doi:10.1111/ene.12329

Risk of non-melanoma skin cancer in myasthenia patients treated with azathioprine

E. G. Pedersen[a], A. Pottegård[b], J. Hallas[b], S. Friis[c], K. Hansen[d], P. E. H. Jensen[e] and D. Gaist[a]

[a]*Department of Neurology, Faculty of Health Sciences, Odense University Hospital and Institute of Clinical Research, University of Southern Denmark (SDU), Odense;* [b]*Institute of Public Health, Clinical Pharmacology, SDU, Odense;* [c]*Danish Cancer Society Research Centre and Department of Public Health, University of Copenhagen, Copenhagen;* [d]*Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Copenhagen; and* [e]*Neuroimmunology Laboratory, DMSC, Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark*

Keywords:
case–control studies, epidemiology, myasthenia, oncology

Received 15 August 2013
Accepted 18 November 2013

Background and purpose: The association between use of azathioprine and risk of non-melanoma skin cancer (NMSC) in patients with myasthenia was evaluated in a nationwide setting. Treatment of autoimmune myasthenia frequently involves long-term exposure to immunosuppressants, including azathioprine. Use of azathioprine increases the risk of NMSC in organ recipients and probably also in patients with other autoimmune disorders. No previous study has specifically investigated the risk of NMSC in myasthenia patients treated with azathioprine.

Methods: This is a case–control study based on Danish population-based registries. Cases were myasthenia patients with a first time diagnosis of NMSC during 2004–2009. Age- and sex-matched controls were selected amongst myasthenia patients with no history of cancer using incidence density sampling. Prior use of azathioprine in cases and controls was assessed through prescription records (1995–2009). Conditional logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (CI) for skin cancer associated with a high cumulative dose (≥150 g) or long-term use (≥5 years) of azathioprine, adjusted for confounders.

Results: Thirty NMSC cases and 360 matched controls were identified. Ever use of azathioprine was associated with a considerably increased risk of NMSC (OR 3.3, 95% CI 1.5–7.3) that was even more apparent in patients with high cumulative dose (OR 4.6, 95% CI 1.7–12.5) or long-term (OR 4.8; 95% CI 1.7–13.6) use of azathioprine.

Conclusion: Azathioprine use in patients with myasthenia is associated with an increased risk of NMSC.

Introduction

Myasthenia frequently requires immunosuppressive treatment. European guidelines on myasthenia treatment recommend the use of azathioprine when long-term immunosuppression is necessary [1]. Azathioprine is converted into 6-thioguanine after ingestion and subsequently incorporated into the DNA of renewing cells, including skin cells [2]. Unlike the native DNA base, 6-thioguanine absorbs UVA resulting in DNA oxidation which constitutes a potential risk of developing skin cancer [2].

Correspondence: D. Gaist, Department of Neurology, Odense University Hospital, Sdr Boulevard 29, 5000 Odense C, Denmark (tel.: +45 6541 2485; fax: +45 6541 3389; e-mail: dgaist@health.sdu.dk).

An elevated risk of skin cancer associated with azathioprine therapy has been reported in recipients of organ transplants [3,4] and in patients with autoimmune disorders other than myasthenia, and the International Agency for Research on Cancer has classified azathioprine as 'carcinogenic to humans' with specific reference to skin cancer (squamous cell carcinoma, SCC) [4]. However, concurrent use of multiple immunosuppressive drugs complicates the interpretation of studies of the carcinogenicity of single agents in organ recipients [4]. A limited number of studies involving autoimmune disorders other than myasthenia have reported an increased risk of non-melanoma skin cancer (NMSC) associated with azathioprine use [5–8]. However, there is evidence that some autoimmune disorders *per se* have an inherent

risk of cancer [9], which may be linked to disease severity rather than immunosuppressive treatment [10]. The results of studies on risk of cancer associated with use of azathioprine in autoimmune disorders other than myasthenia may therefore not necessarily apply to myasthenia patients.

No previous study has specifically addressed the risk of NMSC in myasthenia patients exposed to azathioprine.

