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Diagnostic Role of pANCA and ASCA in Adult Thai Patients with Inflammatory Bowel Diseases

***Petplook E*[1]**
***Viriyataveekul R*[2]**
***Pongprasopchai S*[1]**
***Maneerattanaporn M*[1]**
***Manatsathit S*[1]**
***Rerknimitr R*[3]**

ABSTRACT

Background: Perinuclear antineutrophil cytoplasmic antibodies (pANCA) are the well recognized marker for ulcerative colitis (UC). Antibodies to oligomannosidic epitopes of yeast Saccharomyces cerevisiae (ASCA) are the new seromarker associated with Crohn's disease (CD). While the prevalence of both UC and CD in Thai populations is low, it is interesting to know the role of these seromarkers in such population.

Objective: To assess the value of using pANCA and/or ASCA for the diagnosis of adult Thai IBD patients.

Methods: Serum sample were obtained from 19 patients of CD and 50 patients of UC. Determination of both seromarkers was performed by using the standardized indirect immunofluorescence technique in all sera.

Results: The combination of a positive pANCA test and a negative ASCA test yielded a sensitivity and specificity of 16.0%, 89.5% respectively for UC. The combination of a negative pANCA test and a positive ASCA test yielded a sensitivity and specificity of 42.1%, 62.0% respectively for CD. There was no correlation between the positively of both seromarkers and the clinical features of both diseases.

Conclusion: The prevalence of pANCA in Thai UC patients is much lower than that in the Western population. ASCA and pANCA are not useful diagnostic tools for differentiating UC from CD in adult Thai patients compare with Western population.

Key words : anti-*Saccharomyces cerevisae* antibodies (ASCA), perinuclear antineutrophil cytoplasmic antibodies (pANCA), inflammatory bowel diseases (IBD)

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[1] *Division of Gastroenterology, Department of Internal Medicine, Siriraj Hospital, Faculty of Medicine, Mahidol University, Bangkok, Thailand.*
[2] *Department of Clinical Pathology, Siriraj Hospital, Faculty of Medicine, Mahidol University, Bangkok, Thailand.*
[3] *Gastroenterology unit, Department of Internal Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.*
Address for Correspondence: *Supot Pongprasopchai, M.D. Division of Gastroenterology, Department of Internal Medicine, Siriraj Hospital, Faculty of Medicine, Mahidol University, Bangkok, Thailand.*

INTRODUCTION

Diagnosis of inflammatory bowel diseases (IBD) is based on the detail of history taking, physical examination, radiological, endoscopic, and histopathological studies to guide the appropriate disease management. Based on clinical criteria, IBD can be divided into Crohn's disease (CD) and ulcerative colitis (UC) and by using current diagnostic criteria some patients about 10% of colonic inflammation cannot be classified as having either CD or UC. These patients are categorized as "indeterminate colitis".[1] Thus, the search for a simple method that would allow diagnostic precision to distinguish between the two diseases has resulted in the identification of serological markers. A subset of antineutrophil antibodies, commonly referred to as perineuclear antineutrophil cytoplasmic antibodies (pANCA) has been reported in 30-80% of UC patients and 5-15% in CD patients[2-7]. Antibodies to baker's yeast (*Saccharomyces cerevisiae*, a ubiquitous yeast that is present in different places)[8] have been described in 40-60% of CD patients and 12-20% in UC patients.[9-13] The antigenic target found on the inner side of the nuclear periphery were expressed as pANCA.[14] The antigenic target of anti-*Saccharomyces cerevisae* antibodies (ASCA) have been identified as oligomannosidic epitopes of the yeast cell wall, but their exact pathophysiological role still remains unknown.[8] Inflammatory bowel diseases manifest throughout all ethnic groups, ASCA and pANCA can aid the differentiation between CD and UC, but their prevalence and sensitivity may vary between races depend on the population being studied and the method used for it detection.[3,15,16]

The aim of this study was to evaluate the diagnostic role of ASCA and pANCA in adult Thai IBD patients by using single or combined serological tests. The relationship between serological test results and clinical parameters of both diseases was also studies.

