Journal of Community Hospital Internal Medicine Perspectives

Volume 14 | Issue 3 Article 3

2024

ANCA and ASCA Profiles in a Moroccan Population With Inflammatory Bowel Diseases

Ouadii Abakarim
Faculty of Medicine and Pharmacy, Cadi Ayyad University, Marrakesh, Morocco, ouadii.aba@gmail.com

Raymond Klevor
Faculty of Medicine and Pharmacy, Cadi Ayyad University, Marrakesh, Morocco

Lahcen El Moumou
Biotechnology, Environment and Health Team, Laboratory of Sciences of Health and Environment, High Institute of Nursing Professions and Health Techniques, ISPITS Agadir- Annex Tiznit, Morocco

Raja Hazime
B2S Research Laboratory, Faculty of Medicine and Pharmacy, Cadi Ayyad University, Marrakesh, Morocco

Imane Brahim
Laboratory of Immunology, Center of Clinical Research, University Hospital Mohammed VI, Marrakesh, Morocco

See next page for additional authors

ANCA and ASCA Profiles in a Moroccan Population With Inflammatory Bowel Diseases

Authors

Ouadii Abakarim, Raymond Klevor, Lahcen El Moumou, Raja Hazime, Imane Brahim, Sofia Oubaha, Khadija Krati, and Brahim Admou

RESEARCH ARTICLE

ANCA and ASCA Profiles in a Moroccan Population With Inflammatory Bowel Diseases

Ouadii Abakarim [a,*], Raymond Klevor [a], Lahcen El Moumou [b], Raja Hazime [c,d], Imane Brahim [d], Sofia Oubaha [a,e], Khadija Krati [a,e], Brahim Admou [c,d]

[a] Faculty of Medicine and Pharmacy of Marrakesh, Cadi Ayyad University, Marrakesh, Morocco
[b] Biotechnology, Environment and Health Team, Laboratory of Sciences of Health and Environment, High Institute of Nursing Professions and Health Techniques, ISPITS Agadir-Annex Tiznit, Morocco
[c] Laboratory of Immunology, Center of Clinical Research, University Hospital Mohammed VI, Marrakesh, Morocco
[d] B2S Research Laboratory, Faculty of Medicine and Pharmacy, Cadi Ayyad University, Marrakesh, Morocco
[e] Gastroenterology Department, University Hospital Mohammed VI, Marrakesh, Morocco

Abstract

Introduction: **Diagnosing inflammatory bowel disease (IBD) is hindered by the invasive procedures required for accurate classification as Crohn's disease (CD) or ulcerative colitis (UC). As alternatives, non-invasive tests using anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA) have gained significance. This study evaluated ANCA and ASCA antibody frequencies in IBD and their role in disease characterization in a Moroccan population.**

Methods: **Conducted at Marrakech's Mohammed VI University Hospital from 2014 to 2018, this cross-sectional study included patients with suggestive symptoms or confirmed IBD diagnosis based on clinical, endoscopic, and histological criteria. Immunological investigations detected p-ANCA, c-ANCA, and ASCA using immunofluorescence and immunodot assays.**

Results: **Among 60 participants (mean age: 33.1 ± 11.75 years), the 20−30-year age group was most affected (31.67%). CD, UC, and indeterminate colitis (IC) were diagnosed in 46.67%, 45%, and 8.33% patients, respectively. Gastrointestinal symptoms were prevalent (98.3%), with ANCA+/ASCA-profile in 41% of UC patients versus 11% in CD, and ANCA-/ASCA + profile exclusive to CD (50%). ANCA positivity was significantly associated with UC, rectal syndrome, and inflammatory syndrome, whereas ASCA positivity was significantly associated with CD and König's syndrome ($p < 0.05$).**

Conclusion: **This study highlighted demographic and phenotypic particularities of IBD in a Moroccan population. Non-invasive tests using ASCA and ANCA antibodies offer valuable alternatives to invasive procedures, facilitating personalized management strategies. Variations in ANCA and ASCA profiles provide insights into disease characterization and inform tailored treatment approaches.**

Keywords: Inflammatory bowel disease, Immunological profiles, Crohn's disease, Ulcerative colitis, Morocco

1. Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), pose significant challenges in diagnosis and management. Despite recognized factors such as smoking and appendectomy influencing IBD risk and progression, the mechanisms underlying these associations remain elusive.[1-5] Current diagnostic approaches rely on a combination of clinical, morphological, and histological criteria to accurately classify CD and patients with UC.[6] However, the invasive nature of these diagnostic procedures underscores the need for non-invasive alternatives.

