Prevalence and Significance of Perinuclear Anti-Neutrophil Antibodies (pANCA) in Romanian Patients with Crohn's Disease and Ulcerative Colitis

Carmen Monica Preda[1], Severine Vermeire[2], Paul Rutgeerts[2], Sophie Joosens[2], Mircea Diculescu[1], Cătălin Marica[1] , Mihai Ciocârlan[1], Virginia Mirea[1], Alexandru Oproiu[1]

1) Fundeni Gastroenterology and Hepatology Center, Bucharest, Romania. 2) Erasmus University, Leuven, Belgium

Abstract

Introduction. Antineutrophil cytoplasmic antibodies (ANCA) are known as a serologic marker of immune disturbances in IBD. The most specific are perinuclear ANCA (pANCA). The aim of this study was to investigate their significance for the diagnosis of inflammatory bowel disease (IBD) in Romania. **Material and methods.** A prospective longitudinal study, comprising all patients admitted to our Center in 2000 with ulcerative colitis - UC group (33 patients) and with Crohn's disease - CD group (40 patients). The control group (C) included 22 healthy individuals, with similar age and sex distribution. ANCA was tested in serum by indirect immunofluorescence at Leuven University, Belgium. **Results.** ANCA prevalence in UC group was 12/33 (36.4%), in CD group was 6/40 (15%), while in the C group all sera tested negative (p=0.004). All ANCA antibodies in patients with IBD were perinuclear type. In the UC group, the prevalence of pANCA was higher in females compared to males (52.9% versus 16.7%, p=0.04). The phenotype pANCA+ did not correlate with disease extension, severity, the evolutive form or complications. In the CD group, the phenotype pANCA+, although more frequently found in colonic involvement and in non-obstructive non-fistulizing forms to associate with pANCA +, did not reach statistical significance (p=0.59). A higher severity of CD was associated with higher pANCA titers (p=0.05). **Conclusion.** pANCA prevalence in UC in Romania was lower in comparison with other studies (36.4% versus 50-80%). The highest prevalence was found in females with UC. In CD, pANCA+ was associated with a higher severity. pANCA assessment remains at a research level, further investigations being necessary in order to demonstrate its clinical importance.

Romanian Journal of Gastroenterology
December 2005 Vol.14 No.4, 357-360
Address for correspondence: Preda Carmen Monica
Fundeni Clinical Institute
Centre Gastroenterol Hepatol
Şos.Fundeni, no.258
Bucharest, Romania
Email: preda.carmen@email.ro

Key words

Crohn's disease - ulcerative colitis - perinuclear anti-neutrophil cytoplasmic antibodies

Rezumat

Introducere. ANCA au fost descoperiți de mulți ani ca markeri ai dezechilibrului imunologic în bolile inflamatorii intestinale nespecifice (mai specifici sunt pANCA). Scopul acestui studiu a fost de a investiga semnificația lor în bolile inflamatorii intestinale nespecifice în Romania. **Material și metodă.** Studiu prospectiv longitudinal incluzând pacienții internați în CGEH Fundeni în 2000 cu diagnostic de rectocolită ulcero-hemoragică - lot RCUH (33 cazuri) sau boala Crohn - lot BC (40 cazuri). S-a constituit în aceeași perioadă un lot martor (M), din 22 subiecți sănătoşi, comparabil ca distribuție de vârstă și sex cu loturile studiate. Din serurile recoltate s-au efectuat testări serologice (imunofluorescența indirectă) la Universitatea Leuven, Belgia. **Rezultate.** Frecvența ANCA în lotul RCUH a fost 12/33 (36.4%), în lotul BC 6/40 (15%), iar la subiecții martor toate serurile au fost negative, p=0.004. Toți autoanticorpii ANCA la bolnavii cu boli inflamatorii intestinale nespecifice au fost de tip perinuclear (pANCA). In cadrul lotului RCUH, frecvența pANCA a fost mai mare la femei față de bărbați (52,9% față de 16,7%, p=0,04). Prezența pANCA nu s-a corelat cu extensia bolii, cu severitatea acesteia, forma evolutivă sau prezența complicațiilor. In lotul BC, titrul pANCA pozitiv a fost găsit mai frecvent în localizarea colonică a bolii, şi în formele nonobstructive nonfistulizante, dar fără a se atinge semnificația statistică (p=0,59). Formele de boală Crohn cu o severitate mai mare clinic s-au asociat cu titruri pANCA mai mari (p=0,05). **Concluzii.** In acest studiu prevalența pANCA în rectocolita ulcero-hemoragică este mai redusă față de cea raportată de studii similare din literatură (36,4% față de 50-80%), cu o prevalență mai mare la femeile cu RCUH. In BC, pANCA+ se asociază cu o severitate mai înaltă a bolii. Rămâne o determinare de cercetare, fiind necesară lărgirea studiului pacienților în scopul definirii precise a utilității clinice a acesteia.

