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Serologic Reactivity Reflects Clinical Expression of Ulcerative Colitis in Children

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Background: In contrast to pediatric Crohn's disease (CD), little is known in pediatric ulcerative colitis (UC) about the relationship between disease phenotype and serologic reactivity to microbial and other antigens.

Aim: The aim of this study was to examine disease phenotype and serology in a well-characterized inception cohort of children newly diagnosed with UC during the PROTECT Study (Predicting Response to Standardized Pediatric Colitis Therapy).

Methods: Patients were recruited from 29 participating centers. Demographic, clinical, laboratory, and serologic (pANCA, ASCA IgA/IgG, Anti-CBir1, and Anti-OmpC) data were obtained from children 4–17 years old with UC.

Results: Sixty-five percent of the patients had positive serology for pANCA, with 62% less than 12 years old and 66% 12 years old or older. Perinuclear anti-neutrophil cytoplasmic antibodies did not correspond to a specific phenotype though pANCA ≥ 100 , found in 19%, was strongly associated with pancolitis ($P = 0.003$). Anti-CBir1 was positive in 19% and more common in younger children with 32% less than 12 years old as

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to declare. AP is on the speaker's bureau for Abbvie. PR is a consultant for Shire and Leutpold and a speaker for Abbvie and receives research support from TechLab. MAM and TW have no conflicts to declare. MC is a consultant for Shire, Regeneron, Adare and Receptos and has received research grants from Shire, Regeneron and Receptos. SK is a consultant for Janssen and UCB. LD has received grant support from Abbvie and Janssen. JH is on the advisory board for Janssen and a consultant for Abbvie, Takeda, Lilly, Boehringer-Ingelheim, Allergan, Pfizer and Astra Zeneca. MD is a consultant for Abbvie, Boehringer-Ingelheim, Celgene, Genentech, Janssen, Pfizer, Prometheus Labs, Salix, Shire, Takeda and UCB.

Author Contributions: ES, SD, TW, MC, SK, LD, JH and MD conceived and designed the study. DM, BB, AG, NL, CS, DK, JM, SB, JR, RB, MOH, MP, AO, MH, JN, AS, PR, TW, SK, LD, JH, MD and the PROTECT Study Group acquired data. All authors except the PROTECT Study Group analyzed and interpreted data. ES, SD, AG, NL, JM, TW, SK, LD, JH, and MD drafted the manuscript. All authors except the PROTECT Study Group critically revised the manuscript for important intellectual content. Davis and Marquis performed statistical analysis.

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Abbreviations: IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; pANCA, perinuclear anti-neutrophil cytoplasmic; ASCA, anti-saccharomyces cerevisiae antibody; Anti-CBir1, anti-flagellin; Anti-OmpC, anti-outer membrane C; PUCAI, pediatric ulcerative colitis activity index; HPF, high-power field; Hgb, hemoglobin; Hct, hematocrit; WBC, white blood cell count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ULN, upper limit of normal; VDR, vitamin D receptor.

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compared with 14% 12 years old or older ($P < 0.001$). No association was found in any age group between pANCA and Anti-CBir1. Relative rectal sparing was more common in +CBir1, 16% versus 7% ($P = 0.02$). Calprotectin was lower in Anti-CBir1+ (Median [IQR] 1495 mcg/g [973–3333] vs 2648 mcg/g [1343–4038]; $P = 0.04$). Vitamin D 25-OH sufficiency was associated with Anti-CBir1+ ($P = 0.0009$).

Conclusions: The frequency of pANCA in children was consistent with adult observations. High titer pANCA was associated with more extensive disease, supporting the idea that the magnitude of immune reactivity may reflect disease severity. Anti-CBir1+ was more common in younger ages, suggesting host-microbial interactions may differ by patient age.

Key Words: CBir1, pANCA, PROTECT, serologies, ulcerative colitis

INTRODUCTION

Ulcerative colitis (UC) denotes a phenotype of chronic inflammatory bowel disease (IBD) in which inflammation is localized to the mucosa and extends proximally from the rectum to varying extents of the large intestine. It is thought to result in genetically susceptible individuals, from an inappropriate activation of the immune system and intestinal dysbiosis at the mucosal luminal interface¹. UC is remarkably heterogeneous with respect to age of onset, anatomical extent, disease course, and response to therapies^{2–6}; in general, children have a more severe clinical phenotype than adults with more extensive disease and a greater early need for corticosteroids and biologic therapies^{7,8}.

