Malignancies in inflammatory bowel diseases

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General introduction

General introduction

Inflammatory bowel diseases (IBD) include Crohn’s disease (CD) and ulcerative colitis (UC). These diseases are characterized by chronic recurrent intestinal inflammation that is limited to the colon in UC but may affect any segment of the gastrointestinal tract in the case of CD. In the Netherlands there are currently more than 90,000 IBD patients. IBD has typically a young onset and follows an unpredictable course with periods of remission and relapses. Symptoms vary according to the disease location and may consist of abdominal pain, diarrhoea, bloody stools, weight loss and fatigue.

The medical treatment of IBD aims to induce and maintain prolonged remission and to prevent complications. In order to reach these treatment goals several therapies are available, such as 5-aminosalicylates (5-ASA), thiopurines, methotrexate, TNF alpha inhibitors (anti-TNF agents) and the recently introduced integrin and interleukin IL12/IL23 inhibitors. The expansion of the arsenal of IBD drugs has not resulted in a cure for IBD (yet). As a result, patients are chronically treated with a variety of immunosuppressive medications, during long periods of time in order to prevent long-term complications. Unfortunately, current treatment strategies are not fully sufficient to prevent complications and surgery in IBD patients.

Malignancies in IBD

One of the most serious complications of IBD is cancer development. For example, continuous chronic inflammation increases the risk of colorectal cancer (CRC). This will be discussed further below. In addition, the use of long-term immunosuppressive medication increases the risk of extra-intestinal malignancies.
Drug induced carcinogenesis
Thiopurines and anti-TNF agents have distinct mechanisms of action that may explain their dissimilar role as risk factors for cancer. Anti-TNF agents (infliximab, adalimumab, and golimumab) may increase the risk of cancer through two mechanisms: impaired immunosurveillance and the inhibition of tumor cell necrosis. Nevertheless, anti-TNF use in IBD is only linked to an increased risk of melanoma development, and no overall increased risk of cancer has been established. However, it is difficult to separate the effect of anti TNF therapy from thiopurines, since most IBD patients will be exposed to both drugs.

Thiopurines (azathioprine, 6-mercaptopurine and 6-thioguanine) increase the overall risk of developing cancer in IBD patients (rate ratio 1.41; 95% confidence interval: 1.15 - 1.74). Thiopurines can induce carcinogenesis through 3 different pathways. Firstly, they can induce carcinogenic DNA mutations. Secondly, they impair immunosurveillance of (pre)cancerous cells and thirdly, they reduce the activity of T lymphocytes that prevent chronically virally infected cells (Epstein-Barr virus (EBV) or human papillomavirus (HPV)) to proliferate. The latter is especially relevant for the development of lymphoid malignancies, as well as virus-associated solid tumors, such as squamous cell carcinomas of the cervix and the head and neck area.

Lymphoproliferative disorders
Population-based studies show no or only a minimal increase in the risk of lymphoma development in the general IBD population, however, there is an increased risk associated with thiopurine therapy (SIR 5.7, 95% CI, 3.2 – 10.1). Three types of thiopurine-related non-Hodgkin lymphomas are reported. Two are EBV-related: post-transplant like lymphomas and post-mononucleosis lymphomas. The third type is rare. Hepatosplenic T-cell lymphoma has mainly been reported in male IBD patients younger than 35 years-of-age treated with thiopurine and anti-TNF combination therapy.

Two factors are important in IBD-related lymphoproliferations. Firstly, the importance of local IBD-related inflammation is illustrated by the excess of intestinal lymphoproliferations. Secondly, the decreased immunosurveillance of EBV as evidenced by a high rate of EBV-replication found in IBD lymphomas. These factors show an interesting interplay as EBV is identified in the majority of the intestinal lymphoproliferations suggesting that local inflammation promotes EBV replication.

EBV in lymphoproliferation
An acute EBV infection causes a polyclonal expansion of B lymphocytes containing EBV. In immunocompetent individuals, antigens expressed by the infected B lymphocytes provoke a T lymphocyte immune reaction that eliminates almost all infected B lymphocytes. However, some infected B lymphocytes escape surveillance due to downregulation of antigen expression and this causes a lifelong latent EBV infection of B lymphocytes. Any mucosal biopsy will contain B lymphocytes and therefore may harbour amplifiable EBV DNA.

In thiopurine-induced EBV reactivation, the infected B cell restarts replication to create more infected B cells and ultimately will produce thousands of virions. In case of malignant transformation, neoplastic daughter cells possess the identical viral episomal structure, making the EBV genome a marker for clonality analyses.

Post-transplant like lymphoproliferations
The post-transplant like lymphomas account for most thiopurine-related lymphomas in IBD and are histologically comparable to post-transplant lymphoproliferative disorders (PTLD). PTLD are uncommon B-cell lymphoproliferations, with high mortality when left untreated.

The World Health Organization (WHO) histological classification of PTLDs describes the spectrum of lymphoproliferative
Solid malignancies

Colorectal malignancies

The most frequent malignancy in IBD is directly related to chronic inflammation: CRC. The risk of developing CRC in IBD is increased 1.5 to 2 times compared to that of the general population, with a linear increase over time. Recently, a decrease in colitis associated CRC was observed, supposedly due to better disease control by improved IBD management and more adequate endoscopic surveillance.

There are specific IBD related risk factors for the development of CRC. These factors include chronicity and severity of inflammation, extensive disease, presence of pseudopolyps and strictures, history of colonic dysplasia and co-existing primary sclerosing cholangitis.

Pathogenesis and outcomes

The pathogenesis of colitis-associated CRC resembles the pathogenesis of sporadic CRC, although the timing and frequency of molecular changes are different. Chronic inflammation may cause oxidative stress–induced DNA damage. This results in the activation of oncogenes and the silencing of tumor-suppressor pathways, leading to an accelerated dysplasia-carcinoma sequence. Furthermore, an altered microbiome may contribute to colitis-associated cancer by perpetuating chronic inflammation and/or producing carcinogenic factors.

CRC patients with IBD are younger at cancer diagnosis, more likely to have multiple neoplastic lesions and have an impaired survival compared to sporadic CRC patients.

Surveillance

The increased risk and impaired outcomes of colitis-associated CRC warrant endoscopic colonic surveillance to detect and treat (pre)cancerous lesions. Subsequent subtotal colectomy is an important treatment option in case complete endoscopic treatment is not possible. Although subtotal colectomy reduces the CRC risk, neoplasia of the residual colonic mucosa may still arise. Unfortunately, specific surveillance guidelines for these post-surgical IBD patients are lacking at this time. Further data regarding risk factors, incidence and prevalence of CRC following subtotal colectomy are needed to further develop these guidelines.

Gastric cancer

The incidence of gastric cancer (GC) is declining in the Western world. This may be partially due to the treatment of the carcinogenic Helicobacter pylori (H. pylori). Other risk factors for GC include EBV infection, pernicious anemia, gastric surgery and familial predisposition. No IBD specific risk factors are known, but an increased risk for GC development has been reported for CD patients with a pooled SIR of 2.05 (95% CI 1.06 – 3.97).

GC is classified by Lauren in diffuse and intestinal GC. Two entities with different etiologies, pathogenesis and behavior, but both related to H. pylori. The morphologic differences are attributable to intercellular adhesion molecules, which are preserved in intestinal-type tumors and defective in diffuse types.

The pathogenesis of intestinal GC is poorly understood. However, it follows a multistep progression that is usually initiated by H. pylori infection and follows a well-characterized sequence:
needed to guide clinical decision making.

**Oral cavity and pharyngeal cancer**

In immunosuppressed transplant patients, non-cutaneous head and neck cancer (HNC) incidence is higher compared to the general population. In IBD, data are limited and conflicting. Danish historical cohorts report no increased risk for lip, oral cavity and pharyngeal cancer, while more recent US data show a standardized incidence ratio (SIR) for oral cancer of 9.77 (95% confidence interval (CI), 5.14–16.98).

The main risk factors for HNC are tobacco use, alcohol consumption and HPV infection. HPV associated HNC primarily occur in the (oro)pharynx: the tonsils and tongue base. These cancers generally affect younger patients without a history of excessive alcohol and tobacco use and account for more than half of the oropharyngeal cancers. HPV positive cancers tend to present with regional lymph node metastases and smaller primary tumors and are associated with a better prognosis than HPV negative tumors. In HPV negative squamous cell carcinomas, p53 mutations are very frequent, along with decreased p16 levels. By contrast, HPV positive carcinomas are associated with wild-type p53 and upregulation of p16.

HNC survival is impaired in immunosuppressed transplant patients compared to the general population. In IBD, currently no studies on HPV-involvement, the role of immunosuppression and disease course of HNC are available.

**Management of IBD patients with a present or past malignancy**

The increasing life expectancy and rising IBD prevalence provide new challenges. Due to the aging IBD population, a growing number of IBD patients will develop and may be cured of cancer, re-
This thesis

This thesis is limited to rare malignancies in IBD, which may be related to IBD due to the presence of IBD related inflammation and / or to impaired immunosurveillance due to immunosuppression use.

Although rare, malignant complications show a rising incidence and may occur in all IBD patients. Due to the growing and aging IBD population this will become increasingly important in clinical practice. Balancing the effects and risks of IBD treatment with optimal cancer treatment is very important to achieve the best IBD and cancer outcomes. Therefore, additional information is needed regarding IBD specific risk factors, incidence, clinical course and influence of IBD medical therapy on different malignancies.

Chapter 2 of this thesis will focus on lymphoproliferations, whereas chapter 3 focuses on solid malignancies. Chapter 4 contains the general discussion and future perspectives.

Aims

Lymphoproliferative disorders (chapter 2)
- To assess the utility of histological features in predicting EBV presence in colonic mucosa (chapter 2.1).
- To correlate histopathological assessment (including EBV load and immunoglobulin clonality) with clinical outcomes in IBD patients (chapter 2.1 and 2.2).

Solid malignancies (chapter 3)
- To identify IBD-specific risk factors regarding melanoma, gastric, pharyngeal and oral cavity cancer and colorectal neoplasia following subtotal colectomy (chapter 3.1 to 3.4).
- To compare clinical characteristics, immunosuppression and survival after melanoma, gastric, pharyngeal and oral cavity cancer in IBD patients to unselected ‘non-IBD’ patients with those cancers (chapter 3.1 to 3.3).

Study design

To address these aims we used the following study designs.

Lymphoproliferative disorders (chapter 2)
I: retrospective single-centre cohort study
To assess histologic features and correlate these with EBV load and clinical outcomes, we searched our local pathology database and included all IBD patients who underwent EBV testing in in-
testinal biopsies. All biopsies were classified according to the WHO PTLD classification and the EBV load was scored. For EBV detection we used the golden standard: EBV-encoded RNA in situ hybridization. EBV positive cases were divided into a low and high EBV concentration group. Histological classification was correlated with the EBV concentration and clinical data. Clinical data were collected from patient charts. Reported clinical outcomes included colectomy, need for chemotherapy and mortality.

II: Retrospective multi-centre cohort study
To study the clinical implications of IG-clonality testing, we performed a retrospective analysis in a large multicentre cohort using the EuroClonality database. Both PTLD and IBD related PTLD-like patients were included. IG-gene clonality testing and molecular characterization were performed by the assessment of the IGHV(D)J, -DJ as well as IGK rearrangements. Interpretation of the clonality findings was performed according to the EuroClonality guidelines. Patients were classified EBV-LPD according to the WHO criteria. Clinical data were collected from patient charts and the EuroClonality database. Multivariable analysis was performed to indentify clinical and histological risk factors for poor disease outcome.

Solid malignancies (chapter 3)

III: retrospective case control studies
To identify IBD-specific risk factors and to compare clinical characteristics, we created three nationwide cohorts of IBD patients with a specific malignancy by using PALGA. PALGA is the Dutch nationwide network and registry of histo- and cytopathology. This registry contains pathology reports generated in the Netherlands since 1971 and has complete national coverage since 1991 encompassing all pathology laboratories from all academic and non-academic hospitals in the Netherlands. We used each cohort of specific cancer types and IBD cases for two case control studies: 1: to assess risk factors for cancer development in IBD we compared IBD cases with a specific malignancy to IBD controls without, derived from the IBD South Limburg cohort. 2: to compare clinical characteristics and survival we compared the IBD cases to non-IBD controls with the same specific malignancy. The non-IBD controls were derived from the Eindhoven Cancer Registry (http://www.eindhovencancerregistry.nl), which is part of the Dutch Cancer Registry (IKNL).

IV: Systematic review
To determine risk factors and assess incidence and prevalence of neoplasia in IBD patients following subtotal colectomy, we performed a systematic review and meta-analysis. We reviewed observational studies to collect the available data. Subsequently we determined a pooled incidence and prevalence of CRC in three subgroups: A: patients with a rectal stump, B: patients with an ileorectal anastomosis (IRA) and C: patients with an ileal pouch – anal anastomosis. Finally we determined the risk factors for developing post-colectomy neoplasmia in a pooled model. These data are a solid basis to provide recommendations for endoscopic surveillance in IBD patients after subtotal colectomy.
References


Lymphoproliferations in inflammatory bowel diseases
2.1

Epstein-Barr Virus in Inflammatory Bowel Disease: the spectrum of intestinal lymphoproliferative disorders

Journal of Crohn’s and Colitis, 2015 May; 9 (5): 398 – 403

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Abstract

Background
Inflammatory bowel disease (IBD) patients on thiopurine therapy are at increased risk of Epstein-Barr virus (EBV) associated lymphomas. This virus is frequently detected in the intestinal mucosa of IBD patients and may cause a wide spectrum of lymphoproliferations similar to post-transplantation lymphoproliferative disorders (PTLDs). We aimed to assess whether histological aberrations aid in predicting EBV presence and to correlate histological assessment and EBV load with disease outcome in IBD.

Methods
We included all IBD patients from our centre who underwent EBV testing of intestinal biopsies between January 2004 and October 2013. All biopsies were classified according to the WHO PTLD classification and the EBV load was scored per high-power field (HPF). Clinical data were collected from patient charts. Reported clinical outcomes included colectomy, need for chemotherapy and mortality.

Results
Our cohort included 58 patients: 28 EBV-positive and 30 EBV-negative. An atypical infiltrate was seen more frequently in EBV-positive than in EBV-negative patients (57.1 versus 3.3%; p < 0.001). A high EBV load occurred more frequently in EBV-positive patients undergoing colectomy than in EBV-positive patients without colectomy (50.0 versus 10.0%; p = 0.048). Monomorphic lymphoproliferative disorders, including two overt lymphomas, were present in 10 patients. Reduction of immunosuppression resulted in histological normalization and loss of EBV expression in seven of eight non-lymphoma patients.

Conclusion
The presence of atypical infiltrate in the intestinal mucosa of IBD patients warrants EBV testing. Reduction of immunosuppression is an effective strategy to achieve morphological normalization and loss of EBV. Lymphoproliferation related to IBD appears to have less aggressive clinical behaviour than PTLDs.

Key words:
Inflammatory bowel diseases, Epstein-Barr virus, lymphoma
1. Introduction

The use of immunosuppressive therapy is associated with an increased risk of lymphoproliferation in patients with inflammatory bowel disease (IBD)\(^1\),\(^2\) as well as in post-transplantation patients\(^2\). Post-transplantation lymphoproliferative disorders (PTLDs) are uncommon B-cell lymphoproliferations that are frequently associated with Epstein-Barr virus (EBV).\(^2\),\(^3\) High mortality rates, (up to 80%), have been reported when PTLDs are left untreated.\(^2\),\(^3\)

The World Health Organization (WHO) histological classification on PTLD describes the continuum of lymphoproliferative disorders under immunosuppressive therapy in three categories.\(^4\) This is based on the extent, architectural characteristics and B-lymphocyte cytology of the inflammatory infiltrate.\(^4\)

Similarly, IBD lymphoproliferative disorders are also predominantly EBV-associated\(^5\),\(^6\) and histologically comparable to PTLDs.\(^1\),\(^5\) They are associated with thiopurine therapy, which impairs T-cell activity, leading to decreased immunosurveillance of latent EBV infection.\(^2\),\(^7\) This decreased immunosurveillance can facilitate the reactivation of this oncogenic virus.\(^7\) Indeed, IBD patients on thiopurine therapy have a 3- to 5-fold increased lymphoma risk.\(^1\),\(^8\) This is mainly attributed to the development of primary intestinal lymphomas, which are rare in the general population.\(^9\) EBV-positive cells can be found in the colonic mucosa in up to 60% of IBD patients, predominantly in the inflamed areas.\(^10\),\(^11\),\(^12\) This high percentage is caused by both the increased number of infiltrating B-lymphocytes due to inflammation and the increased EBV replication rate as a result of immunosuppression.\(^11\),\(^13\) The clinical relevance of EBV-positive colon cells in IBD patients remains unclear. No serological or histological predictors of intestinal lymphoma development in IBD patients are currently available.

In transplantation medicine, strategies like regular measurement of EBV load have been developed to detect a “prelymphoma state”.\(^14\) Preventive treatment in this state can reduce the morbidity and mortality of EBV-related lymphoproliferative disorders in PTLD.\(^14\)

Unfortunately, the clinical impact of intestinal EBV and which IBD patients should be tested for intestinal EBV presence remain unclear. Therefore, we aimed to assess the utility of histological aberrations in predicting EBV presence. Furthermore, we aimed to correlate histological assessment and mucosal EBV load with clinical outcomes in IBD patients.

2. Materials and methods

2.1. Patient selection

For this retrospective single-centre cohort study, we initially included all IBD patients at the Radboud University Medical Center (RadboudUMC; Nijmegen, The Netherlands) who underwent EBV testing of ileocolonic mucosal biopsies between January 2004 and October 2013. The RadboudUMC functions as a tertiary referral centre for IBD patients. The local pathology database was used for the patient selection. Search terms used in this query included “Inflammatory Bowel Disease”, “Crohn’s Disease”, “Ulcerative Colitis”, “colitis” or “indeterminate colitis” combined with “Epstein-Barr virus”, “EBV” or “EBV encoded RNA (EBER)”. Inclusion criteria were a confirmed IBD diagnosis, IBD treatment at the RadboudUMC and EBER testing on ileocolonic mucosa at our institution.

Clinical data were retrieved from medical records, including age, gender, EBV serology if available (also before 2004) and IBD characteristics (year of diagnosis, Montreal classification, type and duration of IBD medication and intestinal surgery). Prescribed anti-inflammatory immunosuppressive IBD drugs were recorded, defined as thiopurines, corticosteroids, anti-TNF agents and methotrexate. The number of immunosuppressive drugs at the time of biopsy was also recorded. Clinical endpoints included IBD-related intestinal surgery, lymphoma development and mortality.
2.2. Histopathology
Previously EBV tested ileocolonic biopsies from all enrolled patients were reviewed by one expert gastro-intestinal pathologist (JHJM vK), who was blinded to prior results. Every biopsy was assessed for three histological features: the architecture of the lympho-plasmatic infiltrate in the lamina propria; the presence of atypical, large B-lymphocytes; and third the presence and number of EBER-positive cells. This was performed at the histologically most inflamed site with the most dense atypical lymphocytoplasmic infiltrate.

2.3. Immunohistochemistry
We used immunohistochemistry for the assessment of lymphoplasmatic infiltrate and B-lymphocytes. The lymphoplasmatic infiltrate was scored as normal or atypical in a haematoxylin and eosin (HE) staining (figure 1A-B). Atypical was defined as increased, disorganized lymphoplasmatic infiltrate in the lamina propria.

We visualized B-lymphocytes by immunohistochemistry using an antibody against CD20 (B-lymphocyte antigen Clone: L26; Thermo scientific, USA) and evaluated them as normal or atypical (figure 1C-D). Atypical was defined as the presence of large, atypical CD20-positive B-lymphocytes.

2.4. EBV testing
The gold standard for identifying EBV in biopsies is in situ hybridization for EBV-encoded RNA (EBER-ISH). Standardized EBER-ISH testing (DAKO, Belgium, and Roche, Switzerland) was used for all included mucosal biopsies. Formalin-fixed, paraffin-embedded tissue sections (4 μm) were floated on Superfrost slides. Deparaffinized slides were treated with Proteinase K (DAKO PNA ISH Detection Kit [K5201]). After hybridization with DAKO EBV (EBER) PNA Probe (Y5200) and detection with rabbit anti-FITC/AP (DAKO PNA ISH Detection Kit [K5201]), the EBER PNA probes were visualized with NBT/BCIP (4-Nitro blue tetrazolium chloride/B-Bromo-
Figure 2: Patient selection and categorization.

4-chloro-3-indoyl-phosphate; Roche) followed by counterstaining with Nuclear Fast Red.

EBER-positive cases (Figure 1) were scored quantitatively in the area of the highest EBV concentration per high-power field (HPF, 0.2 mm²) (x400 magnification) and divided into two categories: low EBV concentration (<10 EBER positive cells per HPF) and high EBV concentration (≥ 10 EBER positive cells per HPF).

2.5. Classification
Following WHO guidelines on PTLDs,4 we used three categories to classify the EBV-positive infiltrate in IBD patients: morphologically benign (normal lymphoplasmacytic infiltrate in the lamina propria irrespective of B-lymphocyte morphology); polymorphic lesions (atypical lymphoplasmacytic infiltrate and at most only rare atypical B-lymphocytes); and monomorphic lesions (atypical lymphoplasmacytic infiltrate with numerous large B-lymphocytes). Subsequently, these three categories were correlated with the EBV concentration and clinical data.

2.6. Monomorphic lesions
In the case of monomorphic lesions, we collected all ileocolonic biopsies available at the RadboudUMC from the same patient without prior EBER testing before and after the included first (monomorphic) biopsy. These additional biopsies were also scored for the described histological features in order to study the longitudinal course of lymphoproliferation. Cases with sheets of large B-cells were classified as diffuse large B-cell lymphomas.

2.7. Statistical analysis
Baseline characteristics were expressed as percentages in the case of proportions and as medians with (minimum – maximum) range in the case of continuous data. Categorical variables were analyzed using the χ² test or Fisher’s exact test as appropriate. A p-value of <0.05 was considered statistically significant. Test characteristics,
including sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV), were calculated, using only the first EBV-tested biopsy. We used IBM SPSS version 20.0 for windows (SPSS Inc., Chicago, IL, USA) for statistical analysis.

3. Results

3.1. Patients and biopsies
Initially, we identified 120 patients who underwent EBV testing on intestinal mucosa (Figure 2). Sixty-two patients were excluded due to lack of IBD diagnosis (49 patients) or treatment at other medical centres (13 patients). The remaining 58 patients comprised 16 (27.6%) diagnosed with Crohn’s disease, 41 (70.7%) with ulcerative colitis and one with indeterminate colitis (1.7%). We evaluated the first EBV-tested mucosal biopsy in all 58 included IBD patients; 28 patients were EBV-positive and 30 were EBV-negative (figure 2). All included biopsies involved mucosa with active inflammation on endoscopy and histology. The majority of the biopsies were colonic (n = 53; 91.4%); five biopsies (8.6%) were obtained from the terminal ileum.

3.2. Histopathology and immunohistochemistry
The lymphoid infiltrate was scored in all 58 samples; in 70.7% (n = 41) it was not considered atypical. The B-lymphocytes were normal in 69.0% (n = 40). Normal lymphoid infiltrate and B-lymphocytes were more frequent in the EBV-negative than in the EBV-positive group (83.3% [n = 25/30] versus 32.1% [n = 9/28], p < 0.001). An atypical infiltrate and atypical B-lymphocytes were significantly more frequent in the EBV-positive than in the EBV-negative group (35.8% [n = 10/28] versus 3.3% [n = 1/30]; p < 0.005).

Only nine out of 28 cases with mucosal EBV positivity had a normal lymphoid infiltrate and B-lymphocytes, all with ≤10 EBER-positive cells per HPF.

To evaluate whether the presence of an atypical infiltrate or atypical B-lymphocytes was predictive of mucosal EBV presence, we calculated the sensitivity, specificity, PPV and NPV. For an atypical infiltrate, specificity was 96.7%, sensitivity 57.1%, PPV 94.1% and the NPV 70.7%. These numbers were lower when using atypical B-lymphocytes: 83.3%, 46.4%, 72.2% and 62.5%, respectively.

3.3. Classification
All EBV-positive biopsies were classified into three histological categories according to the WHO classification, as outlined in section 2.5 (Figure 2). Twelve patients had morphologically benign lesions, including three patients with normal lymphoid infiltrate but a few atypical B-lymphocytes. Six patients had polymorphic lesions and 10 had monomorphic lesions. High EBV concentrations were significantly more frequent in the monomorphic group (70.0% (n = 7/10) compared with the benign and polymorphic groups (16.7% [n = 2/12] and 16.7% [n= 1/6]), respectively (p = 0.019). We did not find significant differences in EBV concentrations between the morphologically benign and polymorphic group.

For our analysis we included the three patients with atypical B-lymphocytes and normal infiltrate in the polymorphic group. Comparing the benign, polymorphic and monomorphic lesions again, we now observed a gradual increase in the frequency of high EBV concentrations over the three categories (benign, 0.0% [n = 0/9]; polymorphic, 33.3% [n = 3/9]; monomorphic 70.0% [n = 7/10]; p = 0.009). Patients with morphologically benign lesions had low EBV concentrations (<10 EBV-positive cells per HPF) significantly more frequently than patients with monomorphic lesions.

3.4. Clinical data
3.4.1. Intestinal EBV-positive patients
The median age for having a EBV-positive biopsy was 45 years (range 21-76, SD 14.9). Only one patient had a known serological
EBV status at the start of immunosuppressive therapy. Eighteen out of 28 (64.3%) intestinal-EBV-positive patients underwent intestinal surgery (all colectomy) during follow-up. Patients undergoing surgery had high EBV concentrations significantly more often than patients without surgery (50.0% [n = 9/18] versus 10.0% [n = 1/10], p = 0.048). No mortality was observed in our cohort, with a median follow-up of 58 months (range 8 – 108 months), even though none of our patients with polymorphic lesions and only two with monomorphic lesions received chemotherapy or immunotherapy (Rituximab).

3.4.2. EBV-positive versus EBV-negative patients
The median age at IBD diagnosis was 30.5 years (range 12-75) in the EBV-positive group and 24.5 years (range 13-80) in the negative group (Table 1). Ulcerative colitis was more frequent in the EBV-positive group (82.1% [n = 23]) than in the EBV-negative group (56.7% [n = 17/30], p = 0.052). We found no difference in endoscopic Mayo score between the EBV-positive and -negative ulcerative colitis patients. Seven patients did not receive any treatment (three EBV-positive patients) and an additional six patients did not receive immunosuppressants (two EBV-positive patients). EBV positive patients used corticosteroids and anti-TNF therapy significantly more frequently than EBV-negative patients (corticosteroids, 57.1% [n = 16/29] versus 30.0% [n = 9/30], p = 0.037; anti-TNF therapy 35.7% [n = 10/28] versus 13.3% [n = 4/30], p = 0.047).

We found no significant differences between EBV-positive and -negative patients in the use of 5-aminosalicytes (46.4% [n = 13/28] versus 26.7% [n = 8/30], p = 0.12) and thiopurines (46.4% [n = 13/28] versus 43.3% [n = 13/30], p = 0.81) between EBV positive and negative patients. However, EBV-positive patients used combinations of immunosuppressive drugs more frequently compared with EBV-negative patients (50.0% [n = 14/28] versus 16.7% [n = 5/30], p = 0.025). For detailed information on each enrolled patient, we refer to supplementary file 1.

