Risk factors for lymphoma in patients with inflammatory bowel disease: a case-control study

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ABSTRACT

Background: Subgroups of patients with inflammatory bowel disease (IBD) may have an increased risk of developing lymphoma. We sought to identify factors that were associated with lymphoma in IBD patients.

Methods: Cases and controls were identified though a centralized diagnostic index. We identified 80 adult IBD patients who subsequently developed biopsy-proven lymphoma between 1980 and 2009. For each case, two controls were matched for subtype of IBD, geographic location, and length of IBD follow-up at our institution prior to lymphoma diagnosis. Medical records were abstracted for demographic and clinical information. Conditional logistical regression was used to assess associations between risk factors and the development of lymphoma.

Results: Sixty cases were males (75%) vs. 77 controls (48%). Median age at index date was 59 years for cases and 42 years for controls. Twenty cases (25%) and 23 controls (14%) were receiving immunosuppressive medications at the index date. Four cases (5%) and six controls (4%) were receiving anti-tumor necrosis factor-alpha (anti-TNF-alpha) agents at the index date. In multivariate analysis, age per decade (odds ratio [OR], 1.83; 95% confidence interval [CI], 1.37-2.43), male gender (OR, 4.05; 95% CI, 1.82-9.02) and immunosuppressive exposure at the index date (OR, 4.20; 95% CI, 1.35-13.11) were significantly associated with increased odds of developing lymphoma. Disease severity and use of anti-TNF-alpha agents were not independently associated with developing lymphoma.

Conclusions: In this case-control study, increasing age, male gender and use of immunosuppressive medications were associated with an increased risk of lymphoma in patients with IBD, whereas disease severity and anti-TNF-alpha use were not associated with increased odds.

RÉSUMÉ

Cadre de l'étude: Certains sous-groupes de patients atteints de maladies inflammatoires de l'intestin (MII) peuvent courir un risque accru de développer un lymphome. Nous avons chercher à identifier les facteurs associés avec le lymphome chez les patients avec des MII.

Méthodologie: Les cas et les témoins ont été identifiés à partir d'un répertoire de diagnostics centralisé. Nous avons identifié 80 patients adultes atteints de MII et qui ont dévelopé un lymphome confirmé par biopsie entre les années 1980-2009. Deux témoins étaient appariés pour chaque cas sur le sous-type de MII, la localisation géographique et la durée du suivi pour les MII dans notre centre jusqu'au diagnostic de lymphome. Les dossiers médicaux ont été consultés afin d'obtenir les données démographiques et cliniques. Un modèle de régression logistique conditionel a été utilisé pour évaluer les associations entre les facteurs de risque et le développement d'un lymphome.

Résultats: Parmi les cas retenus, 60 étaient des hommes (75%) vs 77 dans le groupe de témoins (48%). L'âge médian à la date index était de 59 ans pour les cas et de 42 ans pour les témoins. Vingt cas (25%) et 23 témoins (14%) recevaient des médicaments immunosuppresseurs à la date index. Quatre cas (5%) et six témoins (4%) recevaient des anticorps antagonistes du TNF-alpha à la date index. Lors d'une analyse multivariée, l'age par décennie (rapport de cotes [RC]:1,83, intervalle de confiance à 95%[IC]: 1,37-2,43), le sexe masculin (RC:4,05, IC 95%:1,82-9,02) et l'exposition aux médicaments immunosuppresseurs à la date index (RC:4,20, IC 95%: 1,35-13,11) ont été

associés, de façon significative, à un risque accru de developper un lymphome. La sévérité de la maladie et l'utilisation d'anticorps antagonistes du TNF-alpha n'ont pas été associés de manière indépendante au developpement d'un lymphome.

Conclusion: Dans cette étude de cas-témoin, l'age avancé, le sexe masculin et l'utilisation de médicaments immunosuppresseurs ont été associés à un risque supérieur de lymphome chez les patients atteints de MII. Par contre, la sévérité de la maladie et l'utilisation des anticorps antagonistes du TNF-alpha n'ont pas été associés à un risque plus élevé.

PREFACE

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Dedication: I would like to dedicate this thesis to my amazing wife Sabrina, who has supported me in countless ways while I have been a “student” at McGill for the last 15 years.

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1. RATIONALE

Inflammatory bowel disease (IBD) is a chronic inflammatory condition that affects the gastrointestinal tract and is comprised of two major subtypes: Crohn's disease (CD) and ulcerative colitis (UC). IBD likely results from an interplay between genetic predisposition and the initiation of a dysregulated immune response, through exposure to luminal antigens.[1] The incidence of IBD has increased significantly over the last twenty years in Canada and is approximately 16 per 100 000.[2] In fact, Canada has the highest reported worldwide incidence of CD.[2] With the prevalence of IBD estimated to be about 280 per 100 000, approximately 0.5% of the Canadian population has IBD.[2]

Patients with IBD can experience significant morbidity and an impaired quality of life. Patients with CD are at risk of serious complications from ongoing transmural bowel inflammation, including the development of intra-abdominal abscess, internal or extracutaneous fistula formation, peritonitis, and bowel obstruction.[3] These complications will lead to surgical intervention in up to 80% of patients with CD at least once during the course of their disease.[3] In addition, more than 30% of patients with UC will undergo proctocolectomy for either refractory colitis or the development of colorectal cancer.[4]

Treatment with immunosuppressive medications, such as azathioprine (AZA) and its metabolite 6-mercaptopurine (6-MP), and tumor necrosis factor-alpha (TNF-α) monoclonal antibodies, such as infliximab, adalimumab and certolizumab pegol, is effective for inducing and maintaining remission in patients with IBD.[5-9] Earlier and more aggressive medical treatment of IBD with

immunosuppressive and biologic agents has been advocated to alter the natural history of the disease and prevent complications.[2,10,11]

