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Alimentary Tract

Changes in the clinical phenotype and behavior of pediatric luminal Crohn's disease at diagnosis in the last decade ☆,☆☆,★

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ABSTRACT

Background and aims: The aims of this study were to describe the trends in the behavior of pediatric CD during the last decade and to describe the seasonal variation of disease presentation.
Methods: Patients under 18 years old and diagnosed between 2009 and 2019 were included. The clinical, endoscopic, histological, and laboratory data were collected from the medical records. We analyzed the trends of these parameters according to the year and season of diagnosis.
Results: 654 patients were included in the study. The number of incident CD cases increased yearly. Patients diagnosed between 2015 and 2019 were younger at diagnosis (OR 2.53, $p = 0.02$), had more perianal diseases (OR: 2.30, $p < 0.0001$) and more granulomas (OR: 1.61, $p = 0.003$), but fewer eosinophils (OR: 0.35, $p < 0.0001$) and less chronic lymphoplasmacytic infiltrate (OR: 0.56, $p = 0.008$) as compared to the 2009–2014 cohort. There was fewer CD diagnosis during winter. Patients diagnosed in the fall had lower PCDAIs, less failure to thrive and less extensive digestive involvement. Colonic disease was significantly more frequent during summer and fall.
Conclusion: The clinical and histological phenotype of CD has changed over time and there are important seasonal trends in the frequency and severity on disease behavior suggesting possible disease triggers.

1. Introduction

The incidence of Crohn's disease (CD) within the North American pediatric population is among the highest in the world [1] and incidence of CD has been increasing [2–4]. This increase occurs primarily in industrialized countries, where air pollution is high and eating habits trend to westernized. Children living in urban areas are at a greater risk of developing CD than those who live in rural areas because they are less exposed to microbes and more exposed to issues of water sanitation, poor diet, less physical activity, higher antibiotic exposure and atmospheric pollution [2,5].

CD is a multifactorial disease, associated with some genetic markers and environmental factors including diet and gut microbiota [6]. The imbalance between the microbiota and the immune system seems to be associated with a change in intestinal permeability and to disease development [6]. Some studies on the incidence of CD have demonstrated a significant association between

☆ **Summary:** Evolution in the clinical, endoscopic, histological, and laboratory characteristics of pediatric CD during the last decade and seasonal trends of disease presentation.

☆☆ **What's Know on This Subject:** Crohn's disease is a multifactorial disease, associated with some genetic markers and environmental factors including diet, infections and gut microbiota and the incidence of CD has been increasing.

★ **What This Study Adds:** The disease phenotype has changed over time and there are important seasonal trends in disease presentation. Our findings provide interesting avenues for future research, such as identifying the clinical significance of granuloma, vitamin D deficiency and microbiota in pediatric Crohn's disease.

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the development of CD and vitamin D deficiency [7,8]. Also, it has been suggested that patients with an altered gut microbiota have an increased risk of developing CD. Indeed, patients diagnosed during childhood had greater antibiotic exposure prior to the inflammatory bowel disease (IBD) diagnosis [9–11]. Studies of the gut microbiome have demonstrated changes in the composition and bacterial diversity in CD patient, but a causal link has yet to be established [12].

Some studies have shown that CD flares in adult occur mostly during spring and summer [13]. Environmental factors, such as changes in diet, infections most commonly found during those seasons and immune function changes could all trigger digestive inflammation [13].

The main objective of this study was to describe the trends in the clinical phenotype, endoscopic, histological, and laboratory features of pediatric CD during the last decade. The secondary objective of the study was to describe the seasonal variation of disease presentation at diagnosis.

2. Materials and methods

2.1. Study design, participants, procedure

We conducted a retrospective cohort study including all patients diagnosed with luminal CD in a tertiary pediatric hospital (CHU Sainte-Justine, Montreal, Canada). The inclusion criteria were the following: 1. age under 18 years old, 2. CD diagnosis between January 1, 2009 and December 31, 2019, and 3. diagnosis made at CHU Sainte-Justine. The diagnosis of CD was made according to the usual endoscopic and histological diagnostic criteria [14]. Patients with IBD unclassified at initial presentation, with subsequent confirmation of CD, were included. Patients with coexisting significant cardiac, respiratory, neurological or psychiatric disorders at the time of CD diagnosis as well as those with “Crohn’s like disease” linked to other severe immune disorders were excluded. Patients with very early-onset IBD associated with specific genetic mutations and immune deficiencies were excluded. To keep a patient population with luminal involvement, we excluded the patients with isolated orofacial or perianal CD and also the very few patients with isolated proctitis due to their different management from the other patients in the cohort. Eligible patients were identified from the prospective IBD database maintained in our center [15] Data collection was extracted from clinical records. One fourth of the records were reviewed by an independent research assistant to ensure consistency of data collection. The study was approved by the research ethics committee of CHU Sainte-Justine *(2021–2974)*.

2.2. Measures

We considered two outcomes in this study: 1. year of diagnosis and; 2. season at diagnosis. The year of diagnosis was categorized into two groups, patients diagnosed between 2009 and 2014 and patients diagnosed between 2015 and 2019. The seasonality variation was analyzed according to the four seasons categorized by the standard dates of equinoxes and solstices.

The variables of interest were separated into four categories: 1. Clinical; 2. Endoscopic; 3. Histological and 4. Laboratory variables at diagnosis.

