Effects of Disease Activity on Anti–*Saccharomyces cerevisiae* Antibodies

Implications for Diagnosis and Follow-up of Children with Crohn's Disease

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Background: To determine diagnostic accuracy of anti–*Saccharomyces cerevisiae* antibodies (ASCA) in identifying children with inflammatory bowel disease (IBD) and to differentiate Crohn's disease (CD) from other IBD forms; and to determine the effect of medical or surgical treatment and of disease location and activity on ASCA titers.

Methods: Serum samples were obtained from 196 IBD children and 142 controls. ASCA IgA and IgG titers were measured by ELISA. Measurements were repeated during the follow up of CD children.

Results: ASCA titers were significantly higher in CD than in other IBD and in control patients. Combination of IgA and IgG ASCA positivity was highly specific for CD. Medical treatment and disease location did not influence assay results. Significantly lower ASCA titers were obtained in CD children with intestinal resection compared to CD-affected children who did not undergo surgical resection. ASCA titers correlated significantly with disease activity, and children with severe active disease showed higher ASCA values compared to those in remission. A signficant reduction of ASCA was observed during the follow-up of CD children when clinical remission was achieved.

Conclusions: The diagnostic accuracy of ASCA is influenced by disease activity and this suggests an additional use for the follow-up of CD children of this assay.

Key Words: anti–*Saccharomyces cerevisiae* antibody, children, Crohn's disease, disease activity, inflammatory bowel disease

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The incidence of inflammatory bowel disease (IBD) has progressively increased in children in Western countries during the past few decades.[1] The incidence of ulcerative colitis (UC) appears to be greater than that of Crohn's disease (CD) in Italy and in Scandinavian countries but not in the United Kingdom, France, and The Netherlands.[1] In addition, most of the retrospective epidemiological studies do not take into account cases categorized as indeterminate colitis (IC), in which a definitive diagnosis of CD or UC cannot be made owing to overlapping or undefined clinical features.[1] Thus, IBD has become an increasingly important diagnostic consideration in pediatric patients presenting nonspecific intestinal symptoms or extraintestinal manifestations. Unfortunately, there was a median delay period of 5 months from the onset of symptoms to diagnosis, and up to 25% of children apparently suffered symptoms for more than 1 year prior to diagnosis.[2] Furthermore, earlier diagnosis may decrease the long-term morbidity of IBD, which includes delayed puberty and shorter ultimate height, decreased bone density, nutritional deficiencies, and psychologic adjustment to chronic illness.[3–5]

A lot of progress has been made in gut immunology, inflammatory cascades, and genetic susceptibility, yet IBD remains an important challenge to the pediatrician, and clinical investigation of children with suspected IBD follows a conventional pattern that has not substantially changed in the past two decades.[6] Currently, the diagnosis requires a combination of typical clinical signs and symptoms, exclusion of other disorders, plus radiographic, endoscopic, and histologic features consistent with IBD.[7–10] Despite careful clinical evaluation, up to 15% of children with colitis have an indeterminate form, and up to 4% of patients undergoing colectomy with ileoanal anastomosis for treatment of UC are ultimately determined to have CD.[1,11] Furthermore, the diagnosis of a patient initially identified as having UC may, over time, be switched to CD due to an extension of the disease.[11] Therefore, readily available, noninvasive tests are needed to make a timely and accurate diagnosis of IBD. In addition, the course of IBD is one of re-

lapse and remission. Thus, another challenge is the difficulty in predicting relapses or in determining disease activity, and it is important to identify noninvasive markers for monitoring disease activity and the effects of treatment.[12]

Recently, anti–*Saccharomyces cerevisiae* antibodies (ASCA) have been increasingly used for the evaluation of patients with IBD.[13] Immunoglobulin (Ig)A and/or IgG ASCA have been found in 50% to 60% of CD patients, but in only 10% to 15% of UC patients and <5% of healthy controls.[11–14] A high diagnostic specificity for CD (>90%) has been reported, with a sensitivity of about 50%. In addition, positive ASCA titers could be found in unaffected relatives and does not concord in marital pairs, indicating genetic factors or childhood environmental exposure.[15] Finally, some data suggests that ASCA titer may be influenced by disease activity, as it decreases after damaged gut tissue resection.[16] However, more clinical research is needed to determine the real ASCA clinical utility in children.

