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ORIGINAL ARTICLE

Cancer risk for multiple sclerosis patients treated with azathioprine and disease-modifying therapies: an Italian observational study

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Abstract

Background The risk of malignancy associated with sequential disease-modifying therapies (DMTs) for patients with multiple sclerosis (MS) is uncertain. The aim of this study was to analyze the risk of cancer in patients with MS treated with azathioprine (AZA) and the influence of sequential DMTs on the risk.

Method We retrospectively enrolled a cohort of AZA-treated MS patients followed in two Italian centers from 1987 to 2019. The ratio between observed and expected cancers in the Italian general population was calculated as standardized incidence ratio (SIR). Associations between AZA and DMTs and cancer were estimated by Cox proportional hazards model.

Results We identified 500 AZA-treated MS patients, followed for a median time of 9.7 (0.1–45.7) years: 61.8% of them were treated with DMTs. We found 22 cases of cancer (4.4%). The SIR was 1.14 (95% CI 0.98–1.29), not significantly increased in comparison with the general population. However, the risk was significantly higher in the quintiles of age 32–45, SIR 1.21 (95% CI 1.21–1.42), and 46–51, SIR 1.11 (95% CI 1.11–1.32) than in older cases. Age at AZA treatment onset was the only covariate significantly related to cancer incidence (HR = 1.049, 95% CI 1.007–1.093). The exposure to other DMTs did not modify the risk.

Conclusion The risk of malignancy in MS patients after AZA was similar to that of the general population and did not change with other DMTs sequential treatments. The increased risk in the younger ages should be considered in treatment assessment.

Keywords Multiple sclerosis · Cancer · Azathioprine · Disease-modifying therapies

Introduction

Patients with multiple sclerosis (MS) are exposed to chronic treatment with immunoactive disease-modifying therapies (DMTs), several of them with properties of immunosuppressive (IS) agents [1]. The risk of malignancy potentially associated to long-term DMTs treatment is a concern in clinical practice [2, 3], while switching from one to another DMT according to disease activity [4] may expose patients to additive or interactive effects of drugs with different mechanisms of action, modifying their safety profile. Azathioprine (AZA) is an old IS agent widely used in MS since the 1960s [5, 6]. Although replaced over the years by new MS therapies, it is currently indicated for "neurological autoimmune diseases" including MS [7] in Italy and for relapsing MS cases who do not have access to approved DMTs in the USA [8]. Previous studies on the risk of cancer induced by AZA in MS cases have reported conflicting results [2], while the risk associated with sequential DMTs is uncertain [9].

The aim of this study was to analyze the risk of cancer in a cohort of MS patients treated with AZA and to evaluate the influence of other sequential DMTs on the risk.

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Methods

Type of study

This is an observational retrospective study of MS patients followed in two hospital centers of the Italian regions of Lombardia and Veneto.

Study population

Patients with a diagnosis of MS according to McDonald's criteria [10, 11] referring to the participating clinical MS centers, with at least one AZA prescription in the period 1987–2019, were retrospectively identified and included in the study. Data prospectively recorded over the years by the neurologist in charge of patients were anonymized and entered into an ad hoc predefined computerized database. Through medical records reviewing, we collected information on the following: (1) demographic data, MS clinical characteristics, and comorbidities; (2) history of treatment with AZA, duration, cumulative dose, side effects, and reasons for discontinuation; (3) history of other DMTs therapies, time, and duration; and (4) cancer development.

Treatments

Besides AZA, the treatment object of the study, we defined four categories of immunotherapies [1] according to the immunobiological activity: (1) IS agents, interference with DNA synthesis (cyclophosphamide, mitoxantrone, teriflunomide, cladribine); (2) immunomodulating (IMM) agents, immunomodulation of the immune system (interferons -IFNs-, glatiramer acetate, dimethylfumarate); (3) sequestering agents, sequestration of leukocytes (natalizumab, fingolimod); and (4) depleting agents, depletion of immune cells (alemtuzumab, rituximab, ocrelizumab). For the purpose of analysis, the treatments were further grouped as follows: (a) AZA only; (b) IS, sequestrating and depleting agents; and (c) IMM, as specified above.

