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[Intervention Review]

Azathioprine for multiple sclerosis

Ilaria Casetta[1], Gerardo Iuliano[2], Graziella Filippini[3]

[1]Sezione di Clinica Neurologica; Dip.to di Discipline Medico Chirurgiche della Comunicazione e del Comportamento, Universita degli Studi di Ferrara, Ferrara, Italy. [2]U.O. Neurologia, Ospedali Riuniti di Salerno, Salerno, Italy. [3]Neuroepidemiology Unit, Fondazione I.R.C.C.S. - Istituto Neurologico C.Besta, Milano, Italy

Contact address: Ilaria Casetta, Sezione di Clinica Neurologica; Dip.to di Discipline Medico Chirurgiche della Comunicazione e del Comportamento, Universita degli Studi di Ferrara, Corso della Giovecca, 203, Ferrara, 44100, Italy. cti@unife.it.

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ABSTRACT

Background

Azathioprine is the most widely used immunosuppressive treatment in multiple sclerosis (MS). It is an alternative to interferon beta for treating MS also because it is less expensive. Concerns about its safety, mainly a possible increased risk of malignancy, has limited its use.

Objectives

To compare azathioprine versus placebo. To determine the effect of azathioprine on major clinical outcomes, i.e., disability progression and relapses in patients with MS.

Search methods

We searched The Cochrane Multiple Sclerosis Group Trials Register (2006), The Cochrane Central Register of Controlled Trials (*The Cochrane Library* Issue 4, 2006), MEDLINE (PubMed) (1966 to December 2006), EMBASE (1980 to December 2006), Cochrane Database of Systematic Reviews (CDSR - Issue 4, 2006), Database of Abstracts of Reviews of Effectiveness (DARE - searched 28.12.06) Journals and reference lists were hand searched for relevant articles both to benefit and adverse effects. Regulatory agencies were additional sources of information for adverse effects.

Selection criteria

All parallel group randomised controlled trials (RCTs) comparing azathioprine treatment of a least one year duration with placebo for patients with MS. Cohorts, case controls, case series and case reports were also used to assess adverse effects.

Data collection and analysis

Potentially relevant references were evaluated and all data extracted by two independent authors.

Main results

The five trials that met our criteria included 698 patients: data from 499 (71.5%) were available for analysis of relapse frequency at one year's, from 488 (70%) at two years' and from 415 (59.5%) at three years' follow-up. Azathioprine reduced the number of patients who had relapses during the first year of treatment (relative risk reduction [RRR] =20%; 95% CI = 5% to 33%), at two years' (RRR =23%; 95% CI = 12% to 33%) and three years' (RRR =18%; 95% CI = 7% to 27%) follow-up. These results were consistent in sensitivity analysis. There was no heterogeneity among the studies.
Data from only three small trials with a total of 87 patients were available to calculate the number of patients who progressed during the first two to three years. There was a statistically significant benefit (RRR = 42%; 95% CI = 7% to 64%) of azathioprine therapy at three years' follow-up; this result was robust after sensitivity analyses and there was no heterogeneity among the trials.

Gastrointestinal disturbances, bone marrow suppression and hepatic toxicity were greater in the azathioprine group rather than in the placebo group; they were anticipated, and, by monitoring and dosage adjustment, were easily managed. Withdrawals due to adverse effects were few, occurring mostly during the first year of azathioprine treatment and mainly due to gastrointestinal intolerance (5%).
Data from the trials and from cohort and case controls studies available in the literature did not show an increase in risk of malignancy from azathioprine. A possible long-term risk of cancer from azathioprine may be related to a treatment duration above ten years and cumulative doses above 600 g.

Authors' conclusions

Azathioprine is an appropriate maintenance treatment for patients with MS who frequently relapse and require steroids. Cumulative doses of 600 g should not be exceeded in relation to a possible increased risk of malignancy. Considering the trade off between the benefits and harms, azathioprine is a fair alternative to interferon beta for treating MS. A logical next step for future trials would seem the direct comparison of azathioprine and interferon beta. In fact the direct comparison between these two widely used treatments in MS has not been made.

PLAIN LANGUAGE SUMMARY

The effects of the immunosuppressive drug azathioprine (AZA) widely used in multiple sclerosis (MS) before the treatments with interferons or glatiramer acetate

AZA is a possible alternative to interferon beta for treating MS. As concerns have been raised about its safety, mainly due to possible increased risk of cancer, the authors of this review tried to assess the balance between benefits and harms of AZA treatment in MS.
Among the pertinent medical literature only five studies met the methodological quality criteria necessary for their inclusion in this review, comprising a total of 698 participants, with follow-up at one, two and three years.
Taking into account the disability progression and the number of relapses, the authors found evidence that AZA reduced the number of patients who had relapses during the first year of treatment , and at two and three years' follow-up as well. AZA treatment also reduced the number of patients who progressed during the first two to three years of therapy.
Adverse effects such as gastrointestinal disturbances, bone marrow suppression and hepatic toxicity occurred frequently; but they were known and anticipated, thus quite easily managed: withdrawals due to adverse events were few, and mainly due to gastrointestinal intolerance.
Two studies had deaths reported, comprising of four persons in the control group, and eight in the AZA group. These small numbers do not allow a statistical analysis.
Conflicting conclusions on potential risk of cancer in MS patients with long-term AZA treatment have been reported in eight published papers, not considered in the present review because they came from sources other than clinical trials. The presence of patients who developed cancer (three in the AZA and 1 the placebo group) was reported in two out of five studies considered in this review. Numerous studies of AZA treated patient populations other than MS patients are also available. The whole data, however, does not show an increase in risk of malignancy from AZA. Possible long-term risks may be related to a treatment duration above ten years and cumulative doses above 600 g.

BACKGROUND

Typically, the clinical course of multiple sclerosis (MS) is characterised by clinical relapse (relapsing/remitting form (RRMS)), from which recovery may be complete or incomplete, and by progression. In some 80% of relapsing patients, a progressive phase follows within two decades (Weinshenker 1989), and this form is defined as secondary progressive multiple sclerosis (SPMS) by consensus (Lublin 1996). Progression is not preceded by relapses in patients with primary progressive multiple sclerosis (PPMS). Treatment with immunosuppressive and immunomodulating drugs is used trying to reduce frequency of relapses that are thought to be a result of local inflammation and consequent loss of the myelin sheath that normally surrounds axons in the central nervous system. Reduction in relapse frequency does not necessarily predict effects on disability progression.

Azathioprine was created in the mid-1950s (Elion 1993) and by 1960 it had swiftly made the transition from experimentation to bedside use (Rundles 1961). It is a purine antagonist and affects DNA replication and the immune system in a number of ways. It impairs T-cell lymphocyte function and is more selective for T lymphocytes than for B lymphocytes (Patel 2006). A recent study further elucidates the efficacy of azathioprine in chronic inflammatory and autoimmune diseases (Tiede 2003). Because of the favorable therapeutic index of azathioprine over other traditional immunosuppressants like methotrexate and cyclophosphamide, it has been utilised as a corticosteroid sparing agent and as monotherapy to treat several conditions, including severe rheumatoid arthritis, inflammatory bowel disease, myasthenia gravis, malignancies and several autoimmune conditions (Rosman 1973).

Neurologists have been using azathioprine to treat patients with MS for more than 30 years. Although newer immunomodulating drugs, i.e. interferon beta and glatiramer acetate, have been incorporated into clinical practice, the continuing high costs of these medications and their uncertain effects on disability progression have precluded the abandonment of azathioprine. A review of seven clinical studies evaluating the effect of azathioprine on MS up to 1989 concluded it was efficacious in preventing relapses at one, two and three years and had a slight, borderline benefit also on prevention of disability progression at two and three yeas (Yudkin 1991). A post-marketing review comparing the probability to be free from relapses at two years in MS patients treated with interferon beta, glatiramer acetate, or intravenous immunoglobulins with that reported with azathioprine in Yudkin's review, concluded these treatments were equivalent (Palace 1997). Sudlow and Counsell proposed that azathioprine should have been included in the MS risk sharing scheme on the basis that its short-term effects appear similar to those of beta-interferon or glatiramer at a fraction of the cost. The few trials that assessed disability progression with azathioprine found similar reductions to the interferon trials (Sudlow 2003). Using the relative risk of relapse from the analysis by Sudlow and Counsell, researchers at the Sheffield University School of Health estimated that azathioprine was cost saving to the National Health Service, and produced moderate quality of life gains even if the quality of the data was such there was large uncertainty surrounding their estimates (McCabe 2003). The authors conclude that, on current evidence, azathioprine may be a better choice of therapy than glatiramer acetate as the small difference in efficacy does not appear to justify the large price differential. Given the similarity in the effectiveness of these therapies, according to the meta-analysis in Sudlow and Counsell, and the great uncertainty around the estimates of effectiveness, there may be some value in a randomised trial comparing these two therapies (McCabe 2003). A recent study has suggested that azathioprine seems to be effective both on clinical and imaging outcomes (Massacesi 2005). Azathioprine is approved and largely used in Europe for MS treatment (Hommes 2004). However, major concerns facing patients and clinicians regard the quality of the evidence on medium and long term efficacy of azathioprine and its adverse events and a systematic review of the literature is required to establish the role of this treatment in MS.

OBJECTIVES

To assess whether azathioprine prevents the progression of disability in patients with multiple sclerosis.

To assess whether azathioprine increases the probability of remaining free from relapses.

To assess whether azathioprine therapy is a safe treatment.

To evaluate the trade off between the benefits and risks.

METHODS

Criteria for considering studies for this review

Types of studies

All randomised controlled trials lasting at least one year and comparing azathioprine and placebo in patients with MS were eligible for the review. Quasi-randomised, uncontrolled trials or studies where azathioprine has been compared with interventions other than placebo were not included. Both double-blind and single-blind studies were eligible. Since long-term or uncommon adverse events are rarely captured in randomised clinical trials, we evaluated adverse events also from non-randomised studies and observational studies.

Types of participants

Patients of any age and either gender with definite MS according to Poser criteria (Poser 1983), or other recognisable diagnostic criteria, whatever disease severity, were eligible for the review. Any patterns of MS course (relapsing/remitting, relapsing/progressive, secondary progressive or primary progressive) have been considered.

Types of interventions

Administration of any dose of azathioprine versus placebo of at least one year duration. Co-interventions were allowed, i.e. steroids for relapse, as long as the control arm of the randomised clinical trial had the opportunity of receiving equivalent co-intervention.

Types of outcome measures

Primary outcomes

(1) Number of patients who had disability progression during the treatment and follow-up periods. Disability progression is defined as serial in-trial upward changes of 0.5 and 1.0 point on Kurtzke Disability Status Scale (DSS) or its expanded version (EDSS), recorded out of relapse and confirmed at 6 months or later.

(2) Number of patients who experienced relapses during the treatment and follow-up periods. Relapse is defined as the acute or subacute appearance/reappearance of neurological signs and symp-

toms for at least 24 hours, in the absence of fever, infection or concurrent steroid withdrawal.

(3) Average change in DSS or EDSS scores and their standard deviation during the treatment and follow-up periods.

(4) Mean number of relapses and its standard deviation during the treatment and follow-up periods.

(5) Adverse events defined as any untoward medical occurrence in a patient in either of the two arms of the included trials, which did not necessarily have a causal relationship with the treatment, but did, however, result in a dose reduction, discontinuation of treatment, or registration of the event as an adverse event/side effect (Loke 2005).

Secondary outcomes

(1) Number of patients treated with steroids during the first, second and third year from randomisation.
(2) Number of patients hospitalised during the first, second and third year from randomisation.

Search methods for identification of studies

Electronic searches

Trials were identified by searching the Multiple Sclerosis Group's Trials Register (December 2006), Cochrane Central Register of Controlled Trials (CENTRAL) "The Cochrane Library 2006, Issue 4 (Appendix 1), MEDLINE (PubMed) (1966-December 2006)(Appendix 2), EMBASE (1988- December 2006)(Appendix 3).

