SYSTEMATIC REVIEWS AND META-ANALYSES

Fasiha Kanwal, Section Editor

Risk of Lymphoma in Patients With Inflammatory Bowel Disease Treated With Azathioprine and 6-Mercaptopurine: A Meta-analysis

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This article has an accompanying continuing medical education activity on page e49. Learning Objective–Upon completion of this activity, successful learners will be able to evaluate risk of lymphoma in patients using thiopurine medications for inflammatory bowel disease and better apply this knowledge in their discussions with patients in assessing the risk versus benefit of using thiopurines in different demographic groups.

BACKGROUND & AIMS: **Thiopurine therapy for inflammatory bowel disease (IBD) has been associated with increased risk for lymphoma. We estimated the relative risk of lymphoma in patients with IBD exposed to thiopurines and compared relative risk values derived from population-based studies with those from referral center–based studies. We investigated whether active use increased risk compared with past use, and whether sex, age, or duration of use affects risk of lymphoma.**

METHODS: **We searched MEDLINE, EMBASE, and Cochrane databases, as well as conference abstracts and international publications, for the terms "6-MP and lymphoma," "6-mercaptopurine and lymphoma," "thiopurines and lymphoma," "azathioprine and cancer and IBD," "azathioprine and malignancy and IBD," "azathioprine and lymphoma," and "lymphoproliferative and thiopurines." Pooled standardized incidence ratios (SIRs) and 95% confidence intervals (CIs) were estimated. The deviance statistic from Poisson models was used to calculate heterogeneity.**

RESULTS: **Eighteen studies (among 4383 citations) met our inclusion criteria. Overall, the SIR for lymphoma was 4.92 (95% CI, 3.10–7.78), ranging from 2.80 (95% CI, 1.82–4.32) in 8 population studies to 9.24 (95% CI, 4.69–18.2) in 10 referral studies. Population studies demonstrated an increased risk among current users (SIR = 5.71; 95% CI, 3.72–10.1) but not former users (SIR = 1.42; 95% CI, 0.86–2.34). Level of risk became significant after 1 year of exposure. Men have a greater risk than women (relative risk = 1.98; $P < .05$); both sexes were at increased risk for lymphoma (SIR for men = 4.50; 95% CI = 3.71–5.40 and SIR for women = 2.29; 95% CI = 1.69–3.05). Patients younger than 30 years had the highest relative risk (SIR = 6.99; 95% CI, 2.99–16.4); younger men had the highest risk. The absolute risk was highest in patients older than 50 years (1:354 cases per patient-year, with a relative risk of 4.78).**

Abbreviations used in this paper: AZA, azathioprine; CESAME, Cancers Et Surrisque Associé aux Maladies inflammatoires intestinales En France; CI, confidence interval; EBV, Epstein–Barr virus; ENEIDA, Española de Enfermedad Inflamatoria intestinal base de Datos; IBD, inflammatory bowel disease; 6-MP, 6-mercaptopurine; NHL, non-Hodgkin's lymphoma; SEER, Surveillance Epidemiology and End Results; SIR, standardized incidence ratio.

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CONCLUSIONS: **Compared with studies from referral centers, population-based studies of IBD patients show a lower but significantly increased risk of lymphoma among patients taking thiopurines. The increased risk does not appear to persist after discontinuation of therapy. Patients over 50 have the highest absolute risk of lymphoma per year on thiopurines, while men under 35 may also be a high risk group. More study is needed to precisely understand groups highest at risk. The risks of lymphoma and potential benefits of therapy should be considered for all patients with IBD.**

Keywords: Cancer Risk; Ulcerative Colitis; Crohn's Disease; Treatment.

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Immunosuppressive therapies used in the treatment of inflammatory bowel disease (IBD) may increase the risk of lymphoma. Immunosuppressive therapies have been shown to increase the risk for lymphoma after organ transplantation and in other patient groups.[1,2] Our previous meta-analysis demonstrated that the use of thiopurines (azathioprine [AZA] and 6-mercaptopurine [6-MP]) is associated with a 4-fold increased risk of lymphoma in patients with IBD.[3]

Our prior meta-analysis combined multiple referral center–based studies with a single population-based study. Referral center–based studies may include patients with longer disease duration, worse disease severity, or more comorbidities that may confound the association between immunosuppressant use and lymphoma. Population-based studies would be expected to be less at risk for such bias. In addition, because of the dearth of data available in 2005 it was not possible to separate the risk of active use from past use of thiopurines.

Since the original meta-analysis, an additional 7 population-based cohort studies,[4–10] five referral center studies,[11–15] and an update of 1 population-based study[16] have been published regarding the association of thiopurines and lymphoma in IBD. By combining the data from these studies with the data from our prior meta-analysis, we have greater statistical power to examine the relative risk of lymphoma among patients using azathioprine and to assess whether this relative risk differs between population-based and referral-center studies. In addition, we are now able to assess the difference in lymphoma risk with current and former use of thiopurines.

Methods

Inclusion Criteria

Studies needed to meet the following criteria to be included in this meta-analysis: (1) they must have been a cohort study of IBD patients, (2) specifically evaluated cancer as an adverse outcome, and (3) the exposed group must have received AZA or 6-MP and the number of patients exposed to thiopurines must have been reported. Studies that did not meet these criteria were excluded.

Search Strategies

MEDLINE, EMBASE, and the Cochrane database were all searched. The search was performed by using the keywords "6-MP and lymphoma," "6-mercaptopurine and lymphoma," "thiopurines and lymphoma," "azathioprine and cancer and IBD," "azathioprine and malignancy and IBD," "azathioprine and lymphoma," and "lymphoproliferative and thiopurines." There were 4383 citations identified by the search, of which 15 were included.[5–9,11–14,16–21] In addition, a search of abstracts from Digestive Disease Week and the American College of Gastroenterology meetings between 2005 and 2013 with the keywords "lymphoma" and "IBD" found 1 referral-based study[15] and 2 population-based studies[4,10] (Figure 1). Additional data were also extracted from the Spanish collaborative registry Española de Enfermedad Inflamatoria intestinal base de Datos (ENEIDA), the French study Cancers Et Surrisque Associé aux Maladies inflammatoires intestinales en France (CESAME), the referral center database of German patients with IBD administered by the University of Munich, and the

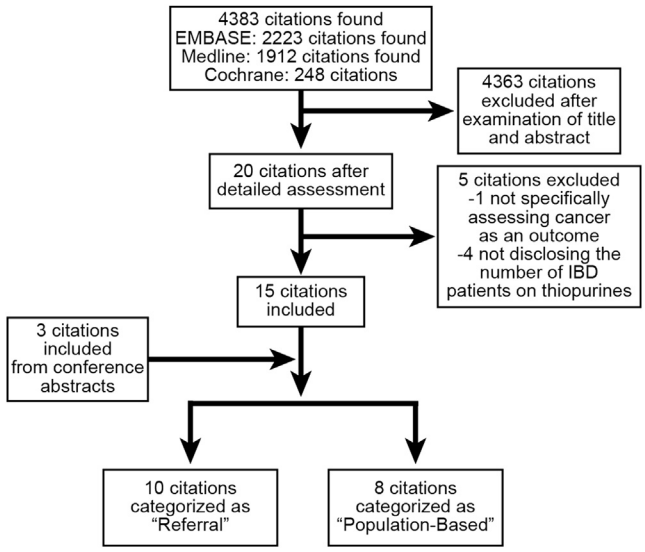

Figure 1. Search strategy.

referral center database of Italian patients with IBD administered by the University of Tor Vergata (Rome).