Table 1: Comparison between EBV-positive and -negative patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>EBV-positive (N = 28)</th>
<th>EBV-negative (N = 30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypical inflammatory infiltrate, n (%)</td>
<td>16 (57.1)</td>
<td>1 (3.3)</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Atypical B lymphocytes, n (%)</td>
<td>13 (46.5)</td>
<td>5 (16.6)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Table 2: Comparison between benign, polymorphic an monomorphic lesions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Benign (N = 12)</th>
<th>Polymorphic (N = 6)</th>
<th>Monomorphic (N = 10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at IBD diagnosis (years), median ± SD</td>
<td>45 ± 16.4</td>
<td>38.5 ± 15.6</td>
<td>27 ± 15.4</td>
<td>0.63</td>
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<td>Male sex, n (%)</td>
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<tr>
<td>IBD type</td>
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<tr>
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<td>3 (25.0)</td>
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<td>2 (20.0)</td>
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<tr>
<td>Age at EBV diagnosis (years), median ± SD</td>
<td>45 ± 15.3</td>
<td>59 ± 12.2</td>
<td>39.5 ± 12.4</td>
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<tr>
<td>EBV load</td>
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<tr>
<td>Low, n (%)</td>
<td>10 (83.3)</td>
<td>5 (83.3)</td>
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<tr>
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<td>3 (50.0)</td>
<td>9 (90.0)</td>
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<tr>
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<td></td>
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<tr>
<td>0.041 *</td>
<td>4 (41.6)</td>
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Table 2: Comparison between benign, polymorphic an monomorphic lesions.

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<th>Variable</th>
<th>Benign (N = 12)</th>
<th>Polymorphic (N = 6)</th>
<th>Monomorphic (N = 10)</th>
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The EBV PCR load in the blood was available for only 27 of our patients (14 with EBV-positive biopsies and with 13 EBV-negative biopsies). In this small group there was no correlation between serum EBV PCR load and the mucosal presence of EBV in biopsies.

### 3.4.3. WHO classification

No differences were found in type of IBD, age at IBD diagnosis or age at EBV diagnosis, between the three histological WHO categories (Table 2). Intestinal surgery was significantly more frequent in patients with monomorphic lesions: 90.0% (n = 9/10) versus 50.0% in patients with benign (n = 6/12) or polymorphic (n = 3/6) lesions. A high EBV load was more frequent in patients with monomorphic lesions than in other groups (monomorphic, 70% [n = 7/10]; benign, 16.7% [n = 1/6]; polymorphic [n = 2/12]; p = 0.019). After an EBV-positive biopsy, immunosuppression was reduced in most patients with monomorphic lesions (90.0%, [n = 9/10] versus 50.0%, [n = 3/6] in patients with polymorphic lesions and 41.6%, [n = 5/12] in those with benign lesions; p = 0.041).

### 3.5. Monomorphic lesions

Ten EBV-positive patients in our cohort were classified as monomorphic (characterized by both atypical infiltrate and atypical B-lymphocytes), based on their first EBV-tested biopsy (index biopsy). In these 10 patients, we identified 39 additional series of biopsies (figure 2), obtained before and after the index biopsy during separate endoscopic procedures, but without prior EBV testing. These 39 series of biopsies were also assessed for histological features. A timeline of EBV status and histological features in patients with a monomorphic infiltrate is presented in supplementary file 2.

In the eight patients with monomorphic histology (excluding the lymphoma patients because they were treated with both chemotherapy and stopping of immunosuppressive therapy), immunosuppression was reduced by the treating physician. Seven patients became EBV-negative following reduction in immunosuppression (median 4 months; range 1-10; SD 3.2) and the remaining patient showed a clear reduction in the quantity of EBV-positive cells. Normalization of the histological features occurred in all patients after 6.5 months (median; range 1-48, SD 17.0), although the endoscopic surveillance frequency was reduced after the patients became EBV-negative. In four patients surgery was planned after the EBV-positive diagnosis, without awaiting the effect of reduced immunosuppression. Three patients in the cohort with monomorphic lesions later underwent a subtotal colectomy due to disease activity. Overt lymphoma was not found in anyone of the resection specimens.

Two patients in this cohort developed overt colonic EBV-associated diffuse large B-cell lymphomas (one due to EBV reactivation during the use of thiopurines, the other after a primary EBV infection). Both patients presented with a colonic perforation and underwent surgery, were treated with chemotherapy (combination of Cyclophosphamide, Hydroxydaunorubicin, vincristine [Oncovin], Prednisone and Rituximab) and reached complete remission (follow-up at 9 and 7 years). In both patients all immunosuppressive medication was stopped and never reintroduced during follow-up.

### 4. Discussion

In the current study, we showed that histological assessment of the inflammatory infiltrate and B-lymphocytes in intestinal biopsies can be used to guide EBV diagnostics in IBD patients. The presence of an atypical inflammatory infiltrate and atypical B-lymphocytes was associated with a high mucosal EBV load (≥ 10 EBER-positive cells per HPF). A high mucosal EBV load was also correlated with subsequent surgery. Despite two lymphoma cases, we observed no mortality in this IBD cohort. Our findings suggest that EBV-associated lymphoproliferative disorders in IBD have a much better outcome compared with EBV-associated PTLDs with similar morphology.
This is the first study that describes the lymphoproliferative spectrum in IBD. Currently, no specific guideline is available for the classification of EBV-associated lymphoproliferations in IBD. We used the WHO PTLD classification to predict EBV presence and to classify the lymphoproliferative disorders in IBD, since PTLDs and lymphoproliferations in IBD show similarities in aetiology and morphology.\(^1\), \(^5\)

Previous studies demonstrated EBV-positive cells in the colonic mucosa in up to 60% of IBD patients.\(^1\), \(^10\), \(^11\), \(^12\) However, in daily practice EBER-ISH is performed infrequently. This suggests that intestinal EBV presence may be underestimated in daily practice. The histology of intestinal biopsies may guide the decision about when to perform EBER-ISH, leading to increased EBV detection.

Monomorphic lesions, characterized by an atypical infiltrate and atypical B-lymphocytes, are accompanied by high EBV loads and high surgery rates. Approximately 68% of the EBV-positive patients can be identified when EBER is performed in cases with atypical infiltrate or atypical B-lymphocytes. In contrast, very few EBV-positive cells are found in benign and polymorphic lesions. Approximately 32% of patients with EBV-positive cells have normal histology. However, these patients may be at low risk of lymphoproliferation due to the lower number of EBV-positive cells. Classification of biopsies with few atypical B-lymphocytes but a normal inflammatory infiltrate as polymorphic lesions instead of benign lesions may result in better differentiation between the three WHO categories in IBD patients.

In line with previous studies,\(^16\), \(^17\) we showed that reduction of immunosuppressants led to regression of both atypical lymphoproliferative disorders and intestinal EBV load. Our data suggest that there is limited need to treat EBV-associated lymphoproliferative disorders with chemotherapy. Reduction of immunosuppressive therapy might be sufficient in most cases. Four out of eight patients with monomorphic lesions in whom immunosuppression was reduced, underwent colectomy before the effect of reduction was evaluated. Interestingly, three of these patients were EBV-negative at histological assessment of the resection specimen.

Two patients with overt EBV-associated diffuse large B-cell lymphoma showed a very good prognosis. These findings are in sharp contrast with the high reported mortality in untreated PTLD patients.\(^2\) No histological predictor of lymphoma development was found in prior intestinal biopsies. However, both lymphoma patients possibly had an additional risk factor for lymphoma development. The first (male) patient had EBV reactivation within 1 year after starting thiopurine therapy. From transplant medicine we know that most PTLD’s occur within the first year after transplantation.\(^2\), \(^18\) This might also apply to IBD, although future research is needed to prove this. The second patient developed a lymphoma during azathioprine therapy just after a primary EBV infection. A primary EBV infection may result in a higher lymphoma risk compared with patients who are EBV-positive before the start of immunosuppressive therapy.\(^1\)

The European Crohn’s and Colitis Organisation recently emphasized the need to determine the prelymphoma state and to develop preventive strategies similar to those for PTLDs.\(^19\) Currently, when there is clinical suspicion of lymphoproliferation in IBD patients, the EBV viral load is measured. However, an IBD flare may result in a temporarily increased EBV viral load unrelated to lymphoproliferation.\(^20\) Despite the small available numbers of EBV PCRs in this study, no correlation was observed between the presence of EBV in the blood and in the biopsies. Large prospective studies are needed to test alternative strategies to screen and treat potential lymphoproliferative disorders in IBD patients, such as the prophylactic antiviral therapy used in transplant medicine.\(^21\)

The results of this study have implications for clinical practice. First, EBER-ISH testing of intestinal biopsies is currently clinically based, but should also be guided by the presence of atypical inflammatory infiltrate and/or atypical B-lymphocytes. Second, reduction of immunosuppression should be considered in IBD patients.
who are diagnosed with EBV-associated lymphoproliferative disorders of all morphologies, except those with overt lymphoma morphology; these patients require additional treatment, which often includes chemotherapy.

Our study has several limitations. First, only patients who underwent EBV testing on clinical or histological grounds were included. Ideally, all IBD patients (who underwent endoscopy with biopsies in the study period) should have been enrolled, but this was not feasible due to the high number and the costs related to testing. Of note, there are currently no guidelines available that indicate when to perform EBV testing on intestinal biopsies. Therefore, most of the included patients had significant disease activity, resulting in a selection bias. Furthermore, due to the retrospective design, disease activity was not uniformly described and no standardized biopsy protocol was used. Future studies with a prospective design, larger patient numbers and systematic collection of data and tissue may provide further insight into the timing and significance of intestinal EBV testing.

In conclusion, we showed that histological assessment of intestinal biopsies is of value in predicting EBV presence in mucosal biopsies from IBD patients. EBER-ISH testing should be considered in IBD patients with atypical inflammatory infiltrate and/or B-lymphocytes. Furthermore, reduction of immunosuppression is an effective strategy to achieve resolution of intestinal EBV. Finally, our data suggest that EBV-driven lymphoproliferative disorders in IBD have a better outcome compared with PTLDs.

Conflict of interest statement
No conflicts of interest exist. There was no relevant funding.

Acknowledgments
JHJMvK, FH, IDN and LHCN designed the study. FH, JHJMvK, LAAPD, WK, DJdJ, PJTAG and IDN participated in revising the manuscript. LHCN, JHJMvK, PJTAG and LAAPD performed the research, LHCN and WK analysed data. LHCN wrote and submitted the manuscript.

This work was presented as an oral poster at the European Crohn’s and Colitis Organisation Congress, Copenhagen, February 2014, as a poster at the Digestive Diseases Week, Chicago, May 2014 and the United European Gastroenterology Week, Vienna, October 2014, and as an oral presentation at the Dutch Gastroenterology and Hepatology Congress (NVGE voorjaarsvergadering), Veldhoven, March 2014.
Lymphoproliferations in inflammatory bowel diseases


## Lymphoproliferations in inflammatory bowel diseases

### Table 1: Detailed information on first biopsy of included EBV-positive (upper panel) and EBV-negative (lower panel) patients.

<table>
<thead>
<tr>
<th>Nr</th>
<th>Gender</th>
<th>Type IBD</th>
<th>Montreal score</th>
<th>Mayo-score</th>
<th>EBV load</th>
<th>Medication at time of biopsy</th>
<th>Surgery</th>
<th>Rescue therapy</th>
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* Lymphoma patient, presentation with colonic perforation; #History of EBV-related diffuse large B-cell lymphoma (DLBCL) in lung / kidney / liver: EBV reactivation after restarting steroids; CMV, cytomegalovirus; CD, Crohn's disease; UC, ulcerative colitis; 5-asa, 5-aminosalicylates; pred, corticosteroids; MTX, methotrexate; Anti-TNF, anti-Tumor Necrosis Factor; Ciclosporin, Cyclosporine
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<th>Biopsy 2</th>
<th>Biopsy 3</th>
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Gray box represents index biopsy. *Lymphoma patient.

Low: < 10 EBER-positive cells per HPF; high, ≥ 10 EBER-positive cells per HPF; negative: no EBER-positive cells per HPF.

Oral medication: A, thiopurine; C, ciclosporin; I, anti-TNF therapy; P, corticosteroid; M, 5-aminosalicylates.
Identification of IG-clonality status as a pre-treatment predictor for mortality in patients with immunodeficiency-associated Epstein-Barr virus-related lymphoproliferative disorders

Haematologica April 2015 100: e152-e154

Walter J.F.M. van der Velden, Loes Nissen, Marieke van Rijn, Jos Rijnstjes, Anton de Haan, Lakshmi Venkatraman, Mark Catherwood, Hongxiang Liu, Hesham El-Daly, Lisette van de Laar, Moniek H.C. Craememhr, J. Han J.M. van Krieken, Wendy B.C. Stevens, Patricia J.T.A. Groenen

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Immunodeficiency-associated Epstein-Barr (EBV)-related lymphoproliferative disorders (EBV-LPD), including the post-transplant lymphoproliferative disorders (PTLD), are aggressive hematologic malignancies which, despite improvements in therapy, including the use of anti-CD20 monoclonal antibody, result in considerable morbidity and mortality.1–3 Retrospective analyses have revealed several clinical risk factors, including therapeutic interventions, that predict outcome in patients with EBV-LPD. However, pre-treatment risk stratification that can be used to guide therapeutic decisions remains difficult and algorithms are lacking.1 Although morphology is regarded to be a cornerstone in therapy decision making, immunoglobulin (IG) clonality might help prognostication. Despite efforts to standardize the pathological classification of EBV-LPD,4 neither histology nor IG clonality has been shown to consistently predict outcome.5–7 Nevertheless, comprehensive IG clonality testing, with a high detection rate of clonality, allows an objective pathological parameter to be re-evaluated in risk stratification of EBV-LPD.8

We performed a retrospective analysis in a large multi-center cohort of 86 patients with EBV-LPD: 62 patients with an EBV-positive PTLD and 24 with another iatrogenic immunodeficiency related EBV-LPD. Patients from 2000–2012 were included in the analysis; patients’ characteristics are presented in Online Supplementary Table S1. IG-gene clonality testing was performed by assessment of the IGH-V(D)J, -DJ as well as IGK (VJ and KDE) rearrangements using the BIOMED2 approach. This IG clonality assay has an unprecedented high detection rate8,9 due to the complementarity of the PCR targets with a sensitivity of each individual PCR of 5%–10% and the specificity of the IG-clonality assay of 94%.10 In this study cohort, 20% of the clonal cases had clonal IGK and/or incomplete IGH-DJ rearrangements without having clonal IGH V(D)J-FR1, 2 or 3 rearrangements. These cases were found in both the EBV-positive PTLD and the iatrogenic immunodeficiency-related EBV-LPD group, and would have gone unnoticed when clonality testing was based only on assessment of the complete IGH rearrangement. Interpretation of the clonality findings was performed according to the EuroClonality guidelines.11 Patients were classified as having either monoclonal or oligo/polyclonal EBV-LPD. Histology was examined by 2 experienced hematopathologists and designated either monomorphous or reactive/polymorphous (no Burkitt- or Hodgkin-type lesion was included) according to the WHO criteria.4 In the majority of the cases, IG clonality was detected in multiple PCRs. There was no clear difference in clonality pattern in monomorphic subtype PTLDs versus the reactive/polymorphic PTLDs, albeit the last group tended to have more cases showing monoclonality with a polyclonal background, although this is not exclusively seen in this group. A statistical analysis was performed to identify pre-treatment risk factors for poor outcome defined as EBV-LPD-related mortality, with an emphasis on pathological features, but also clinical stage according to Ann Arbor, extra-nodal disease, age and underlying diagnosis.

Univariable analysis using the Fisher exact test revealed multiple risk factors for EBV-LPD-related mortality; P<0.05 was considered significant (Table 1). This included PTLD as underlying diagnosis, disease stage II-IV and IG monoclonality, but not age 50 years or over, EBV load at diagnosis, and monomorphic histology. Next we performed a multivariable logistic regression analysis with the four variables having at least a P value of 0.10 in the univariable analysis. In the first model, incorporating all four risk factors, underlying diagnosis was not associated with EBV-LPD-related mortality (P=0.6), but the other three, including age 50 years or over, showed an association with P≤0.1. Analyzing these three risk factors simultaneously in a second model revealed that age and disease stage were significantly related to EBV-LPD-related mortality (Table 1), and that IG-clonality showed a trend (P=0.09). Nevertheless, the high odds ratios at least suggest that the risk factors might all have had an impact and that significance was
Table 1: Univariable and multivariable analysis of risk factors for EBV-LPD related mortality.

<table>
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<tr>
<th>Risk factor</th>
<th>EBV-LPD</th>
<th>OR (95 % CI)</th>
<th>P</th>
<th>OR (95 % CI)</th>
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<td>multivariable</td>
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<tr>
<td>Age &lt; 50 years</td>
<td>13 / 36 (36 %)</td>
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<td>0.08</td>
<td>3.6 (1.2 – 11)</td>
<td>0.03</td>
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<tr>
<td>Age ≥ 50 years</td>
<td>9 / 30 (30 %)</td>
<td>2.3 (0.8 – 7.2)</td>
<td>0.1</td>
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<td>-</td>
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<tr>
<td>Sex Male</td>
<td>17 / 32 (53 %)</td>
<td>5.2 (1.4 – 20)</td>
<td>0.01</td>
<td>3.0 (0.7 – 11.2)</td>
<td>0.11</td>
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<tr>
<td>Sex Female</td>
<td>6 / 32 (19 %)</td>
<td>3.8 (0.7 – 20)</td>
<td>0.11</td>
<td>-</td>
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<tr>
<td>Diagnosis PTLD</td>
<td>20 / 32 (31 %)</td>
<td>2.5 (0.9 – 7.4)</td>
<td>0.13</td>
<td>1.1 (0.4 – 3.1)</td>
<td>0.8</td>
</tr>
<tr>
<td>Iatrogenic EBV-LPD</td>
<td>8 / 32 (25 %)</td>
<td>2.5 (0.9 – 7.4)</td>
<td>0.13</td>
<td>1.1 (0.4 – 3.1)</td>
<td>0.8</td>
</tr>
<tr>
<td>Stage III – IV</td>
<td>21 / 36 (58 %)</td>
<td>14.4 (1.8 – 113)</td>
<td>0.001</td>
<td>11.8 (1.6 – 117.2)</td>
<td>0.02</td>
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<tr>
<td>Extranodal disease Present</td>
<td>12 / 46 (26 %)</td>
<td>5.2 (1.1 – 24.5)</td>
<td>0.03</td>
<td>1.5 (0.3 – 8.7)</td>
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<tr>
<td>Extranodal disease Absent</td>
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<td>2.3 (0.8 – 7.2)</td>
<td>0.1</td>
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<td>Monomorphic</td>
<td>39 / 62 (63 %)</td>
<td>1.0 (0.4 – 2.8)</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Reactive/polomorphic</td>
<td>23 / 62 (37 %)</td>
<td>8.9 (2.1 – 36)</td>
<td>0.02</td>
<td>6.6 (1.8 – 38.2)</td>
<td>0.00</td>
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<tr>
<td>Mono/oligo+polyclonal</td>
<td>10 / 32 (31 %)</td>
<td>14.4 (1.8 – 113)</td>
<td>0.001</td>
<td>11.8 (1.6 – 117.2)</td>
<td>0.02</td>
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P < 0.05 was considered statistically significant. OR: odds ratio. 1Model with four covariates. 2Model with three covariates. 3Data on EBV load only present in 57 patients.

Figure 1: Flowcharts of the clinico-pathological features and patient outcome of patients with EBV-related lymphoproliferative disorders and separated by diagnose subgroups.

The therapy that had been applied mostly is shown in the dark blue boxes. The number of patients who actually received rituximab alone or in combination with chemotherapy (R/R-chemo) are shown in the green boxes.

Because diseases with a high mortality rate require prognostic factors that preferably identify all those at risk for mortality, we next investigated which of the pathological factors had the highest sensitivity and negative-predictive value for EBV-LPD-related mortality. Sensitivity, specificity, negative predictive value and positive predictive value for EBV-LPD-related mortality in the total cohort were 68%, 53%, 83%, 33% for monomorphic morphology and 95%, 30%, 95%, 32% for monoclonal IG-gene status, respectively. When looking at PTLD cases separately, similar results were seen with the sensitivity and negative predictive value of monoclonal IG-gene status exceeding that of monomorphic morphology (95% and 89% vs. 70% and 74%, respectively).

The higher negative predictive value at least suggests that IG-clonality testing performs better than histological examination when used to identify patients that are not at high risk of death. So, patients can be identified that might not require prompt treatment, that is, those with oligo/polyclonal disease. Clonality testing, however, has a similar, but equally low, positive predictive value as histology. Therefore, establishing either monoclonality or monomorphic disease does not necessarily mean that a patient is at high risk for death from EBV-LPD, and therapeutic decision making based on clonality status alone might result in overtreatment.

Realizing the limitations of the multivariable analysis, and the very different clinical context of patient groups defined as either PTLD or another iatrogenic immunodeficiency EBV-LPD, the decision was made to analyze the two groups separately (Online Supplementary Table S1); this precluded comprehensive statistical analysis and, therefore, only descriptive statistics were used.

The subgroup of patients with PTLD consisted of 41 hematopoietic stem cell and 21 solid organ transplant recipients. The distribution of morphological subtypes was similar in the SOT and SCT subgroups with approximately 60%–65% monomorphic disease. In those patients presenting with monomorphic PTLD, 90% (35 of 39)
had been classified as monoclonal EBV-LPD. The 4 monomorphic PTLD cases without monoclonal IG status all had oligoclonal EBV-LPD and displayed similar clinical features to the monoclonal cases. Mortality was high at 36% (14 of 39) despite the use of R/R-chemo (Figure 1A). More specifically, of the 14 patients who died, 13 had monoclonal and one oligoclonal EBV-LPD. In patients with reactive/polymorphic PTLD, the IG-clonality status seemed to be of importance. Monoclonality resulted in an unfavorable outcome with a mortality rate of 33%, which is similar to that seen in the patients with monomorphic PTLD. However, a considerable number of deaths were caused by insufficient treatment. Five patients who died had not received R/R-chemo, probably as a result of inadequate risk assessment based on morphology, age and stage. In contrast, polyclonal reactive/polymorphic PTLD patients had a good outcome with modification of immunosuppression only (Figure 1A).

There were 24 patients with another iatrogenic immunodeficiency EBV-LPD, which involved 22 patients treated for inflammatory bowel disease, e.g. Crohn disease and ulcerative colitis. Extraneal disease involving the diseased colon itself was very common, 72% (16 of 22). Overall, EBV-LPD mortality was 8% (2 of 24). Ann Arbor staging seemed most predictive for outcome (Figure 1B). Stage I disease (n=16) was effectively cured by only modifying immunosuppressive therapy, sometimes complemented by surgical resection; the IG clonality status (44% monoclonal) was not relevant. More advanced stages, II-IV (n=8), which proved monoclonal in 75% of the cases, required treatment with rituximab (R) alone or combined with chemotherapy (R-chemo), but there was still a mortality rate of 25% (2 of 8) (Figure 1B). The 2 patients who had died both had monoclonal disease and succumbed despite use of R/R-chemo.

Our analysis shows that IG-clonality status might be useful in the risk stratification and therapeutic decision making in patients with EBV-LPD in the setting of PTLD. Monoclonal, monomorphic EBV-LPD in PTLD requires early aggressive intervention; polyclonal reactive EBV-LPD may be managed conservatively. In IBD patients with EBV-LPD, low disease stage is more predictive of survival, regardless of whether or not the disease is monoclonal. The fast and full recovery of immunity with reduction of immunosuppressants expected in these patients, who have no additional immunological deficits, seems sufficient to achieve a remission. This contrasts with the situation of PTLD after transplantation where more profound and prolonged immune deficits arise from pre-treatment and conditioning therapy, which precludes control of EBV-LPD by a functional immune system on cessation of immunosuppressants. Reactive/polymorphic and polyclonal PTLD probably reflects an earlier phase of the disease where there might be more time for immune recovery to occur, and so, even in the setting of transplantation, additional therapy can be reserved for those failing modification of immunosuppressive therapy.

Our analysis has several limitations that are related to the retrospective nature of the study and the limited sample size. Nevertheless, our findings appeal for future multicenter prospective studies that incorporate IG-gene clonality testing in a risk stratified approach to PTLD.

Footnotes

- Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.
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References


Some patients received a combination of immunosuppressants.
Hepatosplenic T-cell lymphoma in a 47-year-old Crohn’s disease patient on thiopurine monotherapy


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Abstract

Hepatosplenic T-cell lymphoma (HSTCL) is a rare non-Hodgkin lymphoma with a high mortality rate. Higher incidence is reported in patients with inflammatory bowel disease, specifically in male patients that are younger than 35 years, and have been treated with thiopurine and tumor necrosis factor (TNF)-α inhibitor combination therapy for over 2 years. In this case report we describe a 47-year-old patient with Crohn’s disease (CD) who developed HSTCL after having been treated with thiopurine monotherapy for 14 years. To our best knowledge, only eleven cases exist of patients with CD who developed HSTCL while on thiopurine monotherapy. We report the first patient with CD, older than 35 years, who developed HSTCL while on thiopurine monotherapy. This emphasizes that HSTCL risk is not limited to young men receiving both thiopurines and TNF-α inhibitors.

Key words: Hepatosplenic T-cell lymphoma; Thiopurine; Crohn’s disease; Immunosuppression

Core tip

In this manuscript we provide an overview of all known cases in literature with Crohn’s disease (CD) who developed hepatosplenic T-cell lymphoma (HSTCL) while on thiopurine monotherapy. In addition, we present our case of a patient with CD on thiopurine monotherapy who developed HSTCL at a relatively old age. This emphasizes that HSTCL may develop at all ages, even when the patient is solely on thiopurine monotherapy.

Introduction

Hepatosplenic T-cell lymphoma (HSTCL) is a rare subtype (1%) of peripheral T-cell non-Hodgkin lymphomas[1]. It is an extranodal and systemic neoplasm deriving from cytotoxic T-cells, usually with a gamma-delta (γδ-) T-cell receptor type [2]. These atypical lymphocytes display global infiltration in the splenic red pulp and in the intrasinusoidal space in the liver and bone marrow [3]. As a consequence, patients present with hepatomegaly (77%), splenomegaly (96%), constitutional symptoms (70%), anemia (85%), thrombocytopenia (89%), leukopenia (72%) and liver enzyme abnormalities (46%), in the absence of lymphadenopathy [3]. HSTCL mainly affects male [4] adults with a median age of 20 to 35 years [1,3]. HSTCL has a rapidly progressive course with a mean overall survival less than 16 mo, regardless of the available treatment modalities (chemotherapy, splenectomy, bone marrow or stem cell transplantation) [3].

The incidence of the highly lethal HSTCL is very low in the general population [5]. However, at least 10% of HSTCL arises in inflammatory bowel disease (IBD) patients treated with immunomodulatory therapy (thiopurines and/or tumor necrosis factor (TNF)-α inhibitors)[5]. This results in an increased HSTCL risk in IBD patients compared to the general population, although the absolute risk remains low [1,5]. Especially men younger than 35 years with Crohn’s disease (CD) who are treated with thiopurines and TNF-α inhibitor combination therapy for at least two years are at increased risk [5]. The estimated absolute risk to develop HSTCL in IBD patients treated with combination therapy is 1:22000 in general and 1:3534 for men younger than 35 years old. By contrast, IBD patients on thiopurine monotherapy had an estimated absolute risk of 1:45000 and 1:7404 in general IBD patients and men younger than 35 years old, respectively [4-6]. IBD patients with HSTCL have a poor prognosis with a median survival of seven to eight months [7,8]. In this case report, we present a 47-year-old male CD patient on
thiopurine monotherapy. To our best knowledge, this is the first case report describing an IBD patient on thiopurine monotherapy who developed HSTCL at an age older than 35 years.

Case report

A 47-year-old Caucasian man with CD presented at our hospital with painless icterus, weight loss and malaise, without fever. He had a 33-year history of penetrating CD in the colon (Montreal Classification L2, B3 + P) and was treated with thiopurine monotherapy at presentation. Initial CD treatment had consisted of budesonide and mesalamine which was followed by azathioprine 150-200 mg per day for 14 years. Subsequently, he received infliximab at the age of 33 (three doses, remission induction therapy) and 36 (maintenance therapy for one-and-a-half years, discontinued due to neurologic side-effects). During his course of CD, he underwent both right and left hemicolectomy (at the age of 18 and 30 years, respectively) and received a permanent ileostomy at the age of 41 due to active perianal fistulating disease in the three years before. CD had been in remission in the five years preceding presentation at our hospital.

