Spectrum of ANCA-Associated Disorders According to Serological Phenotype in Routine Care: Retrospective Case Series of 209 Patients

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Abstract: *Objective*: To summarize the experience of three years of positive ANCA (anti-neutrophil cytoplasmic antibodies) testing in a single university based hospital. We describe the clinical features according to ANCA phenotype of patients who did and did not have ANCA- associated vasculitis (AAV).

Methods: We did a review of all samples tested for ANCA in a 3 year-period (2005-2007). Each sample was tested by indirect immunofluorescence (IIF) and enzyme-linked-immunosorbent assay (ELISA). Sera were considered as positive for ANCA testing if either IIF or ELISA for MPO or PR3 antigen specificity was positive. Patients were considered as having AAV on established diagnostic criteria and algorithms.

Results: The positive ANCA population consisted in 209 patients, 54 were classified in the AAV group and 155 patients constituted the "Others" group. The typically most relevant ANCA phenotypes (C-ANCA/anti-PR3+ and P-ANCA/anti-MPO+) were detected in 90 % (49/54) of patients in the AAV group and only 10% (15/155) of the "Others" group ($p < 0.001$). Among the latter none developed AAV during follow-up. Positive IIF alone was found in 4% (2/54) of the AAV group and in 68% (105/155) of the "Others" group ($p < 0.001$). In patients without AAV, positive IIF alone or positive ELISA with negative IIF represented the main ANCA pattern.

Conclusion: In routine clinical practice, most patients with positive ANCA testing do not have AAV. The typical ANCA pattern (C-ANCA/anti-PR3+ or P-ANCA/anti-MPO+) remains a strong predictor of AAV in patients with a high level of suspicion for systemic vasculitis. In other cases, ANCA positivity should be interpreted with extreme caution.

Keywords: ANCA, vasculitis, anti-MPO, anti-PR3, IIF, ELISA, ANCA associated vasculitides.

INTRODUCTION

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed against constituents of primary granules of neutrophils and monocytes' lysosomes. ANCA are strongly associated with small vessel vasculitides: Granulomatosis with Polyangeitis or Wegener's granulomatosis (GPA/WG), Microscopic Polyangiitis (MPA), Eosinophilic Granulomatosis with Polyangiitis or Churg & Strauss syndrome (EGPA/CSS) and idiopathic rapidly progressive glomerulonephritis (iRPGN) [1], diseases commonly referred as "ANCA associated vasculitides" (AAV) [2]. ANCA are not only a serologic marker but could be a major pathogenic factor for pauci-immune small vessel vasculitis [2-4]. Besides being a helpful diagnostic tool for physicians, determination of ANCA levels can also be important for monitoring disease activity, since some studies have shown that relapses are preceded by rises in ANCA levels [5-7]. ANCA testing is now widely ordered in routine clinical practice as a screening tool for the diagnosis of small vessel vasculitis. However if a positive ANCA testing has a high positive predictive value for AAV when used in appropriate situations [8], testing in routine clinical care also has a number of limitations. First, ANCA were until recently not included in the most used classification systems for vasculitides - the 1990 American college of Rheumatology criteria (ACR) [9] and the 1994 Chapel Hill (CCH) consensus definitions [10], which rely both on the result of clinical and pathological investigations for diagnosis of small vessel vasculitides. ANCA are now taken into account in the revised international Chapel Hill consensus conference nomenclature of vasculitides [11]. Secondly, not all patients with small vessel vasculitis have ANCA [12, 13]. Thirdly, ANCA are also found in non vasculitic diseases, such as inflammatory bowel diseases [14], various rheumatic diseases [15], and even in healthy individuals [16]. Consequently, the Consensus Guidelines for ANCA has recommended