Adverse events

An adverse event was defined as an unfavorable outcome that occurred during, or after, the use of a drug, but not necessarily caused by it [12]. An adverse event was defined severe when leading to death; life-threatening; hospitalization; disability or permanent damage; or congenital disability associated with drug exposure before conception or during pregnancy.

Outcome

The outcome was the occurrence of cancer during the follow-up, as recorded in medical records by the neurologists in charge of patients and, if needed, confirmed by direct contact with the physicians involved in cancer treatment. The date of the cancer diagnosis and the type of cancer, defined according to histological findings, treatment (surgery, systemic therapy, radiotherapy), and evolution, were recorded.

Statistical analysis

Descriptive analyses included absolute counts and percentages for categorical variables, means, standard deviation (SD), medians, and range for continuous variables as appropriate. Differences between groups were analyzed using the Chi-square and Mann-Whitney tests. The risk of cancer was analyzed by a binomial logistic regression model, including as covariates age, gender, duration of treatment with AZA (since onset to withdrawal or to last visit, if still ongoing), cumulative dose, age at treatment onset, duration of follow-up, comorbidities, and history of other DMTs in term of type and duration.

The ratio between the total number of observed cancers and the number of expected cancers (O/E) in the Italian general population of the same 5-year age-class and sex was computed by the standardized incidence rate (SIR), considering 5 years age classes for age standardization and referring to expected Italian cases at 2017. The adopted level of statistical significance was $\alpha = 0.05$ (two-sided test; confidence interval (CI) at 95%) [13]. The SIRs by age of incidence in quintiles were also computed and evaluated by the same technique and statistical significance level.

The Cox proportional hazards regression was applied to evaluate the association between the cancer occurrence (dependent variable) and the characteristics of AZA therapy (age at beginning, duration in months, total dose in grams), other DMTs exposure, and number of switches. The starting time for the model computation was the date of the beginning of the AZA therapy; the ending time was the end of the follow-up (censored cases) or the tumor's incidence date (cases with the event). The statistical significance threshold for the model and the assumption of proportionality was set at $p < 0.05$. For the assumption of proportionality and the test of the proportional hazards assumption, if $p \geq 0.05$, we rejected the hypothesis of non-proportionality and accepted the results of the regression. Statistical analyses were performed using IBM SPSS v. 24 and Stata 14.0.

Results

General characteristics of the 500 MS patients included in the study and the occurrence of malignancy during the follow-up are reported in Table 1.

More than 64% of cases were women, and over 85% were from Northern Italy, 70% living either in Lombardia or Veneto. In over 63% of cases, relapsing remitting was the type

Table 1 General characteristics of 500 MS patients and malignancy

Sex: female, *n* (%)	323 (64.6)
Italian area of provenience, *n* (%)	
Nord	428 (85.6)
Centre	21 (4.2)
South and Islands	31 (6.6)
Age of MS onset, years	
Mean (± SD)	30.2 ± 10.7
Median (range)	28.0 (9–62)
Age at AZA onset, years	
Mean (± SD)	41.3 ± 11.6
Median (range)	41 (13–72)
EDSS at AZA onset	
Mean (± SD)	3.7 ± 1.8
Median (range)	3.5 (0–7.5)
Disease course at azathioprine initiation, *n* (%)	
Relapsing -remitting	316 (63.2)
Secondary progressive	138 (27.6)
Primary progressive	46 (9.2)
Disease duration, years	
Mean (± SD)	21.7 ± 10.4
Median (range)	20.8 (3.0–57.0)
Time of follow up since AZA onset, years	
Mean (± SD)	11.3 ± 7.9
Median (range)	9.7 (0.1–45.7)
Age at last follow-up, years	
Mean (SD)	52.4 ± 10.8
Median (range)	52.0 (23.0–83.0)
EDSS at the last follow-up	
Mean (± SD)	5.1 ± 2.3
Median (range)	6.0 (0–10)
Patients with diagnosis of cancer, n (%)	22 (4.4)
Cancer type	
Breast	9
Endometrial	2
Lung	2
Thyroid	2
Multiple myeloma	1
Non-Hodgkin lymphoma	1
Ovary	1
Tongue	1
Melanoma	1
Colon-rectal	1
Basal-cell carcinoma	1
Patients died at the last follow-up, *n* (%)	4 (0.8)

of disease at AZA onset. Median disease duration was 20.8 years (range 3–57), and median follow-up time since AZA onset was 9.7 years (range 0.1–45.7). The median age at last visit was 52 years (range 23–83), and last median EDSS was 6.0 (range 0–10), ≤ 5.5 in 49% and ≥ 7.5 in 18% of cases.