Physical examination of our patient revealed hepatosplenomegaly in the absence of lymphadenopathy, which was confirmed by computed tomography and positron emission tomography (Figure 1). Laboratory testing indicated anemia, thrombocytopenia and liver test abnormalities (Table 1A). Imaging did neither reveal dilated bile ducts nor other (obstructive) abnormalities. Furthermore, viral causes of hepatitis, including Epstein-Barr virus, were excluded by serology. Based on these results, we suspected an hematological malignancy and performed a liver and bone marrow biopsy. Liver biopsy showed sinusoidal and portal infiltration of atypical lymphocytes (Figure 2). Immunophenotyping of both biopsies confirmed a T-cell population with the surface proteins listed in Table 1B. Based on these biopsies, γδ-HSTCL was diagnosed[1].

Figure 1: Positron emission tomography showing hepatosplenomegaly with increased metabolic activity in liver, spleen and bone marrow.

Figure 2: Liver biopsy showing hepatosplenotic T-cell lymphoma.

Left: Hematoxylin and eosin staining (magnification × 100) showing intrasinusoidal and portal infiltration of atypical lymphocytes; Right: CD3 staining (magnification × 100) showing neoplastic cells (appearing in brown colour).
Following the diagnosis of HSTCL, high dose corticosteroids (125 mg per day) were administered, followed by chemotherapy (CHOP regimen; cyclophosphamide, hydroxydoxorubicin, vincristine and prednisone). Despite this treatment, both clinical and biochemical parameters rapidly deteriorated and the patient died 21 d post-diagnosis due to massive esophageal bleeding, secondary to therapy-induced mucositis.

Discussion

In this case report, we presented a male CD patient who developed HSTCL at the age of 47 years and after having been on thiopurine monotherapy during 14 years. Remarkably, this rare malignancy developed at an age older than 35, which is in contrast with previous cases in patients on thiopurine monotherapy. As HSTCL is very rare, and controlled cohort studies are lacking, this case report may contribute to the assessment of HSTCL risk and its relation with immunosuppressive therapies[7].

IBD patients, especially CD patients, are twice more likely to develop any lymphoma, regardless of immunosuppressive treatment[9,10]. The risk to develop HSTCL is also increased in autoimmune disorders like rheumatoid arthritis[11,12], specifically in those patients, treated with TNF-α inhibitors, and in immunocompromised patients with, among others, renal or heart transplant[3].

In IBD patients, thiopurine treatment is associated with a significantly increased overall risk (rate ratio of 1.41) of developing cancer[13], specifically non-melanoma skin cancer, urinary tract cancers and lymphoproliferative disorders (multivariate adjusted hazard ratio of 5.28)[10,14].

More specifically, thiopurines promote development of lymphomas: a recent meta-analysis found an overall standard incidence ratio for lymphoma of 5.7 in IBD patients receiving thiopurines, but not in patients formerly treated with thiopurines or patients who

<table>
<thead>
<tr>
<th>Analysis (Unit)</th>
<th>Results</th>
<th>Reference value</th>
<th>Immunophenotype</th>
<th>Results</th>
<th>Typical HSTCL [2,3]</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count (× 10^9/L)</td>
<td>4.6</td>
<td>4.0-10.0</td>
<td>CD 2 and 3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Platelet count (× 10^9/L)</td>
<td>42</td>
<td>150-400</td>
<td>CD 4 and 5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>7.7</td>
<td>8.5-11.0</td>
<td>CD 7</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Bilirubin (μmol/L)</td>
<td>175</td>
<td>0-17</td>
<td>CD 8</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>265</td>
<td>0-125</td>
<td>CD 16</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>154</td>
<td>0-45</td>
<td>CD 56</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ASAT (IU/L)</td>
<td>250</td>
<td>0-34</td>
<td>T-cell receptor</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ALAT (IU/L)</td>
<td>437</td>
<td>0-44</td>
<td>TIA</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>701</td>
<td>0-247</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALP: Alkaline phosphatase; ASAT: Aspartate transaminase; ALAT: Alanine transaminase; GGT: gamma-glutamyl-transferase; LDH: Lactate dehydrogenase; TIA: T-cell restricted intracellular antigen.
had never used these drugs\textsuperscript{[4]}. The excess risk can be reversed by thiopurine withdrawal. Thiopurine cytotoxicity is mediated by the incorporation of 6-thioguanine during DNA replication in targeted cells, instead of guanine, which ultimately leads to apoptosis\textsuperscript{[10]}.

Previous studies showed a higher absolute risk of developing HSTCL in patients receiving both thiopurines and TNF-\textgreek{a} inhibitors, compared to patients on thiopurine monotherapy\textsuperscript{[4-6]}. Furthermore, a review including 25 IBD patients with HSTCL reported an increased HSTCL risk in those on thiopurine monotherapy compared to patients using TNF-\textgreek{a} inhibitor monotherapy\textsuperscript{[11]}.

Duration of immunosuppressive therapy may also influence the risk of developing HSTCL. For example, more than 80\% of HSTCL cases occur in the first two years after initiation of combination therapy\textsuperscript{[12]}. Median time from initiation of thiopurines to HSTCL development did not significantly differ between patients on thiopurine monotherapy and combination therapy (5.5 years vs 6 years, $P = 0.39$)\textsuperscript{[4]}.

A review of the literature revealed 38 cases of \textgreek{g}HSTCL in patients with CD, including 27 patients on combination therapy and 11 on thiopurine monotherapy (Tables 2 and 3)\textsuperscript{[7,15]}. In contrast to these previous cases, our patient developed HSTCL after a longer period of thiopurine treatment (14 years vs a mean time of 5 years in the previously reported cases) and at an older age (47 years). HSTCL in general mainly affects men with a median age of 20 to 35 years\textsuperscript{[13,4]}. Only 7 CD cases are known to develop HSTCL at an age older than 35, all of them were receiving combination therapy (Table 2)\textsuperscript{[7,15]}. Time to HSTCL development following initiation of thiopurine treatment was reported in three cases, including 5.5, 7.3 and 13.5 years. In addition, all ulcerative colitis patients on thiopurine monotherapy (7 cases) developed \textgreek{g}HSTCL below the age of 35\textsuperscript{[4,5]}. Finally, our patient had a very short survival (21 d) in contrast to previous cases (Table 3) with a median survival of 7-8 mo.

The proven benefit of thiopurines in combination with TNF-\textgreek{a} inhibitors to maintain corticosteroid free clinical remission and mucosal healing should outweigh the risk of serious infections and secondary malignancies, such as untreatable lymphoma\textsuperscript{[16]}. Therefore, the recent published European Crohn’s and Colitis Organisation guideline recommends to limit the duration of combination therapy to 2 years, if possible\textsuperscript{[10]}. In addition, de-escalation of monotherapy (drug cessation or dose reduction) has to be considered to reduce risk of secondary malignancies. Several factors impact this decision, such as disease phenotype and extent, duration of remission, prior surgery, and a history of cancer. Given the prolonged clinical remission in our case, thiopurine withdrawal could have been considered to reduce HSTCL risk, although the extensive, relapsing disease course including surgery called for prolonged therapy\textsuperscript{[17]}.

This case report presents the first IBD patient on thiopurine monotherapy for an extended period of time, who developed a \textgreek{g}HSTCL at an age older than 35 years. This highlights the clinical relevance of knowledge and awareness of HSTCL risk in patients with CD on immunomodulatory therapies.

**Author contributions**

van de Meeberg MM is first author and composed the manuscript; Derikx LAAP and Nissen LHC contributed equally in writing the manuscript; Sinnige HAM, Nooijen P and Schipper DL revised the manuscript; Schipper DL also provided the case.

**Conflict-of-interest statement**

All the authors have no conflicts of interests to declare.
### Table 2: Number of Crohn’s disease cases with hepatosplenic T-cell lymphoma

<table>
<thead>
<tr>
<th>Age</th>
<th>Combination therapy</th>
<th>Monotherapy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 35 year</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>&lt; 35 year</td>
<td>20</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>11</td>
<td>38</td>
</tr>
</tbody>
</table>

### Table 3: Cases of gamma/delta-hepatosplenic T-cell lymphoma in patients with Crohn’s disease on thiopurine monotherapy

<table>
<thead>
<tr>
<th>Index case</th>
<th>Age, sex</th>
<th>Years of thiopurine treatment (type)</th>
<th>Presentation</th>
<th>Treatment</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selvaraj et al. 2013 (AERS 6751796)</td>
<td>47, M</td>
<td>14 (AZT)</td>
<td>HSM, icterus, anemia, thrombocytopenia</td>
<td>Ch. (CHOP)</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Selvaraj et al. 2013 (AERS 7554658)</td>
<td>18, M</td>
<td>&lt; 1 (AZT, 6MP)</td>
<td>NS</td>
<td>NS</td>
<td>7</td>
</tr>
<tr>
<td>Fowler et al. 2010</td>
<td>19, M</td>
<td>6 (AZT)</td>
<td>SM, leukocytopenia</td>
<td>Ch. (NS) + splenectomy</td>
<td>4</td>
</tr>
<tr>
<td>Fowler et al. 2010</td>
<td>22, M</td>
<td>8 (6MP)</td>
<td>SM, night sweats, fever, abdominal tenderness</td>
<td>Ch. (NS)</td>
<td>Survival</td>
</tr>
<tr>
<td>Ochenrider et al. 2010</td>
<td>18, M</td>
<td>5 (6MP)</td>
<td>Fever, night sweats, SM, anemia, thrombocytopenia</td>
<td>Ch. (Pentostatin, ICE) + auto-SCT</td>
<td>7</td>
</tr>
<tr>
<td>Humphreys et al. 2008</td>
<td>27, F</td>
<td>5 (AZT)</td>
<td>Fever, night sweats, pancytopenia, HSM</td>
<td>Interferon-β</td>
<td>&gt; 31</td>
</tr>
<tr>
<td>Zeidan et al. 2007</td>
<td>31, M</td>
<td>6 (6MP)</td>
<td>Chills, SM, fever, pancytopenia</td>
<td>Ch. (CHOP, cytarabine, ESHAP)</td>
<td>7</td>
</tr>
<tr>
<td>Falchook et al. 2006</td>
<td>NS</td>
<td>NS (6MP)</td>
<td>SM</td>
<td>Ch. (NS)</td>
<td>NS</td>
</tr>
<tr>
<td>Mittal et al. 2006</td>
<td>18, M</td>
<td>6 (AZT)</td>
<td>Fever, pancytopenia, HSM</td>
<td>Ch. (IVE, ESHAP, alemtuzumab)</td>
<td>NS</td>
</tr>
<tr>
<td>Navarro et al. 2003</td>
<td>35, M</td>
<td>5.6 (AZT)</td>
<td>Fever, night sweats, HSM, anemia, thrombocytopenia</td>
<td>Ch. (NS) + splenectomy</td>
<td>NS</td>
</tr>
<tr>
<td>Lémann et al. 1998</td>
<td>NS</td>
<td>4 (AZT)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Received one single gift infliximab 51 mo before presentation, therefore considered as TNF-β inhibitor naive.

AERS: Adverse Event Reporting System; AZT: Azathioprine; Ch.: Chemotherapy; CHOP: Cyclophosphamide, hydroxydaunorubicin, vincristine and prednisone; EHSAP: Etoposide, methylprednisolone, cytarabine, cisplatin; ICE: Ifosfamide, carboplatin, etoposide; IVE: Ifosfamide, carboplatin and etoposide; NS: Not specified; (H)SM: (hepato)splenomegaly; SCT: Stem cell transplantation; 6MP: 6-mercaptopurine.
References


Solid malignancies in inflammatory bowel diseases
Impaired gastric cancer survival in patients with inflammatory bowel disease

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** and ***: appendix A and B
Abstract

Background and aims
Both chronic inflammation and reduced immunosurveillance contribute to malignancy development in inflammatory bowel disease (IBD). Previous literature suggests that especially Crohn’s disease patients are at increased risk for developing gastric cancer (GC).

This study aimed to identify risk factors for GC development in IBD and to compare the clinical characteristics of GC to those in IBD to the general population.

Methods
We retrospectively searched the Dutch Pathology Database to identify all Dutch IBD patients with GC between January 2004 and December 2008. Two case-control studies were performed. I: to identify risk factors for GC in IBD, with controls from the IBD South Limburg (IBDSL) population-based cohort; II: to compare GC disease course in IBD patients with the general population. General population data were obtained from the Eindhoven Cancer Registry (ECR).

Results
We included 59 patients with IBD and GC (cases). Cases were significantly older at IBD diagnosis than IBDSL controls (median age 61 year versus 40; p<0.01) and ulcerative colitis (UC) was more frequent in the case group (69.5% versus 51.4%; p<0.01). We found no difference in age at diagnosis, gender, tumor location and tumor differentiation between IBD GC patients and ECR controls. When corrected for confounders and TNM-stage, IBD patients showed impaired survival (p=0.035; HR 1.385).

Conclusions
Survival is significantly reduced in IBD patients compared to the general population in the multivariable analysis of our study, but age at GC diagnosis and TNM-stage were comparable between IBD cases and controls. Elderly onset IBD emerged as a risk factor for GC development in IBD patients, particularly in UC.

Key words:
Inflammatory bowel diseases; gastric cancer; immunosuppressive therapy.
Introduction

Inflammatory bowel disease (IBD), consisting of Crohn’s disease (CD) and ulcerative colitis (UC), is characterized by chronic intestinal inflammation resulting in for example abdominal pain, (bloody) diarrhea, weight loss and/or peri-anal fistula. IBD patients are at increased risk for developing colorectal cancer (CRC) due to chronic intestinal inflammation\textsuperscript{1,2}. In addition, several other malignancies, such as Epstein-Barr virus (EBV) positive lymphomas and non melanoma skin cancer\textsuperscript{3}, also show an increased incidence in IBD patients. Similarly, a recent meta-analysis described an increased risk of gastric cancer (GC) in CD patients\textsuperscript{4}.

The cause of GC development in IBD patients is unclear. The majority of sporadic GC’s develop via an intestinal metaplasia – dysplasia pathway under influence of \textit{Helicobacter pylori} (\textit{H. pylori}) infection\textsuperscript{5}. However, in IBD, prevalence of \textit{H. pylori} is lower compared to the general population\textsuperscript{6,7}. In addition, impaired immunosurveillance\textsuperscript{8} of oncogenic viruses and bacteria has been suggested as a causative factor for cancer development in IBD patients. Ten percent of sporadic gastric intestinal-type adenocarcinoma is associated with gastric EBV infection\textsuperscript{9,10}. It is unknown whether this infection contributes to GC in IBD patients. Furthermore, approximately 5% of CD patients have gastric inflammatory involvement\textsuperscript{11} and local chronic inflammation may influence GC development.

At present it is unclear whether chronic inflammation and/or impaired immunosurveillance play a role in the development of GC in IBD patients. Furthermore, it is unknown whether risk factors, histologic features and clinical course of GC in IBD patients differ from the general population. All of these features are clinically relevant regarding the course of management of immunosuppressive therapies in IBD patients with cancer. In order to elucidate a profile that will allow dissection of the elements that affect the risk for GC in IBD patients we established a nation-wide cohort.

The purpose of this study was to explore potential risk factors for GC development in IBD patients, and to compare the histological features and clinical course of GC in IBD patients with the general population.

Methods

Design and data sources

We studied the clinical course, outcomes and histology of GC in IBD patients. To this end we established a nationwide cohort of IBD patients who developed GC (cases) using PALGA (Dutch nationwide network and registry of histo- and cytopathology)\textsuperscript{12}. Subsequently, cases were included in the following two case control studies:

I Case control study I was performed to identify risk factors for developing GC in IBD patients. Cases were compared with IBD controls, which were randomly selected from the population-based IBD South Limburg Cohort (IBDSL Cohort) in The Netherlands\textsuperscript{13,14}.

II Case control study II was performed to compare clinical characteristics and outcomes of GC in IBD cases to GC in the general population. We used the Eindhoven Cancer Registry (ECR) to extract controls.

This study was approved by the PALGA Privacy Committee and Scientific Council and by the medical ethical committee of the Radboudumc (number 2013/059), The Netherlands.

Selection of cases

In order to identify all IBD patients who were diagnosed with GC between January 2004 and December 2008 in The Netherlands, we performed a PALGA-database search. PALGA has complete national coverage for academic and non-academic hospitals since 1991\textsuperscript{15}. The following search terms were used: “Crohn’s Disease”,...
Ulcerative Colitis”, “Inflammatory Bowel Disease”, “Indeterminate colitis”, “chronic colitis”, “acute colitis”, “lymphocytic colitis”, “necrotizing colitis” or “colitis” combined with “gastric carcinoma”, “gastric dysplasia” or “gastric adenoma”. Our selection strategy ran through 3 stages: 1) selection and exclusion of patients was based on evaluation of the individual pathology reports; 2) intestinal and gastric histologic specimens were reviewed by an expert gastro-intestinal pathologist (I.N.) to confirm both IBD as well as the GC diagnosis; 3) patient charts were evaluated to confirm both diagnoses and to collect additional demographic and clinical data. Patients were excluded when either the IBD or GC diagnosis could not be confirmed, when the IBD diagnosis was established more than 6 months after GC diagnosis or when the GC diagnosis was made before 2004 or after 2008. Only gastric carcinomas were included.

Data collection cases
Two authors (L.N. and E.A.) reviewed anonymized medical charts and extracted both IBD and GC data. The collected data of the cases included the following patient characteristics: date of birth, gender, medical history, alcohol and smoking history.

For IBD, the following variables were collected: type of IBD based on histopathologic evaluation of the histologic specimen, date of IBD diagnosis, IBD phenotype according to the Montreal Classification15, diagnosis of primary sclerosing cholangitis, use of IBD medication (5-aminosalicylates, corticosteroids, thiopurines, methotrexate and biological therapy) and duration of therapy.

For GC the following variables were collected: prior upper endoscopy and histology (with or without gastritis, metaplasia or dysplasia), EBV status, H. pylori (both histological and serological data), family history of GC, use of protonpumpinhibitors, date of GC diagnosis, location, histological classification according to Laurén16 and the World Health Organization (WHO)17, tumor stage according to the TNM classification (6th edition), treatment and overall survival.

Case control study I. Selection of controls from the IBDSL Cohort
IBD controls for the identification of risk factors were randomly selected (using a 1:3 ratio) from the IBDSL Cohort13. The IBDSL cohort is a prospectively followed, population-based cohort, including all new adult IBD cases since 1991. Patients with indeterminate colitis are not included, unless they are classified as UC or CD during a later stage of their disease course. South Limburg is an enclosed geographic area in the southeast of The Netherlands with 605,000 inhabitants and three hospitals (1 university hospital and 2 general district hospitals). As cross-border health care use is limited and migration rates are low, South Limburg provides a good setting for population-based research. The total number of IBD patients in this registry is 2807 IBD patients (40.9% CD, 59.0% UC). This represents 93% of the regional IBD population14.

Case control study II. Selection of controls from the ECR
To compare clinical characteristics and outcome of GC in IBD patients to the general population, we identified controls in the ECR (managed by The Netherlands Comprehensive Cancer Organisation (NCCO)) from January 2004 until December 2008. Since 1989, the ECR prospectively registers all newly diagnosed cancers in the regions “Noord-Brabant” and “Noord-Limburg”, two provinces in the south of The Netherlands, covering an area with 2.3 million inhabitants, encompassing over 95% of all cancers in this region. We used the search term “C16 (stomach)” according to the ICD-0 third edition18. Only carcinomas were included.

The following variables for GC in the general population were collected: gender, age and year of diagnosis, tumor location (cardia or non-cardia), tumor stage according to the TNM classification (6th edition), histologic classification according to the ICD-0 classification18, treatment and follow-up. Obtained histological data were evaluated according to the Laurén16 and WHO classification17.
**Additional histopathological analyses of cases**

To gain insight into the pathogenesis of GC in IBD, we performed additional histopathological stainings in a subset of patients.

We performed EBV-encoded RNA – in situ hybridization (EBER-ISH), the gold standard test for EBV detection\textsuperscript{19}, using formalin-fixed, paraffin-embedded (FFPE) tissues of GC specimens (biopsy or resection)\textsuperscript{20}. Deparaffinized slides were treated with Proteinase K (DAKO PNA ISH Detection Kit[K5201]). After hybridization with DAKO EBV (EBER) PNA Probe (Y5200), and detection with rabbit-anti-FITC/AP (DAKO PNA ISH Detection Kit [K5201]), the EBER PNA probes were visualized with NBT/BCIP (4-Nitro blue tetrazolium chloride/5-Bromo-4-chloro-3-indoxyl-phosphate, Roche) followed by counterstaining with Nuclear Fast Red. Tumors were scored EBV positive or negative. Positive was defined as the presence of EBV in all or in the vast majority of tumor cells.

Immunohistochemical staining of mismatch repair (MMR) proteins was performed on 4-um-thick FFPE GC tissue sections. Slides were stained with antibodies against MLH1 (Pharmingen code: 51-1327gr; dilution 1 : 50), PMS2 (Pharmingen code: 556415; dilution 1 : 80), MSH2 (Oncogene Research Products code: NA26; dilution 1 : 100) and MSH6 (Transduction Laboratories code: G70220; dilution 1 : 250). Staining interpretation was done by the investigators (L.N. and R.v.d.P.) and expert pathologist (I.N.). Staining pattern was assessed as follows: (1) positive – showing nuclear staining in at least some tumor cells; (2) negative – no nuclear staining at all in tumor cells with a positive internal control (staining of normal epithelial, stromal and inflammatory cells); or (3) not assessable – insufficient technical quality to provide an unambiguous result despite repeated assays\textsuperscript{21}.

**Statistics**

First, we compared potential risk factor, clinical characteristics of case control study I and II with univariable analyses. For the univariable analysis, we used a $\chi^2$-test or Fisher exact test (if expected cell counts were <5) for categorical data and independent Student t test or Mann-Whitney U test for continuous data. Variables with a p value of <0.1 in univariable analyses were included the multivariable analyses.

For case control study I, a binary logistic regression analysis with backward elimination of non-significant confounders was performed to determine risk factors for IBD patients to develop GC. The calculated odds ratios (OR) were presented with 95 % confidence interval (95 % CI). This model was always adjusted for the duration of follow up (fixed variable). For cases, follow up was defined as time since IBD diagnosis until GC diagnosis. For controls, follow up was defined as time since IBD diagnosis until death or end of follow up. As medication use in especially the distant past might not be reliable and may be different from current regimes, we did not include medical therapy in the first multivariable analysis. Therefore, we performed a multivariable logistic regression analysis (called sensitivity analysis) including patients with an IBD diagnosis since 1991 in both the case and control group. Patients from the IBDSL cohort with GC were included as cases.

For case control study II, which was performed to compare clinical characteristics and outcomes of GC in IBD cases to GC in the general population, survival plots were derived from Kaplan–Meier curves. Confounder correction was performed with a Cox regression model with forward sampling. A covariate was considered as a confounder when the beta coefficient of the variable of interest changed by 10% or more\textsuperscript{22}. TNM stage was included as fixed variable. All missing values were considered to be at random and were therefore excluded from analyses. For our analysis we used IBM SPSS statistics version 20.0 for windows (SPSS In., Chicago, IL). A p-value of < 0.05 was considered statistically significant.
Results

Selection of cases and controls

With the PALGA search we identified 478 possible cases of GC in IBD patients (Figure 1). After an initial selection based on pathology reports and subsequent biopsy revision, medical record research was performed in 92 patients. In total, 33 patients were excluded: 15 because they had no confirmed diagnosis of IBD, six had no primary gastric carcinoma, six had esophageal carcinoma, three because IBD was diagnosed more than six months after GC diagnosis and three patients were excluded for other reasons. Finally, 59 patients were included with both IBD and GC.

To identify risk factors in IBD patients (case control study I), we randomly selected a control group consisting of 177 IBD patients from the IBDSL cohort (based on a 1:3 ratio).

For case control study II, we selected controls from the ECR. This search yielded 1534 non-IBD GC patients in the general population. We excluded 195 patients (110 lymphomas, 50 gastrointestinal stromal tumors, 30 neuro-endocrine tumors, two sarcomas and three for other reasons), resulting in 1339 GC patients selected from the general population from January 2004 until December 2008.

Case control study I. Risk factors: cases versus IBD controls

Table 1 displays the univariable comparison of potential risk factors for GC development between IBD cases with GC and IBDSL controls. Both age at IBD diagnosis (median age 61 year versus 40; p<0.01) and the number of UC patients (69.5% versus 51.4%; p<0.01) significantly differed between cases and controls. There was a trend towards over-representation of male gender in the case group (p=0.06). We found no difference in disease localization and behavior, IBD related surgery and use of 5-aminosalicylates, methotrexate and cyclosporin. However, use of steroids, thiopurines and
Table 1a / b: Risk factors for the development of gastric cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>A: All IBD cases</th>
<th>B: IBD diagnosis after 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (y), median</td>
<td>43.0</td>
<td>37.0</td>
</tr>
<tr>
<td>Female sex; n (%</td>
<td>88 (48.2)</td>
<td>41 (41.4)</td>
</tr>
<tr>
<td>Ulcerative colitis; n (%)</td>
<td>91 (51.4)</td>
<td>41 (41.4)</td>
</tr>
<tr>
<td>Crohn’s disease; n (%)</td>
<td>86 (48.6)</td>
<td>13 (13.0)</td>
</tr>
<tr>
<td>Proctitis (E1); n (%)</td>
<td>21 (22.2)</td>
<td>7 (17.1)</td>
</tr>
<tr>
<td>Left-sided colitis (E2); n (%)</td>
<td>48 (53.3)</td>
<td>20 (48.8)</td>
</tr>
<tr>
<td>Pancolitis (E3); n (%)</td>
<td>22 (24.4)</td>
<td>14 (34.1)</td>
</tr>
<tr>
<td>Upper digestive (L4) (yes); n (%)</td>
<td>7 (8.1)</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td>5-aminosalicylates; n (%)</td>
<td>155 (88.1)</td>
<td>38 (79.2)</td>
</tr>
<tr>
<td>Steroids; n (%)</td>
<td>116 (65.9)</td>
<td>18 (38.3)</td>
</tr>
<tr>
<td>Thiopurines; n (%)</td>
<td>75 (42.4)</td>
<td>7 (15.2)</td>
</tr>
<tr>
<td>Methotrexate; n (%)</td>
<td>9 (5.1)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Cyclosporin; n (%)</td>
<td>4 (2.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Biologicals; n (%)</td>
<td>38 (21.7)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>IBD related surgery; n (%)</td>
<td>49 (27.7)</td>
<td>13 (26.3)</td>
</tr>
<tr>
<td>Follow up (y), median</td>
<td>8.3</td>
<td>6.4</td>
</tr>
</tbody>
</table>

A: all cases included in the analysis; B: sensitivity analysis: only cases included with IBD diagnosis after 1990

IBD = Inflammatory Bowel diseases; IBD SL = IBD South Limburg cohort; #: only smoking data from Crohn’s disease patients; ##: classified according to Montreal classification

Final multivariable regression model after adjustment for duration of follow up since IBD diagnosis and backward elimination of non-significant variables for the identification of independent risk factors to develop gastric cancer. Similar inclusion periods of IBD diagnosis (since 1991) for cases and controls were used in the sensitivity analysis.

IBD, inflammatory bowel disease; + med: including medication in analysis.

Table 2: Final model of binary logistic regression analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Coefficient β</th>
<th>Odds Ratio (95 % CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases (n=41)</td>
<td>Age at IBD diagnosis</td>
<td>0.081</td>
<td>1.084 (1.043 – 1.126)</td>
<td>0.000</td>
</tr>
<tr>
<td>Sensitivity analysis (n=29)</td>
<td>Age at IBD diagnosis</td>
<td>0.073</td>
<td>1.075 (1.034 – 1.119)</td>
<td>0.000</td>
</tr>
<tr>
<td>All cases (n=18)</td>
<td>Age at IBD diagnosis</td>
<td>0.066</td>
<td>1.068 (1.022 – 1.117)</td>
<td>0.004</td>
</tr>
<tr>
<td>Sensitivity analysis (n=15)</td>
<td>Age at IBD diagnosis</td>
<td>0.048</td>
<td>1.049 (1.012 – 1.088)</td>
<td>0.010</td>
</tr>
<tr>
<td>All cases (n=15)</td>
<td>Age at IBD diagnosis</td>
<td>0.069</td>
<td>1.072 (1.024 – 1.122)</td>
<td>0.003</td>
</tr>
<tr>
<td>No risk factors identified</td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

Final multivariable regression model after adjustment for duration of follow up since IBD diagnosis and backward elimination of non-significant variables for the identification of independent risk factors to develop gastric cancer. Similar inclusion periods of IBD diagnosis (since 1991) for cases and controls were used in the sensitivity analysis.

