0) between exacerbations and those with only partial remissions and hence accumulating neurological deficit. The relapsing-progressive category includes patients with both progression and superimposed attacks. The chronic progressive category includes patients with steady progression without exacerbations

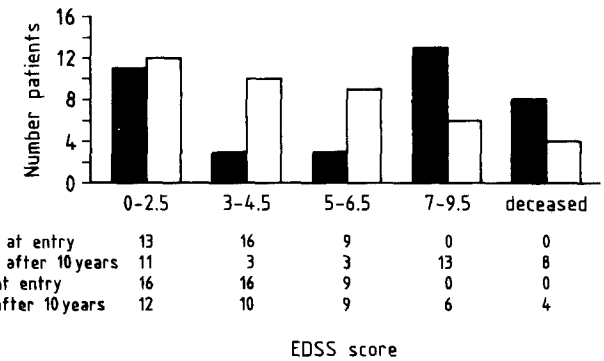

Fig. 1. Number of patients in each EDSS group after 10 years. *P* (chi square) < 0.05. ■ No. IS after 10 years, □ Aza after 10 years

Table 4. Comparison of all 79 multiple sclerosis patients, whose data were obtained concerning duration of treatment and compliance (mean ± SEM); in parentheses probability *P* for identity with the untreated patients' group. Chi-square-test or U-test (Wilcoxon-Mann-Whitney)

	Patients without immuno-suppression	Azathioprine treated patients		
		Good compliance		Compliance bad or unknown
		Treatment > 60 months	Treatment < 60 months	
Number of patients	38	10	16	15
Age (years)	36.9 ± 1.2	32.4 ± 3.0 (0.15)	37.4 ± 1.8 (0.94)	38.9 ± 2.2 (0.46)
Sex (F/M)	23/15	8/2 (0.60)	9/7 (0.99)	9/6 (0.78)
Duration of the disease up to 1.1.1974 (years)	7.8 ± 1.1	7.0 ± 2.2 (0.65)	9.5 ± 1.4 (0.25)	9.8 ± 1.5 (0.16)
EDSS at the beginning	3.6 ± 0.3	2.9 ± 0.2 (0.20)	3.3 ± 0.3 (0.42)	3.8 ± 0.5 (0.92)
EDSS at the end of the follow-up	6.0 ± 0.5	3.3 ± 0.3 (0.027)	4.9 ± 0.6 (0.40)	6.0 ± 0.9 (0.98)

4.9 in Aza-treated patients. This tendency can also be seen for the other parameters of disease activity: the quantified neurological examination (neurostatus) and the ambulation index.

The difference between the groups becomes more clear, if one looks at the distribution of the EDSS at the end of the 10-year follow-up (see Fig. 1). The grouping of the EDSS values is the same as the one used for matching pairs (see above). At the beginning of the follow-up, all patients were able to walk (EDSS ≤ 6.5). Ten years later 6 Aza-treated patients and 13 untreated patients were bound to a wheelchair or bedridden. In addition 4 Aza-treated and 8 untreated patients had died. All deaths were directly or indirectly related to severe physical disability and immobilization due to multiple sclerosis. This difference in distribution is significant in the chi^2-test ($P < 0.05$). Interestingly enough, one can see that at the end of the survey, both groups had about the same number of patients with only minor neurological deficit (EDSS ≤ 2.5; Fig. 1).

As demonstrated in Table 4 the 10 patients with a good compliance and Aza treatment of more than 5 years contribute significantly to the difference between treated and untreated patients at the end of the follow-up. During the 10-year period the mean EDSS for these 10 pa-

tients increased only by 0.5, whereas the increase for patients with less than 5 years Aza treatment was 1.4 and for the group with insufficient or uncertain compliance 2.2.

Discussion

Conclusions based on retrospective studies are always limited by methodological problems [24]. Bad quality of data is an often-mentioned objection against retrospective studies. This could, in our case, concern the criteria for matching pairs, which were compiled according to the clinical records. As we could form pairs out of a sample of 277 patients, we were able to reject cases where the documentation was not sufficient. Another objection could be the inclusion of patients who were not willing or able to come for re-examination at the end of the 10-year period. On the other hand, the information was based on data provided by two or more independent sources (patients themselves, relatives and treating physicians) and thus can be regarded as valid. We are convinced that the error caused by not including these patients would have been more misleading, as we saw that mostly heavily disabled patients could not or did not want to come for re-examination. This group of patients and those who had died in the meantime were clearly over-represented in the untreated group at the end of the 10-year follow-up.