When multiple publications described the same population, the most recent publication was used. Data were abstracted on year of publication, journal, type of study (referral vs population-based), number of patients with IBD, number of patients with Crohn's disease, number of patients with ulcerative colitis, mean/median duration of therapy with AZA and/or 6-MP, mean/median dose of drug, mean/median duration of follow-up, expected number of patients with lymphoma, observed number of patients with lymphoma, and type of lymphoma. Studies were termed referral-based, if they included data on patients from solely one referral center, and population-based studies, which defined by our group previously[22] included IBD patients from a defined geographic area. Population studies all contain data from national databases with multiple geographical sites with the exception of the studies from Peyrin–Biroulet[10] (Olmstead County, MN), Herrinton (northern California),[6] and Lakatos[8] (Veszprem province, Hungary). When patients were listed as having indeterminate colitis, they were counted as having ulcerative colitis. Additional information was obtained from the ENEIDA database for Gisbert, and the CESAME database for Beaugerie. Also additional information was obtained directly from the authors for Van Domselaar,[11] Beigel,[15] Armstrong,[16] Peyrin-Biroulet,[10] Biancone,[13] and Pasternak.[9]

Statistical Analyses

Statistical analysis was performed by using STATA (StataCorp, College Station, TX). All studies were analyzed in the same manner to calculate a pooled relative risk of lymphoma, as done previously.[3] Pooled standardized incidence ratios (SIRs) and corresponding 95% confidence intervals (CIs) were estimated. The SIR is the ratio of observed to expected number of lymphomas that is based on the age and sex of the study population. In this study, the expected number of cases of lymphoma was based on the age-specific and sex-specific rates for the geographic source population in each study cohort. All cases of patients on thiopurines were included for the primary analysis. For the supplementary analysis, only cases of patients solely on thiopurines were included with exclusion of patients ever on methotrexate or anti-TNFs. These included Van Domselaar,[11] Biancone,[13] Beigel,[15] Beaguerie,[5] Herrinton,[6] Lakatos,[8] Khan,[7] and Gisbert.[4]

Expected numbers of lymphomas were obtained from the original studies with the exception of Pasternak et al,[9] Biancone et al,[13] Van Domselaar et al,[11] Armstrong et al,[16] Beigel et al,[15] Freeman et al,[14] Khan et al,[7] and Chaparro et al.[4] In these studies, background data from Spain, Denmark, Germany, Canada, the United Kingdom and Italy were calculated by averaging the sum of the regional incidence of non-Hodgkin lymphoma (NHL) and Hodgkin's disease by using the World Health Organization's Cancer Incidence in Five Continents Database.[23] For Khan, as the data included American patients from 2001–2011, we used the SEER database from the National Cancer Institute (US) for the background rate of Hodgkin's lymphoma and Non-Hodgkin's lymphoma from the years 2001–2010.

Primary raw data were obtained from all studies that had performed age-decile analysis. Five studies were included for analysis: 3 population-based (CESAME/Beaugerie,[5] Gisbert/ENEIDA,[4] and Lewis[24]) and 2 referral center–based trials (Korelitz et al[21] and Fraser et al[19]). In the main analysis, Gisbert,[4] as an outlier, was omitted, while a separate analysis including Gisbert was performed, shown in Supplemental Tables 3 and 4. The SIR was calculated for each age decile (eg, 0–10, 10–20 years). Pooled SIRs and 95% CIs were estimated by summing the observed and expected numbers of lymphomas across studies, stratified by each age decile.

Estimation of pooled SIRs was performed by summation of observed and expected numbers of lymphomas across studies. CIs were calculated assuming a Poisson distribution by using random effects models. To examine for heterogeneity, the deviance statistic from Poisson regression models was examined. Statistically significant heterogeneity was defined as a P value less than .05. To compute the overall heterogeneity in population-based and referral-based studies, all studies were analyzed. In sensitivity analyses, heterogeneity statistics were recalculated after removing those studies with SIR values of 0 (ie, in which no cases of lymphoma were diagnosed).

Funnel plots were generated to examine for evidence of publication bias. The Egger regression asymmetry test, which examines the association between effect size and precision (ie, 1 divided by the standard error of the effect size), was used as a formal test of publication bias. The Begg test was also performed to assess for publication bias.[25]

To assess whether the relationship between thiopurines and the risk of lymphoma differed in referral-based and population-based studies, we computed separate pooled SIRs for these 2 study designs. For analysis of current and past exposure, we created pooled SIRs limited to those studies that provided these data.

Results

Overall, there were 18 studies that met our inclusion criteria (Tables 1 and 2).[4–21]

Relative Risk of Lymphoma

The individual study results and the pooled results stratified by study type are summarized in forest plots in Figure 2. From all pooled data, the total number of observed cases was 93, and the weighted total number of expected cases was 18.92, resulting in SIR of 4.92 (95%

Table 1. Summary of Referral Center Studies Included in the Meta-analysis

	Connell[17]	Farrell[18]	Fraser[19]	Kinlen[20]	Korelitz[21]	Freeman[14]	Van Domselaar[11]	Biancone[13]	Beigel/Steinborn[15]	Ashworth[12]
Year	1994	2000	2002	1985	1999	2002	2009	2011	2011	2012
Setting	Single center	Single center	Single center	Single center	Single center	Single center	Single center	Single center	Single center	Single center
Total no. of IBD patients	755	238[a]	626	321	486	99	370	365	636	1026
% with CD	60	46	43	N/R	67	100	57	70	70	71
% with UC	40	54	57	N/R	33	0	43	30	30	29
Mean/median duration of treatment	12.5 mo	1.82 y	2.26 y	N/R	4.4 y	N/R	N/R	2 y	1.25 y	N/R
Medication studied	AZA	AZA	AZA	AZA	6-MP	AZA, 6-MP	AZA, 6-MP	AZA, 6-MP	AZA, 6-MP	AZA, 6-MP
Mean/median dose or range	2 mg/kg/day	2–2.5 mg/kg/day	1.65 mg/kg/day	N/R	12.5–100 mg/day	N/R	N/R	N/R	N/R	N/R
Mean/median duration of follow-up (*y*)	9	6.9	6.9	N/R	5.9	10	9.4	9.8	2.12	5.44
Type of lymphoma	NHL	NHL	NHL and HD	NHL	NHL and HD	NA	NHL and HD	NHL	NHL and HD	NHL and HD
Expected	0.52	0.05	0.65	0.16	0.61	0.147	0.404	0.524	0.140	0.324
Observed no. of lymphomas	0	2[b]	3	2	3	0	5	2[d]	4[e]	2
SIR[c] (95% CI)	0 (—)	38 (10.4–135)	4.6 (1.6–14)	12.5 (3.5–45)	4.9 (1.7–14)	0 (0–25)	12.4 (5.3–29)	3.8 (1.1–14)	29 (11–73)	6.1 (1.7–22)[f]

All CIs were recalculated by primary statistician for each study independently (A.T.). These differ from Kandiel 2005.
CD, Crohn's disease; HD, Hodgkin's disease; N/R, not reported; UC, ulcerative colitis.
[a]Treated with immunomodulators.
[b]Two additional lymphomas observed in patients treated with methotrexate and cyclosporine.
[c]Because of rounding of expected number of lymphomas, SIR does not exactly equal observed number of lymphomas divided by expected number of lymphomas.
[d]Additional case, and details from direct communication with author, not in paper.
[e]Additional case from published paper with updated data as compared to abstract.
[f]SIR recalculated using data from all patients with thiopurine exposure.

