Clinica Chimica Acta 411 (2010) 1461–1465

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

# The prevalence and diagnostic value of perinuclear antineutrophil cytoplasmic antibodies and anti-*Saccharomyces cerevisiae* antibodies in patients with inflammatory bowel disease in mainland China

Feng Zhou [a,b], Bing Xia [a,b,d,*], Fubing Wang [c], Umid Kumar Shrestha [a,b], Min Chen [a,b], Honglin Wang [a,b], Xianyan Shi [a,b], Zhitao Chen [d], Jin Li [a,b]

[a] Department of Gastroenterology, Zhongnan Hospital, Wuhan University School of Medicine, Wuhan 430071, People's Republic of China
[b] Center for Clinical Study of Intestinal Diseases, Zhongnan Hospital, Wuhan University School of Medicine, Wuhan 430071, People's Republic of China
[c] Department of Medical Laboratory, Zhongnan Hospital, Wuhan University School of Medicine, Wuhan 430071, People's Republic of China
[d] Key Laboratory of Allergy and Immune-related Diseases, Wuhan University School of Medicine, Wuhan 430071, People's Republic of China

ARTICLE INFO

*Article history:*
Received 20 December 2008
Received in revised form 28 May 2010
Accepted 28 May 2010
Available online 4 June 2010

*Keywords:*
pANCA
ASCA
Ulcerative colitis
Crohn's disease
Phenotype

ABSTRACT

*Background:* Perinuclear anti-neutrophil cytoplasmic (pANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) have been studied extensively in Western countries. We determined the prevalence of pANCA and ASCA in the mainland Chinese population and the ability of pANCA and ASCA to discriminate between ulcerative colitis (UC) and Crohn's disease (CD).

*Methods:* Two hundred-six unrelated patients with IBD (UC, n = 152; CD, n = 54), 60 patients with other gastrointestinal diseases, and 80 healthy controls were included. Sera pANCA and ASCA titers were determined by a standardized indirect immunofluorescence technique.

*Results:* The sensitivity, specificity, positive and negative predictive values, and positive likelihood ratio of pANCA were calculated for differentiating UC from healthy controls (43.4%, 96.3%, 95.7%, 47.2%, and 11.7, respectively) and ASCA for differentiating CD from healthy controls and (46.3%, 96.3%, 89.3%, 72.6%, and 12.5, respectively). The combination of pANCA and ASCA did not result in greater diagnostic efficiency than either test alone. pANCA was more frequent in UC with extensive or severe phenotype than others. ASCA was associated with severe CD disease activity.

*Conclusions:* pANCA and ASCA are useful in confirming the diagnosis of IBD and differentiating between UC and CD in an IBD cohort in central China.



## 1. Introduction

Inflammatory bowel disease (IBD) is characterized by idiopathic inflammation of the gastrointestinal tract, and manifested as diarrhea, abdominal pain, rectal bleeding, weight loss, and anemia. IBD consists of two main clinical entities (ulcerative colitis [UC] and Crohn's disease [CD]). Using current diagnostic criteria, approximately 10% of patients will not meet the criteria of either subtype initially and a final diagnosis of unclassified colitis is made [1].

As therapeutic approaches are different, it is essential to make an earlier, more accurate diagnosis of UC or CD, especially when surgery is planned [2]. Various serologic markers have been studied to aid in differentiating patients with IBD over the last several years [3]. Perinuclear anti-neutrophil cytoplasmic (pANCA) and anti–*Saccharomyces cerevisiae* antibodies (ASCA) are the most widely used markers for UC and CD, respectively [4,5].

pANCA are present in 40%–80% of UC patients. pANCA are spontaneously produced by the lamina propria and mesenteric node lymphocytes with the antigenic target located on the inner aspect of the nuclear periphery [6]. pANCA are also present in 15%-25% of patients with CD, in which pANCA are associated with UC-like clinical manifestations [7,8]. ASCA are found in approximately 60% of CD patients and 5%-20% of UC patients [4,8–10]; the antigenic target of ASCA has been identified as a phosphopeptidomannan of the yeast cell wall.