Introduction

Antineutrophil cytoplasmic antibodies (ANCA) were initially described in the serum of patients with Wegener granulomatosis. They are frequently correlated with the disease activity. These antibodies are directed against proteinase 3, a serin-protease with elastinolytic activites, found in azurophil granules of neutrophils and in monocyte lysosomes (1).

The perinuclear anti- neutrophil cytoplasmic antibodies (pANCA) were for the first time described in patients with inflammatory bowel diseases (IBD) and in those with primary sclerosing cholangitis in 1990 (2). This new immunologic marker has been found with increased frequency in patients with ulcerative colitis (UC) (up to 83%), but also in patients with Crohn's disease (CD) (15- 25%) and in those with primary sclerosing cholangitis (up to 88% if it associated an IBD, and 40% in its absence).

There is no correlation between pANCA titer and disease severity or disease extension. pANCA+ phenotype is correlated with the colonic extension in Crohn's disease. Colectomy does not affect the pANCA titer in CD or in UC (3). ANCA presence in a significant proportion of patients with Crohn's disease diminishes their diagnostic value in the differential diagnosis between UC and CD (4).

The aim of this study was to investigate the significance of ANCA in IBD in Romania.

Material and method

We performed a prospective study, comprising all patients with IBD admitted at the Center of Gastroenterology at Fundeni Clinical Institute, Bucharest, in 2000.

There were three groups of patients:

- CD group – 40 patients with CD;
- CU group - 33 patients with UC.
- Control group (C) - 22 healthy individuals, with similar age and sex distribution. (Statistical evaluation showed that this number of controls was valid for a comparison).

We used the following inclusion criteria: the diagnosis defined on endoscopic and histo-pathological and/or radiological criteria, and at least one year duration of the disease.

Excluded from the study were IBD patients on immunosuppressive therapy, which could have modified ANCA titer, although this is controversial (3,4).

All sera were obtained and preserved at minus 20^0C. Blinded sera were sent for serologic assay (ELISA) at Leuven University, in Belgium.

The pANCA assessment was performed by indirect immunofluorescence (IIF). Ethanol-fixated neutrophil slides were used. Sera were incubated at a 1:40 dilution for 30 minutes at room temperature. Fluorescein isothiocyanate-labeled rabbit antihuman IgG immunoglobulin was used. Slides were examined under UV using a Leitz Wetzler Orthoplan microscope. Sera that exhibited fluorescence on IIF titrated to endpoint and classified as perinuclear (pANCA) or cytoplasmic (cANCA). Interference by antinuclear antibodies, which may mimic the pANCA pattern, was ruled out by using formalin-fixed cells.

Titers of serum pANCA were assessed and the results were analyzed using Chi square test, with the aid of Epi Info 6.04 programs. We used the cut-off value: positive if ≥ 1/40. pn = perinuclear; pp = atypical pANCA.

Results

Study population

CD group

The gender distribution was the following: females 25/ males 15. The mean age (± SD) in this group was 37.4 ± 32 years. The distribution of patients after age at diagnosis was : age < 40 years: 67.5% (A1), age > 40 years: 32.5% (A2).

The distribution of patients regarding disease behavior is shown in Fig.1. The subgroups of Crohn's disease were defined according to Vienna classification (6,7).

