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Use of azathioprine for non-thymoma myasthenia and risk of cancer: a nationwide case–control study in Denmark

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Background and purpose: To evaluate the association between the use of azathioprine and risk of cancer in patients with non-thymoma myasthenia gravis (MG) in a nationwide setting.

Methods: Case–control study based on population-based registries. Cases were patients with MG with a first time diagnosis of cancer (except non-melanoma skin cancer) registered during 2000–2009, and controls were patients with MG with no history of cancer. Prior use of azathioprine in cases and controls was assessed through prescription records (1995–2009). We used unconditional logistic regression to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for cancer associated with a high cumulative dose [$\geq$ 1000 defined daily doses (DDD)] or long-term use ($\geq$ 5 years) of azathioprine, compared with never use of the drug and adjusted for potential confounders.

Results: We identified 89 cases and 873 controls. The prevalence of ever use of azathioprine was similar among cases (39.3%) and controls (39.4%). We observed a slightly elevated OR for cancer overall associated with long-term use of azathioprine (1.22; 95% CI: 0.62–2.40, $P = 0.56$). The highest ORs were observed for use of 2000 DDD or more of azathioprine; however, these risk estimates were based on small numbers.

Conclusions: Use of azathioprine in patients with non-thymoma MG may be associated with a slightly increased risk of cancer overall. Larger studies are necessary to address the risk of site-specific cancers.

Introduction

Acquired myasthenia gravis (MG) is an autoimmune disorder involving the neuromuscular junction causing muscle weakness and fatigability [1]. Treatment of MG often includes long-term immunosuppression, for example with glucocorticoids and/or azathioprine [1]. Elevated risk of cancer in patients who have undergone treatment with azathioprine has been reported for patients with inflammatory bowel disease, rheumatoid arthritis and recipients of organ transplants [2–4], and azathioprine has been classified as 'carcinogenic to humans' by the International Agency for Research on Cancer (IARC) [5]. However, use of multiple immunosuppressive drugs complicates the interpretation of studies of the carcinogenicity of single agents in organ recipients [5]. Studies on the risk of cancer associated with use of azathioprine in autoimmune disorders have yielded conflicting results [6,7]. Furthermore, there is evidence that some autoimmune disorders have an inherent risk of cancer due to the disorder *per se* [8], which may be linked to disease severity, rather than immunosuppressive treatment [9].

Relatively few studies have specifically addressed the risk of cancer in patients with MG exposed to

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azathioprine [10–13], and the majority of these studies derive from single clinics and are small, which raises methodological issues. Denmark has an array of nationwide registries that offer unique opportunities to identify patients with MG [14,15], track their use of drugs over long periods of time, and ascertain patients with MG who develop cancer. We therefore used Danish register data to examine the association between azathioprine use in patients with MG and risk of cancer in a nationwide case–control study.

Materials and methods

For the purpose of the present study we used a dataset retrieved from several nationwide registries comprising all cases of first-time ever cancer in the Danish population in 2000–2009 ($N = 267\,117$), and age- and sex-matched controls (eight controls per case) from the background population [16]. Within this dataset and by means of a validated highly sensitive and specific algorithm [14], we identified subjects with a diagnosis of MG prior to their selection as cases or controls, and included them in the present study.

Data sources

We used the following registries in our study.