Methods

Setting

Nationwide Danish registries offer unique opportunities to identify myasthenia patients [11,12], track their use of drugs over long periods and ascertain myasthenia patients who develop cancer including NMSC. These unique data sources were used to examine the association between azathioprine use in myasthenia patients and risk of NMSC in a nationwide case–control study. A data set retrieved from several registries comprising all incident cancer cases in the Danish population in 2000–2009 and age- and sex-matched controls from the background population was used [13]. Within this data set and by means of a validated, highly sensitive and specific algorithm [11], subjects with a diagnosis of myasthenia were identified prior to diagnosis of NMSC or selection as controls.

Using the unique civil registration number assigned to all residents of Denmark since 1968 by the Danish Civil Registration System [14], data from the following registries were linked in our study: (i) the Danish National Patient Registry [15]; (ii) the Danish National Prescription Registry [16]; (iii) the Danish Cancer Registry [17]; and (iv) the Danish Pathology Registry [18]. Data from an *ad hoc* registry, the Antibody Registry, created by merging test results on acetylcholine receptor antibody (AChR-ab) from two laboratories providing services for roughly half the Danish population since 1977 were also used [11]. Our data sources have been described in more detail elsewhere [19].

Identification of myasthenia patients

Data from the above registries were retrieved and our validated method was used [11,12] to classify subjects as myasthenia patients from the date they fulfilled at least two of the following three criteria: (i) a primary diagnosis code of myasthenia 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

Cases were myasthenia patients with a first time diagnosis of histologically verified NMSC in the Cancer Registry during 2004–2009. In this period skin cancers registered at pathology departments were included in the Cancer Registry along with the official reports from the primary or secondary (hospitals) health sector. It was further required that the cases should be without any history of cancer at the date of NMSC diagnosis (index date), have continuous residency in Denmark 10 years prior to the index date, have no history of organ transplantation or HIV/AIDS, and have no history of benign or malignant thymoma. Using risk set sampling [20] and applying the same selection criteria as for cases, each case was matched to 12 controls by age ($\pm$2 years), gender and calendar year. Controls were assigned an index date corresponding to the date of diagnosis for the cases. To account for selection of the same individual as control on more than one occasion, the robust estimator technique was used to widen the confidence intervals (CIs) for odds ratios (ORs) [21]. Cases were eligible as controls before they became cases. Thereby, the odds ratio is an unbiased estimate of the incidence rate ratio [20].

Azathioprine exposure

All information available from the Prescription Registry for cases and controls from 1995 to the index date was retrieved. Based on this information, the use of azathioprine was classified into never use (no prescriptions) or ever use (1+ prescriptions). Azathioprine dispensed within 1 year of the index date was classified as current use. For ever use of the drug, the cumulative dose (total number of dispensed grams) was calculated and a high cumulative dose was defined as a cumulative dispensing of at least 150 g of azathioprine. Also cumulative dose was categorized into three categories (<150 g, 150–299 g, 300+ g) and tested for trend. Treatment duration was estimated assuming that a treatment episode lasted for 150 days from each prescription or until the date of the next azathioprine prescription, whichever came first. Cumulative treatment duration was classified into <5 years and 5+ years.

Analyses

Conditional logistic regression was used to compute crude and adjusted odds ratios (and 95% confidence intervals) for cancer associated with use of azathioprine. Ever use, high cumulative use (≥150 g)

or long-term use (≥5 years) of azathioprine were compared with never use of the drug. The analyses were adjusted for duration of myasthenia (≤10; >10 years) and Charlson Comorbidity Index score [22] (0; ≥1). Age, gender and calendar year were handled by the matching procedure.

In order to express NMSC risk associated with a high cumulative dose of azathioprine use in absolute terms, the 'number needed to treat for one additional patient to be harmed' (NNTH) was estimated using the method proposed by Bjerre *et al*. [23]. The method was modified slightly to express the required exposure as a number of person-years rather than a count of persons. Data from the current study and from a recent epidemiological study on myasthenia in Denmark [12] were used to estimate the incidence rate of NMSC in myasthenia patients who were never users of azathioprine. The NNTH was calculated for the entire myasthenia population and specifically for myasthenia patients aged 50 years or above.

