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Original Contribution

Use of Azathioprine and the Risk of Cancer in Inflammatory Bowel Disease

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Increased risks of lymphoma and skin cancer associated with thiopurine use among patients with inflammatory bowel disease have been shown, but data on the overall cancer risk are limited. We conducted a historical cohort study of 45,986 patients with inflammatory bowel disease (of whom, 5,197 (11%) used azathioprine) in Denmark from 1997 to 2008. We linked registry data on filled drug prescriptions, cancer diagnoses, and covariates and compared rates of overall incident cancer and cancer subgroups between users and nonusers of azathioprine, adjusting for propensity scores. During a median 7.9 (interquartile range: 3.5–12.0) person-years of follow-up, 2,596 incident cases of cancer were detected. Azathioprine use was associated with an increased risk of overall cancer (rate ratio = 1.41, 95% confidence interval: 1.15, 1.74), whereas former use of azathioprine (rate ratio = 1.02, 95% confidence interval: 0.83, 1.25) or increasing cumulative received doses (increase in rate ratio per 365 additional defined daily doses = 1.06, 95% confidence interval: 0.89, 1.27) were not. In subgroup analyses, azathioprine use was associated with increased risk of lymphoid tissue cancer (rate ratio = 2.40, 95% confidence interval: 1.13, 5.11) and urinary tract cancer (rate ratio = 2.84, 95% confidence interval: 1.24, 6.51). In conclusion, azathioprine use was associated with an increased risk of overall cancer in patients with inflammatory bowel disease, although these data cannot establish causality.

immunosuppressive agents; inflammatory bowel diseases; neoplasms; pharmacoepidemiology; registries

Abbreviations: CI, confidence interval; IBD, inflammatory bowel disease; ICD-8, *International Classification of Diseases, Eighth Revision*; ICD-10, *International Classification of Diseases, Tenth Revision*; RR, rate ratio.

More than 1 million people suffer from Crohn's disease and ulcerative colitis (forms of inflammatory bowel disease (IBD)) in North America alone, and studies indicate that the incidence continues to increase in many countries (1, 2). Thiopurines (azathioprine and 6-mercaptopurine) are used for their antiinflammatory properties in the treatment of IBD (3–5), often in combination with other drugs including tumor necrosis factor inhibitors (6). With increasing use and earlier initiation of thiopurines (7–9), a growing number of patients will be exposed to these immunosuppressive drugs over long periods of time, which calls for attention to their safety, including the potential occurrence of cancer (10, 11).

A risk of lymphoma associated with thiopurine treatment in patients with IBD is supported by several studies that have indicated at least a 3-fold increased risk (12–14). Additionally, several recent reports indicate increased risk of skin cancer associated with thiopurine use (15–17). Because thiopurines may induce mutations in human cells and interfere with DNA repair mechanisms (10, 18, 19), these drugs could potentially influence the risk of several types of cancer.

Given this background, estimates of the overall risk of cancer associated with use of thiopurines are needed to support clinical decision making in weighing the benefits against risks of therapy. Although the few studies addressing the overall cancer risk associated with thiopurine treatment in patients with IBD found no significantly increased risk, they had limited power, lacked adequate control groups, presented a limited degree of detail regarding drug exposure, or represented restricted populations (12, 20–23).

We conducted a nationwide registry-based cohort study by comparing rates of overall cancer and of cancer

subgroups in patients with IBD with and without azathioprine treatment.