IBD, inflammatory bowel disease; + med: including medication in analysis.
anti-TNF agents was significantly higher in the control group compared to cases: steroids 65.9% versus 38.3% (p<0.01); thiopurines 42.2% versus 15.2% (p<0.01) and anti-TNF agents 21.7% versus 2.1 (p<0.01), respectively. In Table 1b only cases with an IBD diagnosis after 1990 are included, but the same risk factors (age at IBD diagnosis and IBD type) still emerged. In addition, cases used less 5-aminosalisylic acids (71.9% versus 88.1%; p=0.03).

In the multivariable analysis (Table 2) age at IBD diagnosis and gender were included. Second, medication use (corticosteroids, thiopurines and anti-TNF agents) was added. In the sensitivity analysis, the same factors were included with addition of 5-aminosalisylic acids, as it was a potential confounder (Table 1b). In both the analysis including all cases and in the sensitivity analysis, age at IBD diagnosis was identified as risk factor for GC development in IBD patients. This applied to both UC (OR 1.043 – 1.12; p<0.01 and OR 1.034 – 1.119; p<0.001) and CD (OR 1.012 – 1.088; p=0.01 and OR 1.01 – 1.09; p=0.03).

Case control study II. Clinical characteristics and outcomes: cases versus general population
We found no difference in age at diagnosis, gender, tumor location and tumor differentiation between the IBD patients and general population with GC (Table 3). Although IBD patients presented with a more advanced T-stage (pT3 48.8% versus 28.1%; p=0.01), they had comparable lymph node stages and distant metastases rates. IBD patients were more extensively treated with surgery (57.6% and 32.2%; p=0.01) and chemotherapy (40.8% and 20.6%; p=0.01) at initial treatment.

Most IBD patients with GC presented with an intestinal type GC (59.3%). Histological classification was lacking in 915 out of 1339 GC patients in the general population. Of the remaining 424 patients, 20.3% presented with an intestinal type GC.

We found no survival difference in the univariable analysis (Figure 2A; p=0.53), but when corrected for confounders,
IBD cases showed a poorer survival (Figure 2B; hazard ratio 1.385 (95% confidence interval 1.023 – 1.875)).

At least 19 patients received different (combinations of) IBD medication after GC diagnosis. We found no difference in survival comparing IBD patients with immunosuppressive therapy after GC diagnosis (n=10) versus ECR controls (n=1339; p=0.86).

Histopathology: exploring the etiology of IBD related gastric cancer

Additional staining of the GC was performed in 48 of 59 IBD cases (Figure 3). We excluded 11 cases for analysis due to insufficient amounts of tissue. Two of 48 tumors (4.2%) were EBER positive, four (8.3%) tumors lacked protein expression of MLH1 and PMS2. There were no cases without protein expression of MSH2 and MSH6.

The medical history before the GC diagnosis revealed a prior upper endoscopy in 18 IBD patients (30.5%). Histologic gastritis was present in ten, intestinal metaplasia in nine and dysplasia in one patient. Twenty-one (46.7%) out of 46 patients had a positive history for tobacco use, 21 (56.8%) out of 37 for alcohol use. Fifteen (25.4%) patients were H. pylori positive and six patients had a positive family history of GC.

Discussion

This nationwide cohort study described GC in IBD patients and showed that GC survival was significantly worse in IBD in the multivariable analysis compared to the general population after correction for confounders. Other clinical characteristics were comparable between IBD cases and GC in the general population. Elderly onset IBD emerged as a risk factor for GC development in IBD patients, particularly in UC patients.

There is only limited information available on GC survival in IBD,
partially explained by the low absolute risk to develop gastric cancer in IBD patients. Unlike our results, Shu et al. found no difference in GC survival between IBD patients and the general population. This may be explained by the small number of GC patients with IBD and short follow-up duration (median follow up 5 months) in the latter study.

An impaired prognosis compared to the general population for IBD patients with cancer was previously described for CRC, lymphomas, and bladder cancer. As immunosuppressive therapy may promote tumor progression, we studied the survival of patients with immunosuppression use after GC diagnosis, but found no difference in survival related to immunosuppression use (p=0.86; adjusted for confounders and TNM stage). However, this subanalysis included only 10 IBD patients and should be interpreted with caution.

Unexpectedly, we found that older age at IBD diagnosis is a risk factor for developing GC. With both ageing populations and an increasing IBD prevalence, this group of elderly IBD patients is rapidly expanding. In our univariable comparisons we found less use of medical therapy in the IBD GC cases (with older IBD onset). However, in multivariable analysis no differences in medication use between IBD GC cases and IBD controls were found and therapy-induced immunosuppression seems not linked to GC development in IBD patients in our study. It could be speculated that the genotype and phenotype of elderly onset IBD are associated with a higher cancer risk and thus making these patients more susceptible to (gastric) cancer development.

We analyzed the number of EBV-positive GC’s in IBD patients, but did not find an increase of EBV-positive tumors compared to sporadic GC. Only 2 out of 48 (4.2%) of the GC’s were EBV positive, which is in line with the literature, suggesting that decreased immunosurveillance of EBV is not a causative factor for GC in IBD. Similarly, we considered H. pylori as a causative factor for GC development in IBD, since this infection is present in up to 80% of sporadic GC patients. In our IBD cohort only 25% of GC patients had a positive H. pylori status, which is in line with previous literature. This suggests that it is unlikely that impaired immune surveillance of H. pylori causes and increased risk for GC in IBD. Treatment with 5-aminosalicylates may protect against H. pylori. In the majority of our patients (79.2%) 5-aminosalicylates were part of the treatment strategy. The use of antibiotics in IBD treatment unintentionally resulting in H. pylori eradication, could also explain the lower H. pylori prevalence.

Although gastric inflammation in CD could be a causative factor for gastric carcinogenesis, we found more frequent GC development in UC patients than in CD (69.5 % versus 51.4%; p<0.01). This is in contrast to a previous meta-analysis by Pedersen et al. suggesting that CD patients are at increased risk for GC development. However, the different time frame of this study may explain this difference. While we presented data from a period (2004-2008) that reflects modern treatment strategies, the previous meta-analysis was based on periods between 1950 and 2004. Furthermore, the larger amount of UC patients may be caused by the fact that UC is more prevalent in elderly onset IBD and that elderly onset is the most important risk factor for GC development in IBD.

Recent TCGA (The Cancer Genome Atlas) data demonstrated a new GC classification, based on molecular background of GC’s, dividing GC into EBV positive, microsatellite instable, genomic stable and chromosomal instable tumors. Genomic stable histology represents mainly diffuse tumors, as chromosomal instable tumors represent mainly intestinal histology. Compared to these series, we found an increased percentage of diffuse tumors (35.4% versus 19.7%, Figure 3). Diffuse GC are associated with a CDH1 mutation, which is also important in the epithelial barrier function in UC, but not in CD. This characteristic might contribute to our finding of more UC than CD patients with GC.

Several limitations can be recognized in the current study. We used two prospective databases to answer our research questions,
in contrast to our retrospectively collected data about the GC cases. This may cause an information bias. Furthermore, the limited number of IBD GC cases, although the largest series thus far, may result in type 2 errors. Therefore, the conclusions must be interpreted with caution.

We are also aware that IBD GC cases and IBD controls are obtained from different databases and that the source populations of these databases are not completely identical (the Netherlands versus part of the Netherlands). This can give a selection bias, but IBD cases and controls are treated in the Netherlands according to the same IBD standards. Furthermore, The Netherlands is a small country with limited geographical differences. Unfortunately, the required data needed to address our research questions could not be extracted from one single database, which would have been preferable.

Furthermore, data collection was different for cases and controls, as we studied medical records for GC cases and retrieved GC control data from the ECR database. The imbalance of missing data, for example for TNM stage, and different ways of data ascertainment may impact our results.

The IBDSL cohort only included patients diagnosed since 1991 and excluded patients with a final diagnosis of indeterminate colitis. This may have caused a selection bias and resulted in different treatment regimes due to differences in time frame, as we included IBD patients with GC diagnosed before as cases. Therefore, we performed a sensitivity analyses in which we only included the cases with an IBD diagnosis after 1990. These analyses showed similar results compared to the analyses of the case group as a whole. Furthermore, we included follow up as fixed factor in the analyses to correct for differences in follow up duration.

In conclusion, survival of GC patients in our study was significantly worse in IBD patients compared to the general population in the multivariable analysis, when corrected for confounders. However, age at GC diagnosis and TNM-stage were comparable between IBD cases and controls. Elderly onset IBD emerged as a risk factor for GC development, particularly in UC patients.

Conflicts of interest and Source of funding
None to declare.

Contributorship statement

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Solid malignancies in inflammatory bowel diseases

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3.2
Risk factors and clinical outcomes in patients with IBD with melanoma

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Abstract

Background
Patients with inflammatory bowel disease (IBD) are at increased risk to develop malignant melanoma and this risk may increase with use of anti-tumor necrosis factor (TNF) therapy. Impaired survival of immunosuppressed melanoma patients is reported in transplant and rheumatology patients.

This study aims to (1) identify risk factors for melanoma development in patients with IBD, (2) compare clinical characteristics of melanoma in patients with IBD to the general population, and (3) assess the influence of immunosuppressive medication on survival.

Methods:
We retrospectively searched the Dutch Pathology Database to identify all Dutch patients with IBD with cutaneous melanoma between January 1991 and December 2011.

We then performed two case-control studies. To identify risk factors for melanoma development in IBD, we compared patients with IBD with melanoma to the general IBD population. To compare outcome and survival after melanoma diagnosis, we compared cases with non-IBD melanoma patients.

Results:
We included 304 patients with IBD with melanoma, 1,800 IBD controls, and 8,177 melanoma controls. IBD cases had more extensive IBD (ulcerative colitis: pancolitis: cases 44.5% versus IBD controls without melanoma 28.1%; p < 0.01; Crohn’s disease: ileocolonic disease: cases 57.9% versus controls 48.9%; p = 0.02).

Despite a lower Nodes (N)-stage in patients with IBD (N1+ 8.3% versus 18.2%; p<0.01) with comparable Tumor (T) and Metastasis (M) stages, survival was similar in all three groups, regardless of immunosuppressive or anti-TNF therapy.

Conclusion
This study showed that IBD extent is a risk factor for melanoma development. Despite the lower N-stage in patients with IBD, we could not confirm impaired survival after melanoma in patients with IBD, regardless of anti-TNF with or without thiopurine use.

Key words
inflammatory bowel diseases, melanoma, immunosuppressive therapy
Introduction

Patients with inflammatory bowel disease (IBD) are at increased risk to develop nonmelanoma skin cancer (also called keratinocyte carcinoma)\(^1\) - \(^5\), especially those patients on thiopurine therapy\(^3\) - \(^7\). In addition, a recent meta-analysis showed that the risk for developing cutaneous melanoma is slightly elevated in patients with IBD compared with the general population\(^2\) - \(^8\). Anti-tumor necrosis factor (TNF) therapy may further increase the risk of melanoma development in patients with IBD\(^3\), as is the case in rheumatic arthritis patients on anti-TNF therapy\(^9\). Thiopurine therapy does not seem to affect the risk of melanoma development in IBD\(^3\).

Melanoma is considered an immunogenic malignancy: melanoma tumor antigens can trigger the immune response. Therefore, it is possible that immunosuppression can influence its clinical behavior. Indeed, immunosuppressed patients with cutaneous malignant melanoma were more likely to die of melanoma compared with immunocompetent controls\(^10\) - \(^12\). Overall and melanoma cause-specific 3-year survival were significantly worse in immunosuppressed patients, especially with increasing Breslow thickness\(^13\).

In patients with IBD, additional risk factors for melanoma are unknown. Furthermore, the clinical course of melanoma in patients with IBD remains unclear, as well as the influence of immunosuppressive IBD therapy on the survival. Therefore, we designed a case-control study with 2 specific aims. First, we aimed at identifying IBD-specific risk factors for melanoma development by comparing patients with IBD to patients with IBD without melanoma. Secondly, we researched clinical characteristics and survival after melanoma by comparing patients with IBD with melanoma to unselected ‘non-IBD’ melanoma patients.

Materials and Methods

Study design

We conducted two retrospective case-control studies in The Netherlands (Figure 1).

The first case-control study (I) was performed to identify risk factors for melanoma development in patients with IBD. The second case-control study (II) was performed to compare the clinical course after melanoma. This study was approved by the PALGA Privacy Commission and Scientific Council and by the medical ethical committee of the Leiden University Medical Center (number P13.034), The Netherlands.

IBD case selection

All patients diagnosed with both IBD and cutaneous melanoma between January 1991 and December 2011 in The Netherlands were identified by searching the PALGA-database. PALGA is the Dutch nationwide network and registry of histo- and cyto-pathology, which has complete national coverage for academic and non-academic hospitals since 1991\(^14\). Multiple studies on malignancies in IBD were previously performed using PALGA including gastric cancer, neuro-endocrine tumors, colon cancer and pouch cancer\(^15\) - \(^17\). In the current study, the following search terms were used: “Crohn’s Disease”, “Ulcerative Colitis”, “Inflammatory Bowel Disease”, “terminal ileitis”, “regional ileitis”, “idiopathic colitis”, “chronic idiopathic bowel disease” or “enteritis regionalis” and combined them with “melanoma (primary and metastasis)” or “melanoma in situ”. This search looked for all cases that included any one or more of the first set of search terms and either of the second set of search terms. Based on the PALGA search results, anonymized patient charts in all hospitals were evaluated to confirm diagnoses and to collect additional demographic and clinical data. The IBD diagnosis was based on a combination of clinical, endoscopic, histological and radiographic criteria\(^18\) and classified according the Montreal classification\(^19\).
All patients with IBD with primary cutaneous (in situ or invasive) melanoma were included in the study. Patients were excluded when either the IBD or melanoma diagnosis could not be confirmed, the incidence date of the primary melanoma was before 1991 or after 2011, IBD was diagnosed after melanoma diagnosis, or when the patient was known to have familial melanoma syndrome.

Controls selection

Case-control study I: risk factor identification for melanoma development in patients with IBD

For the identification of melanoma risk factors, 1,800 non-melanoma IBD controls were randomly selected from the IBD South Limburg Cohort (IBDSL Cohort), based on a 1:3 ratio of the first PALGA search results. No matching was performed as we did not want to exclude any potential risk factors.

The IBDSL cohort is a population-based prospectively followed cohort in which all new cases of adult IBD are enrolled since 1991. South Limburg is an enclosed geographic area in the southeast of The Netherlands with 605,000 inhabitants and 3 hospitals. As cross-border health care is limited and migration rates are low, South Limburg provides a good setting for population-based research. In total 2,807 patients with IBD (40.9% Crohn’s disease [CD], 59.0% ulcerative colitis [UC]) are included in the IBDSL Cohort, which represents more than 93% of the IBD population in South Limburg.

Analyses were performed between IBDSL controls and (1) IBD cases with invasive melanoma (2) IBD cases with in situ melanoma and (3) all IBD cases with either invasive melanoma or in situ melanoma, to increase the statistical power.

Case-control study II: melanoma characteristics and clinical course in patients with IBD

To compare clinical characteristics and outcomes of melanoma between patients with IBD and the general population, non-IBD controls were selected from the (Eindhoven) Netherlands Cancer Registry (NCR; managed by the Netherlands Comprehensive Cancer Organisation [NCCO]) from January 1991 until December 2011. Since 1989, the NCR registers the incidence of cancer in the Netherlands, for this study data of the region “Noord-Brabant” and “Noord-Limburg”, two provinces in the south of The Netherlands, covering an area with 2.3 million inhabitants were used. This specific region was used as in this region data on comorbidity (including IBD) was registered. We used the search terms “melanoma” and “melanoma in situ”. Only data on cutaneous (in situ) melanoma were retrieved from the NCR.

Data collection

For IBD cases, the following data were anonymously collected: age, gender, medical history including a diagnosis of primary sclerosing cholangitis, alcohol and smoking history, type of IBD based on histopathologic evaluation, date of IBD diagnosis, IBD phenotype according to the Montreal Classification, use and duration of IBD related immunosuppressive therapy (corticosteroids, thiopurines, methotrexate, cyclosporine and anti-TNF therapy), and previous IBD surgery. For IBD controls, the same variables were collected, although alcohol and smoking history were only partially available and duration of immunosuppressive medication was not available.

The following melanoma variables were collected for both cases and controls: year of melanoma diagnosis, localization, histopathologic evaluation, clinical and pathological tumor stage according the TNM classification (fourth edition until 1992; revised fourth edition 1993 until 1998; fifth edition 1999 until 2002; sixth edition 2003 until 2009), Breslow thickness, Clark level, treatment (surgery, chemotherapy [inclu-
died immunotherapy] and radiotherapy), and overall survival.

IBD cases were anonymously and encrypted linked to the NCR database, for reasons of quality control and completion of collected melanoma data.

Statistical analyses
IBD cases were compared with IBDSL and NCR controls using univariable analyses to identify potential risk factors and to compare clinical characteristics. For the univariable analysis, we used a χ²-test or Fisher exact test (if expected cell counts were <5) for categorical data and independent Student’s t-test or Mann-Whitney U test for continuous data. Variables with a p value of <0.1 in the univariable analyses were included in the multivariable analyses. A p-value of < 0.05 was considered statistically significant. All our analyses were performed using IBM SPSS statistics version 20.0 for Windows (SPSS In., Chicago, IL).

For case-control study I, a binary logistic regression analysis with backward elimination of non-significant confounders was performed to determine risk factors for patients with IBD to develop a melanoma. The odds ratios (ORs) were presented with 95% confidence interval (95% CI). This multivariable model was always adjusted for the duration of follow-up (fixed variable), to correct for differences in follow-up between IBD cases and IBDSL controls. For the IBD cases, follow-up was defined as time since IBD diagnosis until melanoma diagnosis. For controls, follow-up was defined as time since IBD diagnosis until death or end of follow up.

As medication use in especially the distant past might not be reliable and may be different from current regimes, medical therapy was not included in the first multivariable analysis. Therefore, multivariable logistic regression analysis was performed including patients with an IBD diagnosis dating from 1991 in both the case and control group (sensitivity analysis). As UC and CD are classified different according to the Montreal classification, multivariable analyses were performed separately for UC and CD.

For case-control study II survival plots were derived from Kaplan–Meier curves. Hazard ratios (HRs) were calculated. Confounder correction was performed using a Cox regression model with forward sampling. A covariate was considered as a confounder when the beta coefficient of the variable of interest changed by 10% or more.

Results
Selection of cases and controls
Five hundred eighty possible cases of patients with IBD with melanoma were identified (Figure 1), of which 200 were excluded after careful assessment (Figure 1). An additional 76 patients were excluded because IBD was diagnosed after the melanoma diagnosis. In total, 304 patients who developed a melanoma after IBD diagnosis were included (57 in situ and 247 invasive melanoma).

For case-control study I, we randomly selected a nonmelanoma control group consisting of 1800 patients with IBD from the IBDSL cohort (Figure 1).

For case-control study II, the NCR search yielded 8518 non-IBD melanoma patients in the general population (Figure 1). We excluded 341 patients (259: unknown primary site and 82: second melanoma), resulting in 8177 melanoma patients selected from the general population.

Case control study I: risk factor identification for melanoma development in Patients with IBD
IBD extent differed between IBD cases and IBDSL controls (Table 1): pancolitis was more common in comparison with UC controls (cases 44.5% versus controls 28.1%; p < 0.01). CD was more often located in the ileum and colon (cases 57.9% versus controls 48.9%; p = 0.02) and was more often penetrating (35.0% versus 23.6%; p < 0.01). Furthermore, primary sclerosing
### Table 1: Risk factors for melanoma development

<table>
<thead>
<tr>
<th>Variables</th>
<th>IBDSL</th>
<th>Melanoma total</th>
<th>Missing</th>
<th>p-value</th>
<th>melanoma in situ</th>
<th>Missing</th>
<th>p-value</th>
<th>Malig melanoma</th>
<th>Missing</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (y), median (25%; 75%)</td>
<td>39 (28; 54)</td>
<td>41 (28; 57)</td>
<td>0 / 0</td>
<td>0.39</td>
<td>4 / 5 (35; 62)</td>
<td>0 / 0</td>
<td>0.05 *</td>
<td>3 / 9 (28; 55)</td>
<td>0 / 0</td>
<td>0.97</td>
</tr>
<tr>
<td>Female sex; n (%)</td>
<td>983 (53.5)</td>
<td>178 (546)</td>
<td>0 / 0</td>
<td>0.10</td>
<td>3 / 0 (57; 9)</td>
<td>0 / 0</td>
<td>0.51</td>
<td>1 / 5 (50; 7)</td>
<td>0 / 0</td>
<td>0.12</td>
</tr>
<tr>
<td>Smoking (only CD patients); n (%)</td>
<td>131 (97)</td>
<td>6 / 1 (0.4)</td>
<td>20 / 106</td>
<td>0.01 *</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>&lt; 0.01 *</td>
<td>6 / 17</td>
<td>20 / 84</td>
<td>&lt; 0.01 *</td>
</tr>
<tr>
<td>Ulcerative colitis; n (%)</td>
<td>100 (95.0)</td>
<td>178 (7.55)</td>
<td>0 / 0</td>
<td>0.23</td>
<td>3 / 2 (9.3)</td>
<td>0 / 0</td>
<td>0.61</td>
<td>1 / 8 (9.4)</td>
<td>0 / 0</td>
<td>0.26</td>
</tr>
<tr>
<td>Ulcerative colitis; n (%)</td>
<td>0 (0.0)</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>0.07</td>
<td>1 / 7 (12.0)</td>
<td>0 / 0</td>
<td>0.07</td>
<td>1 / 6 (12.0)</td>
<td>0 / 0</td>
<td>0.07</td>
</tr>
<tr>
<td>C reactive protein (mg/L); n (%)</td>
<td>243 (24.4)</td>
<td>19 (11.0)</td>
<td>0 / 0</td>
<td>&lt; 0.01 *</td>
<td>2 / 6 (5.1)</td>
<td>0 / 0</td>
<td>0.07</td>
<td>1 / 17 (10.0)</td>
<td>0 / 0</td>
<td>&lt; 0.01 *</td>
</tr>
<tr>
<td>Colon (Li); n (%)</td>
<td>183 (23.0)</td>
<td>32 (26.4)</td>
<td>4 / 18</td>
<td>0.89</td>
<td>4 / 18 (22.2)</td>
<td>4 / 18</td>
<td>0.89</td>
<td>4 / 18 (22.2)</td>
<td>4 / 18</td>
<td>0.89</td>
</tr>
<tr>
<td>Intestinal dilatation (Li); n (%)</td>
<td>399 (46.9)</td>
<td>70 (37.9)</td>
<td>0 / 0</td>
<td>0.07</td>
<td>1 / 3 (5.1)</td>
<td>1 / 0</td>
<td>0.07</td>
<td>1 / 3 (5.1)</td>
<td>1 / 0</td>
<td>0.07</td>
</tr>
<tr>
<td>Proliferating (B2); n (%)</td>
<td>61 (82)</td>
<td>12 / 8 (38.3)</td>
<td>0 / 1</td>
<td>0.50</td>
<td>2 / 8 (25.0)</td>
<td>0 / 0</td>
<td>0.70</td>
<td>1 / 3 / 1 (33.8)</td>
<td>0 / 0</td>
<td>0.70</td>
</tr>
<tr>
<td>Penetrating (B3); n (%)</td>
<td>100 (23.0)</td>
<td>42 (35.0)</td>
<td>0 / 1</td>
<td>&lt; 0.01 *</td>
<td>1 / 0 (45.5)</td>
<td>0 / 0</td>
<td>0.02 *</td>
<td>1 / 0 (45.5)</td>
<td>0 / 0</td>
<td>0.02 *</td>
</tr>
<tr>
<td>Steroids; n (%)</td>
<td>113 (62.2)</td>
<td>69 (75.1)</td>
<td>12 / 79</td>
<td>0.01 *</td>
<td>2 / 9 (74.1)</td>
<td>12 / 18</td>
<td>0.14</td>
<td>1 / 8 (75.0)</td>
<td>12 / 61</td>
<td>&lt; 0.01 *</td>
</tr>
<tr>
<td>Thromboprophylaxis; n (%)</td>
<td>717 (46.2)</td>
<td>104 (55.6)</td>
<td>17 / 66</td>
<td>0.47</td>
<td>1 / 0 (4.2)</td>
<td>17 / 15</td>
<td>0.73</td>
<td>8 / 4 (20.0)</td>
<td>17 / 45</td>
<td>0.52</td>
</tr>
<tr>
<td>Methotrexate; n (%)</td>
<td>95 (53)</td>
<td>7 / 3 (23.1)</td>
<td>10 / 79</td>
<td>0.36</td>
<td>0 / 0</td>
<td>10 / 17</td>
<td>0.27</td>
<td>7 / 17 (41.7)</td>
<td>10 / 62</td>
<td>0.37</td>
</tr>
<tr>
<td>Cyclosporine; n (%)</td>
<td>36 (15.5)</td>
<td>7 / 3 (23.1)</td>
<td>10 / 87</td>
<td>0.08</td>
<td>1 / 0</td>
<td>10 / 19</td>
<td>0.44</td>
<td>6 / 4 (34.8)</td>
<td>10 / 68</td>
<td>0.06</td>
</tr>
<tr>
<td>Anti-TNF therapy; n (%)</td>
<td>359 (19.7)</td>
<td>37 / 13.2</td>
<td>25 / 60</td>
<td>0.09</td>
<td>6 / 0 (11.0)</td>
<td>25 / 15</td>
<td>0.09</td>
<td>3 / 0 (11.0)</td>
<td>25 / 45</td>
<td>0.19</td>
</tr>
<tr>
<td>IBD related surgery; n (%)</td>
<td>128 / 7 (13.9)</td>
<td>206 / 6.7 (83)</td>
<td>0 / 0</td>
<td>0.21</td>
<td>1 / 8 (32.1)</td>
<td>0 / 0</td>
<td>0.54</td>
<td>1 / 6 (36.4)</td>
<td>0 / 0</td>
<td>0.36</td>
</tr>
<tr>
<td>Duration of follow up since IBD</td>
<td>7.50 (1, 13)</td>
<td>90.0 (4, 17)</td>
<td>0 / 0</td>
<td>&lt; 0.01 *</td>
<td>8.08 (13.2, 55)</td>
<td>0 / 0</td>
<td>0.12</td>
<td>9.00 (4.10)</td>
<td>0 / 0</td>
<td>&lt; 0.01 *</td>
</tr>
</tbody>
</table>

IBD = Inflammatory Bowel Diseases; IBDSL = IBD South Limburg cohort (no melanoma cases); #: only smoking data from Crohn’s disease patients; ##: classified according to Montreal.
cholangitis was more common in cases (3.0% versus 0.7%; p = 0.01) compared with IBDSL controls. We found no difference in IBD-related surgery and use of thiopurines, anti-TNF therapy, methotrexate and cyclosporine between cases and controls. However, use of corticosteroids was significantly higher in cases compared with controls: 75.1% versus 62.2% (p<0.01).

In the multivariable analysis (Table 2), IBD extent was identified as a risk factor for melanoma development in patients with IBD, both in UC (pancolitis OR 3.09; 95% CI 1.670 – 5.727) and CD (ileocolonic disease: OR 1.98; 95% CI 1.009 – 3.882). The sensitivity analysis provided similar results.

In the sensitivity analysis for UC, we found corticosteroid use as risk factor (OR 1.41 – 3.72) and anti-TNF use as a protective factor for melanoma development in UC (OR 0.15 – 0.88) and CD (0.27 – 0.92). This difference was mainly attributed to the in situ melanoma, as this effect was not found for invasive melanoma.

Case control study II: melanoma characteristics and outcome in Patients with IBD

No differences were found in age at diagnosis, sex, tumor location, tumor histopathology, Breslow thickness and melanoma treatment strategy between the IBD cases and controls with melanoma from the general population (Table 3). Patients with IBD presented with a less advanced N-stage (N+ 8.3% versus 18.2%; p < 0.01), but they had comparable T and M stages.