Searching other resources

Reference lists from trials selected by electronic searching were handsearched to identify further relevant trials. Abstracts of MS neurological congresses, conference proceedings, symposia were handsearched (1990-2006). In addition authors and experts were contacted and asked to supply details of any outstanding clinical trials and relevant unpublished materials.
Journals and reference lists were hand searched for articles relevant to adverse effects. Regulatory agencies were additional sources of information for adverse effects: the Australian Adverse Drug Reactions Bulletin (http://www.tga.gov.au/adr/aadrb.htm); the European Public Assessment Reports from the European Medicines Evaluation Agency (http://www.emea.eu.int/#); the UK Current Problems in Pharmacovigilance (http://medicines.mhra.gov.uk/ourwork/monitorsafequalmed/currentproblems/cpprevious.htm); and the US, MedWatch, the Food and Drug Administration Safety information and Adverse Events Reporting Program (http://www.fda.gov/medwatch/elist.htm) (Loke 2005).
No exceptions were made concerning the languages in which the articles had been published.

Data collection and analysis

Selection Of Studies

Two authors (IC,GI) evaluated independently whether the identified trials fulfilled the inclusion criteria. Differences were resolved by consensus. In case consensus could not be reached, GF acted as arbitrator.

Assessment of Study Quality

The two authors independently allocated each included trial into one of three quality categories (protection against bias), based on those described in the Cochrane Handbook for Systematic Reviews of Interventions 4.2.6 (formerly the Reviewers' Handbook) (section 6.7.1) (Higgins 2005):
Category I: Low risk of bias - plausible bias unlikely to seriously alter the results - All the criteria 'met'
Category II: Moderate risk of bias - plausible bias that raises some doubt about the results - One or two criteria scored as 'unclear' or 'not met'
Category III: High risk of bias - plausible bias that seriously weakens confidence in the results - More than two criteria scored as 'unclear' or 'not met'.

The criteria for assessment were:
Generation of the allocation sequence : a) 'Met', if the allocation sequence was generated by a computer or random number table; b) 'Unclear', if the trial was described as randomised, but the method used for the allocation sequence generation was not described; and c) 'Not met', if the allocation sequence was known to the investigators who assigned participants or if the study was quasi-randomised.
Concealment of allocation : a) 'Met', if the allocation of patients involved a central independent office or pharmacy, pre-numbered or coded identical containers which are administered serially to participants, on-site locked computer, sequentially numbered, sealed, opaque envelopes; b) 'Unclear', if the trial was described as randomised, but the method used to conceal the allocation was not described; and c) 'Not met', if the allocation sequence was known to the investigators who assigned participants, e.g. alternation, the use of case record numbers, dates of birth or day of the week, or an open list of random numbers.
Blinded assessment of primary outcome(s) : a) 'Met', if the trial was described as double blind, i.e. both the patiens receiving treatments and the persons responsible for assessing outcomes were unaware of the assigned intervention, and the method of blinding involved identical placebo or active drug; b) 'Unclear', if the trial was described as double blind, but the method of blinding was not described; and c) 'Not met', if the trial was not double blind.
Accounting for withdrawals and dropout rate : a) 'Met', if the numbers and reasons for dropouts and withdrawals in all intervention groups were described and if outcome measures were obtained for 80% to 100% of patients randomised, or for patients who entered the trial; b) 'Unclear', if the report did not specifically state if there had been any dropouts or withdrawals; and c) 'Not met', if the number or reasons for dropouts and withdrawals were not described.
Differences in the authors allocation of studies into quality categories were resolved by consensus.

Data Extraction

Data was extracted and registered onto a standardised form independently by IC and GI. Data was cross-checked and discussed. In case consensus could not be reached, GF acted as arbitrator. Authors of the included trials were asked to provide data of interest , if they had not been reported clearly in the reports.
The following characteristics were recorded for each trial:
- Characteristics of patients including inclusion and exclusion criteria, source of recruitment, criteria for diagnosis, mean or median age, sex ratio, MS course, and disability score at presentation
- Characteristics of interventions including dosages and schedules of azathioprine, type of placebo, cointerventions, duration of treatment and follow-up

- Characteristics and time of outcomes including the prevalence of patients who had relapses or disability progression after the intervention, disability scores and relapse rates. Where disability scales were used it was noted whether or not they were standard scales and whether they have been validated. Assessments of adverse events were noted.
Data was extracted as intention to treat analyses.

Data Synthesis

For binary outcomes, such as the presence or absence of disability progression, the effects of the intervention were expressed as relative risks and absolute risk reductions (ARR) together with 95% confidence intervals. The number needed to treat (NNT) was calculated using the formula 1/ARR. The 95% confidence intervals of the NNT were calculated using the 95% confidence intervals for the ARR. For EDSS means and standard deviations were used to summarise the values in each group.
Relative risks were combined for binary outcomes, standardised mean differences for continuous outcomes. We intended to use a random effects analysis if there was significant (P<0.1) heterogeneity detected in the data. However, a fixed-effects approach was used for data synthesis because no heterogeneity was found among the trials.
Regarding the primary outcome measures, i.e., disability progression and relapses, we included patients with incomplete or missing data in the sensitivity analyses by imputing to them a worst scenario (this last being intention-to-treat analyses):
- Available case analysis: data on only those whose results were known, using as denominator the number of patients who completed the trial;
- Assuming poor outcome (worst): dropouts and withdrawals from both the azathioprine and control groups had the primary outcomes, using as denominator the number of randomised patients.
Characteristics of included and excluded trials (along with their exclusion criteria) have been listed in a summary table.
All results have been organised and processed by the RevMan 4.2 (RevMan) developed by the Cochrane Collaboration.

RESULTS

Description of studies

Fifteen trials that were selected attempted to evaluate azathioprine treatment in MS. Ten trials did not meet the eligibility criteria: one non randomised study (Swinburn 1973); three uncontrolled studies (Silberberg 1973, Aimard 1983, Fratiglioni 1988); one with a follow-up period less than 1 year (Cendrowski 1971); one in which patients had been treated with a concurrent immunosuppressive treatment (Mertin 1980, Mertin 1982); one (Minderhoud 1988) because the results had been incorporated in a study (British & Dutch 1988) included in this review. One study (Zeeberg 1985, Zeeberg 1986) was excluded because available information was not sufficient to define the number of randomised patients, and the number of lost to follow up. Two studies (Patzold 1982, Rosen 1979) were excluded because it was not possible to extract outcome data.

Five trials (British & Dutch 1988, Ellison 1989, Ghezzi 1989, Goodkin 1991, Milanese 1993), that compared azathioprine therapy with placebo and followed up patients for one to three years, were included in this review. Overall, 698 participants had been randomised: 346 to azathioprine and 352 to placebo; the British & Dutch 1988 trial accounted for 354 (51%) participants. The earliest trial was published in 1988 (British & Dutch 1988) and the most recent in 1993 (Milanese 1993).

INCLUSION CRITERIA

All trials included patients with definite MS according to Poser's (British & Dutch 1988, Ellison 1989, Ghezzi 1989, Goodkin 1991) or McDonal's and Halliday's criteria (Milanese 1993). The age range was 15 to 50 years (British & Dutch 1988), 18 to 50 (Ghezzi 1989), 18 to 65 (Goodkin 1991), 18 years or older (Ellison 1989). One study did not report patients' age (Milanese 1993).
One study (Goodkin 1991) enrolled relapsing-remitting patients with mean disease duration of six years and mean entry EDSS of three to four score, while another trial (Ellison 1989) investigated the effect of azathioprine in chronic-progressive patients with disease duration of 13 to 17 years and entry DSS of five to six score. Three trials (British & Dutch 1988; Ghezzi 1989; Milanese 1993) included both relapsing- remitting (67%, 40% and 47.5% respectively) and chronic-progressive (33%, 60% and 52.5%) patients and mean entry EDSS of these patients ranged from two to four score.
Regarding disease activity of relapsing-remitting patients, they had to have had at least one relapse in the previous year (British & Dutch 1988), two or more relapses in the previous 18 months (Goodkin 1991) or two years (Milanese 1993) prior to study entry. In all studies patients had to have had no relapse during the one-month period before randomisation. Chronic-progressive patients had to have had a progression of disability for at least six months (British & Dutch 1988; Ellison 1989), or a steady progression of disability by at least one point on the EDSS scale in the last year (Milanese 1993) prior to study entry. No definition of disease course was reported in one study (Ghezzi 1989).

EXCLUSION CRITERIA

Patients on immunomodulatory drugs or hyperbaric oxygen treatment, with concomitant systemic disease or mental deficit were excluded in one study (British & Dutch 1988). Ellison 1989 considered as exclusion criteria pregnancy or intention to become pregnant within the next three years, infections not under treatment, decubitus ulcers, active coccidiomycosis, neoplastic diseases, and diseases compromising neurological assessment (e.g. deforming arthritis, major amputation, psychoses) as well as treatment with cytotoxic agents within the preceding six months, or steroids within the preceding three months. Goodkin 1991 excluded patients receiving steroids during the month before study entry, immunosuppressant drugs in the year before, or total lymphoid irradiation at any time, and patients who were pregnant, were unwilling to practice acceptable birth control, or suffering from systemic diseases or medical conditions that precluded safe use of azathioprine, or incapable to give informed consent. Ghezzi 1989 excluded patients with disease duration lower than one year and concomitant diseases controindicating immunosuppression. Milanese 1993 excluded patients on immunosuppressive treatment in the year preceding entry into the trial.

INTERVENTIONS

The Ellison 1989 trial was a 3-armed trial including a group receiving azathioprine and methylprednisolone, a group receiving azathioprine and placebo, and a third group receiving placebo. These last two arms were included in the review. Daily dose of azathioprine was 2 mg/kg (Milanese 1993), 2.5 mg/kg (British & Dutch 1988, Ghezzi 1989) or 3 mg/kg (Goodkin 1991). In the Ellison 1989 trial, azathioprine was started at a daily dose of 2.2 mg/Kg body weight. Each month thereafter the dose was increased by 25 mg until the

white blood cell count was maintained between 3,000 to 4,000 or adverse effects were encountered. The daily dose was increased above 4.4 mg/kg when appropriate.

OUTCOMES

All studies reported the number of patients experiencing on-trial relapses, over one year and two years (British & Dutch 1988, Ellison 1989, Goodkin 1991, Milanese 1993), 18 months (Ghezzi 1989), and three years (British & Dutch 1988; Ellison 1989; Milanese 1993). Three trials reported the mean number of relapses at one, two and three years (British & Dutch 1988, Ellison 1989, Milanese 1993). Number of patients with disability progression was reported at one, two and three years by Milanese 1993, at 18 months by Ghezzi 1989, at two years by Goodkin 1991 and at three years by Ellison 1989. Change in disability status score was reported at one and two years by four studies (British & Dutch 1988, Ellison 1989, Goodkin 1991, Milanese 1993), at 18 months by one study (Ghezzi 1989) and at three years by three studies (British & Dutch 1988; Ellison 1989; Milanese 1993).

ADVERSE EVENTS

The number of patients experiencing adverse effects or the number of those who were withdrawn/dropped out because of adverse effects were extracted from four studies (British & Dutch 1988; Ellison 1989; Goodkin 1991; Milanese 1993).

Risk of bias in included studies

The included studies were categorised into one of three quality categories (protection against bias), based on those described in the Cochrane Handbook for Systematic Reviews of Interventions 4.2.6 (section 6.7.1) (Higgins 2005). The results are reported in Additional Figures- Figure 01 (Figure 1).