Patients are increasingly prescribed these medications and concern regarding the potential of malignancy-related sequelae has arisen, particularly the risk of lymphoma and lymphoproliferative disorders (LPD). The relationship between neoplasm and IBD is unclear; chronic immune activation seen in IBD may act as a trigger in the development of neoplasia. In addition, immunosuppressive and anti-TNF-α medications used to treat IBD may also play an inciting role in the development of malignancy. In patients with rheumatoid arthritis (RA), a chronic inflammatory disease of the joints, the association between inflammatory disease and immunosuppressive treatment with lymphoma is well documented, but this association is less clear in patients with IBD.[12-14]

The aim of this study was to identify risk factors that can lead to the development of lymphoma in patients with IBD. By identifying disease and treatment specific risk factors for this serious adverse outcome, clinicians will be able to make informed decisions on the optimal treatment for patients with IBD.

The actual absolute incidence of lymphoma in patients with IBD is in fact quite low and ranges from 0.2 to 0.9 per 1000 patients.[15-17] Therefore assessing risk factors for this relatively rare event is inherently difficult. We elected to conduct a case-control study to assess the association of various demographic, disease-specific, and medication-related factors that may be associated with lymphoma in IBD patients.

2. LITERATURE REVIEW

2.1 Lymphoma (including lymphoproliferative disease)

Lymphoma is broadly classified as a malignancy involving cells of the immune systems, specifically lymphocytes. Lymphoma occurs with clonal proliferation of B or T cells and can result in a heterogeneous group of immune related malignancies. Non-Hodgkin lymphomas (NHL's) represent up to 90% of lymphomas (90%) and Hodgkin's lymphoma account for the remaining 10%. The incidence of lymphoma has increased worldwide since the 1970s, particularly in western countries.[2,18,19] As of 2000, the age standardized incidence of lymphoma has been reported to be approximately 14 per 100 000 in North America.[19]

2.2 Risk of lymphoma with inflammatory bowel disease

The first published case of lymphoma in a patient with IBD occurred in 1928 within a case series on malignancy in patients with UC.[20] Since that time, other case reports, case series and hospital-based studies have described the possible link between IBD and lymphoma.[21-23] Given the retrospective nature of these studies, inferences regarding causality are difficult to make. Lymphoma has been associated with inflammation and autoimmunity in other disease states. For example, systemic lupus erythematosus, Hashimoto thyroiditis, and Sjogren's syndrome have all been shown to be associated with lymphoma.[24] Rheumatoid arthritis has also been shown to be associated with a two-fold increase in lymphoma.[12,13] With this backdrop, chronic immune activation and

inflammation in the gastrointestinal tract may indeed act as a trigger for the development of lymphoma.

Several population-based studies have helped to clarify the risk of lymphoma in patients with IBD and are summarized in **Table 1**.[15,25-34] The largest of these population-based studies, published by Askling et al., involved more than 47 000 Swedish patients with IBD from multiple regional cohorts (the Uppsala cohort, Stockholm County cohort, Stockholm pancolitis register, and the Swedish inpatient register).[34] In this study, no increased risk was found in either UC or CD patients, even after adjustment for extent of disease, hospitalization and surgery was performed.[34]

A large population based cohort using data from the General Practice Research Database (GPRD) from the United Kingdom (8 million patients) found 18 cases of lymphoma in almost 17 000 patients, compared to an expected 13.6 cases (age and sex-matched controls), but this difference was not statistically significant.[31] In addition, seven population based studies from Sweden, Denmark, Italy and Minnesota failed to show a significantly increased risk of lymphoma in IBD.[25,27,29,32,35-37] Finally, in a more recent large French population based study, those patients with IBD who had not received any immunosuppressive or anti-TNF-α medications did not have an increased risk for lymphoma (SIR, 1.45, 95% CI 0.53-3.16). In fact, only one smaller population-based, retrospective, matched cohort study from Manitoba has shown an increased risk of lymphoma in a subgroup of male patients with CD.[30]

In summary, it seems clear that from these population-based studies that a diagnosis of either CD or UC does not increase the risk of lymphoma. Although it is reassuring that a diagnosis of IBD on it's own does not increase the risk of lymphoma, a host of demographic, disease and treatment-specific risk factors may be associated with an increased risk.

2.3 Demographic and disease specific risk factors

In the two largest population-based studies (Sweden and United Kingdom) that assessed the risk of lymphoma in patients with IBD, demographic factors particularly age and gender were matched in controls. In controlling for these demographic factors, the risk of lymphoma associated with these factors cannot be determined. Although age and male gender are known risk factors for the development of lymphoma, their role in IBD is less clear.[38] In a prospective French cohort study, approximately 20 000 IBD patients were followed over the course of 3 years. Age was found to be one of the independent demographic risk factors for lymphoma, with an increased hazard ratio (per year) of 1.06 (95% CI 1.03 - 1.09).[15] Although there was a trend for male gender being a risk factor in the development of lymphoma, the results were not statistically significant (HR 2.32, 95% CI 0.95 – 5.64).[15] In a smaller cohort study from Manitoba that was mentioned previously, male patients with CD were found to have an increased risk of lymphoma resulting in an incidence rate ratio of 3.63 (95% CI, 1.53–8.62).[30]