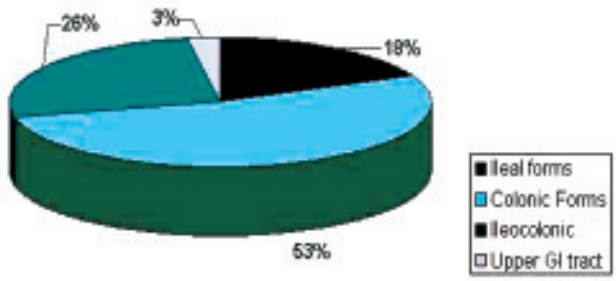

Fig.1 Distribution of patients with Crohn's disease after disease extension.

The activity of Crohn's disease (CDAI) was: median: 210 (min. 22 max. 500).

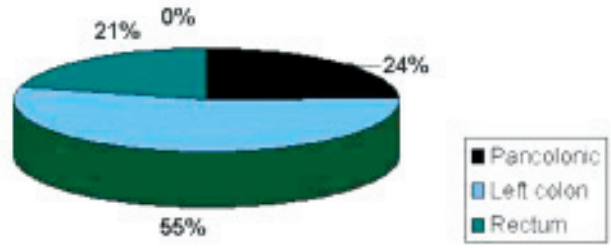

Fig.2 Distribution of patients with ulcerative colitis after disease extension.

UC group

The gender distribution was: females 17/males 16. The mean age (± SD) in this group was 36.5 ± 20 years.

The distribution of patients according to clinical severity (after Truelove) was: mild 66.6% ; moderate 24.2%, and high severity - 9.1% of cases. Regarding the evolutive pattern, this was: 9.1% - acute pattern, 87.9% - chronic remitting pattern, 2% - chronic continuing pattern. The distribution of patients after disease extension is illustrated in Fig.2.

Control (C) group

There were 11 females and 11 males, mean age (± SD) 40.6 ± 10 years.

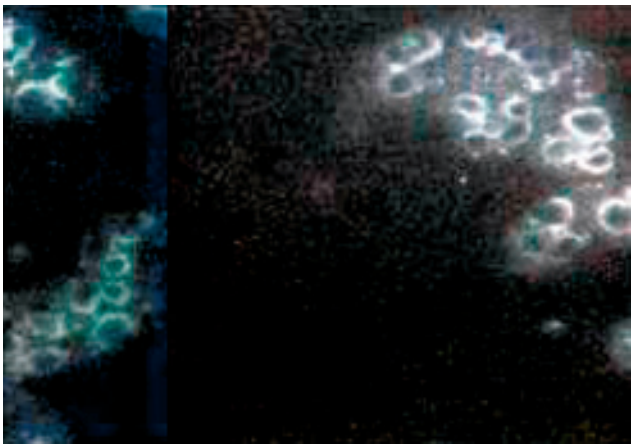

Fig.3 Typical positive perinuclear staining of neutrophils in immunofluorescence with a pANCA positive serum of a patient with ulcerative colitis.

Prevalence of pANCA

Perinuclear fluorescence of neutrophils (Fig.3) on cyto-centrifuge slides was found in 12 of 33 (36.4%) sera of patients with UC (Table I). Titers ranged from 1:40 to 1:160 (Fig. 4). In CD, only 6 of 40 patients had pANCA (15%). Most of the titers were 1: 40, but there was also a serum with a higher titer (1:320).

Table I Prevalence of pANCA in patients with UC, CD and in controls

	Patients (n)	pANCA positive (n)
UC (%)	33	12 (36.4%)
CD	40	6 (15%)
Controls	22	0

In controls, no serum exhibited pANCA activity (p= 0.004).

Performance of pANCA evaluation

Sensitivity of ANCA for the diagnosis of UC versus healthy controls was 36.4%, specificity was 100%. Positive predictive value of ANCA for the diagnosis of UC versus healthy controls was 100%, while negative predictive value was 61.13%.

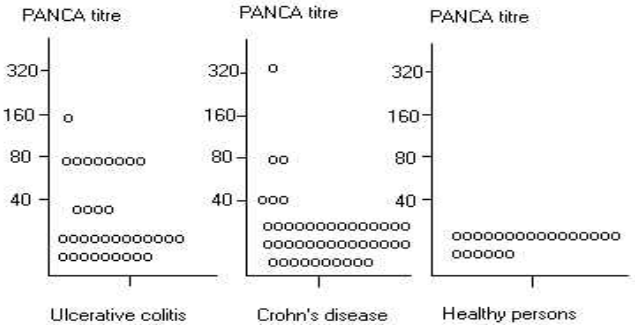

Fig.4 pANCA in patients with ulcerative colitis, Crohn's disease and in controls.