MATERIALS AND METHODS

Patients

This is a cross-sectional study. All patients in this study must be adult Thai patients only (age more than 15 years) and serum samples were obtained from the patients attended at Siriraj Hospital and King's Chulalongkorn Memorial Hospital during January 2006 to January 2007. The identification of patients was made on the basis of a clear diagnosis in the patients who underwent complete diagnostic work up for IBD including clinical manifestation, radiological, endoscopic, and histopathological studies, with compatible clinical courses, and the possibility of infectious colitis was excluded by long duration follow up for at least 6 months. All serum samples were stored at -20 ˚C until assayed and samples were coded for blind analysis. Clinical data regarding demographic information, details of symptoms, patterns of intestinal involvement and extra-intestinal manifestations were obtained by the reviewing of patient records. Each patient's clinical information was collected by investigator unaware of the results of the antibody profiles.

Anti-*Saccharomyces cerevisae* antibodies (ASCA) determination

The ASCA were analyzed by standard indirect immunofluorescence method for immunoglobulin classes IgA and IgG. All test system were performed by using EUROIMMUN® (Germany) and the assay was done according to the instruction manual from the company. Qualitative evaluation of the results of individual sera was determined only positive or negative, and equivocal results were considered as negative. The investigators assessed all slides in a blinded fashion.

Anti-neutrophil cytoplasmic antibodies (ANCA) determination

The pANCA determination was provided by using standard indirect immunofluorescence technique, according to manufacturer's instruction. All sera were first screened on ethanol-fixed slides, which prepared both human neutrophils and primate liver cells substrates, in according to evaluate the possibility of false positive from coexisting anti-nuclear antibodies (ANA) in that sera and subsequently performed formaldehyde-fixed slides to confirm the presentation of pANCA in case of suspecting of false positive test (presence of ANA, atypical ANCA or in case that results were indistinguishable between pANCA or cANCA). An ANCA kit, both ethanol and formaldehyde-fixed slides, used EUROIMMUN® (Germany) as in ASCA test. The results were interpreted qualitatively as positive or negative. The investigators assessed all slides in a blinded fashion.

Statistical analysis

Patients' characteristics were analyzed by descriptive statistics and reported as mean, range, and per-

cent. Sensitivity was defined as the probability of a positive test in a patient with the disease under investigation. Specificity was defined as the probability of having a negative test in a patient without the disease under investigation. Relation between test results and other clinical parameters was determined with Chi-square test or Fisher's exact test as appropriate. Significant level was assigned to any probability when p value less than 0.5.

RESULTS

The patient's demographic features and detail of illness are listed in Table 1-2. A total of 69 serum sample were obtained from the patients diagnosed with IBD. All aged more than 15 years and were Thai population, 19 patients with CD and 50 patients with UC. Among the 19 CD cases, 8 (42.1%) had small bowel lesions, 8 (42.1%) had colonic CD and 3 (15.8%) had both small bowel and colonic CD. The mean duration of symptoms before diagnosis was 17 months, and there were six cases with extraintestinal manifestations, which were apthous oral or genital ulcer and arthritis. In the 49 UC cases, 2 (4.0%) had distal colitis (proctosigmoiditis), 33 (66.0%) had left-sided colitis and 15 (30.0%) had pancolitis. The mean duration of symptoms before diagnosis was 14.4 months, and there were 11 cases with extraintestinal manifestations, which were seronegative spondyloarthropathy, apthous ulcer, arthritis, uveitis, pyoderma gangrenosum and primary sclerosing cholangitis as shown in Table 3.

By means of indirect immunofluorescence assay for pANCA and ASCA, the reactivity of positive sera was detected in the pattern shown in Figure 1. Four-

Table 1. Clinical detail of patients.

	CD patients (%)	UC patients (%)
No of patients	19	50
Gender (male/female) 10/9 18/32		
Mean age at diagnosis (years)	37.2	38.4
range (years)	11-68	9-66
Mean duration of symptom before diagnosis (months)	17.0	14.4
Disease location		
CD: Small bowel	8 (42.2)	
Colon	8 (42.2)	
Small bowel and colon	3 (15.8)	
UC: Distal colon		2 (4.0)
Left side colon		33 (66.0)
Pancolic		15 (30.0)
Previous surgery	6 (31.6)	1 (2.0)
No. of patients with extra-intestinal manifestation	6 (31.6)	11 (22.0)
No. of patients diagnosed with typical histopathologic study	10 (52.6)	24 (48.0)

Table 2. Clinical presentation of patients.

