ORIGINAL RESEARCH

High risk of microscopic colitis after *Campylobacter concisus* infection: population-based cohort study

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ABSTRACT

Objective Microscopic colitis (MC) encompasses the two histopathological distinct entities of collagenous colitis (CC) and lymphocytic colitis (LC). In this Danish population-based cohort study, we examined the risk of MC following stool culture with *Campylobacter concisus*, *C. jejuni*, non-typhoidal *Salmonella* or a culture-negative stool test.

Design We identified patients with a first-time positive stool culture with *C. concisus*, *C. jejuni*, non-typhoidal *Salmonella* or negative stool test, from 2009 through 2013 in North Denmark Region, Denmark, and matched each with 10 population comparisons. All subjects were followed up until 1 March 2018 using Systematised Nomenclature of Medicine codes from The Danish Pathology Register for incident diagnoses of CC and LC. We computed risk and adjusted HRs with 95% CIs for MC among patients and comparisons.

Results We identified 962 patients with *C. concisus*, 1725 with *C. jejuni*, 446 with *Salmonella* and 11 825 patients with culture-negative stools. The MC risk and HR versus comparisons were high for patients with *C. concisus* (risk 6.2%, HR 32.4 (95% CI 18.9 to 55.6)), less for *C. jejuni* (risk 0.6%, HR 3.7 (95% CI 1.8 to 7.7)), low for *Salmonella* (risk 0.4%, HR 2.2 (95% CI 0.5 to 10.8)) and for patients with negative stool testing (risk 3.3%, HR 19.6 (95% CI 16.4 to 23.4)). After exclusion of the first year of follow-up, the HRs were 9.3 (95% CI 4.1 to 20.1), 2.2 (95% CI 0.9 to 5.4), 1.3 (95% CI 0.2 to 11.1) and 5.6 (95% CI 4.6 to 7.2), respectively.

Conclusion A high risk of MC was observed following *C. concisus* in stools. Further studies are needed to elucidate any underlying biological mechanisms.

Significance of this study

What is already known on this subject?

- Microscopic colitis (MC) is a chronic condition of unknown aetiology primarily affecting postmenopausal women.
- The association between gut microbiota, various commensal, enteric and potentially pathogenic micro-organisms and MC is not clear.

What are the new findings?

- A high risk of MC was observed following *Campylobacter concisus* in stools.
- The long-term higher risk of MC in *C. concisus*-positive patients indicates a biological association between *C. concisus* in stools and MC.

How might it impact on clinical practice in the foreseeable future?

- Clinicians should be aware of a higher risk of MC following *C. concisus* in stools.
- Further studies are needed to elucidate any potential underlying biological mechanisms.

INTRODUCTION

Microscopic colitis (MC) is an IBD that affects the large bowel and rectum. There are two main histological forms: collagenous colitis (CC) and lymphocytic colitis (LC). The first case of CC was described by Lindström in 1976,[1] and that of LC was described by Lazenby *et al* in 1989.[2] The term 'microscopic colitis' was introduced by Read *et al* in 1980, referring to 'a subset of patients with chronic diarrhoea of unknown origin'.[3] Patients with CC and LC present with similar symptoms of chronic, non-bloody diarrhoea that may be accompanied by nocturnal diarrhoea, faecal incontinence and mild weight loss.[4 5] No clinical features make it possible to discriminate between the two conditions; therefore, diagnostic differentiation relies on specific histopathological hallmarks. LC is defined by an increased number of surface intraepithelial lymphocytes, and CC is defined by a thickened collagen band underneath the surface epithelium.[6]

The incidence rate of MC is increasing globally, and Denmark has one of the highest incidence rates in the world with a mean annual incidence of 16.4 and 11.1 per 100 000 person-years for CC and LC, respectively.[7 8] The mean age at diagnosis is 60–65 years, with a clear female to male predominance.[7–9] The underlying mechanisms that lead to development of MC are poorly understood. Various factors have been associated with increased risk of MC, including smoking, use of non-steroidal anti-inflammatory drugs (NSAIDs) and proton-pump inhibitors (PPIs).[10 11] The primary biological event leading to MC is likely an abnormal translocation of luminal agents through the mucosal layers, leading to an uncontrolled immune-inflammatory cascade. The role of the gut microbiota and various commensal, enteric and potentially pathogenic micro-organisms is less clear.[5 11]