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that ANCA testing should only be carried out in patients with a clinical history suggestive of GPA/WG or MPA, in order to improve diagnostic accuracy [17]. Thus when used in patients with a suggestive clinical history of small vessel vasculitis, including rapidly progressive glomerulonephritis, pulmonary hemorrhage, cutaneous vasculitis with systemic features, multiple lung nodules, chronic sinusitis, subglottic tracheal stenosis, mononeuritis multiplex, or retro orbital mass, positive ANCA testing may be legitimately used as a substitute for histological features of vasculitis [18]. At the opposite, the discovery of positive ANCA testing in patients without sufficient clinical evidence for small vessel vasculitis raises several questions. What is the clinical significance of positive ANCA testing in such patients? Will some patients with ANCA later develop vasculitis ? Can positive ANCA mislead the clinicians in some cases? What is the spectrum of ANCA-associated disorders beside AAV? Can the ANCA "phenotype" results (combination of indirect immunofluorescence [IIF] and enzyme-linked-immunosorbent assay [ELISA] testing) help? The aim of our study was to summarize the experience of three years of positive ANCA testing in a single university based hospital. We analysed biological and clinical features according to ANCA phenotype of patients with ANCA who did and did not have AAV, according to the current classification criteria.

PATIENTS AND METHODS

A retrospective review of all samples submitted to a single university hospital laboratory for ANCA testing during a 3 year-period (January 2005 to December 2007) was performed. As done routinely in this laboratory, each sample was systematically tested by two standard methods, according to the international guidelines: indirect immunofluorescence (IIF) on ethanol fixed neutrophils (*ANCA kit [ethanol fixed] Binding site, Birmingham, United Kingdom*) and enzyme-linked-immunosorbent assay (ELISA) for PR3 and MPO antigens specificities testing (*anti-MPO, anti-PR3 ELISA [IgG] Euroimmun AG, Lûbeck Germany*). All IIF were read by the same biologist (LT). Positive IIF sera were classified according to three immunostaining patterns: cytoplasmic pattern (C-ANCA) characterised by granular cytoplasmic fluorescence (closely correlated with protease 3 [PR3] antigen); perinuclear pattern (P-ANCA) characterised by perinuclear fluorescence (closely correlated with myeloperoxydase [MPO] antigen); and atypical ANCA pattern (Atyp-ANCA) which includes all other positive IIF, most often the combination of cytoplasmic and perinuclear staining (associated with miscellaneous antigens specificities). The intensity of fluorescence was graded semi-quantitatively (1+ to 3+). In ELISA testing, sera were considered positive if results were superior to the cut-off value established by the manufacturer (20 UR/ml). For IIF positive sera without MPO or PR3 antigens specificities in ELISA testing, other unusual antigens specificities (Lactoferrin, Elastase, Bactericidal Permeability Increasing protein [BPI] and Cathepsin G) were searched for by a semi-quantitative ELISA technique (*ANCA-profile ELISA [IgG] Euroimmun AG, Lübeck, Germany*).

Patients were considered as positive for ANCA testing if either IIF or ELISA for MPO or PR3 antigens specificities was positive. Patients with at least one positive testing and aged over than 15 years were first selected for entry in the study. Their medical records were then reviewed and when available the reason for testing was recorded. The final clinical diagnosis was retained by consensus between at least two experienced clinicians. To avoid the influence of immunosuppressive treatments on ANCA testing results, ANCA phenotype retained for classification for each patient was based on the result obtained at the onset of the disease and before treatment.

The positive ANCA population was then divided in two groups: ANCA-associated vasculitides (AAV) and "Others". Patients with AAV were selected and classified according to the algorithm developed by Watts *et al.* [18] in the following subgroups: EGPA/CSS, GPA/WG, and MPA. This algorithm has been designed for epidemiological purpose. Its aim is to categorize patients with AAV and polyarteritis nodosa (PAN) into single relevant categories, using ACR and Lanham classification criteria, Chapel Hill consensus definitions, ANCA status, and "surrogate" clinical and biological markers for GPA/WG and MPA, leaving as less as possible cases of small vessel vasculitis unclassifiable. "Surrogate" markers allow the inclusion of "limited" or "localized" GPA/WG (such as chronic sinusitis or retro-orbital pseudotumor) when there is no histology but positive ELISA serology for PR3 or MPO. In this algorithm, iRPGN is also classified in the MPA subgroup, as "renal-limited" vasculitis. The remaining patients, without ANCA associated vasculitides, composed the second group labeled "Others". We further divided this group according to clinical categories following Bosh *et al*. [1]: digestive disorders, connective tissue diseases, non systemic vasculitides, systemic vasculitides not typically associated with ANCA, malignancies, infectious

diseases, renal disorders, and miscellaneous disorders.

