Prevalence of Anti-Neutrophil Cytoplasmic Antibodies and Associated Vasculitis in COPD Associated With Alpha-1 Antitrypsin Deficiency

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Prevalence of anti-neutrophil cytoplasmic antibodies and associated vasculitis in chronic obstructive pulmonary disease associated with alpha-1-antitrypsin deficiency: an ancillary study to a prospective study on 180 French patients

Running head: ANCA among alpha-1-antitrypsin deficiency patients

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Alpha-1-antitrypsin (AAT), encoded by the *SERPINA1* gene, is a serine protease inhibitor that has several properties, including the inactivation of neutrophil elastase and proteinase 3 (PR3). In the case of pathogenic mutations, posttranslational folding is affected, which is responsible for the self-association of AAT proteins that become nonfunctional. The PI*S and PI*Z alleles are the most prevalent molecular defects, the respective frequencies of which range from 5-10% and 1-3% in Caucasian populations.[1] AAT deficiency has been associated with chronic obstructive pulmonary disease (COPD) with emphysema and/or bronchiectasis, hepatic cirrhosis or cutaneous panniculitis, as well as systemic inflammatory/autoimmune disorders such as anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (granulomatosis with polyangiitis, GPA, and microscopic polyangiitis, MPA).[1]

Indeed, several studies have shown an overrepresentation of deficient AAT alleles among GPA and MPA patients, with PI*S and PI*Z allele frequencies ranging from 6-10% and 7-8% in France, respectively.[2] The clinical presentation is similar in patients suffering from ANCA-associated vasculitis with or without deficient AAT alleles, although an increased risk of intra-alveolar hemorrhage has been described in case of AAT deficiency.[2] However, very few studies have approached the problem from another perspective by looking for ANCAs and ANCA-associated vasculitis among AAT-deficient patients.[3–7] We formulated the dual hypothesis that COPD patients carrying AAT-deficiency phenotypes would be predisposed to ANCA production and/or ANCA-associated vasculitis, with a higher prevalence of ANCA-associated vasculitis than in the general French population.[8] We therefore tested the sera of AAT-deficient patients from a French AAT deficiency-related COPD cohort for ANCAs and anti-PR3 and anti-MPO antibodies and looked for symptoms suggestive of ANCA-associated vasculitis.

Methods

This was an ancillary study from the French cohort of AAT deficiency-related COPD (CONEDAT, ClinicalTrials.gov NCT00700934), the protocol of which has already been published.[9] Briefly, CONEDAT is a prospective cohort aiming to include all French patients aged ≥18 with pulmonary emphysema confirmed by CT scan associated with an obstructive ventilatory disorder and a serum AAT level below 11 µmol/L or a PI*ZZ phenotype. Clinical data were prospectively collected every 6 months during a five-year period. All patients gave their informed consent to be included in the CONEDAT cohort, which was approved by the institutional review board (IRB Paris Nord – Paris 7). Of note, no patient from the previous study by Audrain *et al*.[5] has been included in this study.

All sera were tested for ANCAs by indirect immunofluorescence (IIF) staining of ethanol-fixed human peripheral blood neutrophils (NOVA Lite®, Inova diagnostics, Werfen) diluted 1/20 and incubated with FITC-labeled goat anti-human immunoglobulin G (Inova diagnostics), and for anti-PR3 and -MPO antibodies by ELISA (ORGENTEC®, ORGENTEC Diagnostika GmbH). All tests were performed in the Department of Immunology, Caen University Hospital.

Using the clinical database, we looked for symptoms suggestive of ANCA-associated vasculitis.

Assuming a prevalence of ANCA-associated vasculitis in the general French population of 0.006%[8] (null proportion p_0) and a prevalence of ANCA-associated vasculitis among AAT-deficient patients of 1% (assumed population proportion p)[3,5] and the null hypothesis: $p_0 = p$, with the inclusion of at least 161 patients, we had a power of 80% to detect a significant difference with an α-risk of 0.05 (Clopper-Pearson exact test, JMP 15.1.0, SAS Institute Inc., Cary, NC, USA). Categorical variables were reported as percentages, and continuous variables were expressed as the mean and standard deviation.

Results

Among the 416 patients included in the CONEDAT study, sera were available for this ancillary study only for 185 COPD-AAT-deficient patients and were tested for ANCAs and anti-PR3 and anti-MPO antibodies. Five patients were excluded because genotyping had not been performed. The characteristics of the population are detailed in Table 1. Among the patients, 157 (87%) had the PI*ZZ phenotype and 14 (8%) had the PI*SZ phenotype. No patient had been diagnosed with ANCA-associated vasculitis.

No patients had a typical perinuclear or cytoplasmic staining in IIF, and 12 (7%) patients showed atypical perinuclear staining; 4 patients (2%) had a noninterpretable fluorescence result because of intense nuclear fluorescence.

No patients tested positive for anti-MPO antibodies. Only one patient (0.6%) was anti-PR3 antibody-positive (112.5 units, normal value <10) with a noninterpretable IIF result. However, he had no signs suggestive of ANCA-associated vasculitis in his history and within the one-year follow-up from his inclusion. Therefore, the prevalence of ANCA-associated vasculitis in our cohort was 0%, which is not different from the estimated prevalence in the general population (0.006%).