Campylobacter species are motile, spiral-shaped or curve-shaped Gram-negative bacteria that can inhabit the GI tract, and *Campylobacter jejuni* is the major cause of bacterial gastroenteritis worldwide.[12] *C. concisus* is a human oral commensal

first described in 1981, when it was isolated from patients with periodontal lesions.[13] *C. concisus* has been associated with diarrhoeal disease,[14 15] and a population-based study from the North Denmark Region found that *C. concisus* was more prevalent than *C. jejuni* in diarrhoeic stool samples.[16] An association of *C. concisus* to Crohn's disease (CD) and UC has been suggested.[17–21] However, a recent population-based study did not support exposure of either *C. concisus* or *C. jejuni* as causal triggers in subsequent development of CD and UC, since culture-negative patients had a similar risk of both conditions at long-term follow-up.[22] Nevertheless, a previous study showed that 12% of *C. concisus*-positive patients were diagnosed with MC during a 6-month follow-up period.[15]

The association of *C. concisus* with MC is unknown, and in this population-based cohort study, we examined the risk of MC following a positive stool culture with *C. concisus*, *C. jejuni*, non-typhoidal *Salmonella* (hereafter *Salmonella*) or culture-negative stool testing against age-matched, gender-matched and calendar time-matched population comparisons.

METHODS

Study population

This population-based cohort study was conducted in the North Denmark Region from 2009 through 2013, as described elsewhere.[22] In brief, the area has a stable urban/rural population of approximately 580 000, which have free access to healthcare. All Danish residents have a unique 10-digit personal identification (CPR) number in the Danish Civil Registration System which enables linkage between health administrative registries at individual level.[23]

During the 5-year study period, the department of clinical microbiology used the filter method for isolation of all *Campylobacter* species from stools, as described elsewhere.[14 16 23] Therefore, culture-negative stools before 2009 or after 2013 could be potential 'false negatives' as stools were not investigated for *C. concisus*.

We used the microbiology laboratory information system (wwLab, Autonik AB, Nyköping, Sweden) to identify patients 15 years or older with a first-time positive stool culture with either *C. jejuni*, *C. coli*, *C. concisus* or *Salmonella* as exclusive pathogenic enteric bacteria.[22] Stools were cultured for a standard panel of enteric bacterial pathogens, including *Campylobacter* species, *Salmonella*, *Vibrio*, *Shigella* species, *Yersinia enterocolitica* and *Clostridium difficile*.[22] *C. jejuni* constitute the majority of thermophilic *Campylobacter* species in Denmark,[24] and differentiation between *C. jejuni* and *C. coli* was not done routinely, only *C. jejuni* hereafter. *Campylobacter concisus* was isolated by the use of the filter technique, whereas other *Campylobacter* species such as *C. curvus*, *C. ureolyticus* and *C. upsaliensis* were also isolated but excluded from further analysis due to low prevalence.[14 16] The date of receipt of stool samples was defined as the index date of stool testing. Finally, we included patients 15 years or older with a first-time negative stool test for the standard enteric panel during the 5-year study period.

For each patient with *C. concisus*, *C. jejuni*, *Salmonella* or a negative stool test, we selected 10 random population comparisons who were alive and without any previous stool testing on the index date. Comparisons were drawn from the Danish Civil Registration System[25] and matched to their individual case by age (in years), sex and residence in the North Denmark Region.

Diagnoses of MC

Diagnosis of MC requires a histopathological examination of a colon tissue sample obtained during a colonoscopy or flexible sigmoidoscopy.[6] The Danish Pathology Register was established in 1990, and since 1997, mandatory pathology data have been reported to this register and coded after the Danish version of the Systematised Nomenclature of Medicine (SNOMED).[8 26] Using the CPR number for all individuals (cases and comparisons), we identified all patients with a first-time diagnosis of either CC (SNOMED code: S62536) or LC (SNOMED code: S62533) from 1 January 1997 to 1 March 2018. For all individuals, we excluded patients with a first-time MC diagnosis (CC or LC) before the positive/negative stool sample.

Comorbidity

We used the Danish National Patient Registry, which includes information about all Danish non-psychiatric inpatient hospital contacts since 1977 and all ambulatory hospital contacts since 1995,[27] for information on all hospital-diagnosed comorbid disease listed in the Charlson Comorbidity Index (CCI).[28] The CCI is a simple, readily applicable, comorbidity scoring system covering many disease categories, such as heart disease or cancer, and assigns points for each condition, with more points associated with more severe disease categories.[28] We measured a score for all individuals and three levels of comorbidity were defined as 'low' (score=0), corresponding to patients with no recorded underlying diseases implemented in the CCI, 'intermediate' (score=1–2) and 'high' (score>2).