Another disease specific risk factor may include duration of disease. In

the above described French cohort of patients, duration of disease (per year) resulted in a hazard ratio of 1.04 (95% CI, 1.00 – 1.08), indicating that perhaps length of disease may indeed increase lymphoma risk.[15]

Inflammation of the gastrointestinal mucosa may also be involved in the "lymphomagenesis" in inflammatory bowel disease.[19] It would therefore follow that severity of disease may then influence the risk of lymphoma as well. This issue has not been assessed adequately in patients with IBD. In the RA literature, it seems clear that an association exists between disease severity and risk of lymphoma.[39,40] To evaluate this risk, a nested case-control study was conducted using a population based cohort of over 11 000 Swedish RA patients.[39] Increased inflammatory activity was associated with an odds ratio of 5.8 (95% CI, 3.1 – 213).[39] Similarly, another nested case-control study from a Swedish cohort of 74 000 patients (378 lymphoma and RA cases and 378 RA controls) demonstrated this increased risk with more severe disease.[41] In this latter study, high disease activity (70-fold) and medium activity (7-fold) were associated with an increased risk when compared to low activity.[41]

In summary, increasing age and a longer duration of disease in patients with IBD have been reported to increase the risk of lymphoma in patients with IBD. Male gender may also increase the risk of lymphoma in patients with IBD. Although increased severity of disease has certainly been shown to be a risk factor for lymphoma in patients with RA, it has not been studied in patents with IBD and is a confounding factor when assessing treatment specific risk factors.

2.4 Treatment specific risk factors

Immunosuppression whether through diseases (primary immunodeficiencies) or through immunosuppressive treatment can be associated with an increased risk of lymphoma.[42,43] In the post-transplant setting, there is extensive literature demonstrating the increased risk of lymphoma with immunosuppression.[43] It would follow that immunosuppression in patients with IBD may indeed increase the risk of lymphoma.

Immunosuppressive medications

Thiopurine analogue immunosuppressive medications such as azathioprine and its metabolite 6-mercaptopurine (6-MP) are effective for the maintenance of remission of patients with IBD.[5,44,45] Endogenous purines are the building blocks of DNA and RNA and these cytotoxic medications limit inflammatory protein synthesis and lymphocyte proliferation.

Several hospital and cohort studies have assessed the risk of lymphoma in patients with IBD treated with immunosuppressive medications and have yielded conflicting results **(Table 2)**.[14,17,31,46-49] The largest population-based study, derived from the UK General Practice Research Database, assessed 1465 patients with IBD on either azathioprine or 6-MP and found no increased risk of lymphoma.[31] A meta-analysis utilizing data from six cohort studies (3891 patients) demonstrated a four-fold higher than expected pooled standardized incidence ratio (SIR 4.2, 95% CI 2.1-7.5) for the development of lymphoma.[17] More recently, a large French prospective observational cohort study confirmed

this degree of risk in patients with ongoing exposure to immunosuppressive medications and reported a multivariate adjusted hazard ratio of 5.28 (2.01-13.9) when comparing those exposed to immunosuppressive medications versus never exposed.[15] Interestingly in this latter study, the increased incidence of lymphoma in patients on immunosuppressive therapy was only seen in patients over the age of 50 (2.58 per 1000 patient-years in those aged 50-65 and 5.41 per 1000 patient-years in those older than 65). In patients under 50, the SIR was 0.37 per 1000 patient-years.

Overall, there is a demonstrated approximate four-fold increased risk of lymphoma in older patients with IBD on immunosuppressive medications, but the effects of disease duration and severity remain confounding factors. Given that patients with severe disease are treated more often with immunosuppressive medications, it is difficult to independently assess these risk factors in patients with IBD.

Epstein-Barr Virus

The link between immunosuppression and the development of lymphoma (post-transplant lymphoproliferative disorder) has been well established in solid organ transplant recipients, and is likely associated with the presence of EBV infection.[35,50-52] Various cases and case-series have described the potential association between the use of thiopurines in IBD and EBV-related lymphoma, with one case report describing regression of a lymphoma after discontinuation of azathioprine.[36,37,53,54] A Mayo Clinic case series identified 18 IBD patients

diagnosed with lymphoma, of whom 6 patients were on thiopurine medications. Five patients (83%) treated with thiopurines had EBV-positive lymphomas, whereas only 2 of 10 patients not on immunosuppressives were found to have an EBV-associated lymphoma.[53] Furthermore, in the French cohort of patients, 12 of 15 patients (80%) receiving immunosuppressive therapy at the time of lymphoma diagnosis were found to have an EBV-positive lymphoma.[15] Finally, in a more recent Dutch cohort of patients, 11 of 12 patients (92%) with EBV-positive lymphomas were on thiopurines, as compared to only 4 out of 21 patients (19%) with EBV-negative lymphoma.[55] These findings certainly suggest an intimate link between immunosuppression in IBD and lymphoma, possibly through a cytotoxic effect on activated T cell and NK cells resulting in a loss of EBV suppression.[24]

Anti-TNF-α medications

TNF-α is a pro-inflammatory cytokine that is elevated in patients with IBD. Treatment with tumor necrosis factor-alpha (TNF-α) monoclonal antibodies, such as infliximab, adalimumab and certolizumab pegol, is effective for inducing and maintaining remission in patients with IBD.[5-9] It is difficult to assess the risk of lymphoma associated with this class of medication as co-administration with immunosuppressive treatment frequently occurs, which has already been shown to increase risk.

