

Impaired Gastric Cancer Survival in Patients with Inflammatory Bowel Disease

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ABSTRACT

Background & 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 an 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 in IBD to those in 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; and 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 years 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 multivariate 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.

Abbreviations: CD: Crohn's disease; EBV: Epstein-Barr virus; EBER: EBV-encoded RNA; EBER-ISH: EBV-encoded RNA – in situ hybridization; ECCO: European Crohn's and Colitis Organization; ECR: Eindhoven Cancer Registry; GC: Gastric cancer; IBD: Inflammatory bowel disease; IBDSL cohort: Inflammatory Bowel Diseases South Limburg cohort; MMR proteins: Mismatch repair proteins; RIVM: Dutch National Institute for Public Health and Environment; UC: Ulcerative colitis.

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. Inflammatory bowel disease patients are at

increased risk for developing colorectal cancer (CRC) due to chronic intestinal inflammation [1, 2]. In addition, several other malignancies, such as Epstein-Barr virus (EBV) positive lymphomas and non melanoma skin cancer [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 [4].

The cause of GC development in IBD patients is unclear. The majority of sporadic GCs develop via an intestinal metaplasia – dysplasia pathway under the influence of a *Helicobacter pylori* (*H. pylori*) infection [5]. However, in IBD, prevalence of *H. pylori* is lower compared to the general population [6,

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7]. In addition, impaired immunosurveillance [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 adenocarcinomas are associated with gastric EBV infection [9, 10]. It is unknown whether this infection contributes to GC in IBD patients. Furthermore, approximately 5% of CD patients have gastric inflammatory involvement [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 daily practice there is great concern that the use of immunosuppressive medication will cause poorer cancer outcomes. 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) [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 [13, 14].

II. Case control study II was performed to compare the 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 Ethics Committee of the Radboud University Medical Center (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 [12]. 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 three 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 Classification [15], 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 proton pump inhibitors, date of GC diagnosis, location, histological classification according to Laurén [16] 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

The IBD controls for the identification of risk factors were randomly selected (using a 1:3 ratio) from the IBDSL Cohort [13]. The IBDSL cohort is a prospectively followed, population-based cohort, including all new adult IBD cases since 1991. Patients with indeterminate colitis were not included, unless they were 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 (one university hospital and two 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 2,807 IBD patients (40.9% CD, 59.0% UC). This represents 93% of the regional IBD population [14].

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 edition [18]. 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 classification [18], treatment and follow-up. Obtained histological data were evaluated according to the Lauren [16] and WHO classification [17].

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 golden standard test for EBV detection [19], using formalin-fixed, paraffin-embedded (FFPE) tissues of GC specimens (biopsy or resection) [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-a-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- μ m-thick FFPE GC tissue sections. Slides were stained with antibodies against MLH1 (Pharmlingen code: 51-1327gr; dilution 1:50), PMS2 (Pharmlingen 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 C.v.d.P.) and the 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 [21].

Statistics

First, we compared potential risk factors, clinical characteristics of case control study I and II with univariate analyses. For the univariate analysis, we used a χ^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 univariate analyses were included in the multivariate analyses.

For case control study I, a binary logistic regression analysis with a 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 multivariate analysis. Therefore, we performed a multivariate 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 [22]. TNM stage was included as a fixed variable. All missing values were considered to be at random and were therefore excluded from analyses. For our analysis we used the 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 (Fig. 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, 6 had no primary gastric carcinoma, 6 had esophageal carcinoma, 3 because IBD was diagnosed more than 6 months after GC diagnosis and 3 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, 2 sarcomas and 3 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 I displays the univariate comparison of potential risk factors for GC development between IBD cases with GC and IBDSL controls. Both age at IBD diagnosis (median age 61 years 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 an over-representation of the male gender in the case group (*p*=0.06). We found no difference in disease location and behavior, IBD related surgery and use of 5-aminosalicylates, methotrexate and cyclosporin. However, use of steroids, thiopurines and anti-TNF agents was