MATERIALS AND METHODS

We conducted a nationwide cohort study between January 1997 and December 2008 by linking individual-level registry data using patients' unique personal identification numbers. All persons living in Denmark who were ≥18 years of age between 1997 and 2008 as identified via the Civil Registration System (24) were eligible. The National Patient Registry (25), which documents all hospitalizations in Denmark since 1977 and all outpatient visits to hospital clinics and emergency departments since 1995, was used to identify patients with IBD. We used *International Classification of Diseases, Eighth Revision* (ICD-8) codes 56300-02 and 56308 and *International Classification of Diseases, Tenth Revision* (ICD-10) code K50 for Crohn's disease; and ICD-8 codes 56319 and 56904 and ICD-10 code K51 for ulcerative colitis. Included in the cohort were patients with a prevalent or incident diagnosis of IBD who had no history of azathioprine use or cancer, who did not use 6-mercaptopurine, and who had no registered diagnosis of heart, lung, liver, or kidney transplant. Incident cancers were identified via the Danish Cancer Registry (26), which documents all cases of cancer in the country, including information on type of cancer, anatomical location, and date of diagnosis; cancers are classified according to the ICD-10 (Web Table 1, available at http://aje.oxfordjournals.org/). Because reporting to the Cancer Registry is mandatory and comes from multiple sources, and because the data go through extensive quality control, the validity of the registry is high and it is thought to be close to complete (26). The primary outcome measure was the rate of overall incident cancer in new users of azathioprine compared with the rate in nonusers. Secondary outcomes included cancer risk according to cumulative received doses, cancer subgroups, sex, and type of IBD. The study was approved by the Danish Data Protection Agency. Ethics approval is not required for registry-based research in Denmark.

Drug exposure

The Prescription Drug Registry (27), established in 1995, contains individual-level information on all prescriptions filled at all Danish pharmacies, including the personal identification number, dispensing date, Anatomical Therapeutic Chemical code, and number of defined daily doses in the prescription. We identified new users of azathioprine (Anatomical Therapeutic Chemical code L04AX01). Exclusion of patients who had filled prescriptions for azathioprine during a washout period of 2 years prior to cohort entry allowed selective inclusion of new users; this reduces the potential for prevalent user bias (28) and permits estimation of exposure duration. In Denmark, 6-mercaptopurine is rarely used to treat IBD; therefore, users of this drug were excluded.

Throughout follow-up, patients' drug exposure status was continuously monitored, with each new prescription counted as corresponding to 6 months of exposed person-time of use. If 2 prescriptions were overlapping, the overlap was disregarded and exposure time was counted from the dispensing day of the most recent prescription to avoid accumulation of exposed person-time. Because specific doses or the number of days' supply are not registered in the Prescription Drug Registry and because of large interindividual differences in the amount of drug taken, we used a common definition of the prescription duration for all participants; we expected 6 months to be a liberal definition that allowed for short pauses between prescriptions. We tested this assumption in a sensitivity analysis in which each prescription counted as 3 months of use. If users did not refill their prescriptions within 6 months after the most recent prescriptions, they were recategorized and started contributing (unexposed) person-time to the former user group. If these former users later refilled their prescriptions, they could be recategorized and again contribute person-time to the user group. Thus, the drug exposure groups were created as time-varying variables with the possibility of moving between the groups; each patient could contribute to several distinct user and former user episodes which, when added, represented the patient's total person-time of use and former use, respectively.

Because azathioprine treatment is unlikely to influence the risk of cancer that develops shortly following the start of use, we allowed for a 6-month lag period immediately following the first prescription to limit the contribution of patients with incipient cancer to the user group. Thus, patients did not contribute person-time to the user group and were instead categorized as a distinct group during the 6-month lag period.

Propensity scores

To adjust for baseline differences in probability of initiating azathioprine treatment, we constructed logistic regression models to estimate propensity scores given the following baseline patient characteristics: year of birth; calendar year; sex; socioeconomic class; degree of urbanization; type of IBD; comorbidities; history of intestinal surgery; history of intestinal, rectal, or anal fistula, abscess, or fissure; and comedications (Web Table 2). After calculating propensity scores, we excluded persons with nonoverlapping probability of azathioprine exposure to limit unmeasured confounding from patients at the extreme ends of the propensity score distribution (trimming) (29).

Statistical analyses

Patients were censored at the date of the first of the following events: cancer diagnosis, loss to follow-up, emigration, death, or the end of the follow-up period (December 31, 2008). By applying Poisson regression models (log-linear regression of the counts of cancer by using the logarithm of the follow-up time as offset), we estimated rate ratios for incident cancer by comparing azathioprine use with no use (SAS, version 9.2, software; SAS Institute, Inc., Cary, North Carolina). Models were adjusted for baseline propensity scores by quintiles as well as the following time-varying covariates: age (in 10-year intervals); calendar year (in

Table 1. Baseline Characteristics of Azathioprine Users and Nonusers in a Nationwide Cohort of Patients With Inflammatory Bowel Disease in Denmark, 1997–2008