Results are reported as mean, median and interquartile range (IQR) or number and percentage (%). Categorical variables were compared using Fisher's exact test and continuous variables using the nonparametric Wilcoxon test or the Mann-Whitney test for pairwise comparisons. To determine how well "typical" ANCA phenotype (association of C-ANCA/anti-PR3+ or P-ANCA/anti-MPO+) when opposed to discordant phenotype, was able to predict occurrence of AAV, we plotted the proportion of true positives against the proportion of false positives, depending on the prediction rule used to classify patients as having a small vessel vasculitis. A 2 × 2 table was established to determine sensitivity and specificity of typical ANCA phenotype for diagnosing AAV in the subgroup of patients with any positive ANCA testing. The positive and negative likelihood ratios were computed. All tests were two-sided, and P values <0.05 were considered statistically significant. Statistical tests were performed with the SPSS 13 software package.

RESULTS

Demographic Features and ANCA Phenotypes

During the three years of the study period, 3135 tests were performed, of these 362 (12%) were positive. The positive ANCA population consisted in 234 patients, of which 25 were excluded because there records were unavailable since they were managed outside the university hospital, leading to 209 patients studied (111 female and 98 male). Mean age was 60, median age was 63 (range 16-87). Patients with positive ANCA originated mainly from the departments of nephrology (102/209; 49%) and internal medicine (41/209; 20%). Fifty four patients (26 %) were classified in the AAV group according to the criteria described above (27 male and 27 female). Mean age was 63, median age 66 (range 15-83). The "Others" group consisted in 155 patients (74%): 84 female and 71 male, mean ages 59, median age 61 (range 16-87). The gender and age distributions were not statistically different between AAV and "Others" groups.

Venn diagram of IIF and ELISA testing results of the ANCA positive population is presented in Figure **1**. Ten different ANCA phenotypes were identified in the studied population according to the combination of IIF and ELISA results. Their distribution in each group is presented in Table **1**.

Among 179 patients (86% of total population) with positive IIF, 52 (29%) had AAV. Among 102 patients (49 % of total population) with positive ELISA, 52 (51%) had AAV. The typically most relevant ANCA phenotypes (C-ANCA/anti-PR3+ or P-ANCA/anti-MPO+) represented 90 % (49/54) of the AAV group but

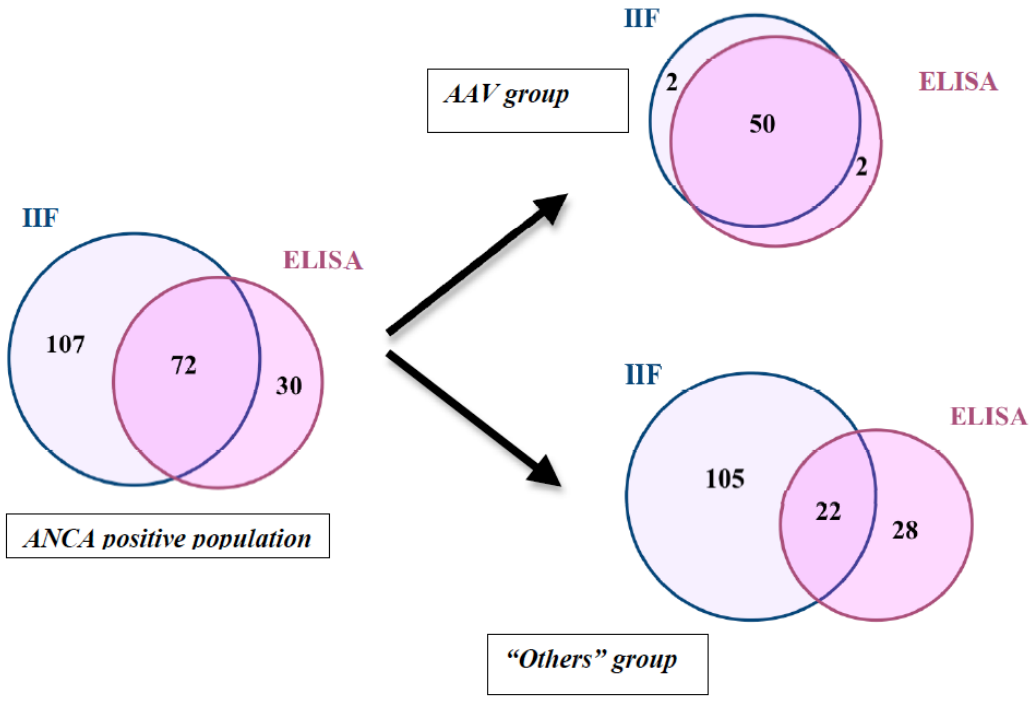

Figure 1: Venn diagram of IIF and ELISA testing in ANCA positive population and AAV and "Others" group.