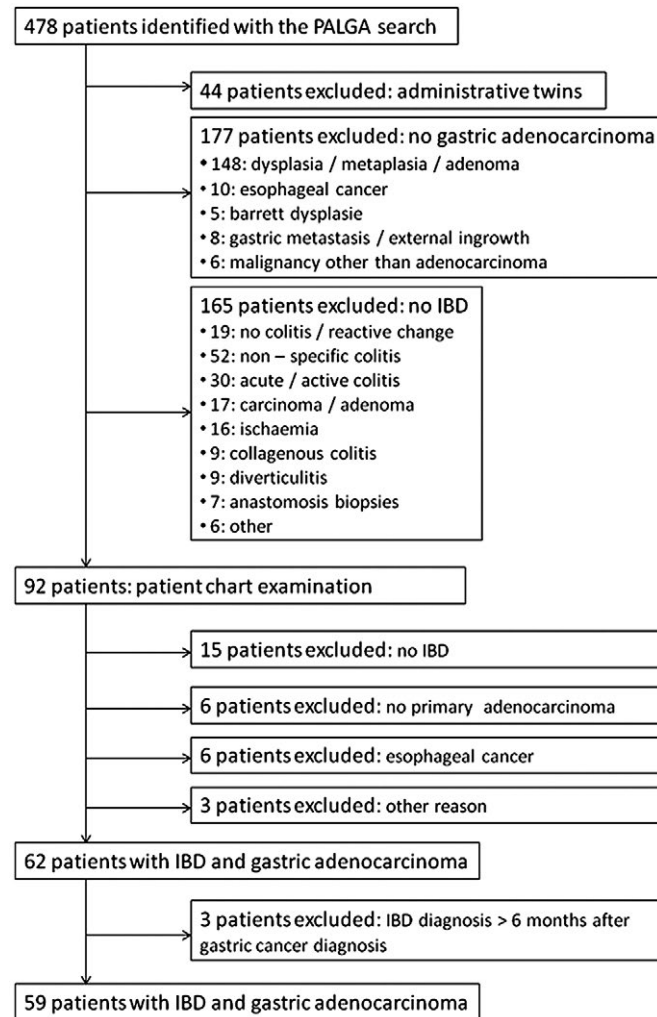


Fig. 1. Flowchart of patient selection.

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 Ib 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-aminosalicylates (71.9% versus 88.1%; $p = 0.03$).

In the multivariate analysis (Table II), 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 the addition of 5-aminosalicylates, as it was a potential confounder (Table Ib). In both the analysis including all cases and in the sensitivity analysis, age at IBD diagnosis was identified as a 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 1.024–1.122, $p < 0.01$).

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 III). 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 univariate analysis (Fig. 2A; $p = 0.53$), but when corrected for confounders, IBD cases evidenced a poorer survival (Fig. 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 (Fig. 3). We excluded 11 cases from analysis due to insufficient amounts of tissue. Two of the 48 tumors (4.2%)