Table 2. Summary of Population-based Studies Included in the Meta-analysis

	Armstrong[16]	Beaugerie[5]	Gisbert[4]	Peyrin Biroulet[10]	Herrinton[6]	Lakatos[8]	Abbas/Khan[7]	Pasternak[9]
Year	2010	2009	2010	2010	2012	2012	2013	2013
Setting	Population-based	Population-based	Population-based	Population-based	Population-based	Population-based	Population-based	Population-based
Total no. of IBD patients	1955	8676	3900	165	N/R[a]	351	4734	10,423
Country	United Kingdom	France	Spain	U.S. (Minnesota)	U.S. (Kaiser, National Data)	Hungary	U.S. (VA National Database)	Denmark
% with CD	N/R	76	70	65	N/R	77	2	48
% with UC	N/R	24	30	35	N/R	23	98	52
Mean/median duration of treatment (*y*)	N/R	N/R	3.53	2.28	N/R	2	0.97	1.9
Medication studied	AZA/6-MP	AZA+6-MP	AZA+6-MP	AZA, 6-MP	AZA, 6-MP	AZA	AZA, 6-MP	AZA
Mean/median dose or range	N/R	N/R	138 mg (15–400)	N/R	N/R	106 mg	N/R	N/R
Mean/median duration of patient follow up (*y*)	6.4	2.97	9.54	4.7	N/R[a]	13	[b]	3.1
Type of lymphoma	N/R	NHL and HD	NHL and HD	NHL	N/R	N/A	NHL	NHL and HD
Expected	1.497	3.58	4.32	0.166	5.63	0.41	6.14	3.52
Observed no. of lymphomas	4	17	4	1	9	0	23	12
SIR[c] (95% CI)	2.7 (1.0–6.8)[d]	4.8 (3.0–7.6)	0.9 (0.4–2.4)	6.0 (1.1–34)	1.6 (0.8–3.0)[a]	0 (0–9.0)	3.75 (2.5–5.62)[e]	3.49 (1.8–4.3)[f]

N/R, not reported; VA, Veterans Affairs.

[a]Number of patients and years of follow-up not reported, but total patient-years of follow-up reported (20,575 patient-years). CI recalculated by using Poisson method.

[b]The product of the follow up time and the number of patients reported in paper not equal to # of patient-years given in text; patient-years used for SIR calculation (25,728 PY).

[c]Because of rounding of expected number of lymphomas, SIR does not exactly equal observed number of lymphomas divided by expected number of lymphomas.

[d]In the original text the odds ratio comparing IBD patients on thiopurines vs without was reported (Odds ratio = 3.22). Recalculated SIR of 2.67 from primary data and in discussions with authors, and the calculated SIR is used for meta-analysis calculation.

[e]Recalculated by using expected numbers of lymphomas from updated data from National Cancer Institute SEER Database (2001–2010).

[f]SIR recalculated using data from all patients with thiopurine exposure.

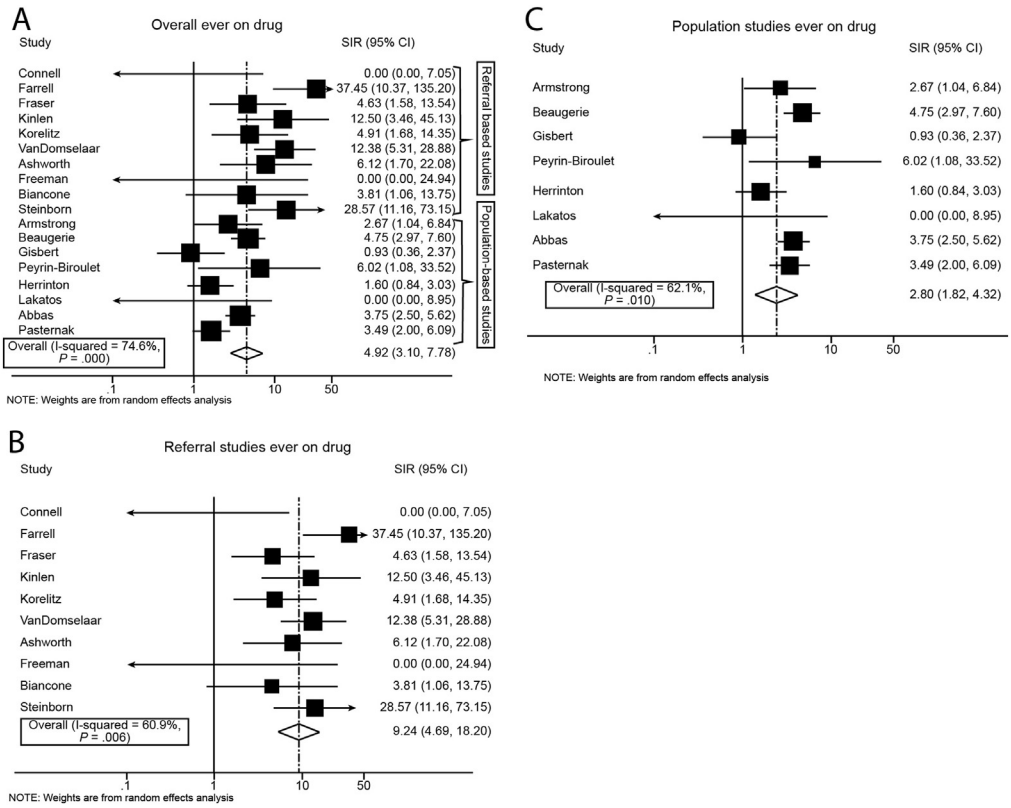

Figure 2. (*A*) Forest plot showing standardized incidence ratios (SIRs) of lymphoma risk among referral center and population-based studies. (*B*) Forest plot of SIR of lymphoma risk in referral center-based studies. (*C*) Forest plot of SIR of lymphoma risk in population based studies.

CI, 3.10–7.78). However, significant heterogeneity was detected among the studies ($P < .001$). Lakatos,[8] Freeman,[14] and Connell[17] appeared to be outliers with SIR of 0. However, when the studies by Lakatos, Freeman, and Connell were excluded, there was still significant heterogeneity ($P < .001$).

The SIR of referral-based studies (SIR = 9.24) and population-based studies (SIR = 2.80) were significantly different ($P < .05$). The heterogeneity was partially but not entirely explained by stratification of the studies according to referral center–based versus population-based. Among the referral center–based studies, the total number of observed cases was 23, and the weighted total number of expected cases was 2.49, resulting in SIR of 9.24 (95% CI, 4.69–18.2) (test for heterogeneity, $P = .0006$, $I^2 = 60.9\%$). Exclusion of the studies by Freeman[14] and Connell et al[17] (all SIR = 0) did not affect heterogeneity (SIR = 10.02; 95% CI, 5.57–18.0; heterogeneity, $P = .029$). Among population-based studies, the total number of observed cases was 70, and the weighted total number of expected cases was 25, resulting in SIR of 2.80 (95% CI, 1.82–4.32). Here, significant heterogeneity remained ($P = .02$, $I^2 = 60.8\%$), even when the study by Lakatos et al[8] was excluded.

Epstein–Barr Viral Positivity

Of the 25 thiopurine-exposed patients in whom Epstein–Barr virus (EBV) positivity was tested, 14 (56%) were EBV-positive. Among those younger than 50 years, 8 of 13 patients (61.5%) were positive for EBV, whereas among those 50 years or older, 6 of 12 (50%) were positive.