Among the myriad serum antibodies (Ab) proposed as diagnostic markers for CD or UC, anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA) have emerged as prominent candidates.[7] These antibodies have potential not only for distinguishing CD from UC but also for refining diagnosis, characterizing disease phenotypes, and predicting disease course.[8]

Received 25 December 2023; revised 17 February 2024; accepted 7 March 2024.
Available online 7 May 2024

* Corresponding author at: University Hospital Mohammed VI, Faculty of Medicine and Pharmacy, Cadi Ayyad University, Marrakesh. Morocco.
E-mail address: ouadii.aba@gmail.com (O. Abakarim).

https://doi.org/10.55729/2000-9666.1347

Despite the existence of numerous antibody markers, the rationale for focusing on ANCA and ASCA lies in their widespread use and established relevance in clinical studies.[9] By examining the ANCA/ASCA profile in patients with IBD, this study aims to fill a crucial gap in the literature by elucidating their frequency and clinical associations within a Moroccan population. This investigation seeks to provide insights into the diagnostic, prognostic, and therapeutic implications of ANCA and ASCA in IBD, thereby contributing to enhanced patient care and management strategies.

2. Methods

2.1. Study design

This descriptive and analytical cross-sectional study was designed to investigate the frequency of anti-neutrophil cytoplasm antibodies (ANCA) and anti-*S. cerevisiae* antibodies (ASCA) in patients with inflammatory bowel disease (IBD).

2.2. Study setting

The study was conducted at the immunology laboratory and gastroenterology department of Marrakech's Mohammed VI University Hospital in Morocco, spanning the period from 2014 to 2018.

2.3. Study participants

Patients exhibiting suggestive clinical symptoms or those with confirmed diagnosis of IBD based on clinical, endoscopic, and histological criteria were enrolled in the study. Patients were admitted to the immunology laboratory for ANCA and ASCA antibody testing.

2.4. Outcome measures

The primary outcome of the study was to establish correlations between immunological results (ANCA and ASCA antibody status) and the clinical and paraclinical characteristics of patients with IBD.

2.5. Study instruments

Clinical data, including demographic characteristics and clinical manifestations, were collected from medical records. Immunological investigations were conducted to detect ANCA and ASCA antibodies using specific assays.

2.6. Immunological investigation

ANCA detection involved indirect immunofluorescence techniques (ANCA ethanol and formaldehyde, Bio-Rad), with a threshold of 1:20. Positive results were further analyzed for anti-myeloperoxidase (MPO) and anti-proteinase 3 (PR3) antigenic specificity using an immunodot assay (BlueDot ANCA, Aesku), with a threshold of 10 index. ASCA detection was performed using an immunodot assay (BlueDot ASCA IGG/IGA, Aesku), with a threshold set at 10 index.

2.7. Data collection

Clinical data were extracted from medical records, encompassing demographic characteristics and clinical manifestations. Symptoms and diagnostic criteria were assessed at the time of initial diagnosis or during the disease.

2.8. Sample size determination

A sample size of 60 patients was calculated for this study on the basis of statistical considerations.

2.9. Statistical analysis

Data analysis involved descriptive and inferential statistics, including Chi-square and cross-tabulation, conducted using the Statistical Package for Social Sciences (SPSS) version 23. A p-value of <0.05 was considered statistically significant.

Ethical approval

Data collection adhered to global ethical guidelines, ensuring confidentiality and protection of patient data. Ethical approval was obtained before the start of the study.