Discussion

A cohort of 180 patients suffering from AAT deficiency-related COPD did not exhibit an increased prevalence of ANCAs, anti-PR3 antibodies, anti-MPO antibodies or ANCA-associated vasculitis. Therefore, although AAT deficiency is statistically overrepresented among ANCA-associated vasculitis patients, this deficiency is likely to be involved, but not as a sufficient or necessary factor, in its pathophysiology. Using previous studies that assessed the prevalence of these different conditions, the null hypothesis is thereby not rejected at a power of 80%.

On the one hand, AAT and AAT polymers affect neutrophil functions (chemotaxis, priming and degranulation), neutrophil proteases and ANCAs (PR3/anti-PR3 interaction, anti-PR3 neutrophil activation and ANCA-stimulated superoxide production) and, on the other hand, neutrophils and ANCAs play a predominant role in ANCA-associated vasculitis.[2] However, the reasons why patients with COPD and AAT deficiency do not appear to have an increased risk of ANCA-associated vasculitis are unknown. Very few studies have searched for ANCAs and ANCA-associated vasculitis among AAT-deficient patients (Table 2).[3–7] Only the study by Audrain *et al*. found an increased prevalence of ANCA positivity compared to 272 PI*MM controls ($p<0.05$), but these ANCAs were directed against neutrophil alpha-granules and neutrophil elastase.[5] Mohammad and Segelmark,[7] thanks to general screening of all newborns in Sweden for AAT deficiency, estimated the annual incidence rate of GPA, MPA and polyarteritis nodosa to be 397 (95% CI 8-787)/1,000,000 homozygous PI*ZZ Swedish individuals.

In addition to these previous studies, we looked for ANCAs in a large cohort of patients by both IIF and specific ELISAs. Additionally, all our patients already suffered from AAT deficiency-related COPD. However, we did not look for the specificity of the atypical ANCAs, as atypical ANCAs are associated with diseases other than ANCA-associated vasculitis. Moreover, MPO and PR3 ELISAs have better specificity than IIF for ANCA-associated vasculitis and have been suggested as the primary screening method for these diseases. We did not include healthy controls, but in a European multicentric study in 2016, ANCAs were detected by IIF in 58-199/924 (6-21%) healthy controls, and anti-PR3 antibodies were detected by different immunoassays in 7-19/924 (1-2%) samples, which is consistent with our results.[10] Additionally, as sera were only available for 185 patients, we cannot exclude a selection bias, especially a survivorship bias.

To conclude, although AAT deficiency is statistically overrepresented among patients with ANCA-associated vasculitis and might be involved in its pathophysiology, the mere presence of AAT deficiency does not seem to expose these patients to an increased risk of ANCA-associated vasculitis. Although based on the results of this relatively small study from a single country that may not reflect experiences in other parts of the world, because of the low prevalence of ANCAs and ANCA-

associated vasculitis symptoms in severe AAT-deficient patients with COPD, systematic screening appears not to be appropriate.

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Table 1. Characteristics of the 180 chronic obstructive pulmonary disease patients with alpha-1-antitrypsin deficiency.

	Results	n
Demographics		
Age, years	50.7 (± 11)	180
Women	68 (38%)	180
Tobacco use	151 (84%)	179
Quantification of tobacco use, pack-year	18.1 (± 12)	136
Follow-up duration, months	65.1 (± 43.7)	180
Body mass index, kg/m²	23 (± 3.7)	173
Associated diseases		
Liver disease	9 (5%)	176
Panniculitis	0	175
Vasculitis	0	180
Bronchiectasis	17 (10%)	170
Alpha-1-antitrypsin phenotype		
Z allele frequency	332 (92%)	360
S allele frequency	15 (4%)	
M allele frequency	6 (2%)	
Other allele frequency*	7 (2%)	

Values are displayed as an absolute number (%) or mean (± standard deviation). * *F, Malton, Mattawa, not determined.*

Table 2. Frequencies of ANCA and ANCA-associated vasculitis among patients with alpha-1-antitrypsin deficiency from literature.

First author, year [ref.]	Country	Sample size	Organ involvement from AAT deficiency	ANCA positivity by IIF, n (%)	Anti-PR3 or anti-MPO antibodies detected by ELISA, n (%)	Number of ANCA-associated vasculitis (%)
Lhotta, 1994 [6]	Austria	47 Pi*ZZ	Liver involvement (n=19) Pulmonary involvement (n=14)	0	0	0
Savige, 1995 [4]	Australia	73 patients (33 PI*MZ, 14 PI*MS, 2 PI*SZ, 1 PI*SS and 23 PI*ZZ)	Unspecified	0	NA	NA
Audrain, 2001 [5]	France	191 Pi*ZZ	Unspecified clinical manifestations or systematic familial inquiry	21 (11%) (c-ANCA: 15; p-ANCA: 6)	Anti-PR3: 0 Anti-MPO: 2 (1%)	2 (1%), but none had anti-PR3 or anti-MPO antibodies
Stone, 2014 [3]	England	651 PI*ZZ or PI*Znull	Unspecified	NA	NA	GPA: 5 (0.8%)
Deshayes, 2020	France	180 patients (157 PI*ZZ, 14 PI*SZ)	Chronic obstructive pulmonary disease	0	Anti-PR3: 1 (0.6%) Anti-MPO: 0	0

AAT: alpha-1-antitrypsin deficiency; IIF: indirect immunofluorescence; PR3: proteinase 3; MPO: myeloperoxidase; ELISA: enzyme-linked immunosorbent assay; NA: not available; c-ANCA: cytoplasmic antineutrophilic cytoplasmic antibody; p-ANCA: perinuclear antineutrophilic cytoplasmic antibody; GPA: granulomatosis with polyangiitis.