Patient Characteristics	Azathioprine Use					
	Users ($n = 5,197$)			Nonusers ($n = 38,772$)		
	No.	%	Mean (SD)	No.	%	Mean (SD)
Age, years			38(16)			47(19)
Male sex	2,500	48		17,367	45	
Type of inflammatory bowel disease						
Crohn's disease	2,500	48		10,454	27	
Ulcerative colitis	2,697	52		28,318	73	
Socioeconomic class						
Employment with unknown, basic, or no qualifications	2,315	45		12,975	33	
Employment with medium-level qualifications	526	10		3,762	10	
Employment with high-level qualifications	394	8		3,112	8	
Self employed/coworking spouse	205	4		1,670	4	
Outside the labor market	1,217	23		7,749	20	
Pensioned	540	10		9,504	25	
Degree of urbanization						
Population density ≤49 inhabitants per km^2	387	7		2,526	7	
Population density 50–99 inhabitants per km^2	1,513	29		11,331	29	
Population density 100–199 inhabitants per km^2	1,107	21		8,293	21	
Population density ≥200 inhabitants per km^2	497	10		4,291	11	
Copenhagen suburbs	1,213	23		8,444	22	
Copenhagen	480	9		3,887	10	
Comorbidity[a]						
Myocardial infarction	30	1		377	1	
Congestive heart failure	33	1		440	1	
Peripheral vascular disease	22	<1		443	1	
Cerebrovascular disease	37	1		669	2	
Dementia	2	<1		85	<1	

Table continues

2-year intervals); and duration of IBD (in 2-year intervals). To account for disease severity, we adjusted for hospitalization for IBD in the last year; aminosalicylate use; oral corticosteroid use; enteral or rectal corticosteroid use; and use of other immunosuppressants (methotrexate, cyclosporine, or cyclophosphamide). We conducted several sensitivity analyses, including estimation of the effects of unmeasured confounders such as smoking, on the main outcome, by using the array approach described by Schneeweiss (30).

RESULTS

From a source population of 4,797,375 persons, we identified 50,085 patients with IBD. Of these, 3,919 patients with a history of cancer, previous azathioprine use, any use of 6-mercaptopurine, or an organ transplant were excluded (Web Figure 1 summarizes the enrollment of patients in the cohort). After the exclusion of 180 patients (including 8 azathioprine users) with nonoverlapping propensity scores, the final cohort included 45,986 patients (7,214 of whom filled at least 1 new prescription for azathioprine during the study period). During the 6-month lag period following the start of use, 2,017 azathioprine-exposed patients were either censored or did not refill prescriptions (not fulfilling the definition of use beyond the lag period). Consequently, 5,197 patients contributed person-time to the user group. Baseline characteristics of users and nonusers of azathioprine are presented in Table 1. The median follow-up in the cohort was 7.9 (interquartile range: 3.5–12.0) years and the median duration of azathioprine use was 1.9 (interquartile range: 0.5–4.2) years.

Overall cancer

Azathioprine use was associated with a significantly increased risk of incident cancer overall compared with no use (Table 2). The increased risk was observed when adjusting only for age (rate ratio (RR) = 1.43, 95% confidence

Table 1. Continued

Patient Characteristics	Azathioprine Use					
	Users ($n = 5,197$)			Nonusers ($n = 38,772$)		
	No.	%	Mean (SD)	No.	%	Mean (SD)
Chronic pulmonary disease	87	2		1,006	3	
Rheumatic disease	59	1		505	1	
Peptic ulcer disease	55	1		553	1	
Diabetes	54	1		767	2	
Hemiplegia or paraplegia	3	<1		45	<1	
Renal disease	10	<1		162	<1	
Liver disease	19	<1		269	1	
HIV/AIDS	0	0		0	0	
Intestinal, rectal, or anal fistula, abscess, or fissure	245	5		544	1	
Intestinal surgery	309	6		1,463	4	
Comedication[b]						
Antidiabetics	84	2		1,210	3	
Anticoagulants	228	4		4,095	11	
Antianemic drugs	426	8		2,696	7	
Cardiovascular drugs	761	15		9,921	26	
Immunosupressants	1	<1		14	<1	
Antiobstructive inhalants for pulmonary use	627	12		5,021	13	
Use of IBD drugs throughout follow-up						
Oral corticosteroids	4,698	90		13,078	34	
Enteral or rectal corticosteroids	3,340	64		11,166	29	
Aminosalicylates	4,401	85		19,996	52	
Immunosuppressants[c]	336	6		574	1	