Case Descriptions

Overall, there were 93 cases of lymphoma, of which 59 were confirmed to be NHL, 8 were confirmed to be Hodgkin's disease, and 26 were not recorded (Supplementary Tables 1 and 2). Two cases of NHL were classified as hepatosplenic T-cell lymphoma. Patients received a median of 18 months of therapy before the diagnosis of lymphoma (range, 1–109 months). Of the 59 NHLs, 19 (32%) originated in the bowel (with 4 in rectum), 14 originated with lymphadenopathy, 5 had disseminated disease, 4 originated in the central nervous system, 3 in the bone marrow, 2 in the liver, 1 in the head/neck area, and 3 were elsewhere. The site was not recorded for 10 patients with confirmed NHL.

Current vs Past vs Never Use of Thiopurines

We explored whether prior exposure to thiopurines increased the risk of the development of lymphoma vs those currently on thiopurines. For this analysis we identified 5 studies that provided separate data for current, past, and never use of thiopurines.[5–7,9,10] The pooled SIRs were 1.06 (95% CI, 0.81–1.40) for never use, 1.42 (95% CI, 0.86–2.34) for prior use, and 5.71 (95% CI,

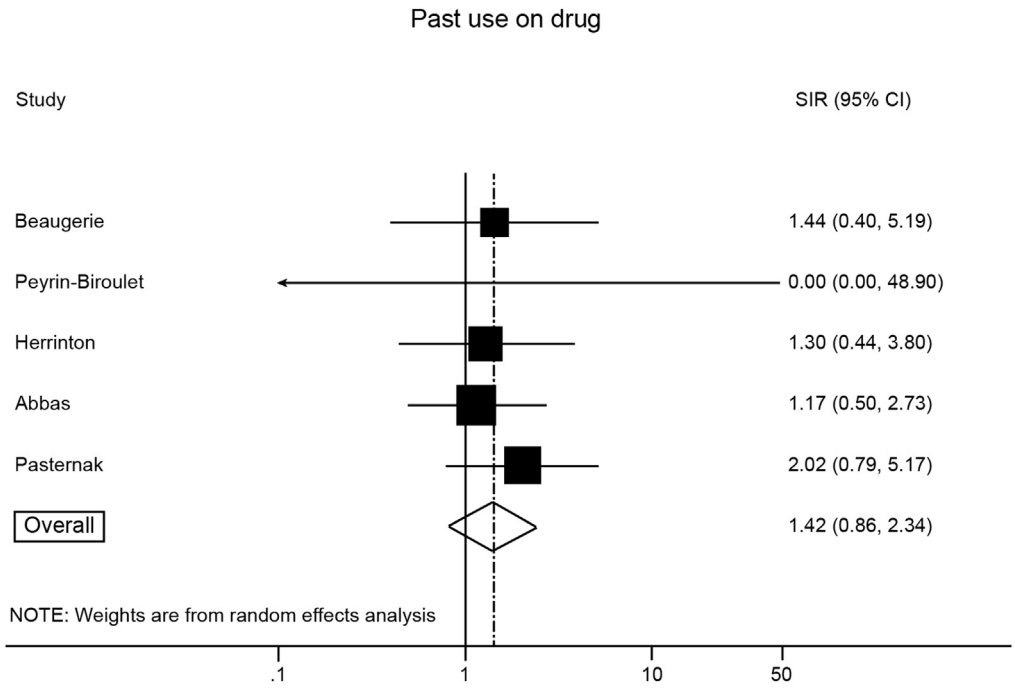

Figure 3. Comparison of SIRs of lymphoma risk stratified by previous or never use of thiopurines. There is no significant heterogeneity. Results are not significantly different from patients who have never used thiopurines.

3.22–10.1) for current use. Figure 3 shows the forest plot for past use of drug.

To measure absolute risk, we computed the number of patients needed to harm, which represents the number of patients needed to be treated for 1 year with thiopurine to cause 1 additional case of lymphoma. In Table 3, the number needed to harm is stratified by age deciles between 0 and 79 years, assuming an increased relative risk of 2, 3, 4, 5, or 6. Assuming a relative risk of lymphoma of 3.0 and using the National Cancer Institute's Surveillance Epidemiology and End Results Database (SEER) data from 2003 to 2007, the number of patients needed to cause 1 additional lymphoma ranges from 4598 in those 20–29 years to 325 in those 70–79 years.[26,27]

Gender and Risk of Lymphoma

We previously reviewed the literature on the effect of gender on the risk of lymphoma in IBD in patients on thiopurines. Earlier we found that there was no effect of gender in terms of distribution of lymphoma in men and women with the exception of hepatosplenic T-cell lymphoma in a systematic review.[28] Of the cases seen, 55.9% of lymphomas were seen in men and 44.1% were seen in women, which was not significant. However, in hepatosplenic T-cell lymphoma, 93.5% of cases were seen in men and only 6.5% in women ($P < .05$).[28]

We reanalyzed this question by using the CESAME and ENEIDA databases, which were both population-based studies. Of our selected citations, we had primary data on gender solely from these 2 databases. A meta-analysis of the CESAME and ENEIDA data (2 studies) from all users of thiopurines revealed that the SIR was elevated in men (SIR = 4.50; 95% CI = 3.71–5.40) and women (SIR = 2.29; 95% CI = 1.69–3.05). Men had approximately twice the relative risk as women (relative risk = 1.98, 95% CI = 1.40–2.84).

Table 3. Number Needed to Treat (Harm) to Cause 1 Additional Lymphoma per Year

Age (*y*)	Lymphoma incidence[a,b]	NNH if relative risk of lymphoma = 2	NNH if relative risk of lymphoma = 3	NNH if relative risk of lymphoma = 4	NNH if relative risk of lymphoma = 5	NNH if relative risk of lymphoma = 6
20–29	7.25	6897	4598	3448	2759	2299
30–39	9.45	5291	3527	2646	2116	1764
40–49	15.6	3205	2137	1603	1282	1068
50–59	29.9	1672	1115	836	669	557
60–69	59.0	848	565	424	339	283
70–79	102.5	488	325	244	195	163

The number needed to harm (NNH) for varying SIRs is presented to put SIR in context to show that absolute risk of lymphoma for a particular patient is typically low, even if relative risk is elevated.

[a]National Cancer Institute. Available at: http://seer.cancer.gov/csr/1975_2007/results_merged/sect_19_nhl.pdf. 2010.

[b]Cases per 100,000 person-years.

Table 4. Combined Meta-analysis of Risk of Lymphoma in IBD Patients on Thiourines per Age-Decile

Age group (*y*)	Observed cases	Expected cases	SIR	95% CI
0–19 (combined)	1	0.091	11.0	0.004–62.8
20–29	4	0.624	6.42	1.67–16.6
30–39	1	1.092	0.92	0–5.25
40–49	2	1.227	1.63	0.15–5.99
50–59	8	1.264	6.33	2.7–12.5
60–69	3	1.140	2.63	0.50–7.79
70–79	5	0.940	5.32	1.68–12.5
80+	0	0.255	0	0–14.4

Age-Decile Analysis

We also explored the possible effect of age on the relative risk for lymphoma by combining raw data from 3 population studies[4,5,24] and 2 referral studies.[19,21] Because of small numbers of events in several age deciles, we combined age groups. As Gisbert[4] was an outlier in our main analysis, we analyzed 2 sets of data: 1 with Gisbert and 1 without. The data including Gisbert[4] is presented in Supplementary Tables 3 and 4, while results presented below omit Gisbert.[4] Of all the studies selected, solely these 5 had primary raw data available, and we therefore evaluated the data from these studies for our age-decile analysis.