Table 1: ANCA Phenotypes in AAV and "Others"

ANCA phenotype	All Patients	AAV	Others
	N=209	N=54 (%)	N=155 (%)
C-ANCA/anti-PR3+	22	18 (32)	4 (3)
C-ANCA/ELISA-	20	2 (4)	18 (12)
P-ANCA/anti-MPO+	42	31 (57)	11(7)
P-ANCA/ELISA-	63	0	63 (41)
Atyp-ANCA/anti-PR3+	5	0	5 (3)
Atyp-ANCA/anti-MPO+	3	1(2)	2 (1)
Atyp-ANCA/ELISA-	24	0	24(15)
IIF-/anti-PR3+	13	1(2)	12(8)
IIF-/anti-MPO+	12	0	12(8)
IIF-/anti-PR3+anti-MPO+	5	1(2)	4(3)

ELISA- denotes the absence of anti-PR3 and anti-MPO antibodies.

only 10% (15/155) of the "Others" group ($p < 0.001$). In this setting, the positive predictive value for AAV of these "typical" phenotypes was then 77%, whereas its negative predictive value was 96%. The sensitivity and specificity for AAV of the phenotype (C-ANCA/anti-PR3+ or P-ANCA/anti-MPO+) in this population of ANCA positive patients were both of 90%. Positive IIF with C-ANCA or P-ANCA pattern but without anti-PR3 or anti-MPO antibodies in ELISA testing was found in 4% (2/54) of the AAV group and in 52% (81/155) of the "Others" group ($p < 0.001$). No patient with C-ANCA pattern had anti-MPO antibodies in ELISA and none of the patients with P-ANCA had anti-PR3 antibodies. Less typical phenotypes (such as Atyp-ANCA alone, positive anti-PR3 and/or anti-MPO associated with Atyp-ANCA pattern or negative IIF) represented 6% (3/54) of the AAV group and 38 % (59 /155) of "Others" group ($p < 0.001$). Among 97 cases with positive ELISA for anti-PR3 or anti-MPO, 51 had AAV and 46 belonged to the "Others" group. Values of anti-PR3 or anti-MPO antibodies were significantly higher in the AAV group (Figure **2**; test U Mann-Whitney, $p < 0.0001$).

ANCA Associated Vasculitis (AAV) Group

The AAV group was composed of 54 patients, 27 GPA/WG (including 5 localized forms: two patients with retro-orbital mass, one with subglottic stenosis and two others with chronic sinusitis), 25 MPA (including 10 iRPGN) and 2 EGPA/CSS. Twenty four cases (44%) fulfilled the ACR criteria, 34 (63%) fulfilled the Chapel Hill criteria, 8 (15%) both. For 43 patients (80% of the AAV group) diagnoses were confirmed by pathologic findings: 38 cases with a crescentic glomerulonephritis at renal biopsy and among them one with interstitial granuloma, 4 lung biopsies showing neutrophilic vasculitis and fibrinoid necrosis, associated for 2 of them with epithelioid granuloma, and one sinus biopsy showing extravascular necrotizing granuloma. Reasons for ANCA testing in AAV patients are presented in Table **2**.

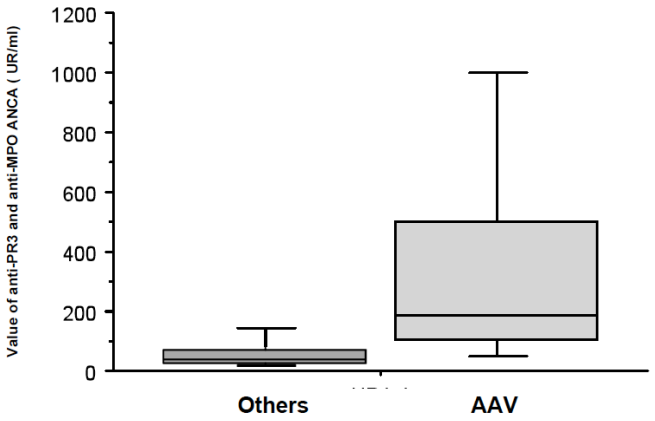

Figure 2: Boxplot illustration of values of anti-PR3 and anti-MPO in AAV (51 patients) and "Others" group (46 patients) (P<0,0001).