The risk of lymphoma with anti-TNF-α has been more extensively researched in the rheumatoid arthritis literature. In data reported by Centocor

(the manufacturer of infliximab), the incidence rate of lymphoma in clinical trials of patients receiving infliximab for any indication (mainly RA) was 0.11 cases per 100 person-years, compared to 0 cases in the placebo groups.[56] This resulted in a standardized incidence ratio (SIR) of approximately 4, when this incidence rate was compared to the expected numbers derived from the US Surveillance Epidemiology and End Results (SEER) registry.[57] Similarly, in clinical and open-label trials of adalimumab (10 050 patients, 12 506 patient-years), the observed incidence in RA patients was 0.12 cases per 100 person-years of follow-up, resulting in a SIR of 3.19 (95% CI, 1.78-5.26) when compared to the SEER data.[58] In contrast, in a US RA database (National Data Bank for Rheumatic Diseases Database) with 13 001 RA patients, the risk of lymphoma was elevated compared to the general population with a SIR of 1.7 (95% CI, 1.3-2.3), but this risk was not associated with infliximab (OR=0.9, 95% CI, 0.4-2.1) or adalimumab (OR=1.3, 95% CI 0.2-10.0) and lends credence to the hypothesis that underlying disease activity in RA may in fact be a risk factor for the development of lymphoma.[24,59]

In a large observational study with greater than 18 000 RA patients in the US, the estimated lymphoma SIR for patients receiving infliximab was 2.6 (95% CI, 1.4-4.5).[60] A second observational study of about 1600 patients from Sweden assessed two cohorts of patients with RA.[61] The first cohort had exposure to anti-TNF-α therapy, while a second cohort was not exposed to biologic therapy and the rates of lymphoma were compared to expected rates of various malignancies. Again, an increased risk was identified in the anti-TNF-α

cohort of patients with a relative risk of 11.5 (95% CI, 3.7-26.9) compared to the expected rate in the population but this calculation was based on only a total of five cases in the cohort.[61] In addition, disease severity was not measured in these studies and as mentioned previously, this is certainly a confounding factor for the development of lymphoma in patients with RA.

Finally, a large meta-analysis of eleven randomized trials of infliximab and adalimumab reported a pooled odds ratio of 2.4 (95% CI, 1.2-4.8) for all malignancies. Although the specific odds ratio for lymphoma was not included, review of the results indicates than ten lymphomas were identified in the anti-TNF-α group but none were reported in the control group.[62,63] Given that disease severity should theoretically be evenly divided in a randomized control study, these results may suggest an increased risk with anti-TNF-α agents above and beyond disease severity.

Overall, multiple studies have shown that rheumatoid arthritis is associated with an increased risk of lymphoma, likely related to increased inflammatory disease activity. Anti-TNF-α medications may increase this risk, but separating disease activity from anti-TNF-α exposure is difficult given most patients with severe inflammatory disease will require these biologic medications. Inferring a similar risk in patients with IBD on anti-TNF-α medications is difficult given that, in contrast to RA, IBD in itself does not confer an increased risk of lymphoma. Another confounding factor that precludes drawing similarities between RA and IBD is that older age is a significant risk

factor for lymphoma and that, on average, IBD patients are significantly younger than patients with RA.

The literature regarding the risk of anti-TNF-α medications in IBD patients is unclear, and is also confounded not only by disease severity but also the concomitant use of immunosuppressive agents.[16,39,41,62,64-66] In a single tertiary care center experience, of 500 patients treated with infliximab (86% on concurrent immunosuppression) two patients (0.4%) developed lymphoma.[65] In a population-based study from Sweden where 212 patients (50% on concurrent immunosuppression) were assessed for adverse events for 28 months, three patients developed lymphoma.[66] This represented an annual incidence of 1.4%, which is much greater than the overall incidence rate of lymphoma in Sweden estimated to be 0.015%.[66]

In contrast, in a more recent single center study, 734 patients who received infliximab for the treatment of IBD over a fourteen-year period (3775 patient-years) were compared to 666 patients (6704 patient-years) who did not receive infliximab.[67] Most patients in both groups received immunosuppression (82% and 58% respectively).[67] Two cases of lymphoma were reported in the infliximab group and three cases were reported in the control group, indicating that there did not appear to be an increased risk of lymphoma.[67] As well, an assessment of an observational Crohn's Therapy, Resource Evaluation and Assessment Tool (TREAT) registry failed to demonstrate an increased risk of lymphoma.[64] The registry follows 6000 patients with CD, of whom approximately half have received infliximab, and contains 15 000 patient-years

of follow-up to date. The incidence of lymphoma was similar in both infliximab exposed and naïve patients (0.06 and 0.05 per 100 patient years respectively) resulting in a non-significant increase in risk (RR, 1.3, 95% CI, 0.4-5.0).[64] In addition, a meta-analysis of 21 placebo-controlled studies in 5356 patients with CD who received biologic medications did not demonstrate an increased risk of malignancy or lymphoma.[68] A second meta-analysis of 26 studies (placebo-controlled trials with biologic agents) with 8905 patients demonstrated an incidence of 6.1 per 10 000 patient-years in those patients exposed to biologic agents.[16] Compared to the general population SEER registry, the SIR was 3.23 (95% CI, 1,5-6.9).[16] The majority of patients were on concurrent immunosuppression (66%) and when compared to the incidence of lymphoma with immunosuppression alone (4 per 10 000 patient years from the Kandiel et al. meta-analysis) the SIR was elevated at 1.7, but it was not statistically significant (95% CI, 0.5-7.1).[16,17] One of the main issues associated with the assessment of adverse events using a meta-analysis of clinical trials is that the follow-up in these patients is relatively short (usually not greater than 1 year) and rare events such as malignancy may not be captured.[69] In addition, the selected population of a clinical trial, which is usually less than 65 years old and with no co-morbid disease, may underestimate the risk of malignancy.[24]

With the available data, a true estimation of the lymphoma risk associated with anti-TNF-α in patients with IBD is difficult. Whether the increased risk described above in selected studies is secondary to anti-TNF-α medications specifically, or related to disease severity or concurrent

immunosuppression, is difficult to determine. In the clinical setting, anti-TNF-α medications are increasingly prescribed in conjunction with immunosuppressive medications, so the exact risk with sole biologic medications may not be as clinically relevant. With the limited and confounded data available, there may not be a significantly increased risk in those patients who are on combined treatment but more research is needed to clearly elucidate the risk.