They were as follows:

British & Dutch 1988: Low risk of bias - random allocation and allocation concealment (information obtained from Prof. Richard Hughes, clinical coordinator), blinding of participants and observers, withdrawals accounted for, withdrawal and drop-out rates 8% (azathioprine) 6% (placebo controls)

Ellison 1989: Low risk of bias - random allocation, allocation concealment, blinding of participants and observers, withdrawals accounted for, withdrawal and drop-out rates 13% (azathioprine) 18% (placebo controls)

Ghezzi 1989: High risk of bias (insufficient information was available in the abstract located and information could not be obtained from the authors). Withdrawal and drop-out rates 26% (azathioprine) 28% (placebo controls)

Goodkin 1991: Moderate risk of bias - random allocation, unclear allocation concealment, blinding of participants and observers, withdrawals accounted for, withdrawal and drop-out rates 7% (azathioprine) 0 (placebo controls)

Milanese 1993: Moderate risk of bias - random allocation, allocation concealment, participants and observers blinded, reasons for withdrawal noted and withdrawal and drop-out rates 26% (azathioprine) 14% (placebo controls).

Analysis of blinding in the Ellison 1989 trial showed that clinician investigators assessing outcomes were well masked during the study but there was a tendency for placebo patients to become unblinded.

The trials reported no evidence of imbalance in baseline characteristics apart from one study where more women than men in the azathioprine group (British & Dutch 1988). In all trials, disability outcome was assessed using Kurzke EDSS, a 10-point ambulation-centred scale (EDSS - Expanded Disability Status Scale where 0 is normal, 3 - mild disability, 6 - cane requirement, 7 - wheelchair use and 10 is death from MS) (Kurtzke 1983). The reporting of adverse events in the trials was mostly adequate. The number of reported adverse events was explicitly related to the number of individuals who experienced them.

Figure 1.

Table 01. Quality assessment

Study	Generation of the allocation sequence	Concealment of allocation	Blinded assessment of primary outcome(s)	Accounting for withdrawals and dropout rate	Withdrawal and drop-out rates: azathioprine	Withdrawal and drop-out rates: placebo	Risk of bias
British & Dutch 1988	Met	Met	Met	Met	8%	6%	Low
Ellison 1989	Met	Met	Met	Met	13%	18%	Low
Ghezzi 1989	Unclear	Unclear	Unclear	Unclear	26%	28%	High
Goodkin 1991	Met	Unclear	Met	Met	7%	0	Moderate
Milanese 1993	Met	Met	Met	Met	26%	14%	Moderate

Effects of interventions

The effect of azathioprine therapy on disability progression

The largest trial (British & Dutch 1988) did not give results as dichotomous outcome and this information could not be obtained from the authors. Three small studies (Ellison 1989; Goodkin 1991; Milanese 1993) reported the number of patients who worsened during follow up; they defined disability progression as a steady worsening of ≥ 0.5 points with a baseline EDSS ≥ 5.5, or a worsening of ≥ 1.0 point with a baseline EDSS < 5.5. The outcome was not defined in the Ghezzi 1989 trial. Only one study (Goodkin 1991) required more than six months of sustained EDSS worsening to classify patient outcome as a progression. The other studies did not report definitions of sustained disability progression. Data from two trials with 87 patients were available to calculate the number of patients who progressed during the first two years (Goodkin 1991; Milanese 1993) and the first three years (Ellison 1989; Milanese 1993) of treatment. The mean rate of progression during the first three years was 61% (range of 46-83%) in the placebo group and 34% (range 30-43%) in the azathioprine group. There was no statistically significant heterogeneity between the trial results both at two years (heterogeneity test [1 degree of freedom] chi squared = 0.07, p=0.80, I^2 = 0%) and at three years (heterogeneity test [1 degree of freedom] chi squared = 1.27, p=0.26, I^2 = 21.5%). There was a statistically significant benefit of azathioprine therapy at three years (relative risk reduction [RRR] = 42%; 95% CI = 7% to 64%). When everybody missing was assumed to have had disability progression in the sensitivity analysis (the worst), the point estimate of the relative risk reduction remained statistically significant (RRR =60%; 95% CI = 11% to 82%). Four patients (95% CI = 2 to 17) needed to be treated with azathioprine for three years to prevent one extra patient from having disability progression for the average baseline risk (61%) observed across the two studies (Ellison 1989; Milanese 1993).

Four trials (British & Dutch 1988; Ellison 1989; Goodkin 1991; Milanese 1993) reported change in disability score as a continuous outcome in 479 patients at two years and three of them (British & Dutch 1988; Ellison 1989; Milanese 1993) reported it in 419 patients at three years. There was a statistically significant reduction in disability score at two years (treatment versus placebo weight mean difference -0.22; 95% CI = -0.44 to -0.00), and a reduction that was not statistically significant at three years (treatment versus placebo weight mean difference -0.25; 95% CI = -0.52 to 0.02). There was no statistically significant heterogeneity between studies.

The effect of azathioprine therapy on relapses

All trials reported the number of patients who had new relapses during the follow up; they evaluated a total of 698 patients. All but one (Ghezzi 1989) of the trials defined relapse. In the Ellison 1989 trial, relapse was a worsening of neurological symptoms and signs lasting more than 24 hours and confirmed by the monitoring neurologist. In the other trials, relapse was an exacerbation of at least five days duration with objective worsening of EDSS by ≥ 0.5 points (Goodkin 1991) or ≥1 point (British & Dutch 1988; Milanese 1993). The outcome was not defined in the Ghezzi 1989 trial. Azathioprine was associated with a significant reduction in the number of pa-

tients who had relapses during the first year compared with placebo (RRR =20%; 95% CI = 5% to 33%), at two years (RRR =23%; 95% CI = 12% to 33%) and at three years (RRR =18%; 95% CI = 7% to 27%). There was no heterogeneity among studies. In a sensitivity analysis assuming the worst, a statistically significant difference between the two groups was confirmed at one year (RRR =34%; 95% CI =6% to 53%), two years (RRR =49%; 95% CI = 26% to 65%), and three years (RRR =50%; 95% CI = 23% to 67%). Nine patients (95% CI = 5 to 33) needed to be treated for one year to prevent one extra patient from having relapse during the first year of treatment for the average baseline risk (56%) observed across the included studies, six patients (95% CI = 4 to 12) needed to be treated for two years for a baseline risk of 71% and seven patients (95% CI = 4 to 17) for three years for a baseline risk of 79% .

Four trials (British & Dutch 1988; Ellison 1989; Goodkin 1991; Milanese 1993) reported change in mean number of relapses as a continuous outcome in 482 patients at two years, and three of them (British & Dutch 1988; Ellison 1989; Milanese 1993) reported it in 417 patients at three years. A significant decrease in mean number of relapses favouring azathioprine was observed at two years (treatment versus placebo weight mean difference [WMD] = -0.22; 95% CI = -0.40 to -0.04); a decrease was also observed at three years (WMD= -0.13; 95% CI = -0.29 to 0.03) but the difference was not statistically significant. There was no statistically significant heterogeneity among studies.

A *dverse effects associated with azathioprine therapy in MS patients*

All trials reported the occurrence of adverse effects, although none specifically categorised their severity. Symptoms and laboratory abnormalities occurred more often within the first year of treatment and resolved after reducing azathioprine dose or discontinuing therapy. Overall, 9% of azathioprine patients and 2% of controls had gastrointestinal disturbances (anorexia, nausea, vomiting, gastric or abdominal pain) that were reported in four trials (British & Dutch 1988; Ghezzi 1989; Goodkin 1991; Milanese 1993). In the first year, withdrawals due to gastrointestinal intolerance were 5% in the azathioprine group in three trials (British & Dutch 1988; Ghezzi 1989; Milanese 1993) and 3% in the Goodkin 1991 trial. In the Ellison 1989 trial, gastrointestinal symptoms did not differ between treated and control groups.

Cutaneous rash was recorded more frequently in azathioprine groups than in controls (5% *vs* 2%); the severity of skin reactions was not specified in any of the trials.

Leukopenia was classed as an adverse effect if total white blood cells were <3000/mm^3 and this was reported in four articles (British & Dutch 1988; Ellison 1989; Goodkin 1991; Milanese 1993). Four of 174 patients (2%) withdrew within the first year due to leukopenia in the British & Dutch 1988 trial; no patient required discontinuance of treatment because of leukopenia in the other trials.

Macrocytic anaemia was reported in two studies (British & Dutch 1988; Ghezzi 1989) and occurred more frequently in patients treated with azathioprine than in controls (3% *vs* 0.4%). Anaemia was responsible for three withdrawals in the Ghezzi 1989 trial. There was a gradual increase in mean corpuscular volume (MCV) in patients receiving azathioprine (British & Dutch 1988; Ellison 1989; Goodkin 1991; Milanese 1993). Thrombocytopenia did not differ between treated and control groups in the British & Dutch 1988 trial (one azathioprine patient and one control). In the Ellison 1989 trial it "was less frequent than leukopenia and anaemia". Thrombocytopenia was not mentioned in the other trials. In the Milanese 1993 study, one patient withdrew after 24 months of treatment due to pancytopenia which remitted after the drug had been withdrawn.

Abnormalities of liver enzymes were the second most frequent haematological adverse events and they were reported in 8% of patients on azathioprine and 1% on placebo (British & Dutch 1988; Ghezzi 1989; Goodkin 1991). It was responsible for one and four withdrawals in the British & Dutch 1988 trial and Ghezzi 1989 trial respectively.

Two trials had deaths reported. There were two deaths during the British & Dutch 1988 trial in the placebo group, both due to pneumonia, and seven in the azathioprine group, due to pneumonia (one), urinary tract infection (two), carcinoma (two), suicidal overdose (one), and accidental cause (one). The three patients in the azathioprine group who died of infections had advanced multiple sclerosis and had stopped taking azathioprine at least 12 months before death. The Ellison 1989 study reported one death by drowning in the azathioprine group, one by suicide and one death by ruptured abdominal aneurysm in the placebo group.

There were no significant differences in other uncommon adverse effects, such as bacterial, viral or fungal infections rate or infections of uncertain etiology, systemic hypertension, hyperglycaemia, pancreatitis or alopecia between the groups.

Malignancy potential

Malignancy was reported in two trials. In the first one (British & Dutch 1988) two patients on azathioprine died, one of ovarian cancer four months after entry to the study and one of bronchogenic carcinoma 18 months after entry to the trial and three months after stopping treatment. In the first patient the cancer was probably present in retrospect before treatment and the other patient was a heavy smoker. Moreover, the solid neoplasms registered during the three-years study were not considered associated with immunosuppression (i.e. skin cancer, leukemia or lymphomas). Cancer and mortality data of all 300 UK patients enrolled in the British & Dutch 1988 trial were prospectively registered and updated to July 2002. There was a small but not significant absolute increase in risk of cancer from a 3-year course of azathioprine of 3.4% (95% CI -2.1 to 9.0%), and little difference in total deaths. (Taylor 2004).

In the second trial (Ellison 1989) one patient with basal cell carcinoma was observed both in the treated and control group. The other three trials reported that no patients developed clinical evidence of malignancy during the study.

Reports of malignancy potential from sources other than clinical trials

Conflicting conclusions on potential risk of malignancy in MS patients with long-term azathioprine treatment have been reported (from sources other than clinical trials) in eight published papers (Lhermitte 1984; Kinlen 1985; Amato 1993; Confavreux 1996; Taylor 2004; Willerding-Mollmann; Knipp 2005; Putzki 2006). Early studies reported increased cancer risks (Lhermitte 1984; Kinlen 1985), but in a case-control study involving 23 cancer cases and 69 controls selected out 1191 MS patients, including two patients receiving the drug for one month or less, the risk was not significantly increased in those treated with azathioprine (OR 1.7, 95% CI 0.6-4.6). A possible risk increase was suggested only by treatment duration beyond ten years or over 600 grams cumulative dose (OR 6.7, 95% CI 1.2-36.1) (Confavreux 1996). Correspondingly, a dose effect was suggested in four cases of myelodysplastic syndrome (MDS) that has been reported so far after long-term treatment with azathio-

prine in MS. The first reported case was a 49-year-old female with a history of MS and on medication with azathioprine over five years amounting to a cumulative dosage of 45 g (Willerding-Mollmann); two cases were treated with cumulative doses of 146 and 627 grams respectively (96 and 123 months) (Knipp 2005); however, it was not reported if these three patients had previously taken other immunosuppressive drugs. The last case was a 39-year old woman with a cumulative dose of 657 grams azathioprine, treatment duration of 98 months and no other previous immunosuppressive therapy (Putzki 2006). No increase in the relative risk of cancer was found in a study on 201 azathioprine-treated MS patients compared to 247 controls (Amato 1993).