During the follow-up, cancer was diagnosed in 22 cases (4.4%): 5 men (lung, colon-rectal, thyroid, cutaneous basal cell, and breast carcinoma) and 17 women (breast cancer in 8 cases, endometrial cancer in 2, ovarian, lung, thyroid, tongue cancer, multiple myeloma, melanoma, non-Hodgkin lymphoma, in one case each). All patients responded to treatment with surgery (16 patients), radiotherapy (6), and/or chemotherapy (4), except one woman aged 60 who died after breast cancer metastasis. Three other cases died: a man aged 66 and a woman aged 49 for respiratory distress, and a woman of 58 years for progressive neurological worsening related to MS.

There were no statistically significant differences in AZA treatment duration (median 38 months, range 0.2–363), cumulative dose (median 133.1 grams, range 0.4–1089), disease duration, age at AZA onset, age at last visit, time of follow-up since AZA onset, EDSS score at treatment onset, and at last follow-up between cases with and without cancer.

At the last follow-up, 91 out 500 cases (18.2%) were in treatment with AZA, while 409 cases (81.8 %) have stopped it at some point of the disease course, 89 of them due to unknown reason. Long-lasting treatment was the main reason to discontinue AZA (33.4 %), followed by lack of efficacy (26.6%) and adverse events (26.9%); serious adverse events occurred in 1.9 % of cases.

In 190 cases (38.2%), AZA was the only treatment used for the disease; 71 cases (14.3%) assumed at least one other treatment pre-AZA but no treatment post-AZA, 172 patients (34.6%) had no treatment pre-AZA and at least one treatment post-AZA, and 64 cases (12.9%) assumed at least one treatment both pre- and post-AZA. There were no differences in treatments between patients with and without cancer, although the former had received more frequently IMM and sequestrating agents, and no one was treated with depleting agents (Table 2). The mean number of switches (2.1 ± 1.2) was similar in the two groups even if 31.8% of patients with cancer switched more than two DMTs compared to 18.5% of those without cancer.

The SIR was 1.14 (95% CI: 0.98–1.29), showing a non-significantly increased risk of cancer of 14% compared to the general population (Supplementary Table S1). However, in the quintiles of age 32–45 and 46–51, the risk was significantly higher: SIR 1.21 (95% CI 1.21–1.42) and 1.11 (95% CI 1.11–1.32), respectively (Table 3).

The Cox proportional hazards regression showed that the only covariate significantly related to the incidence of cancer was the age at the beginning of AZA (hazard ratio (HR) = 1.049, 95% CI, 1.007–1.093) (Table 4).

The cumulative dose of AZA showed a borderline significance, suggesting a very negligible effect. Of note, patients who received DMTs in association with AZA did not show significantly higher HR than those treated only with AZA.

Further analyses were carried out to investigate whether the cancer risk changed according to the sequence of administrating the additional drug (i.e., pre or post-AZA treatment). The HR did not change if DMTs other than IMM or IMM agents were received before AZA (HR DMTs other than IMM = 0.900, CI 0.111–7.330; HR IMM = 0.651, CI 0.147–2.878)

Table 2 History of treatments before and/or after AZA treatment by diagnosis of cancer