Table 1 a/b. Risk factors for the development of gastric cancer

| Variable | IBDSL N = 177 | a: All IBD cases N = 59 | Missing (n) IBDSL / IBD | P-value | b: IBD > 1990 N = 40 | Missing (n) IBDSL / IBD | P -value |
|-----------------------------------------|------------------|----------------------------|----------------------------|---------|-------------------------|----------------------------|----------|
| Age at diagnosis (years), | | | | | | | |
| median | 40.0 | 61.0 | 1 / 0 | < 0.01* | 65.0 | 1 / 0 | < 0.01* |
| Female sex; n (%) | 88 (49.7) | 21 (35.6) | 0 / 0 | 0.06 | 14 (35.0) | 0 / 0 | 0.09 |
| Smoking (no); n (%) | 30 (39.5)* | 25 (42.4) | 15 / 13 | 0.11 | 15 (50.0) | 15 / 10 | 0.32 |
| IBD type | | | | | | | |
| Ulcerative colitis; n (%) | 91 (51.4) | 41 (69.5) | 0 / 0 | < 0.01* | 29 (72.5) | 0 / 0 | < 0.01* |
| Crohn's disease; n (%) | 86 (48.6) | 13 (22.0) | | | 8 (20.0) | | |
| Indeterminate colitis; n (%) | 0 (0.0) | 5 (8.5) | | | 3 (7.5) | | |
| Ulcerative colitis** | | | | | | | |
| Proctitis (E1); n (%) | 20 (22.2) | 7 (17.1) | 1 / 0 | 0.49 | 7 (24.1) | 1 / 0 | 0.28 |
| Left-sided colitis (E2); n (%) | 48 (53.3) | 20 (48.8) | | | 11 (39.7) | | |
| Pancolitis (E3); n (%) | 22 (24.4) | 14 (34.1) | | | 11 (39.7) | | |
| Crohn's disease ## | | | | | | | |
| Ileum (L1); n (%) | 25 (29.1) | 3 (23.1) | 0 / 0 | 0.56 | 2 (25.0) | 0 / 0 | 0.80 |
| Colon (L2); n (%) | 21 (24.4) | 5 (38.5) | | | 3 (37.5) | | |
| Ileocolonic (L3); n (%) | 40 (46.5) | 5 (38.5) | | | 3 (37.5) | | |
| Upper digestive (L4) (yes); n (%) | 7 (8.1) | 2 (15.4) | 0 / 0 | 0.40 | 1 (12.5) | 0 / 0 | 0.53 |
| Non stricturing/penetrating (B1); n (%) | 44 (51.2) | 8 (61.5) | 0 / 0 | 0.49 | 6 (75.0) | 0 / 0 | 0.28 |
| Stricturing (B2); n (%) | 27 (31.4) | 3 (23.1) | 0 / 0 | 0.53 | 2 (25.0) | 0 / 0 | 1.00 |
| Penetrating (B3); n (%) | 23 (26.7) | 6 (46.2) | 0 / 0 | 0.19 | 3 (37.5) | 0 / 0 | 0.68 |
| Medication | | | | | | | |
| 5-aminosalicylates; n (%) | 155 (88.1) | 38 (79.2) | 1 / 11 | 0.11 | 23 (71.9) | 1 / 8 | 0.03* |
| Steroids; n (%) | 116 (65.9) | 18 (38.3) | 1 / 12 | < 0.01* | 8 (25.8) | 1 / 9 | < 0.01* |
| Thiopurines; n (%) | 75 (42.4) | 7 (15.2) | 0 / 13 | < 0.01* | 3 (10.0) | 0 / 10 | < 0.01* |
| Methotrexate; n (%) | 9 (5.1) | 1 (2.1) | 0 / 11 | 0.37 | 0 (0.0) | 0 / 8 | 0.36 |
| Cyclosporin; n (%) | 4 (2.3) | 0 (0.0) | 0 / 11 | 0.24 | 0 (0.0) | 0 / 8 | 1.00 |
| Biologicals; n (%) | 38 (21.7) | 1 (2.1) | 2 / 11 | < 0.01* | 0 (0.0) | 2 / 8 | < 0.01* |
| IBD related surgery; n (%) | 49 (27.7) | 13 (23.6) | 0 / 0 | 0.61 | 10 (26.3) | 0 / 0 | 0.86 |
| Follow up (y), median | 8.3 | 10.9 | 6 / 0 | 0.03* | 6.4 | 6 / 0 | 0.18 |

A: all cases included in the analysis; B: sensitivity analysis: only cases included with IBD diagnosis after 1990; IBD: Inflammatory Bowel Diseases; IBDSL: IBD South Limburg cohort; #: only smoking data from Crohn's disease patients; ##: classified according to Montreal classification.

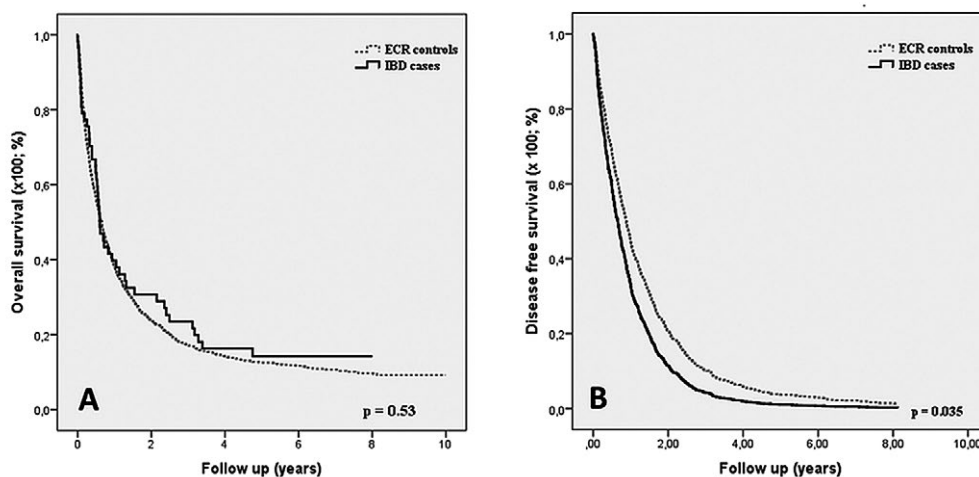


Fig. 2. Overall survival curves of IBD cases and the general population following gastric cancer diagnosis. A: Univariate analysis: Kaplan-Meier curve; B: Multivariate analysis (correction for TNM correction): Cox model (hazard ratio 1.385). IBD: inflammatory bowel diseases; ECR: Eindhoven Cancer Registry.