Statistical analysis

All statistical analyses were conducted using STATA software V.15. First, we followed up all individuals from the index date until the diagnosis of MC (CC or LC), death or emigration out of Denmark, 1 March 2018, whichever occurred first. We created contingency tables to describe demographics for each group and their matched comparisons. To account for competing risk of death, we used 'stcompet' in STATA to construct cumulative incidence curves for MC, including subanalysis for CC and LC, for patients with *C. jejuni*, *C. concisus*, *Salmonella* and negative stool cultures, and for each comparison group. HRs with 95% CIs for first diagnosis of MC from the index date and until the end of follow-up among cases versus their matched comparisons were obtained using Cox proportional hazards regression analyses. In secondary analyses, we computed HRs for first diagnosis of MC from 1 year after the index date and until end of follow-up. Crude regression analyses were controlled for age, sex and calendar time due to the matched design, and in adjusted analyses, we further controlled for comorbidity by the CCI scores. In all Cox models, we modelled the cause-specific hazard of MC to account for the competing risk of death, and we stratified on matched sets to account for the matched design.

Patient and public involvement

Patients at the individual level were not involved in the present study.

RESULTS

Demographic characteristics

The initial cohort comprised 990 *C. concisus*-positive, 1733 *C. jejuni*-positive and 446 *Salmonella*-positive patients and 31 658 matched population comparisons. Of these, 45 patients with *C. concisus*, *C. jejuni* or *Salmonella* with previous MC, their 369 comparisons and further 53 comparisons with previous

Table 1 Baseline characteristics of patients with *Campylobacter concisus*, *C. jejuni, Salmonella* or negative stool cultures and their age-matched, sex-matched and calendar time-matched comparisons, in the North Denmark region, 2009–2013

	C. concisus (n=962)	Comparisons (n=9593)	*C. jejuni* (n=1725)	Comparisons (n=17 202)	*Salmonella* (n=446)	Comparisons (n=4441)	Culture-negative (n=11 825)	Comparisons (n=117 916)
Female, n (%)	556 (57.8)	5540 (57.8)	835 (48.4)	8319 (48.4)	207 (46.4)	2060 (46.4)	6900 (58.4)	68 736 (58.3)
Age (years), median (IQR)	57.1 (36.4–71.7)	57.0 (36.4–71.6)	40.3 (25.5–55.8)	40.4 (25.6–55.9)	46.2 (28.8–60.9)	46.2 (28.9–60.8)	56.5 (37.2–71.9)	56.5 (37.1–71.9)
Hospital requisition,* n (%)	362 (37.6)	–	432 (25.0)	–	128 (28.7)	–	4542 (38.4)	–
Comorbidity,† n (%)								
0	547 (56.9)	6851 (71.4)	1299 (75.3)	14 020 (81.5)	318 (71.3)	3519 (79.2)	6345 (53.7)	84 606 (71.8)
1–2	265 (27.5)	2114 (22.0)	342 (19.8)	2669 (15.5)	94 (21.1)	772 (17.4)	3328 (28.1)	26 197 (22.2)
>2	150 (15.6)	628 (6.6)	84 (4.9)	513 (3.0)	34 (7.6)	150 (3.4)	2152 (18.2)	7113 (6.0)

*Data on place of stool culture requisition (primary care or hospital) were missing for 1.6% (192) of patients with negative culture.
†Conditions included in the Charlson Comorbidity Index.

MC were excluded. The initial cohort also comprised 11 963 patients with culture-negative stools and their 119 542 matched comparisons, of which 138 patients with culture-negative stools and pre-existing MC, their 1380 comparisons and further 246 comparisons with pre-existing MC were excluded. Thus, the final study cohort comprised 962 *C. concisus*-positive, 1725 *C. jejuni*-positive, 446 *Salmonella*-positive patients, and their 31 236 matched comparisons, and 11 825 patients with culture-negative stools and their 117 916 matched comparisons (see table 1).

The *C. concisus*-positive patients had a median age of 57.1 years (IQR 36.4–71.7) and higher CCI scores than *C. jejuni*-positive and *Salmonella*-positive patients. The cohort of *C. concisus*-positive patients consisted of 556 (57.8%) women and 406 (42.2%) men, which were quite similar to the age distribution, sex ratio and CCI scores of those of the culture-negative cohort (see table 1). The median age of the *C. jejuni*-positive cohort was 40.3 years (IQR 25.5–55.8) and consisted of 835 (48.4%) women and 890 (51.6%) men. The median age of the *Salmonella*-positive cohort was 46.2 years (IQR 28.8–60.9) and consisted of 207 (46.4%) women and 239 (53.6%) men. Approximately, three quarters of *C. jejuni*-positive and *Salmonella*-positive patients had no hospital-diagnosed comorbidities. Of the remaining quarter, the majority were classified in the intermediate CCI group. Data of comorbidity by age group are available in online supplementary table 1.