In a recent comparative study of serologic markers in Chinese and Caucasian patients with IBD in Hong Kong, the combination of pANCA and ASCA testing improved the differentiation between UC and CD [11]. However, no data are available regarding the prevalence of pANCA and ASCA in the mainland Chinese population. Therefore, the purpose of this study was to determine the prevalence of pANCA and ASCA in an IBD patient cohort from central China and the accuracy in

*Abbreviations:* pANCA, perinuclear antineutrophil cytoplasmic antibodies; ASCA, anti–*Saccharomyces cerevisiae* antibodies; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; MPO, myeloperoxidase.

* Corresponding author. Department of Gastroenterology, Zhongnan Hospital, Wuhan University School of Medicine, Wuhan 430071, Hubei Province, People's Republic of China. Tel.: +86 27 67813061; fax: +86 27 67812892.
*E-mail address:* bingxia2004@yahoo.com.cn (B. Xia).

0009-8981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2010.05.041

diagnosis and differentiation between UC and CD, either alone or in combination. The relationship between pANCA and ASCA with the clinical parameters of UC and CD was evaluated as well.

## 2. Materials and methods

### 2.1. Subjects

Two hundred six unrelated IBD patients (CD, n = 54; female-to-male ratio, 23:31; mean age, 38.7 ± 14.8 y; mean duration of disease, 4.5 ± 4.9 y; UC, n = 152; female-to-male ratio, 66:86; mean age, 42.1 ± 13.6 y; mean duration of disease, 3.8 ± 4.4 years) under care at the Zhongnan Hospital of Wuhan University between April 2004 and April 2009 were enrolled in this study. The diagnosis of UC and CD was made by clinical, radiologic, endoscopic, and histologic examinations in accordance with the Lennard-Jones criteria [12]. Only patients with UC or CD for a duration>6 months were included in the study, and those patients with infections and other recognized causes of inflammation were excluded. The clinical characteristics of all patients are shown in Table 1. UC patients were classified as follows: proctitis, left-sided colitis, or extensive colitis; and the severity based on the Truelove and Witts criteria [13]. The clinical activity of CD patients was measured according to the Crohn's Disease Activity Index (CDAI) [14]. CD patients were subgrouped by the Montreal classification [15], age at diagnosis (A1, ≤16 years; A2, 17~40 years; and A3, >40 years), disease behavior (B1, non-stricturing, non-penetrating; B2, structuring; B3, penetrating), and disease location (L1, terminal ileum; L2, colon,;L3, ileocolon; L4, upper gastrointestinal tract). Eighty age- and gender-matched healthy controls (female-to-male ratio, 34:46; age, 43.3 ± 11.6 years) were recruited from healthy blood donors and volunteers from the hospital personnel. We also studied sera from 60 patients (female-to-male ratio, 26:34; age, 42.5 ± 15.2 years) with other gastrointestinal diseases (OGDs). The OGD group consisted of patients with non-IBD gastrointestinal inflammation, like diverticulitis, infectious gastroenteritis, ischemic colitis, pseudomembranous colitis, and intestinal vasculitis, as well as patients with liver cirrhosis, a disease known to be accompanied by increased intestinal permeability.

The study was approved by the Ethics Committee of Wuhan University Zhongnan Hospital. The nature of the study was explained to each participant before an informed consent was signed.