Table II. Final model of binary logistic regression analysis.

| Model | Variable | Coefficient β | Odds Ratio (95 % CI) | P-value |
|-------------------------------------------------------------------------|----------------------------|---------------------|-----------------------|---------|
| Ulcerative colitis <i>All cases (n=41)</i> | Age at IBD diagnosis | 0.081 | 1.084(1.043 – 1.126) | 0.000 |
| Ulcerative colitis (> 1991) <i>Sensitivity analysis (n=29)</i> | Age at IBD diagnosis | 0.073 | 1.075 (1.034 – 1.119) | 0.000 |
| Ulcerative colitis (>1991; + med) <i>Sensitivity analysis (n=29)</i> | Age at IBD diagnosis | 0.066 | 1.068 (1.022 – 1.117) | 0.004 |
| Crohn's disease <i>All cases (n=18)</i> | Age at IBD diagnosis | 0.048 | 1.049 (1.012 – 1.088) | 0.010 |
| Crohn's disease (>1991) <i>Sensitivity analysis (n=15)</i> | Age at IBD diagnosis | 0.069 | 1.072 (1.024 – 1.122) | 0.003 |
| Crohn's disease (>1991; + med) <i>Sensitivity analysis (n=15)</i> | No risk factors identified | | | |

Final multivariate regression model after adjustment for the duration of the 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.

were EBER positive, 4 (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%). Histologically, gastritis was present in 10, intestinal metaplasia in 9 and dysplasia in 1 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 6 patients had a positive family history of GC.

This nationwide cohort study described GC in IBD patients and showed that GC survival was significantly worse in IBD in the multivariate analysis, compared to the general population after correction for confounders, although IBD patients presented with smaller GCs. 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. [23] 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 [24-26], lymphomas, and bladder cancer [23]. As immunosuppressive therapy may promote tumor progression [23, 27-29], 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 sub-analysis 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

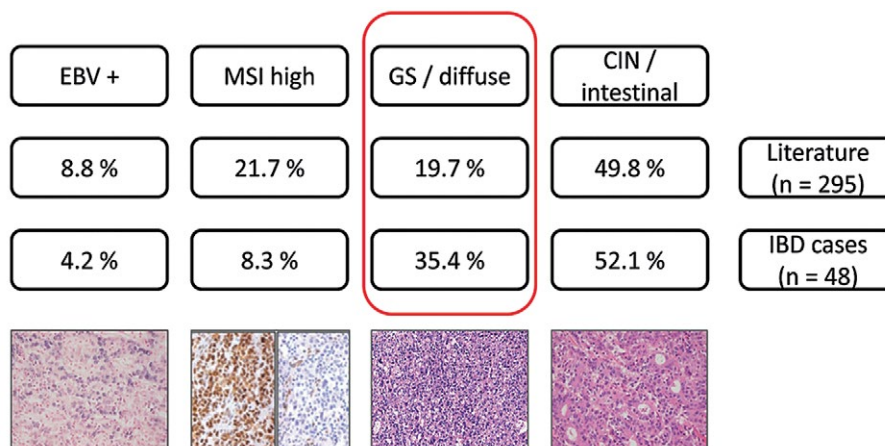


Fig. 3. Gastric cancer histology of IBD patients, including additional staining. Magnification: x200. EBV: Epstein Bar virus; MSI: microsatellite instability; GS: genomic stable; CIN: chromosomal instable. Reference [33].

Table III. Univariate comparison of gastric cancer characteristics between cases (IBD patients who developed gastric cancer) and controls (gastric cancer patients in the general population; ECR).