The heterogeneity of IBD has prompted a search for a marker or combination of markers to distinguish IBD from non-IBD, differentiate IBD subtypes (UC versus CD), anticipate disease outcomes, and predict response to therapies. A number of studies from the early 1990s onward have evaluated various serological immune markers to fill this role^{9–12}. Perinuclear anti-neutrophil cytoplasmic antibody (pANCA) has historically been linked to a UC phenotype, and anti-saccharomyces cerevisiae antibody (ASCA) has been associated with a classic CD behavior. Anti-flagellin (Anti-CBir1) reactivity, on the other hand, has been described to be more prevalent in younger-onset CD, characterized by a colitis phenotype¹³, but its prevalence in pediatric UC remains undefined.

The PROTECT Study (Predicting Response to Standardized Pediatric Colitis Therapy) was initiated in 2012 to systematically examine treatment responses of children and adolescents newly diagnosed with UC. This multicenter inception cohort was designed to examine the demographic, clinical, laboratory, endoscopic, histologic, and serologic factors that are associated with initial response to therapy. This study tests the hypothesis that serologic reactivity is related to disease phenotype, histology, and laboratory values in children with newly diagnosed UC. While this relationship has been studied thoroughly in pediatric CD, the same rigorous studies do not exist in pediatric UC, and an improved understanding of the prevalence and associations with these serologic markers would better inform diagnosis, disease classification, and, potentially, therapy.

MATERIALS AND METHODS

Patient Population

Potential study patients were recruited from the clinical practices of the 29 centers participating in PROTECT. Children

between the ages of 4 and 17 years inclusive with a clinical history consistent with colonic inflammation (eg, any combination of diarrhea, bleeding, abdominal pain) were eligible for study. Complete demographic, clinical, laboratory, and serologic data were obtained along with diagnostic esophagogastroduodenoscopy and ileocolonoscopy. Eligibility required disease extent beyond the rectum, a baseline Pediatric Ulcerative Colitis Activity Index (PUCAI)¹⁴ score of ≥ 10 , no previous therapy for colitis, and a stool culture negative for enteric bacterial pathogens (Salmonella, Shigella, Campylobacter, *E. coli* 0157:H7) and *Clostridium difficile* toxin. Patients were all newly diagnosed with UC, and this was a clinical, endoscopic, and histologic diagnosis of UC using previously established criteria. Exclusionary criteria included any clinical, endoscopic, radiologic, or histologic evidence of CD.

Age

Age was evaluated by years in categories of 4–6, 7–10, 11–13, and 14–17 years old to assess trends in seropositivity across childhood and adolescence.

Clinical Disease Activity

Clinical activity was determined by the PUCAI (range 0–85) and endoscopic activity by the Mayo endoscopy subscore¹⁵. PUCAI < 10 denoted inactive disease/remission, 10–34 denoted mild, 35–64 denoted moderate, and ≥ 65 denoted severe disease. The baseline PUCAI was defined as the last PUCAI on or before the treatment start date, and the majority ($> 60\%$) occurred on the scope date itself.

Endoscopic Assessment

Endoscopic evaluation included assessment of mucosal inflammation noting granularity, loss of vascular pattern, small superficial ulcers, mucopurulent exudate, and a line of demarcation between abnormal and normal colon in a patient whose colitis did not extend to the cecum. Note was made whether there was patchiness to the endoscopic appearance or relative rectal sparing.

Disease Extent

Disease extent was classified as proctosigmoiditis, left-sided colitis (to the splenic flexure), extensive colitis (to the hepatic flexure), and pancolitis (to the cecum). In analyses, we combined extensive colitis, pancolitis, and cases where a limited examination was performed due to severity of disease. Visual

evidence of inflammation, not histology, was used to determine disease extent.