In summary, a diagnosis of IBD does not specifically increase the risk of lymphoma, but the specific risk with various demographic and clinical factors are somewhat less clear. Exposure to immunosuppressive medication likely increases the risk of lymphoma, but the risk with anti-TNF-α agents is less clear and needs further investigation. With this backdrop of information, using a case-control methodology, we attempted to identify independent demographic and clinical factors that were associated with lymphoma in patients with IBD. Given the low absolute incidence of lymphoma, a case-control study is the most cost-effective strategy for identifying possible risk factors. Using this study design, we will not only be able to control for known risk factors, but using multiple variable analysis, we will be able to adjust for various demographic and clinic risk factors.

3. METHODS

Cases

The Mayo Clinic Rochester diagnostic index was searched for patients diagnosed with UC or CD and cross-referenced against a list of patients diagnosed with various LPD (using the terms lymphoma, lymphoproliferative and hemophagocytic syndrome). Additional databases consisting of the Mayo Clinic Rochester pathology tissue registry and the Lymphoma Registry, in the Division of Hematology, were searched and cross-referenced to ensure all lymphoma cases were identified from the IBD reference database. Patients who had undergone liver transplantation for primary sclerosing cholangitis and any patients who had received immunosuppressive therapy for an indication other than IBD were excluded from the study.

The IBD diagnosis was confirmed by reviewing the patient's medical history, radiology and pathology, as well as ensuring the Mayo physician agreed with and documented the diagnosis. The lymphoma diagnosis was confirmed if there was tissue confirmation of the diagnosis by a Mayo pathologist. Cases were defined as having both a confirmed IBD and lymphoma diagnosis. In addition, in order to more clearly assess the role of various IBD clinical factors in the development of lymphoma, the initial diagnosis of IBD must have occurred at least 90 days prior to the diagnosis of lymphoma. Cases in which the confirmed Mayo IBD diagnosis was made less than 90 days prior to the diagnosis of lymphoma, but the initial IBD diagnosis (at another institution) was

documented by the Mayo physician as being greater than 90 days prior to lymphoma diagnosis, were included in the study (26 cases).

The study period ranged from January 1980 to December 2009. All patients included in the study provided authorization for medical record review for research purposes, and the study was reviewed and approved by the Mayo Clinic Institutional Review Board.

Matched Controls

Two matched controls that did not have a confirmed diagnosis of lymphoma were selected for each case. Controls required an initial Mayo Clinic diagnostic visit for IBD within approximately five calendar years of the case. Controls were matched to cases on subtype of IBD (CD or UC), geographic area of residence (Olmsted County; six surrounding counties; balance of Minnesota; five-state area including Wisconsin, Illinois, Iowa, South Dakota, and North Dakota; and elsewhere), and finally on the length of IBD follow-up at Mayo Clinic (interval between the confirmed Mayo IBD diagnosis and the diagnosis of the lymphoma). This last matching criterion was selected, as opposed to the total duration of IBD (from initial IBD diagnosis to lymphoma diagnosis), to avoid information bias regarding medication exposure. Although total duration of IBD may be associated with an increased risk of lymphoma and the matching criteria only partially controls for this factor, the potential for bias using simple disease duration may have invalidated any association between

medication exposure and lymphoma (one of the primary associations we wished to assess).

In one instance, only one control could be identified for the case (unable to match within five calendar years and inadequate length of follow-up). In 7 instances, controls with a similar length of Mayo Clinic follow-up could not be identified, and controls with the longest available follow-up were instead selected. Matching according to type of IBD and geographic location was exact.

Data Abstraction

Medical records were abstracted for complete demographic and clinical information. For each case, clinical data were abstracted from the first Mayo Clinic visit for IBD though the date of diagnosis of the lymphoma. For each of the two matched controls, clinical data were abstracted from the first Mayo Clinic visit for IBD until the earliest clinical visit (index date) that provided an equal interval to length of follow-up for the corresponding matched case. The following demographic and clinical factors were abstracted retrospectively for both cases and controls: age, gender, smoking status (current, previous or never), duration, extent, and severity of disease (remission, mild-moderate or severe-fulminant) as per previously published clinical criteria.[70,71] Additional factors abstracted were concomitant medications (current, previous or no exposure), and dose, duration and type of medication: oral 5-aminosalicyates (ASA), oral corticosteroids (defined as ≥ 20 mg/day), immunosuppressive therapy (AZA, 6-MP or methotrexate), or anti-TNF-α therapy (infliximab or

adalimumab). Medication exposure was carefully abstracted in both cases and controls and all available documentation from the medical record was assessed. In addition, for cases, the details regarding the lymphoma and the results of Epstein-Barr virus (EBV) immunostaining (if available) were abstracted from the chart.