Clinical variables included clinical symptoms (such as diarrhea, abdominal pain, etc.), extra-intestinal manifestations, age at diagnosis, sex; weight, height and BMI z-scores; Pediatric Crohn’s Disease Activity Index (PCDAI, either continuous or categorized) [16,17]; Paris classification [18], perianal involvement (including any of the following: inflammatory fissures, perianal fistulas, abscesses clinically detected at the physical examination or pelvic magnetic resonance imaging), and presence of penetrating perianal lesions (exclusively perianal abscesses and fistulas clinically detected at the physical examination or pelvic magnetic resonance imaging).

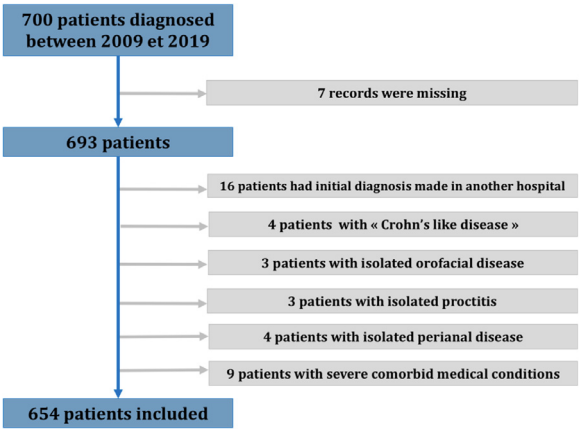

Fig. 1. Flowchart of the cohort.

Endoscopic variables included the simple endoscopic score for CD (SES-CD) [19]. The endoscopy reports were completed consistently and endoscopic photos were available, which allowed the retrospective calculation of the score. Histological variables included the presence of architectural distortions, moderate to severe lymphoplasmacytic infiltrate and moderate to severe signs of active inflammation (cryptitis, foveolitis, cryptic abscesses, ulcerations) on biopsies, as well as the presence of granulomas, increase in the number of eosinophils above normal values [20,21] and increase in lymphoid follicles at diagnosis.

Endoscopy protocol has remained constant over the past decade and the team of three pathologists has remained the same. Each patient had at least one biopsy in each digestive segment (ileum, ascending-transverse-descending colon, sigmoid and rectum).

Laboratory variables included: hemoglobin, serum albumin, vitamin D, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and fecal calprotectin (FC) levels at diagnosis.

2.3. Data analysis

Data were analyzed for the cohort as a whole and, according to diagnostic periods (2009–2014 and 2015–2019) and seasons (winter, spring, summer and autumn). The categorical variables were analyzed in bivariate analysis by the chi-square method and the continuous variables were analyzed in bivariate analysis by Student’s t tests or non-parametric tests by the Wilcoxon test. An ordinal logistic regression model was used to investigate the associations between patient’s characteristics and the year at diagnosis (odds ratios, OR).

3. Results

3.1. The CHU Sainte-Justine CD cohort

Seven hundred and nine patients were diagnosed with CD between January 1st 2009 and December 31st 2019 and 654 were included in the study. Fig. 1 displays the flowchart of the cohort. The clinical, endoscopic, histological and laboratory baseline characteristics at diagnosis are displayed in Table 1. The median age at diagnosis was 14.0 years (interquartile range (IQR): 11.5–15.6). The median PCDAI at diagnosis was 35.0 (IQR: 25.0–45.0) and the median SES-CD at diagnosis was 11.0 (IQR: 7.0–18.0). Fifty-five percent of patients were male, 28.1% had ileal disease, 22.8% had colonic disease and 47.5% had ileocolonic disease. Most patients had mod-

Table 1
Patients characteristics at diagnosis.

Total, Nb.	654
CLINICAL DATA	
Age, median (IQR), years	14.0 (11.5–15.6)
Sex, Nb. (%)[a]	
Male	360 (55.1)
Female	294 (45.0)
Weight z-score, median (IQR)	−0.6 (−1.5–0.2)
Height z-score, median (IQR)	−0.2 (−0.9–0.5)
BMI z-score, median (IQR)	−0.7 (−1.6–0.2)
PCDAI, median (IQR) ($n = 604$)[b]	35.0 (25.0–45.0)
PCDAI, Nb. (%)	
Mild (10–27.5)	207 (34.3)
Moderate (30–37.5)	171 (28.3)
Severe ($\geq$ 40)	226 (37.4)
sPCDAI, median (IQR)	40.0 (30.0–50.0)
Symptoms at diagnosis, Nb. (%)	
Abdominal pain	590 (90.2)
Asthenia	296 (45.3)
Fever	131 (20.0)
Diarrhea	458 (70.0)
Rectal bleeding	293 (44.8)
Vomiting	128 (19.6)
Weight loss	413 (63.2)
Paris Classification[c]	
Age at diagnosis, Nb. (%)	
A2	42 (6.4)
A1b	534 (81.7)
A1a	78 (11.9)
Location of digestive involvement, Nb. (%)	
L1	184 (28.1)
L2	149 (22.8)
L3	311 (47.6)
L4a isolated	1 (0.2)
L4b isolated	5 (0.8)
L4ab isolated	4 (0.6)
Upper digestive tract involvement, Nb. (%)	
No	228 (34.9)
L4a	289 (44.2)
L4b	46 (7.0)
L4ab	91 (13.9)
Disease phenotype, Nb. (%)	
B1	555 (85.0)
B2	52 (8.0)
B3	33 (5.1)
B2B3	13 (2.0)
Presence of inflammatory perianal involvement[d]**, Nb. (%)**	205 (31.4)
Presence of perianal abscesses/fistulas, Nb. (%)	123 (18.8)
Extra-intestinal manifestations, Nb. (%)	
Autoimmune hepatitis	4 (0.6)
Primary sclerosing cholangitis	11 (1.7)
Aphthous stomatitis	158 (24.2)
Arthralgia	122 (18.7)
Arthritis	37 (5.7)
Erythema nodosum	22 (3.4)
Pyoderma gangrenosum	0 (0.0)
Uveitis	4 (0.6)
Skin rash	37 (5.7)
ENDOSCOPIC AND HISTOLOGICAL DATA[e]	
Coloscopy at diagnosis, Nb. (%)	635 (97.1)
Upper digestive endoscopy at diagnosis, Nb. (%)	583 (89.1)
SES-CD[f]**, median (IQR)** ($n = 511$)	11.0 (7.0–18.0)
Presence of architectural distortions, Nb. (%)	487 (77.7)
Presence of moderate to severe lymphoplasmacytic infiltrate, Nb. (%)	523 (83.4)
Signs of moderate to severe active inflammation, Nb. (%)	395 (63.0)
Presence of granulomas, Nb. (%)	259 (41.3)
Increased eosinophils, Nb. (%)	357 (56.9)
Presence of lymphoid follicles, Nb (%)	377 (60.1)
LABORATORY DATA[g]	
Hemoglobin, median (IQR), g/L ($n = 626$)	117.0 (107.0–127.0)
Anemia at diagnosis[h]**, Nb (%)**	374 (59.7)
Albumin, median (IQR), g/L ($n = 610$)	32.25 (28.0–37.0)
Total vitamin D, median (IQR), nmol/L ($n = 136$)	55.0 (42.0–70.0)