When data were combined for patients ages 0–29, 30–59, and 60+ years, SIRs were 6.99 (95% CI, 2.99–16.4) for those ages 0–29, 3.07 (95% CI, 1.71–5.50) for those 30–59, and 3.43 (95% CI = 1.46–6.79) for those older than 60. However, combining groups for ages 0–39, 40–59, and 60+ eliminated differences between age groups (SIR = 3.3, 4, and 2.6, respectively), whereas combining all those older than 50 resulted in SIR of 4.78. In the general population in those older than 50, the absolute risk of lymphoma is 1:1694 per patient per year[26,27,29] (SEER data weighted by population distribution of the U.S. census), and with a relative risk of 4.78, the absolute risk in IBD patients on thiopurines is 354 per patient per year (Tables 4 and 5).

In terms of our analysis including Gisbert, no significant differences in risk were seen in those under 50. However in those 50 and older, there was a decreased risk observed when Gisbert was included and in those over 60, the increase in risk was no longer significant. The SIR in those over 50 was 1.81 (95% CI = 1.07–2.87), while in those over 60 it was 1.33 (95% CI = 0.60–2.53). See Supplementary Tables 3 and 4 for age-decile results, and grouped results, respectively.

Table 5. Combined Meta-analysis of Risk of Lymphoma in IBD Patients on Thiopurines, Binned 0–29, 30–59, and 60+ Years of Age

Age group (*y*)	Observed cases	Expected cases	SIR	95% CI
0–29	5	0.715	6.99	2.99–16.4
30–59	11	3.583	3.07	1.71–5.50
60+	8	2.33	3.427	1.46–6.79

Analysis of Duration on Risk of Lymphoma

To examine the effect of duration of exposure to thiopurines on risk of lymphoma, we combined extant data from Chaparro et al[4] and Khan et al[7]. Data on duration were only available with these 2 studies. In those with less than 1 year of thiopurine exposure, SIR was 1.39 (95% CI, 0.60–3.24), in those with 1–2 years of exposure, SIR was 4.31 (95% CI, 1.85–10.1), in those with 2–3 years of exposure, SIR was 3.08 (95% CI, 1.05–9.00), and in those with >3 years of exposure, SIR was 4.84 (95% CI, 2.88–8.11). Other than 1 case of lymphoma that developed within 2 months of treatment, which may have been concurrent, there was a lag time of at least 8 months before onset of lymphoma (Figure 4).

Bias Analysis

The Begg and Egger tests were performed to assess for bias for current users, past users, and all users. All current and past use were not significant (Supplementary Figure 1).

Analysis With Exclusion of Patients With Anti-TNF Use

We also performed an analysis with all of the above with removal of patients with anti-TNF exposure, if possible, with exception of the duration analysis, as for that analysis no information on patients without anti-TNF use was available. Studies assessing patients prior to August 1998 did not receive anti-TNF therapy, as this was before FDA approval of Infliximab, the first anti-TNF medication approved for IBD. The studies with this information included Van Domselaar,[11] Herrinton,[6] Biancone,[13] Beigel,[15] Beaugerie,[5] Gisbert,[4] Lakatos,[8] and Khan.[7] None of the results were significantly different from the primary analysis. See Supplementary Figure 3 for forest plots for the analysis excluding anti-TNF use for all studies, population based studies, and referral based studies.

Discussion

We demonstrated that patients with IBD who are taking thiopurines have a nearly 6-fold higher incidence of lymphoma when compared with the general population. In addition, we determined that use of thiopurines does not appear to result in a persistent elevated incidence of lymphoma after the medication is discontinued,

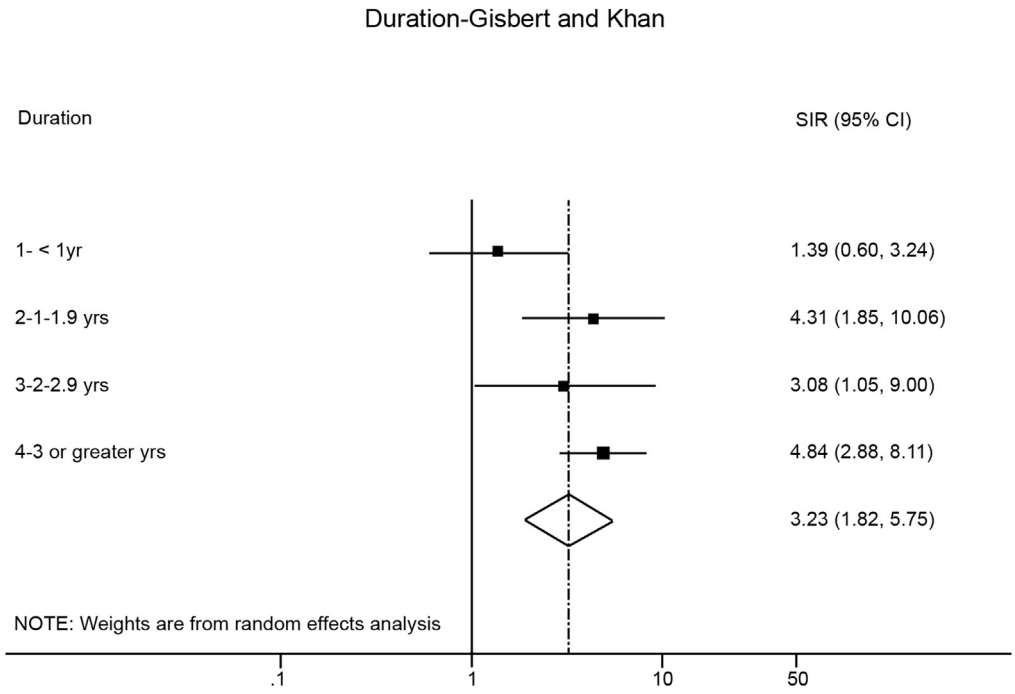

Figure 4. Comparison of SIRs between patients stratified by duration on thiopurine therapy. There was no significant heterogeneity present.

suggesting that immunosuppression rather than direct DNA damage may be more of a factor in the development of excess lymphomas. This is consistent with the large proportion of EBV-associated lymphomas among patients treated with thiopurines.

It is important to emphasize that although the relative risks of lymphoma in active users are moderate, there remains a very low absolute risk of lymphoma for any given patient. If one interpolates the risk on the basis of our extant data, as shown in Table 3, the risk to any patient younger than 50 (with the exception of men younger than 30) remains less than 1 in 2000 per year, which is still quite low. In contrast, those older than 50 may have an absolute risk closer to 1:350 per year, which requires more caution in prescribing long durations of thiopurine treatment in this population.

In a subanalysis of 2 studies, men, more so than women, had an elevated risk of lymphoma when on thiopurines. Data on gender from primary raw databases were solely available from these 2 studies. Our results should be interpreted with caution because it was limited to 2 studies; we cannot rule out a relative risk of lymphoma of less than 3.05 among women. Among the general population, lymphoma is more common in men than in women.[26,27] Bernstein et al[30] previously reported an increased risk of lymphoma among men with IBD but not women as well. In addition, from the SEER database, men also show a higher baseline risk of lymphoma as compared with women, with men showing an incidence of NHL of 26 cases per 100,000 person-years as compared with 17 cases per 100,000 person-years.[26] In Hodgkin's lymphoma, there are 3.2 cases per 100,000 person-years in men and only 2 cases per 100,000 person-years in women.[27]

In terms of duration of exposure, it is clear that exposure for more than 1 year of treatment while on active therapy significantly increases the risk of lymphoma. Although few cases have been seen with less than 12 months of treatment, SIR for less than 1 year of therapy may be influenced by patients using the drug for extremely short amounts of time (eg, 1–2 months, and not tolerating the drug because of adverse effects). Thus, the lack of an observed increased risk within 1 year of initiating therapy may be falsely reassuring.