Abbreviations: HIV/AIDS, human immunodeficiency virus/acquired immunodeficiency syndrome; IBD, inflammatory bowel disease.
[a] As captured by the Danish National Patient Registry (which documents hospitalizations and outpatient hospital visits) within 2 years prior to cohort entry.
[b] As captured by the nationwide Danish Prescription Drug Registry within 2 years prior to cohort entry.
[c] Methotrexate, cyclosporine, or cyclophosphamide.

interval (CI): 1.17, 1.74), and in the full model when also adjusting for propensity scores, concomitant medication for IBD, disease duration, and hospitalization for IBD (RR = 1.41, 95% CI: 1.15, 1.74). Patients who discontinued azathioprine were followed for an additional median 2.9 (interquartile range: 0.9–5.8) years after discontinuation; there was no significant risk of cancer in these former users of azathioprine compared with nonusers (RR = 1.02, 95% CI: 0.83, 1.25; Table 2). There was no significantly increased risk of cancer during the 6-month lag period (23 azathioprine-exposed cases; RR = 0.82, 95% CI: 0.54, 1.26) compared with nonusers.

Rate ratios for the association between azathioprine use and cancer were similar in men and women (Table 2). Associations between azathioprine use and cancer were observed in both patients with Crohn's disease and those with ulcerative colitis, but reached significance only in those with Crohn's disease (Table 2).

The risk of cancer did not increase significantly with increasing cumulative received doses; the increase in adjusted rate ratio per 365 additional received defined daily doses was 1.06 (95% CI: 0.89, 1.27; Table 3).

Cancer subgroups

There were significantly increased risks of urinary tract cancer and of lymphoid tissue cancer associated with azathioprine use (Table 4). There were no other significant associations, but an elevated rate ratio with the lower boundary of the confidence interval just below 1 was observed for the subgroup of cancer of the female genital organs. In the subgroup of urinary tract cancer, 6 of the 7 azathioprine-exposed cases had renal cancer and 1 had cancer of the renal pelvis; the 108 nonuser cases represented 58 bladder cancers, 40 renal cancers, 8 cancers of the renal pelvis, 1 ureteral cancer, and 1 urethral cancer. Among the 9 azathioprine-exposed

Table 2. Use of Azathioprine and Risk of Incident Cancer Overall in a Nationwide Cohort of Patients With Inflammatory Bowel Disease in Denmark, 1997–2008

	Person-Years of Follow-Up	Incident Cancer Cases, No.	Age-Adjusted Incidence Rate Per 100,000 Person-Years	Age Adjusted		Fully Adjusted[a]	
				RR	95% CI	RR	95% CI
Overall							
Users	13,849	104	163	1.43	1.17, 1.74	1.41	1.15, 1.74
Former users	18,771	107	111	0.98	0.81, 1.19	1.02	0.83, 1.25
Nonusers	304,992	2,362	114	1	Referent	1	Referent
Women							
Users	6,987	51	122	1.54	1.16, 2.03	1.42	1.06, 1.91
Former users	10,665	60	82	1.03	0.79, 1.34	0.99	0.75, 1.31
Nonusers	168,549	1,199	79	1	Referent	1	Referent
Men							
Users	6,862	53	207	1.30	0.99, 1.71	1.40	1.04, 1.87
Former users	8,107	47	147	0.92	0.69, 1.24	1.06	0.78, 1.44
Nonusers	136,443	1,163	160	1	Referent	1	Referent
Crohńs disease							
Users	7,337	58	201	1.63	1.25, 2.14	1.53	1.14, 2.05
Former users	9,011	51	133	1.08	0.81, 1.44	1.08	0.78, 1.48
Nonusers	88,488	632	123	1	Referent	1	Referent
Ulcerative colitis							
Users	6,512	46	125	1.18	0.88, 1.59	1.25	0.92, 1.69
Former users	9,761	56	93	0.87	0.67, 1.14	0.95	0.72, 1.25
Nonusers	216,504	1,730	106	1	Referent	1	Referent

Abbreviations: CI, confidence interval; RR, rate ratio.