(*continued on next page*)

Table 1 (*continued*)

C-reactive-protein, median (IQR), mg/L (n = 600)	25.6 (6.6–57.5)
Erythrocyte sedimentation rate, median (IQR), mm/h (n = 550)	32.0 (20.00–44.0)
Fecal calprotectin, median (IQR), ug/g (n = 186)	1281.0 (515.0–2100.0)

[a] All percentages are colum proportions. [b] Only continuous variables that are not available for all patients in the cohort have specified sample size. [c] Crohn's disease localized to the distal one-third of the ileum ± limited caecal involvement is defined as L1, isolated colitis as L2, and ileocolonic disease as L3. Upper gastrointestinal tract involvement is designated L4a when proximal to the ligament of Treitz and L4b if distal to the ligament of Treitz but proximal to the distal one-third of the ileum. Disease behavior is classified as: inflammatory (B1), structuring (B2), penetrating (B3); or structuring and penetrating (B2B3). A1a CD includes patients aged less than 10 years-old, A1b CD includes patients aged between 10 and 17 years old and A2 CD includes patients aged more than 17 years old [d] Inflammatory perianal involvement includes inflammatory fissures in addition to perianal fistulas and abscesses clinically detected at the physical examination or pelvic magnetic resonance imaging. [e] Findings on histology including those on OGD and colonoscopy. [f] Including only patients who had a complete successful colonoscopy at diagnosis (visualization of the rectum to the ileum), n = 511 [g] Measure of the laboratory parameter closest to the date of diagnosis within +/- 1 month. [h]Anemia was determined based on the hemoglobin values, gender and age of patients.

erate to severe lymphoplasmacytic infiltration, architectural distortions and moderate to severe active inflammation at diagnosis. Granulomas were found in 41.3% of patients and 56.9% had increased tissue eosinophils at diagnosis. Most patients had anemia, hypoalbuminemia, vitamin D insufficiency and elevated CRP, ESR and FC levels at diagnosis *(*Table 1*)*. In patients who had a vitamin D blood level at diagnosis, 80.9% of them had a 25–hydroxy vitamin D level lower than 75 nmol/L [22].

3.2. Number and phenotypes of CD diagnoses over the decade

The annual number of CD cases has increased over the past decade. Between 2009 and 2014, the average number of CD was 54 cases per year while from 2015 to 2019 this number increased by 20% reaching an annual number of 67 cases (p = 0.01). Table 2 displays the clinical, endoscopic, histological and laboratory characteristics according to the year of diagnosis. Indeed, patients diagnosed between 2015 and 2019, compared to patients diagnosed between 2009 and 2014, had a lower age at diagnosis, more perianal fistulas and/or abscesses, more granulomas, less increased eosinophils and lower lymphoplasmacytic infiltrate on biopsies.

Yearly analysis also displayed an increase in the rate of perianal fistulas/abscesses, granulomas, and younger age at diagnosis. The presence of granulomas on biopsies was associated with greater perianal involvements and younger age at diagnosis. Perianal fistulas and abscesses at diagnosis were present in 27.0% of patients with granulomas and in 14.1% of patients without granulomas (OR = 2.25, 95% CI [1.51; 3.36], $p < 0.001$). The median age at diagnosis in patients with granulomas was 13.5 years (IQR = 10.6–15.5) while in patients without granulomas the median age at diagnosis was 14.4 years (IQR = 12.2–15.8) (OR = 0.90, 95% CI [0.85; 0.95], p = 0.0002).

The rate of increased eosinophils and moderate to severe lymphoplasmacytic infiltrate decreased over the years. The presence of eosinophils on biopsies was associated with more moderate to severe lymphoplasmacytic infiltrate. Patients with increased eosinophils had moderate to severe lymphoplasmacytic infiltrate in 87.7% of cases while this proportion dropped to 77.8% in patients without eosinophils (OR = 2.03, 95% CI [1.33; 3.11], p = 0.001). There was no correlation between increased eosinophils and age at diagnosis, as well as with perianal fistulas and abscesses.