Statistical Analysis

It was estimated that with 100 cases and 2 controls per case, there would be approximately 80% power to detect an odds ratio of greater than 2.5, assuming the medication exposure occurred in at least 20% of the controls. Only 80 cases were identified, and as such the power to detect an odds ratio of 2.5 or greater was 73% (or 80% for an odds ratio of 2.7). The descriptive summary statistics are reported for the overall set of cases and controls and do not reflect the matched-pair nature of the data.

The associations of case-control status with individual demographic, disease-specific and medication-related factors (listed above) were assessed using univariate conditional logistic regression. Results are reported in the form of odds ratios (OR) with 95% confidence intervals (CI). Multiple variable conditional logistic regression models were also examined including anticipated clinically important and/or statistically significant factors from the univariate analyses in several postulated models. Two *a priori* specified sensitivity analyses were completed. The first excluded 26 cases whose Mayo IBD diagnosis was less than 90 days prior to the lymphoma diagnosis and the

second excluded the 12 patients that underwent remote proctocolectomy for UC but subsequently developed lymphoma.

4. RESULTS

Demographic and Clinical Characteristics of Cases and Controls

Eighty patients with a confirmed diagnosis of IBD and lymphoma were identified during the time period between January 1980 and December 2009. Cases were fairly evenly divided by subtype of IBD, with 44 cases (55%) diagnosed with CD and the remaining 36 cases (45%) diagnosed with UC. The median interval between initial IBD diagnosis and lymphoma diagnosis was 13.5 years (interquartile range [IQR], 4.8-26.1 years), whereas the median duration from the first Mayo IBD diagnosis and lymphoma diagnosis was 3.5 years (IQR, 8 days-12.8 years). The predominant lymphoma subtype, non-Hodgkin's lymphoma, was seen in 69 cases (86%). Seven cases (9%) were classified as Hodgkin's lymphoma, and two cases each (2.5%) were classified as hemophagocytic syndrome (HPS) and as plasmacytoma. Twenty-four cases (30%) had primary intestinal involvement of the lymphoma. Baseline demographic and clinical characteristics of the cases and controls are shown in **Table 3**.

Risk Factors for Lymphoma

In the univariate analyses, age (per decade) (OR, 1.72; 95% CI, 1.38-2.14) and male gender (OR, 3.15; 95% CI, 1.71-5.80) were significantly associated with increased odds for lymphoma. Current exposure to immunosuppressive medications was also significantly associated with an increased odds of lymphoma (OR, 2.33; 95% CI, 1.08-5.05). Current exposure

to anti-TNF-α agents was not significantly associated with an increased odds for lymphoma. Current exposure to 5-ASA medications was significantly associated with a reduced odds for lymphoma (OR, 0.50; 95% CI, 0.26-0.93). Severity of IBD, cigarette smoking and family history of lymphoma were not significantly associated with the development of lymphoma (**Table 4**).

In multiple variable analyses, age (per decade) (OR, 1.83; 95% CI, 1.37-2.43) and male gender (OR, 4.05; 95% CI, 1.82-9.02) were strongly associated with the development of lymphoma (**Table 5**). The association with current exposure to immunosuppressive medications was stronger and remained significant (OR, 4.20; 95% CI, 1.35-13.11). Current exposure to 5-ASA medications was no longer significantly associated with lymphoma in multiple variable model, and the association between exposure to anti-TNF-α medications and lymphoma remained non-significant. Current or previous exposure to cigarette smoking was inversely associated with lymphoma development (OR, 0.43; 95% CI, 0.20-0.92).

As mentioned previously, two *a priori* sensitivity analyses were performed to evaluate the validity of the above results. In the first analysis, we excluded 26 cases in which the confirmed Mayo IBD diagnosis was made less than 90 days prior to the diagnosis of lymphoma. The results were similar when these patients were excluded and age, male gender and exposure to immunosuppressive medications remained significantly associated with lymphoma in univariate analyses. In multiple variable analyses, the point estimates for these variables were similar and remained significant (**Table 6**). In

the second *a priori* sensitivity analysis, we excluded 12 patients who had undergone remote proctocolectomy for UC and subsequently developed lymphoma. These patients had no exposure to immunosuppressive or biologic medications after surgical management. Again, age, male gender and current immunosuppressive use remained significantly associated with lymphoma (**Table 6**).

EBV Status

Out of the 80 patients we identified with IBD and lymphoma, 20 patients (25%) were exposed to immunosuppressive medications at lymphoma diagnosis, whereas 4 patients (5%) were exposed to anti-TNF-α medications. Twelve out of the 20 patients who developed lymphoma on immnosuppressive therapy had undergone EBV testing and 9 (75%) were found to have an EBV-positive lymphoma. Out of the 4 cases exposed to anti-TNF-α medications, 3 had undergone EBV testing and all were found to have an EBV-positive lymphoma. Both patients on combination therapy underwent EBV testing and were found to have an EBV-positive lymphoma (see **Table 7**).

5. DISCUSSION

It is clear that certain subgroups of patients with IBD have an increased risk of developing lymphoma.[69] Using the Mayo Clinic diagnostic index we were able to identify 80 patients with IBD who subsequently developed lymphoma, the largest case-control study to date. We assessed the association of various demographic, disease-specific, and medication related factors in IBD patients who develop lymphoma.

Both increasing age and male gender were demographic factors that were significantly associated with higher odds of developing lymphoma. Although increasing age and male gender are well known risk factors for the development of lymphoma in the general population, our results clearly indicate that there is an increased risk in the IBD population, which we were able to specifically quantify. In multivariate analysis, age (per decade) resulted in an OR of approximately 2; stated another way, the odds of developing lymphoma in the setting of IBD doubles every 10 years. In addition, male gender was associated with an increased odds of greater than 4. In older, male patients, these increased odds of developing lymphoma should be carefully weighed when prescribing immunosuppressive medications, which have their own inherent risk.