3. Results

The study included 60 patients, with a mean age of 33.1 ± 11.75 years (range: 16−64 years), showing a slight female predominance (sex-ratio M/F = 0.87). The most affected age groups were 20−30 years (31.67%, n = 19) and 30−40 years (30%, n = 18) (Table 1). Among these patients, 28 (46.67%), 27 (45%), and 5 (8.33%) were diagnosed with CD, ulcerative colitis (UC), and indeterminate colitis (IC), respectively.

Gastrointestinal symptoms were predominant, observed in 98.3% of cases, followed by general

Table 1. Distribution of patients according to demographic and clinical characteristics.

Category of IBD	CD n = 28 (46.7%)	UC n = 27 (45%)	IC n = 5 (8.3%)	Total
Demographic characteristics				
Mean age in years [ranges]	31.8 [16–52]	35.1 [18–64]	28 [18–38]	33.1 [6–64]
Male-to-female ratio	1.15	0.8	0.25	0.87
Clinical manifestations	n (%)	n (%)	n (%)	n (%)
General signs	25 (89.3)	18 (66.7)	5 (100)	48 (80)
Physical decline	21 (75)	11 (40.7)	3 (60)	35 (58.3)
Fever	3 (10.7)	1 (3.7)	0	4 (6.7)
Edema	1 (3.6)	0	0	1 (1.7)
Skin pallor	13 (46.4)	14 (52)	3 (60)	30 (50)
Digestive signs	27 (96.4)	27 (100)	5 (100)	59 (98.3)
Chronic diarrhea	26 (92.8)	24 (88.8)	3 (60)	53 (88.3)
Abdominal pain	20 (71.4)	14 (50)	4 (80)	38 (63.3)
Rectal syndrome	10 (35.7)	21 (77.7)	1 (20)	32 (53.3)
Koenig's syndrome	12 (42.8)	2 (7.4)	1 (20)	15 (25)
Rectal bleeding	1 (3.6)	12 (44.4)	2 (40)	15 (25)
Anoperineal lesions	14 (50)	4 (14.8)	4 (80)	22 (36.7)
Extra-digestive signs	14 (50)	8 (29.6)	2 (40)	24 (40)
Arthritis	13 (46.4)	8 (29.6)	2 (40)	23 (38.3)
Erythema	1 (3.6)	3 (11.1)	1 (20)	5 (8.3)
Eye redness	4 (14.3)	0	0	4 (6.7)
Biological manifestations				
Anemia (%)	11 (39.3)	13 (48.1)	2 (40)	26 (43.3)
Inflammatory syndrome (%)	17 (60.7)	17 (63)	4 (80)	38 (63.3)
Localization				
Ileal (%)	3 (5)	0	0	3 (5)
Ileoceacal (%)	14 (23.3)	0	0	14 (23.3)
Colitis (%)	3 (5)	0	0	3 (5)
Left colitis (%)	0	5 (8.3)	0	5 (8.3)
Proctosigmoiditis (%)	1 (1.7)	2 (3.3)	0	3 (5)
Proctitis (%)	2 (3.3)	10 (16.7)	0	12 (20)
Pancolitis (%)	3 (5)	11 (18.3)	0	14 (23.3)

symptoms (80%), joint signs (38.3%), ocular signs (66.7%), and cutaneous signs (5%) (Table 1).

3.1. ANCA and ASCA frequencies

Of the patients, 33.3% (n = 20) tested positive for ANCA, predominantly exhibiting a perinuclear pattern (60%, n = 12), and less frequently a cytoplasmic pattern (10%, n = 2). Among ANCA-positive cases, 20% (n = 4) had PR3-ANCA, and 15% (n = 3) had both MPO- and PR3-ANCA. ASCA was positive in 31.7% (n = 19) of patients, with predominance of the IgA (36.8%) and IgG isotypes (31.6%). ANCA positivity was significantly associated with UC (p = 0.0003), rectal syndrome (p = 0.018), and inflammatory syndrome (p = 0.02), whereas ASCA positivity was significantly associated with CD (p = 0.0002) and König's syndrome (p = 0.037) (Table 2).