PCDAI, and SES-CD, disease location and behavior, symptoms, height, weight and BMI z-scores at diagnosis and baseline laboratory markers such as albumin, hemoglobin, C-reactive protein, sedimentation rate did not change over time (apart from small variations due to changes in the laboratory techniques over the years).

3.3. Seasonal variations in the number and severity of pediatric CD at diagnosis

There was a trend towards fewer diagnoses of pediatric CD in winter (134 cases (20.5%)) compared to other seasons of the year (average of 174 CD diagnoses per season (26.5%)) (p = 0.03).

Table 3 presents the different patients clinical, endoscopic, histological and laboratory characteristics according to the season at diagnosis. Patients diagnosed in the fall had a median PCDAI of 30.0 (IQR = 22.5–37.5) significantly lower than the median PCDAI of 37.5 (IQR = 27.5–47.5) of patients diagnosed in winter, spring, or summer ($p < 0.001$). This seasonal trend was also found within each year of the study period. Patients diagnosed in the fall had less growth failure than other patients: the median body mass index (BMI) z-score was −0.6 (IQR = −1.4–0.4) and the median weight z-score was −0.4 (IQR=−1.3–0.5) compared to respectively −0.8 (IQR = −1.7–0.1) (p = 0.004) and −0.7 (IQR = −1.5–0.1) (p = 0.008) in patients diagnosed in the three other seasons. Patients diagnosed in fall also had less fever (13.4% versus 22.7%, p = 0.007), weight loss (55.6% versus 66.2%, p = 0.01) and fatigue at diagnosis (36.4% versus 48.8%, p = 0.004). All these seasonal trends were found for each of the two study periods and for most of each year.

Ileal location occurred in 32.9% of patients diagnosed in spring and fall, while for other seasons, this rate was 23.7% (p = 0.01). Colonic location occurred in 27.3% of patients diagnosed in summer and fall, versus 18.2% of cases in winter and spring (p = 0.01). Finally, the ileocolonic location represented only 35.7% of patients diagnosed in fall, while patients diagnosed during the other 3 seasons of the year had L3 CD in 53.2% of cases (p = 0.0002). All the disease location trends related to seasonality were also found for each of the two study periods and for the majority of each year.

There was no correlation between disease location and weight and BMI z-scores, the presence of fever, asthenia or weight loss at diagnosis However, there was a modest correlation between disease location and PCDAI at diagnosis. Patients with ileal CD (L1) had a mean PCDAI of 33.3, those with colonic CD (L2) had a mean PCDAI of 35.1 and those with ileo-colic CD (L3) had a mean PCDAI of 37.6 (p = 0.004). Likewise, patients diagnosed in fall had both less extensive and severe CD

The highest vitamin D levels in patients occurred in summer and fall (Fig. 2). Indeed, the median vitamin D level was 60.0 nmol/L in summer and fall (IQR = 50.00–78.0) compared to 47.0 nmol/L (IQR = 35.0–58.0) in winter-spring (p = 0.003). Vitamin D levels at diagnosis were inversely correlated with PCDAI (Pearson correlation coefficient = −0.19, p = 0.03) and SES-CD (Pearson correlation coefficient = −0.20, p = 0.04).

4. Discussion

Many CD patients had elevated inflammatory markers, hypoalbuminemia, anemia and growth failure at diagnosis. These results are similar to what has previously been shown in the literature [23]. However, we found in our cohort more ileal CD than colonic CD which is different from other cohorts. Indeed some studies have reported that 16% of patients had ileal diseases, 28% of patients had colonic diseases and 53% of patients had ileocolonic diseases [23,24]. The rate of ileal CD has been shown to increase with age

Table 2
Bivariate analysis of patients clinical, endoscopic, histological and laboratory characteristics at diagnosis according to the year of diagnosis.

