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Fistulizing pattern in Crohn's disease and pancolitis in ulcerative colitis are independent risk factors for cancer: A single-center cohort study

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Abstract

Background & Aims: The combined role of immunomodulators (IMM) and clinical characteristics of Inflammatory Bowel Disease (IBD) in determining the cancer risk is undefined. The aim was to assess whether clinical characteristics of IBD are independent risk factors for cancer, when considering thiopurines and anti-TNFs use.
Methods: In a single-center cohort study, clinical characteristics of IBD patients with IBD duration $\geq$1 year and $\geq$2 visits from 2000 to 2009 were considered. Tests for crude rates and survival analysis methods were used to assess differences of incidence of cancer between groups. The methods were adjusted for the time interval between diagnosis and immunomodulatory treatments.
Results: IBD population included 1222 patients :615 Crohn's disease (CD), 607 ulcerative colitis (UC). Cancer was diagnosed in 51 patients (34 CD,17 UC), with an incidence rate of 4.3/1000 pt/year. The incidence rate of cancer was comparable between CD and UC (4.6/1000 pt/year vs 2.9/1000 pt/year ;p=n.s.). Cancer most frequently involved the breast, the GI tract, the skin. Lymphoma was diagnosed in CD (1HL,1NHL,0 HSTCL). Risk factors for cancer included

Abbreviations CD, Crohn's Disease; UC, ulcerative colitis; IBD, inflammatory bowel disease; IMM, immunomodulators; AZA, azathioprine; 6-MP, 6-mercaptopurine; MTX, methotrexate; Anti-TNFs, anti-tumor necrosis factor alpha; EIM, extraintestinal manifestations; GI, gastrointestinal; OR, odds ratio; HR, hazard ratio; HSTCL, hepatosplenic T cell lymphoma.

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older age at diagnosis of IBD (CD: HR 1.25;95%CI 1.08–1.45; UC:HR 1.33;95%CI 1.15–1.55 for an increase by 5 years; p=0.0023; p=0.0002), fistulizing pattern in CD (HR 2.55; 95%CI 1.11–5.86, p=0.0275), pancolitis in UC (HR 2.79;95%CI 1.05–7.40 p=0.0396 *vs* distal). IMM and anti-TNFs did not increase the cancer risk in CD, neither IMM in UC (anti-TNFs risk in UC not feasible as no cases observed).

Conclusions: Fistulizing pattern in CD, pancolitis in UC and older age at diagnosis of IBD are independent risk factors for cancer.

1. Introduction

Thiopurines and anti-TNFs show a proven efficacy in Inflammatory Bowel Disease (IBD).[1] Thiopurines have been used since late 1960s in IBD.[2,3] An increased risk of lymphoma has been suggested in IBD patients treated with azathioprine (AZA) and 6-mercaptopurine (6-MP),[4–8] particularly in young patients with Crohn's Disease (CD).[9] Nevertheless, the absolute risk of lymphoma by using thiopurines in IBD appears to be small and the benefits have been reported to overwhelm the lymphoma risk.[1,9,10] This issue is however still debated, particularly due to the recent use of combined treatment with anti-TNFs.[1,11] As the first trial using Infliximab in IBD has been published in 1995,[12] the long-term outcome of patients treated with anti-TNFs is under investigation. Current evidences suggest that anti-TNFs with no thiopurines do not increase the cancer risk,[1,13–19] despite few discrepant findings.[20] Differently, evidences consistently indicate that combined treatment with thiopurines and anti-TNFs significantly increases the lymphoma risk in IBD.[1,21,22] Growing evidences describe the development of a rare hepatosplenic T cell lymphoma (HSTCL) after combined treatment with thiopurines and anti-TNFs, particularly in young male patients with CD.[1,21–26] This issue assumes relevance also in relation to the proven efficacy of this combined treatment, particularly in young patients with a severe IBD course.[1]

Despite several studies investigated the cancer risk associated with the use of immunomodulators (IMM) use in IBD, the role of clinical characteristics of IBD in determining this risk has been less extensively investigated. CD has been associated with lymphoma.[6–10,27] non-melanotic skin cancers[28] and breast cancer[29] even in patients with no history of IMM. Long-standing colitis and sclerosing cholangitis have been reported to increase the risk of colon cancer in IBD.[30] Most of these observations derive from monocentric studies, thus accounting for the observed discrepant findings.[31]

By our knowledge, few studies investigated the cancer risk when using IMM and/or biologics in patients with different clinical characteristics of IBD. This issue may currently assume relevance due to the worldwide use of IMM in young IBD patients, highly responsive to ISS and anti-TNFs.

On the basis of these observations, we aimed to assess, in a single-center cohort study, the role of clinical characteristics of IBD in determining the cancer risk in a cohort of IBD patients under regular follow up. In particular, we aimed to assess whether characteristics of IBD and/or of the host represent independent risk factors for cancer. The role of IMM drugs in determining the cancer risk has been also investigated in relation to clinical characteristics of IBD.