Table 2: Reasons for ANCA Testing in AAV Patients

Reasons for testing	No
rapidly progressive glomerulonephritis	38
pulmonary hemorrhage	15
cutaneous vasculitis	3
multiple lung nodules	7
chronic sinusitis, otitis	8
subglottic tracheal stenosis	1
mononeuritis multiplex	7
retro-orbital mass	2

ANCA phenotypes in patients with AAV are detailed in Table **3**. The ANCA phenotype of GPA/WG group was C-ANCA/anti-PR3+ for 18/27 (67%) patients, and P-ANCA/anti-MPO+ for 7/27 (26%). No GPA/WG case was negative in ELISA at disease onset. Two GPA/WG were IIF negative at onset but had high levels of anti-PR3 by ELISA and in both cases subsequent IIF tests also showed C-ANCA. MPA was diagnosed in 25 patients. Twenty two MPA cases (88%) were P-ANCA/anti-MPO+. Only two MPA cases were negative in ELISA at onset, for one of them a later test became positive and for the other, anti-elastase antibodies were found. One case with MPA was Atyp-ANCA/anti-MPO+ at onset became P-ANCA/anti-MPO+ later. The two patients with a diagnosis of EGPA/CSS were P-ANCA/anti-MPO+.

"Others" Group

One hundred and fifty five patients without criteria of AAV constituted the "Others" group. This population was highly heterogeneous in terms of diagnose and

Table 3: ANCA Phenotype in AAV Cases

IIF	No	intensity	No	ELISA	WG	MPA	CSS
C-ANCA	20	+	6	anti-PR3+	6		
				anti-MPO+			
				anti-PR3-/anti-MPO-			
		++	5	anti-PR3+	5		
				anti-MPO+			
				anti-PR3-/MPO-			
		+++	9	anti-PR3+	7		
				anti-MPO+			
				anti-PR3-/MPO-		2	
P-ANCA	31	+	6	anti-MPO+	2	3	1
				anti-PR3+			
				anti-PR3-/anti-MPO-			
		++	11	anti-MPO+	3	7	1
				anti-PR3+			
				anti-PR3-/anti-MPO-			
		+++	14	anti-MPO+	2	12	
				anti-PR3+			
				anti-PR3-/anti-MPO-			
Atyp-ANCA	1	+	1	anti-MPO+		1	
				anti-PR3+			
				anti-PR3-/anti-MPO-			
		++	0	anti-MPO+			
				anti-PR3+			
				anti-PR3-/anti-MPO-			
		+++	0	anti-MPO+			
				anti-PR3+			
				anti-PR3-/anti-MPO-			
IIF -	2		2	anti-MPO+			
				anti-PR3+	1		
				anti-PR3+/anti-MPO+	1		
TOTAL	54		54		27	25	2

ANCA phenotypes. We classified the cases in eight subgroups according to the final medical diagnosis retained after reviewing the medical records: (1) *digestive disorders*: 11 patients (7% of patients of "Others" group) of whom 5 had ulcerative colitis and 4 autoimmune hepatitis or primary biliary cirrhosis; (2) *connective tissue diseases*: 29 patients (19%) of whom 16 has systemic lupus erythematosus, 7 rheumatoid arthritis, and 3 primary Sjögren's syndrome; (3) *non systemic vasculitides* (localized vasculitides without systemic involvement): 6 patients (4%) of whom 5 had isolated cutaneous vasculitis and 1 non systemic vasculitic neuropathy; (4) *systemic vasculitides other than ANCA-associated systemic vasculitides*: 6 patients (4%) including 3 cases of giant cell arteritis; (5) *malignancies*: 5 patients (3%); (6) *infectious diseases*: 23 patients (15%); (7) *renal disorders*: 40 patients (26%); (8) *miscellaneous disorders*: 35 patients (23%). Only few unambiguous clinical indications for ANCA testing were found in this group, the two main reasons for testing being arthralgia and acute renal failure (Table **4**).