1 The Civil Registration System, which has assigned a unique civil registration number to all residents of Denmark since 1968 [17]. Use of the civil registration number ensures unambiguous linkage between population-based registries. The Civil Registration System also holds continuously updated information on residency, migration and vital status.
2 The Danish National Patient Registry, which holds information on discharges (since 1977) and outpatient visits (since 1995) from all hospitals in Denmark. Patient-specific data include contact dates, and codes for hospital departments and diagnoses. Diagnosis codes are classified according to International Classification of Diseases (ICD) versions 8 (prior to 1994) and 10 (1994 to present) [18].
3 The Danish National Prescriptions Registry holds information on all prescriptions presented at community pharmacies in Denmark since 1995 [19]. For each prescription, the date the drug was dispensed and a full account of the dispensed product including the anatomical therapeutic code (ATC) [20] are recorded. The indication and prescribed dose are not available in the Prescription Registry.
4 The Danish Cancer Registry has recorded incident cases of cancer since 1943. Cancer diagnoses were recorded according to the ICD for Oncology from 1977 to 2003 (ICD-O-3), and ICD-10 since 2004 [21].
5 The Antibody Registry was created by merging data on acetylcholine receptor antibody (AChR-ab) results from the Neuroimmunology Laboratory at Rigshospitalet, Copenhagen University Hospital, collected in 1977–2008, and the laboratory at Odense University Hospital (2000–2008). Both laboratories provide in-service, as well as analyses of blood samples from other hospitals, general practitioners and privately practicing neurologists. Together, the two laboratories provide service for roughly half of the Danish population [14].
6 The Danish Pathology Registry was established in 1999. All pathology departments in Denmark report data on all pathology specimens to the registry. Information on each specimen includes diagnoses based on the Danish Systematized Nomenclature of Medicine (SNOMED) codes [22].

Identification of patients with MG

We identified all discharges and outpatient visits with a diagnosis code of MG (ICD-8 733.9; ICD-10 G70.0) in the Patient Registry (1977–2009). From the Prescription Registry, we retrieved all information on dispensed prescriptions on pyridostigmine (ATC N07AA02) in 1995–2009. In the Antibody Registry, subjects with a recorded positive AChR-ab test were identified, and the first date of a positive test was noted.

Using our validated method [14], we classified subjects as patients with MG if they fulfilled at least two of three criteria: (i) a primary diagnosis code of MG in the Patient Registry; (ii) at least two pyridostigmine prescriptions in the Prescription Registry; and (iii) a positive AChR-ab test.

Case ascertainment and selection of controls

Eligible cases and controls should fulfill the following criteria: (i) date of MG diagnosis prior to the index date, i.e. the date of cancer diagnosis for cases and date of selection for controls; (ii) no history of cancer (except non-melanoma skin cancer) prior to index date; (iii) residency in Denmark for 10 consecutive years prior to the index date; (iv) no history of organ transplantation or HIV/AIDS (see Appendix 1 for codes). We excluded subjects with a history of benign or malignant thymoma according to data from the Pathology Registry (1995–2009), the Patient Registry (1977–2009; see Appendix 1 for codes) or the Cancer Registry ($N = 42$).

Cases were patients with MG with a first-time diagnosis of cancer (except non-melanoma skin cancer) in the Cancer Registry during the period 1 January 2000 –31 December 2009. Using incidence density sampling, controls were selected by assigning all subjects with

MG a random index date between 1 January 2000 and 31 December 2009. Cases were eligible as controls if selected prior to the date of cancer diagnosis. Thereby, the odds ratio (OR) is an unbiased estimate of the incidence rate ratio [23]. To increase the likelihood of obtaining controls within the period of interest, we sampled controls twice and accounted for this by widening the confidence intervals (CIs) by use of the robust estimator [24].

Assessment of azathioprine exposure

We retrieved all information available from the Prescription Registry for cases and controls from 1995 to the index date. Based on this information we classified use of azathioprine into never use (no prescriptions) or ever use (1+ prescriptions). The latter group was further categorized according to the total number of prescriptions: 1–10; 11–20; 20+. For ever use of azathioprine, we calculated the cumulative dose as the total number of defined daily doses (DDD; 1 DDD =150 mg azathioprine) dispensed, and classified azathioprine use into the following categories: < 500 DDD; 500–999 DDD; 1000–1999 DDD; 2000–2999 DDD; and 3000+ DDD. We defined a high cumulative dose of azathioprine as a cumulative dose of 1000 DDD or more. We also estimated the total treatment duration. A treatment episode was considered to last for the duration of each prescription (set to 150 days), or until the date of the next azathioprine prescription, whichever came first. For each subject, the cumulative duration of treatment episodes was added and classified into the following categories: < 2; ≥ 2 to < 5; ≥ 5 to < 10; and ≥ 10 years. We defined long-term use as duration of treatment of 5 or more years.