The aims of this study were to investigate the efficacy of ASCA in identifying children with IBD, to differentiate CD from the other major forms of IBD, and to determine the effect of different therapeutic interventions, disease location, and activity on ASCA titers in a large cohort of IBD patients and controls.

MATERIALS AND METHODS

Study Population

Serum was obtained from children attending different Italian centers of pediatric gastroenterology involved in a nationwide multicenter study approved by the IBD working group of the Italian Society of Pediatric Gastroenterology and Hepatology (SIGEP).

The identification of patients for this study was made on the basis of a clear diagnosis in patients who underwent a complete diagnostic workup for IBD including clinical, radiologic, endoscopic, and histologic evaluation.[10] Whenever possible, a new serum sample was obtained from the same consenting patient to evaluate the modification in the ASCA titers during the course of the disease. All serum samples were stored at –20°C until assayed and was coded for a blind analysis. Furthermore, serum was collected from a control group. These children did not shown evidence or familial history of IBD, immune-mediated disorders, or immunodeficiencies. Each patient's clinical information was collected by investigators unaware of the results of the antibody profiles. IBD children were classified as CD or UC according to their diagnosis established by clinical, endoscopic, histologic, and radiographic standard criteria.[10] A third group consisted of patients whose endoscopic and histologic diagnosis was that of IC, based on the Chong criteria,[17] and confirmed after at least 1-year follow-up. Disease activity for the groups with CD was calculated by the Pediatric Crohn's Disease Activity Index (PCDAI)[18] and those with UC by a previously established clinical score.[19]

The study protocol and consent form were approved by the Ethics Committee at the Faculty of Medicine of the University 'Federico II' of Naples.

Anti–*Saccharomyces cerevisiae* Antibodies Determination

Immunoglobulin A and IgG ASCA were determined by ELISA according to the instruction manuals (Medizym ASCA, ALIFAX, MEDIPAN Diagnostic, Selchow, Germany), as previously described.[13,20] The cutoff for ASCA IgA and IgG positivity was set at 20 U/mL as determined by the company on the basis of the results in well-defined patients with CD.

Statistical Analysis

Statistical analysis was performed using the SPSS software version 10.0.7 for Windows. In addition to the sensitivity and specificity test, the statistical analysis was based on the study of the differences between groups concerning IgA and IgG ASCA levels in IBD children and in controls. The χ^2 test was used to compare qualitative variables between groups. Analysis of variance (ANOVA) was used to compared IgA and IgG ASCA titers among groups. Adjustment for multiple comparisons was made using the Bonferroni correction or the Dunnet's T3 test. The Student's *t* test was used to evaluate the differences in ASCA titers in patients who had undergone intestinal resection and during the follow-up of CD children. A p value <0.05 was considered significant. Linear regression analysis was used to evaluate correlations.

RESULTS

Patient Population

Sera were collected from 329 subjects divided in 4 groups: controls, CD, UC, and IC children. Informed consent was obtained from each subject. The control group consisted of a total of 142 children, all with no family history of IBD and no immune-mediated disorders or immunodeficiencies: 98 with extraintestinal disorders (mainly patients with respiratory tract infection), and 44 with non-IBD intestinal illness. This subgroup consisted of children with acute viral gastroenteritis (n = 16), irritable bowel syndrome (n = 8), gastroesophageal reflux (n = 6), *Helicobacter pylori* gastritis (n = 5), allergic colitis (n = 3), pseudomembranous colitis, polyposis, and acute self-limiting colitis (n = 2 each). The demographic characteristics of the study groups were similar, and are reported in Table 1. Further clinical characterization of IBD patient groups is shown in Table 2.

Anti–*Saccharomyces cerevisiae* Antibodies Determination

The IgA and IgG ASCA titers in IBD and non-IBD children are reported in Figure 1. Comparison of ELISA results

TABLE 1. Demographic Data in Controls and in CD, UC, and IC Children

	Controls	CD	UC	IC
n	142	102	47	38
Sex				
Male	82	60	24	20
Female	60	42	23	18
Age (years)				
Mean	8.1	12.9	10.8	9.9
SD	3.3	4.1	3.8	3.5
Range	1–17	1–19	2–18	1–19

CD, Crohn's disease; UC, ulcerative colitis; IC, indeterminate colitis.