Numerous studies of various patient populations treated with azathioprine for other autoimmune or immune-mediated diseases are also available (McEwan 1972; Anstey 2004; Knipp 2005; Patel 2006; Masunaga 2007). There was no increase in the rate of malignancies in a large number of azathioprine-treated, nontransplant patients, compared with placebo-treated controls (McEwan 1972). Reports of skin malignancies in patients receiving long-term azathioprine monotherapy for autoimmune diseases are rare, suggesting that the risk, if it exists, is small (Anstey 2004). Recent findings from a large meta-analysis suggest that the administration of azathioprine in patients with either Chron disease or ulcerative colitis probably does not confer a significantly increased risk of malignancy compared with patients with inflammatory bowel disease who are not receiving azathioprine (Masunaga 2007). However, the risk of lymphoma in one study of patients with rheumatoid arthritis (RA) who underwent long-term treatment with azathioprine was one case per 1000 patients years (Silman 1988); in another study of RA patients, the attributable malignancy risk was rougly 5.7 per 1000 patient-years at risk after azathioprine intimation (Jones 1996). In these studies a time- and dose-dependency association was reported. An important consideration is also that RA patients had a considerably higher risk (above 15-fold) of malignancy than that of the general population. This may be attributable to mechanisms inherent to their rheumatological diseases and/or to other DNA-damaging drugs such as cyclophosphamide (Knipp 2005).

The report obtained from the website of the Australian Adverse Drug Reactions Bulletin (ADRAC 2006) lists 414 cases of pancreatitis of which 33 (8%) reported with azathioprine, but it does not state for what condition the drug had been taken. The report indicates that a causal association has not been firmly established, but a drug-induced cause should be considered when other causes have been reasonably excluded. 'At risk' groups include patients receiving other immunomodulatory agents. There is insufficient information available on the course of pancreatitis once azathioprine had been stopped.

SECONDARY OUTCOMES

The effect of azathioprine therapy on steroids sparing

The trials included in this review did not report the number of patients who were treated with corticosteroids during the follow up period. The mean number of relapses treated with steroids was smaller in azathioprine patients than in placebo controls at one year (0.27±0.07 *vs* 0.39±0.07), two (0.23±0.04 *vs* 0.32±0.06) and three (0.19±0.04 vs 0.23±0.04) years (British & Dutch 1988). Similarly, the number of exacerbations treated with steroids was lower in azathioprine patients than in controls (19/28 *vs* 39/48) in the Goodkin 1991. Corticotrophic hormone (ACTH) was used 23 times in the treated group (9 times for progression) and 49 times in the placebo group (12 times for progression) in the Ellison 1989 study. The other trials did not report data on steroid use.

T *he effect of azathioprine therapy on patients' hospitalisation sparing*

No data were available on this outcome.

DISCUSSION

The largest randomised trial (British & Dutch 1988) showed a significant protective effect of azathioprine against recurrence of relapses during the first, second and third year of treatment. The other four small trials evaluating relapse outcome at one to three years did not demonstrate any statistically significant benefit of azathioprine over placebo. The reason for one trial giving a positive result whilst the others have been negative has been discussed between the authors of this review. Azathioprine treatment has a moderate effect on relapses at 12, 24 and 36 months and none of the negative trials have sufficient power to detect this difference. The relative risk reduction (20% at one year, 23% at two years and 18% at three years) in the proportion of patients who relapsed for those treated with azathioprine therapy is however statistically significant and is consistent in sensitivity analysis. When the mean number of relapses was considered, a significant decrease in the average relapse count was observed at two years. The continuous relapse measure should be interpreted with caution however; relapse counts follow a positive asymmetric distribution (standard deviations tend to increase with increasing mean values across studies) rather than approximating the normal function, as it is assumed by the weighted mean difference analysis.

Investigators in three small trials with a total of 87 patients reported that fewer patients had disability progression during two to three years of azathioprine treatment than controls (the difference was statistically significant at three years). There was also a difference between the two groups when the EDSS disability scores were measured as continuous outcome at two to three years. There were more trials with a total of 479 patients available for this continuous outcome, but a change in disability score has less clinical meaning than the proportion of patients progressed. Most of the studies did not use a validated definition for measurement of progressive disability in the trial context; only one (Goodkin 1991) clearly reported that this outcome was confirmed at more than six months. Unremitting disability occurs in the progressive phase of the disease, but changes in short-term disability that occur frequently in relapsing-remitting patients may reflect random variation, measurement error and remitting relapses. Confirmation of disability worsening at six months to one year is essential for meaningful disability results mainly in relapsing-remitting patients (Ebers 2006; Kremenchutzky 2006).

Gastrointestinal disturbances, bone marrow suppression and hepatic toxicity were greater in the azathioprine group than placebo group; they were anticipated, and, by monitoring and dosage adjustment, were easily managed. Withdrawals due to adverse effects were few, occurring mostly during the first year of azathioprine treatment and mainly due to gastrointestinal intolerance (5%).
The inhibitory effect of azathioprine on the immune system could, on theoretical grounds, lead to an increased rate of malignancy with long-term therapy. In fact, concerns about increased cancer

risk have been one major issue that has limited its use. Data from the trials considered in this review, including data from a prospective 15 years-follow-up of cancer risk and death in participants to the largest randomised trial (British & Dutch 1988; Taylor 2004) did not show an increase in risk of cancer from a 3-year course of azathioprine. Evidence in the literature is available from long-term cohort studies and case-control studies in different fields, including multiple sclerosis and other autoimmune or immune-mediated diseases. The results of all these studies indicate that the long-term risk of cancer from azathioprine may be dose-related. There is a possible increased risk related to treatment duration above ten years (cumulative doses above 600 g).

In summary, there is RCT evidence in support of azathioprine in preventing recurrence of relapses, compatible with the two to three year time-frame of placebo-controlled trials. Publication bias is unlikely to have influenced our results because we made a thorough effort to trace unpublished studies and because the majority of trials did not show statistically significant reduction in the proportion of patients who had relapses on their own. Finally, if one ranks studies by their size, the largest one is that showing a statistically significant treatment effect on its own. Any effect of azathioprine on prevention of disability progression is uncertain because of inadequacies in the available data. In fact, this outcome is harder to define due to the limited expected progression of disability over the short period of the available trials.
Results of this review should be considered by those who have been skeptical about the effectiveness of azathioprine, mostly on the ground of a harmful effect in terms of malignancy.

AUTHORS' CONCLUSIONS

Implications for practice

Azathioprine is an appropriate maintenance treatment for patients with multiple sclerosis who frequently relapse and require steroids. Cumulative doses of 600 g should not be exceeded in relation to a possible increased risk of malignancy. Neurologists should make patients aware of the possible increased risk of malignancy related to long-term (above ten years) treatment.
Considering the trade off between the benefits and harms, azathioprine is a fair alternative to interferon beta for treating multiple sclerosis.

Implications for research

The number of RCTs on azathioprine so far conducted is small but provides sufficient statistical power to detect a moderate but worthwhile benefit from treatment in multiple sclerosis. A logical next step for future trials would seem to be the direct comparison of azathioprine and interferon beta. In fact, the direct comparison between these two widely used treatments in multiple sclerosis has not been made. Future trials should focus on appropriate outcome to measure effects on disability.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

British & Dutch 1988

Methods	The randomisation sequence was generated for each centre by the trial statistician and the packs of trial tablets were issued to individual pharmacies labelled with a code. The aza and placebo tablets were identical. Blindness: double. Design: parallel group. Duration: 3 years treatment/follow-up. Intention to treat analysis: performed.
Participants	RRMS N= 236 (67%); SPMS N= 67 (18%) ; PPMS N= 51 (15%) . N= 354 (azathioprine 174; placebo 180). N= 332 followed patients at 3 years (azathioprine 161; placebo 171). Sex: 207 females, 147 males. Age: mean 39 years (azathioprine); mean 38 years (placebo). Disease duration: mean 9 years. EDSS: mean 3.7(1.5) (azathioprine); mean 3.7 (1.6) (placebo). Exclusion criteria: patients on other immunomodulatory drugs or hyperbaric oxygen treatment, or patients with concomitant systemic disease and mental deficit. UK and Holland. 20 Centers.
Interventions	1. Azathioprine 2.5 mg/kg/day (to the nearest 25 mg). Control: placebo. 2. PLacebo
Outcomes	Mean change in EDSS score, Kurtzke functional scales and ambulation Index at 1, 2 and 3 years.

British & Dutch 1988 *(Continued)*

	Mean (SE) number of relapse per patient per year at 1, 2 and 3 years.
Notes	23 (6.5%) people were lost to 3 years follow-up (14 azathioprine, 9 placebo); reasons - 3 persons (azathioprine) died of a cause unrelated to MS; 16 persons (11 azathioprine, 5 placebo) declined to attend; 2 persons (placebo) emigrated; reasons not reported for 2 persons (placebo). Drop-outs/withdrawals: 1st year 47 (35 azathioprine, 12 placebo); 2nd year 16 (6 azathioprine, 10 placebo); 3rd year 11 (5 azathioprine, 6 placebo). People who dropped-out were included in analysis. Supported by the Medical Research Council. Wellcome Research laboratories supplied AZA and placebo tablets.

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Low risk	A - Adequate

Ellison 1989

Methods	Allocation: randomised (3 arms) - blocks of 4 patients. Allocation masked: patient sequence was the order of presenting the initial prescription to the pharmacy. Blindness: double. Design: parallel group. Duration: 3 years treatment/follow-up. Intention to treat analysis: performed.
Participants	MS patients in progressive phase N=65 (31 azathioprine and placebo; 34 placebo). N= 54 (83%) included in analysis (26 azathioprine and placebo; 28 placebo). Sex: 32 females, 22 males. Age at onset: mean 31 years (azathioprine); mean 33 years (placebo). Disease duration: mean 16.7 years (azathioprine); mean 12.6 years (placebo). DSS: mean 5.6 (1.3) (azathioprine); mean 5.5 (1.0) (placebo). Exclusion criteria: pregnancy or pregnancy planned within the next 3 years; men wishing to father offpring during the next 3 years. Infections not under treatment. Pressure ulcers. Active coccidiomycosis, past or present neiplastic diseases, diseases that compromise neurological assessment (deformimg arthritis, major amputations, psycoses) Cytotoxic therapy within the preceding 6 months, steroids within the preceding 3 months, relapses within the 3 months before. USA 1 centre
Interventions	1. Azathioprine started at 2.2 mg/kg/day (to the nearest 25 mg) up to above 4.4 mg/kg/day until the white blood cell count was mantained between 3000 to 4000 or adverse effects were encountered . Placebo i.v. preparation was added. 2. Placebo
Outcomes	Mean difference DSS score at 3 years (ending minus baseline). Number of patients who worsened defined as a change in DSS over 3 years Number of relapses for patient at 1, 2 and 3 years. Frequency of adverse events.
Notes	13 (19%) people were lost to 3 years follow-up (7 azathioprine, 6 placebo); reasons - 3 persons (1 azathioprine, 2 placebo) died by drowing, suicide, and ruptured abdominal aneurysm respectively ; 7 persons dropped out (3 azathioprine, 4 placebo); 3 persons (azathioprine) withdrew for causes unrelated to MS. Drop-outs/withdrawals: 25 (13 azathioprine, 12 placebo). People who dropped-out were included in analysis.