The prescribed dose was not available in the prescription data. We calculated an estimated daily dose (DDD/day) as the cumulative dose in DDD dispensed divided by the total treatment duration estimated as described above, and classified this measure into < 0.5 DDD/day, ≥ 0.5 to < 1.0 DDD/day and ≥ 1 DDD/day.

Analyses

We used unconditional logistic regression to compute crude and adjusted ORs (and 95% CI) for cancer associated with the use of azathioprine. In all analyses, use of azathioprine was compared with never-use. In the regression model, we included sex, age (< 50, 50–59, 60–69, 70–79, > 80 years), and use of prednisolone or aspirin (ATC codes in Appendix 1). Exposure to the potential confounder drugs was defined as a cumulated dose of at least 500 DDD within 5 years prior to the index date. Furthermore, we included history of chronic obstructive pulmonary disease (COPD), as a proxy measure for heavy tobacco smoking, and diabetes in the model (see Appendix 1 for codes). We also included the Charlson Comorbidity Index (CCI) score [25] (CCI scores: 0, 1 and 2+). Finally, duration of MG, defined as duration in years from onset (first of the following events: date of diagnosis code; first pyridostigmine prescription; or positive AChR-ab test) to index date was also included in the model (categories: ≤ 5, 6–10, > 10 years).

To further evaluate the potential confounding influence from tobacco smoking, we examined risk estimates for the composite end-point of the following cancers known to be related to tobacco smoking: cancers of buccal cavity and pharynx, oesophagus, stomach, colorectum, liver, pancreas, nasal cavity and paranasal sinuses, larynx, lung, cervix, ovary, kidney, renal pelvis or ureter, urinary bladder or myeloid leukaemia [26]. In addition, we examined the potential modifying effect of gender by stratifying the analyses by gender.

Finally, we conducted the following sensitivity analyses: (i) the main analyses were repeated with long-term exposure defined as 500+ DDD and 1500+ DDD, respectively; (ii) subjects with MG were identified without the use of antibody data (as these were not nationwide); and (iii) the procedure for control selection was replaced with frequency matching within strata of sex, age in 10-year age-groups and duration of MG (time in years from onset to index date).

All analyses were performed using Stata Release 12.1 (StataCorp, College Station, TX, USA).

The study was approved by the Danish Data Protection Agency and the Danish Medicinal Agency.

Results

We identified 89 patients with MG with incident cancer (cases) and 873 controls during the study period, 2000–2009. Cases were more likely to be male (52.8% vs. 39.7%) and older (median age 73 vs. 65 years) than controls (Table 1). Duration of follow-up for MG, i.e. length between MG onset and index date, was similar between cases and controls. Both COPD and diabetes were more prevalent in the case group, and overall co-morbidity measured by the CCI was also higher in cases compared with controls.

Ever use of azathioprine was comparable among cases (35; 39.3%) and controls (344; 39.4%). However, cases were exposed to higher cumulative doses of azathioprine both measured as a continuous (median 783 DDD vs. 583 DDD) and a dichotomous variable

Table 1 Characteristics of cases and controls

	Cases ($N = 89$) *n* (%)	Controls ($N = 873$) *n* (%)
Sex		
Men	47 (52.8)	347 (39.7)
Women	42 (47.2)	526 (60.3)
Age, years		
< 50	10 (11.2)	189 (21.6)
50–59	13 (14.6)	143 (16.4)
60–69	14 (15.7)	182 (20.8)
70–79	27 (30.3)	189 (21.6)
80+	25 (28.1)	170 (19.5)
Duration of myasthenia, years[a]		
≤ 5	25 (28.1)	279 (32.0)
> 5 and ≤ 10	24 (27.0)	196 (22.5)
> 10	40 (44.9)	398 (45.6)
Exposure to drugs[b]		
Aspirin	20 (22.5)	142 (16.3)
Prednisolone	28 (31.5)	230 (26.3)
Co-morbidity		
CCI[c]		
0	29 (32.6)	523 (59.9)
1	38 (42.7)	222 (25.4)
2+	22 (24.7)	128 (14.7)
COPD[c]	16 (18.0)	39 (4.5)
Diabetes[d]	19 (21.3)	108 (12.4)