[a] Adjusted for baseline propensity scores and the following time-varying covariates: age (in 10-year intervals); calendar year (in 2-year intervals); disease duration (in 2-year intervals); inflammatory bowel disease hospitalizations in the last year; and use of aminosalicylates, oral corticosteroids, enteral or rectal corticosteroids, and other immunosuppressants (methotrexate, cyclosporine, cyclophosphamide).

cases in the lymphoid tissue cancer subgroup, there were 6 non-Hodgkin lymphomas, 2 Hodgkin lymphomas, and 1 multiple myeloma; among the 80 nonexposed cases, there were 43 non-Hodgkin lymphomas, 28 multiple myelomas, 6 Waldenström macroglobulinemias, and 3 Hodgkin lymphomas.

Sensitivity analyses

First, we extended the lag period to 2 years, which was intended to limit the influence of misclassification by incipient cancer developing shortly after initiation of azathioprine use and to restrict the exposed cohort to patients who were most adherent to therapy; the association between azathioprine use and cancer persisted with a slightly higher rate ratio (Table 5). Because we used a 6-month lag period, a bias may have been introduced as the result of misclassification of cancer cases that ought to have been attributed to the user or nonuser categories. We therefore reclassified the 6-month lag period to the nonuser group; this had no impact on the estimates (Table 5). Reclassifying the 6-month lag period to the user group decreased the rate ratio, but the association was still significant (Table 5). Because patients with newly diagnosed IBD are likely to undergo an extensive clinical evaluation, a detection bias for cancer might operate immediately following diagnosis. We therefore introduced a lag period so that follow-up in the entire cohort started 1 year after the diagnosis of IBD; this had no impact on the estimates (Table 5). Next, we used an alternative definition of drug use, with each new prescription counting as 3 months of use instead of 6 months of use. This was intended to limit the misclassification of unexposed time periods as exposed by reducing potential gaps between prescriptions; the rate ratio remained unchanged (Table 5). We additionally introduced data on the use of tumor necrosis factor inhibitors (by using data from the National Patient Registry) as a time-varying covariate in the model; the estimate was unchanged (Table 5). Our principal cohort included both prevalent and incident IBD, and previous azathioprine users were excluded. Therefore, the inclusion of the patients with prevalent IBD without azathioprine treatment may have introduced a bias as these patients may have had lower propensity for azathioprine exposure. For that reason, we restricted the cohort to patients with incident IBD; results were similar to those of the original analysis (Table 5). Next, we tested the impact of the lymphoid tissue and urinary tract cancer subgroups on the overall estimate; a significant association

Table 3. Associations Between Azathioprine Use and Risk of Incident Cancer Overall According to Cumulative Received Doses, Nationwide Cohort of Patients With Inflammatory Bowel Disease in Denmark, 1997–2008

Cumulative Defined Daily Doses	Person-Years of Follow-Up	Incident Cancer Cases, No.	Fully Adjusted[a]		*P* Value[b]
			RR	95% CI	
0–365	5,286	40	1.43	1.04, 1.97	
366–730	4,518	25	1.11	0.75, 1.66	0.53
731–1,095	2,254	22	1.80	1.17, 2.75	
≥1,096	1,791	17	1.54	0.95, 2.50	

Abbreviations: CI, confidence interval; RR, rate ratio.

[a] Adjusted for baseline propensity scores and the following time-varying covariates: age (in 10-year intervals); calendar year (in 2-year intervals); disease duration (in 2-year intervals); inflammatory bowel disease hospitalizations in the last year; and use of aminosalicylates, oral corticosteroids, enteral or rectal corticosteroids, and other immunosuppressants (methotrexate, cyclosporine, cyclophosphamide).