Younger patients may be infected with EBV around the same time as they receive thiopurines, and this particular subset of patients may represent a group at higher risk for treatment with thiopurines. In addition, the risk of EBV may be underestimated; an analysis of the CESAME data shows a risk of 2.9 cases per 1000 person-years for men younger than 35 years at risk for fatal primary EBV infection.[31] In addition, patients having stem cell transplants are at up to 20% risk of post-transplant lymphoproliferative disorder, with the risk increasing with increased immunosuppression.[32]

When we analyze the risk by age group, when dividing the population into 3 cohorts, 0–29, 30–59, and 60+ years, we see an elevated risk in 0- to 29-year-olds of approximately 7 times (SIR = 6.99). It should be noted that dividing these cohorts differently (eg, 0–39, 40–59, 60+) causes all age groups to have similar relative risks of about 3–4. The different results in the age-decile analysis with Gisbert must also be considered. One possible explanation for the lower risk seen in older patients might be that there was a skewed, lower number of cases in Gisbert, and as an outlier, not predictive of true risk in the older patient cohort. Indeed, multiple analyses have shown an increased risk in those over 50.[15,46] Both results should be considered with caution, and addition of further studies in the future will help clarify this issue further.

In addition we evaluated the effect of excluding patients on anti-TNF therapy, and did not see a significant difference in any of the analyses performed when compared to our primary analyses including all patients on thiopurines.

If there is truly a greater risk in those younger than 30, assuming there is at least 2-fold greater risk in active users, that men have a roughly double relative risk than women (eg, approximately 9 vs 5, with average SIR of 7), and that men have 1.5-fold higher absolute risk as compared with women,[26,27] the absolute risk in men younger than 30 currently taking thiopurines may be as high as 1 per 500–1000 person-years, much higher than expected for those of this age group. From the results of a single database (CESAME), the risk was also seen to be substantially higher in young men (2.9 per 1000 patients per year in men younger than 35).[31] Because of the disparate results that are based on combining different age groups as noted above, these data should be interpreted with a high degree of caution, and more data are critically needed to more precisely understand the risks to men younger than 35.

There also exists a small (<1:20,000 person-years) but real chance of development of hepatosplenic T-cell lymphoma, nearly uniformly present in men younger than 35 years.[28] Although rare, without allogeneic stem cell transplantation, it has typically been rapidly fatal.[33] This is in contrast to the other types of NHL, where more than 75% of patients younger than 45 are alive after 5 years after intensive chemotherapy.[27] It should be noted that in another systematic review of hepatosplenic T-cell lymphoma, 14% of non-IBD cases and 20% of IBD cases had EBV positivity, indicative of a possibly different etiology than other NHL or Hodgkin's disease seen with immunomodulator use.[34]

The relative risk estimates were higher in referral center studies than in population-based studies. However, even in population-based studies, there was a significant 2.9-fold higher incidence of lymphoma among patients treated with thiopurines compared with the general population. The underlying IBD seen in the referral center population may be more severe than that seen in the population as a whole. Therefore, seemingly higher relative rates of lymphoma in referral studies may be confounded by the severity of disease.[3] This is analogous to rheumatoid arthritis, where many studies have shown that the presence of rheumatoid arthritis itself confers an elevated risk of lymphoma as compared with the general population.[35–38] Furthermore, the more severe the rheumatoid arthritis, the greater the risk of lymphoma.[39] Alternatively, patients with other comorbidities are more often found in referral centers, and these (such as obesity) may also raise the risk of lymphoma. Future studies focusing on other risk factors for lymphoma among patients with IBD could help to clarify this issue.

Approximately 30% of all lymphomas were seen in the gastrointestinal tract. Most Hodgkin's lymphomas present as mediastinal masses. The presentation of NHL is highly variable. Estimates of gastrointestinal involvement in NHL range from 5% to as much as 60% with more advanced disease.[40–42] Some data suggest that EBV-positive extranodal mucosa-associated lymphomas might be related to the post-transplant state of immunosuppression.[43] A study[44] of 200,000 organ transplant recipients showed 11.8-fold increased risk in the renal transplant population, with preferential site of lymphoma near the area of the transplant and gastrointestinal tract involvement in approximately 15% of the patients. Current data do not allow for a distinction of whether an excess of gastrointestinal tract involvement in patients with IBD is related to the underlying disease process or differential detection.

Both referral based studies ($P = .006$, $I^2 = 60.9\%$), and population based studies ($P = .02$, $I^2 = 60.8$) had significant heterogeneity. Although there is no consensus, some consider values greater than 50% as moderately heterogeneous and those greater than 75% as having high heterogeneity.[45]

The definitions of referral and population-based studies are important. Here we define population-based studies as those gathering data from IBD patients from a defined geographic area, as our group defined previously.[22] We define referral center studies as those including IBD patients from solely one referral center. It is not always clear from the literature whether a study is truly population-based. For example, the populations included in the Kaiser Permanente Northern California study[6] and from the General Practice Research Database[16] can be thought of as population representative because they do not capture everyone within a geographic area but rather a representative sample. Likewise, how well studies such as CESAME,[5] which includes a large number of centers across a country, represent the overall population of the country is uncertain. Nondifferential misclassification bias would result in an attenuated estimate of the relative risk of lymphoma and could be more common in population-based studies relying on administrative data.

The comprehensiveness of this meta-analysis is limited to the available published data and therefore is subject to publication bias. Small studies, particularly those with negative results, may go unpublished. We first attempted to account for this with a search of major scientific meeting abstracts. Second, we used specific inclusion criteria to focus our meta-analysis on cohort studies that examined IBD, evaluated cancer as an outcome of interest, and focused on patients who received AZA or 6-MP. Studies that were not specifically evaluating cancer as a primary event were not included, because these studies are often less complete and can result in further misclassification bias.[3] Third, we performed statistical testing (through Begg and Egger tests) to look for evidence of bias from unpublished studies. Statistical testing did not reveal publication bias.

The calculation of expected lymphoma rates used in each study depends on estimates of the background rate of lymphoma in the population. For some of the studies

included in this meta-analysis, it was necessary to estimate expected cancer incidence rates from smaller regions than the actual geographic area included in the study.[23] This was true for the studies derived from Spain,[4,11] Italy,[13] and Hungary.[8] These studies rely on data taken from a select number of local regions within the country and extrapolated to the larger Spanish population. For example, the referral center described by Van Domselaar et al[11] is located in Madrid, which is not a region included in the background population data used to calculate the expected number of lymphomas. For this to bias our results, there would need to be substantial regional variation in the incidence of lymphoma within Spain, for example.

In conclusion, our data suggest that current thiopurine use of at least 1 year may increase the risk of lymphoma nearly 6-fold. However, the increased risk appears to revert back to the baseline risk after thiopurines are discontinued, suggesting that immunosuppression is the key factor associated with increasing the incidence of lymphoma. The absolute risk is highest in those older than 50. Young male patients (younger than 30–35) may also be a high-risk population. For patients of all ages and genders, the risk of lymphoma needs to be weighed against the potential benefits of therapy. Further work is needed to understand how this tradeoff of potential benefit and harm varies by age, particularly in the era of combination immunosuppression therapy.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2014.05.015.