	Patients diagnosed between 2009 - 2014 inclusively	Patients diagnosed between 2015 - 2019 inclusively	OR[a] [95% CI]	*p* Value
Total, Nb.	319	335		0.01[b]
CLINICAL DATA				
Age, median (IQR), years	14.1 (11.6–15.8)	13.9 (11.3–15.5)	0.96 [0.91; 1.01]	0.07
Sex, Nb. (%)[c]				
Male	176 (55.2)	184 (54.9)	1	0.95
Female	143 (44.8)	151 (45.1)	1.01 [0.74; 1.38]	
Weight z-score, median (IQR)	−0.6 (−1.5–0.2)	−0.59 (−1.5–0.3)	1.03 [0.92; 1.16]	0.57
Height z-score, median (IQR)	−0.2 (−0.8–0.5)	−0.3 (−1.0–0.5)	0.92 [0.80; 1.06]	0.24
BMI z-score, median (IQR)	−0.7(−1.7–0.1)	−0.8 (−1.6–0.4)	1.05 [0.94; 1.18]	0.37
PCDAI, median (IQR)	35.0 (27.5–45.0)	35.0 (25.0–42.5)	0.99 [0.98; 1.01]	0.31
PCDAI, Nb. (%)				
Mild (10–27.5)	99 (31.9)	108 (36.7)	1	0.33
Moderate (30–37.5)	95 (30.7)	76 (25.9)	0.73 [0.49; 1.10]	
Severe (≥ 40)	116 (37.4)	110 (37.4)	0.87 [0.60; 1.27]	
Symptoms at diagnosis, Nb. (%)				
Abdominal pain	292 (91.5)	298 (89.0)	0.75 [0.44; 1.26]	0.27
Asthenia	151 (47.3)	145 (43.3)	0.85 [0.62; 1.16]	0.30
Fever	75 (23.5)	56 (16.7)	0.65 [0.44; 0.96]	0.03
Diarrhea	228 (71.5)	230 (68.7)	0.87 [0.63; 1.22]	0.43
Rectal bleeding	136 (42.6)	157 (46.9)	1.19 [0.87; 1.62]	0.28
Vomiting	69 (21.6)	59 (17.6)	0.78 [0.53; 1.14]	0.20
Weight loss	204 (64.0)	209 (62.4)	0.94 [0.68; 1.29]	0.68
Paris Classification[d]				
Age at diagnosis, Nb. (%)				
A2	29 (9.1)	13 (3.9)	1	0.02
A1b	250 (78.4)	284 (84.8)	2.53 [1.29; 4.98]	
A1a	40 (12.5)	38 (11.3)	2.12 [0.96; 4.67]	
Location of digestive involvement, Nb. (%)				
L1	87 (27.3)	97 (29.0)	1	0.67
L2	78 (24.5)	71 (21.2)	0.82 [0.53; 1.26]	
L3	149 (46.7)	162 (48.4)	0.98 [0.68; 1.41]	
L4a isolated	0 (0.0)	1 (0.3)	–	
L4b isolated	3 (0.9)	1 (0.3)	–	
L4ab isolated	2 (0.6)	3 (0.9)	–	
Upper digestive tract involvement, Nb. (%)				
No	125 (39.2)	103 (30.8)	1	0.14
L4a	134 (42.0)	155 (46.3)	1.41 [0.99; 2.00]	
L4b	19 (6.0)	27 (8.1)	1.73 [0.91; 3.28]	
L4ab	41 (12.9)	50 (14.9)	1.48 [0.91; 2.41]	
Disease phenotype, Nb. (%)				
B1	267 (83.7)	288 (86.2)	1	0.19
B2	33 (10.3)	19 (5.7)	0.53 [0.30; 0.96]	
B3	14 (4.4)	19 (5.7)	1.26 [0.62; 2.56]	
B3B3	5 (1.6)	8 (2.4)	1.48 [0.48; 4.59]	
Presence of inflammatory perianal involvement[e], Nb. (%)	84 (26.3)	121 (36.1)	1.58 [1.13; 2.21]	0.007
Presence of perianal abscesses/fistulas, Nb. (%)	40 (12.5)	83 (24.8)	2.30 [1.52; 3.48]	<0.001
Extra-intestinal manifestations, Nb. (%)				
Aphthous stomatitis	84 (26.3)	74 (22.1)	0.79 [0.55; 1.14]	0.21
Arthralgia	65 (20.4)	57 (17.1)	0.80 [0.54; 1.19]	0.27
Arthritis	16 (5.0)	21 (6.3)	1.27 [0.65; 2.47]	0.49
Erythema nodosum	13 (4.1)	9 (2.7)	0.65 [0.27; 1.54]	0.32
Skin rash	9 (2.8)	28 (8.4)	3.14 [1.46; 6.77]	0.004
ENDOSCOPIC AND HISTOLOGICAL DATA[f]				
SES-CD[g], median (IQR)	12.0 (9.0–17.0)	11.0 (6.0–19.0)	1.00 [0.98; 1.02]	0.82
Presence of architectural distortions, Nb. (%)	250 (80.7)	237 (74.8)	0.71 [0.49; 1.04]	0.08
Presence of moderate to severe lymphoplasmacytic infiltrate, Nb. (%)	271 (87.4)	252 (79.5)	0.56 [0.36; 0.86]	0.008
Signs of moderate to severe active inflammation, Nb. (%)	198 (63.9)	197 (62.2)	0.93 [0.67; 1.28]	0.65
Presence of granulomas, Nb. (%)	110 (35.5)	149 (47.0)	1.61 [1.17; 2.22]	0.003
Increased eosinophils, Nb. (%)	216 (69.7)	141 (44.5)	0.35 [0.25; 0.48]	<0.001
Presence of lymphoid follicles, Nb (%)	178 (57.4)	199 (62.8)	1.25 [0.91; 1.72]	0.17

[a] OR to have a diagnosis in 2015–2019. [b] The p-value for the numbers of total diagnoses was determined by comparing the number of diagnoses per year between 2009 and 2019. All other p-values were calculated from comparing patient characteristics across the two periods of the decade. [c] All percentages are column proportions [d] Crohn's disease localized to the distal one-third of the ileum ± limited caecal involvement is defined as L1, isolated colitis as L2, and ileocolonic disease as L3. Upper gastrointestinal tract involvement is designated L4a when proximal to the ligament of Treitz and L4b if distal to the ligament of Treitz but proximal to the distal one-third of the ileum. Disease behavior is classified as: inflammatory (B1), structuring (B2), penetrating (B3); or structuring and penetrating (B2B3). A1a CD includes patients aged less than 10 years-old, A1b CD includes patients aged between 10 and 17 years old and A2 CD includes patients aged more than 17 years old. [e]Inflammatory perianal involvement includes inflammatory fissures in addition to perianal fistulas and abscesses clinically detected at the physical examination or pelvic magnetic resonance imaging. [f] Findings on histology including those on OGD and colonoscopy. [g] Including only patients who had a complete successful colonoscopy at diagnosis (visualization of the rectum to the ileum), $n = 511$.

Table 3
Bivariate analysis of patients clinical, endoscopic, histological and laboratory characteristics at diagnosis according to the season of diagnosis.