Table 4: Reasons for ANCA Testing in "Others" Group

Reasons for testing	No
rapidly progressive glomerulonephritis	2
pulmonary hemorrhage	0
cutaneous vasculitis	7
multiple lung nodules	0
chronic sinusitis, otitis	2
subglottic tracheal stenosis	0
mononeuritits multiplex	4
retro-orbital mass	0
acute renal failure	49
arthralgia	36
unexplained inflammatory syndrome	25
central neurological disturbances	14
weight loss	12
respiratory distress	11
routine follow-up (patients with renal graft)	8
eye disorders	7
digestive disorders	4
unknown	5

In the "Others" group, only *15 patients (10%) had the "typical" phenotype of AAV:* 4 patients (3%) were C-ANCA/anti-PR3+ and 11 patients (7%) were P-ANCA/anti-MPO+. Of these 15 patients, 10 had two or more positive IIF and ELISA testing and the value of ELISA was higher than 100 UR/ml for 6 patients. Two patients were initially considered as having AAV on the result of ANCA testing but clinical features were judged insufficient to retain this diagnosis after a thorough review of their clinical records.

One hundred and five patients (68% of the "Others" group) had a positive IIF without anti-PR3 or anti-MPO in ELISA testing. Eighteen patients were C-ANCA/anti-PR3-, mostly suffering from infectious and renal disorders, of them 3 patients were positive for anti-BPI. Sixty three patients (41%) were P-ANCA/anti-MPO-, of them 53 patients were tested for characterization of other antigens specificities. Anti-cathepsin G antibodies were found in one patient, and anti-elastase antibodies in three others. Anti-nuclear antibodies (ANA) were also searched in 61 patients, and were found in 39 patients (64% of the P-ANCA/anti-MPO-population tested for ANA). This subgroup with P-ANCA/anti-MPO- and positive ANA which likely represents a "false positive" of the IIF technique was composed of patients with digestive diseases, connective tissue diseases and miscellaneous renal disorders. Thirty one patients had Atyp-ANCA, and for 11 of them, antigens specificities were found: 7 were either positive in anti-PR3 or anti-MPO antibodies, one had anti-PR3 and anti-lactoferrin antibodies, one patient had anti-cathepsin G antibodies, and three patients anti-BPI antibodies. We considered that none of these patients had vasculitis. Among the 20 patients having Atyp-ANCA without positive ELISA testing, 11 were positive for ANA.

Twenty eight patients (18% of the "Others" group) had negative IIF, of which 12 had positive anti-PR3 antibodies, 12 positive anti-MPO antibodies, and 4 had both. This group consisted in patients with heterogeneous disorders including infectious or renal disorders and malignancies. Most of them had only one ANCA testing, with low value of ELISA, and none of them developed systemic vasculitis during follow-up. One patient with chronic aortic dissection had anti-cathepsin G, anti-BPI, anti-elastase, anti-PR3 and anti-MPO antibodies.

DISCUSSION

Strenths and Limits of the Study

The aim of our study was to describe the characteristics of a large population of patients with positive ANCA found in the routine clinical practice of a university hospital, and to provide an overview of clinical cases without vasculitic disorders and/or

atypical ANCA phenotypes. We retrieved cases from a single laboratory, and all IIF were read by the same biologist, thus increasing the reliability of IIF method, which is dependent on individual interpretation, as opposed to ELISA testing which relies on a reproducible technique. Although we used a retrospective design, diagnoses were ascertained from a thorough chart review taking in account follow up data when available. The diagnosis of AAV was retained according to a precise and consensual algorithm [18]. However, our study has several limitations. First, we must admit that in the "Others" group, despite a rigorous chart review, some patients may actually have had unrecognized small vessel vasculitis. Second, we acknowledge that the precise reason for ANCA testing cannot be correctly recorded in a retrospective study. Third, and despite our attempt, we cannot assert that we have delimited the wide spectrum of ANCA associated disorders. For example, no case of propylthiouracil- or carbimazole-induced ANCA vasculitis was found in our series, even if one third of patients with hyperthyroidism treated with such drugs have ANCA [19]. Conversely, we must recognize a bias toward the overrepresentation of patients with renal disease, due to the fact that ANCA testing is part of routine investigations of renal failure in the department of Nephrology.