2. Materials and Methods

2.1. Study Population

In a single-center cohort study, clinical records of all IBD patients under regular follow up at our tertiary IBD referral Unit from 2000 to 2009 were reviewed. Clinical characteristics of IBD patients were prospectively recorded and defined according to current guidelines.[1,22,32] Inclusion criteria: a) diagnosis of IBD[1]; b) IBD duration $\geq$1 year including at least 2 visits at our referral center; c) no history of cancer before the diagnosis of IBD; d) no thiopurines and/or anti-TNFs use before the diagnosis of IBD; e) detailed clinical characteristics considered in the analysis. The following variables were reported in a database and considered: age at diagnosis of IBD, type of IBD (CD *vs* UC), IBD site/duration, CD behaviour (inflammatory, fistulizing, fibrostricturing), smoking habits, IBD surgery (yes/no), family history of IBD, extraintestinal manifestations (EIM), perianal disease, IMM use (AZA, 6-MP; methotrexate, MTX), including the date of administration (year), treatment duration and combination with anti-TNFs (Infliximab, Adalimumab, Certolizumab). For anti-TNFs, the dose and number of administrations[1] were reported. In order to analyze the effect of IMM on the incidence of cancer, IMM use was considered for patients treated with AZA, 6-MP and/or MTX for $\geq$3 months and anti-TNFs use for patients with $\geq$1 treatment. In patients with cancer, parameters considered included: date and patients' age (year) at time of diagnosis of cancer and site/histotype of cancer. In order to assess the role of IBD characteristics and to optimize control of potential confounding variables, the analysis of the incidence of cancer was carried out separately in UC and CD. As our study population includes patients with a diagnosis of IBD made both before and after the year 2000, it is a mixed cohort (prevalent and incident cohort respectively). Limits and observations supporting the absence of a substantial selection bias are detailed in the statistical analysis.

2.2. Statistical analysis

Differences in terms of characteristics between groups were assessed by the Wilcoxon-test (continuous variables) and by the Chi-square test (categorical variables). Associations between clinical characteristics and treatments were further investigated in a multivariate logistic regression. Crude overall incidence of cancer in subgroups was estimated in terms of rate per 1000 pt-years to adjust for different

lengths of individual follow-up (thus e.g. a rate equal to 4 means that assuming a constant average risk of developing cancer, 4 out of 1000 patients develop cancer during 1 year of follow-up), and compared by a test according to Miettinen. A further analysis of the association between prognostic factors and onset and timing of cancer was performed analyzing the cancer-free survival, defined as the time interval between diagnosis and onset of cancer or death, censoring patients alive with no prior occurrence of cancer at the date of last follow-up. Since the deaths without prior cancer were only 4/1 out of 38/18 events respectively in CD and UC, this analysis is equivalent to the analysis of cancer occurrence over time. Overall survival was defined as the time interval between diagnosis of IBD and death, censoring patients alive at the date of last follow-up. For descriptive purposes, cumulative survival probability for both endpoints was estimated by the Kaplan–Meier method and differences in subgroups were assessed in univariate analysis by the Log-Rank test. We aimed to investigate the role of clinical characteristics and of IMM in the development of cancer. At this purpose, since these treatments were administered after the diagnosis of IBD, in order to avoid time bias, a Cox regression for cancer-free survival with time-dependent covariates for IMM and anti-TNFs drugs was applied. The following candidate adjustment factors were identified on the basis of their known or suspected relations to either treatment or cancer onset: IBD site (CD: ileum only, ileum-colon, colon only; UC: distal, subtotal, pancolitis), CD pattern (inflammatory, fistulizing, fibrostricturing), smoking habits (yes/no), surgery for IBD (yes/no), family history of IBD (yes/no), EIM (yes/no), perianal disease (yes/no) and age at diagnosis of IBD (continuous variable). It was checked that the presence of the latter variable (Age at $t=0$) with a linear effect in the Cox model was appropriate to take into account the increase of risk of developing cancer with aging. Current age ($Age(t)=Age(0)\pm t$) indeed showed no major departure from the linearity. Model selection was implemented step-wise without the use of any automatic procedure nor of strict significance thresholds. The final models exclude for parsimony variables that, if included, would have a significance level $p\geq 0.3$. As the role of IMM represents a key issue of the study, the related variables were included in the final models, and it was checked the absence of any interaction between them and the other variables. Finally, in order to check the robustness of the conclusions in relation to the possible length bias which could arise from the use of a "prevalent case" subpopulation, the multivariate analysis was repeated by applying a left truncation for the time to cancer onset, imposing that the time from diagnosis to either failure (cancer or death) or to last follow-up had to be longer than the maximum between time 0 and the time interval between diagnosis of IBD and 2000. This analysis showed very similar results, thus confirming the validity of the conclusions.

3. Results

3.1. Clinical Characteristics

Table 1, shows clinical characteristics of the 1222 IBD patients fulfilling the inclusion criteria, including 615 patients with CD and 607 with UC. CD and UC populations were comparable in terms of family history of IBD and duration of IMM treatment (Table 1). Differently, the median patients' age and the median age at diagnosis of IBD was lower in CD

Table 1 Clinical characteristics of the 1222 IBD patients with no history of cancer before the diagnosis of IBD.