	CD (n = 19)	UC (n = 50)
Presence of mucus-bloody stool	13	42
Presence of watery stool	5	5
Presence of tenesmus	1	9
Presence of abdominal pain	13	21
Presence of systemic symptoms	17	23

(systemic symptoms : fever, weight loss, anorexia, nausea and vomiting)

Table 3. Extraintestinal manifestation in studied patients.

	CD (n = 19)	UC (n = 50)
Oral ulcer	5	4
Genital ulcer	1	0
Arthritis	1	2
Primary sclerosing cholangitis	0	1
Pyoderma gangrenosum	0	1
Seronegative spondyloarthropathy	0	4
Uveitis	0	1

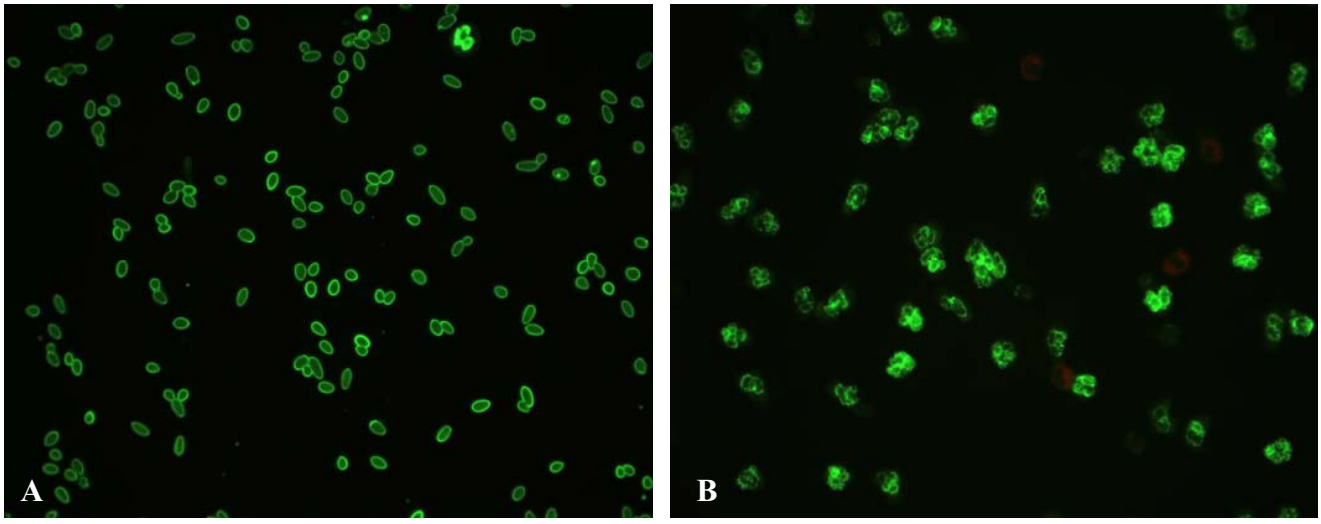

Figure 1. A: positive ASCA
B: positive typical pANCA

Table 4. Test results for CD and UC patients.
Values are number (%), sensitivity (%) and specificity (%).

	CD (n = 19)	UC (n = 50)	Sensitivity	Specificity
ASCA-IgA +	7 (36.8)	13 (26.0)	36.8	74.0*
ASCA-IgG +	9 (47.4)	23 (46.0)	47.4	54.0*
ASCA-IgA + / ASCA-IgG +	7 (31.6)	11 (22.0)	36.8	78.0*
ASCA + (Any of IgA or IgG)	9 (47.4)	25 (50.0)	47.4	50.0*
pANCA +	3 (15.7)	14 (28.0)	28.0	84.2†
pANCA + / ASCA -	2 (16.5)	8 (16.0)	16.0	89.5†
pANCA - / ASCA +	8 (42.1)	19 (54.0)	42.1	62.0*
pANCA + / ASCA +	1 (5.3)	6 (12.0)	12.0	94.7†
pANCA - / ASCA -	8 (42.1)	17 (34.0)	42.1	66.0*