We found no differences in the univariable (Kaplan Meier) survival analyses for all cases (p = 0.42), in situ (p = 0.63) and malignant melanoma (p = 0.68). Additional (multivariable) analyses for males, females, Breslow thickness > 2 or 4 mm and IBD diagnosis showed no difference.

We compared IBD cases on immunosuppressive medication after melanoma diagnosis with NCR controls. In the univariable analysis, we found no difference in survival in patients with IBD with melanoma using corticosteroids (n = 97; p = 0.93) and a better

### Table 2: Final model of binary logistic regression analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All melanoma</strong>&lt;br&gt;Ulcerative colitis (all cases = 178)</td>
<td>Montreal E3 disease</td>
<td>3.09 (1.67 – 5.73) *</td>
</tr>
<tr>
<td>Crohn’s Disease (all cases = 121)</td>
<td>Montreal L2 disease</td>
<td>2.62 (1.21 – 5.68) *</td>
</tr>
</tbody>
</table>
| &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &n
survival of patients with IBD using anti-TNF (n=35; p=0.026 Figure 2A) after melanoma diagnosis. In addition, we detected a trend towards improved survival in patients using thiopurines (n = 83; p = 0.058; Figure 2B). In the multivariable analysis, we found no difference in survival in patients with IBD using anti-TNF (Hazard Ratio (HR) 0.32; 95% CI 0.08-1.27) and thiopurines (HR 0.72; 95% CI 0.37 – 1.31).

Finally, we compared the survival of IBD cases who did and did not use immunosuppressive medication after melanoma diagnosis. Patients (141 of 269) used immunosuppression after melanoma diagnosis; 23 / 141 (16.3%) had recurrent disease, compared with 13 / 128 patients with IBD (10.2%) without immunosuppressive medication use after IBD diagnosis (p = 0.14).

Univariable, we found no difference in survival for patients using corticosteroids (p = 0.41), a trend toward better survival in patients with IBD taking thiopurines (p = 0.06; Figure 2D) and a better survival of patients using anti-TNF (p = 0.038; Figure 2C). In the multivariable analysis, no differences in survival for patients with IBD using anti-TNF were observed (HR 0.16, 95% CI 0.02 – 1.21). The HR of patients with IBD using thiopurines was 0.55 in the multivariable analysis (95% CI 0.25 – 1.23).

Discussion

This nationwide cohort study describes risk factors for and the clinical course of melanoma in patients with IBD. The results indicate that a more extensive IBD phenotype is a risk factor for melanoma development in patients with IBD. Histology, location, and survival of melanoma in patients with IBD are similar compared with the general population. The use of anti-TNF therapy and/or thiopurines did not impair survival after melanoma.

It has been described in other cohorts of immunosuppressed (transplant) patients have that survival after melanoma diagnosis is

<table>
<thead>
<tr>
<th>All melanoma patients</th>
<th>IBD patients N = 304</th>
<th>NCR patients N = 8177</th>
<th>Missing / NA (n)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (y), median (25 %; 75%)</td>
<td>54 (43, 66)</td>
<td>55 (42, 67)</td>
<td>0 / 0</td>
<td>0.53</td>
</tr>
<tr>
<td>Female sex; n (%)</td>
<td>178 (58.6)</td>
<td>4779 (58.4)</td>
<td>0 / 0</td>
<td>0.97</td>
</tr>
<tr>
<td>In situ melanoma; n (%)</td>
<td>57 (19.3)</td>
<td>1533 (19.3)</td>
<td>7 / 200</td>
<td>0.99</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentigo maligna</td>
<td>35 (13.6)</td>
<td>862 (15.2)</td>
<td>4 / 0</td>
<td>0.86</td>
</tr>
<tr>
<td>Nodular</td>
<td>40 (15.5)</td>
<td>823 (14.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial spreading</td>
<td>173 (67.1)</td>
<td>3798 (66.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10 (3.9)</td>
<td>193 (3.4)</td>
<td></td>
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</tr>
<tr>
<td>Not otherwise specified</td>
<td>42</td>
<td>2501</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head / neck; n (%)</td>
<td>49 (16.3)</td>
<td>1569 (19.2)</td>
<td>9 / 28</td>
<td>0.49</td>
</tr>
<tr>
<td>Trunk; n (%)</td>
<td>107 (35.7)</td>
<td>2849 (34.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm / shoulder; n (%)</td>
<td>68 (22.7)</td>
<td>1603 (19.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg / hip; n (%)</td>
<td>76 (25.3)</td>
<td>2132 (26.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breslow thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1</td>
<td>131 (56.5)</td>
<td>3064 (53.5)</td>
<td>72 / 2451</td>
<td>0.84</td>
</tr>
<tr>
<td>1.01 - 2</td>
<td>51 (22.0)</td>
<td>1329 (23.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.01 – 4</td>
<td>30 (12.9)</td>
<td>816 (14.3)</td>
<td></td>
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</tr>
<tr>
<td>&gt; 4</td>
<td>20 (8.6)</td>
<td>517 (9.0)</td>
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<tr>
<td>TNM stage</td>
<td></td>
<td></td>
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<tr>
<td>T-stage</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IS</td>
<td>57 (19.3)</td>
<td>1534 (19.4)</td>
<td>8 / 583</td>
<td>0.94</td>
</tr>
<tr>
<td>T1 – T3</td>
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</tbody>
</table>

* p < 0.05; IBD = inflammatory bowel diseases; NCR = Netherlands Cancer Registry.
impaired compared with the general population, especially for patients with higher Breslow thickness (> 1.5 mm). These data previously suggested the need for treatment adaptation in patients with IBD after a diagnosis of melanoma, by decreasing immune suppression. However, this is not supported by this study, as we found no differences in survival between IBD cases and the general population, also when specifically assessed for immunosuppression. Similar survival results were previously described in a smaller study of patients with IBD (n = 97), although this previous study had a longer inclusion period and shorter median follow up. However, our results suggesting similar survival between IBD and non-IBD melanoma patients must be interpreted with caution for several reasons. First, the differences with studies in transplant patients may be explained by the limited number of patients with IBD developing a melanoma and by the lower doses, shorter treatment duration and different combinations of immunosuppressive therapy in patients with IBD versus transplant patients. Most patients with IBD discontinue or switch immunosuppressive therapy, mainly because of side effects or loss of response, while transplant patients are in general lifelong on immunosuppressive therapy. Moreover, a significant percentage of patients with IBD is treated without immunosuppressive therapy. Second, Penn showed that the risk of recurrence is highest in the first 2 years after malignancy diagnosis in (renal) transplant patients. As the moment of starting immunosuppressive therapy after melanoma diagnosis varied widely in our study, this may have also influenced our data, also because it was not possible in this study to specify exactly when patients started or stopped certain immunosuppressive medication, because of the retrospective study design. Third, our finding that thiopurine or anti-TNF therapy did not affect survival may be explained by a selection bias: the fear of a (faster) recurrence implies that these treatments might have been started in patients with a favorable risk profile only.

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found to be a risk factor for melanoma development. IBD extent could be a risk factor because more extensive immune activation may be present, which might contribute to melanoma development. Although IBD extent and medication use are not the same, IBD extent could be a surrogate marker for more immunosuppression use, which was difficult to verify during chart reviews and therefore maybe underreported. Because IBDSL data are recorded prospectively, underreporting will occur less likely. This might also explain why anti-TNF therapy is even found as a protective factor in the sensitivity analysis.

This study has several limitations. To test our hypothesis, we used three different databases that were constructed in different ways. Our data search is for example retrospective, while the IBDSL, PALGA and the NCR collect data prospectively. The IBDSL cohort only included patients diagnosed since 1991. Our PALGA study group included patients with diagnosis before that date and also the total follow-up since diagnosis of IBD is longer. This may have resulted in different treatment regimes due to differences in time frame. Underreporting of immunosuppressiva in our retrospective PALGA cohort can be an explanation for the results. Because IBDSL data are prospectively, underreporting will occur less likely. Unfortunately, there is no single database available that could answer our research questions sufficiently.

We performed a sensitivity analyses in which only medication of patients diagnosed with IBD after 1990 was included. Follow-up was included as fixed factor in the analyses to correct for differences in follow-up duration, which were caused by differences in inclusion period. Furthermore, we did not have information about skin type, number of sun burns and melanoma mitotic index because it was not included in the registries and was often not recorded in the medical charts. Finally, for a number of variables in the analyses, there are considerable numbers of missing values. For these variables, results should be interpreted cautiously.

The major strength of this study is the large cohort of IBD and melanoma patients, showing reassuring data on survival. This can influence clinical decision making for patients with IBD with melanoma in daily practice, especially with the current rising incidence and prevalence of both IBD and melanoma. For patients with IBD who develop or had melanoma in the past, treatment decisions still require close collaboration between gastroenterologists, dermatologists, and oncologists, and they must be based on a thorough knowledge of the individual case, including the IBD activity, concomitant therapy and melanoma stage. In conclusion, this first study on the clinical course of melanoma in IBD shows no differences in the clinical characteristics of melanoma in patients with IBD and the general population, besides a lower N stage in patients with IBD. Overall survival and survival in patients with IBD treated with thiopurine or anti-TNF therapy were not impaired. Although these survival data from our nation-wide cohort are reassuring for daily practice, treatment choices remain dependent on individual risk assessment by treating physicians and patients.

Acknowledgements
The authors would like to thank Prof. Dr. L.A.L.M. Kiemeneij for his epidemiological and statistical advice.

The authors have no conflict of interest to disclose.
References


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Solid malignancies in inflammatory bowel diseases

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3.3

Risk factors and clinical outcomes of head and neck cancer in inflammatory bowel disease: a nationwide cohort study.

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10) Department of Gastroenterology and Hepatology, Jeroen Bosch Ziekenhuis, Den Bosch, The Netherlands
Abstract

Background
Immunosuppressed inflammatory bowel disease (IBD) patients are at increased risk to develop extra-intestinal malignancies. Immunosuppressed transplant-patients showed increased incidence of head and neck cancer with impaired survival.

This study aims to identify risk factors for oral cavity (OCC) and pharyngeal carcinoma (PC) development in IBD, to compare clinical characteristics in IBD to the general population and to assess the influence of immunosuppressive medication on survival.

Methods
We retrospectively searched the Dutch Pathology Database to identify all IBD patients with OCC and PC between 1993 and 2011. Two case-control studies were performed: we compared cases to the general IBD population to identify risk factors and we compared cases to non-IBD cancer patients for outcome analyses.

Results
We included 66 IBD patients and 2141 controls with OCC; 31 IBD patients and 1552 controls with PC, and 1800 IBD controls. Age at IBD diagnosis was a risk factor for OCC development, for Crohn’s disease (CD) (Odd’s Ratio (OR) 1.04; 95%-confidence interval (CI) 1.02–1.07) and ulcerative colitis (UC) (OR 1.03; 95%-CI 1.01–1.06). For PC this applied for UC (OR 1.05; 95%-CI 1.01–1.06).

IBD OCC cases showed an impaired survival (p=0.018), in PC survival was similar. There was no effect of immunosuppression on survival. Human papillomavirus(HPV) testing of IBD cases revealed 52.2% (12/23) HPV positive oropharyngeal carcinomas(OPC).

Conclusion
This study shows that IBD is associated with impaired OCC survival. Higher age at IBD diagnosis is a risk factor for OCC development. We found no influence of immunosuppression on survival. 52.2% of OPC in IBD contained HPV.

Key words
Inflammatory Bowel Diseases, head and neck cancer, pharyngeal carcinoma, oral cavity carcinoma, immunosuppressive therapy.
Introduction

Inflammatory bowel disease (IBD) is mainly diagnosed in young people and characterized by chronic relapsing intestinal inflammation. The majority of the IBD patients need long term treatment with immunosuppressive medication to control the disease and prevent complications. Furthermore, they have an increased risk to develop both intestinal and extra-intestinal malignancies (EIM). Immunosuppressive therapy can cause DNA damage and decrease immune surveillance, subsequently increasing EIM risk. Reduced immune surveillance of the human papillomavirus (HPV) for example is one of the contributing factors for the increased incidence of cervical cancer in patients with immunosuppression.

HPV is associated with head and neck cancers (HNC) as well, suggesting that immunosuppression use in IBD patients may also impact HNC risk in these patients. HPV associated HNC primarily occur in the (oro)pharynx of young patients without a history of excessive exposure to alcohol and tobacco and account for more than one-half of cancers of the oropharynx in the USA. However, the reported prevalence seems to vary between countries. In recent studies on the prevalence in the Netherlands show lower prevalence. In immunosuppressed transplant patients, the incidence of HNC is doubled compared to the general population. The outcome is worse in this group of immunosuppressed patients, which may be associated with high-dose immunosuppression.

Conflicting data on HNC risk in IBD are reported. Danish historical cohorts show no increased risk for lip, oral cavity and pharyngeal cancer, while more recent US data show a standardized incidence ratio (SIR) for oral cancer of 9.77 (95% confidence interval (CI), 5.14–16.98) in IBD patients. Various reports advocate regular oral screening. Currently, studies on risk factor for HNC development and HNC outcome in IBD patients are lacking. We hypothesized a worse outcome for oral cavity cancer (OCC) and pharyngeal cancer (PC) in IBD patients treated with immunosuppression in comparison with the general population.

In this study we aimed to: (I) identify risk factors in IBD patients that contribute to the development of OCC and PC and (II) compare clinical characteristics, outcome and survival of OCC and PC in IBD patients with the general population.

Methods

Study design

This study consisted of two retrospective case-control studies. Cases included IBD patients who developed OCC or PC and were selected through PALGA, the Dutch nationwide network and registry of histo- and cytopathology.

Case-control study I:
in order to identify risk factors for the development of OCC and PC in IBD patients we compared IBD cases with HNC versus IBD controls. Controls were randomly extracted from a population based IBD cohort in The Netherlands (IBD South Limburg, IBDSL).

Case-control study II:
in order to compare clinical characteristics and outcomes of OCC and PC between patients with and without IBD, we compared IBD cases with HNC to patients with HNC from the general population (controls). Controls were extracted from the Eindhoven Cancer Registry (ECR), a part of the Netherlands cancer registration (NCR; managed by The Netherlands Comprehensive Cancer Organisation (NCCO)).

The study was approved by the Privacy Commission and Scientific Council of PALGA, by the Medical Ethics Review Committee of the Radboudumc, Nijmegen, The Netherlands (Registration number 2013/211) and by the Medical Ethics Review Committee of the IBD-SL.
Case selection
To identify all Dutch IBD patients with HNC from January 1, 1993 until December 31, 2012, a search was performed in the national pathology database PALGA. PALGA has nationwide coverage since 1991 and covers all academic and non-academic Dutch pathology laboratories. Search terms for IBD included: “ulcerative colitis”, or “Crohn’s disease”, or “indeterminate colitis”, or “chronic idiopathic inflammatory bowel disease”. These terms were combined with search terms for HNC including: “head - neck”, or “pharynx”, or “mouth”, or “oral cavity “, or “lip”, or “tongue”, or “tooth”, or “tonsil”, or “adenoid”.

An initial selection of cases was made based on pathology reports. Subsequently, medical charts were investigated for definitive in- or exclusion. All IBD patients with primary OCC and PC were included in the study. Exclusion criteria were: OCC and PC in situ, lymphoma, diagnosis of IBD > 3 months after OCC or PC diagnosis, OCC or PC diagnosis before 1993 or after 2012 and no confirmed diagnosis of IBD.

Case control study I: controls from the IBDSL cohort
For the identification of risk factors, we randomly included IBD controls diagnosed between 1991 and 2011 from the population based IBDSL cohort.

The IBD South-Limburg (IBDSL) cohort comprises adult IBD patients that were diagnosed between 1991 and 2011 in the South-Limburg area of the Netherlands. The South-Limburg area is a well-defined geographic region in the southeast of the Netherlands, bordered by Belgium, Germany and narrowly to the rest of the Netherlands in the north. Its geographic isolation results in a low net migration rate of 2.1 per 1,000 inhabitants per year, favoring population research. A recent completeness check showed that 93% of all eligible IBD patients in the South-Limburg area is currently registered in the IBDSL cohort. For detailed information on the IBDSL cohort, we refer to the cohort profile.

We randomly included 1800 patients with an IBD diagnosis between 1991 and 2011, similar to our previous studies. An unmatched study design was chosen as we had a relatively large number of cases allowing adjustment for possible confounders and to avoid missing potential risk factors.

Case control study II: controls from the ECR
In order to compare clinical characteristics and outcome of HNC in IBD patients to the general population, we identified controls with OCC and PC from the ECR. The ECR is managed by the Netherlands Comprehensive Cancer Organisation (NCCO) and prospectively registers all newly diagnosed cancers in The Southeast of The Netherlands since 1989. It covers an area with 2.3 million inhabitants (about 15% of the Dutch population), encompassing over 95% of all cancers in this region (http://www.eindhovencancerregistry.nl). The search terms used in this registry were C00, C02, C03, C04, C05, C06, C09, C10, C11, C12, C13 and C14 according to the ICD-0 third edition. We included all OPC and PC controls in the period from January 1, 1993 until December 31, 2012. In situ cancers and lymphoma’s were excluded.

Data extraction
Three authors (L.N., L.D. and A.J.) reviewed anonymized medical charts of IBD cases and extracted both IBD and HNC data.

The collected data for the IBD cases included the following patient characteristics: date of birth, gender, medical history, alcohol, smoking history, height and weight. For IBD, the following variables were collected: type of IBD based on histopathologic evaluation of the histologic specimen, date of IBD diagnosis, IBD phenotype according to the Montreal Classification, diagnosis of primary sclerosing cholangitis, use of IBD medication (5-aminosalicylates, corticosteroids, thiopurines, methotrexate, calcineurin inhibitors and anti-TNF therapy), duration of therapy and number and type of surgery. IBD diagnosis was based on a combination of clinical, en-
oscopic, histological and radiographic criteria. For IBD controls from the IBDSL same variables were collected, although alcohol and smoking history were only partially available and duration of immunosuppressive medication was not available.

HNC characteristics included for cases and controls: date of diagnoses, location, tumor stage according to the TNM classification (7th edition), previous HNC or radiation, differentiation grade, primary treatment, (local) recurrence and overall survival.

IBD cases were anonymously and encrypted linked to the Dutch Cancer Registry database, for reasons of quality control and completion of collected HNC data.

**HPV detection and genotyping**

**Sample preparation**
DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tissue sections (4 μM) with the EZ1 robot (Qiagen, Germany, with the DNA tissue kit of Qiagen) according to standard procedures and used for PCR analysis. A negative water control was included with each batch of 10 samples.

**Immunohistochemistry**
For immunostaining an anti-p16 monoclonal antibody was used (clone G 175–405; BD Pharmingen, San Diego, CA) at a dilution of 1:10. As positive control, a cervical carcinoma tissue specimen with high p16 expression was used. The p16 immunohistochemistry was scored positive if a strong nuclear and cytoplasmic staining was present in >70% of the malignant cells. All other staining patterns were scored as negative.

**HPV-DNA detection and typing**
Broad-spectrum HPV-DNA amplification was performed using a short-PCR-fragment assay (HPV SPF10-LiPA25, version 1; Labo Bio-medical Products B.V, Rijswijk, Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame of HPV genotypes. HPV genotyping was performed using a cocktail of 9 conservative probes in a micro titer hybridization assay, the DNA enzyme immunoassay (DEIA). The samples positive for HPV by DEIA were subsequently analyzed with the line probe assay (LiPA25) by reverse hybridization with type-specific probes for HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74. The LiPA strips were visually inspected and interpreted following the standardized reference guide. Phocine Herpesvirus (PhHV) was used as an internal control for amplification.

**Statistics**
For both case control studies we assessed potential risk factors, HNC characteristics and/or outcomes between cases and controls with univariable analyses. X² test or Fisher exact test (if suitable) were used for categorical data and independent Student t test or Mann-Whitney U test were used for continuous data. Variables with a P-value of <0.1 in univariable analyses were included in a multivariable model.

For case control study I, we performed a multivariable logistic regression model with backward sampling. This model was adjusted for the duration of follow up (fixed variable). Follow up was defined as the time since IBD onset until the date of HNC diagnosis (cases) or the end of follow up or dead (controls). The model was made separately for UC and CD patients to identify independent risk factors for HNC development. As medication use in especially the distant past might not be reliable and may be different from current regimes, we did not include medical therapy in the primary multivariable analysis. Therefore, we performed a secondary multivariable logistic regression analysis (called sensitivity analysis) including patients with an IBD diagnosis since 1991 in both the case and control group. Medical therapy was included in this logistic regression model.
For case control study II, we made Kaplan Meier survival curves and performed log rank analysis. Subsequently confounder correction was performed with a Cox regression model with forward sampling. A covariate was considered as a confounder when the beta coefficient of the variable of interest changed by 10% or more. As the clinical behavior (and TNM classification) is different for PC sub-sites (oro-, naso- and hypopharynx), TNM stage and survival were analyzed separately.

A p-value of <0.05 was considered statistically significant. All statistical analyses were performed with IBM SPSS statistics version 20.0 (SPSS Inc, Chicago, IL).

Results

Cases and controls

The initial PALGA search yielded 760 IBD and cancer cases. First, we excluded 466 patients who did not present with either an OCC or a PC (Figure 1). Second, 197 patients were excluded who did not have confirmed IBD in their medical records, resulting in 31 patients with PC and 66 with OCC.

For case control study I, 1800 IBD controls were randomly selected from the IBDSL. For case control study II, we included 2141 patients with OCC and 1552 with a PC from the ECR (Figure 2).

Case control study I: risk factors for OCC and PC development in IBD patients

OCC

The univariable comparison between IBD cases and IBDSL controls (Table 1) showed that cases were older at IBD diagnosis (median 53.5 versus 39.0 years; p<0.01) and were more frequently male (66.7% versus 46.5%; p<0.01). UC cases had more extensive disease (Montreal E3 disease: 50.0% versus 28.1%; p<0.01) and CD patients had more frequent Montreal L4 disease (20.0% versus 3.6%; p=0.037).
## Table 1: Univariable comparison of potential risk factors between IBD patients with HNC (cases) and IBDSL controls

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<th>Pharynx N = 31</th>
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<th>p-value</th>
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<td>Age at diagnosis (y), median</td>
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<td>253 (37.5)</td>
<td>9 (30.0)</td>
<td>1 / 122</td>
<td>0.45</td>
<td>0 (0.0)</td>
<td>3 / 122</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>345 (51.2)</td>
<td>25 (9.2)</td>
<td>6 (20.0)</td>
<td>0.07</td>
<td>10 (30.0)</td>
<td>1 (30.0)</td>
<td>0.35</td>
</tr>
<tr>
<td>Alcohol use (current &amp; past); n (%)</td>
<td>46 (69.7)</td>
<td>46 (69.7)</td>
<td>- / -</td>
<td>-</td>
<td>17 (55.9)</td>
<td>- / 11</td>
<td>-</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis; n (%)</td>
<td>13 (0.7)</td>
<td>1 (1.5)</td>
<td>4 / 20</td>
<td>0.36</td>
<td>0 (0.0)</td>
<td>10 / 20</td>
<td>0.10</td>
</tr>
<tr>
<td>IBD type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis; n (%)</td>
<td>1004 (55.8)</td>
<td>34 (52.4)</td>
<td>0 / 0</td>
<td>0.586</td>
<td>14 (46.7)</td>
<td>0 / 0</td>
<td>0.79</td>
</tr>
<tr>
<td>Indeterminate colitis; n (%)</td>
<td>796 (44.2)</td>
<td>31 (47.7)</td>
<td>0 / 0</td>
<td>0.586</td>
<td>16 (53.3)</td>
<td>0 / 0</td>
<td>0.79</td>
</tr>
<tr>
<td>Ulcerative colitis##</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proctitis (E1); n (%)</td>
<td>240 (24.4)</td>
<td>0 (0.0)</td>
<td>0 / 30</td>
<td>&lt;0.01*</td>
<td>1 (7.7)</td>
<td>1 / 30</td>
<td>0.21</td>
</tr>
<tr>
<td>Pancolitis (E3); n (%)</td>
<td>472 (47.5)</td>
<td>17 (50.0)</td>
<td>0 / 20</td>
<td>0.06</td>
<td>7 (23.1)</td>
<td>0 / 20</td>
<td>0.06</td>
</tr>
<tr>
<td>Crohn's disease##</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum (L1); n (%)</td>
<td>223 (12.4)</td>
<td>5 (16.1)</td>
<td>0 / 10</td>
<td>0.26</td>
<td>1 (7.1)</td>
<td>0 / 10</td>
<td>0.26</td>
</tr>
<tr>
<td>Colon (L2); n (%)</td>
<td>312 (17.3)</td>
<td>10 (32.3)</td>
<td>0 / 10</td>
<td>0.06</td>
<td>5 (16.1)</td>
<td>1 / 10</td>
<td>0.21</td>
</tr>
<tr>
<td>Ileocolonic (L3); n (%)</td>
<td>389 (21.6)</td>
<td>36 (13.4)</td>
<td>0 / 10</td>
<td>0.06</td>
<td>8 (25.8)</td>
<td>1 / 10</td>
<td>0.06</td>
</tr>
<tr>
<td>Upper digestive (L4); n (%)</td>
<td>65 (3.6)</td>
<td>65 (3.6)</td>
<td>0 / 10</td>
<td>0.06</td>
<td>3 (10.3)</td>
<td>1 / 10</td>
<td>0.06</td>
</tr>
<tr>
<td>Stenosing (B2); n (%)</td>
<td>263 (14.6)</td>
<td>12 (40.0)</td>
<td>0 / 10</td>
<td>0.06</td>
<td>5 (16.1)</td>
<td>1 / 10</td>
<td>0.06</td>
</tr>
<tr>
<td>Peneetrating (B3); n (%)</td>
<td>188 (10.4)</td>
<td>9 (30.0)</td>
<td>0 / 10</td>
<td>0.06</td>
<td>5 (16.1)</td>
<td>1 / 10</td>
<td>0.06</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-aminosalicylates; n (%)</td>
<td>1605 (88.2)</td>
<td>50 (87.7)</td>
<td>0 / 10</td>
<td>0.26</td>
<td>14 (81.8)</td>
<td>9 / 13</td>
<td>0.27</td>
</tr>
<tr>
<td>Steroids; n (%)</td>
<td>1323 (68.8)</td>
<td>44 (71.4)</td>
<td>0 / 10</td>
<td>0.26</td>
<td>16 (71.4)</td>
<td>5 / 13</td>
<td>0.33</td>
</tr>
<tr>
<td>Thalidomide; n (%)</td>
<td>717 (39.8)</td>
<td>30 (62.0)</td>
<td>0 / 10</td>
<td>0.26</td>
<td>10 (60.0)</td>
<td>5 / 13</td>
<td>0.33</td>
</tr>
<tr>
<td>Methotrexate; n (%)</td>
<td>93 (5.1)</td>
<td>1 (1.9)</td>
<td>0 / 10</td>
<td>0.26</td>
<td>4 (2.4)</td>
<td>0 / 10</td>
<td>0.26</td>
</tr>
<tr>
<td>Cyclosporin; n (%)</td>
<td>26 (1.4)</td>
<td>4 (1.8)</td>
<td>0 / 10</td>
<td>0.26</td>
<td>6 (3.5)</td>
<td>0 / 10</td>
<td>0.26</td>
</tr>
<tr>
<td>Anti-TNF therapy; n (%)</td>
<td>390 (20.9)</td>
<td>63 (21.5)</td>
<td>0 / 10</td>
<td>0.26</td>
<td>4 (2.4)</td>
<td>0 / 10</td>
<td>0.26</td>
</tr>
<tr>
<td>IBD related surgery; n (%)</td>
<td>1284 (71.7)</td>
<td>43 (62.2)</td>
<td>0 / 8</td>
<td>0.26</td>
<td>19 (63.3)</td>
<td>1 / 8</td>
<td>0.32</td>
</tr>
<tr>
<td>Duration of follow up since IBD diagnosis (y), median</td>
<td>7.00</td>
<td>9.00</td>
<td>0 / 30</td>
<td>0.01*</td>
<td>11.00</td>
<td>0 / 30</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**Note:** HNC = head and neck cancer; IBD = inflammatory bowel disease; IBDSL = IBD South Limburg cohort; CD = Crohn's disease; ## according to the Montreal classification. Smoking data of IBDSL only available for CD patients.
In the multivariable logistic regression model age at IBD diagnosis remained an independent risk factor to develop OCC in both UC (OR 1.03, 95% CI 1.01 – 1.06) and CD (OR 1.04, 95% CI 1.02 – 1.07) (Table 2). For CD, Montreal L4 disease (OR 1.10, 95% CI 1.04 – 1.17) was an additional risk factor.