Regarding the location of stool culture requisition (primary care or hospital), more than one-third of *C. concisus*-positive and culture-negative patients and one-fourth of *C. jejuni*-positive and *Salmonella*-positive patients, respectively, had their stool examined for bacterial enteric pathogens either during hospitalisation or in an outpatient setting.

Overall, the median follow-up time was 2064 days (IQR 1714–2795) for all individuals. A small proportion of all individuals (1.2%) emigrated during the study period. One hundred eighty-seven (19.4%) *C. concisus*-positive patients, 85 (5%) *C. jejuni*-positive patients, 27 (6%) *Salmonella*-positive patients and 3031 (25.6%) culture-negative patients died during follow-up.

MC-positive patients following *C. concisus*, *C. jejuni* or *Salmonella* infection or negative stool testing

No patients or comparisons with pre-existing classical IBD (CD and UC) were later diagnosed with MC. In total, 459 (3.1%) of 14 958 patients who had their stools investigated for bacterial enteric pathogens were diagnosed with MC during follow-up. Three-hundred and twenty-two patients (70%) were subsequently diagnosed with CC and 137 (30%) with LC.

Overall, MC was more frequently diagnosed among women (n=343) than men (n=116), corresponding to a female:male ratio of 3.0:1.0. For CC, there was an even higher female predominance with a female:male ratio of 3.5:1.0, whereas for LC, the ratio was 2.2:1.0. The median age at the time of an MC diagnosis was 67.7 years, varying from 69.2 years in cases of CC and 65.7 for LC.

Among patients with *Campylobacter* in stools, 60 (6.2%) *C. concisus*-positive patients (female:male, 46:14) were diagnosed with MC (CC, n=46; LC, n=14) and 11 (0.6%) *C. jejuni*-positive patients (female:male, 8:3) were diagnosed with MC (CC, n=7; LC, n=4) during follow-up. Only two (0.4%) *Salmonella*-positive patients (female:male, 1:1) were diagnosed with MC (CC, n=1; LC, n=1) during follow-up, whereas 386 (3.3%) culture-negative patients (female:male, 288:98) were diagnosed with MC (CC, n=268; LC, n=118).

The numbers of MC-positive patients during follow-up (after the index date) in the matched population comparison groups were as follows: comparisons to *C. concisus* 26/9593 (0.3%) (female:male, 16:10) (CC, n=17; LC, n=9), to *C. jejuni* 29/17 202 (0.2%) (female:male, 15:14) (CC, n=14; LC, n=15), to *Salmonella* 9/4441 (0.2%) (female:male, 6:3) (CC, n=4; LC, n=5) and to culture-negatives 259/117 916 (0.2%) (female:male, 179:80) (CC, n=159; LC, n=100).

HRs for MC following *C. concisus*, *C. jejuni* or *Salmonella* infection or negative stool testing

Cox regression analyses showed a higher HR for MC among *C. concisus*-positive and *C. jejuni*-positive patients and for patients with a negative stool test during the whole follow-up period compared with matched population comparisons (see table 2). There were only a few *Salmonella*-positive patients, and based on these limited data, we cannot make any conclusion, and this will not be further detailed here. In general, there was a higher HR of MC for all age groups and for both sexes among patients with stool tests versus comparisons. For all estimates, there were no major differences between the crude HR and the comorbidity-adjusted HR, and in the main text, we only reported results for the latter. For *C. concisus*-positive patients, the HR of MC was 32.4 (95% CI 18.9 to 55.6) for the whole period and 9.3 (95% CI 4.1 to 20.1) when we excluded the first year of observation. The HR was 3.7 (95% CI 1.8 to 7.7) for *C. jejuni*-positive patients during the first, which dropped to 2.2 (95% CI 0.9 to 5.4) when we excluded the first year after the positive stool sample. Similarly, but less pronounced, the culture-negative patients had an HR of 19.6 (95% CI 16.4 to 23.4) during the

Table 2 Risk of MC for the whole time period and for the remaining time period after exclusion of the first year among patients with *Campylobacter concisus*, *C. jejuni*, *Salmonella* and culture-negative patients

