

ANTI-SACCHAROMYCES CEREVISIAE (ASCA) IgG AND IgA ANTIBODIES IN INFLAMMATORY BOWEL DISEASE

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ABSTRACT

Objective: Anti-*Saccharomyces cerevisiae* antibodies are significantly more prevalent in patients with Crohn's disease than in patients with ulcerative colitis (UC) or normal healthy controls. The objective of this study was to evaluate the performance of two newly developed ELISA assays for detection of ASCA IgG and IgA antibodies in specimens from Crohn's disease, UC, and other non-inflammatory bowel disease patients.

Methods: Three laboratories evaluated the performance of the QUANTA Lite™ ASCA IgG and IgA ELISAs on panels of clinical specimens assembled in their laboratories. A combined total of 137 Crohn's disease, 72 UC, 393 normal control, and 75 other potentially cross-reactive specimens were tested.

Results: The results obtained on the combined panel of Crohn's specimens showed that the ASCA IgG and ASCA IgA ELISAs interpreted 72.3% (94/130) and 45.8% (60/131) of the specimens as positive respectively. The sensitivity increased to 76.7% (99/129) when the results of the IgG and IgA ASCA ELISAs were combined. For UC specimens, 16.3% and 4.3% of the specimens were interpreted as ASCA IgG and IgA positive respectively. Of 393 healthy control specimens, 4.4% (17/383) and were interpreted as positive by the ASCA IgG and 2.1% (8/389) by the ASCA IgA ELISA. None of the controls were positive for both ASCA IgG and IgA. While none of the 63 Viennese UC specimens were positive for both ASCA IgG and IgA, two of the 9 Iowa UC specimens were both IgG and IgA ASCA positive. One of these specimens was from a patient with acute colitis. A total of 11 specimens were either ASCA IgG or IgA positive in the cross-reactivity panel. Reactive specimens included specimens which were positive for gliadin IgG (2/8), autoimmune hepatitis type 1 (4/12), alcoholic hepatitis/alcoholic cirrhosis (3/3), chronic hepatitis C (1/6), and granulomatous hepatitis/bladder cancer (1/1). When both ASCA IgG and IgA were positive, the specificity was 99.6% (463/465) for the combined panel of 465 ulcerative colitis and controls and was 99.1% (535/540) for all non-Crohn's disease specimens (UC, controls, and non-Crohn's disease sera).

Conclusion: This study confirms the high prevalence of ASCA antibodies in specimens from Crohn's disease patients and the very high specificity for the combination of IgG and IgA ASCA results. The availability of these assays will allow more widespread testing for ASCA and further definition of the clinical utility of these assays.

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD), a general term used to describe diseases that cause chronic inflammation of the intestine. In CD inflammation commonly occurs in the lower part of the distal ileum, although it may affect any part of the digestive tract. The inflammation in CD extends deeply into the affected tissue, in contrast to ulcerative colitis, where inflammation is located in the top layers of the lining of the colon and rectum. Inflammation in CD is asymmetrical and segmental, with areas of both healthy and diseased tissue, in contrast to ulcerative colitis where inflammation is symmetrical and uninterrupted from the rectum proximally. Although there are many theories concerning the etiology of CD and UC, none have been proven. Since many symptoms of CD and UC are similar, diagnosis is often difficult and relies on detailed clinical evaluation, the results of endoscopic, histologic, and radiographic examination, and the exclusion of other disorders. Diagnosis of IBD pediatric patients can often be particularly difficult because of nonspecific symptoms early in the disease. While an accurate differential diagnosis is possible with most patients, 10-15% of patients have an indeterminate form with features common to both CD and UC. With time, many of these patients are eventually classified as either CD or UC. The development of accurate, standardized, reproducible, and non-invasive serological tests could provide clinicians a valuable tool to assist in the assessment and diagnosis of IBD patients. Furthermore, accurate serological tests may assist clinicians make earlier diagnoses in pediatric patients.

Approximately 10 years ago, it was found that anti-*Saccharomyces cerevisiae* antibodies (ASCA) were significantly more prevalent in patients with CD compared to patients with ulcerative colitis (UC) or healthy controls. These antibodies, which can include antibodies of both the IgG and IgA classes, appear to be directed against mannose sequences in the cell wall of *Saccharomyces cerevisiae*. The presence of IgG or IgA ASCA have been shown to have a high specificity for CD.

In the present study, we report the results of testing a large, blinded panel of highly-characterized clinical specimens from patients with CD, UC, and healthy controls with recently developed commercial ASCA IgG and ASCA IgA ELISA kits.