Main Results

Our results show that in routine clinical practice the majority of ANCA positive patients do not have and won't develop AAV. They confirm that the "typical" phenotype (C-ANCA/anti-PR3+ or P-ANCA/anti-MPO+) has a high positive predictive value for AAV and that the absence of these phenotypes carries a high negative predictive value against AAV. Most of the patients without AAV present with the phenotype IIF-/ELISA+ or IIF+/ELISA-. When ELISA is positive without IIF, ELISA titres can be helpful in distinguishing subjects with or without AAV, since we have shown that they are significantly higher in the AAV group than in "Others" patients.

ANCA Testing

ANCA testing provides a simple tool to support the diagnosis of vasculitis. However, in routine clinical practice, an overwhelming proportion of samples submitted for ANCA testing turns out to be negative [20-23]. In our study, only 12% of samples tested were positive. Most of clinical studies on ANCA have been made to evaluate the diagnostic performance of ANCA for idiopathic vasculitides [24, 25] or to describe the clinical spectrum and prognosis of AAV according to ANCA phenotypes and titres [5, 20]. If numerous studies report cases of ANCA positive patients without AAV in their study population [21, 26-29], and others the incidence of positive ANCA in non AAV disease [15, 30-32], few studies used the ANCA serology as entry point for analysis [22, 23, 33-35]. Furthermore, in many of the early ANCA studies, IIF was the only method utilized. Besides ours, few studies have provided a complete description of a positive ANCA population determined by the two recognized methods of ANCA testing (IIF and ELISA), irrespective of the final diagnosis [22, 34]. In such studies, the ANCA positive populations are dependent on the ANCA detection methods [36, 37] and on the population in which the tests were applied. IIF method is known to have higher sensitivity than ELISA testing [19, 21] but IIF assays are not antigen specific and their interpretation depends on the subjective analysis of fluorescence pattern. Ten per 100 of ANCA positive serum samples in patients with GPA/WG or MPA can be demonstrated only by IIF and 5% of serum samples are positive only in ELISA [17]. The international consensus statement on testing and reporting of ANCA [17], and a metaanalysis of the diagnostic performance on ANCA testing [23] suggest that the combination of IIF and ELISA testing for PR3 and MPO should be performed on all samples. The combination of these two methods leads to a specificity for AAV of 99% and a sensitivity of 73% for GPA/WG and 67% for MPA for the combinations C-ANCA/anti-PR3 or P-ANCA/anti-MPO [24]. Although we chose to test systematically by the two methods all sera in our laboratory, some studies have suggested that neither sensibility nor specificity was improved if ELISA and IIF testing were applied to every samples compared to using ELISA alone [28, 38].

Prevalence of AAV in ANCA Positive Patients

We have reviewed the medical records of all patients in which ANCA were detected in a 3-year period and found that only 54 patients (26%) had one of the AAV. In studies with a similar design, Edgar *et al.* found 27% of AAV in a series of 301 ANCA positive patients [34], only 15% of AAV were found in a Spanish series [33], 12.5% in a Tunisian series [35], 33% in a Slovenian series [22], and 20.5% in a Greek series [23]. Discordantly, AAV was considered as present in 85% of ANCA positive Chinese patients, but this finding relied only on the request forms received by the laboratory [37]. We chose to select the patients with AAV according to the algorithm proposed by Watts *et*

al. [18], in which positive ANCA allow the classification of cases with localized forms of vasculitis without histological proof as AAV cases. We found that in routine clinical practice the "typical" phenotype (C-ANCA/anti-PR3+ or P-ANCA/anti MPO+) had a positive predictive value for AAV of 77%, and a negative predictive value of 96%. Strictly speaking, we should not have calculates sensitivity, specificity and predictive values of ANCA positivity, since ANCA testing was included in the gold standard for classification of cases in the AAV group. However, ANCA contributed to the final diagnosis in only 4 patients (7 %) of the group ("localized" GPA/WG without histological proof). The combination of IIF and ELISA positivity is strongly suggestive for AAV in ANCA positive patients with positive predictive value ranging from 75 to 88% [21, 34]. Conversely, the absence of the "typical" phenotype is a fair argument to exclude ANCA associated vasculitis. In our study, only five patients in the AAV group (9%) did not have the "typical" phenotype, which is consistent with other studies (15% for Stone *et al.* [21], 3% for Pradhan *et al.* [27], 8% for Calvo Romero *et al.* [33]).