*Sensitivity and specificity for CD
†Sensitivity and specificity for UC
All test had no statistically significant ($p > 0.05$)

teen of 50 (28.0%) UC patients and 3 of 19 (15.7%) CD patients showed the presence of pANCA. There was no statistically significant difference in the positive rate of pANCA in UC patients compared to CD patients. Nine of 19 (47.4%) CD patients and 25 of 50 (50.0%) UC patients showed the presence of ASCA. The subclasses of ASCA-IgA/IgG positive rate in CD and UC patients were 7/9 (36.8%/47.4%) and 13/23 (26.0%/46.0%) respectively. There was no statistically significant difference in the positive rate of either or both classes of ASCA in CD patients compared to UC patients ($p = 0.530$ and $p = 0.566$, respectively). The sensitivity and specificity for ASCA positive and combination of pANCA negative/ASCA positive for diagnosis of CD and pANCA positive and the combination of pANCA positive/ASCA negative for diagnosis of UC were showed in Table 4. The prevalence of ASCA in small bowel involvement CD was 5/19 (26.4%), colonic CD was 4/19 (21.1%) and CD with both small bowel and colonic involvement was 1/19 (5.3%). In the CD group there were 6 cases which need surgical treatment (3 for internal perforation, 2 for bleeding, and 1 for stenosis) and ASCA were positive in 4/6 (66.7%) cases, but all of these showed pANCA negative.

The analysis between the presence of both pANCA and ASCA with any clinical parameters of both CD and UC showed no significant correlation (data

not shown). All of CD patients were negative pANCA test and UC patients with ASCA positive had no particular features compared with UC patients who were ASCA negative.

DISCUSSION

In the present study, we assessed the diagnostic role of pANCA and ASCA in a group of adult Thai IBD patients where the diseases is scanty with the low incidence among Asians compared with the incidence in Western countries.[17] In Thailand, there are only four studies on ulcerative colitis and none had studied the role of both pANCA and ASCA.[18-21] This is the first study in Thailand that evaluate the role of these tests in Thai IBD patients and using the more precise laboratory technique for the interpretation of pANCA by means of standard formaldehyde-fixed slides indirect immunofluorescene method.

Our percentage from serum samples of patients with CD which were ASCA positive are compare with the data reported in Western countries.[22,23] Overall sensitivity and specificity of ASCA for diagnosis CD are 47.4% and 50.0% respectively. The specificity for the diagnosis of CD in this study was much lower than the previous report.[9-13,22,23] After combining the two tests of pANCA negative and ASCA positive, sensitivity (42.1%) and specificity (62.0%) were not improved as much as we expected. These tests could not be used in clinical practice to differentiate between CD and UC.

In this study we found that pANCA were positive in only 28% of UC patients and three of CD patients were test positive. This is contrast to previous study in Thailand[21] and other studies in Western countries[2-7,24-27], even in combination of both pANCA positive and ASCA negative, the sensitivity was still too low to be used in clinical practice for differentiation between UC and CD (sensitivity 16.0%). The possible explanation of these results may be the difference in laboratory method used in this study. This study determined the presence of pANCA in sera by screening of ANCA by indirect immunofluorescene with substrates contained both human neutrophils and primate liver cells that was able to show the component of ANA effect on primate liver cells which may be misinterpret as positive ANCA test and pANCA was confirmed by formaldehyde-fixed slides again, compared with previous study in Thailand that used only ethanol-fixed slides.[21] This may explain why the percentage of pANCA positive in UC patients dropped from 39% in previous study to 28% in this study and made all the test panel much lower in sensitivity and specificity until these tests seem to have no diagnostic role in this population as confirmed by the meta-analysis of studies reporting on pANCA and ASCA in IBD that pANCA is less useful in Asian population than the Caucasian population.[28]

In the present study, there was no significant correlation between the presence of pANCA or ASCA and the extent of disease involvement, the presence of extra-intestinal complications or any particular clinical features. Because the presence of pANCA and ASCA does not reflect the disease activity or severity, serial assessment is not necessary in both CD and UC patients.[23,29,30]

In conclusion, this study has shown that the prevalence of pANCA and ASCA in Thai patients with CD and UC were different and lower than that of a Western population. The sensitivity of both tests was poor to fair. Both pANCA and ASCA could not be served as useful diagnostic tools for CD and UC in this part of the world where these diseases are uncommon. While infectious colitis is much more common.

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