Characteristics	Crohn's disease (n=615; 50.3%)	Ulcerative Colitis (n=607; 49.7%)	P-value
Gender			
Females	318 (52%)	264 (44%)	0.0048
Age (years) [a]	43 (16–83)	45 (14–90)	<0.02
Age at diagnosis of IBD (years) [a]	30 (11–79)	36 (10–83)	<0.0001
IBD duration (years) [a]	9 (1–46)	7 (1–47)	<0.0001
Family history of IBD			
Yes	92 (15%)	82 (14%)	0.52
Smoking habits			
Yes	302 (49%)	216 (36%)	<0.0001
Extraintestinal manifestations			
Yes	355 (58%)	229 (38%)	<0.0001
Previous surgery			
Yes (≥1)	326 (53%)	55 (9%)	<0.0001
Duration of ISS (months) [a]	24 (3–184)	24 (3–144)	0.88
IMM (with or without anti-TNFs)			
Yes	207 (34%)	90 (15%)	<0.0001
Anti-TNFs (with or without IMM)			
Yes	152 (25%)	46 (8%)	<0.0001
IMM only			
Yes	105 (17%)	63 (10%)	0.0009
Anti-TNFs only			
Yes	50 (8%)	19 (3%)	0.0002

Abbreviations: IBD=Inflammatory Bowel Disease; IMM=Immunomodulators.
[a] Median and range.

than in UC ($p<0.02$ and $p<0.0001$, respectively) (Table 1). The median IBD duration was higher in CD than in UC ($p<0.0001$). When compared with UC, our CD population showed a higher proportion of females ($p=0.0048$), smokers, frequency of EIM and previous surgery ($p<0.0001$ for all) (Table 1). The median follow up of patients was 11 years [95% CI = 10–12] for CD and 7 years for UC [95% CI 8–10].

The overall survival was comparable between CD and UC, thus indicating that this variable did not significantly affect our findings.

Among the 615 CD patients, lesions involved the ileum in 444 (72%), the colon in 53 (9%) and both the ileum and colon in 118 (19%) patients. In CD, the prevalent pattern was inflammatory in 236 (43%), fibrostricturing in 229 (37%) and fistulizing in 123 (20%) patients. Perianal disease was observed in 158 (26%) patients. Among the 607 UC patients, UC was distal in 457 (75%), subtotal in 68 (11%) and total in 82 (14%) patients.

3.2. Immunomodulators and Clinical Characteristics of IBD

In order to assess the role of IMM in determining the cancer risk, the use of these drugs has been analyzed in relation to clinical characteristics of IBD.

IMM use was observed in a higher percentage of CD *vs* UC patients (IMM only: $p=0.0009$; anti-TNFs only: $p=0.0002$; IMM with or without anti-TNFs: $p<0.0001$; anti-TNFs with or without IMM: $p<0.0001$) (Table 1). CD and UC patients were comparable in terms of duration of IMM ($p=0.88$) (Table 1) and number of anti-TNFs treatments (median: 8, range 1–62 *vs* 5, range 1–35 in CD *vs* UC, respectively; $p=0.5$).

Multivariate logistic regression analysis was used to assess the associations between IMM and clinical characteristics of IBD (Table 2). IMM use was less frequent in patients with older age at diagnosis of IBD (OR for an increase by 5 years: OR = 0.86, $p<0.0001$;OR = 0.88, $p=0.003$ for CD and UC, respectively) and more frequent in patients with EIM ($p=0.001$ for CD and $p<0.0001$ for UC, respectively). In CD, IMM use was more frequent in patients with a fibrostricturing or fistulizing *vs* inflammatory pattern ($p=0.02$ and $p=0.001$, respectively) and with ileo-colonic *vs* ileal CD ($p=0.044$), being comparable between patients with colonic vs ileal CD ($p=0.72$). IMM use was more frequent in patients with subtotal or total *vs* distal UC ($p=0.007$ and $p=0.032$), being not associated with surgery in IBD ($p=0.48$, $p=0.095$ for CD and UC, respectively) (Table 2). Anti-TNFs use was less frequent in patients with older age at diagnosis of IBD (for an increase by 5 years: $p<0.001$ for CD and $p<0.0001$ for UC) and more frequent in patients with EIM (CD: $p<0.0001$; UC: $p=0.03$). In UC, but not in CD, anti-TNFs use was more frequent in patients with previous surgery ($p=0.0031$ and $p=0.58$). As shown in Table 2, anti-TNFs use in CD was more frequent in patients with perianal disease ($p=0.004$) and with a fibrostricturing or fistulizing *vs* inflammatory pattern ($p=0.021$ and $p<0.001$, respectively). Anti-TNFs use was not associated with IBD site in CD (colon *vs* ileum only: $p=0.66$; ileum-colon *vs* ileum only: $p=0.21$), while it was more frequent in patients with subtotal or pancolitis *vs* distal UC ($p<0.0001$ and $p=0.05$, respectively) (Table 2).