**PC**

We included 31 IBD cases with PC: 25 with oropharyngeal carcinoma (OPC) and 6 with hypopharyngeal carcinoma (HPC) (figure 1). The univariable comparison between cases and IBDSL controls only showed a difference in tobacco use in CD patients (100% versus 62.5%; p<0.01).

In the multivariable logistic regression model (Table 2) no risk factors remained for CD. For UC, age at IBD diagnoses was an independent risk factor (OR 1.05, 95% CI 1.08 – 1.06).

The sensitivity analysis revealed a protective effect of 5-aminosalicylates for OPC in UC patients (OR 0.02, 95% CI 0.01 – 0.09; Table 2).

**Case control study II: OCC and PC cases versus ECR controls**

**OCC**

IBD cases had a lower median age at OCC diagnosis (60.5 versus 65.0 years; p=0.02) compared to controls (Table 3). OCC cases had less frequent well-differentiated tumors (12.2% versus 27.5%; p=0.03) and there was a trend towards a lower TNM stage (p=0.05). Overall survival was similar in the univariable analysis (p=0.30; supplementary file 1). However, adjusted for confounders, IBD cases showed a worse survival (p=0.02; Figure 3). Immunosuppressive therapy did not negatively affect overall survival following OCC (p=0.43; supplementary file 1).

**PC**

Despite a lower T-stage (T1-T2: 75.0% versus 47.5%; p=0.02; Table 4) in IBD patients with OPC, a comparable TNM stage (p=0.06) and

---

**Table 2: Final multivariable logistic regression model after adjustment for confounders and follow up: independent risk factors for HNC development**

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Coefficient β</th>
<th>Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cavity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Age at IBD diagnosis</td>
<td>0.03</td>
<td>1.03 (1.01 – 1.06)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Male sex</td>
<td>1.03</td>
<td>2.79 (1.08 – 7.18)</td>
<td>0.03</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>Age at IBD diagnosis</td>
<td>0.04</td>
<td>1.04 (1.02 – 1.07)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Oropharynx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Age at IBD diagnosis</td>
<td>0.05</td>
<td>1.05 (1.00 – 1.09)</td>
<td>0.01</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>5-aminosalicylates</td>
<td>-4.03</td>
<td>0.02 (0.01 – 0.09)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>No risk factors identified</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 continued**

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Coefficient β</th>
<th>Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases (n=31)</td>
<td>Age at IBD diagnosis</td>
<td>0.04</td>
<td>1.04 (1.02 – 1.07)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Sensitivity analysis (n=20)</td>
<td>Age at IBD diagnosis</td>
<td>0.05</td>
<td>1.05 (1.00 – 1.09)</td>
<td>0.01</td>
</tr>
<tr>
<td>Sensitivity analysis (n=9)</td>
<td>5-aminosalicylates</td>
<td>-4.03</td>
<td>0.02 (0.01 – 0.09)</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Note: IBD = inflammatory bowel disease
Table 3: Univariable comparison of oral cavity characteristics between IBD cases and controls from the general population with oral cavity

<table>
<thead>
<tr>
<th>Variable</th>
<th>IBD patients</th>
<th>ECR patients</th>
<th>Missing (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis (y), median</td>
<td>60.50</td>
<td>65.00</td>
<td>0 / 0</td>
<td>0.02*</td>
</tr>
<tr>
<td>Female sex; n (%)</td>
<td>22 (33.3)</td>
<td>810 (37.8)</td>
<td>0 / 0</td>
<td>0.26</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral cavity</td>
<td>57 (86.4)</td>
<td>1506 (70.3)</td>
<td>0 / 0</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Lip</td>
<td>9 (13.6)</td>
<td>635 (29.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>SCC</td>
<td></td>
<td>N = 66</td>
<td>N = 2141</td>
</tr>
<tr>
<td></td>
<td>62 (95.4)</td>
<td>2045 (96.5)</td>
<td>1 / 0</td>
<td>0.50</td>
</tr>
<tr>
<td>Staging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T stage oral cavity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1; n (%)</td>
<td>33 (60.0)</td>
<td>619 (42.0)</td>
<td>2 / 31</td>
<td>0.07</td>
</tr>
<tr>
<td>T2; n (%)</td>
<td>12 (21.8)</td>
<td>401 (27.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3; n (%)</td>
<td>3 (5.6)</td>
<td>135 (7.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4; n (%)</td>
<td>7 (12.7)</td>
<td>340 (23.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N stage oral cavity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO; n (%)</td>
<td>39 (74.2)</td>
<td>1270 (71.7)</td>
<td>2 / 80</td>
<td>0.87</td>
</tr>
<tr>
<td>N1; n (%)</td>
<td>7 (11.3)</td>
<td>184 (10.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2; n (%)</td>
<td>9 (14.5)</td>
<td>300 (16.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3; n (%)</td>
<td>0 (0.0)</td>
<td>17 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M stage (yes) oral cavity #</td>
<td>1 (1.8)</td>
<td>17 (1.2)</td>
<td>1 / 121</td>
<td>0.51</td>
</tr>
<tr>
<td>TNM – stadium oral cavity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stadium I, n (%)</td>
<td>27 (50.9)</td>
<td>454 (33.8)</td>
<td>4 / 162</td>
<td>0.05</td>
</tr>
<tr>
<td>Stadium II, n (%)</td>
<td>7 (13.2)</td>
<td>231 (17.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stadium III, n (%)</td>
<td>7 (13.2)</td>
<td>162 (12.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stadium IV, n (%)</td>
<td>12 (22.6)</td>
<td>497 (37.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNM – stadium lip #</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stadium I, n (%)</td>
<td>7 (100.0)</td>
<td>248 (87.3)</td>
<td>2 / 351</td>
<td>1.00</td>
</tr>
<tr>
<td>Stadium II, n (%)</td>
<td>0 (0.0)</td>
<td>20 (7.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stadium III, n (%)</td>
<td>0 (0.0)</td>
<td>8 (2.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stadium IV, n (%)</td>
<td>0 (0.0)</td>
<td>8 (2.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery (yes); n (%)</td>
<td>60 (90.9)</td>
<td>1834 (85.7)</td>
<td>0 / 0</td>
<td>0.23</td>
</tr>
<tr>
<td>Chemo (yes); n (%)</td>
<td>1 (1.5)</td>
<td>69 (3.2)</td>
<td>0 / 0</td>
<td>0.44</td>
</tr>
<tr>
<td>Radiotherapy (yes); n (%)</td>
<td>23 (34.8)</td>
<td>750 (35.0)</td>
<td>0 / 0</td>
<td>0.98</td>
</tr>
</tbody>
</table>

OSCC = Oral squamous cell carcinoma; IBD = Inflammatory bowel disease; ECR = Eindhoven Cancer Registry; SCC = squamous cell carcinoma; *# according to the 7th TNM edition

Table 4: Univariable comparison of pharynx carcinoma characteristics between IBD cases and controls from the general population with pharynx carcinoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>IBD patients</th>
<th>ECR patients</th>
<th>Missing (n)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharynx and hypopharynx</td>
<td></td>
<td></td>
<td>N = 31</td>
<td>N = 1552</td>
</tr>
<tr>
<td>Age at diagnosis (y), median</td>
<td>59.00</td>
<td>61.00</td>
<td>0 / 0</td>
<td>0.65</td>
</tr>
<tr>
<td>Female sex; n (%)</td>
<td>12 (38.7)</td>
<td>432 (27.8)</td>
<td>0 / 0</td>
<td>0.22</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>0 (0.0)</td>
<td>158 (9.4)</td>
<td>0 / 23</td>
<td>0.09</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>25 (80.6)</td>
<td>1095 (64.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>6 (19.4)</td>
<td>434 (25.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>SCC</td>
<td></td>
<td>30 (96.8)</td>
<td>1459 (94.0)</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>2 (9.3)</td>
<td>88 (6.9)</td>
<td>9 / 275</td>
<td>0.69</td>
</tr>
<tr>
<td>Moderate</td>
<td>11 (50.0)</td>
<td>725 (56.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>9 (40.9)</td>
<td>464 (36.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oropharynx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T stage #</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 – T2; n (%)</td>
<td>18 (75.0)</td>
<td>505 (47.5)</td>
<td>0 / 33</td>
<td>0.02*</td>
</tr>
<tr>
<td>T3 – T4; n (%)</td>
<td>7 (25.0)</td>
<td>557 (52.5)</td>
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</tr>
<tr>
<td>N stage #</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO; n (%)</td>
<td>12 (50.0)</td>
<td>363 (34.8)</td>
<td>0 / 25</td>
<td>0.49</td>
</tr>
<tr>
<td>N1; n (%)</td>
<td>3 (12.5)</td>
<td>160 (15.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2; n (%)</td>
<td>8 (33.3)</td>
<td>469 (45.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3; n (%)</td>
<td>1 (4.2)</td>
<td>51 (4.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M stage (yes) #</td>
<td>1 (4.2)</td>
<td>44 (4.4)</td>
<td>1 / 55</td>
<td>0.96</td>
</tr>
<tr>
<td>TNM – stadium #</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stadium I – IV, n (%)</td>
<td>9 (37.5)</td>
<td>222 (21.6)</td>
<td>1 / 65</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T stage #</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1; n (%)</td>
<td>0 (0.0)</td>
<td>39 (9.3)</td>
<td>1 / 14</td>
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</tr>
<tr>
<td>T2 – T3; n (%)</td>
<td>4 (80.0)</td>
<td>231 (55.0)</td>
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<tr>
<td>T4; n (%)</td>
<td>1 (20.0)</td>
<td>150 (35.7)</td>
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<tr>
<td>N stage #</td>
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<tr>
<td>NO; n (%)</td>
<td>4 (80.0)</td>
<td>109 (26.7)</td>
<td>1 / 1</td>
<td>0.11</td>
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<tr>
<td>N1; n (%)</td>
<td>1 (20.0)</td>
<td>75 (18.3)</td>
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<tr>
<td>N2; n (%)</td>
<td>0 (0.0)</td>
<td>188 (46.0)</td>
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<tr>
<td>N3; n (%)</td>
<td>0 (0.0)</td>
<td>37 (9.0)</td>
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<tr>
<td>M stage (yes) #</td>
<td>0 (0.0)</td>
<td>24 (6.0)</td>
<td>1 / 34</td>
<td>1.00</td>
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<tr>
<td>TNM – stadium #</td>
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<tr>
<td>Stadium I, n (%)</td>
<td>0 (0.0)</td>
<td>11 (2.6)</td>
<td>1 / 18</td>
<td>0.03*</td>
</tr>
<tr>
<td>Stadium II – III, n (%)</td>
<td>4 (80.0)</td>
<td>109 (26.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stadium IV, n (%)</td>
<td>1 (20.0)</td>
<td>296 (71.2)</td>
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<tr>
<td>Oropharynx and hypopharynx</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Surgery (yes); n (%)</td>
<td>14 (46.7)</td>
<td>348 (22.4)</td>
<td>1 / 0</td>
<td>&lt;0.01</td>
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<td>Chemo (yes); n (%)</td>
<td>2 (6.7)</td>
<td>276 (17.8)</td>
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<td>0.15</td>
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<td>Radiotherapy (yes)</td>
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<td>1235 (79.6)</td>
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<td>Previous Malignancy</td>
<td>12 (41.4)</td>
<td>342 (22.0)</td>
<td>2 / 0</td>
<td>0.01*</td>
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</table>

IBD = Inflammatory bowel disease; ECR = Eindhoven Cancer Registry; SCC = squamous cell carcinoma; # according to the 7th TNM edition
survival (p=0.50) was found compared to controls. Immunosuppressive therapy did not negatively affect overall survival following OPC (p=0.10 and p=0.07; supplementary file 2A). We found no difference in survival between HPV positive and negative OPCs (p=0.45).

IBD patients with HPC (n=6) had a lower TNM stage (p=0.03; Table 4) and a better overall survival compared to the general population (p=0.01; supplementary file 2B).

Both OPC and HPC cases underwent more frequently surgery (46.7% versus 22.4%; p<0.01; Table 4).

**Histopathology and HPV analysis**

We obtained tissue from 48 of 66 identified OCC cases for further analyses. All cases were squamous cell carcinomas, and five of 48 tumors (10.4%) were p16 positive, suggesting the possibility of HPV-related cancers. Subsequent HPV testing of these five cases revealed three cases with HPV 16 infection and two cases without HPV detection. In addition, we obtained tissue from 27 out of 31 PC patients for further analyses (figure 4). The p16 positive cases (n = 11) were all HPV positive and were all oropharyngeal of origin. Of the OPC 12 of 23 (52.2%) were HPV positive, most HPV 16 (10/12; 83.3%). In 8 of 12 HPV-positive cases we were informed about medication use: 6 used immunosuppressive therapy before HNC diagnosis (4/6 at least thiopurines).
In this nationwide study we found that IBD patients with OCC have an impaired survival compared to the general population, adjusted for TNM stage. Higher age at IBD diagnosis was a risk factor for OCC development in IBD and for PC in UC. Proximal CD localization was another risk factor for OCC development. Furthermore, we found that 52.2% of IBD associated oropharyngeal cancers were HPV positive compared to 30% in general Dutch population. Immunosuppression did not impact survival of HNC in IBD.

We showed that 52.2% of the OPCs in IBD were HPV positive, which is in line with the reported prevalence of HPV positive OPCs in the (international) general population. However, the reported prevalence seems to vary between countries and in recent studies on the prevalence in the Netherlands lower figures (30%) have been reported. Differences in HPV prevalence between IBD patients with and without immunosuppression could not be determined due to the limited number of cases. In the general population patients with HPV-positive tumors have better outcomes, but in the IBD cases no difference in survival was observed. As OPC incidence is increasing mainly due to the increased incidence of HPV positive OPCs, the potential impact of prophylactic HPV vaccines is of interest. This especially applies for immunosuppressed patients on thiopurine therapy, as reduced immunosurveillance may cause persisting HPV infections.

In line with colorectal cancer in IBD, OCC in IBD arise at a younger age (median 60.5 versus 65.0 years; p = 0.021) and has an impaired prognosis. Furthermore, immunosuppressive therapy may promote tumor progression and impair survival. However, we found no difference in tumor stage and overall survival in our cohort regardless of immunosuppression use. These results must be interpreted with caution due to the limited number of IBD patients with HNC. Furthermore, clinicians fear the negative impact of immunosuppression on cancer outcomes thus immunosuppression may only be used in patients with a favorable risk profile. Finally, Penn showed that the risk of recurrence for (renal) transplant patients is highest in the first two years after malignancy diagnosis. Our data may have been influenced by wide variations in the commencement of immunosuppressive therapy after cancer diagnosis.

We indentified two risk factors for HNC development in IBD. First, proximally localized disease in CD patients (Montreal L4; including oral cavity inflammation) (OR 1.103; 95% CI 1.040 – 1.170; p = 0.028). This may be related to upper gastrointestinal IBD-related inflammation. Furthermore, we identified older age at IBD diagnosis as a risk factor for both OCC and PC development. This is an interesting observation that is in line with other cancer types in IBD, although our study does not provide an explanation for this observation.

To date, this is the largest, systematically collected series of OCC and PC in IBD patients. However, our study also comes with several limitations, such as the retrospective nature of the study. Especially in cases that were enrolled in early years, it was challenging to collect accurate and complete information. Therefore, we also performed a sensitivity analysis, enrolling only cases diagnosed with IBD after 1990. This did not show immunosuppressive therapy as a risk factor for OCC or PC development. Second, we used three different databases that were constructed in different ways to address our hypothesis. Our data search is retrospective, while the IBDSL and the cancer registry (including ECR) collect prospectively. Unfortunately there is no single database available that could answer our research questions sufficiently. Third, important data on tobacco and alcohol use, the main risk factors for HNC development, were incomplete for the ECR and IBDSL. As HPV presence is correlated inversely with tobacco (and alcohol) consumption, this would provide important additional information.

Some authors have suggested oral screening for all IBD patients, especially those who are starting immunosuppression. As the incidence of HNC in IBD in general is low and...
no well defined premalignant lesions analogous to cervical intraepithelial neoplasia in cervical cancer have been established yet, we would recommend increased awareness for HNC in IBD rather than active screening, especially in elderly onset IBD.

In conclusion we found that IBD patients with OCC may have a reduced survival compared to the general population. Proximal disease localization in CD is a risk factor for OCC development. The majority (52.2%) of IBD associated oropharyngeal cancers was HPV positive. Immunosuppression did not impact incidence and survival of HNC in IBD.

Acknowledgements
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Conflicts of Interest and Source of Funding
none.

Funding
none.

References:


**confounder correction, including TNM stage
IMS = immunosuppressive therapy (defined as use of steroids, and/or thiopurines, and/or anti-TNF therapy, and/or Metotrexate, and/or calcineurin inhibitor use); IBD = Inflammatory bowel disease; ECR = Eindhoven Cancer Registry; HNC = head and neck cancer

**confounder correction, including TNM stage
IMS = immunosuppressive therapy (defined as use of steroids, and/or thiopurines, and/or anti-TNF therapy, and/or Metotrexate, and/or calcineurin inhibitor use); IBD = Inflammatory bowel disease; ECR = Eindhoven Cancer Registry; HNC = head and neck cancer
Appendix
A: PALGA group**
E.E.C. de Jonge (Groene Hart Ziekenhuis, Gouda), R. Natte (Hagaziekenhuis, Den Haag) E.W.P. Nijhuis (Onze Lieve Vrouwe Gasthuis, Amsterdam), C. Peutz – Kootstra (Maastricht University Medical Center, Maastricht), J.J.T.H. Roelofs (Amsterdam Medical Center, Amsterdam), S.M. Willems (University Medical Center Utrecht, Utrecht), A.P. Willig (Laurentius Hospital, Roermond).

B: IBD and HNC group***
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3.4

Neoplasia risk after colectomy in inflammatory bowel disease patients – A systematic review and meta-analysis

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Abstract

Colorectal neoplasia can still develop after colectomy for inflammatory bowel disease. However, data on this risk are scarce, and there have been few conclusive findings, so no evidence-based recommendations have been made for postoperative surveillance. We conducted a systematic review and meta-analysis to determine the prevalence and incidence of and risk factors for neoplasia in patients with inflammatory bowel disease who have undergone colectomy, including the permanent-end ileostomy and rectal stump, ileorectal anastomosis (IRA), and ileal pouch-anal anastomosis (IPAA) procedures.

We searched PubMed, Embase, Web of Science, and Cochrane Library through May 2014 to identify studies that reported prevalence or incidence of colorectal neoplasia after colectomy or specifically assessed risk factors for neoplasia development. Studies were selected, quality was assessed, and data were extracted by 2 independent researchers.

We calculated colorectal cancer (CRC) prevalence values from 13 studies of patients who underwent rectal stump surgery, 35 studies of IRA, and 33 studies of IPAA. Significantly higher proportions of patients in the rectal stump group (2.1%; 95% confidence interval [CI], 1.3%–3.0%) and in the IRA group (2.4%; 95% CI, 1.7%–3.0%) developed CRC than in the IPAA group (0.5%; 95% CI, 0.3%–0.6%); the odds ratio (OR) for CRC in the rectal stump or IRA groups compared with the IPAA group was 6.4 (95% CI, 4.3–9.5). A history of CRC was the most important risk factor for development of CRC after colectomy (OR for patients receiving IRA, 12.8; 95% CI, 3.31–49.2 and OR for patients receiving IPAA, 15.0; 95% CI, 6.6–34.5).

In a meta-analysis of published studies, we found the prevalence and incidence of CRC after colectomy to be less than 3%; in patients receiving IPAA it was less than 1%. Factors that increased risk of cancer development after colectomy included the presence of a residual rectum and a history of CRC. These findings could aid in development of individualized strategies for post-surgery surveillance.

Introduction

Although the magnitude of the colorectal cancer (CRC) risk in inflammatory bowel disease (IBD) patients is still under debate, it is well-established that both ulcerative colitis (UC) and Crohn’s disease (CD) patients with colonic involvement have an increased risk to develop CRC. It is one of the most detrimental complications of IBD, with significant morbidity and an associated mortality rate of approximately 15%.1 To reduce CRC risk, endoscopic surveillance guidelines have been developed that allow detection and potential removal of precancerous lesions. This strategy might reduce the increased CRC incidence in IBD patients and improve mortality rates.2 However, IBD surveillance guidelines have been written on the basis of research in patients with an intact colon.3–4

Although therapeutic options have expanded during the last decade, bowel surgery still plays an important role in the management of IBD. Indeed, the cumulative risk of intestinal surgery for UC patients is 25%–30% and even higher for CD patients (70%–80%).5–6 For UC or extensive colorectal CD, staged restorative proctocolectomy is the surgical treatment of choice. The series of surgical procedures start with subtotal colectomy and ileostomy with a residual rectum left in situ. This initial approach will keep options for reconstructive surgery open.1 Ileal pouch-anal anastomosis (IPAA) is the preferred reconstructive procedure after colectomy in UC patients.1 In patients with extensive colonic CD, ileorectal anastomosis (IRA) is the first restorative option to consider.8 For several reasons including comorbidities and concerns about fertility, treating physicians and patients may reconsider restorative surgery, and these patients usually continue with a permanent ileostomy and rectal stump.
Colectomy, with or without reconstructive surgery, substantially reduces the risk to develop colorectal neoplasia. However, neoplasia of the residual rectum or ileoanal pouch may still arise and is associated with a poor prognosis. The latter underlines the importance of preventative strategies such as endoscopic surveillance. In recent years, data have expanded regarding prevalence, incidence, and risk factors for colorectal neoplasia after colectomy. Lack of a comprehensive approach and interpretation of these data has led to the absence of endoscopic surveillance recommendations for these patients. Thus, integrated data on CRC risk in postsurgical IBD patients are needed to further aid development of surveillance guidelines. We therefore conducted a systematic review and meta-analysis that aimed to determine prevalence, incidence, and risk factors regarding colorectal dysplasia and cancer after colectomy in 3 groups of IBD patients including (1) patients with a permanent ileostomy and rectal stump, (2) patients with IRA, and (3) patients with IPAA.

Methods

Search strategy
Medline, Embase, the Cochrane Library, and Web of Science were independently searched with the help of a clinical librarian until May 2014 by 2 authors (L.D., L.N.) to identify studies that evaluated the colorectal neoplasia risk after colonic resection in IBD patients. We used the medical subject headings (MeSH) “Inflammatory bowel disease” OR “Ulcerative colitis” OR “Crohn’s disease”, combined with “Surgical anastomosis” OR “Colectomy” OR “Restorative proctocolectomy” and combined with “Colorectal neoplasms” OR “Rectal neoplasms” OR “Colon neoplasms” OR “Anus neoplasms”. Simultaneously, a title/abstract search was performed with similar search terms and synonyms. The full search strategy is described in Supplementary Table 1. No restrictions regarding language, year of publication, or publication type were imposed. A manual search for references in the initially selected articles (Figure 1) was performed to identify additional relevant articles. The reporting checklist proposed by the Meta-analysis of Observational Studies in Epidemiology was used as a guideline in this systematic review and meta-analysis.

Inclusion and exclusion criteria
Studies were eligible for inclusion if the authors reported a series of IBD patients who underwent colonic resection and if occurrence of postoperative neoplasia in the residual rectum or pouch was described. In addition, we included studies that specifically assessed risk factors for neoplasia development after colectomy. Studies including patients with a Hartmann procedure or segmental resection and studies not defining the total IBD patient group were excluded. Furthermore, we excluded case reports, case series, studies including < 20 patients, and conference abstracts because these might not be representative for the target population. In case of duplicate publication or similar data from same institutions, the most recent and complete data sets were considered. Multiple studies from one institution were both considered if less than 25% of the inclusion years overlapped.

Quality assessment of retrieved articles
On the basis of the guidelines for critically appraising studies of prevalence or incidence, we composed a list of parameters for quality judgment. These comprise whether the study was single center or population based, the number of patients (more or less than 100; a calculated sample size of 114 patients would be needed to show a prevalence of 5% with an error rate of 4%; a smaller sample size would give a higher risk of bias), duration of follow-up (cutoff mean or median 1 year), proctectomy or pouch excision rate, whether a clear pathologic classification system was used for grading neoplasia, whether the study was retrospective or prospective,
and whether the study was consecutive. Two authors (L.D., L.S.) independently determined a quality score for each study, with a maximum of 7 points. Disagreement was resolved by discussion and consensus with a third reviewer (F.H.).

**Data extraction**

Different parameters were independently extracted by 2 authors (L.D., L.S., in consensus with F.H.) from the original articles including demographics, IBD characteristics, neoplasia prevalence and incidence, and risk factors such as a history of preoperative colorectal neoplasia, primary sclerosing cholangitis (PSC), pouchitis, and type of pouch anastomosis. IBD characteristics included the type and duration of IBD. For each group of IBD patients (rectal stump, IRA, and IPAA), the prevalence of colorectal neoplasia was calculated by dividing the cases by the total patient group at risk. Patients with either adenocarcinoma, including carcinoma in situ, or dysplasia were included as cases. The first group with a rectal stump was defined as the group of patients who underwent a co-locolic resection including the hepatic and splenic flexure and who received a permanent ileostomy. A rectal or rectosigmoidal stump was still in situ and at risk for neoplasia development. Patients who were lost to follow-up, postoperatively deceased, or undergoing secondary reconstruction of IRA or IPAA were not included in this group. The IRA and IPAA groups included all patients who underwent IRA or IPAA in 1 or more stages, respectively. Patients with an ileosigmoidal or cecorectal anastomosis were also included in the IRA group. In accordance with the rectal stump group, patients who were lost to follow-up or postoperatively deceased were excluded from the IRA and IPAA groups.

**Statistics**

We performed a meta-analysis to estimate pooled prevalences and cumulative incidences of colorectal neoplasia after colectomy. Random-effect models were used because of heterogeneity of studies.
We assessed publication bias with the visual inspection of a funnel plot and used the Egger test to analyze funnel plot asymmetry. Subsequently, we compared prevalences between subgroups (for example UC versus CD) with a logistic regression model calculating odds ratios (ORs).

To analyze potential risk factors for developing CRC after colectomy, ORs were separately calculated for each study and subsequently pooled with a random-effect model. Risk factors that comprised continuous data were analyzed in a pooled model by calculating a weighted mean difference.

To compare equality of follow-up duration between the 3 groups, we used one-way analysis of variance. Correlations between duration of follow-up and prevalence were analyzed with Spearman correlation coefficient. A P value less than 0.05 was considered statistically significant. All statistical analyses were performed by using StatsDirect Statistical Software version 2.8.0 (StatsDirect, Sale, Cheshire, UK), Statistical Analysis Software version 9.2 (SAS Institute, Cary, NC), or IBM SPSS statistics version 20.0 (SPSS Inc, Chicago, IL).

Results

Study selection
The systematic study selection flowchart is depicted in Figure 1. Sixteen, 68, and 56 articles were included in the rectal stump group, the IRA group, and the IPAA group, respectively. Because of duplicate data, 2 studies in the rectal stump group, 32 studies in the IRA group, and 18 studies in the IPAA group were not used for prevalence calculations (references for excluded studies are listed in Supplementary Material). For both the rectal stump group and the IRA group we included 1 article and for the IPAA group 5 articles that specifically assessed risk factors.

Study characteristics and quality assessment
Summarized quality scores for all included studies are depicted in Supplementary Tables 2–4 for the rectal stump group, IRA group, and IPAA group. Full quality assessment is displayed in Supplementary Tables 5–7.