ANCA Associated Vasculitis (AAV) Group

We found that 26% of GPA/WG had anti-MPO antibodies, which is slightly higher than in others reports (24% for Hagen *et al.* [25], 13% for the WG etanercept group [40], 11% for Vizjak *et al.* [22]). In our study, no MPA had anti-PR3 and 92 % had anti-MPO (27% and 58% for Hagen *et al.* [25], 6% and 83% for Vizjak *et al.* [22] respectively). Even if ANCA are less frequently found in localized forms of GPA/WG [40], we found 5 such cases in our series, one with anti-MPO antibodies, four others with anti-PR3. All these patients received immunosuppressive treatment but none of them became ANCA negative during follow up. In this subgroup, ANCA testing contributed to the diagnosis except for a patient with subglottic stenosis for whom a pathological confirmation was obtained.

ANCA Positive Patients who do not have AAV

In our study, they represented 74% of ANCA positive patients (the "Others" group), mainly composed of patients with renal disorders (26%), connective tissue diseases (19%) and miscellaneous disorders (35%). Most of these patients (133/155: 86%) were positive for ANCA testing by only one method: 108 (68%) were only positive in IIF (among them 24 have atypical ANCA), 28 (18%) were only positive in ELISA. Moreover many ANCA tests were requested by the clinicians for patients without clinical features suggestive of AAV [17]. The restriction of prescription to patients meeting the 1999 guidelines for ANCA testing aims to decrease the number of tests without missing cases of AAV and to increase the specificity of ANCA testing [8, 41, 42]. A strict obedience to these guidelines would have reduced ANCA testing from 155 to 15 in this group.

In ANCA positive patients without AAV, the "typical" phenotype (C-ANCA/anti-PR3+ or P-ANCA/anti-MPO+) was observed in only 15 patients, and none of these developed AAV with a median follow up of 35 months. Although C-ANCA/anti-PR3+ is considered as a highly specific features of AAV [28], 4 patients of the "Others" group were C-ANCA/anti-PR3+ but only one of them could have had AAV. One other had been exposed to silica known to induce ANCA and sometimes vasculitis [43]. One had Sjögren's syndrome, which has been associated with anti-PR3 antibodies in some cases [21, 44]. Two retrospective studies retrieved respectively 18 and 19 patients with C-ANCA/anti-PR3+ without AAV, only one of these patients having later developed systemic vasculitis [44, 45]. Prolonged infections such as bacterial endocarditis may mimic AAV, and many of such cases have C-ANCA/anti-PR3+ [46]. We found 11 P-ANCA/anti-MPO+ patients without AAV suffering from miscellaneous disorders. Russell *et al.* [28] described 18 such patients with non necrotising vasculitides, renal disorders and connective tissue diseases.

In IIF method, ANA can be misinterpreted as P-ANCA because of perinuclear fluorescence. To avoid this pitfall, it is recommended to test PR3 and MPO antigens by ELISA [47], and to perform IIF on formalin fixed slides [48]. In the routine practice of our laboratory, samples were not tested on formalin-fixed neutrophil substrate. As a result, among the 63 patients with P-ANCA positivity alone, 39 patients positive for ANA at least could be considered as "false positive" cases and most suffered from connective tissue diseases. In a large cohort of connective tissue diseases, P-ANCA were always associated with the presence of ANA [32]. C-ANCA pattern in IIF is believed to be highly specific for GPA/WG [49]. However, in our study, 90% of C-ANCA were found in the non AAV group, mainly in infectious diseases and in various renal disorders. As described in the literature [47], we found atypical ANCA pattern in IIF in inflammatory bowel disease patients and other autoimmune diseases, mainly systemic lupus erythematosus. Atypical ANCA pattern can be also induced by high level of ANA.

In conclusion, if ANCA testing remains an unequally useful tool in the diagnostic and monitoring of patients with small vessel vasculitides, we must be aware of the high number of other diseases which can be associated with positive ANCA. Further prospective studies are needed to determine the usefulness of ANCA testing in disorders such as specific infections [45], and the clinical relevance of antibodies directed toward other antigens than PR3 and MPO.

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