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Conflicts of interest
These authors disclose the following: James Lewis received consulting fees from Pfizer Inc; consulting fees, Advisory Committees or Review Panels from Centocor; consulting fees from Shire Pharmaceuticals Group, Millennium Pharmaceuticals, AstraZeneca, GlaxoSmithKline, Dark Canyon Laboratories, and Roche Pharma AG; and grant/research support from Allos Therapeutics, Takeda Pharmaceutical, and Amgen. Laurent Beaugerie received salary for speaking and teaching from Ferring Pharmaceuticals Inc, Schering-Plough Corp, and Merck. Edward V. Loftus received consulting fees, Advisory Committees or Review Panels from Pfizer Inc, Abbott, UCB Pharma, and Shire Pharmaceuticals Inc; and grant/research support from Genentech Inc, Braintree Laboratories Inc, Abbott, UCB Pharma, Bristol-Myers Squibb Co, Centocor, Inc, Takeda Pharmaceutical Company Ltd, and Amgen. Gary Lichtenstein received consulting fees from Bristol-Myers Squibb Co, Ferring Pharmaceuticals Inc, Centocor/Orthobiotech, Inc, Millennium Research Group, Procter & Gamble Pharmaceuticals, Prometheus Laboratories Inc, Salix Pharmaceuticals, Inc, Warner Chilcotte, Schering-Plough Corp, Shire Pharmaceuticals Inc, and Wyeth Pharmaceuticals; and received grant/research support from Abbott Laboratories, Centocor/Orthobiotech, Inc, Elan Pharmaceuticals, Inc, Procter & Gamble Pharmaceuticals, Prometheus Laboratories Inc, Salix Pharmaceuticals, Inc, Shire Pharmaceuticals Inc, UCB, Inc, and Warner Chilcotte. The remaining authors disclose no conflicts.

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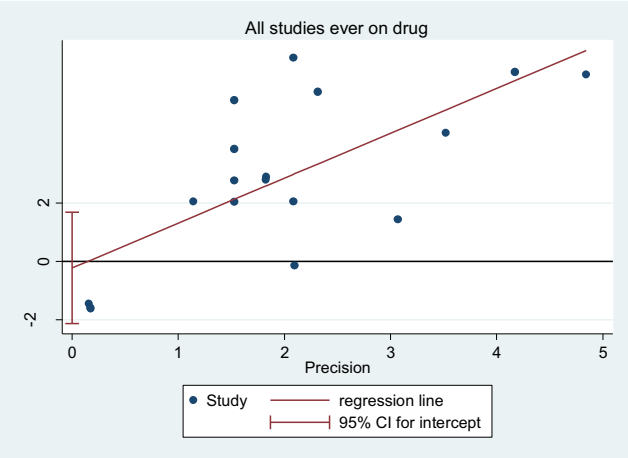
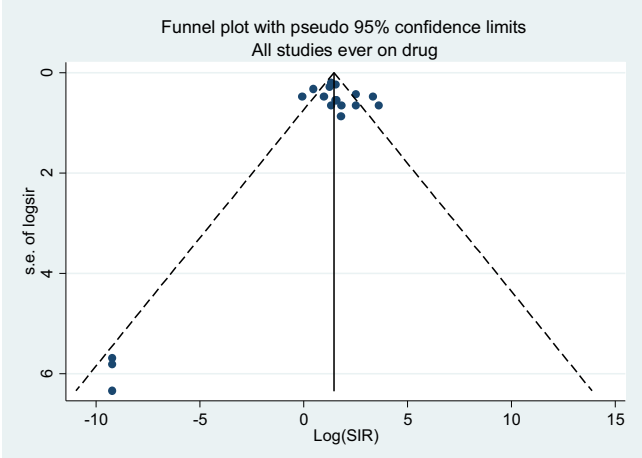
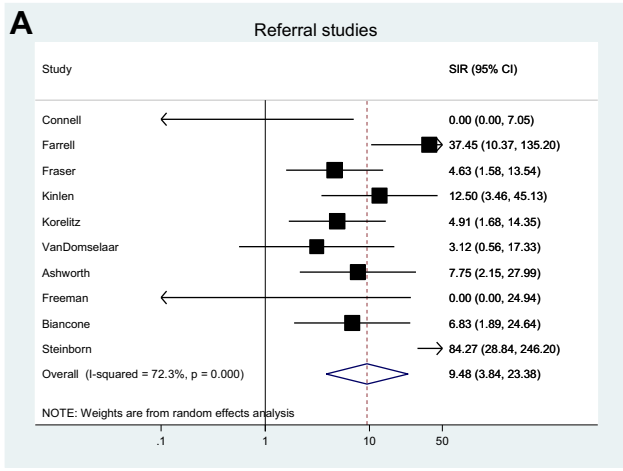
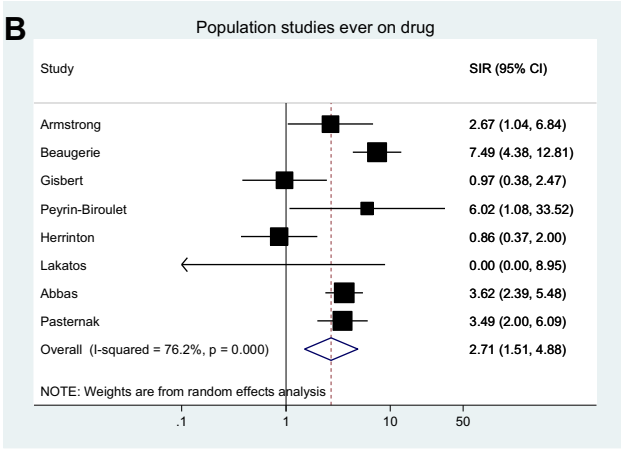


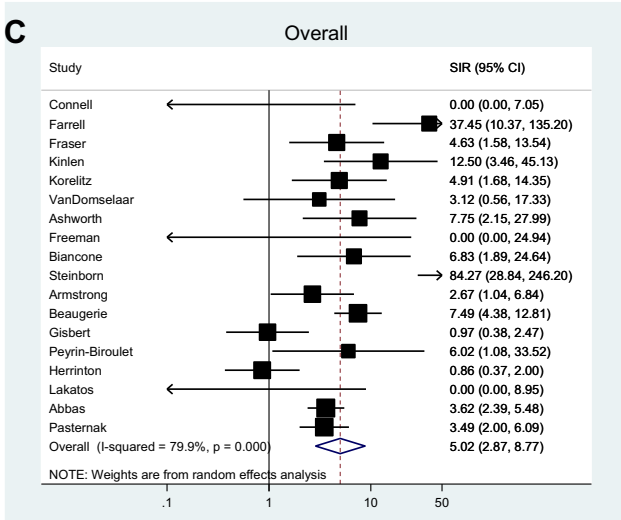
Supplementary Figure 1. Egger bias plot, all studies. No significant bias present. SND, standard normal deviate.



Supplementary Figure 2. Funnel bias plot, all studies. No significant bias present. s.e., standard error.







Supplementary Figure 3. (*A*) All studies excluding users of anti-TNF agents when reported. (*B*) Referral center studies excluding users of anti-TNF agents when reported. (*C*) Population based studies excluding users of anti-TNF agents when reported.