Several supplementary analyses were performed: (i) cases were classified by type of NMSC [SCC versus basal cell carcinoma (BCC)]; (ii) analyses were stratified by age (<70 vs. ≥70 years) and sex; (iii) the study base was expanded to 2000–2009, thus including cases and controls from 2000 to 2003 (Danish Cancer Registry data on NMSC from 2000 to 2003 incomplete); (iv) analyses were stratified by use of prednisolone (<5 g vs. ≥5 g); (v) analyses included use of 'other immunosuppressants' as a confounder in a separate model. 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. Approval from an ethics committee was not necessary according to Danish law.

Results

Thirty patients with NMSC (cases), i.e. 22 cases of BCC and eight of SCC, and 360 controls were identified in the study period 2004–2009. Cases were more frequently exposed to azathioprine (53% vs. 30%) or other immunosuppressants (20% vs. 5%), and a larger proportion had suffered from myasthenia for >10 years (73% vs. 51%) compared with controls (Table 1).

Ever use of azathioprine was associated with an increased risk of NMSC (adjusted OR 3.3, 95% CI 1.5–7.3), and this risk further increased in analyses restricted to current use of azathioprine (OR 4.7, 95% CI 1.8–12.0). A high cumulative dose (above 150 g) of azathioprine was associated with an adjusted OR for NMSC of 4.6 (95% CI 1.7–12.5), and a statistically significant trend was present for increasing cumulative doses (<150 g, 150–300 g, 300+ g) of azathioprine ($P = 0.003$ for trend) (Table 2). In subanalyses defining azathioprine use according to treatment duration, use of azathioprine for 5 or more years was associated with a two-fold higher risk estimate (OR 4.8, 95% CI 1.7–13.6) than azathioprine use for <5 years (OR 2.4, 95% CI 0.9–6.3). In analyses by subtype of NMSC, ever use of azathioprine was more strongly associated with SCC (OR 8.8, 95% CI 1.1–69.6) compared with BCC (OR 2.4, 95% CI 0.9–6.1), and this pattern was further apparent in analyses restricted to high cumulative dose of azathioprine (SCC: OR 16.9, 95% CI 1.7–159.4; BCC: OR 3.1, 95% CI 0.8–11.3), although based on small numbers. The remaining supplementary analyses produced results similar to those of the main analyses (data not shown).

The incidence rate of NMSC amongst myasthenia patients who were never users of azathioprine was 4.6 per 1000 person-years (based on 14 unexposed cases). The corresponding NNTH was 60 person-years (95% CI 19–309 person-years), i.e. one additional NMSC was induced for each 60 person-years of azathioprine exposure. For subjects above 50 years, the corresponding value was 30 person-years (95% CI 11–82 person-years). The limited numbers of NMSC cases amongst study subjects below 50 years did not allow computation of an NNTH estimate.

Table 1 Characteristics of subjects in a study of risk of non-melanoma skin cancer in patients with myasthenia in Denmark

	Cases ($N = 30$)	Controls ($N = 360$)
Sex		
Men	10 (33)	120 (33)
Women	20 (67)	240 (67)
Age, years		
Median (interquartile range)	70 (62–79)	70 (60–79)
Duration of myasthenia, years[a]		
≤10	8 (27)	176 (49)
>10	22 (73)	184 (51)
Charlson index score[b] of comorbidity		
0	15 (50)	178 (49)
≥1	15 (50)	182 (51)
Ever use of immunosuppressants[c]		
Azathioprine	16 (53)	107 (30)
Prednisolone/prednisone	19 (63)	176 (49)
Other immunosuppressants[d]	6 (20)	17 (5)

Numbers are percentages unless otherwise stated. [a]Time period since onset of myasthenia according to register data. [b]Based on Patient Registry data. [c]Based on Prescription Registry data. [d]ATC codes in parenthesis: methotrexate (L04AX03), ciclosporine (L04AD01), tacrolimus (L04AD02), cyclophosphamide (L01AA01), mycophenolate mofetil (L04AA06).