Histology

Centralized histologic examination was performed on a single rectal biopsy by one of the authors (MC) who was blinded to clinical data via a standardized scoring system that was created for PROTECT to evaluate degree of inflammation and architectural changes, and this scoring system has been previously published¹⁶. Of note, chronic features were scored as either present or absent, and these included ulcer/erosion, surface villiform changes, basal plasmacytosis, basal lymphoid aggregates, Paneth cell metaplasia, and crypt architectural abnormalities.

Laboratory Assessment

Hemoglobin (Hgb), hematocrit (Hct), white blood cell count (WBC), serum albumin, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were recorded from local site standard-of-care assessments, as available, within 4 weeks prior to initial UC treatment and not more than 2 days after initiating treatment. For some sites, the standard of care for milder disease did not include laboratory studies before a colonoscopy, and so the baseline window for participants with a mild PUCAI was extended to 8 weeks. Plasma albumin was measured at a central laboratory by ELISA per manufacturer's instructions (Cell Biolabs, Inc., San Diego, CA) for participants with no available local serum value. We report observed values of all laboratory studies with the exception of C-reactive protein, which we report with respect to the upper limit of normal (ULN) for the local laboratory. Fecal calprotectin was determined using an ELISA (Bühlmann Laboratories AG, Schönenbuch, Switzerland) from stool samples collected before colonoscopy cleanout or at least 2 days after colonoscopy, but not more than 3 days after initial UC treatment^{17,18}. Vitamin D 25-OH was performed centrally from plasma collected at baseline, and the level was defined as "sufficient" (≥ 30 ng/mL), "insufficient" (20–<30 ng/mL), or "deficient" (<20 ng/mL)¹⁹.

Serology

Serologic determination of pANCA, ASCA IgG and IgA, Anti-CBir1, and anti-outer membrane C (Anti-OmpC) was performed at Cedars-Sinai Hospital, Los Angeles, California, utilizing previously published methods²⁰. Perinuclear anti-neutrophil cytoplasmic antibody was considered high-titer at a level of ≥ 100 EU/mL^{21–23}.

Statistical Analysis

Demographic, clinical, and serologic characteristics were compared across age categories: 4–6, 7–10, 11–13, and 14–17 years old. Patients were grouped as pANCA positive versus negative, pANCA titer negative versus positive, <100 versus

positive and ≥ 100 , and Anti-CBir1 positive versus negative. Groups were compared using a χ^2 test or Fisher exact test for categorical assessments, a Mantel-Haenszel χ^2 test for ordinal categorical assessments, a *t* test for normally distributed continuous assessments, and a Wilcoxon rank-sum test for continuous assessments with skewed distributions. Age-adjusted least-squares-mean titer values by disease location were obtained by analysis of covariance. All statistical tests were 2-tailed. There was no adjustment for multiple comparisons; *P* values are for descriptive purposes. Analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC).

Manuscript Preparation

The manuscript was written by the PROTECT Study Publication committee and subsequently approved by all authors.

ETHICAL CONSIDERATIONS

Informed consent or assent was obtained in all cases, and local investigational review boards at all investigative sites approved the study. This study was registered with [clintrials.gov](https://www.clinicaltrials.gov/ct2/show/study/NCT01536535) (NCT01536535).

RESULTS

Study Population

A total of 431 subjects were confirmed to have UC, and 399 (93%) had serology performed. Baseline demographic, clinical, and laboratory characteristics—categorized by age—of the 399 patients are shown in [Table 1](#).

Serologic Immune Markers

Perinuclear anti-neutrophil cytoplasmic antibody (pANCA)