4. ASCA and ANCA profiles

Regarding disease subtypes, the ANCA-positive/ASCA-negative profile was more prevalent in UC

Table 2. ANCA subtypes and ASCA isotypes according to the clinical category of IBD.

IBD categories	CD n = 28 (100%)	UC n = 27 (100%)	IC n = 5 (100%)
ANCA-positive	5 (17.8)	15 (55.6)	0
p-ANCA	1 (3.6)	11 (40.7)	0
c-ANCA	0	2 (7.4)	0
MPO specificity	0	0	0
PR3 specificity	0	4 (14.8)	0
Both MPO and PR3	1 (3.6)	2 (7.4)	0
Undetermined specificities	3 (10.7)	2 (7.4)	0
ASCA-positive	16 (57.1)	3 (11.1)	0
ASCA-IgA	6 (21.4)	1 (3.7)	0
ASCA-IgG	5 (17.8)	1 (3.7)	0
Undetermined specificities	5 (17.8)	1 (3.7)	0

patients (41%) than in CD patients (11%), whereas the ANCA-negative/ASCA-positive profile was exclusively found in CD patients (50%). ANCA/ASCA double negative status was observed in all IC patients (Table 3).

Information regarding the confirmation of IBD diagnosis and the duration of the disease at study entry, as well as treatment regimens and the mean time to symptom control, were not specifically addressed in this study and would require additional investigation or data collection beyond the scope of this cross-sectional analysis.

5. Discussion and conclusion

Our study observed a noteworthy prevalence of inflammatory bowel diseases (IBD), predominantly Crohn's disease (CD), and ulcerative colitis (UC) within our patient cohort. This observation aligns with the existing literature, which commonly reports a higher incidence of CD than that of UC.[10-12] However, it is essential to note that few studies have shown a predominance of UC, highlighting the variability in disease distribution across different populations.[13]

The mean age of our patients (33.1 years) corresponds with that of previous studies indicating that IBD primarily affects young adults without a significant gender predilection.[14,15] Manifestations of IBD, such as gastrointestinal symptoms and extra-digestive manifestations, were consistent with the literature, with diarrhea and abdominal pain being the most prevalent complaints.[16-18]

Studies have consistently demonstrated a high frequency of ASCA and ANCA antibodies in CD and UC, respectively, suggesting their potential diagnostic utility.[19,20] Our findings support this finding, indicating a statistically significant association between ANCA/ASCA profiles and CD/UC diagnosis. Specifically, the ASCA-positive/pANCA-negative and pANCA-positive/ASCA-negative profiles were highly indicative of CD and UC, respectively.[21]

In our study, ANCA positivity was more common in patients with UC, whereas ASCA positivity was predominantly observed in patients with CD, consistent with the literature.[22–25] These antibody profiles aid in disease differentiation and classification. However, it is crucial to recognize that although statistically significant associations were observed, the sensitivity and specificity of these antibody markers, which are the gold standard for IBD diagnosis, were not evaluated in this study, which warrants further investigation.

The association between sociodemographic factors and ANCA/ASCA positivity was not statistically significant in our study, which is consistent with previous findings.[13,16,24,26] However, we observed a statistically significant association between ANCA/ASCA positivity and specific clinical manifestations, such as diarrhea and disease severity, which contradicts some previous studies.[10,13,24] These discrepancies underscore the need for further research to elucidate the clinical implications of ANCA and ASCA in IBD.

6. Clinical value of ANCA and ASCA in IBD

In total, the clinical relevance of ANCA and ASCA in IBD lies in the following facts:

- Joint research of ANCA and ASCA is useful to optimize the diagnosis of CD and UC, where the clinic is especially atypical[28];
- The existence of an ANCA-negative/ASCA-positive profile favors the diagnosis of CD, and conversely, an ANCA-positive/ASCA-negative profile favors the diagnosis of UC.[28]

Table 3. IBD patients characteristics clustered according to ANCA and ASCA results.