Variable	All cases (*n* 500)	No cancer (*n* 478)	Cancer (*n* 22)	*p* Value*
Patients treated with other DMTs, *n* (%)				
Yes	307 (61.8)§	293 (61.7)	14 (63.6)	0.854 #
No	190 (38.2)	182 (38.3)	8 (36.4)	
Number of patients in DMTs, *n* (%)*				
IMM agents	256 (51.5)	243 (51.2)	13 (59.1)	0.467 #
IS agents	148 (29.8)	141 (29.7)	7 (31.8)	0.831 #
Sequestering agents	47 (9.5)	44 (9.3)	3 (13.6)	0.493 #
Depleting agents	5 (1.0)	5 (1.1)	0 (0)	0.629 #
Duration of DMTs (AZA excluded), months				
Mean (± SD)	64.9 ± 55.3	64.6 ± 55.4	71.1 ± 53.0	0.612 °
Median (range)	48.0 (1–264)	48.0 (1–264)	64.5 (2–161)	
Duration of IMM agents, months				
Mean (± SD)	57,4 ± 49.4	57.6 ± 49.6	51.8 ± 48.2	0.700°
Median (range)	47.0 (1–264)	46.0 (1–264)	52.5 (1–161)	
Duration of IS agents, months				
Mean (± SD)	20.2 ± 20.6	20.3 ± 21.0	18.7 ± 7.5	0.473°
Median (range)	12.5 (1–120)	12.0 (1–120)	19.0 (8–30)	
Duration of Sequestering agents, months				
Mean (± SD)	49.7 ± 35.0	49.4 ± 35.7	53.0 ± 29.1	0.769°
Median (range)	44.5 (1–120)	44.0 (1–120)	55.0 (23–81)	
Duration of Depleting agents, months				
Mean (± SD)	7.4 ± 8.0	7.4 ± 8.0	0 ± 0	--
Median (range)	5.0 (1–20)	5.0 (1–20)	0 (0–0)	
Number of DMTs switches, *n*				
Mean (± SD)	2.1 ± 1.2	2.1 ± 1.2	2.6 ± 1.5	0.124 °
Median (range)	2.0 (1–8)	2.0 (1–8)	2.5 (1–6)	
Number of patients according to DMTs switches number, *n* (%)				
Zero	190 (38.2)	182 (38.3)	8 (36.4)	0.454 #
One	128 (25.8)	124 (26.1)	4 (18.2)	
Two	84 (16.9)	81 (17.1)	3 (13.6)	
Three or more	95 (19.1)	88 (18.5)	7 (31.8)	

* Comparison between cancer and no cancer groups: #Chi-square test; independent samples Mann-Whitney's *U* test

§ Uncertain data: 3 cases

Abbreviations: *DMTs* disease-modifying therapies, *IMM* immunomodulating, *IS* immunosuppressor

The agents included in each category are specified in Methods

or after AZA (HR DMT = 0.712, CI 0.168–3.013; HR IMM = 0.521, CI 0.129–2.106)

Discussion

In this retrospective cohort of 500 MS patients exposed to AZA during the course of their disease, we found an increased risk of cancer of 14% compared to the general population that was not statistically significant.

Our findings are in agreement with the results of several previous studies [14–18] (Supplementary Table S2). Other authors reported a higher risk of cancer in MS patients treated with IS agents, including AZA, compared to those not exposed [16, 19, 20], and related to the dosage and the duration of treatment [19–22].

We found that the incidence of cancer was significantly higher in the range of age 32–51 years than in older cases, suggesting a possible higher susceptibility in young patients. In a previous study involving a Sicilian cohort of MS patients, most of them treated with DMTs, a significantly higher risk of cancer was found in men aged 20–50 years and in women over 50 years [14]. Higher age at treatment initiation was identified as a risk factor for malignancies in MS patients treated with mitoxantrone while sex showed no influence [23].

Table 3 SIR by age of incidence (quintiles) and main AZA treatment characteristics

Incident age (quintiles)	Cases	F/M	SIR	95% CI		Median age at AZA onset (min-max)	Median AZA duration in months (min-max)	Median AZA total dose in grams (min-max)
32–45	5	4/1	1.212	1.208	1.416	29 (18–39)	27 (1–174)	121.5 (1.5–522)
46–51	4	3/1	1.115	1.111	1.319	37 (26–45)	39.5 (1–70)	129.7 (3–306)
53–55	5	4/1	0.558	0.554	0.562	41 (25–50)	36 (14–92)	108 (52.5–486)
56–58	4	4/0	0.398	0.394	0.402	46 (41–51)	13.5 (12–108)	136.5 (36–396)
65–70	4	2/2	0.468	0.464	0.472	46 (40–69	36 (19–63)	139.5 (57–189)
Total	22	17/5	1.138	0.982	1.294	41 (18–69)	34 (1–174)	108 (1.5–522)

These data suggest that aging may interact with several factors predisposing to, or influencing, malignancies development, including the interaction between immune system and cancerogenesis.