	HR* (95% CIs)					
	Whole period			First year excluded		
	MC risk (%) (n/N)	Crude HR* (95% CI)	Adjusted HR*† (95% CI)	MC risk (%) (n/N)	Crude HR* (95% CI)	Adjusted HR*† (95% CI)
C. concisus	6.2 (60/962)	30.4 (18.1 to 51.0)	32.4 (18.9 to 55.6)	1.6 (13/835)	7.9 (3.7 to 16.6)	9.3 (4.1 to 20.1)
Female	8.3 (46/556)	37.2 (19.7 to 70.2)	39.7 (20.4 to 77.2)	2.1 (10/476)	7.8 (3.4 to 18.0)	8.0 (3.4 to 19.1)
Male	3.4 (14/406)	18.9 (7.6 to 46.8)	22.4 (8.2 to 61.4)	0.8 (3/359)	3.8 (1.0 to 14.8)	4.5 (1.0 to 19.9)
Age, 15–49 years	0.8 (3/385)	9.7 (1.9 to 47.9)	12.5 (2.1 to 75.2)	0.3 (1/381)	3.2 (0.3 to 30.4)	4.5 (0.4 to 49.5)
Age, 50+ years	9.9 (57/577)	34.3 (19.7 to 59.8)	36.6 (20.5 to 65.4)	2.6 (12/454)	6.9 (3.2 to 14.5)	7.2 (3.3 to 15.6)
C. jejuni	0.6 (11/1725)	3.6 (1.8 to 7.3)	3.7 (1.8 to 7.7)	0.4 (6/1697)	2.2 (0.9 to 5.3)	2.2 (0.9 to 5.4)
Female	1.0 (8/835)	5.2 (2.2 to 12.2)	5.1 (2.0 to 12.6)	0.5 (4/818)	2.6 (0.8 to 7.7)	2.4 (0.7 to 7.7)
Male	0.3 (3/890)	2.0 (0.6 to 7.1)	2.1 (0.6 to 7.5)	0.2 (2/879)	1.4 (0.3 to 5.9)	1.4 (0.3 to 6.3)
Age, 15–49 years	0.3 (4/1144)	4.9 (1.5 to 16.3)	3.6 (1.0 to 13.2)	0.3 (3/1141)	3.7 (1.0 to 13.9)	2.4 (0.6 to 20.5)
Age, 50+ years	1.2 (7/581)	3.1 (1.3 to 7.4)	3.6 (1.5 to 8.9)	0.5 (3/556)	1.3 (0.4 to 4.5)	2.6 (0.5 to 5.4)
Salmonella	0.4 (2/446)	2.2 (0.5 to 10.2)	2.2 (0.5 to 10.8)	0.2 (1/438)	1.2 (0.2 to 9.9)	1.3 (0.2 to 11.1)
Female	0.5 (1/207)	1.6 (0.2 to 13.6)	1.2 (0.1 to 13.1)	0 (0/204)	–	–
Male	0.4 (1/239)	3.3 (0.3 to 32.0)	2.6 (0.2 to 26.6)	0.4 (1/234)	3.3 (0.3 to 32.0)	2.6 (0.2 to 26.6)
Age, 15–49 years	0 (0/252)	–	–	0 (0/251)	–	–
Age, 50+ years	1.0 (2/194)	3.3 (0.7 to 16.3)	3.3 (0.6 to 17.4)	0.5 (1/187)	1.6 (0.2 to 13.6)	1.8 (0.2 to 15.9)
Culture-negative	3.3 (386/11 825)	20.1 (16.8 to 23.9)	19.6 (16.4 to 23.4)	1.0 (102/10 168)	5.9 (4.6 to 7.6)	5.6 (4.6 to 7.2)
Female	4.2 (288/6900)	21.3 (17.3 to 26.2)	20.9 (16.9 to 25.8)	1.3 (77/5962)	5.5 (4.1 to 7.3)	5.3 (4.0 to 7.1)
Male	2.0 (98/4925)	17.1 (12.3 to 23.7)	16.5 (11.8 to 23.1)	0.6 (25/4206)	4.2 (2.6 to 6.8)	3.9 (2.4 to 6.4)
Age, 15–49 years	1.0 (46/4836)	11.6 (7.6 to 17.9)	11.2 (7.2 to 17.3)	0.3 (15/4756)	3.8 (2.1 to 6.9)	3.8 (2.1 to 7.0)
Age, 50+ years	4.9 (340/6989)	22.1 (18.2 to 26.8)	21.7 (17.8 to 26.4)	1.6 (87/5412)	5.4 (4.2 to 7.1)	5.2 (3.9 to 6.8)

*HR estimates for patients with *C. concisus*, *C. jejuni*, *Salmonella* and negative stool cultures versus their age-matched, sex-matched and calendar time-matched population comparisons.
†Adjusted for comorbidity.
MC, microscopic colitis.

whole period, which dropped to a HR of 5.6 (95% CI 4.6 to 7.2) after exclusion of the first year (see table 2).

For the whole period *C. concisus*-positive patients had a higher HR for MC (1.9 (95% CI 1.4 to 2.5)) than patients with negative stool cultures (reference); however, the HR dropped to 1.6 (95% CI 0.9 to 2.9) after exclusion of the first year. For *C. jejuni*, the HR of MC was lower than that for patients with culture-negative stools (see online supplementary table 2).