| Variable | IBD patients N = 59 | ECR patients N = 1339 | Missing (n) IBD / ECR | P-value |
|----------------------------------|------------------------|--------------------------|--------------------------|----------|
| Age at diagnosis (years), median | 73.0 | 71.0 | 0 / 0 | 0.67 |
| Female sex; n (%) | 21 (35.6 %) | 517 (38.6 %) | 0 / 0 | 0.64 |
| Tumor location | | | | |
| Cardia (vs non-cardia); n (%) | 12 (20.3 %) | 313 (23.4 %) | 0 / 0 | 0.59 |
| Histology | | | | |
| Lauren – intestinal | 35 (59.3 %) | 86 (20.3 %) | 0 / 915 | < 0.01* |
| WHO Tubular; n (%) | 28 (49.1) | 84 (19.8) | 2 / 915 | < 0.01 * |
| Papillar; n (%) | 3 (5.3) | 7 (1.7) | | |
| Mucinous; n (%) | 3 (5.3) | 48 (11.3) | | |
| Poorly cohesive; n (%) | 23 (40.4) | 338 (79.7) | | |
| Differentiation | | | | |
| Good & moderate / poor; n (%) | 19(32.2) / 40(67.8) | 298 (30.5) / 679 (69.5) | 0 / 362 | 0.78 |
| Tumor stage | | | | |
| T stage | | | | |
| T1; n (%) | 1 (2.3) | 95 (12.7) | 16 / 591 | 0.01* |
| T2; n (%) | 10 (23.3) | 271 (36.2) | | |
| T3; n (%) | 21 (48.8) | 210 (28.1) | | |
| T4; n (%) | 11 (25.6) | 172 (23.0) | | |
| N stage (histological) | | | | |
| N0; n (%) | 16 (53.3) | 212 (40.4) | 29 / 814 | 0.39 |
| N1; n (%) | 9 (30.0) | 222 (42.2) | | |
| N2; n (%) | 5 (16.7) | 78 (14.9) | | |
| N3; n (%) | 0 (0.0) | 13 (2.5) | | |
| M (yes) | 22 (37.3 %) | 508 (46.0 %) | 0 / 234 | 0.19 |
| TNM – stage | | | | |
| Stage 1; n (%) | 7 (12.7) | 178 (17.6) | 4 / 326 | 0.30 |
| Stage 2; n (%) | 12 (21.8) | 139 (13.7) | | |
| Stage 3; n (%) | 9 (16.4) | 140 (13.8) | | |
| Stage 4; n (%) | 27 (49.1) | 556 (54.9) | | |
| Initial treatment | | | | |
| Any (yes) | 43 (72.9 %) | 798 (59.6 %) | 0 / 0 | 0.04* |
| Surgery (yes) | 34 (57.6%) | 546 (40.8%) | 0 / 0 | 0.01* |
| Chemotherapy (yes) | 19 (32.2 %) | 276 (20.6%) | 0 / 0 | 0.01* |
| Radiotherapy | 5 (8.5 %) | 102 (7.6 %) | 0 / 0 | 0.81 |

IBD: inflammatory bowel disease; ECR: Eindhoven cancer registry; WHO: World Health Organisation

and an increasing IBD prevalence [30], this group of elderly IBD patients is rapidly expanding. In our univariate comparisons we found less use of medical therapy in the IBD GC cases (with older IBD onset). However, in the multivariate analysis no differences in medication use between IBD GC cases and IBD controls were found and therapy-induced immunosuppression seems not to be 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, thus making these patients more susceptible to (gastric) cancer development.

We analyzed the number of EBV-positive GCs 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 GCs

were EBV positive, which is in line with the literature [10], 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 [31]. 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 an increased risk for GC in IBD. Treatment with 5-aminosalicylates may protect against *H. pylori* [7]. 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. [4], 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 number UC patients may be caused by the fact that UC is more prevalent in elderly onset IBD [32] 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 [33], based on the molecular background of GCs, 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%, Fig. 3). Diffuse GCs are associated with a *CDH1* mutation, which is also important in the epithelial barrier function in UC [34], 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. The limited number of IBD GC cases, although the largest series thus far, may result in type 2 error. 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 the time frame, as we included IBD patients with GC diagnosed before as cases. Therefore, we performed a sensitivity analysis in which we only included cases with an IBD diagnosis after 1990. These analyses showed similar results compared to the analysis of the case group as a whole. Furthermore, we included the follow up as a fixed factor in the analyses to correct for differences in the follow up duration.

CONCLUSIONS

The survival of GC patients in our study was significantly worse in IBD patients compared to the general population in the multivariate 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.

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Authors' contribution: I.N., F.H., D.d.J. and L.N. designed this study. L.N., E.A., L.D. and C.P. performed the research. L.N., L.D. and W.K. analyzed the data. L.N. wrote the manuscript. M.P. and T.H. supplied data from the IBDSL cohort, L.O. supplied PALGA data and R.V. supplied ECR data.

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APPENDIX

The working groups

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