The mean quality score of selected studies in the rectal stump group was 2.3 out of 7. All studies included retrospective, single-center cohort studies. Sample sizes were insufficient, and none of the studies mentioned the pathologic classification system that was used to evaluate rectal neoplasia. Proctectomy rates differed between 46% and 95%, resulting in a reduced number of patients at risk to develop rectal neoplasia. However, there was no correlation between proctectomy rates and cancer prevalence (P = 0.510).

Studies included in the IRA group had better overall quality, with average quality score of 4.0. This was mainly caused by better documentation and longer duration of follow-up, lower proctectomy rate, and larger number of included patients per study. Higher proctectomy rates were not correlated with lower cancer prevalence (P = 0.311).

Included articles on the IPAA group had a mean quality score of 3.0. The difference in quality score with the IRA group might be attributed to absence of the pouch excision rate, which was not reported in most articles. No correlation between follow-up duration and prevalence was observed (P = 0.515).

Ileostomy and rectal stump
Prevalence and incidence.
A pooled analysis including 1011 IBD patients demonstrated a carcinoma prevalence of the rectal stump of 2.1% (95% confidence interval [CI], 1.3–3.0; Supplementary Table 2, Supplementary Figures 1 and 4). This value represents the prevalence in a variable duration of reported follow-up between 0.25 and 40 years. None of the included studies evaluated dysplasia development. One study specifically assessed the cumulative rectal cancer incidence in
UC patients with a rectal stump or secondary IRA (constructed in 2 stages), resulting in an incidence of 12.6% after 24 years after surgery.9

**Risk factors.**

Only 1 study assessed risk factors for the development of rectal stump cancer.10 This retrospective case-control study included 12 rectal stump carcinomas and 18 control patients without rectal stump neoplasia and identified PSC and IBD duration until subtotal colectomy as risk factors. The study design of this case-control study was not sufficient to identify a history of colorectal neoplasia as risk factor because these patients were excluded from the control group.

We detected no difference in carcinoma prevalence of the rectal stump between UC and CD (2.2%, 95% CI, 1.3%–3.4% versus 2.1%, 95% CI, 0.6%–4.4%; OR 1.4, 95% CI, 0.4–5.0, p = 0.574).

**Ileorectal anastomosis**

**Prevalence and incidence.**

A pooled rectal carcinoma prevalence of 2.4% (95% CI, 1.7%–3.3%) was calculated in the IRA group, including 2762 patients with a variable duration of reported follow-up between 1 and 35 years (Supplementary Table 3, Supplementary Figures 2 and 5). Development of rectal dysplasia after IRA was described in 16 studies including 1425 patients, resulting in a pooled dysplasia prevalence of 2.5% (95% CI, 1.2%–4.2%). Because the year of publication and the duration of follow-up may influence the prevalence, we performed subgroup analysis on the basis of these variables. There was a statistically significant lower carcinoma prevalence (OR, 2.3; 95% CI, 1.3–4.1; p = 0.003) in studies published after 1990 (1.6%; 95% CI, 2.3%–4.3% versus 0.7%; 95% CI, 0.2%–1.6%) with OR of 10.3 (95% CI, 2.5–41.9; p = 0.001).

A forest plot evaluating prior CRC as a risk factor to develop rectal carcinoma after IRA construction is displayed in Figure 2A. Three studies were available for meta-analysis because they reported prior CRC both in the patients who developed rectal carcinoma and in the patients who did not.11, 12, 15 A pooled OR of 12.8 (95% CI, 3.3–49.2) favors prior CRC as risk factor to develop rectal carcinoma in patients with IRA. This is further supported by another study that described rectal neoplasia after subtotal colectomy in 17 CD patients with a history of CRC.16 Six of 17 patients (28.6%) developed rectal carcinoma after subtotal colectomy, which is significantly higher than a pooled prevalence of 2.4% (P < 0.001). A history of colorectal dysplasia could not be assessed as risk factor because of insufficient data.

Three studies reported a cumulative incidence of rectal carcinoma in the IRA group after onset of IBD.11–13 A pooled analysis showed cumulative incidences of 0%, 5% (95% CI, 3.0%–7.5%), and 10% (95% CI, 7.0%–12.0%) after 10, 20, and 25 years after IBD onset, respectively. One study estimated cumulative incidences after IRA construction. After 5, 10, 15, and 20 years, respectively, cumulative incidences were 0%, 2%, 5%, and 14% for rectal carcinoma and 7%, 9%, 20%, and 25% for rectal dysplasia.14

**Risk factors.**

UC, a history of CRC, and IBD duration emerged as risk factors for developing rectal carcinoma after IRA construction. None of the included studies specifically evaluated PSC as a risk factor. UC patients, including patients with indeterminate colitis, were more likely to develop rectal carcinoma after IRA construction compared with CD patients. A higher pooled carcinoma prevalence of the rectum was estimated in UC patients versus CD patients (3.2%, 95% CI, 2.3%–4.3% versus 0.7%, 95% CI, 0.2%–1.6%) with OR of 10.3 (95% CI, 2.5–41.9; p = 0.001).
A longer duration of IBD also predisposes development of rectal carcinoma after colectomy and IRA. Others have reported an increasing risk over time in which none of the 22 rectal carcinomas after IRA developed within IBD duration of 10 years (3534 patient-years of follow-up). Beyond 10 years, the risk was 1 in 185 patient-years between 10 and 20 years of IBD duration and 1 in 117 patient-years in patients with IBD history of more than 20 years. Moreover, 1 study showed that patients who developed rectal cancer had a statistically significant longer duration of IBD compared with patients who did not develop rectal cancer (P = 0.030). Nine studies reported IBD duration until rectal carcinoma development, and none of the 49 patients developed cancer within 10 years of IBD duration.

Ileal pouch-anal anastomosis

Prevalence and incidence.

The pooled prevalence of carcinoma in the ileoanal pouch was 0.5% (95% CI, 0.3%–0.6%; Supplementary Table 4, Supplementary Figures 3 and 6). This analysis included 8403 patients with a variable duration of follow-up. Thirty-one articles including 7647 patients reported pouch dysplasia development, resulting in a pooled pouch dysplasia prevalence of 0.8% (0.5%–1.3%). Even studies that only included high-risk patients, such as patients with chronic pouchitis, prior CRC, long pouch duration (> 8 years), or PSC, showed relatively low pouch neoplasia prevalence (0.9%–4.6%).

Three studies reported cumulative incidences of pouch carcinoma after IPAA construction, resulting in a pooled cumulative incidence of 0.4% (95% CI, 0.1%–0.9%), 0.9% (95% CI, 0.2%–1.9%), 1.4% (95% CI, 0.04%–3.0%), 2.7% (95% CI, 2.1%–3.4%), and 3.4% (95% CI, 2.8%–4.0%) after 5, 10, 15, 20, and 25 years, respectively. Cumulative incidences of pouch dysplasia were reported in 2 of these studies. A pooled analysis showed cumulative incidences of pouch dysplasia after IPAA of 0.6% (95% CI, 0.2%–1.2%), 0.9% (95% CI, 0.8%–1.8%), 1.5% (95% CI, 1.2%–1.9%), and 3.0% (95% CI, 2.0%–5.0%) after 5, 10, 15, and 20 years, respectively.

Risk factors.

Risk factors for pouch neoplasia development are a history of colorectal neoplasia and IBD duration. There was insufficient evidence available to evaluate a stapled anastomosis, PSC, and pouchitis as risk factors.

Prior colorectal neoplasia is the most important risk factor for developing pouch neoplasia. A pooled analysis including 5216 patients showed that patients with prior CRC had a statistically significant increased risk to develop pouch carcinoma (OR, 15.0; 95% CI, 6.6–34.5; Figure 2B) compared with patients without a history of CRC. Moreover, an analysis excluding patients with prior CRC showed that prior colorectal dysplasia was also a risk factor for developing pouch carcinoma (OR, 4.4; 95% CI, 1.9–10.1; Supplementary Figure 7). A systematic review of all described pouch carcinoma cases in IBD patients reported that 57.1% of these cases (28 of 49) had prior colorectal neoplasia.

IBD duration might also be considered as a risk factor because patients who developed pouch neoplasia had a significantly longer IBD duration before pouch construction compared with patients who did not develop pouch neoplasia in the univariate analysis of the 2 largest cohort studies. A pooled analysis of these 2 studies including 4403 patients showed that patients with pouch neoplasia had 5.1 years (95% CI, 2.5–7.6) longer IBD history before pouch construction (P < 0.001). Mean pouch duration before cancer was 10.8 7.3 years in all cases described in the literature.

Patients with a hand-sewn anastomosis with mucosectomy carry a higher risk to develop pouch carcinoma compared with patients with a stapled anastomosis as shown in a pooled meta-analysis (OR, 2.9; 95% CI, 1.3–6.6; Supplementary Figure 8). However, no statistical difference was reached when comparing stapled and hand-sewn anastomosis for pouch neoplasia development (OR, 1.7; 95% CI, 1.0–3.1; Supplementary Figure 9).

Less conclusive evidence is available regarding the role of PSC and pouchitis in pouch neoplasia development. One small
A study including 22 patients with IPAA found that patients with PSC had a higher risk to develop atrophic pouch mucosa. Pouchitis was associated with atrophic pouch mucosa development in 2 other studies. These patients with PSC or pouchitis might indirectly carry an increased risk to develop pouch neoplasia because atrophic pouch mucosa is associated with pouch neoplasia development. By contrast, both PSC and pouchitis were not identified as risk factors for pouch neoplasia development in the 2 largest IBD cohorts with IPAA (n = 120028 and n = 320327).

Comparison of rectal and pouch neoplasia in the rectal stump, ileorectal anastomosis, and ileal pouch-anal anastomosis

A summary of the prevalence, incidence, and risk factors for each group is shown in Figure 3. Pooled prevalences of both rectal carcinoma and rectal dysplasia in patients with a residual rectum (rectal stump or IRA) were significantly higher compared with pouch carcinoma and pouch dysplasia (IRA and rectal stump carcinoma versus pouch carcinoma, OR, 6.4, 95% CI, 4.3–9.5, p < 0.001; IRA carcinoma versus pouch carcinoma, OR, 7.1, 95% CI, 4.8–10.7, p < 0.001; IRA dysplasia versus pouch dysplasia, OR, 3.3, 95% CI, 2.1–5.2, p < 0.001; rectal stump carcinoma versus pouch carcinoma, OR, 4.5, 95% CI, 2.5–7.9, p = 0.049). Prevalence of rectal carcinoma in the rectal stump group versus the IRA group did not show significant differences (OR, 0.6, 95% CI, 0.4–1.0, p = 0.074). Because the duration of follow-up after colectomy might influence the prevalence, we compared this between the 3 groups. No differences in follow-up duration were observed between the rectal stump, IRA, and IPAA groups (p = 0.544). In addition, no increasing trend of pooled prevalences over time was observed when analyzed per 5-year mean or median duration of follow-up (Figure 4).
Discussion

One of the key findings that can be derived from our systematic review is a low overall carcinoma prevalence and incidence in IBD patients after (reconstructive) colonic surgery. The cancer prevalence appeared to be dependent on the type of surgery and was highest in IRA patients (2.4%), followed by patients with a rectal stump (2.1%), and lowest in IPAA patients (0.5%). Prior CRC was the most important risk factor for developing rectal or pouch carcinoma (IRA group: OR, 12.8; IPAA group: OR, 15.0). Furthermore, we identified IBD duration and a diagnosis of UC as risk factors.

The calculated prevalences and cumulative incidences of rectal and pouch carcinoma need to be placed in perspective. The lifetime incidence for developing CRC in the general population approaches 5%. Although the cumulative incidence of rectal carcinoma after IRA is based on only 1 study, 5% equals the cumulative rectal carcinoma risk 15 years after IRA construction. The pooled cumulative incidence of pouch carcinoma 25 years after IPAA construction (3.4%) is below the general lifetime CRC risk. None of the reported rectal carcinomas in the IRA group developed within 10 years after IBD onset. Pouch carcinomas developed after mean 10.8 years after IPAA. Furthermore, for proper interpretation of prevalences and incidences we should take a declining CRC risk over time into account because of improved IBD treatment strategies and advanced endoscopic procedures. This may have resulted in lower CRC prevalences and incidences for rectal stump, IRA, and IPAA patients in recent years. Moreover, prevalences and incidences may even be lower because mainly single-center studies rather than population-based cohorts were available for analysis.

A history of colorectal neoplasia before IRA or IPAA surgery is the most important risk factor for subsequent development of rectal and pouch carcinoma (IRA: OR, 12.8; IPAA: OR, 15.0). This is underlined by a shorter pouch duration before cancer diagnosis in IPAA patients with prior dysplasia or cancer compared with those...
without prior pouch neoplasia.\textsuperscript{31} Furthermore, the majority of the carcinomas in the IPAA group arose from the rectal mucosa rather than from the ileal pouch mucosa.\textsuperscript{31} Therefore, it could be speculated that residual colonic mucosa is the main contributor to an increased risk to develop colorectal neoplasia, especially in patients with prior colorectal neoplasia.

As a corollary, one may hypothesize that the total amount of colorectal mucosa in situ may correlate with the subsequent risk to develop rectal or pouch carcinoma. The significantly lower cancer prevalence in the IPAA group compared with the groups with a rectum in situ fuels this hypothesis. In line with this, patients with a complete colon in situ may bear an even higher risk for colorectal neoplasia. This is supported by other authors who showed a lower risk of CRC per patient-year in patients after IRA compared with patients with an intact colon.\textsuperscript{52} On the other hand, patients with a stapled anastomosis, leaving a few centimeters rectal mucosa in situ, were not carrying a higher risk compared with patients with a hand-sewn anastomosis with mucosectomy. The presence of residual colonic mucosal islands that remain even after “complete” mucosectomy might form an explanation for this latter observation.\textsuperscript{57}

UC patients had approximately 10-fold increase in risk to develop rectal carcinoma after IRA construction in comparison with CD patients. This might suggest an association with the inflammatory process, because the rectum is more frequently involved in UC patients. On the other hand, pouchitis, inflammation of the pouch, was not identified as a risk factor. However, pouchitis is variable and often poorly defined, making it difficult to assess this potential risk factor.

Our findings may impact clinical practice because they could provide guidance in developing a postsurgical endoscopic surveillance strategy. Similar to the guidelines for CRC screening, direct evidence regarding the benefit of colorectal surveillance is not available.\textsuperscript{3, 4} To this end, the identified risk factors may assist in recommendations on surveillance. The current British surveillance guidelines distinguish low-risk (no high-risk factors) and high-risk groups (PSC, prior colorectal neoplasia, atrophic mucosa) after colectomy and recommend surveillance intervals of 5 years and 1 year, respectively.\textsuperscript{4} On the basis of our findings, we believe that the presence of a residual rectum after surgery is the major determinant for cancer development. Furthermore, the cancer risk is determined by a history of preoperative colorectal neoplasia, the duration of IBD, and a UC rather than a CD diagnosis. All these factors should be assessed by the clinician and taken into account in a postoperative surveillance strategy. IPAA patients, especially those without prior colorectal neoplasia, have a low cancer risk, and a very limited surveillance program might be sufficient for these patients.

One of the limitations of this review is that the included studies have a high risk of bias, especially those in the rectal stump group. Most studies were retrospective single-center studies introducing selection and recall bias. Furthermore, neoplasia development was often one of the secondary outcomes, and study heterogeneity was significant across studies. For example, some studies offered routine surveillance after colectomy, whereas other studies only performed an endoscopic procedure on indication. In addition, the included studies had a highly variable duration of follow-up, and the year of publication of the included studies varied between 1956 and 2014, which may also introduce bias. In older studies, diagnosis of IBD, detection of dysplasia and carcinoma, and IBD treatment differed from current practice. More recent treatment strategies such as thiopurines and biologicals may have decreased the burden of chronic colonic inflammation and have led to a reduction of cancer risk. In addition, advancing endoscopic visualization techniques may have further reduced cancer rates over time. Indeed, we observed statistically lower carcinoma prevalence in studies published after 1990.

In conclusion, we found significantly lower carcinoma prevalence in the IPAA group (0.5%) compared with the rectal stump
group (2.1%) and IRA group (2.4%). A history of CRC was the most important risk factor, with 15.0-fold (IPAA) and 12.8-fold (IRA) increase in risk. Furthermore, IBD duration and UC rather than a diagnosis of CD emerged as risk factors for rectal or pouch neoplasm. These findings may aid in developing individualized postsurgical endoscopic surveillance strategies to optimize prevention of CRC development in IBD patients.

References

### Solid malignancies in inflammatory bowel diseases

3.4

**Rectal stump.**


**Ileorectal anastomosis.**


17. Khoubchanti IT, Staski J Jr, Nedwich A. Prospective surveillance by rectal biopsy following ileorectal anastomosis for inflammatory disease.

**Supplementary Material.** Reference lists of excluded duplicate publications by same institutions.


### Ileal pouch-anal anastomosis.


Supplementary Figure 1. Forest plot displaying pooled carcinoma prevalence in the rectal stump of IBD patients.

Supplementary Figure 2. Forest plot displaying pooled carcinoma prevalence for IBD patients with IRA.
Supplementary Figure 3. Forest plot displaying pooled carcinoma prevalence for IBD patients with IPAA.

Supplementary Figure 4. Funnel plot analyzing publication bias of prevalence studies regarding rectal cancer in IBD patients with a rectal stump. Visual inspection of the funnel plot does not indicate that some low prevalence studies are missing. Indeed, asymmetry of the plot is confirmed with the Egger test. However, because prevalence cannot extend below “0”, some asymmetry of the funnel plot may be expected. In addition, there are no outliers.

Supplementary Figure 5. Funnel plot analyzing publication bias of prevalence studies regarding rectal cancer in IBD patients with IRA. Visual inspection of the funnel plot does not indicate publication bias, although the Egger test showed some asymmetry of the funnel plot. Many low prevalence studies are included contradicting publication bias.
Supplementary Figure 6. Funnel plot analyzing publication bias of prevalence studies regarding pouch cancer in IBD patients with IPAA. Both visual inspection of the funnel plot and the Egger test showed no indication for publication bias.

Supplementary Figure 7. Forest plot displaying effect of colorectal dysplasia before colectomy on development of IPAA neoplasia. I² (inconsistency) = 0%.

Supplementary Figure 8. Forest plot displaying effect of type of anastomosis on development of IPAA carcinoma. I² (inconsistency) = 0.1%.

Supplementary Figure 9. Forest plot displaying effect of type of anastomosis on development of IPAA neoplasia. I² (inconsistency) = 0%.
<table>
<thead>
<tr>
<th>Author/Year of publication</th>
<th>Center</th>
<th>Inclusion period</th>
<th>Type of study</th>
<th>Rectal cancer/ total (%)</th>
<th>Proctectomy rate (%)</th>
<th>Duration to proctectomy since colectomy</th>
<th>Duration of follow up since colectomy</th>
<th>Overall quality assessment</th>
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IC, indeterminate colitis; NS, not stated.
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<th>Rectal cancer / total patients at risk (%)</th>
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IC, indeterminate colitis; NS, not stated

Supplementary Table 3. Overview of included articles that assessed prevalence of pouch carcinoma after restorative proctocolectomy with IPAA in IBD patients.
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<th>Author/Year of publication</th>
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**Supplementary Table 4.** Overview of included articles that assessed prevalence of pouch cancer after restorative proctocolectomy with IPAA in IBD patients.

**IC, indeterminate colitis; NS, not stated**
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### References for Supplementary Figures and Tables


General discussion

Both life expectancy and inflammatory bowel disease (IBD) prevalence show an increasing trend in recent decades. Consequently, a growing number of IBD patients will develop and may be cured of cancer, resulting in new clinical challenges and questions concerning IBD management. Balancing the effects and risks of IBD treatment versus optimal cancer treatment is very important in order to achieve the best IBD and cancer outcomes for every individual patient. Therefore it is of crucial importance to establish the impact of IBD and immunosuppression on the development, clinical course and recurrence of malignancies. At this time, data on this topic are very limited and treatment decisions are made on expert opinion rather than evidence-based, resulting in a case-by-case approach.

This thesis consists of two parts. Chapter 2 focuses on lymphoproliferations and aims to correlate histopathological assessment and clonality analyses with clinical outcomes. Chapter 3 includes solid malignancies. The objective was to identify IBD-specific risk factors for specific malignancies in IBD, and to compare the clinical course of these malignancies in IBD with the general population. This general discussion addresses these two parts combined with a focus on interpretation of results, future perspectives, as well as identification of knowledge gaps that are possible topics for further research.

In Table 1 an overview of the aims, main findings and conclusions of this thesis are presented.
Table 1: Aims, main findings and conclusions (part 1)

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| 2. Lymphoproliferations | 2.1     | • Assess utility of histological aberrations in predicting EBV presence  | • Atypical infiltrate more frequent in EBV+ patients: warrants EBV testing  
• High EBV load more frequent in EBV+ patients undergoing colectomy  
• IMS reduction effective in decreasing and loss of mucosal EBV  
• Prognosis lymphoproliferation in IBD seems better than in PTLD | • Selection bias: EBV testing on clinical or histological grounds  
• Single tertiary centre  
• What is the influence of IBD activity?  
• WHO PTLD classification applicable for IBD |
|               |         | • Correlate histological assessment and EBV load with clinical outcomes |                                                                 |                                                                                             |                                                                                               |
|               | 2.2     | • Correlate IG clonality to clinical outcomes for risk stratification in PTLD and IBD-related lymphoproliferations | • IG-clonality might be useful in risk stratification in PTLD  
• In IBD low disease stage is more predictive of survival, regardless of clonality  
• IMS reduction effective treatment of IBD lymphoproliferations | • Retrospective  
• Limited sample size |                                                                                               |
| 3. Solid Malignancies | 3.1     | • Explore risk factors for GC development in IBD  
• Compare histological features and clinical course of GC in IBD to general population | • Elderly onset IBD is risk factor for GC development in IBD  
• GC survival is reduced in IBD patients  
• IMS did not impact GC survival  
• Hp prevalence 25 % in IBD GC, EBV+ in 4.2 % of IBD GC | • IMS duration and timing related to GC important to assess IMS influence  
• Three different databases  
• Propensity bias? |                                                                                               |
|               | 3.2     | • Identify IBD-specific risk factors for melanoma development  
• Compare clinical course of melanoma in IBD to general population | • IBD extent is risk factor for melanoma development in IBD  
• No impaired survival after melanoma in IBD, independent of IMS use | • No information about skin type, sun burns and mitotic index  
• Propensity bias?  
• IMS duration and timing related to melanoma important to assess IMS influence  
• Retrospective: under-reporting of IMS use? |                                                                                               |
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| 3: Solid Malignancies | 3.3 | • Identify risk factors for development of OCC and PC in IBD  
• Compare clinical course of OCC and PC in IBD to general population  
• Assess influence of IMS on clinical course of OCC and PC in IBD | • Higher age at IBD diagnosis risk factor for OCC and PC development (PC in UC)  
• Proximal CD localization risk factor for OCC development  
• Impaired OCC survival in IBD compared to general population  
• Majority of IBD associated oropharyngeal cancers are HPV positive  
• IMS did not impact OCC and PC survival | • HPV prevalence PC in line with general population  
• HPV related to timing and duration of IMS?  
• Data influenced by wide variations in commencement of IMS after cancer diagnosis?  
• Awareness instead of regular oral screening |
| 3.4 | • Determine prevalence, incidence and risk factors regarding CRC following colectomy in 3 groups of IBD patients (permanent ileostomy and rectal stump; IRA and IPAA) | • Prevalence and incidence of CRC after colectomy < 3%  
• Prevalence and incidence of CRC after IPAA < 1%  
• History of CRC most important risk factor for CRC development after colectomy  
• IBD duration and a diagnosis of UC other risk factors | • Pouchitis and PSC emerged not as risk factors  
• Post-colectomy screening only in high risk patients?  
• Included studies had highly variable follow-up duration and publication year, many single centre studies |

EBV: Epstein-Barr virus; IBD: Inflammatory bowel diseases; IMS: Immunosuppression (including steroids, thiopurines, metotrexate and anti-TNF alpha); WHO: World Health Organization; Ig: Immunoglobulin; PTLD: post-transplantation lymphoproliferative disorder; GC: Gastric cancer; OCC: Oral cavity cancer; PC: Pharyngeal carcinoma; UC: Ulcerative colitis; CD: Crohn’s disease; HPV: Human papilloma virus; CRC: Colorectal carcinoma; IRA: Ileorectal anastomosis; IPAA: Ileal pouch-anal anastomosis; PSC: Primary sclerosing cholangitis.
Lymphoproliferations

Results
We studied a cohort of 58 IBD patients who were evaluated for colonic Epstein-Barr virus (EBV) presence, through histopathological testing. The World Health Organization post-transplant lymphoproliferative disorders (PTLD) classification was used to classify IBD-related lymphoproliferations. An atypical inflammatory infiltrate and presence of atypical B-lymphocytes were significantly more common in EBV positive colonic mucosa. Monomorphic lesions contained a higher EBV load and these patients needed surgery more often. Reduction of immunosuppression was an effective strategy to achieve morphological normalization and loss of EBV.

This cohort was also part of a multicentre cohort study in which we studied Immunoglobulin (Ig) clonality assessment in IBD patients with EBV positive lymphoproliferations. In IBD patients, Ig clonality analyses were not useful for clinical course risk stratification, and low disease stage of the lymphoproliferation was more predictive of survival. There was no mortality in this cohort with IBD-related lymphoproliferations.

Implications
Based on our study, histopathological assessment can guide EBV diagnostics as well as classification of IBD-related lymphoproliferations. An atypical inflammatory infiltrate and/or presence of atypical B-lymphocytes warrants EBV testing. At this moment, the body of evidence to guide treatment decisions is limited at best and patients are treated on a case-by-case base. Our classification provides objective criteria for lymphoproliferations in IBD, enabling more standardized research and treatment evaluation. Especially for patients with monomorphic lesions (atypical inflammatory infiltrate and presence of atypical B-lymphocytes) at the very least immunosuppressive therapy should be reduced in order to prevent lymphoma development.

Future perspectives
EBV-positive cells can be found in the inflamed colonic mucosa of the majority of IBD patients. At this time we are unable to distinguish inflammation-driven EBV presence that will resolve upon anti-inflammatory treatment and the EBV presence that will cause lymphoproliferations. Nor do we know if and where there is a turning point in ‘physiologic’ EBV presence in inflamed mucosa and the origin of lymphoproliferations. Further research is needed to answer these questions.

In PTLD preventive treatment strategies exist to prevent lymphoma development. In IBD there are none. There are even suggestions that pre-emptive treatment with Rituximab has a
negative influence on the IBD course. We need further research to identify markers of IBD-related lymphoproliferations and criteria that help in clinical risk stratification and decision making.

Finally, prospective research in a larger cohort with an interobserver agreement study is needed to confirm our results.

Solid malignancies

Studies guiding the management of IBD after a specific cancer are very scarce. Due to this paucity of data, a case-by-case approach is advocated\(^2\,3\). We performed studies focusing on cancer-specific clinical course, consisting of case-control studies on gastric cancer (chapter 3.1), melanoma (chapter 3.2), oral cavity cancer and pharyngeal cancer (chapter 3.3) and of a systematic review on neoplasia in IBD patients following subtotal colectomy (chapter 3.4).

Case control studies

Results

Gastric cancer (chapter 3.1)

We studied 63 IBD patients who developed gastric cancer (GC) and found impaired survival for IBD patients. No differences in survival related to immunosuppression use were observed. Elderly onset IBD emerged as a risk factor for GC development. Finally, histopathological assessment showed no increased incidence of EBV and *Helicobacter pylori* (Hp) positive GC in IBD.

Melanoma (chapter 3.2)

We included 304 IBD patients who developed melanoma and found that melanoma survival in IBD patients is similar compared to the general population. The use of immunosuppressive therapy did not impair survival following melanoma. A more extensive IBD (pancolitis in UC; ileal and colonic involvement in CD) is a risk factor for melanoma development in IBD patients.

Oral cavity and pharyngeal cancer (chapter 3.3)

We evaluated 66 patients with oral cavity cancer (OCC) and 31 with pharyngeal cancer (PC). Survival analyses showed that the overall survival in OCC is impaired and in PC similar compared to the general population. Use of immunosuppressive medication did not affect survival after cancer diagnosis. For both OCC and PC development in IBD elderly onset IBD was a risk factor. For OCC, proximal disease activity was also a risk factor. Histopathological assessment showed human papillomavirus (HPV) presence in 52% of the oropharyngeal cancers in IBD patients, comparable with the general population.

