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Original Article

Clinical significance of anti-*sacchromyces cerevisiae* antibody in Crohn's disease: a single-center study

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Abstract: Objectives: To evaluate the clinical significance of serum anti-Saccharomyces cerevisiae antibodies (ASCA) IgG and IgA for diagnosis of Crohn's disease (CD), and the stability over the course of the disease of its clinical behavior. Methods: Total 51 patients with CD were recruited to as CD group. Control group included 22 healthy subjects. ASCA-IgG and -IgA in serum samples were detected using ELISA. Sensitivity, specificity and positive predictive value (PPV) of ASCA-IgG and IgA were calculated. These patients were followed up for 5 years. Results: ASCA-IgG and ASCA-IgA were significantly different between the CD group and the control group. Among the 51 CD patients, the sensitivity of ASCA IgG was 45.1%, and specificity and PPV were both 100%. The sensitivity of ASCA IgA was 35.3%, specificity was 91% and PPV was 90%. The sensitivity of ASCA-IgG was significantly increased in the patients with complications than the patients without complications (56.3% vs. 26.3%). During the follow-up, those ASCA-IgG-positive CD patients might be more likely to need surgical treatments compared to the ASCA-IgG-negative patients. Conclusions: ASCA IgG could be a useful predictive tool in diagnosis of CD, and the clinical value of ASCA-IgG detection is higher than that of ASCA-IgA.

Keywords: ASCA, Crohn's disease, diagnosis, serological biomarkers, surgery

Introduction

Crohn's disease (CD) is a principal type of inflammatory bowel disease (IBD). It is a common disease in Western countries, and has exhibited a gradually increasing incidence in China in recent years [1, 2]. Based on a number of hospital case reports, the CD-related morbidity is approximately 1.4/105, which may even be an underestimate [3]. More recently, the disease has become one of the main reasons for common digestive system diseases and chronic diarrhea [4, 5]. Patients with CD are most commonly young adults. The disease brings a great impact to the social well-being and the personal life quality of working-age adults affected, and consequently draws great attention from society in general.

Currently, anti-saccharomyces cerevisiae antibody (ASCA) is one of the most commonly used serologic antibody markers for diagnosis of IBD, including immunoglobulin (Ig) IgG and IgA, which appear to be specifically directed against mannose sequences of mannan present in the cell wall of saccharomyces cerevisiae. Positive-staining of both ASCA-IgA and ASCA-IgG are increased in 50% to 80% of CD patients, in less than 10% of ulcerative colitis (UC) patients and in less than 5% of apparently normal healthy individuals where the specificity of diagnosis of CD is 90% [6, 7]. However, there are only a few published reports describing the positive rate of serological ASCA in CD [8, 9]. Gologan S *et al.* have found that higher titers of ASCA IgA and IgG are related to a younger age at diagnosis and more aggressive phenotypes in Romanian CD patients [10]. A combination of tumor necrosis factor superfamily member 15 and ASCA-IgA is of value for prediction of stenosis/perforating phenotype in CD patients with TNFSF15 [11]. Differences in the sensitivity and specificity of ASCA-IgG and ASCA-IgA with CD have rarely been studied in China.

Therefore, one purpose of this study was to explore the sensitivity, specificity, and positive or negative predictive values (PPV, NPV) of

Table 1. Overview of the characteristics of the CD patients

	CD (n = 51)	Control (n = 22)
Gender		
Male	30	14
Female	21	8
Age (year)		
Mean	32.8	35.6
Range	10-60	24-60
A1 (≤ 16)	4	NA
A2 (17 ≤ A2 ≤ 40)	33	
A3 (> 40)	14	
Disease location		NA
L1 (ileum alone)	13	
L2 (colon alone)	16	
L3 (ileocolon)	22	
Disease behavior		NA
B1 (nonstricturing, nonpenetrating)	31	
B2 (stricturing)	16	
B3 (penetrating)	4	
p*	19	
With complication*	32	
Disease reactivity		NA
Active stage	40	
Remissive stage	11	

NA = not applicable; p* = patients with perianal disease during B1, B2, and B3; with complication * = patients with complication, including B2, B3, and perianal disease.