Sensitivity of ANCA for the diagnosis of UC versus CD was 36.4%, specificity was 85%.Positive predictive value of ANCA for the diagnosis of UC versus CD was 70.82%, while negative predictive value was 57.2%.

Correlation of pANCA with clinical features in UC

pANCA titer was more frequently positive in female patients (p = 0.04) (Table II).

Table II Prevalence of pANCA in patients with UC, with regard to gender

	Male patients n (%)	Female patients n (%)
pANCA+	3 (18.8%)	9 (52.9%)
pANCA -	13 (81.2%)	8 (47.1%)

pANCA positivity did not predict disease extension, severity, or evolutive form.

Patients with complications such as erythema nodosum or arthritis were pANCA negative, while one single case with pyoderma gangrenosum was pANCA positive, in a high titer (1:160).

Only one patient out of 33 with UC had undergone colectomy 3 years before the study, and he was pANCA negative.

Correlation of pANCA with clinical features in CD

In CD, pANCA positive titers were associated with ileocolonic and colonic extension of the disease (p=0.05). Severity of CD correlated well with pANCA+ titers: pANCA were positive for a median CDAI 315, and negative for a CDAI 200.

There was no statistically significant association between pANCA titer and gender, age at diagnosis, evolutive pattern of CD, or presence of complications.

Discussion

pANCA were reported in the literature with a variable prevalence in patients with UC ranging from 33% to 84% (1, 4-5, 8-16).

In Estonian patients (population with lower incidence of IBD compared to West-European studies), ANCA were detected in 29 of 59 cases (49%) with UC, in 4 of 17 (24%) cases with CD and in 4 of 111 (4%) healthy controls (5). The staining in IIF was predominantly perinuclear (pANCA). For both diseases, there was no correlation between ANCA presence and duration or extension of the disease. Positive sera were screened using ELISA method for anti-proteinase 3 antibodies, anti-myeloperoxidase antibodies, anti-lactoferrin antibodies and anti-catepsin G antibodies. Only 18% of sera (14 out of 76) tested positive. Anti-proteinase 3 antibodies were detected in 5 cases with UC and in one case with CD, mostly of cANCA type. Anti-myeloperoxidase antibodies were not detected. Anti-lactoferrin antibodies were found in 6 UC patients and one CD patient: one UC and one CD serum were pANCA positive, one UC had both ANCA types, while 2 were negative. Anti-catepsin G anti-bodies were detected in 3 sera of UC patients and 2 sera of CD patients: one UC serum and one CD serum were pANCA

positive. Three patients with UC and one with CD were ELISA positive for two different antigens (5).

In the study published by Colombel et al, the prevalence of ANCA in UC was 48%, in CD 7%, and in the control group 0. Almost all patients had pANCA, like in our study. The specificity of pANCA was not directed against either myeloperoxidase, proteinase 3 or lactoferrin in patients with IBD, IBD being thus associated with a new subset of pANCA. Preliminary results are indicating that this pANCA subset may be directed against catepsin G, a chymotrypsin-like protease, located inside the primary granules of neutrophils in some UC patients (16).

In our study, the prevalence of ANCA in UC was lower: 36.4% and pANCA+ titer did not predict disease extension, disease severity, and its evolutive form. These differences might be due to ethnic particularities, technical differences in preparing the antigen, or the use of different detection systems such as IIF or fixed neutrophil ELISA.

Our study also supports the finding that for both UC and sclerosing cholangitis, the ANCA prevalence and titer do not correlate with activity of the disease or with disease extension. A stimulating effect over in vitro neutrophil function was not reported for ANCA in UC (4,8,16).

Although pANCA do not contribute to disease pathogenesis, they are important markers, and not an epiphenomenon secondary to colonic inflammation. This fact was demonstrated by the high specificity of ANCA for UC, compared to infectious colitis and other types of colitis, by lack of relationship with IBD activity, and by ANCA persistence after colectomy (5).