**Table 1**
Clinical data of patients with UC and CD, and healthy and non-IBD controls.

| Characteristic | UC | CD | Healthy Controls | Non-IBD Controls |
|---|---|---|---|---|
| | n = 152 | n = 54 | n = 80 | n = 60 |
| Female/ male | 66/86 | 23/31 | 34/46 | 26/34 |
| Mean age, (y) | 42.1 ± 13.6 | 38.7 ± 14.8 | 43.3 ± 11.6 | 42.5 ± 15.2 |
| Range | 7-82 | 17-69 | 18-65 | 15-72 |
| Disease duration: mean (yr) | 3.8 ± 4.4 | 4.5 ± 4.9 | | |
| Disease location (%): UC | | | | |
| Extensive | 56(36.8%) | | | |
| Left side | 57(37.5%) | | | |
| Proctitis | 39(25.7%) | | | |
| Severity of the disease (%): | | | | |
| Mild | 53(34.9%) | | | |
| Moderate | 78(51.3%) | | | |
| Severe | 21(13.8%) | | | |
| Disease location (%): CD | | | | |
| L1: Terminal ileum | | 12(22.3%) | | |
| L2: Colon | | 20(37.0%) | | |
| L3: Ileocolon | | 20(37.0%) | | |
| L4: Upper GI | | 2(3.7%) | | |
| Clinical disease activity (%): CDAI | | | | |
| Mild | | 16(29.7%) | | |
| Moderate | | 26(48.1%) | | |
| Severe | | 12(22.2%) | | |
| Disease behavior (%): CD | | | | |
| B1: Non-stricturing, non-penetrating | | 26(48.1%) | | |
| B2: Stricturing | | 17(31.5%) | | |
| B3: Penetrating | | 11(20.4%) | | |

### 2.2. Methods

Sera were collected from the peripheral blood of IBD patients, and healthy and non-IBD controls, and stored at -70 °C until assayed. The serologic technicians were not aware of the clinical details of the patients and the controls at the time of the determination of serologic markers.

## 3. Indirect Immunofluorescence Assays

Commercially available kits were used to detect pANCA and ASCA (Euroimmun, Lübeck, Germany) by indirect immunofluorescence assays. All slides were evaluated by 2 independent observers; in case of a difference in opinion, a third observer was decisive.

Determination of pANCA titers were performed according to standard techniques using ethanol-fixed human neutrophil granulocyte BIOCHIP slides (Euroimmun). Sera were diluted 1:10 in phosphate buffered saline (PBS) and incubated on the BIOCHIP slides for 30 min at room temperature, then washed twice in PBS-Tween solution for 5 min, and incubated according to the manufacturer's instructions with 20 µl fluorescein-labeled goat anti-human IgG. Following incubation for 30 min at room temperature, the slides were washed and embedded by cover glasses. Examination and classification were performed under ultraviolet light (UV) using a Leica indirect immunofluorescence microscope (Wetzler, Germany). Sera that exhibited fluorescence on indirect immunofluorescence were titrated to endpoint and classified as perinuclear (pANCA) or cytoplasmic (cANCA). Interference by anti-nuclear antibodies which might mimic the pANCA pattern was ruled out by using formalin fixation. Because we should also consider that there are several types of pANCA tests, a pANCA-positive test typically reflects antibodies to myeloperoxidase (MPO) and occurs in association with vasculitis. Formalin fixation renders the neutrophil cell membrane permeable to antibodies, but does not solubilize proteins, such as MPO, thus the perinuclear reaction obtained with MPO–pANCA is abolished and converted to a cytoplasmic reaction pattern. Sera were considered to be positive for pANCA if specific perinuclear fluorescence was present at a titer of ≥1:32.

The indirect immunofluorescence procedure for ASCA was identical to that used for pANCA. Sera were incubated with a *Saccharomyces cerevisiae* smear substrate covering slides of BIOCHIP (Euroimmun). After two washes, the slides were incubated with 20 µl fluorescein-labeled goat anti-human IgG and IgA to detect ASCA-IgG and -IgA, respectively. Specific reactivities at a titre ≥1:1000 for IgG and ≥1:100 for IgA were regarded as positive staining.

## 4. Statistical Analysis

The sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were calculated to determine the predictive power of pANCA and ASCA-IgG and -IgA, and the combination of the 2 markers, to distinguish among UC, CD, and healthy controls. The relationship between serologic markers and clinical parameters was studied using a chi-square test or Fisher's exact test, as appropriate. The odds ratio and 95% confidence intervals (CI) are presented for significant predictors. The threshold for statistical significance was $P<0.05$. All statistical analyses were performed using SPSS11.5 (SPSS, Inc., Chicago, IL).