[b] Test for homogeneity.

between azathioprine use and overall cancer persisted when these 2 subgroups were excluded from the analysis (Table 5). When accounting for time since discontinuation in former azathioprine users, we found that the risk of cancer was similar at 0–5.9 months (adjusted RR = 1.06, 95% CI: 0.66, 1.69), 6–11.9 months (adjusted RR = 1.17, 95% CI: 0.69, 1.99), and ≥12 months (adjusted RR = 0.99, 95% CI: 0.77, 1.26) since discontinuation. When assessing the influence of other drugs on the association between azathioprine and overall cancer, we found no significant interactions with concomitant use of aminosalicylates ($P = 0.67$), oral corticosteroids ($P = 0.42$), or tumor necrosis factor inhibitors ($P = 0.69$). Additionally, instead of adjusting for propensity scores, we used propensity score matching. Patients were matched 1:1 on the second decimal of the propensity score with additional adjustment for time-varying covariates; the rate ratio for the association between azathioprine use and cancer remained significant and tended towards increased risk (Table 5). Finally, the effect of a potential unmeasured confounder or combination of confounders was modeled on the overall cancer outcome by assuming a wide range of combinations of confounder prevalences in the exposed group and strengths of the association between the confounder and cancer. For example, if a confounder was present in 30% of the exposed group and 20% of the unexposed group, and if the confounder increased the risk of cancer 3-fold, the observed estimate of 1.43 would have been biased by 14% and the confounder-adjusted estimate would be 1.25 (Web Table 3). At confounder prevalences of 40% in the exposed group and 20% in the unexposed group, the confounder-adjusted estimate decreased from 1.43 to 1 when the confounder-cancer relative risk was higher than 4.5 (Web Table 3).

DISCUSSION

This nationwide historical cohort study of more than 45,000 patients with IBD found an increased rate of overall cancer among users of azathioprine compared with nonusers. In subgroup analyses, this study confirmed previous findings of an increased risk of lymphoid tissue cancer associated with azathioprine use and additionally found a significantly increased risk of urinary tract cancer. Because of the lack of association between former use of azathioprine and cancer or increasing cumulative dose of azathioprine and cancer, these findings should be interpreted with caution.

Our finding of an approximately 40% increased rate of cancer associated with azathioprine use in patients with IBD is novel in comparison with previous studies that primarily aimed to investigate this question. The hitherto largest report, a case-control study of primary care data from the United Kingdom, found no significantly increased risk of overall cancer associated with azathioprine use (odds ratio = 1.08, 95% CI: 0.78, 1.51 for ever use vs. never use) (12). Although our main estimate is within the confidence intervals of the United Kingdom study, there are differences between the studies that could explain the different findings. The United Kingdom study did not quantify duration of azathioprine exposure in detail and may have classified former users as exposed, which would bias the results towards the null. In contrast, we estimated exposure time prospectively and treated drug use as a time-varying variable, accounting for treatment gaps and discontinuation, which may have provided greater precision. Additionally, the United Kingdom study detected only prescriptions issued in primary care, whereas our study covered all prescriptions in Denmark. This likely provided greater sensitivity to detect drug use. Finally, our analyses were based on 104 azathioprine-exposed cancer cases compared with 41 in the United Kingdom study. On the other hand, our findings are in line with data from the French CESAME cohort study of 19,486 patients with IBD; although this study primarily investigated the association between thiopurine use and lymphoproliferative disorders, it reported a 2% incidence of overall cancer in thiopurine users compared with 1% in those who had never used thiopurines ($P = 0.0016$) (13). Other studies, although having found no significantly increased risk of cancer associated with thiopurines, have had limited power, have lacked adequate control groups, have been restricted to single centers, or have provided limited detail with regard to drug exposure (20–23).

Our study confirmed a previously shown risk of lymphoma associated with azathioprine use in patients with IBD (12–14). Although the risk increase was less pronounced than in previous studies, the upper limit of the confidence intervals was above 5, which is consistent with previous data (12–14). We found no significantly increased risk of skin cancer, in contrast with the body of previous data; 2 nested case-control studies and a prospective cohort study have found increased risk of skin cancer associated with thiopurine use in patients with IBD (15–17), and 1 cohort study failed to find an association (31). However, our analysis of skin cancer was based on a limited number of exposed cases, with the upper limit of the confidence intervals above

Table 4. Associations Between Azathioprine Use and Subgroups of Cancer, Nationwide Cohort of Patients With Inflammatory Bowel Disease in Denmark, 1997–2008