With regards to clinical factors, disease severity in patients with IBD has not been thoroughly assessed as a risk factor for the development of lymphoma. In the RA literature, there seems to be a strong association between disease activity and the risk of lymphoma.[7,8] In our study increased disease severity,

when comparing mild-moderate or severe-fulminant disease to those patients in remission, was not associated with an increased odds of developing lymphoma.

The potential risk of lymphoma associated with exposure to immunosuppressive or biologic medications is a topic of great concern to both patients and clinicians, given the exponential increase in their use in IBD patients over the last decade. Given previous studies have indicated that ongoing medication exposure (and not previous exposure) is associated with an increased risk of lymphoma, we compared current exposure to previous or no exposure.[15] In this study, there was an approximate 4-fold increase in the odds of developing lymphoma in patients with IBD. This increased to 10-fold when patients with remote proctocolectomy and no post-surgical exposure to immunosuppressive medications were excluded from the analysis. These odds ratios are similar in range to the SIR reported in other studies (the odds ratio approximates the relative risk when the event in question is rare). There is conflicting data regarding the risk associated with anti-TNF-α exposure in IBD patients. In our study, exposure to anti-TNF-α medications was not associated with an increased odds of lymphoma in patients with IBD. Although these data regarding biologic medications are somewhat reassuring, given the limited exposure to these agents in this cohort of patients, no definite conclusions regarding the safety of these medications can be made. Finally, in terms of EBV as a risk factor for lymphoma, our study showed that when assessed, a significant proportion of patients (75% to 100%) had an EBV-positive lymphoma

on immunosuppressive or biologic therapy. These findings are remarkably similar to previously published studies detailed previously. [15,53,55]

There are several potential limitations to our study. The study is retrospective in nature, and various exposures could be subject to misclassification or information bias. Severity of disease was divided into broad categories (remission, mild-moderate and severe-fulminant) to ease with accurate classification and minimize this bias. With regards to medication exposure, we controlled for the length of Mayo Clinic follow-up as to minimize possible information bias by having more detailed medication exposure for cases as opposed to controls.

As we partially controlled for duration of disease (length of Mayo Clinic follow-up), we were unable to assess this variable as a risk factor. In fact, in the French cohort of patients, a mildly increased risk of lymphoma with an increased duration of disease was demonstrated (hazard ratio of 1.04 per year of IBD, 95% CI: 1.00-1.08).[15] Given that this association appears to be quite weak, it is unlikely that the overall results of this study would have been affected had we fully controlled for the length of disease. In addition, we did not specifically assess the duration of treatment exposure. In fact, literature from the post-transplant population and from the French cohort study demonstrate that the risk does not seem to increase over time.[15]

As with any case-control study, we can only calculate odds ratios, which are not as clinically intuitive or useful as standardized incidence ratios or relative risks, but in cases where the absolute incidence is low (IBD and lymphoma), the

odds ratio can approximate the relative risk. Lastly, given the limited number of patients exposed to anti-TNF-α or combination therapy, we cannot conclude with certainty that there is no risk with these types of treatment. In fact, the risk with anti-TNF-α or combination therapy may be similar to that seen with immunosuppressive therapy.[15] If the sample size were greater, testing for effect modification may have been useful to further clarify the risk of lymphoma with anti-TNF-α medications.

6. CONCLUSIONS

In summary, our study supports previously published literature on the risk factors associated with lymphoma in patients with IBD. It is important to note that the majority of IBD patients who developed lymphoma in this study had no current exposure to immunosuppressive or anti-TNF-α medications. In fact, the actual absolute incidence of lymphoma in patients with IBD is quite low and ranges from 0.2 to 0.9 per 1000 patients.[15-17] Increasing age, male gender and ongoing exposure to immunosuppressive medications result in increased odds of developing lymphoma, whereas severity of disease was not associated with an increased risk. The risk and benefit of immunosuppressive medications should be carefully weighed prior to initiation, particularly in older, male patients. EBV certainly plays an important role in the development of lymphoma in IBD patients, but further prospective studies need to be completed to better elucidate this risk.

7. TABLES

Table 1: Overall risk of lymphoma in patients with IBD (population-based studies)

Reference	Setting	Patients	SIR (95% CI)
Ekbom et al.[26]	Uppsala, Sweden	CD: 1655	0.4 (0-2.4)
		UC: 3121	1.2 (0.5-2.4)
Persson et al.[27]	Stockholm, Sweden	CD: 1251	1.4 (0.4-3.5)
Karlen et al.[28]	Stockholm, Sweden	UC: 1547	1.2 (0.3-3.5)
Loftus et al.[29]	Olmstead County, USA	CD: 216	2.4 (0.1-13)
		UC: 238	0 (0-6.4)
Palli et al.[30]	Florence, Italy	CD: 231	2.5 (0.3-9)
		UC: 689	1.8 (0.2-6.5)*
Bernstein et al.[31]	Manitoba, Canada	CD: 2857	2.4 (1.2-5)
		UC: 2672	1.0 (0.5-2.2)
Lewis et al.[32]	United Kindom (UK)	CD: 6605	1.4 (0.5-3.4)
		UC: 10391	1.2 (0.7-2.1)
Winther et al.[33]	Denmark	UC: 1160	0.5 (0.1-1.8)
Jess et al.[34]	Denmark	CD: 374	None observed
Askling et al.[35]	Multiple Swedish	CD: 20120	1.3 (1.0-1.6)
	cohorts	UC: 27559	1.0 (0.8-1.3)