We showed that the prevalence of pANCA in CD was 15%, and pANCA titer was related to disease severity, and colonic or ileocolonic extension.

pANCA assessment remains a research tool, and can not be recommended for routine clinical diagnosis. If we could identify the antigen specificity, the diagnostic potential may be improved. One potential clinical use for ANCA might be the prediction of pouchitis in patients with ileal-pouch anal anastomosis (17).

ANCA are probably a marker of the immunologic disturbance which underlies UC and sclerosing cholangitis. More important, ANCA may be a marker of genetic susceptibility for UC and for sclerosing cholangitis. This was first proved by the increased prevalence of ANCA in healthy, not affected relatives of patients with UC. pANCA were found in 25-30% of the relatives of patients with UC or sclerosing cholangitis (8).

Conclusion

pANCA prevalence in UC Romanian patients was lower in comparison with other studies (36.4% versus 50-80%) The highest prevalence was found in females with UC. In CD, pANCA+ is associated with a higher disease severity.

References

1. Dalekos GN, Manoussakis MN, Goussia AC, Tsianos EV, Moutsopoulos HM. Soluble interleukin-2 receptors, anti-neutrophil cytoplasmic antibodies, and other autoantibodies in patients with ulcerative colitis. Gut 1993; 34: 658-664.
2. Saxon A, Shanahan F, Landers C, Ganz T, Targan SR. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. J Allergy Clin Immunol 1990; 86: 202-210
3. Shanahan F. Neutrophil autoantibodies in inflammatory bowel disease: are they important? Gastroenterology 1994;107: 586-589
4. Bansi DS, Fleming KA, Chapman RW. Importance of antineutrophil cytoplasmic antibodies in primary sclerosing cholangitis and ulcerative colitis: prevalence, titre and IgG subclass. Gut 1996; 38:384-389
5. Kull K, Salupere R, Uibo R, Ots M, Salupere V. Antineutrophil cytoplasmic antibodies in Estonian patients with inflammatory bowel disease. Prevalence and diagnostic role. Hepato-gastroenterology 1998; 45: 2132- 2137.
6. Sachar DB, Andrews HA, Farmer RG, et al. Proposed classification of patient subgroups in Crohn's disease. Gastroenterol Int 1992:5.
7. Gasche C, Scholmerich J, Brynskov J, et al. A simple classification of Crohn's disease : report of the Working Party for the World Congress of Gastroenterology, Vienna 1998. Inflamm Bowel Dis 2000; 6: 8-15.
8. Seibold F, Weber P, Klein R, Berg PA, Wiedmann KH. Clinical significance of antibodies against neutrophils in patients with inflammatory bowel disease and primary sclerosing colangitis. Gut 1992; 33: 657-662.
9. Duclos B. Maladie de Crohn, rectocolite hemorragique: des anticorps et des hommes. Gastroenterol Clin Biol 1995; 19: 865- 866.
10. Quinton J-F, Sendid B, Reumaux D, et al. Anti- Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic antibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut 1998; 42: 788- 791.
11. Ruemmele FM, Targan SR, Levy G, et al. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. Gastroenterology 1998; 115: 822-829.
12. Hoffenberg EJ, Fidanza S, Sauaia A. Serologic testing for inflammatory bowel disease.J Pediatr 1999;134: 447-452.
13. Shanahan F, Duerr RH, Rotter JI, et al. Neutrophil antibodies in ulcerative colitis: familial aggregation and genetic heterogeneity. Gastroenterology 1992; 103: 456-461
14. Duerr RH, Targan SR, Landers CJ, et al. Anti-neutrophil cytoplasmic antibodies in ulcerative colitis. Comparison with other colitides/ diarrheal illnesses. Gastroenterology 1991; 100:1590-1596
15. Yang P, Jarnerot G, Danielsson D, Tysk C, Lindberg E. p-ANCA in monozygotic twins with inflammatory bowel disease. Gut 1995; 36: 887-890
16. Colombel JF, Reumaux D, Duthilleul P, et al. Antineutrophil cytoplasmic antibodies in inflammatory bowel diseases. Gastroenterol Clin Biol 1992; 16: 656-660.
17. Fleshner PR, Vasiliauskas EA, Kam LY, et al. High level perinuclear antineutrophil cytoplasmic antibody (pANCA) in ulcerative colitis patients before colectomy predicts the development of chronic pouchitis after ileal pouch-anal anastomosis. Gut 2001;49:671-677.