Cancer Subgroup	Incident Cancer Cases, No.	Adjusted[a]	
		RR	95% CI
Lip, oral cavity, and pharynx			
Users	4	1.69	0.57, 5.00
Former users	5	1.70	0.63, 4.60
Nonusers	60	1	Referent
Digestive organs (noncolorectal)			
Users	11	1.70	0.89, 3.22
Former users	10	1.05	0.53, 2.07
Nonusers	235	1	Referent
Colorectal			
Users	12	1.36	0.75, 2.49
Former users	8	0.71	0.34, 1.46
Nonusers	380	1	Referent
Respiratory/intrathoracic organs			
Users	12	1.09	0.60, 1.98
Former users	20	1.22	0.75, 1.99
Nonusers	329	1	Referent
Skin[b]			
Users	11	1.67	0.86, 3.21
Former users	11	1.25	0.64, 2.42
Nonusers	162	1	Referent
Breast[c]			
Users	8	0.80	0.39, 1.64
Former users	9	0.53	0.27, 1.05
Nonusers	338	1	Referent
Female genital organs			
Users	8	2.01	0.92, 4.37
Former users	10	1.49	0.72, 3.10
Nonusers	111	1	Referent
Male genital organs			
Users	12	1.26	0.69, 2.32
Former users	16	1.43	0.83, 2.46
Nonusers	257	1	Referent
Urinary tract			
Users	7	2.84	1.24, 6.51
Former users	6	1.73	0.70, 4.24
Nonusers	108	1	Referent
Lymphoid tissue			
Users	9	2.40	1.13, 5.11
Former users	5	0.88	0.33, 2.33
Nonusers	80	1	Referent
Hematopoietic tissue			
Users	3	1.01	0.30, 3.40
Former users	3	0.66	0.19, 2.27
Nonusers	71	1	Referent
Other[d]			
Users	7	1.31	0.60, 2.88
Former users	4	0.55	0.20, 1.53
Nonusers	229	1	Referent

Abbreviations: CI, confidence interval; RR, rate ratio.

[a] Adjusted for baseline propensity scores and the following time-varying covariates: age (in 10-year intervals); calendar year (in 2-year intervals); disease duration (in 2-year intervals); inflammatory bowel disease hospitalizations in the last year; and use of aminosalicylates, oral corticosteroids, enteral or rectal corticosteroids, and other immunosuppressants (methotrexate, cyclosporine, cyclophosphamide).

[b] Excludes basal cell carcinoma.

[c] Estimated in women only.

[d] Bone and articular cartilage; mesothelial and soft tissue; central nervous system including eye; endocrine glands; cancers of ill-defined, secondary, and unspecified sites; cancers of independent multiple primary sites.

3, and the lack of a significant association could thus reflect limited power. Furthermore, our subgroup analysis of skin cancer is not directly comparable with previous reports because it included melanoma and squamous cell carcinoma, whereas the other studies analyzed squamous cell and basal cell carcinomas. We found an almost 3-fold significantly increased risk of urinary tract cancer among azathioprine users. Although the number of exposed cases was small, 6 of the 7 azathioprine-exposed cases had renal cancer and none had bladder cancer, which appears disproportionate when considering that, in nonusers, fewer than 40% had renal cancer and more than half had bladder cancer. However, given the absence of previous data to support this, the possibility of a chance finding must be considered as an explanation. In analyses according to type of IBD, the risk of overall cancer was significantly increased in patients with Crohn's disease but not in patients with ulcerative colitis. Because the confidence intervals in these secondary analyses were largely overlapping, these differential findings could reflect limited power. However, another possibility is that azathioprine is differentially associated with cancer according to type of IBD; this merits further investigation.