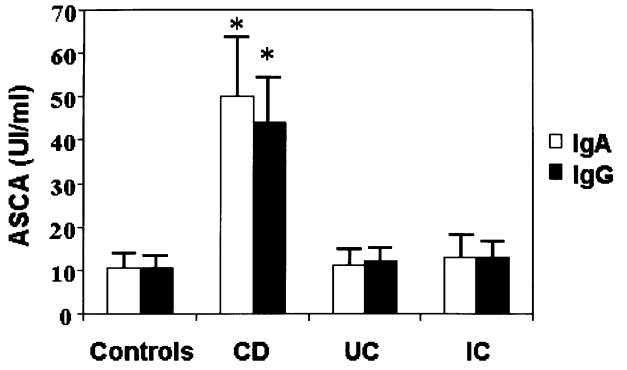

FIGURE 1. IgA and IgG ASCA values in the different study groups. Dare are means ± 95% CI. *$p < .001$, CD versus UC, IC, or controls.

showed that the mean ASCA levels were significantly higher in CD than in UC, IC, and control groups. The combination of IgA and IgG ASCA positivity was highly specific for CD, with 60 out of 102 (58.8%) patients with CD being IgA or IgG positive, compared with only 5 out of 47 (10.6%) with UC, and 3 out of 38 (7.8%) with IC (Table 3). Positive titers of ASCA IgA or IgG were found in 5 out of 142 control children (3.5%). The combination of IgA and IgG ASCA positivity was observed in none of the UC patients and controls, and in only 1 out of 38 IC patients.

Influence of Medical and Surgical Therapy

In CD children, the use of different medical therapeutic options: anti-inflammatory (mesalamine or sulfasalazine) (n = 30, 14 with active disease), immunosuppressive drugs (methylprednisolone or azathioprine) (n = 34, 19 with active disease), and nutritional therapy (n = 4, all with active disease) did not have an impact on ASCA assay results compared with those obtained in untreated patients (n = 34, 23 with active disease and 8 with an unknown PCDAI at the time of serum sampling).

Among CD children, 13 out of 102 (12.7%) had undergone an intestinal resection. Ten of these, all in clinical remission (PCDAI score ≤ 10), showed negative ASCA titers (mean interval between surgery and testing = 2.5 years). On the other hand, 3 patients, all with moderate active disease (PCDAI score between 11 and 30), were positive for ASCA IgG or IgA (mean interval between surgery and testing = 35 days). However, the mean ASCA titers were significantly lower in CD patients with intestinal resection compared with CD children who did not undergo surgical resection (29.6 ± 9.5 vs 44.2 ± 5.3 and 23.6 ± 7.0 vs 52.6 ± 6.7, IgA and IgG ASCA, respectively, means ± 95% CI, $p < 0.05$).

Influence of Disease Location

No significant relationship was found between ASCA titers and the site of CD inflammation (small versus large

TABLE 2. Clinical Characteristics of IBD Children

	CD	UC	IC
n	102	47	38
Mean disease duration (months)	26.8	21.8	22.5
Location			
Small bowel	36		
Colon	12	47	38
Small bowel and colon	54		
Activity			
Active/inactive	53/41*	26/21	25/13

CD, Crohn's disease; UC, ulcerative colitis; IC, indeterminate colitis.
*In 8 CD patients, the PCDAI score was unknown at the time of serum sampling.

TABLE 3. Diagnostic Accuracy of ASCA To Distinguish Between CD, UC, IC, and non-IBD Children

CD vs Other IBD + Non-IBD	Sensitivity (%)	Specificity (%)	P.P.V. (%)	N.P.V. (%)
IgA+	48/102 (47.1)	220/227 (96.9)	48/55 (87.3)	220/274 (80.3)
IgG+	58/102 (56.9)	215/227 (94.7)	58/70 (82.9)	215/259 (83.0)
IgA+ or IgG+	60/102 (58.8)	210/227 (92.5)	60/77 (77.9)	210/252 (83.3)
IgA+ and IgG+	46/102 (45.1)	225/227 (99.1)	46/48 (95.8)	225/281 (80.1)

CD, Crohn's disease; UC, ulcerative colitis; IC, indeterminate colitis; P.P.V., positive predictive value; N.P.V., negative predictive value.