We observed that a non-significant higher number of cancer patients switched more than two DMTs compared to those without cancer, in agreement with previous studies showing a higher risk of cancer in MS patients switching more than two [14] or more than three DMTs [19], without association with a specific agent. However, multivariate Cox regression analyses showed that in our cases, the risk was related only to AZA age at onset and did not change when other DMTs were administered in combination with AZA, or according to the type and sequence of administering (i.e., pre- or post-AZA treatment).

However, whether the sequential exposure to other DMTs approved over time and reflecting the evolution of therapeutic armamentarium increases the risk of carcinogenesis remains a clinically relevant question. Long-term safety update for chronic therapies is further necessary in clinical setting [9], observational studies being the design of choice for the detection of rare, delayed, and/or unexpected adverse effects of drugs.

As a matter of fact, both old and new immunoactive agents have different potential to cause cancer, requiring different surveillance and monitoring [2, 23–27]. Actually, several drugs authorized for MS were long used for cancer (methotrexate, cladribine, rituximab), and some are currently evaluated for their possible anti-tumor effect (fingolimod, teriflunomide, dimethylfumarate) [3].

Our study has several limitations. Firstly, the incompleteness of data due to the retrospective study design and the possible loss to follow-up, although the included patients were prospectively followed and any effort was made to update their data. On the other hand, just because these patients were closely surveilled according to specific medical treatment

Table 4 Effects of therapeutic and individual characteristics on cancer incidence: Cox proportional hazards regression

Variables	Hazard ratio	95% Confidence interval		*p* Value
		Lower limit	Upper Limit	
Other DMTs treatments				
No. (ref. cat.)	1			
DMTs treatments other than IMM*	0.217	0.026	1.827	0.160
IMM	0.511	0.135	1.937	0.323
Number of shifts	1.257	0.868	1.822	0.226
Duration (months)	0.980	0.953	1.009	0.168
Total dose (grams)	1.004	0.996	1.012	0.315
Age at AZA start (years)	1.049	1.007	1.093	0.023

Cases in the analysis = 494. Censored cases = 472. Cancer incidence cases = 22. Time interval considered: onset of the AZA treatment - end of follow-up/cancer incidence date. *DMTs treatments other than IMM were IS, sequestrating, and depleting agents. *IMM* immunomodulating agents

protocols, early diagnosis of asymptomatic cancer was possible. Secondly, the small number of tumors and the population's geographical distribution did not allow to draw any conclusions about specific types of tumor. Thirdly, potential confounders such as smoking, family history of cancer, or alcohol use have not been evaluated as unavailable in medical records, while the impact of the new depleting agents has been poorly assessed due to their recent marketing approval.

We believe that despite these limitations, the results of our study may provide a certain assurance to clinicians that even after a prolonged treatment with AZA, the risk of malignancy was similar to that of the general population and did not change with other sequential DMTs. The finding of an increased risk in the younger age groups requires further investigation as well as careful evaluation in treatment decisions.

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Authors' contributions Loredana La Mantia: Conceptualization; Methodology; Original Draft Preparation; Writing, Reviewing and Editing Data; Data Collection and Data Interpretation; Supervision. Maria Donata Benedetti: Methodology and Statistical Analysis; Writing, Reviewing and Editing; Data Collection and Data Interpretation, Visualization, Supervision. Milena Sant: Methodology and Statistical Analysis, Data Interpretation, Critical Revision. Roberto Lillini: Statistical analysis; Writing, Reviewing and Editing; Visualization. Alessia d'Arma: Statistical Analysis, Data Management, Visualization. Sonia Di Tella: Statistical Analysis, Visualization. Laura Mendozzi, Antonio Marangi, Marco Turatti, and Domenico Caputo: Data Collection and Revision. Marco Rovaris: Data Collection and Critical Revision.

Declarations

Ethics approval The project has been approved by the Institutional Board (Number 1404201691626).

Patient consent for publication Patients signed a consent for research purposes.

Competing interests The authors declare no competing interests.

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