Our study had additional strengths and weaknesses. We used nationwide registries; our results are therefore not influenced by bias resulting from selective inclusion of hospitals, health insurance systems, or age groups. The majority of patients with IBD in Denmark are identified via the National Patient Registry; completeness of the registration of IBD was estimated to be 94%, and the validity of registered

Table 5. Sensitivity Analyses of Association Between Azathioprine Use and Risk of Cancer, Nationwide Cohort of Patients With Inflammatory Bowel Disease in Denmark, 1997–2008

Analysis	Person-Years of Follow-Up	Incident Cancer Cases, No.	Adjusted[a]	
			RR	95% CI
Alternative lag periods following start of exposure				
2-year lag period	8,700	70	1.55	1.21, 1.98
6-month lag period reclassified to nonuser group	13,849	104	1.42	1.16, 1.75
6-month lag period reclassified to user group	17,332	127	1.26	1.04, 1.52
Lag period (1-year lag period introduced following diagnosis of IBD)	13,530	102	1.42	1.15, 1.76
Alternative definition of drug use (each prescription accounts for 3 months of use)	11,405	86	1.39	1.11, 1.75
Additional time-varying covariate in model (additionally adjusted for TNF inhibitor use)	13,849	104	1.45	1.18, 1.78
Alternative definition of cohort (restricted to incident IBD)	8,277	61	1.47	1.12, 1.93
Alternative definition of overall cancer (lymphoid and urinary tract cancer subgroups excluded)	13,849	85	1.31	1.04, 1.65
Alternative covariate adjustment (propensity score-matched (1:1) cohort)	13,674	104	1.72	1.34, 2.22

Abbreviations: CI, confidence interval; IBD, inflammatory bowel disease; RR, rate ratio; TNF, tumor necrosis factor.

[a] All estimates except the estimate of the propensity score-matched cohort analysis were adjusted for baseline propensity scores and the following time-varying covariates: age (in 10-year intervals); calendar year (in 2-year intervals); disease duration (in 2-year intervals); inflammatory bowel disease hospitalizations in the last year; and use of aminosalicylates, oral corticosteroids, enteral or rectal corticosteroids, and other immunosuppressants (methotrexate, cyclosporine, cyclophosphamide).

diagnoses was estimated to be 97% for Crohn's disease and 90% for ulcerative colitis when using a pathology registry as a reference (32); unpublished observations from a more recent incidence study found that more than 90% of patients were diagnosed in hospitals (33). A series of sensitivity analyses, including an analysis taking into account tumor necrosis factor inhibitor use, demonstrated the robustness of the main findings. The median follow-up of azathioprine exposure in our study was less than 2 years, and the development of cancer is a slow process. Therefore, our analyses may have underestimated the risk. However, the analysis of cancer risk according to cumulative received defined daily doses showed a relatively constant increase in the risk over time. If confirmed, these data may be consistent with a hypothesis suggesting that azathioprine accelerates the development of clinically detectable cancer rather than inducing cancer per se. Alternatively, this finding, together with the absence of risk associated with former use, might indicate that patients taking azathioprine receive increased medical attention and therefore have their cancers diagnosed earlier. Although this is the largest study to date, the number of exposed cases was limited, and we could therefore not estimate risks of several cancer subgroups with precision.

This observational study compared cancer rates between users and nonusers of azathioprine. A meta-analysis on extraintestinal cancer risk among patients with IBD has suggested an increased risk of a number of cancer types (34). An underlying increased risk of cancer in patients with IBD may be, as shown for colorectal cancer (35), related to the severity of disease, the degree of disease activity, and hence the probability of being treated with azathioprine. Therefore, an important question is whether increased disease activity rather than azathioprine treatment itself may have influenced the risk of cancer and thereby introduced confounding by indication. We addressed this potential limitation. First, to account for the probability of patients receiving azathioprine, we adjusted for propensity scores. Furthermore, although data did not include laboratory markers of inflammation or information on symptoms and signs, proxies for disease activity were controlled for by including hospitalizations for IBD and concomitant use of IBD medications as time-varying variables in the multivariate model. However, if the severity of the underlying disease in the present study was not fully captured by baseline propensity scores and adjustments for time-varying variables, the excess risk of overall cancer in azathioprine users may be more limited. It should also be noted that the increased risk of cancer was constant over cumulative doses of exposure, which could indicate confounding by indication. However, the absence of significant associations between the other 2 groups that also had indications for azathioprine treatment (the lag period group and the former user group) and cancer suggests that indication for treatment may not have influenced the association between azathioprine and cancer.

In conclusion, this large cohort study of patients with IBD found an approximately 40% increased rate of cancer associated with azathioprine use. Although these data do not establish causality, they may inform decision making when

weighing the significant clinical benefits (3–5) against risks of azathioprine treatment in patients with IBD.

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