The most important methodological problem of any retrospective study is that no random assignment of the compared therapies can be achieved. By matching pairs, we were able to control for some well-known intervening variables such as age, sex, duration of the disease and disability at entry. However, the influence of other unknown parameters, which 10 years ago might have influenced the therapeutic decisions, could not be evaluated.

One inbalance which we observed at the end of the study concerns the course of the disease at the beginning of the follow-up period. Patients with chronic progressive and relapsing progressive course were significantly over-represented in the untreated patient group. This may have influenced the comparison of the treatments as some authors suppose that chronic progressive courses have a worse prognosis [4, 22]; others could not confirm this [3, 20]. It has been assumed that the higher age at the onset in chronic progressive patients may be the main reason for a bad prognosis [15]. Both age and duration of the disease were quite comparable in the study groups. If we compare the subgroup of 12 pairs of patients with the same disease course at the beginning of the follow-up, the tendency of the data is the same as in the whole group of 37 pairs (data not shown), although the difference in favour of Aza does not reach significance in this small group.

Whether the efficacy of Aza treatment would have been improved by a better compliance and longer drug intake cannot be answered by this study. It is remarkable that the duration of treatment and also the compliance show a great variation in our patients. At the end of the follow-up, patients with long treatment duration and good compliance were by far less disabled as compared with others. This could have two different reasons: maybe the patients who spontaneously had less or only mild relapses continued on medication because they attributed their well-being to the Aza treatment, or maybe Aza was indeed effective in slowing down progression and reducing relapse rate. Observations of other authors [2, 7, 23] and our own observations of relapses with a latency of some months after the discontinuation of Aza [16] as well as theoretical considerations on the duration of the effect of Aza support the second view.

In accordance with other authors who have described a decrease of relapse rates under Aza therapy [1, 6, 7, 19, 23], we found a significant tendency in the Aza-treated group to develop courses with fewer relapses. The fact that relapsing/remitting patients in the not immunosuppressively treated group mostly kept to their relapsing course of disease over the 10 years shows that this cannot be explained only by the natural evolution of the disease.

Table 5. Long-term studies on the effect of Aza in multiple sclerosis

Reference	Patients included	Patients evaluated	Control group	Course of the disease	Therapy dose; duration	Follow-up	Results	Comment
[19, 23]	102	67 (66%)	No	Remitting, remitting-progressive	3 mg/kg/day; more than 5 years	>10 years	Relapse rate decreased, 59% stabilized; high rel. rate after end of therapy	No control, long follow-up
[14]	211	145 (69%)	No	Remitting: 97 Progressive: 48	2.5 mg/kg/day; variable	9.5 years	RR: 65% stable, 35 % worse CP: 35% stable, 65% worse	No control groups
[1]	277	175 (63%)	Historical	All	150 mg Aza/day; mean duration about 4 years	9 years	Relapse rate reduced, less progression	Large group with the longest but incomplete follow-up

As prospective controlled trials lasting 5 or 10 years have not yet been conducted in multiple sclerosis and do not seem to be feasible, one has to rely on information from retrospective studies or extrapolations from short-term trials. The present matched pairs analysis was intended to provide some additional information on the long-term effects of Aza treatment in multiple sclerosis. Compared with three other retrospective studies on Aza treatment, also covering long follow-up periods (see Table 5), this study differs by its matched pairs design which provides for a control group and by the very low rate of patients lost to follow-up.

The conditions defined for this study did not allow for efficient blinding of patients and investigators. All the same the resulting bias may not have influenced our results significantly because the main differences were seen in the rather clear-cut categories "wheelchair-bound/bedridden (EDSS: 7.0–9.5)" and "deceased (EDSS: 10)". The impression emerging from our data is that Aza may have tempered especially severe courses but did not halt the slow progression of the disease. One subgroup of patients would possibly have had a mild course, even without therapy (see left columns, Fig. 1). However, with the existing criteria, it is not possible to differentiate these patients from others in advance.

The (small) beneficial effect of Aza must carefully be weighed against possible harm in the long run. A review of the literature and our own observations in 202 multiple sclerosis patients followed for a mean period of 15 years after Aza treatment initiation ([11]; manuscript in preparation) indicate that the risks of this therapy are rather low.