Ellison 1989 *(Continued)*

	Blindness: 47% of patients, 37% of neurologists (who performed neurological examinations and recorded the results at 3-months intervals and when a relapse was suspected), and 90% of monitoring neurologists (who assessed patients for non-MS problems including adverse effects and provided standard care) guessed the assigned treatment. Supported by USPHS grants and University grants. Wellcome Company supplied AZA and appropriate placebo; Upjohn Company provided methylprednisolone and placebo.

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Low risk	A - Adequate

Ghezzi 1989

Methods	Allocation: randomised- no further information. Blindness: single. Design: parallel group. Intention to treat not done. Duration: 18 months treatment/follow-up.
Participants	RRMS N= 74 (40%); SPMS N= 111 (60%). N= 185 (azathioprine 93; placebo 92). N= 135 (73%) included in analysis (azathioprine 69; placebo 66). Baseline characteristics available for 135 patients: Sex: 88 females, 47 males. Age at onset: mean 26 years (RRMS); mean 30 years (SPMS). Disease duration: mean 5 years (RRMS); mean 7 years (SPMS). EDSS: mean 2.1 (range 1-5) (RRMS); mean 3.7 (range 1-7) (SPMS). Exclusion criteria: disease duration lower than 1 year and concomitant diseases controindicating immunosuppression.
Interventions	1. Azathioprine 2.5 mg/kg/daily. 2. No treatment.
Outcomes	Mean number of relapses in the two groups at 18 months. Patients defined worsened in relation to the difference between final and initial Kurtzke EDSS score.
Notes	50 (27%) people were lost to 18 monhs follow-up (24 azathioprine, 26 placebo); reasons - 13 persons (azathioprine) had adverse effects and 1 person (unclear about the treatment status) had surgical operation. No other data available. People who dropped-out were not included in analysis.

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Unclear risk	B - Unclear

Goodkin 1991

Methods	Allocation: randomised; random number tables used - no further information. Blindness: double. Design: parallel group.

Goodkin 1991 *(Continued)*

	Intention to treat not done. Duration: 2 years treatment/follow-up.
Participants	Diagnosis: RRMS N= 59 (100%) N= 54 (azathioprine 29; placebo 25). N= 52 (96%) included in analysis (azathioprine 27; placebo 25). Sex: 40 females,19 males. Age at onset: mean 30 years. Disease duration: mean 6 years. EDSS: mean 3.2 (1.2) (azathioprine); mean 3.7 (1.6) (placebo). Exclusion criteria: immunosuppressive therapy for 1 year prior to the study, total lymphoid irradiation at any time. Pregnancy, unwilling to practice birth control, systemic ilnesses, unable to give informed consent. USA 1 centre.
Interventions	1. Azathioprine 3 mg/kg/daily 2. Placebo
Outcomes	Primary outcomes: mean relapse rate at 1 and 2 years. Mean change in EDSS score at 2 years. Secondary outcomes: time to first relapse, percentage of patients with at least one relapse at 1, 2 years, time to EDSS deterioration sustained for > 2 months, change in mean ambulation index score, time to deterioration of at least 1 point on ambulation index score > 2 months, time to deterioration of 20% or more in baseline nine-hole-peg test (9HPT) or in box-and-block test (BBT) for > 2 months, percentage of groups experiencing such deterioration in 9HPT or BBT, patient's subjective assessment of treatment, examining physician's assessment. * EDSS deterioration: worseniing of 0.5 or more if EDSS at entry > 5; of 1 or more if baseline EDSS < 5.5.
Notes	7 (12%) people were lost to 2 years follow-up (3 azathioprine, 4 placebo); reasons - 4 persons (1 azathioprine, 3 placebo) denied entry; 1 person (placebo) developed severe relapse before entry; 1 person (azathioprine) refused follow-up; and 1 person (azathioprine) was a protocol breach. People who did not enter the study or who dropped-out were not included in analysis. Blinding: at the end of follow-up, 56% of patients and 85% of the psysicians did not guess therapy. Supported by National Multiple Sclerosis Society. Drugs supplied by Wellcome Company. Recruitment period: 1983 to 1989.

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Unclear risk	B - Unclear

Milanese 1993

Methods	Allocation: randomised- central randomisation, provided by Wellcome Italia, with random code numbers. Masking of allocation unclear. Blindness: double. Design: parallel group. Intention to treat: performed. Duration: 3 years treatment/follow-up.
Participants	Diagnosis: RRMS N=19 (47.5%); SPMS N=10 (25%); PPMS N=11 (27.5%). N= 40 (azathioprine 19; placebo 21). N= 33 followed patients at 3 years (azahioprine 14; placebo 19). All included in survival analysis. Sex: not reported Mean age: at onset: azathioprine 29.5 years (6.5 SD); Placebo 29.6 (8.6 SD). Mean disease duration: azathioprine 92.2 months (50.4 SD); placebo 87.8 (44.9 SD).

Milanese 1993 *(Continued)*

	EDSS: mean 3.4 (1.7) (azathioprine); 3.1 (1.1) (placebo) . Relapse rate: azathioprine 0.69 (0.77 SD); placebo 0.5 (0.58 SD). Exclusion criteria: immunoprogressive treatment in the year preceding the study. Italy 1 Centre.
Interventions	1. Azahioprine 2 mg/kg/die (no more than 2.5 mg/kg/die). 2. Placebo (lactose) in identical form (50 mg tablets).
Outcomes	Annual relapse rate. Number of patients experiencing at least one relapse at 1, 2, and 3 years. Mean change in EDSS at 1 and 2 and 3 years. Number of patient remaining stable (no deterioration by 1 EDSS point or more if EDSS at entry was 5 or less or by 0.5 point or more if initial EDSS was >5).
Notes	7 (17.5%) people were lost to 3 years follow-up (5 azathioprine, 2 placebo); reasons - not reported. Drop-outs/withdrawals: 21 people (12 azathioprine, 9 placebo); reasons - 5 persons (4 azathioprine, 1 placebo) had adverse events; 13 persons (7 azathioprine, 6 placebo) required the double-blind regimen to be interruped but reasons were not reported; 2 persons(1 azahioprine, 1 placebo) had change of residence; and 1 person (placebo) due to wife's pregnancy. People who dropped-out were included in analysis. AZA and placebo tablets in identical form were supplied by Wellcome Company.

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment?	Low risk	A - Adequate

RRMS: relapsing remitting MS.
SPMS: secondary progressive MS;.
PPMS: primary progressive MS.
DSS = Kurtzke Disability Status Scale.
EDSS = Kurtzke Expanded Disability Status Scale.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Aimard 1983	Uncontrolled study.
Cendrowski 1971	Follow-up period less than one year.
Fratiglioni 1988	Uncontrolled study.
Mertin 1980	Patients had been treated with a concurrent immunosoppressive treatment.
Mertin 1982	Patients had been treated with a concurrent immunosoppressive treatment.
Minderhoud 1988	54 patients who were incorporated in the British and Dutch 1988 trial were included in this review.
Patzold 1982	It was not possible to extract outcome data.
Rosen 1979	It was not possible to extract outcome data.
Silberberg 1973	Uncontrolled study.

Study	Reason for exclusion
Swinburn 1973	Non randomised study.
Zeeberg 1985	Available information was not sufficient to define the number of randomised patients, and the number of losses to follow up.
Zeeberg 1986	Available information was not sufficient to define the number of randomised patients, and the number of losses to follow up.

DATA AND ANALYSES

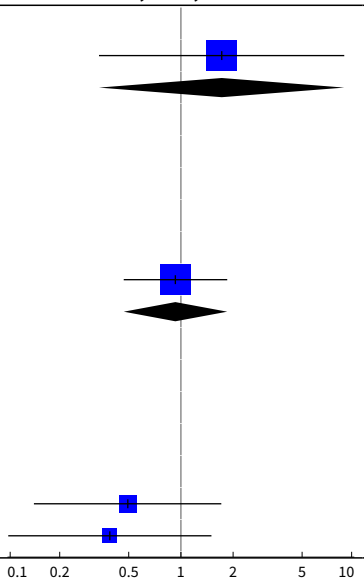
Comparison 1. Azathioprine versus placebo

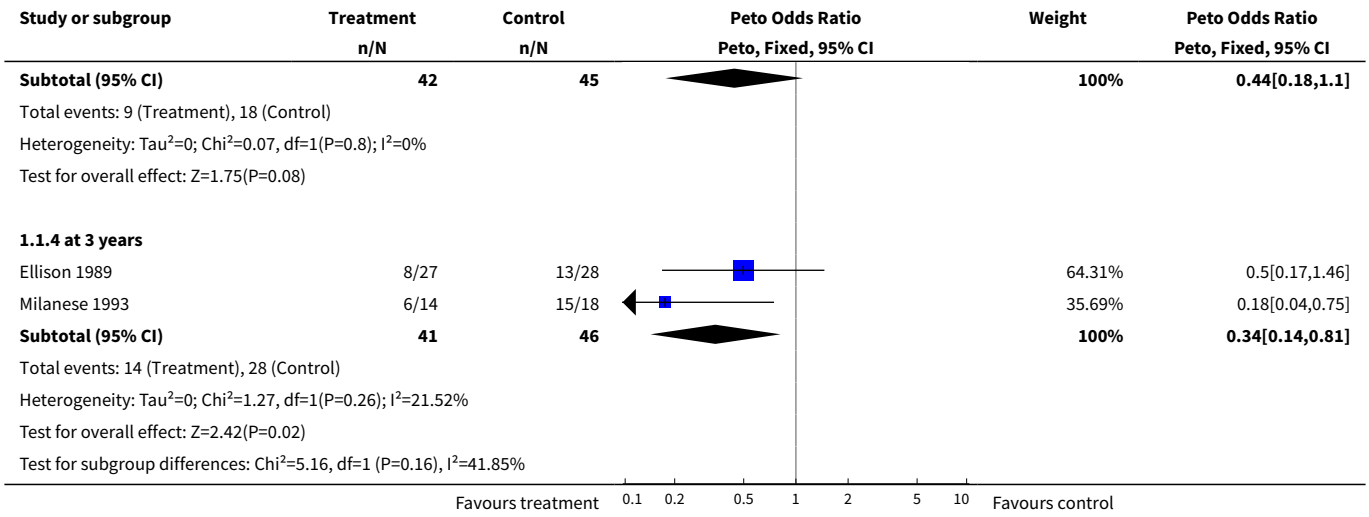
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Patients who had disability progression	4		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
1.1 at 1 year	1	37	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.72 [0.34, 8.75]
1.2 at 18 months	1	135	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.93 [0.47, 1.85]
1.3 at 2 years	2	87	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.44 [0.18, 1.10]
1.4 at 3 years	2	87	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.34 [0.14, 0.81]
2 Patients who had disability progression (worst)	4		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
2.1 at 1 year	1	40	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.92 [0.47, 7.90]
2.2 at 18 months	1	185	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.89 [0.50, 1.60]
2.3 at 2 years	2	92	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.71 [0.30, 1.66]
2.4 at 3 years	2	105	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.40 [0.18, 0.89]
3 Change in EDSS disability score	5		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
3.1 at 1 year	4	486	Mean Difference (IV, Fixed, 95% CI)	-0.00 [-0.17, 0.17]
3.2 at 18 months	1	135	Mean Difference (IV, Fixed, 95% CI)	-0.08 [-0.43, 0.27]
3.3 at 2 years	4	479	Mean Difference (IV, Fixed, 95% CI)	-0.22 [-0.44, 0.00]
3.4 at 3 years	3	419	Mean Difference (IV, Fixed, 95% CI)	-0.25 [-0.52, 0.02]
4 Patients with at least one relapse	5		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 at 1 year	4	499	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.63 [0.44, 0.90]
4.2 at 18 months	1	135	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.26 [0.64, 2.50]
4.3 at 2 years	4	488	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.47 [0.32, 0.69]
4.4 at 3 years	3	415	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.49 [0.31, 0.75]
5 Patients with at least one relapse (worst)	5		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
5.1 at 1 year	4	513	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.66 [0.47, 0.94]
5.2 at 18 months	1	185	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.11 [0.62, 1.99]
5.3 at 2 years	4	513	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.51 [0.35, 0.74]
5.4 at 3 years	3	459	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.50 [0.33, 0.77]
6 Mean number of relapses	4		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
6.1 at 1 year	4	488	Mean Difference (IV, Fixed, 95% CI)	-0.15 [-0.35, 0.05]
6.2 at 2 years	4	482	Mean Difference (IV, Fixed, 95% CI)	-0.26 [-0.43, -0.10]
6.3 at 3 years	3	417	Mean Difference (IV, Fixed, 95% CI)	-0.13 [-0.29, 0.03]