3.3. Cancer in IBD Patients

Cancer after the diagnosis of IBD was diagnosed in 51 out of the 1222 IBD patients, including 34 with CD and 17 with UC (Table 3). The incidence rate of cancer in IBD was 4.3/1000 pt/year. The frequency of cancer in IBD patients was 4.2% (51/1222), in CD 5.5% (34/615) and in UC 2.8% (17/607). The incidence rate of cancer was 4.6/1000 pt/year in CD and 2.9/1000 pt/year in UC. This difference was not significant ($p=0.138$), also when considering the occurrence of

Table 2 Multivariate logistic regression analysis assessing the associations between immunomodulators and clinical characteristics in IBD.

VARIABLE	IMM use			Anti-TNFs use		
	OR	95% CI	P	OR	95% CI	P
Crohn's disease						
Age	0.86	0.79–0.93	<0.0001	0.86	0.78–0.94	<0.001
Fibrostricturing vs inflammatory pattern	1.71	1.09–2.68	0.02	1.83	1.09–3.06	0.021
Fistulizing vs inflammatory pattern	2.55	1.50–4.32	0.001	2.83	1.58–5.08	<0.001
Perianal disease	1.22	0.80–1.84	0.35	1.90	1.22–2.95	0.004
Colonic vs ileal CD	1.13	0.56–2.28	0.72	0.84	0.37–1.87	0.66
Ileo-colonic vs ileal CD	1.64	1.01–2.65	0.044	1.40	0.82–2.40	0.21
Extraintestinal Manifestations	2.01	1.38–2.92	<0.001	3.71	2.35–5.84	<0.0001
Surgery	1.17	0.76–1.80	0.48	0.87	0.54–1.42	0.581
Ulcerative colitis						
Age	0.88	0.81–0.96	0.003	0.67	0.58–0.79	<0.0001
Subtotal vs distal UC	2.39	1.27–4.52	0.007	6.25	2.84–13.75	<0.0001
Total vs distal UC	1.96	1.06–3.61	0.032	2.27	0.99–5.25	0.05
Extraintestinal manifestations	2.65	1.66–4.24	<0.0001	2.02	1.05–3.88	0.03
Surgery	1.83	0.90–3.71	0.095	3.93	1.59–9.74	0.003

IMM = Immunomodulators; CD = Crohn's Disease; UC = Ulcerative Colitis.

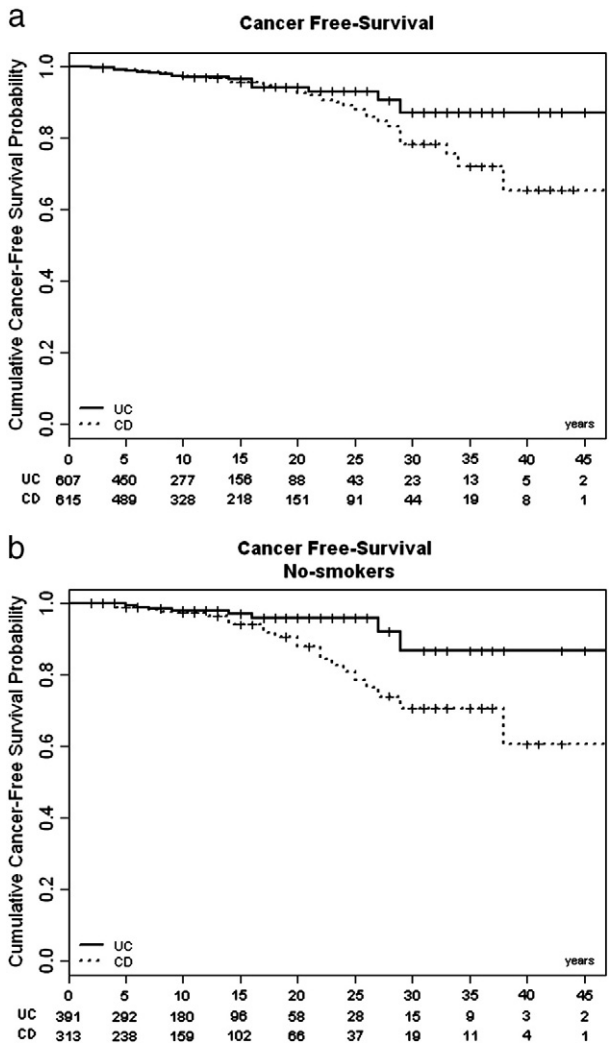

Figure 1 *Panel a.* Cancer-free survival analysis showing that the occurrence of cancer over time did not significantly differ between CD and UC patients (Log-Rank test for the cancer-free survival p=0.21). Accordingly, the incidence rate of cancer did not significantly differ between CD and UC (4.6/1000 pt/year *vs* 2.9/1000 pt/year in UC, respectively; p=0.138).*Panel b.* When the analysis was restricted to non-smokers, the incidence of cancer was significantly higher in CD *vs* UC (6.2/1000 pt/year *vs* 1.8/1000 pt/year, p=0.0053 for the crude rates, p=0.0107 for the cancer-free survival probabilities).

cancer over time (Log-Rank test for the cancer-free survival p=0.21) (Fig. 1a). When the analysis was restricted to non-smokers, the incidence of cancer was higher in CD than in UC (6.2/1000 pt/year *vs* 1.8/1000 pt/year, p=0.0053 for the crude rates p=0.0107 for the cancer-free survival probabilities) (Fig. 1b).