For MC diagnoses by histological subtype, see table 3. When using cumulative incidence curves estimates, we saw a steep increase in MC incidences shortly after the index date for *C. concisus*-positive patients and culture-negative patients (see figure 1A). The two curves deviated continuously beyond the first year so that approximately 7.2% of *C. concisus*-positive patients and 3.4% of culture-negative patients had an MC diagnosis after 9 years. For *C. jejuni*-positive patients, the MC incidence was much lower, and the MC incidence remained close to zero for all population comparisons. When we examined CC separately, we saw the same pattern as for MC (figure 1B). Therefore, the steep increase in MC was mainly powered by CC. The LC incidence curves showed a steep increase shortly after the index date for *C. concisus*-positive patients and culture-negative patients, and remained almost the same during follow-up.

DISCUSSION

This is the first population-based cohort study investigating the risk of MC after a positive stool sample with either *C. concisus*, *C. jejuni* or *Salmonella* and after a culture-negative stool test. We observed a marked increased HR of MC in *C. concisus*-positive patients during follow-up, compared with the age-matched and gender-matched background population. The increased risk after *C. concisus* infection was highest during the first year from the index date (date of stool testing) but remained increased up

Table 3 Risk and HRs with 95% CIs for CC and LC

	CC		LC	
	Risk % (n/N)	HR* (95% CI)*	Risk % (n/N)	HR* (95% CI)*
Campylobacter concisus	4.8 (46/962)	40.3 (20.8 to 78.1)	1.5 (14/962)	23.3 (8.2 to 65.7)
C. jejuni	0.4 (7/725)	4.6 (1.8 to 11.6)	0.2 (4/725)	3.1 (1.0 to 9.9)
Salmonella	0.2 (1/446)	2.3 (0.2 to 22.7)	0.2 (1/446)	2.2 (0.2 to 20.4)
Culture-negative	2.3 (268/11 825)	22.1 (17.7 to 27.6)	1.0 (118/11 825)	15.2 (11.2 to 20.4)

*HR estimates for patients with *C. concisus*, *C. jejuni*, *Salmonella* and negative stool cultures versus their age-matched, sex-matched and calendar time-matched population comparisons. Adjusted for comorbidity (Charlson Comorbidity Index scores of 0, 1–2 and >2).
CC, collagenous colitis; LC, lymphocytic colitis.