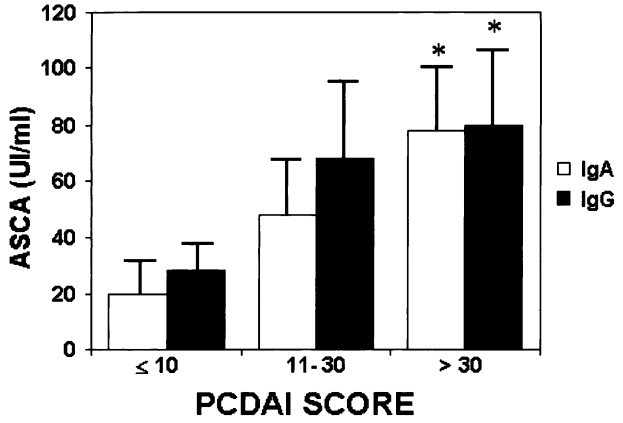

FIGURE 2. Effect of disease activity (determined by the PCDAI score) on IgA and IgG ASCA titers in CD children. Dare are means ± 9 5% CI. *$p < 0.001$, patients with a PCDAI score > 30 versus children with a PCDAI score ≤ 10.

bowel involvement, or both). However, patients with active CD limited to the colon tended to have lower IgA and IgG ASCA titers compared with others with CD involving only the small intestine ($p = 0.86$).

Influence of Disease Activity

To evaluate the effect of disease activity on ASCA titers in CD patients, we divided the children in subgroups according to the PCDAI score determined at the time of serum sampling. Thus, we obtained the following three subgroups: (1) remission (PCDAI score ≤ 10, $n = 41$); (2) moderate activity (PCDAI score between 11 and 30, $n = 28$); (3) severe activity (PCDAI score > 30, $n = 25$). Eight CD patients were excluded from this evaluation because the PCDAI score was unknown at the time of serum sampling. As reported in Figure 2, an increasing IgA and IgG ASCA titer pattern was obtained in agreement with disease activity. Children with severe active CD (PCDAI score > 30) showed significantly higher values compared with patients in clinical remission (PCDAI score ≤ 10). On the contrary, no significant difference in ASCA titers was observed between patients in clinical remission (PCDAI ≤ 10) and patients with moderate disease activity (PCDAI 11–30). In addition, as shown in Figure 3, ASCA titers showed a significant correlation with the PCDAI score. Moreover, as reported in Table 4, the diagnostic accuracy of ASCA for CD was highly improved when we considered patients with active disease.

Determination of Anti–*Saccharomyces cerevisiae* Antibodies During the Follow-up of CD Children

To investigate further the influence of disease activity on the ASCA titers, we performed a double determination in 20 CD patients. The first was done during the active phase of CD and the second, after 10 weeks of exclusive nutritional therapy