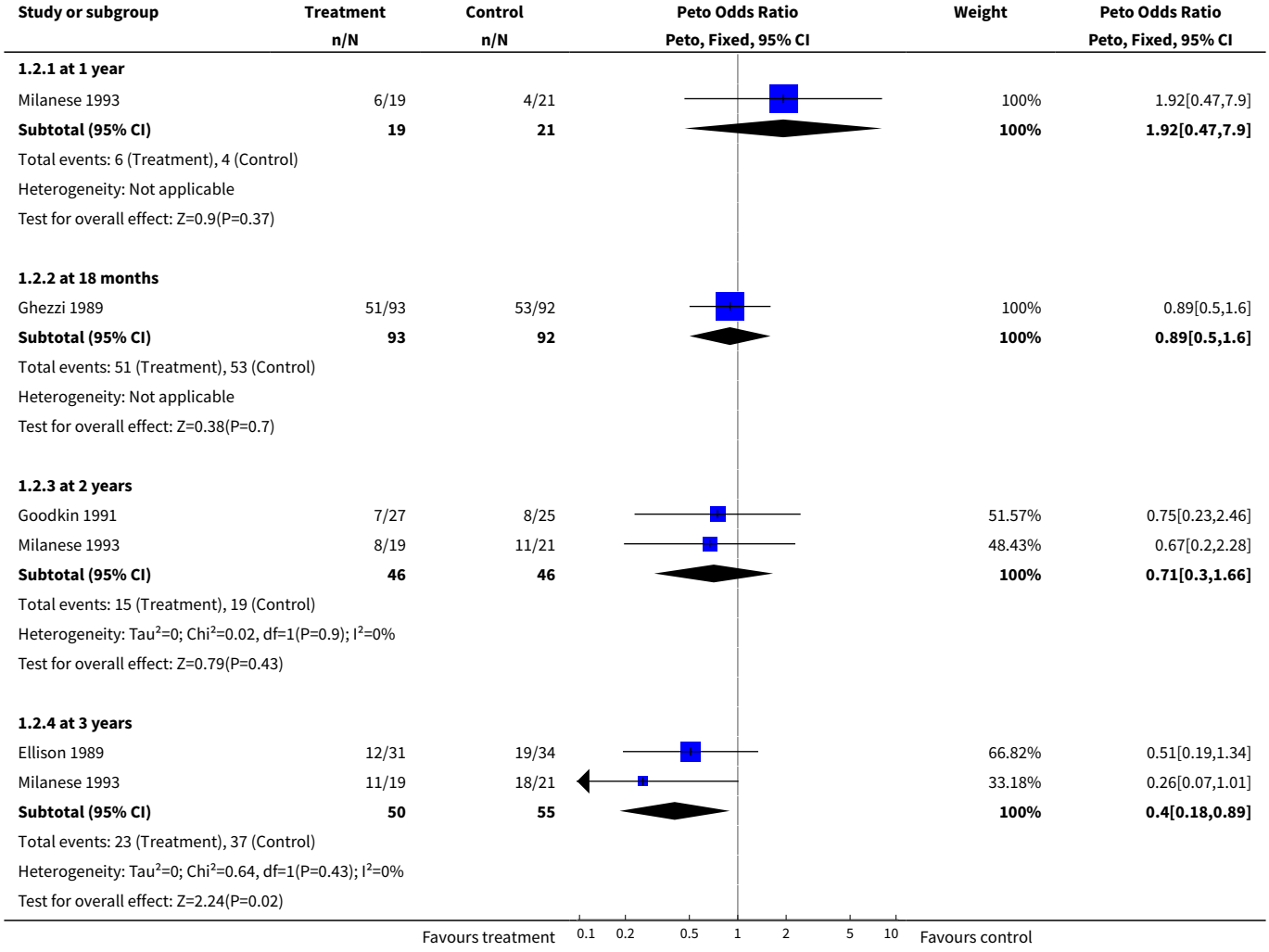
Analysis 1.1. Comparison 1 Azathioprine versus placebo, Outcome 1 Patients who had disability progression.

Study or subgroup	Treatment n/N	Control n/N	Peto Odds Ratio Peto, Fixed, 95% CI	Weight	Peto Odds Ratio Peto, Fixed, 95% CI
1.1.1 at 1 year					
Milanese 1993	4/17	3/20		100%	1.72[0.34,8.75]
Subtotal (95% CI)	**17**	**20**		**100%**	**1.72[0.34,8.75]**
Total events: 4 (Treatment), 3 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.65(P=0.51)					
1.1.2 at 18 months					
Ghezzi 1989	27/69	27/66		100%	0.93[0.47,1.85]
Subtotal (95% CI)	**69**	**66**		**100%**	**0.93[0.47,1.85]**
Total events: 27 (Treatment), 27 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.21(P=0.83)					
1.1.3 at 2 years					
Goodkin 1991	5/27	8/25		53.96%	0.49[0.14,1.71]
Milanese 1993	4/15	10/20		46.04%	0.39[0.1,1.5]

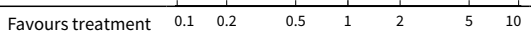




Analysis 1.2. Comparison 1 Azathioprine versus placebo, Outcome 2 Patients who had disability progression (worst).

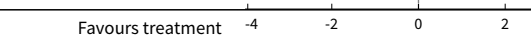


Study or subgroup	Treatment n/N	Control n/N	Peto Odds Ratio Peto, Fixed, 95% CI	Weight	Peto Odds Ratio Peto, Fixed, 95% CI
Test for subgroup differences: Chi²=4.4, df=1 (P=0.22), I²=31.78%					



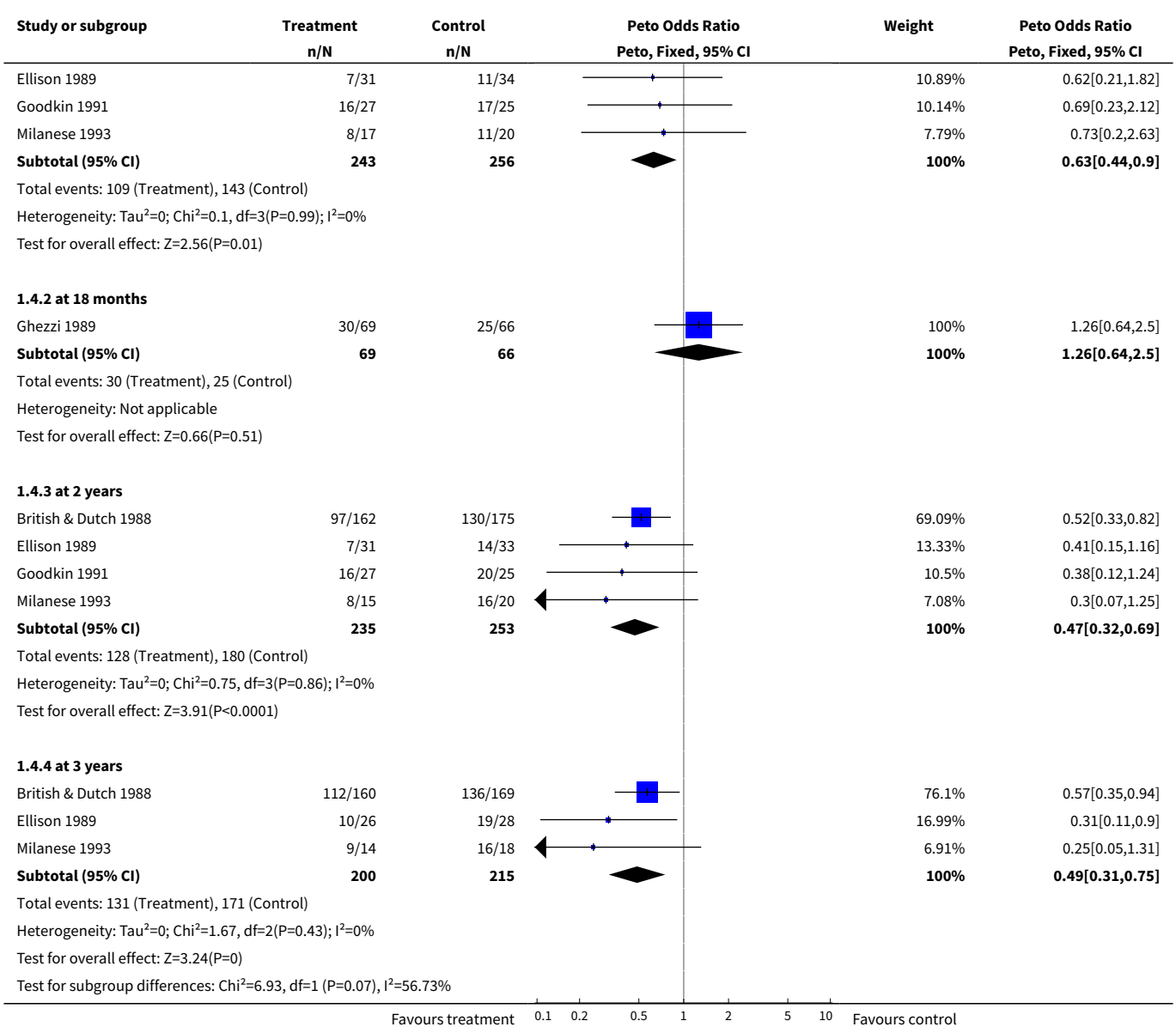
Analysis 1.3. Comparison 1 Azathioprine versus placebo, Outcome 3 Change in EDSS disability score.