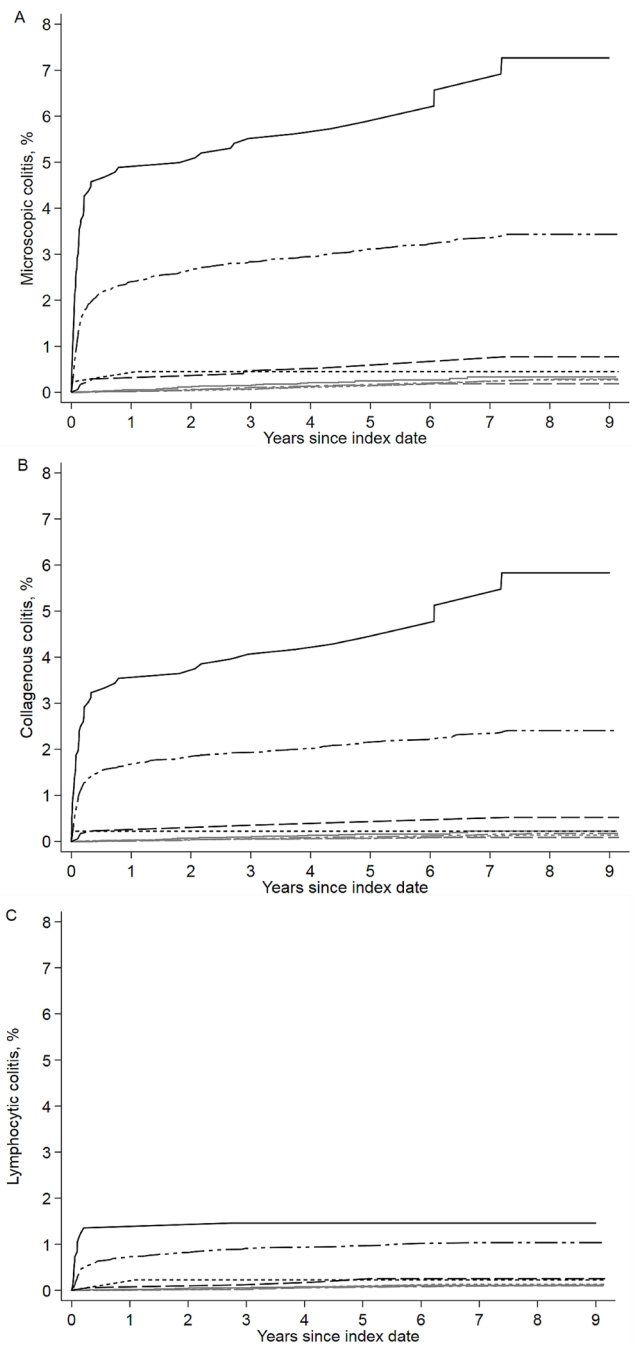

Figure 1 Cumulative incidence curves showing first-time diagnosis of microscopic colitis (A) and divided into collagenous colitis (B) and lymphocytic colitis (C) in patients as a function of time since the first positive culture with *Campylobacter concisus* (black solid line), *C. jejuni** (black long-dashed line), *Salmonella* (black short-dashed line) or the first culture-negative stool test (black long-dashed/short-dashed line). Comparisons are shown in grey. *Approximately 95% *C. jejuni* and 5% *C. coli*.

to 9 years after the positive stool sample. In a direct comparison to patients with a culture-negative stool sample, the HR of MC was almost twice as high following *C. concisus* in stool during the whole follow-up period, suggesting an association between *C. concisus* infection and MC. The increased risk was most pronounced for the CC subtype. For *C. jejuni*, the HR of MC was significantly lower compared with culture-negatives, indicating that *C. jejuni* infection is not to be associated with an increased risk for MC.

A diagnosis of MC requires a histopathology report consistent with lymphocyte proliferation or collagen bands in gut biopsies, combined with a typical clinical presentation of chronic, watery diarrhoea. Svensson *et al* found a high validity for an MC diagnosis from Swedish pathology reports, with a positive predictive value (PPV) of 95% for CC and 85% for LC.[29] In Denmark, registration of pathology data in the Danish Pathology Register has been mandatory since 1997, and therefore, the PPV for MC in the Danish register should be high.[26]

Detection bias may in part explain part of the association between any stool test and MC, since clinical criteria for diagnosing MC include a negative stool test to exclude any infectious aetiology of diarrhoea. This observation corresponds to previous findings of similarities in temporal risk patterns for classical IBD following positive or negative stool tests.[30] In a recent study, we reported similarities in the classical IBD risk patterns following *Campylobacter* in stools and for culture-negative stools, but a possible association between *C. concisus* infection and CD could not be sustained.[22]

Throughout the last decades, the incidence of MC has increased. Tong *et al* conducted a meta-analysis which showed pooled incidence rates of 4.14 and 4.85 per 100 000 person-years for CC and LC, respectively.[9] During 1995–2015, Bergmann *et al* performed a Swedish nationwide cohort study based on biopsy reports, which showed that CC constituted 33% and LC constituted 67% of all MC cases.[31] In contrast, Bonderup *et al* conducted a 10-year pathology-based nationwide Danish cohort study, which showed that CC constituted 61% and LC 39% of MC cases.[8] Our cohort had a higher prevalence of CC, constituting 70% of MC cases. Moreover, the high risk of MC after *C. concisus*, and to a lesser extent after negative stool testing, seemed to be powered by CC, but we have no explanation for the higher prevalence of CC in our cohort. Our MC cohort was otherwise comparable to previous cohorts in relation to age at the time of MC diagnosis and gender distribution.

We did not have registry-based data available on smoking or medication, including use of NSAID. A recent study by Burke *et al* showed an increased risk of MC by 2.6 in postmenopausal women using hormonal replacement therapy, and the risk increased with longer duration of use.[32] Exogenous reproductive hormones have previously been linked to the incidence and progression of other inflammatory bowel disorders, and this new finding strongly suggests an association between exogenous hormone use and incident MC, but the underlying mechanisms are unclear.[32–34]

In a previous study by Nielsen *et al*, clinical data were evaluated from 139 patients infected with *C. concisus* and compared with 187 patients infected with *C. jejuni*.[15] The use of intestinal anti-inflammatory agents, glucocorticoids and antineoplastic agents did not differ between the two groups. However, the use of PPIs was higher in the *C. concisus* group (18.4%) compared with the group of patients infected with *C. jejuni* (10.2%), with a relative prevalence proportions of 1.8 (95% CI 1.1 to 3), so there was a possibility for confounding by this well-characterised risk factor. The survey also showed that 80% of patients infected with *C. concisus* compared with 32% of patients infected with *C. jejuni* reported diarrhoea for longer than 2 weeks.[15] Moreover, 55% of patients infected with *C. jejuni* were treated with antibiotics, most often ciprofloxacin, compared with only 31% of patients infected with *C. concisus*, but whether antibiotic therapy may have affected the risk of MC is unclear.