Perinuclear anti-neutrophil cytoplasmic antibody (pANCA) was positive in 65% (260 of 399) of patients. The percentage of pANCA positive was lower in ages 4–6 (44%, 11 of 25) than ages 7–17 (67%, 249 of 34; *P* = 0.022) (See [Fig. 1](#)). The prevalence of pANCA was comparable across the other age groups: 7–10 (69%), 11–13 (63%), and 14–17 (68%) years old (*P* = 0.63). Positive pANCA did not correspond to a specific phenotype, (ie, there was no significant difference in extent of disease, PUCAI, Mayo score, rectal sparing, or basal plasmacytosis) ([Table 2](#)). However, average age-adjusted pANCA titer was somewhat higher for extensive pancolitis (least squares mean 60.0; 95% CI, 55.3–64.7) compared with proctosigmoiditis (39.2; 95% CI, 21.7–56.7) and left-sided disease (48.1; 95% CI, 34.4–61.5; *P* = 0.028). A pANCA titer ≥ 100 , as observed in 19% of the patients, was strongly associated with pancolitis. All but 3 of the 76 (96%) with pANCA ≥ 100 had extensive colitis, as compared with 81% of the remaining 324 with pANCA

TABLE 1: Demographic and Clinical Features of Study Population

	All patients N = 399	4–6 years N = 25	7–10 years N = 65	11–13 years N = 111	14–17 years N = 198	P
Age years, mean (SD)	12.7 ± 3.3					
Gender (% female)	198 (50%)	15 (60%)	43 (66%)	54 (49%)	86 (43%)	0.010
Non-white race	66/391 (17%)	6/25 (24%)	14/60 (23%)	15/109 (14%)	31/197 (16%)	0.29 ^b
Hispanic	36/395 (9%)	2/24 (8%)	10/65 (15%)	6/110 (5%)	18/196 (9%)	0.17 ^b
Family History of IBD						0.13 ^b
None	335 (84%)	20 (80%)	56 (86%)	96 (86%)	163 (82%)	
UC only	40 (10%)	0	6 (9%)	12 (11%)	22 (11%)	
CD only	13 (3%)	3 (12%)	2 (3%)	2 (2%)	6 (3%)	
Mixed	11 (3%)	2 (8%)	1 (2%)	1 (1%)	7 (4%)	
BMI Z-score	-0.2 ± 1.3	-0.2 ± 1.2	-0.5 ± 1.4	-0.2 ± 1.3	-0.2 ± 1.3	0.26
Proctosigmoiditis	24 (6%)	1 (4%)	4 (6%)	6 (5%)	13 (7%)	0.37
Left-sided colitis	41 (10%)	2 (8%)	5 (8%)	8 (7%)	26 (13%)	
Extensive/Pancolitis ^a	334 (84%)	22 (88%)	56 (86%)	97 (87%)	159 (80%)	
PUCAI, mean (SD)	49.9 ± 19.9	44.4 ± 21.3	51.8 ± 20.4	50.3 ± 19.1	49.8 ± 20.1	0.47
PUCAI 10–30	95 (24%)	9 (36%)	13 (20%)	26 (23%)	47 (24%)	0.49
35–60	172 (43%)	9 (36%)	27 (42%)	47 (42%)	89 (45%)	
≥65	132 (33%)	7 (28%)	25 (38%)	38 (34%)	62 (31%)	
Mayo Endoscopy score						0.32
Grade 1	53 (13%)	5 (20%)	9 (14%)	13 (12%)	26 (13%)	
Grade 2	210 (53%)	14 (56%)	37 (57%)	54 (49%)	105 (53%)	
Grade 3	136 (34%)	6 (24%)	19 (29%)	44 (40%)	67 (34%)	
Hospitalized at baseline	154 (39%)	9 (36%)	28 (43%)	47 (42%)	70 (35%)	0.54

^aExtensive/Pancolitis/U reassessable due to severity of disease were all included in this group.

^bFisher exact test comparing age groups.

<100 ($P = 0.001$) and 83% of the 139 who were pANCA negative ($P = 0.005$). However, the PUCAI and Mayo endoscopy subscores were not significantly different between groups with pANCA ≥ 100 and pANCA <100.

Anti-flagellin (Anti-CBir1)

Anti-CBir1 was positive in 19% (77 of 399) of patients and more commonly positive in younger children. The percentage of Anti-CBir1 positive decreased steadily with age (Spearman rank correlation = -0.25 , $P < 0.001$), with the highest in ages 4–6 (44%) and lowest in ages 14–17 (10%) (Fig. 1). Table 3 characterizes the associations of Anti-CBir1 with phenotype and baseline laboratory studies. More limited disease (proctosigmoiditis or left-sided colitis) was more common in Anti-CBir1-negative patients (18% vs 9%, $P = 0.041$). However, relative rectal sparing was more common in Anti-CBir1-positive patients (16% vs 7%, $P = 0.023$), and basal plasmacytosis on rectal biopsy was less common (38% vs 56% $P = 0.016$). There was no association between pANCA and Anti-CBir1 in any age group.