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References

1. Aimard G, Confavreux C, Ventre JJ, Guillot M, Devic M (1984) Etude de 213 cas de sclérose en plaques traités per l'azathioprine de 1967 à 1982. Rev Neurol (Paris) 139:509–513
2. British and Dutch Multiple Sclerosis Azathioprine Trial Group (1988) Double-masked trial of azathioprine in multiple sclerosis. Lancet II:179–183
3. Clark VA, Detels R, Visscher BR, Valdiviezo NL, Malmgren RM, Dudley JP (1982) Factors associated with a malignant or benign course of multiple sclerosis. JAMA 248:856–860
4. Confavreux C, Aimard G, Devic M (1980) Course and prognosis of multiple sclerosis assessed by the computerized data processing of 349 patients. Brain 103:281–300
5. Ellison GW, Myers LW (1979) A review of systemic nonspecific immunosuppressive treatment of multiple sclerosis. Neurology 29:132–139
6. Frick E, Angstwurm H, Strauss G (1974) Immunosuppressive Therapie der multiplen Sklerose. 2. Mitteilung: Kritischer Bericht über eigene Erfahrungen. Münch Med Wochenschr 116: 1987–1992
7. Frick E, Angstwurm H, Blomer R, Strauss G (1977) Immunsuppressive Therapie der Multiplen Sklerose. 4. Mitteilung: Behandlungsergebnisse mit Azathioprin und Antilymphozytenglobulin. Münch Med Wochenschr 119:1111–1114
8. Hauser SL, Dawson DM, Lehrich JR, Beal MF, Kevy SV, Propper RD, Mills JA, Weiner HL (1983) Intensive immunosuppression in progressive multiple sclerosis. N Engl J Med 308:173–180
9. Hughes RA (1986) Immunological treatment of multiple sclerosis. II. J Neurol 233:66–68
10. IFMSS (1985) Minimal record of disability for multiple sclerosis. National Multiple Sclerosis Society, New York
11. Kappos L, Stolle U, Rohrbach E, Haubitz I, Mertens HG (1988) The impact of long term azathioprine on survival and incidence of malignancies in multiple sclerosis. J Neurol 235 [Suppl]:S94
12. Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33:1444–1452
13. Kurtzke JF (1985) Epidemiology of multiple sclerosis. In: Koetsier JC (ed) Handbook of clinical neurology, vol 47. Demyelinating diseases. Elsevier, Amsterdam, pp 259–287
14. Lhermitte F, Marteau R, Roullet E, Saxce H de, Loridan M (1984) Traitement prolongé de la sclérose en plaques par l'azathioprine à doses moyennes. Bilan de quinze années d'éxperience. Rev Neurol (Paris) 140:553–558
15. Matthews WB, Acheson ED, Batchelor JR, Weller RO (1985) McAlpine's multiple sclerosis. Churchill Livingstone, Edinburgh
16. Mertens HG, Dommasch D (1977) Long term study of immunosuppressive therapy in multiple sclerosis, comparison of periods of the disease before and during treatment. In: Delmotte P, Hommes OP, Gonsette R (eds) Immunosuppressive treatment in MS. European Press, Gent, pp 198–211
17. Mertin J (1985) Drug treatment of patients with multiple sclerosis. In: Vinken PJ, Bruyn GW (eds) Handbook of clinical neurology, vol 3. Elsevier, Amsterdam, pp 187–212
18. Mertin J, Rudge P, Kremer M, Healey MJ, Knight SC (1982) Double-blind controlled trial of immunosuppression in the treatment of multiple sclerosis: final report. Lancet II:351–354
19. Oger J, Deugnier Y, Hinault P, Sabouraud O (1977) Experience of long term immunosuppressive therapy in multiple sclerosis. In: Delmotte P, Hommes OR, Gonsette R (eds) Immunosuppressive treatment in multiple sclerosis. European Press, Gent, pp 100–112
20. Patzold U (1985) Multiple Sklerose: Verlauf und Therapie. Ergebnisse einer prospektiven Untersuchung. Thieme, Stuttgart
21. Patzold U, Hecker H, Pocklington P (1982) Azathioprine in treatment of multiple sclerosis. Final results of a 4½-year controlled study of its effectiveness covering 115 patients. J Neurol Sci 54:377–394
22. Poser S, Bauer HJ, Poser W (1982) Prognosis of multiple sclerosis. Results from an epidemiological area in Germany. Acta Neurol Scand 65:347–354
23. Sabouraud O, Oger J, Darcel F, Madigand M, Merienne M (1984) Immunosuppression au long cours dans la sclérose en plaques: evaluation des treaitements commencés avant 1972. Rev Neurol (Paris) 140:125–130
24. Schleselman JJ (1982) Case-control studies. Design, conduct, analysis. Oxford University Press, New York
25. Schumacher GA, Beebe G, Kibler RF, Kurland LT, Kurtzke JF, McDowell F, Nagler B, Sibley WA, Tourtellotte WW, Willmon TL (1965) Problems of experimental trials of therapy in multiple sclerosis: report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. Ann NY Acad Sci:552–568
26. Waksman BH (1988) Autoimmunity in demyelinating disease. Ann NY Acad Sci 540:13–24