Implications

Our nationwide studies contribute to the knowledge about the clinical course of malignancies in IBD. As a corollary they may aid in guiding IBD management after a specific cancer diagnosis.

In line with multiple recent publications\(^4\,5\) we found no evidence for a negative influence of immunosuppressive therapy on the cancer course. Therefore our data may suggest that immunosuppressive therapy can still be used after GC, melanoma, OCC and PC diagnosis. Furthermore, survival of melanoma and PC in IBD is not impaired. Although these data may be reassuring in daily practice, they have to be interpreted with great caution for a number of reasons. The first is the possible presence of selection bias. Solid data in transplant medicine have shown that immunosuppressive therapy can negatively influence the clinical course and recurrence of malignancies. IBD clinicians may be aware of these risks and do not prescribe immunosuppressive drugs to IBD patients with a malignancy with high risk of recurrence, positively influencing the outcomes of the retrospective studies. Secondly, the moment of starting immunosuppressive therapy after cancer diagnosis varied widely. As the influence of immunosuppression is greatest in the two years after cancer diagnosis\(^6\), this may have influenced our data. Thirdly, not all IBD patients use immunosuppressive therapy and the number of
patients who actually used it after cancer diagnosis in our studies was limited. Fourthly, the number of studied patients with a diagnosis of cancer and using immunosuppressive therapy is too small to draw robust conclusions regarding the risk of cancer recurrence. Therefore, we still advocate the case-by-case approach for IBD patients with a malignancy: balancing the effects and risks of IBD treatment with optimal cancer treatment to achieve the best IBD and cancer outcomes.

Elderly onset IBD emerged in our GC, OCC and PC studies as a risk factor for malignancy development. Although the explanation is not elucidated yet, it may be related to the aging of the immune system: aging-related reduced immunosurveillance might increase the risk for malignancy development\(^7\). With the growing aging population it is important that clinicians are especially aware of cancer development in elderly onset IBD patients.

**Strengths and limitations**

These cancer specific studies are the largest series on the clinical course of specific malignancies in IBD. The comparable retrospective design of our case-control studies explains the corresponding limitations. Due to the retrospective character of the studies, it was not possible to assess the exact dose, duration and time between cancer diagnoses and (re)start of (immunosuppressive) therapy. Therefore it was impossible to draw firm conclusions on this subject. We considered linking our patients with the Dutch pharmacology database, but this database was only available for a small part of the Netherlands.

In our studies we used three different databases: the Dutch pathology database PALGA\(^8\), the Eindhoven Cancer Registry and the IBD South Limburg cohort\(^9\). This may have caused biases as the design and definitions between the three databases varied. Unfortunately, we could not answer our research questions using a single database.

At the start of this PhD trajectory we also planned to calculate the relative risk (RR) for IBD patients to develop a specific tumor. Despite our efforts, it remained difficult to determine the extent of the Dutch IBD population: at present and in the past.

**Future perspectives**

To determine the risk of developing a specific cancer in IBD patients and to establish the effects of immunosuppressive therapy on the risk and the clinical course of that specific cancer, large prospective databases are needed. As malignancies in IBD are relatively rare, there are large databases needed which include sufficient cases with a specific malignancy to draw conclusions that can guide clinical decision-making. In these databases the timing, dose, duration and time between cancer diagnoses and start of (immunosuppressive) therapy should be accurately registered. Furthermore, the effect of the cancer treatment on the IBD activity should be observed to assess the influence of cancer therapy on IBD. With these data we can achieve optimal IBD and cancer management.

**Systematic review (chapter 3.4)**

**Results**

The colorectal cancer (CRC) prevalence for patients with a rectal stump was 2.1\%(95% confidence interval (CI) 1.3\%-3.0\%), 2.4\% for patients with an ileorectal anastomosis (95% CI 1.7\%-3.0\%) and 0.5\% for patients with an ileal pouch – anal anastomosis (IPAA; 95% CI 0.3\%-0.6\%). In all three groups, the most important risk factor for developing CRC post colectomy was a prior CRC. Other risk factors were IBD duration and ulcerative colitis rather than Crohn’s disease. Pouchitis and primary sclerosing cholangitis did not emerge as risk factors.
Implications
Surveillance guidelines for IBD patients after subtotal colectomy are lacking. This may result in ineffective screening in these patients with intervals that are either too long or too short. Our data suggest that high-risk patients, with a history of CRC, should be screened more frequently after subtotal colectomy. In contrast, in the absence of risk factors, surveillance interval may be reduced to 5 years. Furthermore, as UC is another risk factor, surveillance intervals may be different for UC and CD. Finally, IPAA patients without a CRC history may no longer need endoscopic surveillance.

Strengths and limitations
As malignancies in IBD are relatively rare, large cohorts are needed to yield enough power to detect differences and risk factors. To increase statistical power, this review and meta-analysis was performed with pooled data from several cohorts. Nevertheless, this also introduced bias because the included studies had highly variable follow-up duration and year of publication. As the publication year varied by more than 50 years, treatment options, treatment guidelines, surveillance strategies and endoscopic techniques were very different between studies. Furthermore, many of the included studies were single centre studies.

Future perspectives
To assess the benefit of surveillance following subtotal colectomy, we need large prospective studies with long-term follow-up, to standardize our policies, and to follow widely accepted guidelines. To further optimize this surveillance, we need more knowledge about the pathophysiology of progression of low grade dysplasia to high grade dysplasia and factors influencing this process. This can aid in optimizing surveillance strategies.

Conclusions
Cancer development is one of the most serious complications in IBD. A growing number of IBD patients will develop and may be cured of cancer, providing new clinical challenges and questions. In this thesis we correlated histopathological assessment with clinical outcomes in IBD. Furthermore, we assessed IBD-specific risk factors and clinical outcomes for solid malignancies and determined cancer risks after colectomy.

We found that histopathological assessment can guide EBV diagnostics and that IMS reduction is an effective treatment for IBD-related lymphoproliferations. Survival is reduced in IBD for GC and OCC, but not for melanoma and PC. In our studies, immunosuppression (including anti-TNF alpha) did not influence survival in solid malignancies. A prior CRC is the most important risk factor for developing a post-colectomy CRC.

As data on malignancies in IBD are limited, these findings may guide clinical decision making for patients with the studied malignancies. As our studies are retrospective, prospectively collected data are needed to further optimize IBD and cancer management.
References


Inflammatory bowel diseases (IBD) include Crohn’s disease (CD) and ulcerative colitis (UC) and are characterized by chronic recurrent intestinal inflammation. The medical treatment of IBD aims to induce and maintain prolonged remission and to prevent complications. One of the most serious complications of IBD is cancer development. Continuous chronic intestinal inflammation increases the risk of intestinal malignancies, such as colorectal cancer (CRC). Moreover, the risk of extra-intestinal malignancies is increased by the use of immunosuppressive medication.

Both life expectancy and IBD prevalence are increasing. Consequently, a growing number of IBD patients will develop cancer, resulting in new clinical challenges and questions concerning IBD management. Balancing the effects and risks of IBD treatment versus optimal cancer treatment is very important in order to achieve the best IBD and cancer outcomes. Therefore, it is very important to establish the impact of IBD and immunosuppression on the development, clinical course and recurrence of malignancies. Currently, data on this topic are limited and treatment decisions are expert based rather than evidence-based, resulting in a case-by-case approach.

This thesis covers mainly two subjects. Chapter 2 focuses on lymphoproliferations and aims to assess the utility of intestinal histologic features in predicting EBV presence and to correlate histopathological assessment with clinical outcomes in IBD patients. Chapter 3 includes studies on solid malignancies and aims to identify IBD-specific risk factors for particular malignancies and to compare the clinical characteristics of these malignancies in IBD patients to unselected non-IBD patients. This includes also the influence of immunosuppressive therapy on clinical characteristics.
Lymphoproliferations
Post-transplant lymphoproliferative disorders (PTLD) are proliferations of B-cells that develop as a consequence of immunosuppression use in transplant patients and are frequently associated with the Epstein-Barr virus (EBV). PTLDs are classified according to the histological World Health Organization (WHO) PTLD classification. Lymphoproliferative disorders in IBD are histologically comparable to PTLD and are also predominantly EBV associated. Immunosuppressive IBD medication causes decreased immunosurveillance of EBV and facilitates reactivation of this oncogenic virus with subsequently (intestinal) lymphoproliferation development.

Chapter 2.1 aimed to assess whether histological aberrations aid in predicting intestinal EBV. We retrospectively included 58 IBD patients from our tertiary hospital with prior EBV testing on their intestinal biopsies. An atypical inflammatory infiltrate was more frequent in EBV-positive than in EBV-negative patients (57.1 versus 3.3%; \( p < 0.001 \)). This implicates that the presence of an atypical infiltrate in the intestinal mucosa of IBD patients warrants EBV testing. Reduction of immunosuppression is an effective strategy to achieve morphological normalization and loss of EBV. Furthermore, the histological EBV load was correlated with clinical outcomes. EBV-positive patients undergoing colectomy had more frequent a high EBV load compared to EBV-positive patients without colectomy. We also studied the use of the WHO PTLD classification in IBD related lymphoproliferations. This classification is suitable for IBD associated lymphoproliferations, enabling more standardized research and treatment evaluation.

Immunoglobulins (Ig) are antibodies produced by B-cells and are used by the immune system to neutralize pathogens such as pathogenic viruses. The aim of Chapter 2.2 was to correlate Ig clonality with clinical outcomes of lymphoproliferations in both PTLD and IBD. This may lead to pre-treatment risk stratification to guide therapeutic decisions. We found no clear difference in clonality pattern in monomorphic subtype PTLDs versus the reactive/polymorphic PTLDs. A statistical analysis was performed to identify pre-treatment risk factors for poor outcome (mortality). In PTLD, Ig-clonality might be useful for risk stratification. In IBD lower disease stage is more predictive of survival.

Both Chapter 2.1 and 2.2 indicate that IBD related lymphoproliferations appear to have less aggressive clinical behaviour than PTLDs, what is reassuring for daily IBD practice. Reduction of immunosuppression is an appropriate treatment, especially for lower stages of lymphoproliferations, and should be considered by treating physicians.

Chapter 2.3 presents a case-report with a CD patient who developed a hepatosplenic T-cell lymphoma (HSTCL). HSTCL is rare, not EBV related non-Hodgkin lymphoma with a high mortality rate. Higher incidence is reported in IBD patients, specifically in male patients younger than 35 years, treated with thiopurine and anti-TNF alpha combination therapy for over 2 years. This case is unusual as the presented patient is 47 years old and developed HSTCL after 14 years thiopurine monotherapy. This emphasizes that HSTCL risk is not limited to young men receiving combination therapy.

Solid malignancies
Studies guiding IBD management after a specific cancer are scarce and a case-by-case approach is advocated. In the second part of this thesis, specific malignancies in IBD are studied, aiming:

I: to assess IBD specific risk factors for cancer development (Chapter 3.1 - 3.4).
II: to compare clinical characteristics and survival between IBD cases with a specific malignancy versus non-IBD controls with the same malignancy (Chapter 3.1, 3.2 and 3.3).
IBD patients with a specific malignancy (IBD cases) were identified using PALGA, the Dutch pathology database. This registry contains pathology reports generated in the Netherlands since 1971 and has complete national coverage since 1991 encompassing all pathology laboratories from all hospitals in the Netherlands.

For the first (I) aim we compared IBD cases with a specific malignancy to random IBD controls, derived from the population based IBD South Limburg (IBDSL) cohort. South Limburg is an enclosed geographic area in the southeast of The Netherlands with 605,000 inhabitants and three hospitals. The total number of IBD patients in this registry is 2807 IBD patients representing 93% of the regional IBD population.

For the second (II) aim, controls were derived from the Eindhoven Cancer Registry (ECR; maintained by the Netherlands Comprehensive Cancer Organization). Since 1989, the ECR prospectively registers all newly diagnosed cancers in the regions “Noord-Brabant” and “Noord-Limburg”, encompassing over 95% of all cancers in this region.

Two factors are important in the development of malignancies in IBD: local inflammation and immunosuppression use (causing reduced immunosurveillance). In the malignancies we studied, we hypothesized that at least one of these factors might be involved. Local inflammation may be important for gastric cancer (GC) and oral cavity cancer (OCC) development (beside colorectal cancer). Both EBV and *H. pylori* are pathogens that increase the GC risk and their activity may be influenced by immunosuppression. In addition to EBV, human papillomavirus (HPV) is also important in (oro)pharyngeal cancer development. Finally melanoma was studied, as anti-TNF therapy increases the risk for melanoma development.

In chapter 3.1 63 IBD patients with GC were included. Elderly onset IBD emerged as a risk factor for GC development and survival in IBD patients was impaired. Histopathological assessment showed no increased incidence of EBV and Helicobacter pylori (Hp) positive GC in IBD.

In chapter 3.2 we included 304 IBD patients with cutaneous melanoma. A more extensive IBD (pancolitis in UC; ileal and colonic involvement in CD) is a risk factor for melanoma development in IBD patients. Melanoma survival in IBD patients is similar compared to the general population.

In chapter 3.3 we studied two head and neck cancer localizations: OCC and pharyngeal cancer (PC). 66 IBD patients with OCC were included, showing that the overall survival of OCC in IBD is impaired. 31 IBD patients with PC were included, with similar survival compared to the general population.

For both OCC and PC development in IBD elderly onset IBD was a risk factor. For OCC, proximal disease activity was also a risk factor.

Histopathological assessment of (O)PC showed HPV presence in 52 % of the oropharyngeal cancers in IBD patients, comparable with the general population.

In chapter 3.1, 3.2 and 3.3 we also studied the influence of immunosuppressive therapy on survival. We observed no differences in survival related to immunosuppression use after cancer diagnosis. Although these data may be reassuring in daily practice, they have to be interpreted with great caution for a number of reasons. First, selection bias may have occurred since clinicians may have prescribed immunosuppressive medication to patients with a favourable risk profile. Second, the moment of starting immunosuppressive therapy after cancer diagnosis varied widely. As the influence of immunosuppression is greatest in the two years after cancer diagnosis, this may impact our findings. Third, the number of IBD patients using immunosuppressive therapy after cancer diagnosis was limited.

Chapter 3.4 includes a systematic review and meta-analysis to determine risk factors and assess CRC risk in IBD patients following subtotal colectomy. We studied three groups: patients
with a rectal stump, patients with an ileorectal anastomosis (IRA) and patients with an ileal pouch–anal anastomosis (IPAA). We calculated CRC prevalences based on 13 studies about CRC development in the rectal stump, 35 IRA studies and 33 IPAA studies.

The CRC prevalence was 2.1% in patients with rectal stump, 2.4 % in IRA patients and lowest in IPAA patients (0.5 %). Prior CRC was the most important risk factor for developing CRC following subtotal colectomy (IRA group: odds ratio 12.8; IPAA group: odds ratio 15.0). Furthermore, IBD duration and a diagnosis of UC rather than CD were identified as risk factors. Pouchitis and primary sclerosing cholangitis did not emerge as risk factors. These data may help in developing endoscopic surveillance recommendations for IBD patients after subtotal colectomy.

In conclusion, in this thesis we correlated histopathological assessment with clinical outcomes in IBD. We found that histopathological assessment can guide EBV diagnostics and that immunosuppression reduction is an effective treatment for IBD-related lymphoproliferations. Furthermore, we assessed IBD-specific risk factors and clinical outcomes for solid malignancies and determined cancer risks after colectomy. Survival is reduced in IBD for GC and OCC, but not for melanoma and PC. In our studies, immunosuppression did not influence survival in solid malignancies. As data on malignancies in IBD are limited, these findings may guide clinical decision making for patients with the studied malignancies. As our studies are retrospective, prospectively collected data are needed to further optimize IBD and cancer management.
stuk 3 bevat studies over solide maligniteiten. Het doel is om IBD specifieke risicofactoren te identificeren voor bepaalde maligniteiten, om het klinische beloop van maligniteiten bij IBD te vergelijken met het beloop bij niet-IBD patiënten. Hierin bestu-deren we ook de invloed van immunsuppressieve therapie op de klinische karakteristieken.

Lymfoproliferaties
Post-transplantatie lymfoproliferatieve aandoeningen (PTLD) zijn proliferaties van B-cellen, die zich ontwikkelen als het gevolg van het gebruik van immunsuppressieve medicatie bij transplantatie patiënten. Ze zijn vaak Epstein-Barr virus (EBV) geassocieerd. PTLDs worden geclassificeerd volgens de histologische World Health Organization (WHO) PTLD classificatie. IBD gerelateerde lymfoproliferaties zijn histologisch vergelijkbaar met PTLD en ook voornamelijk geassocieerd met EBV. Immunsuppressieve medicatie vermindert de immunosurveillance van EBV waardoor er reactivatie van dit oncogene virus kan ontstaan. Dit kan vervolgens leiden tot de ontwikkeling van (intestinale) lymfoproliferaties.

Hoofdstuk 2.1 heeft als doel het beoordelen of histologische afwijkingen kunnen helpen bij het voorspellen van intestinale EBV aanwezigheid. Hiervoor includeerden we retrospectief 58 IBD patiënten uit ons tertiaire ziekenhuis, waarbij reeds eerder EBV diagnostiek verricht was op intestinale biopten. Een atypisch ontstekingsinfiltraat kwam frequenter voor bij EBV-positieve patiënten dan bij EBV-negatieve patiënten (57.1 versus 3.3%; p < 0.001). Dit impliceert dat de aanwezigheid van een atypisch ontstekingsinfiltraat in de intestinale mucosa van IBD patiënten EBV diagnostiek rechtvaardigt. Het reduceren van immuunsuppressieve medicatie is effectief om morfologisch herstel en verlies van EBV aanwezigheid te bereiken.

Tevens werd de histologische EBV concentratie gecorreleerd met klinische uitkomsten. EBV-positieve patiënten die een colectomie ondergingen, hadden vaker een hoge EBV concentratie in vergelijking met patiënten die geen colectomie ondergingen. Ook bestudeerden we of het gebruik van de door de wereldgezondheidsorganisatie (WHO) vastgestelde PTLD classificatie ook geschikt was voor gebruik bij IBD gerelateerde lymfoproliferaties. Het gebruik van de WHO PTLD classificatie is geschikt om te gebruiken bij IBD gerelateerde lymfoproliferaties. Hierdoor kan er meer gestandaardiseerd onderzoek en behandeling verricht worden.

Immuunglobulines (Ig) zijn antilichamen die door de B-cel geproduceerd worden en gebruikt worden in het immuunsysteem om pathogenen (zoals EBV) te neutraliseren. Het doel van hoofdstuk 2.2 was het correleren van immuunglobuline (Ig) clonaliteit met klinische uitkomsten, ten behoeve van risico stratificatie. Dit kan mogelijk helpen bij pre-treatment risicostratificatie en bij het nemen van behandel beslissingen. We vonden geen verschil in clonaliteitspatroon tussen monomorfe en reactieve of polymorfe PTLDs. Een statistische analyse werd verricht om risicofactoren voor slechter uitkomst (mortaliteit) te bepalen. Bij PTLD patiënten kan het bepalen van Ig-clonaliteit nuttig zijn ter risico stratificatie, maar niet voor IBD. Bij IBD was voornamelijk een lager ziekte stadium voorspellend voor overleving.

Zowel hoofdstuk 2.1 en 2.2 laten zien dat IBD gerelateerde lymfoproliferaties een minder agressief klinisch beloop lijken te hebben dan PTLD. Dit is geruststellend voor de dagelijkse IBD praktijk. Het reduceren van immuunsuppressie is een geschikte behandeling voor IBD geassocieerde lymfoproliferaties, met name in de lagere stadia.

Hoofdstuk 2.3 presenteert een case-report van een ZvC patient die een hepatosplenisch T-cell lymfoom (HSTCL) ontwikkelt. Een HSTCL is een zeer zeldzame, niet EBV gerelateerde, non-Hodgkin lymfoom met zeer hoge mortaliteit. Dit lymfoom komt vaker voor bij IBD patiënten, met name bij mannen onder de 35 jaar, die...
langer dan twee jaar behandeld zijn met combinatie therapie van thiopurines en anti-TNF alpha. Deze casus is ongewoon, omdat de gepresenteerde patiënt 47 jaar oud is en HSTCL ontwikkelde na 14 jaar thiopurine monotherapie. Dit laat zien dat HSTCL ontwikkeling niet beperkt is tot jonge mannen die combinatie therapie krijgen.

**Solide maligniteiten**

Studies die de IBD behandeling na specifiek tumoren bestuderen zijn zeer schaars. Derhalve wordt gepleit voor een ‘case-by-case’ benadering. In het tweede deel van dit proefschrift, worden specifieke maligniteiten bij IBD patiënten bestudeerd met als doel: I: het bestuderen van IBD specifieke risicofactoren voor het ontwikkelen van een specifieke maligniteit (hoofdstuk 3.1 – 3.4). II: het vergelijken van klinische karakteristieken en overleving van IBD cases met een specifieke maligniteit met niet-IBD patiënten met die maligniteit (hoofdstuk 3.1, 3.2 en 3.3).

IBD patiënten met een specifieke maligniteit (IBD cases) zijn geïdentificeerd met behulp van PALGA, de Nederlandse pathologie database. Deze database bevat pathologie verslagen sinds 1971 en heeft nationale dekking van alle ziekenhuizen in Nederland sinds 1991. Voor het eerste doel (I) vergeleken we IBD cases met een specifieke maligniteit met random IBD controles, die we verkregen uit het population-based IBD Zuid-Limburg (IBDSL) cohort. Zuid-Limburg is een ingesloten gebied in het Zuid-Oosten van Nederland met 605.000 inwoners en 3 ziekenhuizen. Het totaal aantal IBD patiënten in de database is 2807, wat 93 % van regionale IBD populatie vertegenwoordigt.

Voor het tweede doel (II) verkregen we controles uit de Eindhoven Cancer Registry (ECR; onderdeel van Integraal Kankercentrum Nederland). Sinds 1989, registreert de ECR alle nieuw gediagnosticeerde maligniteiten in “Noord-Brabant” en “Noord-Limburg” en bevatten meer dan 95 % van alle maligniteiten in dit gebied.

**De twee factoren die het belangrijkst zijn in de ontwikkeling van maligniteiten bij IBD zijn lokale ontsteking en het gebruik van immuunsuppressie (wat de immuunsurveillance vermindert).** Van de maligniteiten die in dit proefschrift worden beschreven, was de hypothese dat tenminste 1 van deze 2 factoren betrokken kon zijn. Lokale inflammatie zou, behalve bij een colorectaalcarci-

noom, een belangrijke factor kunnen zijn bij de ontwikkeling van een maagcarcinoom of een mondholte carcinoom. Zowel EBV als H. pylori zijn pathogenen die het risico op de ontwikkeling van een maagcarcinoom vergroten. Hun activiteit kan beïnvloed worden door het gebruik van immuunsuppressie. In aanvulling op EBV is het humaan papillomavirus (HPV) een belangrijke factor in de ontwikkeling van (oro)pharynx carcinoom. Als laatste hebben we het melanoom bestudeerd, omdat het bekend is dat het risico hierop verhoogd wordt door het gebruik van anti-TNF therapie.

In hoofdstuk 3.1 includeerden we 63 IBD patiënten met maagcarcinoom. Het ontwikkelen van IBD op oudere leeftijd bleek een risicofactor voor het ontwikkelen van maagcarcinoom bij IBD. De overleving van IBD patiënten met maagcarcinoom was slechter dan van controles. Histologische beoordeling liet geen toename van incidentie van Helicobacter pylori of EBV positieve tumoren zien bij IBD patiënten.

In hoofdstuk 3.2 werden 304 IBD patiënten met een cutaan melanoom geïncludeerd. Uitgebreidere IBD (pancolitis bij CU en zowel colon als ileum betrokkenheid bij ZvC) is een risicofactor voor melanoom ontwikkeling bij IBD patiënten. De overleving van melanomen is vergelijkbaar tussen IBD patiënten en niet IBD patiënten.

Hoofdstuk 3.3 beschrijft 2 tumoren in het hoofd-hals gebied: mondholte carcinomen (OCC) en pharynx carcinomen (PC). Er werden 66 patiënten met OCC en IBD geïncludeerd, waarbij de overleving van IBD patiënten verminderd is. Ook werden 31 patiënten met PC en IBD geïncludeerd, waarbij geen verschil gevonden werd in overleving tussen IBD patiënten.
en niet-IBD controles. Voor zowel OCC als PC bleek het ontwikkelen van IBD op hogere leeftijd een risicofactor. Bij OCC bleek ook proximale ziekte activiteit een risicofactor te zijn.

Histologische beoordeling liet zien dat 52% van de oropharyngeale carcinomen HPV positief waren, wat vergelijkbaar is met het percentage HPV positieve tumoren in de algemene bevolking.

In hoofdstuk 3.1, 3.2 en 3.3 werd ook de invloed van immuunsuppressieve medicatie op overleving bestudeerd. Er werden geen verschillen gevonden als gevolg van het gebruik van immuunsuppressieve medicatie na maligniteit diagnose. Hoewel deze bevindingen enerzijds heel geruststellend zijn voor de dagelijkse praktijk, is het belangrijk dat deze data zeer voorzichtig geïnterpreteerd worden. Dit heeft meerdere redenen: ten eerste kan er een selectiebias zijn. Artsen zijn zich bewust van de mogelijke risico’s van immuunsuppressieve medicatie, waardoor ze dit alleen voorschrijven bij patiënten met maligniteiten met een laag risico profiel. Ten tweede is het moment van starten van immuunsuppressieve medicatie van belang, omdat de (negatieve) invloed van immuunsuppressie het grootst is in de twee jaar na de maligniteit diagnose. In onze studie was er ruime variatie in tijd tussen maligniteit en starten van immuunsuppressieve medicatie. Ten derde, het totaal aantal patiënten wat immuunsuppressie gebruikte na maligniteit diagnose was beperkt.

Het laatste artikel in hoofdstuk 3.4 bevat een systematische review en meta-analyse met als doel het bepalen van risicofactoren en het absolute risico op het ontwikkelen van een CRC na subtotale colectomie. Er werden 3 groepen bestudeerd: patiënten met een rectum stomp, patiënten met een ileorectale anastomose en patiënten met een ileoanale pouch (IPAA). Berekende CRC prevalenties waren gebaseerd op 13 studie over rectum stomp, 35 IRA studies en 33 IPAA studies. De CRC prevalentie was 2.1% bij patiënten met een rectum stomp, 2.4% bij IRA patiënten en 0.5% bij IPAA patiënten. Het eerder gehad hebben van een CRC was de belangrijkste risicofactor om een CRC te ontwikkelen na subtotale colectomie (IRA patiënten: odds ratio 12.8; IPAA patiënten: odds ratio 15.0). Verder bleken IBD duur en CU meer dan ZvC risicofactoren voor de ontwikkeling van CRC en niet pouchitis primair scleroserende cholangitis. Deze data helpen bij het opstellen voor surveillance aanbevelingen bij IBD patiënten met een subtotale colectomie.

In conclusie hebben we in dit proefschrift de histopathologische beoordeling gecorreleerd met klinische uitkomsten bij IBD. Histologische beoordeling kan leiden tot inzet van EBV diagnostiek. Het reduceren van immuunsuppressieve medicatie is een effectieve behandeling voor IBD geassocieerde lymfoproliferaties.

Verder bestudeerden we IBD specifieke risicofactoren en de klinische uitkomsten bij solide tumoren. Ook bepaalden we het CRC risico na subtotale colectomie. De overleving bij IBD patiënten is verminder bij maagcarcinomen en OCC, maar niet bij melanomen en PC. In onze studies was er geen negatieve beïnvloeding van survival door het gebruik van immuunsuppressie.

Omdat data over maligniteiten bij IBD beperkt zijn, kunnen deze resultaten besluitvorming in de dagelijkse klinische praktijk beïnvloeden bij IBD patiënten met de bestudeerde maligniteiten. Omdat onze studies retrospectief zijn, zijn prospectieve data hard nodig om de behandeling van IBD patiënten met maligniteiten te optimaliseren.
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List of publications