Fecal calprotectin was higher in the Anti-CBir1-negative group (median: 2634 mcg/g, interquartile range [IQR]:

1324–4022]) compared with the Anti-CBir1-positive group (median: 1495 mcg/g [IQR: 973–3333], $P = 0.046$), despite similar Mayo endoscopy subscores, ESR, and CRP. Additionally, both the median 25-OH vitamin D level ($P = 0.0002$) and the percentage of patients with sufficient levels of 25-OH vitamin D ($P = 0.0009$) were strikingly higher in the Anti-CBir1-positive group. This relationship between vitamin D sufficiency and Anti-CBir1 positivity remained significant when the patients were stratified by age group ($P = 0.005$) (Table 3).

Anti-outer membrane C (Anti-OmpC) and Anti-saccharomyces cerevisiae antibody (ASCA)

Anti-OmpC and ASCA IgA/IgG were positive in only a small number of our pediatric UC patients, 5% and 2%, respectively.

DISCUSSION

In our large, rigorously phenotyped inception cohort of children with UC, we have shown that 65% of children newly diagnosed with UC have a positive pANCA titer. This is similar to the rate of pANCA positivity in adults, which has been shown to be 60%–65%^{10–12, 24–28}. We also demonstrated in our

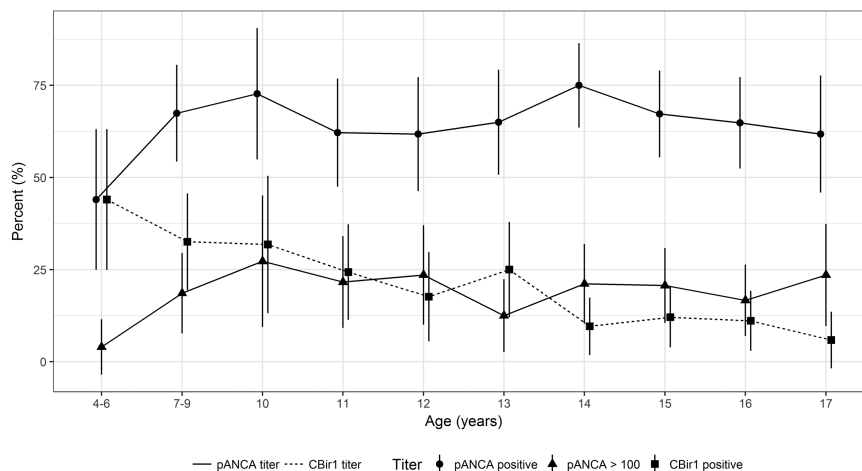


FIGURE 1. Relationship of serologic reactivity and age in children newly diagnosed with UC. Bars represent 95% confidence intervals for the percent seropositive per age category.

pediatric population that there is an association between high titer pANCA (≥ 100) and more extensive disease; yet, interestingly, we found that the PUCAI and Mayo endoscopic subscores were not significantly different between high and low titer pANCA groups. Previous studies have shown that pANCA titers can be used to risk stratify patients into immunologically distinct subgroups²⁰. In a pediatric study from 1998, Ruemmele et al observed differences in the titer of pANCA within subgroups of IBD, with equivalently high titers in UC-like CD with pancolitis (median of 68.2 EU/mL) and UC (median of 57.7 EU/mL)⁹. This stratification by pANCA titer has been further explored in adult UC, with Fleshner et al showing that a pre-operative pANCA >100 titer was a risk factor for the development of chronic pouchitis after ileal pouch-anal anastomosis²³ and White et al reporting an association between high titer pANCA and both pancolitis and backwash ileitis²⁹. Our observed association of higher pANCA titer with more extensive endoscopic disease activity corroborates this relationship between magnitude of immune reactivity and extent of disease activity.