C. concisus has been isolated throughout the GI tract of patients with CD, UC and healthy controls.[35] However, our data suggest that *C. concisus* in the gut has a stronger long-term effect compared with *C. jejuni*, probably due to a more persistent exposure of *C. concisus* to the intestinal mucosa. In a previous study, *C. concisus* and *C. jejuni* were equally involved with postinfectious IBS (PI-IBS) as one-fourth of *C. concisus* positive patients and one-fifth of *C. jejuni* positive patients reported PI-IBS after 6 months of follow-up.[36] We can only speculate on whether persistent exposure of endogenous *C. concisus* strains differs in pathogenic potential compared with transmission of exogenous *C. concisus* strains. Kirk *et al* examined 104 genomes of *C. concisus* isolated from different anatomical sites (saliva, intestinal biopsies and faeces) of patients with CD, UC and healthy controls and found no association with clinical phenotype.[17] The genomic variation was more related to source of isolation, and this supports the hypothesis that *C. concisus* could be considered a pathobiont, exerting pathogenic activity only when the intestinal environment is suitable.[17] The finding of *C. concisus* in stools could reflect an undiagnosed dysbiosis, defined as a reduction in faecal bacterial diversity owing to a shift in the balance between commensal and potentially pathogenic microorganisms, facilitating the colonisation of *C. concisus* in patients with underlying MC. An altered composition of the intestinal microbiota may lead to exposure of bacterial antigens that trigger an inflammatory cascade.[11]

The intestinal microbiota in MC has been sparsely characterised. Fischer *et al* reported a reduction of *Akkermansia* species,[37] and Krogsgaard *et al* reported a lower alpha diversity in patients with MC compared with controls; interestingly though, this is no longer evident after budesonide treatment for 8 weeks.[38] A high abundance of the genus *Faecalibacterium* has been found in patients with CC at baseline compared with controls.[38] This is in contrast to one of the most consistent changes in patients with classical IBD where *Faecalibacterium prausnitzii* is often reduced.[39 40] Ovesen *et al* confirmed a reduced gut bacterial diversity in MC, with dysbiosis of several species, notably an increase in *Prevotella* abundance.[41] Higher *Prevotella* abundance may indeed lead to a Th17-related immune response, rendering some *Prevotella* strains are inflammophilic pathobionts.[42] Whether this is reinforced by the presence of *C. concisus* in unknown.

Watery diarrhoea is the key symptom in MC, and the pathophysiological explanation includes sodium malabsorption in the distal colon. Barmeyer *et al* discovered, by activation of key effector cytokines, inhibition of the upregulation of epithelial sodium channels in response to aldosterone in sigmoid biopsies from patients with LC.[43] The same research group also showed an epithelial barrier dysfunction with downregulation of epithelial tight junctions, through downregulation of claudin-4, claudin-5 and claudin-8, and redistribution of claudin-5 and claudin-8 off the tight junction, which contributes to diarrhoea by a leak-flux mechanism.[44] Quite recently, Nattramilarasu *et al* also showed that *C. concisus* caused epithelial sodium channel dysfunction, as well as claudin-8-dependent barrier dysfunction, both of which contribute to Na^+ malabsorption and the clinical manifestation of watery diarrhoea.[45]

The main strengths of our study are the population-based design, long-term follow-up and complete registration of all cases. However, there are some limitations. First, it may be difficult to distinguish between MC and *C. concisus* infection in the clinical setting, since both conditions may present with prolonged watery diarrhoea. We had no information regarding the clinical context in which the stool sample was obtained, that is, whether it was suspicion of MC or an episode of gastroenteritis, precluding an assessment of potential differences, which might bias our findings. Undiagnosed patients with MC may have more frequent notification of enteric infections because of ascertainment bias, although the magnitude of this potential bias is unknown. Second, stool testing does not necessarily identify all possible pathogenic infections, and thus, potential novel pathogens not part of the then clinical microbiological testing regimen used at the time could confound our cohorts. Third, we had no direct link to registry-based data on smoking and use of NSAIDs, PPIs or menopausal hormone therapy, all previously described to influence MC development. Finally, we lack clinical data describing any inappropriate host response to *Campylobacter* infection, which could be involved in the MC pathogenesis.

In conclusion, this is the first population-based cohort study showing an increased risk of MC after *C. concisus* infection. The risk was more pronounced during the first year but remained increased up to 9 years after the positive stool sample. In a direct comparison versus patients with a culture-negative stool samples, the HR of MC was almost double following *C. concisus* in stools. The higher risk indicates that a biological association between *C. concisus* infection and MC is plausible. While studies elucidating the underlying mechanisms are needed, our results indicate that clinicians should be aware of a higher risk of MC following *C. concisus* in stools.

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