Table 2. Comparison of ASCA-IgG and-IgA between CD group and control group

	ASCA-IgG	ASCA-IgA
CD group (n = 51)	23	18
Control group (n = 22)	0	2
χ^2 value	14.49	5.31
P-value	< 0.01	< 0.05

ASCA-IgG and ASCA-IgA for the detection of CD at a single center in eastern China. The current study also aimed to gain greater appreciation of the clinical relevance of both ASCA-IgG and ASCA-IgA and to assess the evolution over time of behavior of CD. Results of this study suggest that positive-staining of serum ASCA is associated not only with the location of the disease but also the clinical behavior of CD. However, there was no clear distinction between ASCA-IgG and ASCA-IgA for either the disease location or clinical behavior.

Patients and methods

Patients

The study protocol was approved by the ethics committee of Sir Run Run Shaw Hospital with obtainment of written informed consent from each participating patient. The study recruited a total of 51 hospitalized patients with CD in the Sir Run Run Shaw hospital from July 2008 to October 2009. The diagnosis of CD was made by considering the clinical characteristics, colonoscopy results, imaging studies, surgical findings and pathology results in accordance with the diagnostic criteria of the Society of Gastroenterology of the Chinese Medical Association [3]. The exclusion criteria were: patients with IBD requiring the use of corticosteroids and immunosuppressive agents within one month prior to the study; those patients presenting with SLE or rheumatoid arthritis; patients with stool culture showing fungus or mycelium; patients who were actively using NSAIDs, PPI, or were dependent on alcohol consumption [12]. The 51 CD patients were regularly followed up in our gastroenterology department for 5 years. We then reviewed the medical notes of these patients. The study also collected a total of 22 age and sex-matched normal healthy control subjects following physical examination at the Sir Run Run Shaw hospital from January 2008 to December 2009. Colonoscopy found no abnormality in any control subject. The exclusion criteria for the control group were similar to that described for the CD patient group. Moreover, the patients with irritable bowel syndrome were also excluded from the normal control group.

Detection of ASCA IgG and IgA immunoglobulin isotypes

Venous blood sample extracted from each participant was centrifuged within 3 h of collection and then stored at -20°C until assayed. A standard enzyme-linked immunosorbent assay (ELISA) technique was employed to detect the ASCA-specific IgG and IgA antibodies in accordance with the manufacturer's instructions (Inova Diagnostic Inc. CA, USA). ASCA was determined using QUANTA lite™ASCA (S. cerevisiae) IgG 708865, and QUANTA lite™ASCA (S.

Table 3. The positive detection rates of various ASCA types in CD group

ASCA types	Sensitivity (%)	Specificity (%)	PPV (%)
ASCA IgG	45.1	100	
ASCA IgA	35.3	91	90
ASCA-IgG/IgA*	60.8	91	93.9
ASCA-IgG + IgA*	21.6	100	

Positive predictive value, PPV; ASCA-IgG/IgA* = ASCA IgG or ASCA IgA; ASCA-IgG + IgA* = both ASCA IgG and ASCA IgA.

cerevisiae) IgA 708870 semi-quantitative ELISA kits (INOVA, USA).

Statistical analysis

The sensitivity, specificity and positive predictive value (PPV) of the CD group and control group were analyzed using diagnostic analysis. The differences of data except the age range between the two groups were compared by a Chi-square analysis. The age range was analyzed by using non-parametric test. *P*-value < 0.05 was considered significant. All of the statistical analyses was performed using the SPSS 16.0 software program (SPSS, Inc., Chicago, Ill., USA).

Results

Clinical characteristics of CD patients and healthy control subjects

Clinical characteristics of the 51 CD patients and the 22 control subjects were shown in **Table 1**, including gender distribution, age, disease location, disease behavior, disease reactivity according to the Montreal classification criteria [13], which was recommended in 2005 by the World Gastroenterology Conference. Age and gender distribution did not significantly differ between the CD group and the control group ($P > 0.05$). The clinical activity of CD was evaluated by the Best CDAI scoring system [14]. Among the 51 patients presenting with CD, 2 cases received surgical therapy during the experiment because of aggravated disease.