* for non-Hodgkin lymphoma only

Table 2: Risk of lymphoma in patients on immunosuppressive medications

Reference	Setting	Number of pts	Observed cases	Expected cases	SIR (95% CI)
Kinlen	UK (SC)	321	2	0.16	12.5 (1.2-46)
Connell et al.	London (SC)	755	0	0.52	0
Korelitz et al.	New York City, USA (SC)	486	3	0.61	4.9 (0.9-14.5)
Farrell et al.	Dublin, Ireland (SC)	238	2	0.05	37.5 (3.5-138)
Lewis et al.	GPRD, UK (PB)	1465	2	0.64	1.6 (0.001-9)
Fraser et al.	Oxford, UK(SC)	626	3	0.65	4.6 (0.9-13.7)
Kandiel et al.	Pooled meta-analysis	3891	11	2.63	4.2 (2.1-7.5)
Beaugerie et al.	France (PB)	19486	15	2.19	6.86 (3.8-11.31)

(SC): Single center study, (PB): Population based study

Table 3: Baseline characteristics of lymphoma cases and controls

Variables	Odds Ratio	95% CI	p-value
Age (per decade)	1.72	1.38 - 2.14	<0.001
Male gender	3.15	1.71 - 5.80	<0.001
Severity (vs. remission)			
Mild/moderate	0.97	0.50 - 1.89	0.93
Severe/fulminant	0.58	0.25 - 1.24	0.15
Smoking (vs. never)			
Current/previous	0.67	0.38 - 1.19	0.17
Family history of lymphoma	7.12	0.79 - 64.38	0.08
Current medications at index date (vs. previous or no exposure)			
5-ASA	0.49	0.26 - 0.93	0.03
Corticosteroids	0.93	0.47 – 1.81	0.82
Immunosuppressive	2.33	1.08 - 5.05	0.03
Anti-TNF-α	1.38	0.36 - 5.29	0.64

Table 4: Univariate assessment of risk factors for lymphoma

Variables	Odds Ratio	95% CI	p-value
Age (per decade)	1.72	1.38 - 2.14	<0.001
Male gender	3.15	1.71 - 5.80	<0.001
Severity (vs. remission)			
Mild/moderate	0.97	0.50 - 1.89	0.93
Severe/fulminant	0.58	0.25 - 1.24	0.15
Smoking (vs. never)			
Current/previous	0.67	0.38 - 1.19	0.17
Family history of lymphoma	7.12	0.79 - 64.38	0.08
Current medications at index date (vs. previous or no exposure)			
5-ASA	0.49	0.26 - 0.93	0.03
Corticosteroids	0.93	0.47 – 1.81	0.82
Immunosuppressive	2.33	1.08 - 5.05	0.03
Anti-TNF-α	1.38	0.36 - 5.29	0.64

Table 5: Multiple variable assessment of risk factors for lymphoma

Variables	Odds Ratio	95% CI	p-value
Age (per decade)	1.83	1.37 – 2.43	<0.001
Gender			
Female	1.0	reference	
Male	4.05	1.82 – 9.02	<0.001
Severity (vs. remission)			0.67 (overall)
Remission	1.0	reference	
Mild/moderate	0.71	0.29 – 1.73	0.45
Severe/fulminant	0.64	0.22 – 1.88	0.42
Smoking (vs. never)			
Never	1.0	reference	
Current/Previous	0.43	0.20 – 0.92	0.03
Medication use at index date			
Use of 5-ASA			
No or past use	1.0	reference	
Currently used	0.55	0.25 – 1.23	0.14
Use of Immunosuppressive			
No or past use	1.0	reference	
Currently used	4.20	1.35 – 13.11	0.01
Use of Anti-TNF-α			
No or past use	1.0	reference	
Currently used	2.04	0.32 – 12.79	0.45

Table 6: Sensitivity analyses, multiple variable assessments of risk factors for lymphoma

	Excluding Mayo IBD and lymphoma (< 90 days)		Excluding remote proctocolectomy patients	
Age (per decade)	1.88	1.34 – 2.63	1.76	1.30 – 2.38
Gender				
Female	1.0	reference	1.0	reference
Male	3.56	1.25 – 10.10	4.82	1.87 – 12.42
Severity (vs. remission)				
Remission	1.0	reference	1.0	reference
Mild/moderate	0.98	0.35 – 2.77	1.05	0.41 – 2.72
Severe/fulminant	2.02	0.44 – 9.26	0.82	0.27 – 2.55
Smoking (vs. never)				
Never	1.0	reference	1.0	reference
Current/Previous	0.60	0.23 – 1.60	0.46	0.20 – 1.08
Medication use at index date				
Use of 5-ASA				
No or past use	1.0	reference	1.0	reference
Currently used	0.62	0.21 – 1.80	0.77	0.33 – 1.82
Use of Immunosuppressive				
No or past use	1.0	reference	1.0	reference
Currently used	9.90	1.97 – 49.80	5.25	1.57 – 17.49
Use of Anti-TNF-α				
No or past use	1.0	reference	1.0	reference
Currently used	0.06	<0.01 – 2.17	4.45	0.59 – 33.68

Table 7: EBV status of patients with lymphoma on immunosuppressive or biologic therapy

Concurrent therapy	N (%)
Immunosuppressive (n=20)	
EBV positive	9 (45)
EBV negative	3 (15)
EBV unknown	8 (40)
Anti-TNF-α (n=4)	
EBV positive	3 (75)
EBV unknown	1 (25)
Combination therapy (n=2)	
EBV positive	2 (100)

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