[a]Time period between onset of MG and index date.
[b]500 DDD or more within 1–5 years prior to index date according to Prescription Registry data.
[c]Based on Patient Registry data.
[d]Based on Patient and Prescription Registry data.
CCI, Charlson Co-morbidity Index; COPD, chronic obstructive pulmonary disease.

(high cumulative dose: 15.7% vs. 12.4%). In multivariable analyses, long-term (5+ years) use of azathioprine (OR: 1.22; 95% CI: 0.62–2.40, $P = 0.56$) was associated with a slightly increased risk of overall cancer compared with never use of azathioprine, whereas the risk estimate for a high cumulated dose of azathioprine (> 1000 DDD) was close to unity (OR: 1.06; 95% CI: 0.52–2.16, $P = 0.86$) (Table 2 & 3). Use of additional categories of cumulative dose indicated a higher risk of cancer in subjects exposed to 2000 DDD or more of azathioprine, whereas longer (10+ years) duration of use did not further increase the risk (Table 4). We also found that a high cumulated dose of azathioprine was associated with a slight increase in the risk of non-tobacco-related cancers, but not of tobacco-related cancers (Table 2). However, the statistical precision was limited in the subcategory analyses. The results were similar in men and women for both length and dose of azathioprine (data not shown).

In general, the number of cases was too small to provide precise risk estimates for site-specific cancers associated with use of azathioprine. Even so, we found that both a high cumulated dose (OR: 2.45; 95% CI: 0.44–13.64, $P = 0.31$) and long-term duration of azathioprine use (OR: 2.43 95% CI: 0.44–13.50, $P = 0.31$) were associated with an increased risk of lymphoma. However, these analyses were based on only two cases of lymphoma and did not allow a meaningful evaluation of type of lymphoma.

The sensitivity analyses all produced comparable results to those of the main analyses (data not shown).

Discussion

We found that a high cumulative dose and long-term treatment with azathioprine may be associated with a small increase in the risk of overall cancer in patients with non-thymoma MG.

According to some studies, myasthenia *per se* may influence the risk of cancer [27,28], which could make the use of population controls problematic. To avoid this potential source of bias, it is important that studies of cancer risk associated with azathioprine use are conducted within cohorts of patients with MG, an approach also used by others [10–13]. In addition, this approach addresses better the pertinent clinical issue: what are the potential trade-offs in initiating azathioprine in a patient with MG? In the largest of these studies, which included 212 cases with extrathymic cancer and 2258 controls from multiple centres, ever use of azathioprine was associated with a slightly increased risk of cancer (OR: 1.2; 95% CI: 0.9–1.7) [10]. Two other studies reported no increase in risk of overall cancer with use of azathioprine in patients

Table 2 Use of high cumulative dose of azathioprine and cancer risk in patients with myasthenia

Cancer site	Cases High/Never[a]	Controls High/Never[a]	OR (95% CI) Crude	OR (95% CI) Adjusted[b]
All malignancies	14/54	108/529	1.27 (0.69–2.33)	1.06 (0.52–2.16)
Tobacco-related cancers	6/26	108/529	1.13 (0.47–2.74)	0.82 (0.30–2.30)
All non-tobacco-related cancers	8/28	108/529	1.40 (0.62–3.16)	1.22 (0.48–3.06)

[a]Use of 1000+ DDD of azathioprine (1 DDD = 150 mg)/never use of azathioprine.
[b]Adjusted for age, sex, duration of MG, CCI, COPD, diabetes, and use of prednisolone or aspirin.
CI, confidence interval; OR, odds ratio.