Supplementary Table 1. Available Data on Individual Cases of Lymphoma in Thiopurine-treated IBD Patients in Referral Centers

Author/patient no.	Age (*y*)	Sex	IBD subtype	Duration of AZA/6-MP therapy (*mo*)	Lymphoma type	Location of NHL	EBV positivity	Active thiopurine use?
Farrell/#1	53	M	UC	14	NHL	Bowel	N/R	N/R
Farrell/#2	34	F	CD	9	NHL	Bowel	N/R	N/R
Fraser/#1	43	M	UC	72	NHL	Bowel	N/R	N/R
Fraser/#2	19	M	UC	4	HD	—	N/R	N/R
Fraser/#3	52	F	UC	94	NHL	Other	N/R	N/R
Kinlen/#1	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
Kinlen/#2	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
Korelitz/#1	69	M	CD	40	NHL	Bowel	N/R	N/R
Korelitz/#2	53	M	CD	6	NHL	CNS	N/R	N/R
Korelitz/#3	50	M	CD	87	NHL	Other	N/R	N/R
Van Domselaar/#1	55	M	CD	28	NHL	Head/neck	–	N/R
Van Domselaar/#2	47	F	UC	17	NHL	Bowel	+	N/R
Van Domselaar/#3	41	M	CD	20	NHL	Bowel	+	N/R
Van Domselaar/#4	70	M	UC	17	NHL	Bowel	+	N/R
Van Domselaar/#5	33	F	CD	12	NHL	N/R	–	N/R
Ashworth/#1	12	M	CD	22	NHL	N/R	–	Yes
Ashworth/#2	18	M	UC	28	HD	N/R	–	Yes
Biancone/#1	36	F	CD	72	NHL	N/R	N/R	N/R
Biancone/#2	42	M	CD	12	NHL	N/R	N/R	Yes
Steinborn/Seiderer/#1	38	M	CD	0.5	NHL	N/R	N/R	N/R
Steinborn/Seiderer/#2	56	M	CD	2	NHL	N/R	N/R	N/R
Steinborn/Seiderer/#3	40	M	UC	60	HD	N/R	N/R	N/R
Steinborn/Siederer/#4	24	M	CD	60	NHL-HSTCL	N/R	N/R	N/R

CD, Crohn's disease; CNS, central nervous system; HD, Hodgkin's disease; HSTCL, Hepatosplenic T-cell lymphoma; N/R, not reported; UC, ulcerative colitis.

Supplementary Table 2. Available Data on Individual Cases of Lymphoma in Thiopurine-treated IBD Patients in Population-based Studies

Author/patient no.	Age (*y*)	Sex	IBD subtype	Duration of AZA/6-MP therapy (*mo*)	Lymphoma type	Location of NHL	EBV positivity	Active thiopurine use?
Lewis/Armstrong/#1	47	M	UC	10	HD	—	N/R	N/R
Armstrong/#2	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
Armstrong/#3	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
Armstrong/#4	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
Beaugerie/#1	20	M	CD	3	NHL	Bowel	+	Yes
Beaugerie/#2	22	F	CD	1	NHL	LN	–	Yes
Beaugerie/#3	25	F	CD	8	HD	LN	+	Yes
Beaugerie/#4	26	M	CD	4	NHL	LN	+	Yes
Beaugerie/#5	37	F	CD	3	NHL	Disseminated	+	Yes
Beaugerie/#6	52	M	CD	16	HD	Lung/liver	+	Yes
Beaugerie/#7	54	F	CD	3	NHL	LN	+	Yes
Beaugerie/#8	55	F	CD	13	NHL	Bowel	–	Yes
Beaugerie/#9	56	M	CD	1	NHL	LN	–	Yes
Beaugerie/#10	60	M	UC	2	NHL	Brain	+	Yes
Beaugerie/#11	60	M	CD	3	NHL	Rectum	+	Yes
Beaugerie/#12	76	M	CD	5	NHL	LN	+	Yes
Beaugerie/#13	78	M	UC	10	NHL	Bone marrow	–	Yes
Beaugerie/#14	79	M	UC	9	NHL	Brain	+	Yes
Beaugerie/#15	79	F	CD	7	NHL	Colon	–	Yes
Beaugerie/#16	56	M	CD	N/R	NHL	Bowel	–	No
Beaugerie/#17	75	F	UC	N/R	NHL	Thorax	N/R	No
Gisbert/#1	68	M	N/R	41	HD	N/R	N/R	No
Gisbert/#2	52	M	N/R	11	NHL	N/R	N/R	Yes
Gisbert/#3	27	F	N/R	8	HD	N/R	–	Yes
Gisbert/#4	35	M	N/R	54	NHL–HSTCL	N/R	N/R	Yes
Peyrin–Biroulet/#1	29	M	UC	37	NHL	Rectal	+	Yes
Khan/#1	70	M	UC	8.4	NHL	Colon	N/R	Yes
Khan/#2	61	M	UC	73	NHL	Small bowel	N/R	Yes
Khan/#3	54	M	UC	48	NHL	Skin	N/R	Yes
Khan/#4	77	M	UC	46	NHL	Rectum	N/R	Yes
Khan/#5	50	M	UC	109	NHL	Disseminated	N/R	Yes
Khan/#6	63	M	UC	19	NHL	Liver and spleen	N/R	Yes
Khan/#7	38	M	UC	8.4	NHL	Small bowel	N/R	Yes
Khan/#8	76	M	UC	1.2	NHL	Disseminated	N/R	Yes
Khan/#9	49	M	UC	83	NHL	LN	N/R	Yes
Khan/#10	81	M	UC	34	NHL	Disseminated	N/R	Yes
Khan/#11	73	M	UC	44	NHL	LN	N/R	Yes
Khan/#12	70	M	UC	70	NHL	LN	N/R	Yes
Khan/#13	41	M	UC	91	NHL	LN	N/R	Yes
Khan/#14	71	M	UC	18	NHL	Colon	N/R	Yes
Khan/#15	42	M	UC	24	NHL	Brain	N/R	Yes
Khan/#16	66	M	UC	44	NHL	Disseminated	N/R	Yes
Khan/#17	76	M	UC	20	NHL	LN	N/R	Yes
Khan/#18	72	M	UC	76	NHL	LN	N/R	Yes
Khan/#19	54	M	UC	18	NHL	LN	N/R	No
Khan/#20	77	M	UC	36	NHL	Rectum	N/R	No
Khan/#21	49	M	UC	32	NHL	Bone marrow	N/R	No
Khan/#22	67	M	UC	17	NHL	Bone marrow	N/R	No
Khan/#23	44	M	UC	62	NHL	LN	N/R	No

Data on individual patients were not available for studies by Herrinton, Lakatos, and Pasternak.

CD, Crohn's disease; HD, Hodgkin's disease; HSTCL, Hepatosplenic T-cell lymphoma; N/R, not reported; UC, ulcerative colitis.

Supplementary Table 3. Combined Meta-analysis of Risk of Lymphoma in IBD patients on Thiourines per Age-Decile (Gisbert Included)

Age group	Observed cases	Expected cases	SIR	95% Confidence interval
0–19 (combined)	1	0.099	10.1	(0.004–58.1)
20–29	5	0.858	5.83	(1.84–13.7)
30–39	2	2.022	0.99	(0.09–3.64)
40–49	2	2.938	0.68	(0.06–2.50)
50–59	9	3.142	2.86	(1.30–5.46)
60–69	4	3.406	1.17	(0.31–3.04)
70–79	5	2.667	1.87	(0.59–4.41)

Supplementary Table 4. Combined Meta-analysis of Risk of Lymphoma in IBD patients on Thiopurines, binned 0–29, 30–59 and 60+ years of age (Gisbert Included)

Age group	Observed cases	Expected cases	SIR	95% Confidence interval
0–29	6	0.957	6.27	(2.26–13.7)
30–59	13	8.102	1.60	(0.85–2.75)
60+	9	6.786	1.33	(0.60–2.53)