Qualitative determination of ASCA-IgG and -IgA of the CD group as compared to the normal control group

It was observed that there was a statistically significant difference in the ASCA-IgG and -IgA between the CD group and the control group ($P < 0.05$, **Table 2**). Excluding the possible interference effect of the subtype classification, we could get positive detection rates of various ASCA types in the 51 patients of the CD group (**Table 3**).

Comparison between the CD subtype groups

By comparing the qualitative levels of ASCA-IgG and ASCA-IgA of each CD subtype group, we found that there was not significant difference in neither ASCA IgG positive rate nor the ASCA IgA positive rate between these CD subtype groups ($P > 0.05$, **Table 4**). There was also no significant difference between the active stage group and the remission stage group ($P > 0.05$, **Table 4**).

Relationship between ASCA and complications in the CD group

Complications were identified in 32 of the 51 CD patients (including those with B2, B3, p) and 18 cases with positive ASCA-IgG. The sensitivity of ASCA-IgG was found to be 56.3%, and both the specificity and PPV were found to be 100%. In addition, there was a significant difference for ASCA-IgG, ASCA IgG or ASCA IgA between the patients with or without complications ($P < 0.05$, **Table 5**). However, ASCA IgA did not demonstrate any remarkable differences in any group ($P > 0.05$, **Table 5**).

Relationship between the ASCA-IgG and surgery in the CD group after 5 years

Among the 51 patients, there were 23 patients who were positive of ASCA-IgG and 18 patients who were positive of ASCA-IgA. After following-up for 5 years, we found that there were a total of 14 patients who suffered from surgery, meanwhile there were 11 (11/23) ASCA-IgG-positive patients, and 6 (6/18) ASCA-IgA-positive patients, respectively (**Table 6**). Over the 5 years, it was found that those patients who were positive of ASCA-IgG in the CD group, appeared to be more likely to need surgical treatments as compared to the patients who were not positive of ASCA-IgG ($\chi^2 = 8.73$, $P < 0.01$, **Table 6**). However, the significant difference was not found for the ASCA-IgA-positive patients compared to the patients who were not positive of ASCA-IgA (**Table 6**).

Table 4. Positive detection rates of various ASCA subtypes in CD group

ASCA types		Sensitivity (%)		Specificity (%)		PPV (%)	
		ASCA-IgG	ASCA-IgA	ASCA-IgG	ASCA-IgA	ASCA-IgG	ASCA-IgA
A	A1	75.0	25.0	100	91.0	100	33.3
	A2	36.4	30.3	100	91.0	100	83.3
	A3	57.1	50.0	100	91.0	100	77.8
L	L1	38.5	30.8	100	91.0	100	66.7
	L2	37.5	31.3	100	91.0	100	71.4
	L3	57.1	42.9	100	91.0	100	81.8
B	B1	45.2	29.0	100	91.0	100	81.8
	B2	50.0	50.0	100	91.0	100	80.0
	B3	25.0	25.0	100	91.0	100	33.3
	p	47.4	100	91.0	91.0	100	80.0
Active stage		47.5	40.0	100	91.0	100	88.9
Remission stage		36.4	18.2	100	91.0	100	50.0

A, age range; L, disease location; B, disease behavior.

Table 5. The relationship between ASCA level and complications in the CD group

	CD patients with complications (n = 32)	CD patients without complications (n = 19)	χ^2 value	*P*-value
ASCA-IgG	18	5	4.31	< 0.05
ASCA-IgA	11	7	0.03	> 0.05
ASCA-IgG/IgA*	23	8	4.43	< 0.05
ASCA-IgG + IgA*	7	3	0.03	> 0.05

ASCA-IgG/IgA* = ASCA IgG or ASCA IgA; ASCA-IgG + IgA* = both ASCA IgG and ASCA IgA.