Study or subgroup	Treatment N	Treatment Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
1.3.1 at 1 year							
British & Dutch 1988	164	0.1 (1.1)	170	0.1 (1)		55.66%	0.07[-0.16,0.3]
Ellison 1989	30	0 (0.8)	33	0.1 (0.8)		18.32%	-0.09[-0.48,0.3]
Goodkin 1991	27	0 (1.1)	25	0 (1.1)		8.05%	0[-0.59,0.59]
Milanese 1993	17	0.2 (0.6)	20	0.4 (0.6)		17.97%	-0.14[-0.54,0.26]
Subtotal ***	**238**		**248**			**100%**	**-0[-0.17,0.17]**
Heterogeneity: Tau²=0; Chi²=1.05, df=3(P=0.79); I²=0%							
Test for overall effect: Z=0.03(P=0.97)							
1.3.2 at 18 months							
Ghezzi 1989	69	0.5 (1.1)	66	0.6 (1)		100%	-0.08[-0.43,0.27]
Subtotal ***	**69**		**66**			**100%**	**-0.08[-0.43,0.27]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.45(P=0.65)							
1.3.3 at 2 years							
British & Dutch 1988	160	0.3 (1.3)	170	0.4 (1.3)		63.57%	-0.12[-0.39,0.15]
Ellison 1989	30	0.2 (1.1)	32	0.5 (1.1)		15.63%	-0.25[-0.8,0.3]
Goodkin 1991	27	0.2 (1.4)	25	0.4 (1.4)		8.6%	-0.25[-1,0.5]
Milanese 1993	15	0.2 (0.9)	20	0.8 (1)		12.19%	-0.66[-1.29,-0.03]
Subtotal ***	**232**		**247**			**100%**	**-0.22[-0.44,0]**
Heterogeneity: Tau²=0; Chi²=2.43, df=3(P=0.49); I²=0%							
Test for overall effect: Z=1.95(P=0.05)							
1.3.4 at 3 years							
British & Dutch 1988	161	0.6 (1.5)	171	0.8 (1.6)		65.33%	-0.18[-0.51,0.15]
Ellison 1989	26	0.4 (1)	28	0.5 (1.1)		23.47%	-0.11[-0.66,0.44]
Milanese 1993	14	0.3 (0.9)	19	1.2 (1.5)		11.21%	-0.92[-1.72,-0.12]
Subtotal ***	**201**		**218**			**100%**	**-0.25[-0.52,0.02]**
Heterogeneity: Tau²=0; Chi²=3.09, df=2(P=0.21); I²=35.26%							
Test for overall effect: Z=1.8(P=0.07)							
Test for subgroup differences: Chi²=3.51, df=1 (P=0.32), I²=14.56%							

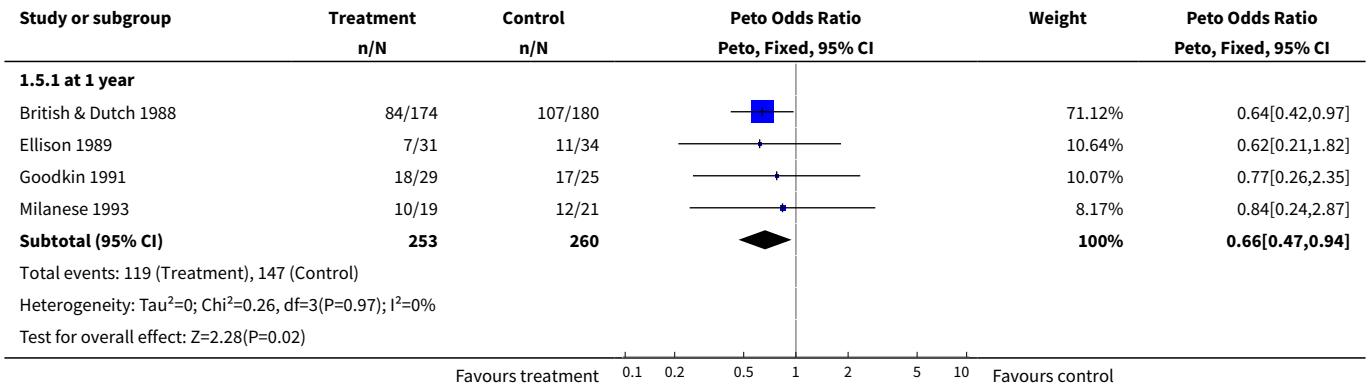


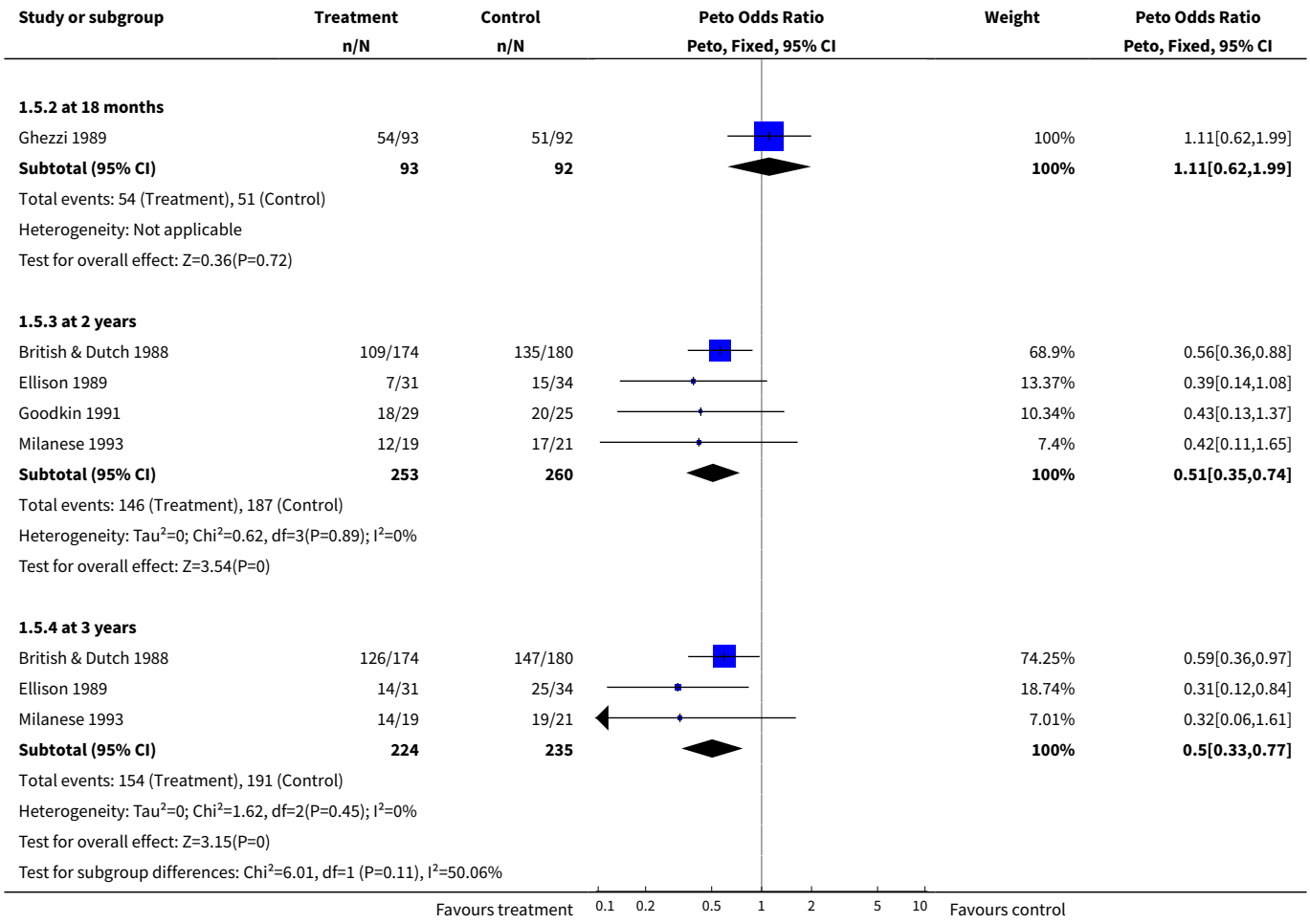
Analysis 1.4. Comparison 1 Azathioprine versus placebo, Outcome 4 Patients with at least one relapse.

Study or subgroup	Treatment n/N	Control n/N	Peto Odds Ratio Peto, Fixed, 95% CI	Weight	Peto Odds Ratio Peto, Fixed, 95% CI
1.4.1 at 1 year					
British & Dutch 1988	78/168	104/177		71.17%	0.61[0.4,0.93]

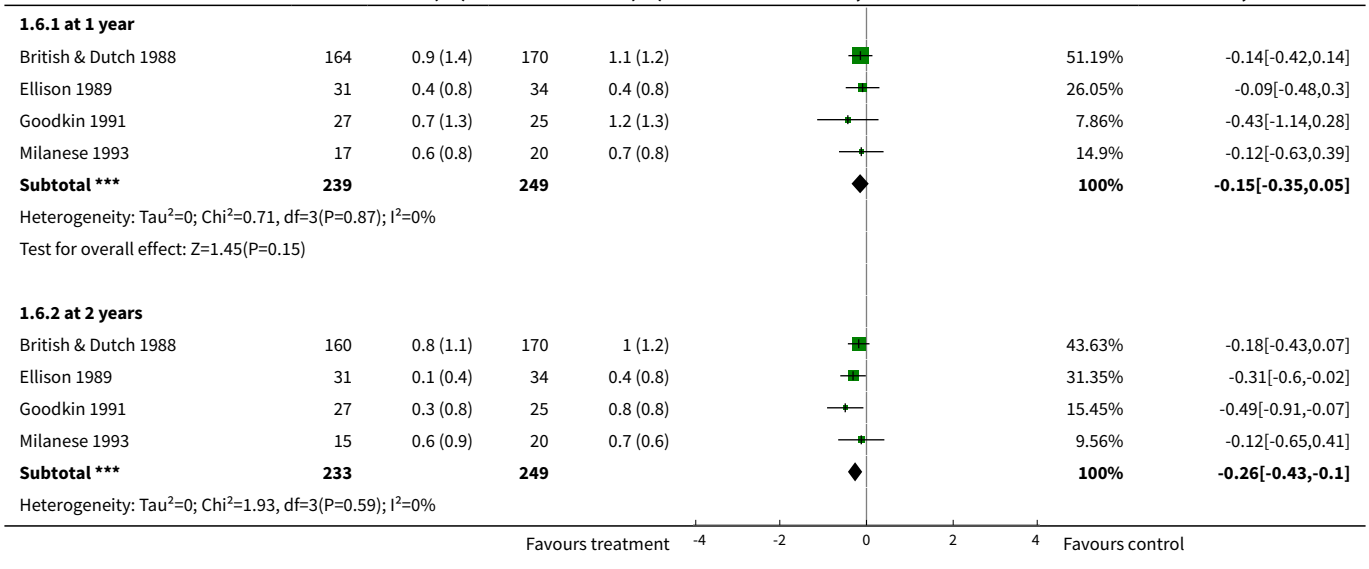


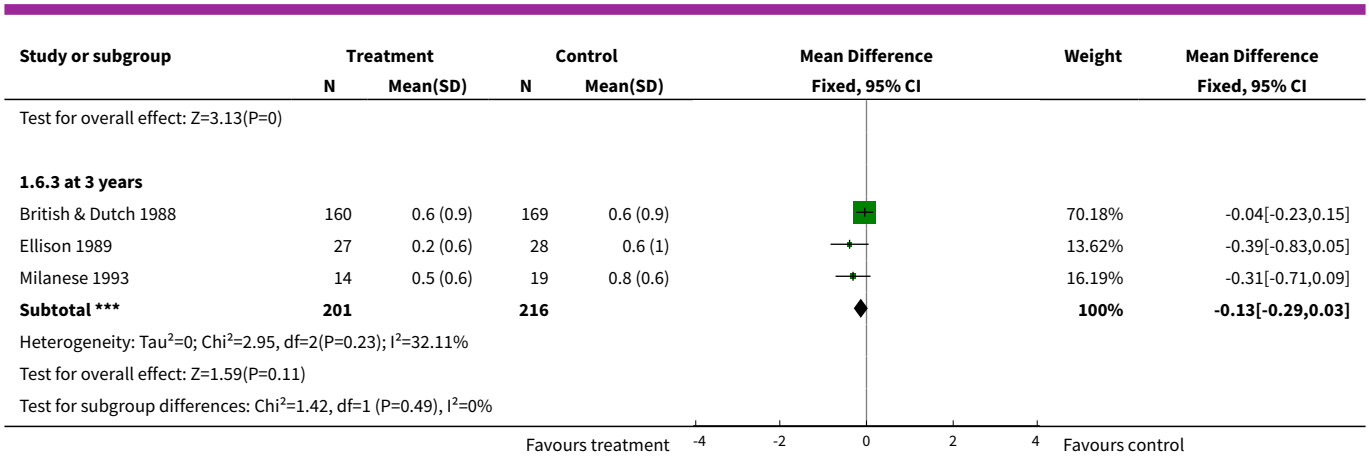
Analysis 1.5. Comparison 1 Azathioprine versus placebo, Outcome 5 Patients with at least one relapse (worst).