Table 3 Long-term exposure to azathioprine and cancer risk in patients with myasthenia

Cancer site	Cases 5+ years/Never[a]	Controls 5+ years/Never[a]	OR (95% CI) Crude	OR (95% CI) Adjusted[b]
All malignancies	16/54	109/529	1.44 (0.80–2.58)	1.22 (0.62–2.40)
Tobacco-related cancers	8/26	109/529	1.49 (0.67–3.34)	1.15 (0.46–2.89)
All non-tobacco-related cancers	8/28	109/529	1.39 (0.62–3.13)	1.22 (0.49–3.01)

[a]Use of azathioprine.
[b]Adjusted for age, sex, duration of MG, CCI, COPD, diabetes, and use of prednisolone and aspirin.
CI, confidence interval; OR, odds ratio.

Table 4 The association between azathioprine use and risk of cancer in patients with myasthenia

Use of azathioprine	Cases	Controls	OR (95% CI) Crude	OR (95% CI) Adjusted[a]
Never use	54	529	1 (reference)	1 (reference)
Ever use				
Cumulative dose, DDD[b]				
< 500	16	162	0.97 (0.55–1.71)	0.90 (0.49–1.67)
500–999	5	74	0.66 (0.26–1.71)	0.61 (0.24–1.56)
1000–1999	8	79	0.99 (0.46–2.16)	0.85 (0.34–2.15)
2000–2999	4	25	1.57 (0.53–4.66)	1.29 (0.42–4.01)
3000+	2	4	4.90 (1.43–16.78)	3.13 (0.68–14.42)
DDD/day[b]				
< 0.5	14	153	0.90 (0.50–1.62)	0.81 (0.43–1.50)
≥ 0.5, < 1.0	19	176	1.06 (0.63–1.79)	0.92 (0.51–1.67)
≥ 1	2	15	1.31 (0.29–5.86)	1.71 (0.32–9.08)
No. prescriptions				
1–10	12	119	0.99 (0.52–1.88)	0.83 (0.42–1.63)
11–20	2	56	0.35 (0.08–1.49)	0.46 (0.11–1.96)
20+	21	169	1.22 (0.73–2.03)	1.05 (0.59–1.88)
Duration of use, years				
< 2	14	141	0.97 (0.54–1.77)	0.88 (0.46–1.68)
≥ 2, < 5	5	94	0.52 (0.21–1.31)	0.51 (0.19–1.36)
≥ 5 and < 10	13	84	1.52 (0.79–2.92)	1.23 (0.58–2.60)
≥ 10	3	25	1.18 (0.40–3.47)	1.21 (0.32–4.60)

[a]Adjusted for age, sex, duration of MG, CCI score, COPD, diabetes, and use of prednisolone or aspirin.
[b]Defined daily dose (1 DDD = 150 mg azathioprine).
CI, confidence interval; OR, odds ratio.

with MG [11,12]. In a small cohort study of patients with MG from a single centre, cancer occurrence was compared in those exposed ($N = 144$) and not exposed ($N = 44$) to azathioprine [13]. The authors reported a relative risk of cancer of 0.28 (0.04–1.44) associated with azathioprine exposure and found no association with cumulative dose of the drug.

Only one previous study has provided risk estimates of the effect of duration of immunosuppressive treatment on the risk of cancer in patients with MG. In that study, long duration (> 10 years) of immunosuppressive treatment (azathioprine or prednisolone) increased the risk of cancer (OR: 2.2; 95% CI: 0.91–5.72) [29]. Small numbers ($n = 18$), however, prevented meaningful analyses of azathioprine use. All of the aforementioned studies included patients with MG with thymoma that were excluded in our study. Although this and other differences in methodology and setting complicates comparisons between studies, we conclude that our results are in line with those of previous studies regarding the risk of overall cancer associated with azathioprine use in patients with MG.

The main strength of the present study was the use of nationwide registries with complete coverage and continuously updated data on all Danish residents – a study design that eliminated recall bias, minimized selection bias and provided a large sample compared with single-centre studies. Also, use of the Cancer Registry enabled us to identify incident cancer cases with minimal misclassification.