	Diagnosis in **fall**	Diagnosis in **winter**	Diagnosis in **spring**	Diagnosis in **summer**	*p* Value
Total, Nb.	187	134	165	168	0.03
CLINICAL DATA					
Age, median (IQR), years	14.2 (11.8–16.0)	14.0 (11.5–15.7)	14.0 (11.3–15.5)	13.6 (11.3–15.4)	0.67
Sex, Nb. (%)[a]					
Male	96 (51.3)	79 (59.0)	87 (52.7)	98 (58.3)	0.40
Female	91 (48.7)	55 (41.0)	78 (47.3)	70 (41.7)	
Weight z-score, median (IQR)	−0.4 (−1.4–0.5)	−0.8 (−1.44–0.2)	−0.6 (−1.4–0.3)	−0.8 (−1.5–0.0)	0.05
Height z-score, median (IQR)	−0.2 (−0.9–0.6)	−0.1 (−0.8–0.6)	−0.2 (−0.9–0.6)	−0.4 (−0.9–0.3)	0.19
BMI z-score, median (IQR)	−0.6 (−1.4–0.4)	−0.9 (−1.8–0.0)	−0.66 (−1.6–0.2)	−0.9 (−1.7–0.0)	0.03
PCDAI, median (IQR)	30.0 (22.5–37.5)	37.5 (27.5–47.5)	35.0 (27.5–47.5)	37.5 (25.0–50.0)	<0.001
PCDAI, Nb. (%)					
Mild (10–27.5)	82 (47.4)	31 (25.6)	45 (29.2)	49 (31.4)	0.0002
Moderate (30–37.5)	48 (27.8)	37 (30.6)	49 (31.8)	37 (23.7)	
Severe (≥ 40)	43 (24.9)	53 (43.8)	60 (39.0)	70 (44.9)	
Symptoms at diagnosis, Nb. (%)					
Abdominal pain	163 (87.2)	117 (87.3)	152 (92.1)	158 (94.1)	0.08
Asthenia	68 (36.4)	58 (43.3)	92 (55.8)	78 (46.4)	0.004
Fever	25 (13.37)	33 (24.6)	37 (22.4)	36 (21.4)	0.05
Diarrhea	125 (66.8)	91 (67.9)	121 (73.3)	121 (72.0)	0.50
Rectal bleeding	92 (49.2)	56 (41.8)	64 (38.8)	81 (48.2)	0.16
Vomiting	37 (19.8)	20 (14.9)	35 (21.2)	36 (21.4)	0.48
Weight loss	104 (55.6)	84 (62.7)	110 (66.7)	115 (68.5)	0.05
Paris Classification[b]					
Age at diagnosis, Nb. (%)					
A2	16 (8.7)	8 (6.0)	4 (2.4)	14 (8.3)	0.02
A1b	150 (80.2)	118 (88.1)	140 (84.9)	126 (75.0)	
A1a	21 (11.2)	8 (6.0)	21 (12.7)	28 (16.7)	
Location of digestive involvement, Nb. (%)					
L1	61 (32.6)	31 (23.1)	52 (31.5)	40 (23.8)	0.01
L2	56 (30.0)	27 (20.2)	27 (16.4)	39 (23.2)	
L3	65 (34.8)	76 (56.7)	83 (50.3)	87 (51.8)	
L4a isolated	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	
L4b isolated	3 (1.6)	0 (0.0)	1 (0.6)	0 (0.0)	
L4ab isolated	2 (1.1)	0 (0.0)	2 (1.2)	1 (0.6)	
Upper digestive tract involvement, Nb. (%)					
No	71 (38.0)	47 (35.1)	63 (38.2)	47 (28.0)	0.43
L4a	80 (42.8)	57 (42.5)	68 (41.2)	84 (50.0)	
L4b	13 (7.0)	9 (6.7)	15 (9.1)	9 (5.4)	
L4ab	23 (12.3)	57 (42.5)	68 (41.2)	28 (16.7)	
Disease phenotype, Nb. (%)					
B1	156 (83.4)	121 (90.3)	141 (85.5)	137 (82.0)	0.62
B2	15 (8.0)	8 (6.0)	13 (7.9)	16 (9.6)	
B3	12 (6.4)	4 (3.0)	6 (3.6)	11 (6.6)	
B3B3	4 (2.1)	1 (0.8)	5 (3.0)	3 (1.8)	
Presence of inflammatory perianal involvement[c]**, Nb. (%)**	52 (27.8)	45 (33.6)	45 (27.3)	63 (37.5)	0.13
Presence of perianal abscesses/fistulas, Nb. (%)	34 (18.2)	24 (17.9)	25 (15.2)	40 (23.8)	0.23
Extra-intestinal manifestations, Nb. (%)					
Aphthous stomatitis	35 (18.7)	34 (25.4)	45 (27.3)	44 (26.2)	0.22
Arthralgia	29 (15.5)	21 (15.7)	39 (23.6)	33 (19.6)	0.19
Arthritis	8 (4.3)	5 (3.7)	8 (4.9)	16 (9.5)	0.09
Erythema nodosum	2 (1.1)	2 (1.5)	10 (6.1)	8 (4.8)	0.03
Skin rash	14 (7.5)	8 (6.0)	7 (4.2)	8 (4.8)	0.56
ENDOSCOPIC AND HISTOLOGICAL DATA[d]					
SES-CD[e]**, median (IQR)**	10.0 (6.0–18.0)	11.0 (8.0–17.0)	11.0 (6.0–18.0)	13.0 (9.0–20.0)	0.08
Presence of architectural distortions, Nb. (%)	145 (80.1)	101 (78.3)	122 (75.8)	119 (76.3)	0.76
Presence of moderate to severe lymphoplasmacytic infiltrate, Nb. (%)	143 (79.0)	109 (84.5)	136 (84.5)	135 (86.5)	0.27
Signs of moderate to severe active inflammation, Nb. (%)	123 (68.0)	77 (59.7)	104 (64.6)	91 (58.3)	0.25
Presence of granulomas, Nb. (%)	71 (39.2)	53 (41.1)	69 (42.9)	66 (42.3)	0.91
Increased eosinophils, Nb. (%)	101 (55.8)	76 (58.9)	95 (59.0)	85 (54.5)	0.81
Presence of lymphoid follicles, Nb (%)	115 (63.5)	81 (62.8)	98 (60.9)	83 (53.2)	0.22