Analysis 1.6. Comparison 1 Azathioprine versus placebo, Outcome 6 Mean number of relapses.

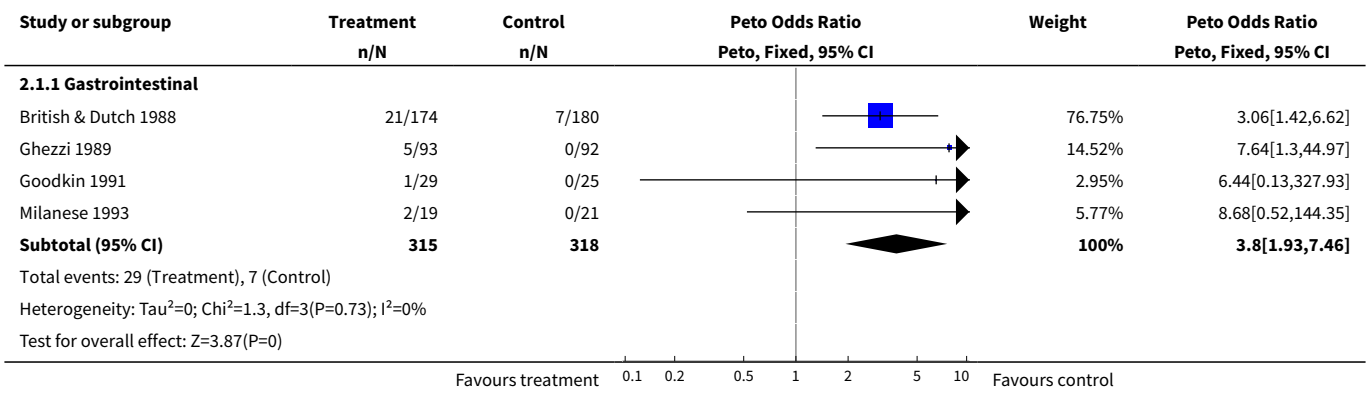


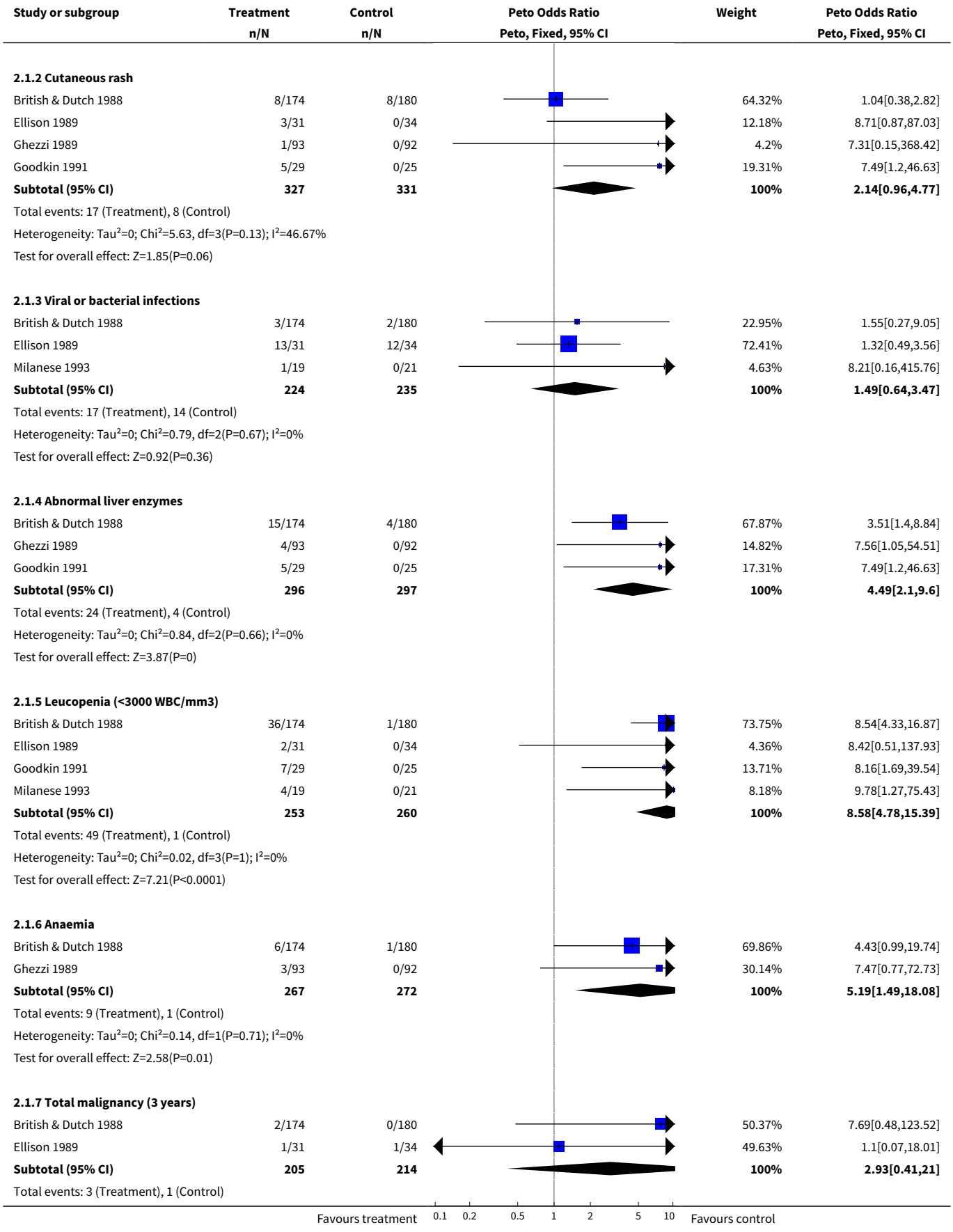


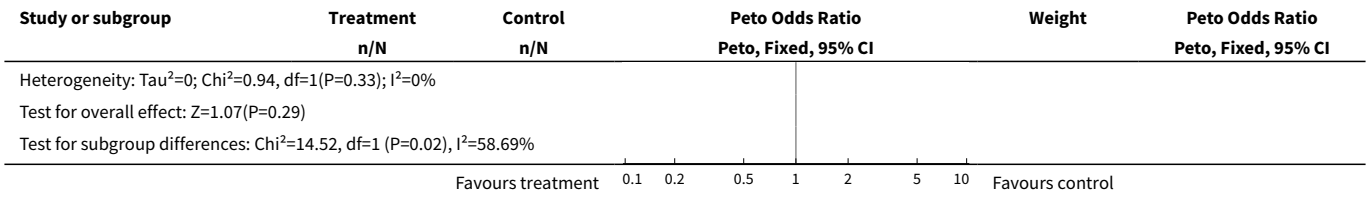
Comparison 2. Azathioprine versus placebo

Outcome or subgroup title	No. of studies	No. of partici-pants	Statistical method	Effect size
1 Adverse effects	5		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
1.1 Gastrointestinal	4	633	Peto Odds Ratio (Peto, Fixed, 95% CI)	3.80 [1.93, 7.46]
1.2 Cutaneous rash	4	658	Peto Odds Ratio (Peto, Fixed, 95% CI)	2.14 [0.96, 4.77]
1.3 Viral or bacterial infections	3	459	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.49 [0.64, 3.47]
1.4 Abnormal liver enzymes	3	593	Peto Odds Ratio (Peto, Fixed, 95% CI)	4.49 [2.10, 9.60]
1.5 Leucopenia (<3000 WBC/mm3)	4	513	Peto Odds Ratio (Peto, Fixed, 95% CI)	8.58 [4.78, 15.39]
1.6 Anaemia	2	539	Peto Odds Ratio (Peto, Fixed, 95% CI)	5.19 [1.49, 18.08]
1.7 Total malignancy (3 years)	2	419	Peto Odds Ratio (Peto, Fixed, 95% CI)	2.93 [0.41, 21.00]

Analysis 2.1. Comparison 2 Azathioprine versus placebo, Outcome 1 Adverse effects.







APPENDICES

Appendix 1. CENTRAL search strategy

#1"multiple sclerosis"
#2MeSH descriptor Multiple Sclerosis explode all trees
#3"Demyelinating disease*"
#4MeSH descriptor Demyelinating Diseases, this term only
#5"transverse myelitis"
#6MeSH descriptor Myelitis, Transverse, this term only
#7"neuromyelitis optica"
#8"optic neuritis"
#9MeSH descriptor Optic Neuritis explode all trees
#10"encephalomyelitis acute disseminated"
#11MeSH descriptor Encephalomyelitis, Acute Disseminated explode all trees
#12"devic"
#13MeSH descriptor Azathioprine explode all trees
#14azathioprine
#15azatioprina
#16immuran
#17imuran
#18imurel
#19aza
#20(#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12)
#21(#13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19)
#22(#20 AND #21)

Appendix 2. MEDLINE (PubMed) search strategy

((("Multiple Sclerosis"[mh]) OR ("Myelitis, Transverse"[mh:noexp]) OR ("Demyelinating Diseases"[mh:noexp]) OR ("Encephalomyelitis, Acute Disseminated"[mh:noexp]) OR ("Optic Neuritis"[mh])) OR ((("multiple sclerosis") OR ("neuromyelitis optica") OR ("transverse myelitis") OR (encephalomyelitis) OR (devic) OR ("optic neuritis")) OR ("demyelinating disease*") OR ("acute disseminated encephalomyelitis"))) AND ((randomized controlled trial[pt]) OR (controlled clinical trial[pt]) OR (randomized[tiab]) OR (placebo[tiab]) OR (drug therapy[sh]) OR (randomly[tiab]) OR (trial[tiab]) OR (groups[tiab])) NOT ((animals[mh]) NOT (animals[mh]) AND (human[mh])) AND (("Azathioprine"[Mesh]) OR (azathioprine) OR (azathioprina) OR (immuran) OR (imuran) OR (imurel) OR (aza))

Appendix 3. EMBASE (EMBASE.com) search strategy

(('azathioprine'/exp) OR (azathioprine:ab,ti) OR (azatioprina:ab,ti) OR (immuran:ab,ti) OR (imuran:ab,ti) OR (imurel:ab,ti) OR (aza:ab,ti)) AND ((('encephalomyelitis'/exp) OR ('demyelinating disease'/exp) OR ('multiple sclerosis'/exp) OR ('myelooptic neuropathy'/exp) OR ('multiple sclerosis':ti,ab) OR ('neuromyelitis optica':ab,ti) OR (encephalomyelitis:ab,ti) OR (devic:ti,ab)) AND (('crossover procedure'/exp) OR ('double blind procedure'/exp) OR ('single blind procedure'/exp) OR ('randomized controlled trial'/exp) OR (random*:ab,ti) OR (factorial*:ab,ti) OR (crossover:ab,ti) OR (cross:ab,ti AND over:ab,ti) OR (placebo:ab,ti) OR ('double blind':ab,ti) OR ('single blind':ab,ti) OR (assign*:ab,ti) OR (allocat*:ab,ti) OR (volunteer*:ab,ti))) AND [embase]/lim AND [humans]/lim

WHAT'S NEW

Date	Event	Description
22 August 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

All the authors conceived the idea and development of the project, posed the questions of the research project, designed the protocol, and assessed quality of the trials. Ilaria Casetta and Gerardo Iuliano abstracted data and analysed the results. Vanna Pistotti run the search strategy. All the three authors contributed to write the text of the review.

DECLARATIONS OF INTEREST

None.

INDEX TERMS

Medical Subject Headings (MeSH)

Azathioprine [adverse effects] [*therapeutic use]; Immunosuppressive Agents [adverse effects] [*therapeutic use]; Multiple Sclerosis [*drug therapy]; Neoplasms [chemically induced]; Randomized Controlled Trials as Topic

MeSH check words

Humans