Our study has some potential weaknesses that should be considered. We used an algorithm to identify subjects for our study in population-based registries, which raises the question of the validity of the register diagnosis. In a validation study of the algorithm, the register diagnosis of MG had a positive predictive value of 97% and a false-positive rate of only 3% [14]. Therefore, we regard the register diagnosis of MG as highly valid.

The National Prescription Registry has collected nationwide data only since 1995. Therefore, the medication histories of our subjects spanned 5–15 years, depending on their index dates. This left truncation of Prescription Registry data may give rise to two potential problems. First, subjects who stopped using azathioprine prior to 1995 were misclassified as never users in our study. Second, the duration of use may be underestimated in subjects with azathioprine use prior to 1995. Such misclassifications most likely would produce a conservative misclassification bias, i.e. reduce the association between azathioprine use and cancer risk. However, supplementary analyses only including subjects with at least 10 years of medication history, i.e. index date 2005–2009, produced similar point estimates to those of the total sample,

indicating that this source of misclassification had little influence on our results (data not shown).

Thymoma, detected in approximately 10% of patients with MG, has been linked to both an increased severity of MG [1,30], a factor that could influence the use of immunosuppressants, and an increased risk of non-thymoma cancer [31,32]. We handled this potential confounder by excluding subjects with thymoma from our study population. We achieved virtually complete coverage for all types of thymoma by combining data from the Cancer, Patient and Pathology Registries. We cannot rule out some degree of misclassification for thymoma cases diagnosed prior to 1997 where identification in our study was based on Cancer and Patient Registry data only, particularly because the Cancer Registry data on thymoma are incomplete for benign thymoma [33], but we believe that the magnitude of this misclassification is small.

A small minority of patients with myasthenia, previously classified as antibody negative, have been shown to have antibodies against a kinase (MUSK). In our experience, the incidence of MUSK-positive myasthenia is very low in Denmark. Therefore, lack of information on MUSK status in our study only represents a minor problem.

We observed a slightly increased risk of overall lymphoma (Hodgkin's and non-Hodgkin's) associated with azathioprine use, whereas the limited statistical precision did not allow a solid evaluation of the risk of non-Hodgkin's lymphoma – the type of lymphoma classified as causally related to use of azathioprine according to the IARC [5]. The broad confidence intervals surrounding our point estimates of risk of lymphoma clearly indicate that our study was underpowered to identify risks by cancer site.

In conclusion, we found that azathioprine may be associated with a slight increase in overall cancer in patients with MG. We hope to be able to overcome some of the sample size limitations of the present study in future studies based on updated data sets from Denmark or, if feasible, in collaborative projects involving data from other Nordic countries, where the health care and registry settings are highly comparable.

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Disclosure of conflict of interest

The authors declare no financial or other conflict of interests.

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Appendix 1

List of codes used in the analysis

Hospital discharges codes
Myasthenia
ICD-8: 73309
ICD-10 DG700
Chronic obstructive pulmonary disease
ICD-8: 490.00, 491.00, 491.01, 491.03
ICD-10: J42, J43, J44
Diabetes
ICD-8: 249.00, 249.09, 250.00, 250.09
ICD-10: E10-E14
Thymoma[a]
ICD-8: 226.19, 194.29
ICD-10: DD38.4, DC37.9
Organ transplantation
ICD-8: 997.70
ICD-10: DZ940-4, DZ948-9
Operation classification: KKAS, KTQA, KJJC, KJLE
HIV, AIDS
ICD: DB20-4
Anatomical Therapeutic Classification Codes (ATC)
Pyridostigmine
N07AA02
Aspirin
B01AC06, N02BA01, N02BA51
Anti-diabetics
A10
Prednisone/Prednisolone
H02AB06, H02AB07
Pathology Registry codes (SNOMED)
Thymoma[a]
M85800, M85801, M85803, M85804, M85806, M85807, M85811, M85821, M85831, M85841, M85851

[a]Used to identify patients with thymoma prior to index date who were subsequently excluded from the sample.