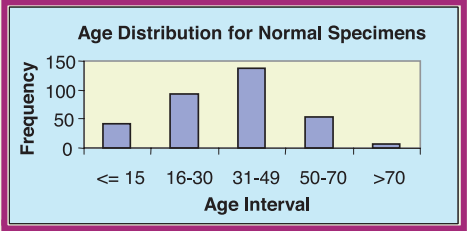
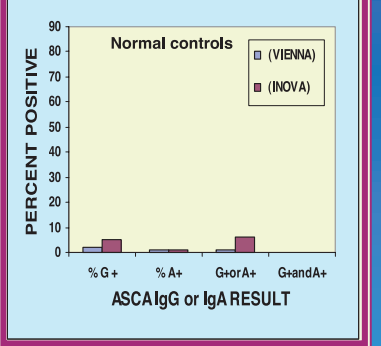
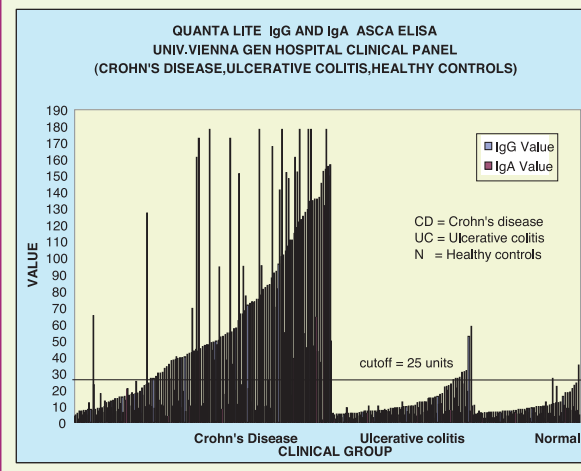
Methodology

QUANTA Lite™ ASCA ELISA

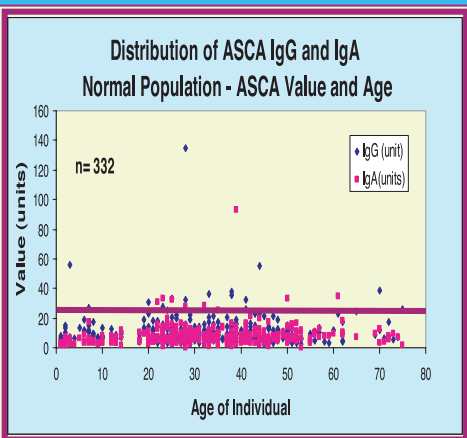
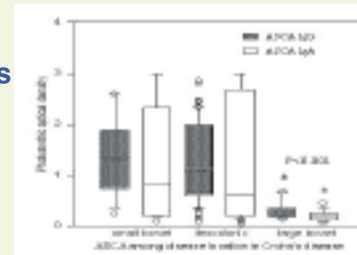
The QUANTA Lite™ ASCA IgG and IgA ELISA assays use partially purified and disrupted *Saccharomyces cerevisiae* antigen bound to the wells of color-coded 96 well polystyrene microwell plates. Patient specimens are run at a 1:101 dilution. The ELISA assays use pre-diluted controls, single point antigen specific calibration, 30 minute room temperature incubations, ready-to-use conjugate, and single vial TMB substrate solution. Results are expressed in arbitrary units.

Normal Range

A combined panel of 393 specimens collected from healthy individuals residing in California, New York, and Vienna was tested with the QUANTA Lite™ ASCA ELISA kit to establish a normal range for the assay.



ASCA Values and Disease Location



ASCA Cross-reactivity Study

CLINICAL GROUP	N=	ASCA IgG Pos	ASCA IgA Pos	IgG+ or IgA+	IgG+ and IgA+
Gliadin IgA pos.	12	0	0	0	0
Gliadin IgG pos.	8	2	0	2	0
Transglutaminase IgA	7	0	0	0	0
Antinuclear antibody (ANA) pos	8	0	0	0	0
Autoimmune hepatitis (AIH), type1	15	1	3	4	0
H. pylori positive	10	0	0	0	0
alcohol cirrhosis ascites	1	0	1	1	0
alcoholic hepatitis	2	2	2	2	2
chronic Hep C	6	1	0	1	0
chronic liver dis	2	0	0	0	0
Granulomatous hepat s/p BCG im.Rx for blad.ca.	1	1	1	1	1
Hepatitis NOS	1	0	0	0	0
non infectious Gastroenteritis	1	0	0	0	0
Esoph varices w/ bleed	1	0	0	0	0
total	75	7	7	11	3

(not exclude equivocal results from calc.)

SUMMARY OF RESULTS FOR CLINICAL SPECIMENS

	Crohn's Disease				Ulcerative Colitis				Normal Controls						
	N=	IgG pos	IgA pos	G+ or A+	IgG pos	IgA pos	G+ or A+	G+and A+	N=	IgG pos	IgA pos	G+ or A+	G+and A+		
Vienna	115	73.9%	42.7%	75.5%	40.0%	63	15.3%	0.0%	15.3%	0.0%	48	2.2%	2.1%	4.5%	0.0%
Iowa	22	63.2%	61.9%	84.2%	40.9%	9	28.6%	37.5%	42.9%	22.2%					
INOVA											345	4.7%	2.0%	6.9%	0.0%
% Pos(-eq)	137	72%	45.8%	76.7%		72	16.7%	4.3%	18.2%	3.0%	393	4.4%	2.1%	6.4%	0.0%

Conclusions

- This study confirms the high prevalence of ASCA in patients with Crohn's disease compared to those with ulcerative colitis and healthy controls.
- None of 393 normal control specimens were positive for both IgG and IgA ASCA.
- Approximately 76% of the Crohn's disease patients were positive for ASCA IgG or IgA. There is clearly a group of clinically-defined Crohn's disease patients who do not appear to have IgG or IgA ASCA in levels different than non-Crohn's disease patients.
- Two of 8 gliadin IgG positive specimens were found to be ASCA IgG positive. The significance of this observation will be tested on a larger population clinically characterized specimens.
- The finding that 2 of 2 alcoholic hepatitis patients were ASCA IgG and IgA positive is intriguing. This finding, along with the gliadin observations, may point to common features in altered intestinal function and the development ASCA.