Table 2 Use of azathioprine and risk of non-melanoma skin cancer in patients with myasthenia in Denmark

Azathioprine use	Cases (N = 30)	Controls (N = 360)	Odds ratio (95% confidence interval)	
			Crude	Adjusted[a]
Never use	14	253	1 (reference)	1 (reference)
Ever use	16	107	2.8 (1.3–6.0)	3.3 (1.5–7.3)
High cumulative dose[b]	9	30	5.6 (2.2–14.4)	4.6 (1.7–12.5)
Cumulative dose, g[c]				
<150	7	77	1.8 (0.6–5.2)	2.3 (0.9–6.2)
150–300	5	17	6.5 (2.1–19.7)	5.6 (1.6–19.3)
300+	4	13	5.5 (1.2–25.0)	3.9 (0.8–19.3)
Treatment duration, years				
<5	7	72	1.9 (0.7–5.0)	2.4 (0.9–6.3)
5+	9	35	4.9 (1.8–13.3)	4.8 (1.7–13.6)

[a]Adjusted for duration of myasthenia and Charlson Comorbidity Index. [b]Use of 150 g or more of azathioprine versus never use of azathioprine. [c]Test for trend: P = 0.003.

Discussion

Our main finding was a considerably increased risk of NMSC associated with azathioprine use in patients with non-thymoma myasthenia. The highest risk estimates were found for a cumulative dose of azathioprine above 150 g or for duration of azathioprine treatment for 5 or more years. The association of azathioprine with skin cancer has not previously specifically been investigated in patients with myasthenia, although it has been reported in other patient populations, i.e. recipients of organ transplants and patients with inflammatory bowel disease disorders [5,7].

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 and minimized selection bias. Use of the Cancer Registry enabled us to identify incident histologically verified NMSC cases with minimal misclassification.

Our study has some potential weaknesses. An algorithm was used to identify study subjects through registries, which raises the question of the validity of the diagnosis. This may be particularly pertinent in patients with ocular myasthenia, where AChR-ab status is negative in up to 50% of cases. Furthermore, it is conceivable that pyridostigmine prescription renewal may be less frequent in patients with ocular myasthenia, amongst whom pyridostigmine is not as effective as in generalized myasthenia. These aspects may infer selective under-ascertainment of cases of ocular myasthenia by our algorithm. However, ocular myasthenia comprises only approximately 10%–15% of myasthenia cases, and there is no evidence on differential cancer risk according to type of myasthenia. Furthermore, in a validation study of the algorithm, the register diagnosis of myasthenia had a positive predictive value of 97% and a false positive rate of only 3% [11]. Therefore, the register diagnosis of myasthenia is regarded as highly valid.

The Cancer Registry has a slight under-reporting of NMSC (≈3%), especially of SCC [24]. It is not believed that this small percentage of under-reporting affects our estimates, especially since there is no reason to believe that under-reporting was associated with azathioprine use. In fact, our setting, with national reporting of skin biopsy results from hospitals, private dermatologists and plastic surgeons, and general practitioners, offers unique opportunities for this type of study.

The National Prescription Registry has been in operation since 1995. Subjects who stopped using azathioprine before 1995 were therefore misclassified as never users in our study. Also, cumulative dose and length of use may have been underestimated for some subjects. However, these biases are probably conservative, i.e. the misclassification reduced the observed association between azathioprine use and risk of NMSC.

Due to the observational design of our study, the possibility cannot be excluded that inadequately measured confounders influenced our results. Importantly, no information was available on exposure to sunlight, the most important risk factor for NMSC [4,7]. However, there is little reason to believe that patients with azathioprine use are more exposed to sunlight than non-users of the drug.

It was found that use of azathioprine was associated with a three- to four-fold increased risk of NMSC which in absolute terms corresponds to one excess case of NMSC for every 60 years of high cumulative treatment (>150 g). Our finding of a considerably increased risk of NMSC associated with azathioprine use in patients with myasthenia may be causal, and underscores the importance of clinicians advising patients on vigilant sunscreen use and

avoidance of prolonged sunlight exposure in order to prevent NMSC. Regular dermatological screening of patients using azathioprine should also be considered in high risk patients, e.g. those with long-term use and/or high cumulative azathioprine dose.

Acknowledgements

Savværkejer Jeppe Juhl og hustru Ovita Juhls Mindelegat fund is a private fund that supported the study.

Disclosure of conflicts of interest

Drs Pedersen, Hallas, Jensen and Gaist report no disclosures. Anton Pottegård reports no disclosures. Dr Hansen receives royalties from ThermoFisher Scientific. Dr Friis teaches at courses in pharmacoepidemiology at the Danish Association of the Pharmaceutical Industry.

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