Beyond phenotypic predictions, pANCA titers have been linked to response to therapy. Importantly, Sandborn et al showed in a 1996 retrospective study of 56 UC patients with left-sided disease that those who were pANCA-positive were more likely to have treatment-resistant disease (90% vs 62%)³⁰. And in a larger 2007 study of 100 IBD patients starting infliximab (IFX) therapy, it was confirmed that patients who were pANCA+/ASCA- had lower early clinical response—55% vs 76%³¹. In children, Dubinsky et al in 2010 reported that pANCA positivity is independently associated with primary nonresponse to anti-TNF α therapy in both CD and UC (odds ratio [OR]: 5.4; $P = 0.01$)³². Subsequent long-term analysis of the PROTECT cohort will be required to determine if a relationship exists between pANCA titer and response to various therapies and ultimate outcomes.

Unlike pANCA, the microbial antibodies, ASCA, Anti-OmpC, and Anti-CBir1 are more common in CD than UC with prevalence in CD of 34%–76%, 17%–24%, and 55%–56%, respectively^{13, 25, 33, 34}. Our data confirmed that antibodies to ASCA and Anti-OmpC are uncommon in pediatric UC. Importantly, although Anti-CBir1 reactivity is negatively associated with UC in adults^{35, 36}, 19% of our population was Anti-CBir1-positive. This might raise suspicion about the diagnosis of UC in our study population; however, observation of the study cohort for 1 to 3 years has resulted in no change in diagnosis of any of the study patients, suggesting that Anti-CBir1 is more common in pediatric UC than previously appreciated. In part, this might be due to the fact that Anti-CBir1 reactivity is more common with younger age, as demonstrated now in our pediatric UC cohort and previously in pediatric CD¹³.

From a phenotypic perspective, Anti-CBir1 reactivity was associated with rectal sparing, more limited disease extent, lower frequency of basal plasmacytosis on rectal biopsy, and lower fecal calprotectin. Spiliadis et al in 1987 were the first group to report 12 UC patients with relative endoscopic rectal sparing from their 27-year experience³⁷. Glickman et al in 2004 described that histologically patchy disease and relative rectal sparing occurred in 21% and 26%, respectively, of 73 pediatric UC patients; interestingly, they also had a comparison group of adults that had neither rectal sparing nor patchy disease. In this same group, another 16% had only very subtle histologic findings of chronic inflammation³⁸, supporting a prior study by Washington et al, who showed no evidence of crypt architectural distortion in 34% of biopsies in new-onset UC³⁹. Rajwal et al reported that, in general, relative rectal sparing is more common in those younger than 10 years old⁴⁰. This is compatible with our observations that Anti-CBir1 was associated with younger age, as well as relative-rectal sparing. It is unclear as to whether there are prognostic implications of rectal sparing as it relates to therapeutic response or surgical

TABLE 2: Relationship of pANCA to Clinical and Laboratory Features in Children Newly Diagnosed with UC