	ANCA-positive		ASCA-positive	
	n = 20 (33.3%)	*P* value	n = 19 (31.7%)	*P* value
Demographic characteristics				
Male-to-female ratio	0.66	0.630	1.11	0.528
Clinical manifestations				
Digestive signs				
Abdominal pain	10 (26.3)	0.691	11 (28.9)	0.426
Rectal syndrome	15 (44.1)	0.018	8 (23.5)	0.121
Koenig's syndrome	4 (26.7)	0.630	8 (53.3)	0.037
Rectal bleeding	6 (40)	0.423	3 (20)	0.329
Anoperineal lesions	8 (22.2)	0.738	17 (47.2)	0.727
Extra-digestive signs	10 (29.4)	0.517	11 (32.4)	0.473
Biological manifestations				
Anemia	9 (34.6)	0.667	6 (23)	0.251
Inflammatory syndrome	8 (21)	0.020	11 (28.9)	0.551

- These autoantibodies should minimize invasive investigations and are particularly useful in cases where endoscopic procedures are difficult or contraindicated.[27]
- They can help detect certain severe forms with a more aggressive course of the disease, whether for CD or UC.[29]

However, the emergence of new biomarkers, such as calprotectin and lactoferrin, may impact the diagnostic utility of ANCA and ASCA in IBD diagnosis.[28]

In conclusion, our study provides valuable insights into the prevalence and clinical implications of ANCA and ASCA antibodies in IBD in the Moroccan population. Although our findings support the diagnostic value of these antibodies, further research with larger sample sizes and diverse populations is warranted to validate our results and better define the ANCA/ASCA profile in the entire IBD population. In addition, future studies should assess the sensitivity and specificity of these antibody markers in relation to the gold standard for IBD diagnosis to strengthen their clinical utility.

Data availability statement

The data used to support the findings of this study have been included in the manuscript.

Ethical approval

Data collection was performed in accordance with the global rules of ethics relating to the confidentiality and protection of patients' personal data.

Patient consent statement

Written informed consent was obtained from the patients to publish this report in accordance with the journal's patient consent policy. We hereby transfer, assign, or otherwise convey all copyright ownership, including all rights incidental thereto, exclusively to the journal if such work is published.

Funding statement

This research received no specific grant from any funding agency in the public, commercial, or not for-profit sectors.

Conflicts of interest

We have no conflicts of interest to declare.

Acknowledgments

We thank all the clinical staff who participated in elaborating this work.