(*continued on next page*)

Table 3 (*continued*)

	Diagnosis in **fall**	Diagnosis in **winter**	Diagnosis in **spring**	Diagnosis in **summer**	*p* Value
LABORATORY DATA[f]					
Hemoglobin, median (IQR), g/L	119.0 (110.0–129.0)	118.0 (106.0–129.0)	117.0 (108.0–126.0)	114.5 (105.0–123.0)	0.02
Anemia at diagnosis[g]**, Nb (%)**	100 (56.2)	72 (55.8)	97 (61.0)	105 (65.6)	0.24
Albumin, median (IQR), g/L	34.0 (29.0–37.0)	32.0 (27.5–37.0)	32.0 (28.0–36.0)	32.0 (28.0–37.0)	0.28
Total vitamin D, median (IQR), nmol/L	57.0 (48.0–73.0)	48.5 (34.5–62.5)	46.0 (35.0–58.0)	65.0 (53.0–78.0)	0.003
C-reactive-protein, median (IQR), mg/L	20.3 (4.6–55.0)	30.6 (9.7–57.8)	28.9 (8.0–61.2)	30.3 (8.3–56.2)	0.44
Erythrocyte sedimentation rate, median (IQR), mm/h	28.0 (18.0–39.0)	31.5 (20.0–45.0)	36.0 (25.0–46.0)	32.0 (22.0–45.0)	0.003
Fecal calprotectin, median (IQR), ug/g	1517.0 (540.0–1800.0)	887.0 (300.0–887.0)	1015.0 (365.0–2100.0)	1414.0 (859.0–2100.0)	0.26

[a] All percentages are column proportions. [b] Crohn's disease localized to the distal one-third of the ileum ± limited caecal involvement is defined as L1, isolated colitis as L2, and ileocolonic disease as L3. Upper gastrointestinal tract involvement is designated L4a when proximal to the ligament of Treitz and L4b if distal to the ligament of Treitz but proximal to the distal one-third of the ileum. Disease behavior is classified as: inflammatory (B1), structuring (B2), penetrating (B3); or structuring and penetrating (B2B3). A1a CD includes patients aged less than 10 years-old, A1b CD includes patients aged between 10 and 17 years old and A2 CD includes patients aged more than 17 years old. [c]Inflammatory perianal involvement includes inflammatory fissures in addition to perianal fistulas and abscesses clinically detected at the physical examination or pelvic magnetic resonance imaging. [d]Findings on histology including those on OGD and colonoscopy. [e] Including only patients who had a complete successful colonoscopy at diagnosis (visualization of the rectum to the ileum), $n = 511$. [f] Laboratory data measured closest to date of diagnosis, within +/- 1 month of diagnosis. [g] Anemia was determined based on the hemoglobin values, gender and age of patients.

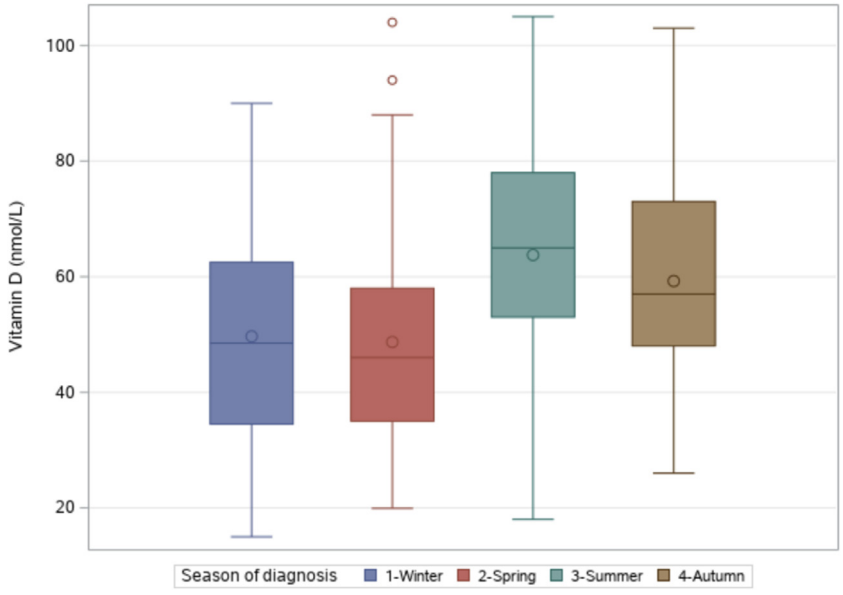

Fig. 2. Vitamin D[a] blood levels at diagnosis according to the season of diagnosis.
[a] 25-Hydroxy Vitamin D3 level.

in CD and the median age is higher in our cohort as we excluded patients younger than 4 years old which may explain these differences [24].

Over the past decade, there has been an increase in the number of new diagnoses of luminal CD in children. IBD experts from the four pediatric tertiary centers in Quebec have documented an increase of CD in each center (unpublished data). The phenotype of luminal CD in children has changed over the decade, there was a significant increase in the proportion of patients with granulomas at diagnosis and perianal fistulas and abscesses and the mean age at diagnosis has dropped.