Table 6. The relationship between the ASCA-IgG and surgery in the CD group after 5 years

	Patients needing surgery (n)
ASCA-IgG (+) CD group (n = 23)	11
ASCA-IgG (-) CD group (n = 28)	3
χ^2 value	8.73
P-value	< 0.01
ASCA-IgA (+) CD group (n = 18)	6
ASCA-IgA (-) CD group (n = 33)	8
χ^2 value	0.13
P-value	> 0.05

Discussion

In the last 2 decades, incidence and prevalence of CD have been elevated rapidly in Asia [15]. It is a common disease in young adults. ASCA may play a significant role in the diagnosis, activity, classification, treatment response and follow-up of patients with CD [16, 17]. In our study, the QUANTA Lite™ASCA (S. cerevisiae) IgG/IgA kit assisted us to make a qualitative judgment for each group by ELISA. It has been reported that ASCA is particularly useful for differentiation of CD from UC [18]. Similarly, the study also found that ASCA-IgG/IgA has a high specificity and PPV for diagnosis of CD in the general population, and this was especially true for ASCA IgG.

However, both ASCA-IgG and ASCA-IgA suffered from a low sensitivity. Moreover, this result is very similar to many recent European and American studies [19, 20]. Therefore, we suggest that ASCA-IgG is not suitable as an indicator for population screening. However, ASCA-IgG may possess certain qualities to aid in guiding the diagnosis of CD, which might be partly due to its high specificity and PPV.

A study by Peeters *et al.* [21] also suggests that the expression levels of ASCA may exhibit a degree of correlation with the CD location. Specifically, ASCA exhibits a tighter relationship with disease manifestation in anatomical locations of the stomach, duodenum and small intestine than with colonic disease. In addition, ASCA appears to be associated with more severe phenotype, and all patients with the severe phenotype of CD require surgery at an average of 9 years of follow-up. Many studies have shown that ASCA is associated with the clinical behavior of CD, and follow-up examinations of CD patients with high ASCA titers have found that they continued to develop a complex phenotype later, or more likely, require surgical treatments [10, 22-24]. Other studies have also found that it is possible to make predictions on the occurrence and development of IBD before diagnosis in previous years by measuring ASCA and pANCA [18, 25].

Hitherto, the results of our study suggest that there was not statistically significant difference between the active and remission stages of CD. But we found a significant difference in the ASCA-IgG between the CD patients with or without complications compared to those patients not presenting with a complication. This result also suggests that ASCA-IgG/IgA is significantly different between the CD patients with or without complications. However, it is important for us to consider the possibility that this may be due to the existence of ASCA-IgG. This observation prompts us to investigate the possibility of developing a severe clinical phenotype in ASCA-IgG-positive patients later. After 5 years following-up, we found those ASCA-IgG-positive CD patients might be more likely to need surgical treatments because of the progress of the disease. In consistence with these results, Forcione *et al.* have hinted that positive ASCA status might predict an increased risk of early surgery [26]. Moreover, a recent meta-analysis indicates that positive ASCA status is a risk factor for requirement for surgery in CD patients [22]. Assessment of ASCA-IgG may play a role in the prevention and prediction of CD, thus providing clinicians an opportunity to adjust and refine the treatment of the disease.

The study has some limitations. Firstly, the sample size of our study is small. Further studies with a larger number of patients are warranted to validate the findings of the study. Secondly, we do not recommend that monitoring of ASCA IgG is suitable as an indicator for population screening, and equally for assessing the activity of disease. But the value of detecting ASCA-IgG alone is much greater than detecting ASCA IgA alone or a combination of ASCA-IgG and ASCA-IgA. Consequently, it could be useful in the diagnosis of CD.

ASCA-IgG may be useful in diagnosis of CD, predicting whether the disease will later develop into a more complex phenotype and stratifying clinical subtypes of CD. It is therefore feasible to establish a stable serological detection system for ASCA IgG in the laboratory for assessing the development and prognosis of CD in China.

Disclosure of conflict of interest

None.

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