References

1. Loftus Jr EV, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Crohn's disease in Olmsted Country, Minnesota, 1940−1993: incidence,prevalence, and survival. *Gastroenterology*. 1998;114(6):1161−1168.
2. Loftus EV. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology*. 2004;126(6):1504−1517.
3. Podolsky DK. Inflammatory bowel disease. *N Engl J Med*. 2002; 347(6):417−429.
4. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*. 2008;134(2):577−594.
5. Calkins BM. A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig Dis Sci*. 1989;34(12):1841−1854.
6. Andersson RE, Olaison G, Tysk C, Ekbom A. Appendectomy and protection against ulcerative colitis. *N Engl J Med*. 2001; 344(11):808−814.
7. Heresbach D, Heresbach-Le Berre N, Ramee MP, Semana G, Gosselin M, Bretagne JF. Fréquence et valeur pronostique du granulome épithélioïde au cours des maladies inflammatoires chroniques de l'intestin. *Gastroenterol Clin Biol*. 1999;23: 1376−1385.
8. Pierre Desreumaux, Arnaud Bourreille, Jean-Frédéric Colombel. Physiopathologie des MICI: "qu'en est-il des tests diagnostiques et prédictifs non invasifs?". *Gastroentérol Clin Biol*. 2003;27. N° SUP 3 - mars.
9. Zhou Guangxi, Song Yang, Yang Wenjing, et al. ALCA and many more: are They useful in the diagnosis of inflammatory bowel disease? *Dig Dis*. 2016;34(1−2):90−97.
10. Loste MTA, Bonilla G, Moraleja I, et al. Presence of anti-proteinase 3 antineutrophil cytoplasmic antibodies (Anti-PR3 ANCA) as serologic markers in inflammatory bowel disease. *Clin Rev Allergy Immunol*. 2013;45:109−116.
11. Musleh M, Triplett D, Akram S. Prognostic significance of anti-Saccharomyces cerevisiae antibody (ASCA) in Southwestern Ohio Veteran IBD population. *Gastroenterology AGA*. 2015;148(4):S-251.
12. Smids C, Horjus THCS, Groenen MJM, van Koolwijk EHM, Wahab PJ, van Lochem EG. The value of serum antibodies in differentiating inflammatory bowel disease, predicting disease activity and disease course in the newly diagnosed patient. *Scand J Gastroenterol*. 2017;52(10):1104−1112.
13. Mokhtarifar A, Ganji A, Sadrneshin M, et al. Diagnostic value of ASCA and atypical p-ANCA in differential diagnosis of inflammatory bowel disease. *Middle East J Digest Dis*. 2013;5(2): 93−97.
14. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology*. 2011;140(6):1785−1794.
15. Baños FJ, Vélez MHR, Arango JFF, et al. Phenotypes and natural history of inflammatory bowel disease (IBD) in a referral population in Medellín, Colombia. *Rev Colomb Gastroenterol*. 2010;25(3):238−249.
16. Hwang V, Ross U, Mahadevan. Micronutrient deficiencies in inflammatory bowel disease: from A to zinc. *Inflamm Bowel Dis*. 2012;18(10):1961−1981.
17. Hart AL, Ng SC. Crohn's disease. *Medicine*. 2015;5(43): 282−290.
18. Castel D, Faure C, Veyrac M. Evaluation thérapeutique. Traitement de la maladie de Crohn : évaluation clinique. *Revue d'évaluation sur le médicament*. 2001;XXII(6):5−15.
19. Joossens S, Reinisch W, Vermeire S, et al. The value of serologic markers in indeterminate colitis: a prospective follow-up study. *Gastroenterology*. 2002;122:1242−1247.
20. Vermeire S, Peeters M, Vlietinck R, et al. Anti-Saccharomyces cerevisiae antibodies (ASCA), phenotypes of IBD, and intestinal permeability: a study in IBD families. *Inflamm Bowel Dis*. 2001;7(1):8−15.
21. Wang ZZ, Shi K, Peng J. Serologic testing of a panel of five antibodies in inflammatory bowel diseases: diagnostic value and correlation with disease phenotype. *Biomed Rep*. 2017;6(4): 401−410.

22. Landers CJ, Cohavy O, Misra R, et al. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology*. 2002;123:689−699.
23. Targan SR, Karp LC. Defects in mucosal immunity leading to ulcerative colitis. *Immunol Rev*. 2005;204:296−305.
24. Reese GE, Constantinides VA, Simillis C, et al. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol*. 2006;101:2410−2422.
25. Mow WS, Vasiliauskas EA, Lin YC, et al. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology*. 2004;126:414−424.
26. Zholudev A, Zurakowski D, Young W, Leichtner A, Bousvaros A. Serologic testing with ANCA,ASCA, and anti-OmpC in children and young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. *Am J Gastroenterol*. 2004;99:2235−2241.
27. Gologan S, Iacob R, Preda C, et al. Higher titers of anti-Saccharomyces cerevisiae antibodies IgA and IgG are associated with more aggressive phenotypes in Romanian patients with Crohn's disease. *J Gastrointestin Liver Dis*. 2012;21:39−44.
28. Escande A. Anticorps associés aux maladies inflammatoires chroniques de I'Intestin. *Rev Fr Lab*. 2002;(341):35−40.
29. Peeters M, Joossens S, Vermeire S, Vlietinck R, Bossuyt X, Rutgeerts P. Diagnostic value of anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic auto-antibodies in inflammatory bowel disease. *Am J Gastroenterol*. 2001;96:730−734.