The majority of cancers in IBD involved the gastrointestinal (GI) tract (CD: n=4, 1 small bowel, 3 colon cancer; UC: n=2, both colon cancer) or the breast (CD:n=9; UC: n=3) (Table 3). Lymphoma was observed in 2 CD patients: 1 NHL (F, 36 year, after 72 months treatment with AZA, no anti-TNFs) and 1 HL (M, 50 year, never treated with IMM). No HSTCL in IBD and no lymphoma in UC were reported. No statistical comparison was feasible in terms of different types

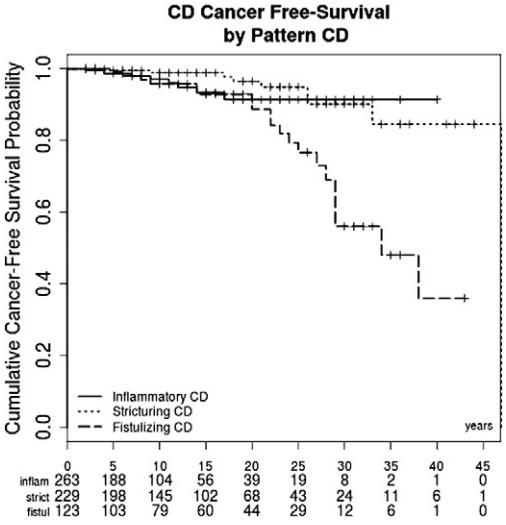

Figure 2 Cancer-free survival analysis showing that the incidence of cancer was significantly higher in patients with fistulizing CD when compared to patients with inflammatory or fibrostricturing CD (overall differences for the cancer-free survival curves: p=0.001).

of cancer between CD *vs* UC due to the small number of specific histotypes observed.

3.4. Risk factors for cancer in CD

The crude incidence rates of onset of cancer and the cancer-free survival probabilities were computed and also compared in subgroups, for a descriptive purpose. In CD, the incidence of cancer was higher in patients with fistulizing CD when compared to patients with inflammatory or fibrostricturing CD (rates: 9.3/1000 pt/year *vs* 3.8/1000 pt/year; p=0.03 and *vs* 2.4/1000 pt/year; p=0.0012, respectively). Differences for the cancer-free survival curves when considering CD pattern are shown in Fig. 2 (p=0.0012). As expected, the incidence of cancer was more frequent in smokers than in no smokers (6.2/1000 pt/year *vs* 3.2/1000 pt/year, p=0.026; from the comparison of cancer-free survival probabilities, RR=2.09, p=0.0241; Log-Rank test p=0.039). The incidence rates of cancer were higher in patients with CD involving the ileum-colon (rate=7.8 per 1000 pt/year) compared to the ileum (rate=3.7;p=0.028) but not to the colon (rate=5.3;p=0.25). However, this difference appeared to have taken place in the long term. The incidence of cancer in CD patients with or without perianal disease was at limit of statistical significance (6.7 *vs* 3.5 per 1000 pt/year; p=0.051). For both ileo-colonic and perianal disease, no statistically significant higher incidence of cancer was detected by the Log-Rank test (p=0.21 for both).

All the other tested variables appeared not to significantly influence the incidence rates of cancer, including: IBD surgery (5.1/1000 pt/year *vs* 3.4/1000 pt/year, p=0.75; Log-Rank test p=0.77), EIM (5.4/1000 pt/year *vs* 3/1000 pt/year, p=0.14; Log-Rank test p=0.25), gender (females 5.1/1000 pt/year *vs* males 4/1000 pt/year p=0.76; Log-Rank test p=0.46). Difference due to Age $\geq$40 year at diagnosis of CD compared to $\leq$40 didn't reach statistical significance