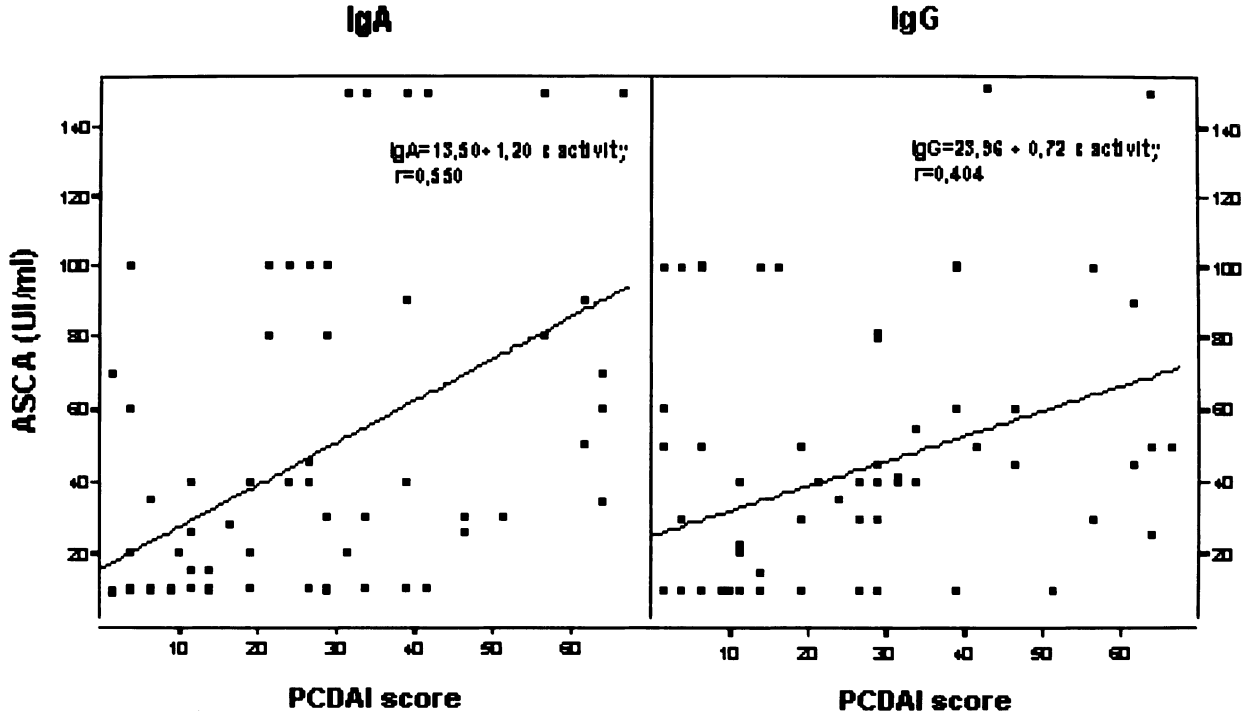

FIGURE 3. Linear regression function showing a significant correlation between disease activity (determined by the PCDAI score) and both IgA and IgG ASCA in CD children ($p < 0.05$).

TABLE 4. Modifications of the Diagnostic Accuracy of ASCA To Distinguish Between CD Versus non-IBD Children According to PCDAI

	Sensitivity (%)	Specificity (%)	P.P.V. (%)	N.P.V. (%)
PCDAI ≤10 (n = 41)				
IgA+	6/41	140/142	6/8	140/175
	(14.6)	(98.6)	(75.0)	(80.0)
IgG+	12/41	139/142	12/15	139/168
	(29.3)	(97.9)	(80.0)	(82.7)
IgA+ or IgG+	12/41	137/142	12/17	137/166
	(29.3)	(96.5)	(70.6)	(82.5)
IgA+ and IgG+	6/41	142/142	6/6	142/177
	(14.6)	(100.0)	(100.0)	(80.2)
PCDAI 11–30 (n = 28)				
IgA+	16/28	140/142	16/18	140/152
	(57.1)	(98.6)	(88.9)	(92.1)
IgG+	18/28	139/142	18/21	139/149
	(64.3)	(97.9)	(85.7)	(93.3)
IgA+ or IgG+	19/28	137/142	19/24	137/146
	(67.9)	(96.5)	(79.2)	(93.8)
IgA+ and IgG+	15/28	142/142	15/15	142/155
	(53.6)	(100.0)	(100.0)	(91.6)
PCDAI >30 (n = 25)				
IgA+	21/25	140/142	21/23	140/144
	(84.0)	(98.6)	(91.3)	(97.2)
IgG+	23/25	139/142	23/26	139/141
	(92.0)	(97.9)	(88.5)	(98.6)
IgA+ or IgG+	24/25	137/142	24/29	137/148
	(96.0)	(96.5)	(82.8)	(99.3)
IgA+ and IgG+	20/25	142/142	20/20	142/147
	(80.0)	(100.0)	(100.0)	(96.6)

CD, Crohn's disease; PCDAI, Pediatric Crohn's Disease Activity Index; P.P.V., positive predictive value; N.P.V., negative predictive value.

with a polymeric formula (Modulen, Nestlè, Italy), when the clinical remission was achieved (PCDAI score ≤ 10). The results are reported in Figure 4. A significant reduction of both IgA and IgG ASCA titers was observed for all patients.

DISCUSSION

The onset of pediatric IBD is frequently insidious and presents nonspecific or overlapping symptoms, which often results in missed or delayed diagnosis as well as in the overuse of invasive diagnostic tests.[1] The central immunologic contribution to the pathogenesis of IBD stimulated the search for antibodies and associated antigens in the hope that this would reveal a specific pathogenic mechanism for IBD. However, at the present time, IBD-associated antibodies have not been found to mediate pathogenic events.[21] Although such antibodies are not of direct pathogenic importance, their level of specificity for either UC or CD suggests that they cannot be considered as entirely nonspecific secondary responses to mucosal injury or inflammation.[21] Instead, they suggest an immune dysfunction or reflect a cross-reactivity with environmental factors, including intestinal microflora.[22] Another potential role for IBD associated antibodies is their use in substratifying unclear disease phenotypes like IC as either UC or CD or in classifying disease subtypes with different genetic associations.[1] The ASCA and perinuclear antineutrophil cytoplasmic antibodies (pANCA) have been the two most intensely studied antibodies in the substratification of IBD patients.[13]

We used a well-established ELISA ASCA assay that had recently been evaluated in a large comparative study.[13] Our results confirm that ASCA are helpful in the diagnosis of pediatric CD showing a high specificity and positive predictive values in discriminating CD versus other IBD forms. Furthermore, in agreement with other reports,[15,22] our results suggest that both medical therapy and disease location did not influence ASCA titers. However, children with CD involving the small intestine tended to have higher ASCA titers compared with other CD patients, but larger populations of children are needed to draw conclusions on this issue.