	pANCA-	pANCA+	<i>P</i>	pANCA + and		<i>P</i> comparing pANCA-, <100, ≥100
	N = 139	N = 260		< 100	pANCA ≥ 100	
Age, mean (SD)	12.5 ± 3.6	12.8 ± 3.1	0.29	12.7 ± 3.2	13.1 ± 2.8	0.41
pANCA (%) stratified by age categories	—	—	0.22	—	—	0.26
4–6	14/25 (56%)	11/25 (44%)	0.022	10/25 (40%)	1/25 (4%)	0.03 ^b
7–10	20/65 (31%)	45/65 (69%)	—	31/65 (48%)	14/65 (22%)	—
11–13	41/111 (37%)	70/111 (63%)	—	49/111 (44%)	21/111 (19%)	—
14–17	64/198 (32%)	134/198 (68%)	—	94/198 (47%)	40/198 (20%)	—
Disease extent (%)	—	—	0.60	—	—	0.004
Proctosigmoiditis	12 (9%)	12 (5%)	—	11 (6%)	1 (1%)	—
Left-sided colitis	12 (9%)	29 (11%)	—	27 (15%)	2 (3%)	—
Extensive/Pancolitis ^a	115 (83%)	219 (84%)	—	146 (79%)	73 (96%)	—
PUCAI, mean (SD)	48.3 ± 20.6	50.8 ± 19.6	0.23	50.6 ± 20.1	51.4 ± 18.3	0.45
PUCAI score (%)	—	—	0.24	—	—	0.47
10–30	41 (29%)	54 (21%)	—	39 (21%)	15 (20%)	—
35–60	53 (38%)	119 (46%)	—	81 (44%)	38 (50%)	—
≥65	45 (32%)	87 (33%)	—	64 (35%)	23 (30%)	—
Mayo Endoscopic Sub-score (%)	—	—	0.50	—	—	0.55
Grade 1	23 (17%)	30 (12%)	—	21 (11%)	9 (12%)	—
Grade 2	69 (50%)	141 (54%)	—	104 (57%)	37 (49%)	—
Grade 3	47 (34%)	89 (34%)	—	59 (32%)	30 (39%)	—
Relative rectal sparing (%)	13/139 (9%)	23/259 (9%)	0.88	13/183 (7%)	10/76 (13%)	0.30
Basal plasmacytosis (%)	56/111 (50%)	112/210 (53%)	0.62	74/151 (49%)	38/59 (64%)	0.12
Hgb (g/dL)	N = 130	N = 246	0.59	N = 176	N = 70	0.53
Mean (SD)	11.5 ± 2.4	11.4 ± 2.1	—	11.5 ± 2.1	11.2 ± 2.1	—
ESR (mm/hr)	N = 126	N = 236	0.043	N = 170	N = 66	—
Median (IQR)	22 (11, 35)	26 (12, 47)	—	23 (11,41)	34 (22,55)	<0.0001
Albumin (g/dL)	N = 139	N = 258	0.39	N = 182	N = 76	—
Mean (SD)	3.7 ± 0.7	3.6 ± 0.7	—	3.7 ± 0.7	3.6 ± 0.7	0.43
Calprotectin (mcg/g)	N = 82	N = 146	0.20	N = 108	N = 38	—
Median (IQR)	2023 (1083,3975)	2676 (1378,3928)	—	2932 (1414,4036)	1754 (1088,3544)	0.17
CRP or hsCRP (%)	N = 103	N = 192	—	N = 142	N = 50	—
>ULN	40 (39%)	92 (48%)	0.13	63 (44%)	29 (58%)	0.08
>2xULN	27 (26%)	64 (33%)	0.21	45 (32%)	19 (38%)	0.32
CBir1 positive (%)	33 (24%)	44 (17%)	0.10	28 (15%)	16 (21%)	0.14

^aExtensive/Pancolitis /Unassessable due to severity of disease were all included in this group. ^b Comparison of pANCA for age <7 vs age ≥7.

outcomes. Additionally, the CBir1 antigen has a high degree of homology to bacterial flagellins, resembling those from enteric microbiota including *Butyrivibrio*, *Roseburia*, *Thermotoga*, and *Clostridium* species²⁰. Further examination of Anti-CBir1, in conjunction with microbiome data, is warranted.

Lower levels of the damage-associated molecular pattern proteins S100A8/S100A9, collectively called fecal calprotectin, in the Anti-CBir1-positive group might be explained by the relationship between the innate and adaptive immune system.

Fecal calprotectin mediates a number of different antimicrobial functions, and since some patients are low fecal calprotectin expressers, this could theoretically lead to mitigation of the microbiota adherent to the mucosa and altered triggering of Anti-CBir1 antibodies^{17, 25, 34}.