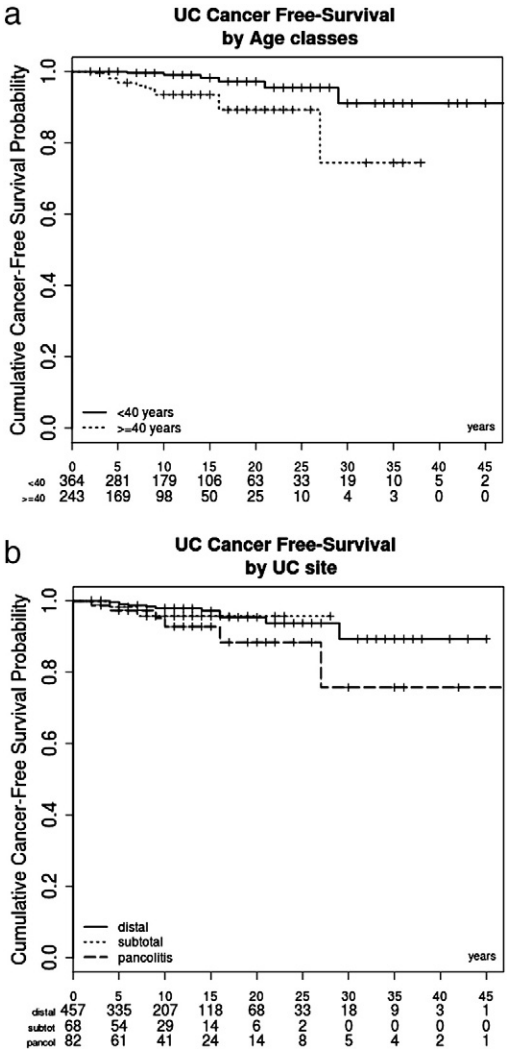

Figure 3 Panel a. Cancer-free survival analysis showing that in UC cancer was significantly more frequent in patients ≥40 years *vs* <40 years old (from the comparison of cancer-free survival probabilities, Log-Rank test p=0.0011; RR=4.6).Panel b. The impact of UC extent was not statistically significant (Log-Rank test p=0.110) due to similarity between distal and subtotal UC (rates per 1000 pt/year: distal 2 pt/year, subtotal 3.4 pt/year p=0.52). Nevertheless, the difference was statistically significant between both distal UC vs pancolitis (rates per 1000 pt/year: distal 2 pt/year, pancolitis 6.8 pt/year p=0. 0.0156) and between subtotal UC *vs* pancolitis (rates per 1000 pt/year: distal 3.4 pt/year, pancolitis 6.8 pt/year p=0.045). Multivariate Cox model also showed a significant association between pancolitis and an increased cancer risk [pancolitis *vs* distal UC: HR=2.79 (95% CI 1.05–7.40); p=0.0396].

(6.7 *vs* 4 per 1000 pt/year, p=0.075 Log-Rank test p=0.24). However, this assessment is limited by the use of this arbitrary cutpoint, as supported by the below reported findings from the multivariate analysis.

The incidence of cancer did not differ between CD patients with or without IMM and/or anti-TNFs use (rates per 1000 pt/year: 3.5 in patients never treated with IMM or anti-TNFs; 3.8 in patients with IMM use; p=0.81; 2.7 in patients with anti-TNFs use p=0.93; 3.4 in patients with both IMM and anti-TNFs use p=0.9).

A multivariate Cox model for the risk of developing cancer or dying was used to assess the independent prognostic role of each factor. Among the tested variables, only older age at the diagnosis of CD and a fistulizing pattern showed a significant association with an increased cancer risk [HR with 95%CI: Age: HR=1.25 (1.08–1.45) for an increase by 5 year, p=0.0023; fistulizing *vs* inflammatory pattern HR=2.55 (1.11–5.86), p=0.0275)]. Smoking was associated with both the pattern (p=0.03) and the site (p=0.0419), which were in turn associated (p<0.0001).

Multivariate Cox model showed that, differently from CD pattern, smoking and CD site were not significantly associated with the risk of cancer or death. Differently from CD pattern, smoking and CD site in a multivariate Cox model were not significantly associated with the risk of cancer or death. Neither IMM use nor anti-TNFs use were found to be significantly associated with the risk of cancer or death (p=0.44 and p=0.84 respectively).

3.5. Risk Factors for Cancer in UC

As for CD, the incidence of cancer was higher in patients with older age at diagnosis of UC (≤40 *vs* >40: 1.3/1000 pt/year *vs* 5.9/1000 pt/year; p=0.0026; from the comparison of cancer-free survival probabilities, RR=4.6, p=0.0011) (Fig. 3, panel a). The impact of UC extent was not significant (Log-Rank test p=0.110) due to similarity between distal and subtotal UC (rates per 1000 pt/year: distal 2 pt/year, subtotal 3.4 pt/year p=0.52). Nevertheless, the difference was significant between distal UC and pancolitis (rates per 1000 pt/year: distal 2 pt/year, pancolitis 6.8 pt/year p=0.0.0156) and between subtotal UC *vs* pancolitis (rates per 1000 pt/year: distal 3.4 pt/year, pancolitis 6.8 pt/year; p=0.045) (Fig. 3, panel b). The incidence of cancer was higher in smokers than in no smokers, but this was not significant (4.7/1000 pt/year *vs* 1.8/1000 pt/year; p=0.056; Log-Rank test p=0.19). In UC, no differences were observed between patients with or without surgery (2.7/1000 pt/year *vs* 2.9/1000 pt/year; p=0.81;Log-Rank test p=0.85), EIM (2.3/1000 pt/year *vs* 3.3/1000 pt/year; p=0.86; Log-Rank test p=0.91) or females vs males (2.1/1000 pt/year *vs* 3.4/1000 pt/year; p=0.36; Log-Rank test p=0.16). The incidence of cancer did not differ between UC patients with or without IMM (2.4/1000 pt/year *vs* 2.5/1000 pt/year; p=0.91). The incidence of cancer in UC patients with no anti-TNFs use was 3.1/1000 pt/year (95% CI 1.9-5/ 1000 pt/year), while no cancer was observed in UC patients treated with anti-TNFs, due to the small number of treated patients (n=46) or to the limited follow up length.