In contrast to constant pANCA values after colectomy in UC patients, it has been suggested that ASCA titers may be reduced after intestinal resection in CD patients.[16] Our results support these findings, but it could be important to note that all but 3 CD children who underwent intestinal resection were in clinical remission when ASCA values were determined and this suggests the possible influence of disease activity on

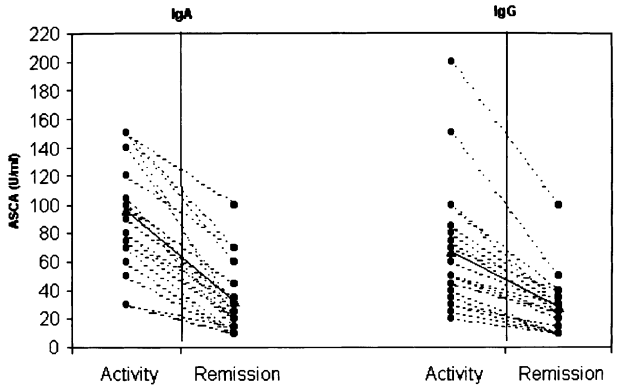

FIGURE 4. Modifications of ASCA IgA and IgG titers during the follow up of CD children. A double determination of ASCA titers was performed in 20 CD patients: the first was done during the active phase of CD (PCDAI score > 30) and the second, after 10 weeks of nutritional therapy, with a polymeric formula, when a clinical remission was achieved (PCDAI score ≤ 10). A significant reduction of both IgA and IgG ASCA titers was observed ($p < 0.05$).

ASCA titers. Moreover, in our study at least two lines of evidence suggest a further relationship between disease activity and ASCA values. The first is represented by the significantly higher titers obtained in severe active CD compared with those measured in patients in clinical remission. The second is the decreasing pattern of ASCA titer observed in all CD children tested serially when the disease was active and then when clinical remission was achieved. Finally, linear regression analysis showed a significant correlation between ASCA titers and PCDAI score. These findings are seemingly in contrast with a previous observation showing higher but not statistically significant IgG ASCA titer in children with active CD compared with those observed in subjects in remission.[16] However, it is possible that the smaller population of active CD patients previously analyzed (21/119, 18.0%), compared with the larger number of the present observation (53/94, 56.3%) could account for this discrepancy. The relationship between disease activity and ASCA titers, observed in this study, suggests that this assay could be used to evaluate disease activity in CD children. The course of CD is one of relapses and remissions. The evaluation of disease activity using a specific marker may help in the differential diagnosis of nonspecific symptoms. Measuring ASCA, together with standard parameters and emerging activity markers such as calprotectin in stools[23] and nitric oxide in rectal dialysate fluid,[24] could be useful in monitoring the disease course and response to treatment in CD children. In addition, it has been recently suggested that high levels of both IgA and IgG ASCA titers could be associated with a greater necessity for small bowel surgery, because of stricturing or perforating complications, in CD patients.[7] Similar observations were reported in patients with mutations in the CARD15/NOD2 gene who are more likely to develop fibrostenosing disease of the small bowel.[25] These considerations could have important implications for disease subtype stratification.

Finally, the influence of disease activity on ASCA titers could contribute to an improvement of the diagnostic accuracy of this assay. Considering all CD patients together, we obtained a low sensitivity, similar to that obtained in other studies.[11,13,16] On the contrary, when we compared only active CD patients with controls or others IBD forms, we obtained a significant improvement in sensitivity (up to 96%), without a significant decrease in specificity. Thus, the sensitivity of ASCA is higher in children with active CD, which represents the major diagnostic dilemma for pediatricians.

In conclusion, together with a high diagnostic specificity and sensitivity for active forms of CD in pediatric patients, ASCA antibodies could provide another noninvasive tool for guiding medical management of CD children.

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