The relationship between higher, more often sufficient levels of 25-OH vitamin D and Anti-CBir1-positive serologies is intriguing. It is recognized that vitamin D is an important component of both the innate and adaptive immune

TABLE 3: Relationship of CBir1 to clinical and laboratory features of children newly diagnosed with UC

	CBir1+ N = 77	CBir1- N = 322	P
Age, mean (SD)	10.9 ± 3.6	13.1 ± 3.1	<0.0001
CBIR (%) stratified by age categories			<0.0001
4–6	11/25 (44%)	14/25 (56%)	—
7–10	21/65 (32%)	44/65 (68%)	—
11–13	25/111 (23%)	86/111 (77%)	—
14–17	20/198 (10%)	178/198 (90%)	—
Disease extent (%)			0.041
Proctosigmoiditis	0 (0%)	24 (7%)	—
Left-sided colitis	7 (9%)	34 (11%)	—
Extensive /Pancolitis ^a	70 (91%)	264 (82%)	—
PUCAI, mean (SD)	45.7 ± 21.4	51.0 ± 19.4	0.052
PUCAI score (%)			0.095
10–30	25 (32%)	70 (22%)	—
35–60	30 (39%)	142 (44%)	—
≥65	22 (29%)	110 (34%)	—
Mayo Endoscopic Sub-score (%)			
Grade 1	13 (17%)	40 (12%)	0.18
Grade 2	42 (55%)	168 (52%)	—
Grade 3	22 (29%)	114 (35%)	—
Relative rectal sparing (%)	12/76 (16%)	24/322 (7%)	0.023
Basal plasmacytosis (%)	22/58 (38%)	146/263 (56%)	0.015
Hgb (g/dL)	11.0 ± 2.2	11.5 ± 2.2	0.063
Mean (SD)	N = 75	N = 301	—
ESR (mm/hr)	25 (16, 49)	24 (12, 40)	0.33
Median (IQR)	N = 70	N = 292	—
Albumin (g/dL)	3.7 ± 0.8	3.6 ± 0.7	0.26
Mean (SD)	N = 77	N = 320	—
Calprotectin (mcg/g)	1495 (971, 3333)	2634 (1324, 4022)	0.046
Median (IQR)	N = 35	N = 193	—
CRP or hsCRP (%)			
>ULN	24 (44%)	108 (45%)	0.85
>2xULN	17 (31%)	74 (31%)	0.99
	N = 55	N = 240	—
25-OH Vitamin D (ng/mL)	30.9 (27.2,38.0)	27.6 (23.0,33.4)	0.0002
Median (IQR)	N = 75	N = 317	—
25-OH Vitamin D (%)			0.0009
<20 Deficient	5 (7%)	37 (12%)	—
20-< 30 Insufficient	25 (33%)	159 (50%)	—
≥30 Sufficient	45 (60%)	121 (38%)	0.005 ^b
pANCA positive (%)	44 (57%)	216 (67%)	0.10

^aExtensive/Pancolitis /Unassessable due to severity of disease were all included in this group.

^bP value for association of CBir1 with 25-OH vitamin D ≥30 stratified by age-group based on a Mantel-Haenszel χ^2 test.

systems. Immune cells produce both the vitamin D receptor (VDR) and the enzymes to convert vitamin D₃ into its active 1,25(OH)₂D₃ form, and this active form can modulate numerous cell types, including activated T- and B-cells,

macrophages, and dendritic cells, leading to an observed antimicrobial effect^{41, 42}. Additionally, vitamin D affects intestinal barrier function, and VDR^{-/-} mice have been observed to have disruptions in their tight junctions, leading to increased

susceptibility to DSS injury⁴³. It is possible that vitamin D mediates the immune response in such a way that alters the adaptive anti-flagellin response, leading to production of Anti-CBir1.

Our study has several limitations. Due to its multicenter nature, there may have been site-specific differences in clinical scoring despite prespecified guidelines in the PROTECT study protocol. Mayo endoscopy su-scores were guided at each site by standardized photographs. Histologic examination was done by a central reader (MHC). There was also a larger acceptable window for attaining baseline labs, which included the serological assessment, before treatment in those with mild disease (8 weeks vs 4 weeks); however, it is unlikely that there would have been large shifts in titers in this mild population over this short interval. Given the exploratory nature of our study, unadjusted *P* values were used.

In summary, our study greatly extends the knowledge base of serologic expression in children with UC. Future work will be required to examine the relationship of serologic reactivity to colonic community microbial profiling as well as rectal gene expression. These relationships may provide a platform to better understand disease pathogenesis and perhaps response to therapy.

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