A multivariate Cox model for the risk of developing cancer or dying was used to assess the independent prognostic role of each factor. Among tested variables, only older age at diagnosis of UC and pancolitis were associated with an increased cancer risk [age: HR=1.33 (95% CI 1.15–1.55) for an increase by 5 years, p=0.0002; pancolitis *vs* distal UC: HR=2.79 (95% CI 1.05–7.40); p=0.0396]. IMM use was not significantly associated with the risk of cancer or death (p=0.13).

4. Discussion

The widespread use of IMM in IBD is rising concern about their potential cancer risk. In 2003, HSTCL in a young IBD patient treated with AZA has been described.[33] Since then, a growing number of cases have been described in IBD, particularly in young male CD patients treated with thiopurines and anti-TNFs.[1,23–26,34] Almost 10% of all HSTCL described worldwide developed in IBD patients treated with IMM.[1,23–26,33,34] As the first trial using anti-TNFs in IBD has been published in 1995,[12] the long-term outcome of treated patients is undefined. Thiopurines with no anti-TNFs have been associated with an increased lymphoma risk in CD, although the absolute risk appears to be small.[9,10] An increased risk of non-melanotic skin cancer has also been reported in IBD patients treated with thiopurines.[35] In 2004, Fiocchi[36] postulated for the first time a relation between anti-TNFs and cancer.[37] In 2006, in a multicenter, matched-control study we reported a comparable incidence of cancer in a cohort of 808 CD patients treated or not with Infliximab matched for clinical variables (median follow up 25 months).[13] A longer follow up of the same cohort (median 74 months) confirmed these findings.[18] Since 2006, several studies supported that anti-TNFs with no IMM do not increase the cancer risk in IBD,[1,13–19,22] with few conflicting findings.[20,38] Longer follow up of anti-TNFs treated patients are however required to assess this risk. Moreover, whether clinical characteristics of IBD patients treated with IMM and/or anti-TNFs may influence the cancer risk is undefined. This issue was therefore investigated in the present study.

In our IBD population, independent risk factors for cancer included fistulizing pattern in CD and pancolitis in UC. These clinical characteristics are frequently associated with a severe course responsive to IMM and/or anti-TNFs. Although these treatments have been suggested as potential risk factors for cancer, clinical characteristics of IBD, rather than IMM, appeared to increase the cancer risk in our study.

Older age at diagnosis of IBD also appeared to be a significant risk factor for cancer. This factor represents in general the effect of current age, including age at time of diagnosis of cancer. IMM and anti-TNFs use was less frequent in older patients, thus further supporting that advanced age represents an independent risk factor for cancer. This finding, together with recent observations showing that advanced age is an independent risk factor for severe infections and mortality using anti-TNFs,[19] further supports a proper selection of patients eligible for treatment with biologics. The observed not statistically significant difference for the cancer-free survival curves in patients with age $\geq$ *vs* $\leq$40 years at diagnosis of CD does not imply that older age at diagnosis did not represent a significant risk factor. The cut-off of 40 years is indeed arbitrary. Differently, multivariate Cox model showed that older age at diagnosis of CD was significantly associated with an increased cancer risk, further supporting that older age at diagnosis of IBD represented a significant risk for cancer.

The observed higher incidence of cancer in patients with fistulizing CD and with pancolitis in UC was not related to an older age, as data were corrected for this variable. No cancer along fistulous tracts as described in case reports,[39] were detected in CD.

A high incidence of cancer was observed in our IBD population (4.3/1000 pt/year). A variable incidence of cancer has been shown in IBD, due to different study designs, IBD populations and referral centres.[28–31,38–41] Tertiary referral centres most often indeed include patients with a severe course at higher cancer risk related to IBD, to IMM and/or to radiation exposure. Although radiation exposure represents a risk factor for cancer in IBD,[42] we did not consider this variable due its difficult reliable quantification. As expected, smoking was a risk factor for cancer in CD. The same finding was not observed for UC, most likely due to the relatively small

Table 3 Histotypes of cancer in the 51 IBD patients with diagnosis of cancer after the diagnosis of IBD.

Cancer	Crohn's disease (n = 34/615)	Ulcerative Colitis (n = 17/607)
Breast adenocarcinoma	9	3
Gastrointestinal tract	4	2
Colon cancer	3	2
Small Bowel cancer	1	0
Lymphoma	2 (1 HL, 1 NHL)	0
Others	19	12
Non melanotic skin cancer	1	0
Melanoma	1*	2
Thyroid cancer	3	2
Renal cancer	3	1
Prostatic cancer	2	3
Ovarian cancer	2	0
Uterine cervical cancer	1	0
Bladder cancer	2	0
Testicular cancer	2	0
Laryngeal cancer	2	0
Lung cancer	0	2
Tongue cancer	0	1
Schwannoma	0	1

*1 CD patient with 2 melanomas.

number of smokers in our UC population. In non-smokers, the incidence of cancer was higher in CD than in UC. Our study population included a comparable number of CD and UC patients. However, when compared with UC, CD patients showed a longer IBD duration and a younger age, although not clinically relevant (median, years: 43 vs 45 for CD and UC). The percentage of patients using IMM and/or anti-TNFs was also higher in CD vs UC. While the higher percentage of patients using anti-TNFs in CD may be related to the earlier use of anti-TNFs in CD, the same does not apply for IMM. Therefore, IBD course appeared more severe in CD, thus accounting for the higher incidence of cancer among non-smokers CD *vs* UC patients. As comparison between CD and UC did not represent the aim of the study, CD and UC patients were not matched for clinical variables.