These results demonstrate a strong association between the presence of granulomas and young age at diagnosis, as well as the presence of perianal fistulas or abscesses. Little information is available on the pathophysiological process of granuloma formation, but they may play a role as sites of antigen presentation to memory *T*-cells [25]. A recent meta-analysis in adults showed that patients with granulomas had more hospitalizations and more severe and extensive diseases along the digestive tract [26]. However, the clinical significance of granulomas in pediatrics remain unknown and the increase in the proportion of patients with granulomas with time has never been documented. We hypothesize that some specific environmental exposures have change over the decade that could have led to an increase in the proportion of CD characterized by the presence of granulomas and the associated clinical features listed above, such as changes in antibiotic exposure, diet, level of physical activity and microbiota composition.

These changes in environmental exposure could explain the significant decrease in the presence of eosinophils and moderate to severe lymphoplasmacytic infiltrate in intestinal biopsies over the decade. We found a strong association between these two histologic findings. We hypothesize that this distinction in the evolution of histologic characteristics over the decade in patients may be associated with a particular phenotypic profile of CD. Patients with

eosinophilia on digestive biopsies at diagnosis may have specific cytokine expression. The interleukins IL-5 and IL-13 are known to play an important role in the pathophysiology of eosinophilic esophagitis [27] and it would be interesting to verify whether they are upregulated in CD with increased tissue eosinophils.

In terms of diagnostic trends according to seasons, data from the present study indicate that the onset of CD occurs primarily during the warm months (spring, summer and fall), suggesting possible disease flare-up triggers. This trend had been previously described in a cohort of adult patients, but never in pediatrics [13]. An important cofounder which must be considered is that children with long lasting symptoms may only seek medical attention in the spring or summer rather than during winter due to the school year.

The study showed that the many patients had, regardless of the season of diagnosis, severe vitamin D deficiency. Several cohort studies have shown that vitamin D deficiency was associated with higher incidence of CD [7,8]. We hypothesize that vitamin D deficiency for long periods during winter would promote the development of the first flare of the disease [28] subsequently diagnosed later in the next three seasons because of the diagnosis delay of 4.9 – 9.0 months for the majority of patients as recently published [29–31]. In addition to increase in the CD incidence, vitamin D deficiency has also been associated with the severity of CD at diagnosis [32–34]. Past studies provide strong evidence that deficient vitamin D signaling may contribute to the dysregulation of intestinal innate immunity of CD [28] The current study also highlights the inverse correlation between vitamin D levels and severity of CD (PCDAI and SES-CD scores) at diagnosis. Likewise, CD diagnosed in fall had a less severe phenotype. We hypothesize that high levels of vitamin D during summer and fall has a protective effect on the severity of CD in development.

We also hypothesize that certain triggering events during the transition from cold to hot season would be a cornerstone for CD activity. CD is associated with inappropriate immune activation manifested by an increase in the production of immune-inflammatory cells and mediators. The immune function decreases in winter when there is less production of pro-inflammatory cytokines and an increased TH1 / TH2 ratio [35–37]. Also, bacterial antigens may contribute to digestive inflammation in CD by modulating gut immune response [38–40]. Bacterial colitis caused by *Campylobacter jejuni* or *Salmonella spp* are more frequent in spring and summer and it has been shown that newly diagnosed CD patients have a higher likelihood of having positive test results to those bacteria species around the diagnostic period [41,42]

Also, the results of this study show that newly diagnosed CD with ileal location were more frequent in spring and fall, while colonic location was more frequent in summer and fall. Summer is associated with a high prevalence of some specific bacterial colitis which could cause imbalance in the colonic microbiota. It has been shown that the Firmicutes to Bacteroidetes ratio in the microbiota changes over seasons and was significantly higher in summer and fall in certain populations [43]. Seasonal variations in the microbiota composition could be one explanation of the seasonal trends in disease location.

The limitations of the work stem from the retrospective nature of the study. Not all patients had assays of all inflammatory biomarkers at diagnosis, especially for FC during the first half of the decade since that test was not readily available then. Also, the large majority of disease activity scores (PCDAI, SES-CD) were calculated retrospectively.

Even though the team of three pathologists has remained constant throughout the decade, there may be inter-observer variability that couldn't be quantified. However, we analyzed the histological findings according to each pathologist and we found that the increase in the proportion of patients with granulomas and the decrease in the proportion of patients with moderate to severe inflammatory lymphoplasmacytic infiltrate over the decade was observed by each of the three pathologists. However, the presence of increased eosinophils, there appears to be greater variability. Indeed, for the cohort as a whole, one of the three pathologists reported an increase in eosinophils in 33.5% of their cases, while the other two pathologists reported an increase in eosinophils in 61.9% of their cases. The pathology reports did not contain the eosinophilic count in each digestive segment, and the increase in the number of eosinophils was left to the pathologist's interpretation. However, despite these differences, for all pathologists we noticed a significant decrease in the proportion of patients with increased eosinophils over the decade. Also, taking a single biopsy per segment for the may limits digestive histological findings.

The use of MRI became more common in the second half of the decade. This could therefore act as a confounder in the increased incidence of perianal fistulas and abscesses. However, in Table 2, we notice that the clinical obvious perianal disease (including inflammatory fissures and penetrating diseases) also increased over time.

The study focuses on the analysis of a very large cohort of children with CD. The large sample size allowed us to identify several significant temporal associations and these results have produced new knowledge. Our findings provide interesting avenues for future research, such as identifying the clinical significance of granulomas in pediatric CD and microbiota on disease activity.

Declaration of Competing Interest

None declared.

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Supplementary materials

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