Clinical characteristics and risk factors were comparable to the general IBD population,[1,32] thus supporting our findings. Nevertheless, a limit of the present study arises from the relatively small number of events observed (cancer or death). This may have affected the multivariate analysis particularly for UC, as a high number of variables were considered. Therefore, among variables not included in the models there could be some with an independent impact on the outcome, not detectable from our analysis due to non-strong or time-varying effect or to correlation with other variables.

The observed histotypes of cancer were expected, more frequently involving the breast, the GI tract or skin.[28–30,35,40,41] The number of cases (IBD and cancer) observed allowed the assessment of the overall cancer risk and not of the risk of specific histotypes. The small number of observed events (IBD and cancer) did not allow any comparison between the risk of the various histotypes of cancers in IBD *vs* a reference healthy and/or IBD population, as this analysis would be statistically underpowered (Table 3). Breast cancer showed the higher frequency, as expected in the general non-IBD population[43] and in CD.[29] Among GI cancers, both colon and small bowel cancers were observed in CD, while in UC only colon cancers were observed, as expected.[40,41] Almost two/third of IBD patients were on mesalazine, involved in the observed reduced frequency of intestinal cancer in IBD.[44] As expected, lymphoma was observed only in CD. IMM and/or anti-TNFs did not increase the cancer risk, in agreement with evidences suggesting that, differently from lymphoma, the overall cancer risk is not increased by using these drugs in IBD.[1,13–19,22,45] As for CD, ISS (with no anti-TNFs) appeared not to increase the cancer risk in UC. Nevertheless, the low p-value observed by multivariate analysis does not exclude a possible significant risk when considering a higher number of cases. As above discussed, the major limit of the present study is indeed represented by few cases observed (IBD and cancer n = 51: 34 in CD, 17 in UC). As our study population includes patients with a diagnosis of IBD both before and after the year 2000, it is a mixed cohort (prevalent and incident cohort respectively). Nevertheless, the first subgroup does not completely meet the traditional definition of "prevalent cohort", as it includes a small number of patients with cancer diagnosed before the year 2000 too. It can not therefore be ruled out that other patients with a diagnosis of IBD before 2000 who had a fatal cancer before the year 2000 were not included in the study. Thus, among patients with a diagnosis of IBD before 2000 the cases with longer time-to-failure could be over-represented. However, as the "incident cases" (diagnosis after 2000) represent the 45% of the study population, they may provide sufficient information regarding the diagnosis of cancer during the first years after the diagnosis of IBD. In addition, the similarity of the characteristics of the study population to the general IBD population suggests a substantial absence of relevant selection bias. This conclusion was confirmed by a further analysis where we applied left truncation for the time to cancer onset (imposing that the time from the diagnosis of IBD to either failure or to last follow-up had to be longer than the maximum between time 0 and the time interval between diagnosis of IBD and 2000), which showed very similar results.

Present findings indicating that IMM did not significantly increase the cancer risk in our IBD population is in agreement with recent observations suggesting that the anti-inflammatory effect of IMM may reduce the cancer risk in IBD.[46] Findings from the CESAME study indeed suggest that IBD patients receiving thiopurins have a 3.5-fold decreased risk of colorectal advanced neoplasia, thus supporting that the anti-inflammatory effect of thiopurins on colonic mucosa has a greater impact on the risk of colorectal cancer that the putative deleterious effect of drug-induced immunosuppression[46] This hypothesis is further supported by a recent Dutch study,[47] indicating that thiopurins protect against the development of advanced colorectal neoplasia in IBD (adjusted HR 0.10, 95% CI 0.01 to 0.75), while the effect of 5-ASA appears less pronounced (adjusted HR 0.56, 95% CI 0.22 to 1.40 (47) In the present study, the cancer risk using anti-TNFs in UC could not be appropriately assessed as no cases (UC and cancer using anti-TNFs) were observed among the relatively small number of treated patients (n = 46). This observation appeared not related to the follow up length, comparable between treated and untreated patients. Blocking TNF α has been suggested as protective for the colitis-associated colon cancer in animal models and in UC.[48,49] However, statistical analysis suggests that anti-TNFs did not represent a protective factor for cancer in UC, due to the small number of tested patients in our study.

Present findings suggest that fistulizing pattern in CD and pancolitis in UC represent independent risk factors for any cancer in IBD. These clinical characteristics of IBD are associated with a more frequent need of thiopurines and anti-TNFs, and their combined use has been shown to increase the lymphoma risk. Indication for IMM should therefore be carefully considered on a case-by case basis in IBD patients with a higher cancer risk related to characteristics of the disease, including a fistulizing pattern in CD and pancolitis in UC, particularly in patients with older age at diagnosis of IBD.

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