Letter to the Editor

ANCA subclasses in IgG4-related disease and ANCA-associated vasculitis

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IgG4-related disease (IgG4-RD) is a pathological condition characterised by fibro-inflammatory lesions in one or many organs, with peculiar histological features, such as a lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis, and tissue eosinophilia [1–3] as well as elevation of serum IgG4 concentration, which has been proposed as a biomarker of IgG4-RD. However, a high serum IgG4 level can also be detected in other autoimmune conditions, such as ANCA-associated vasculitis (AAV), the most similar to IgG4-RD in terms of organ involvement. In fact, co-existence of the two conditions appears to be particularly frequent. It was previously demonstrated that sera from patients with AVV-IgG4-RD overlap contain IgG4-ANCA autoantibodies [4]. However, IgG4-ANCA autoantibodies are present at high titre also in AAV [5–8], and the identification of this specific subclass of autoantibody does not identify by itself an overlap. Moreover, little is known about the subclass distribution of ANCA autoantibodies in AAV-IgG4-RD overlap patients.

We evaluated the distribution of ANCA subclasses with a sensitive ANCA-MPO subclass ELISA in a cohort of 20 IgG4-RD ANCA-negative patients and in a case of biopsy-proven AAV-IgG4-RD overlap, compared to 35 ANCA-MPO-positive AAV.

Clinical and serological features of the patients are summarised in Table I.

According to the comprehensive diagnostic criteria for IgG4-RD [9], 13 patients had a definitive diagnosis, three had probable diagnosis, and four presented a possible diagnosis. Eight patients presented multiorgan involvement.

One patient with biliary tract involvement (lymphoplasmacytic infiltrate rich of eosinophils, abundant IgG4-positive plasma cells) developed a rapidly progressive glomerulonephritis. Renal histology showed a crescentic glomerulonephritis and a tubulointerstitial nephritis, with IgG4+ plasma cells and eosinophils. Thus, AAV-IgG4-RD overlap was diagnosed.

According to the classification criteria of AAV (CCHC revision 2012), out of the 35 ANCA-MPO+ patients 10 were diagnosed with granulomatosis with polyangiitis (GPA), nine were eosinophilic granulomatosis with polyangiitis (EGPA), and 16 were microscopic polyangiitis (MPA).

In the AAV-IgG4-RD overlap patient, IgG2-ANCA and IgG3-ANCA were detected (Figure 1). In the IgG4-RD cohort, seven patients were positive

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Table I. Demographic and clinical features. Where not specified, values are expressed as median (IQR)

Parameter	IgG4-RD (n = 20)	AAV (n = 35)
Age (mean ± SD)	60 ±11	66 ±11
Sex (M : F)	13 : 7	23 : 12
IgG anti-MPO (% U, < 18)	Absent	61 (43.75–92.25)
IgG4 (mg/dl, < 135)	205 (103–547)	NA
CRP (mg/dl, < 0.5)	0.67 (0.3–2)	2.495 (0.825–5.433)
ESR (mm/h, < 35)	34 (20–47)	45 (31–82.5)
Eosinophils (cells/µl, < 500)	195 (97–280)	248.3 (135–1597)
Creatinine (mg/dl, nv 0.5–0.9)	0.94 (0.77–1.09)	2.4 (1.01–3.11)
BVAS		7 (4–12)
IgG4-RD Responder Index	5 (4–9)	

NA – not available.
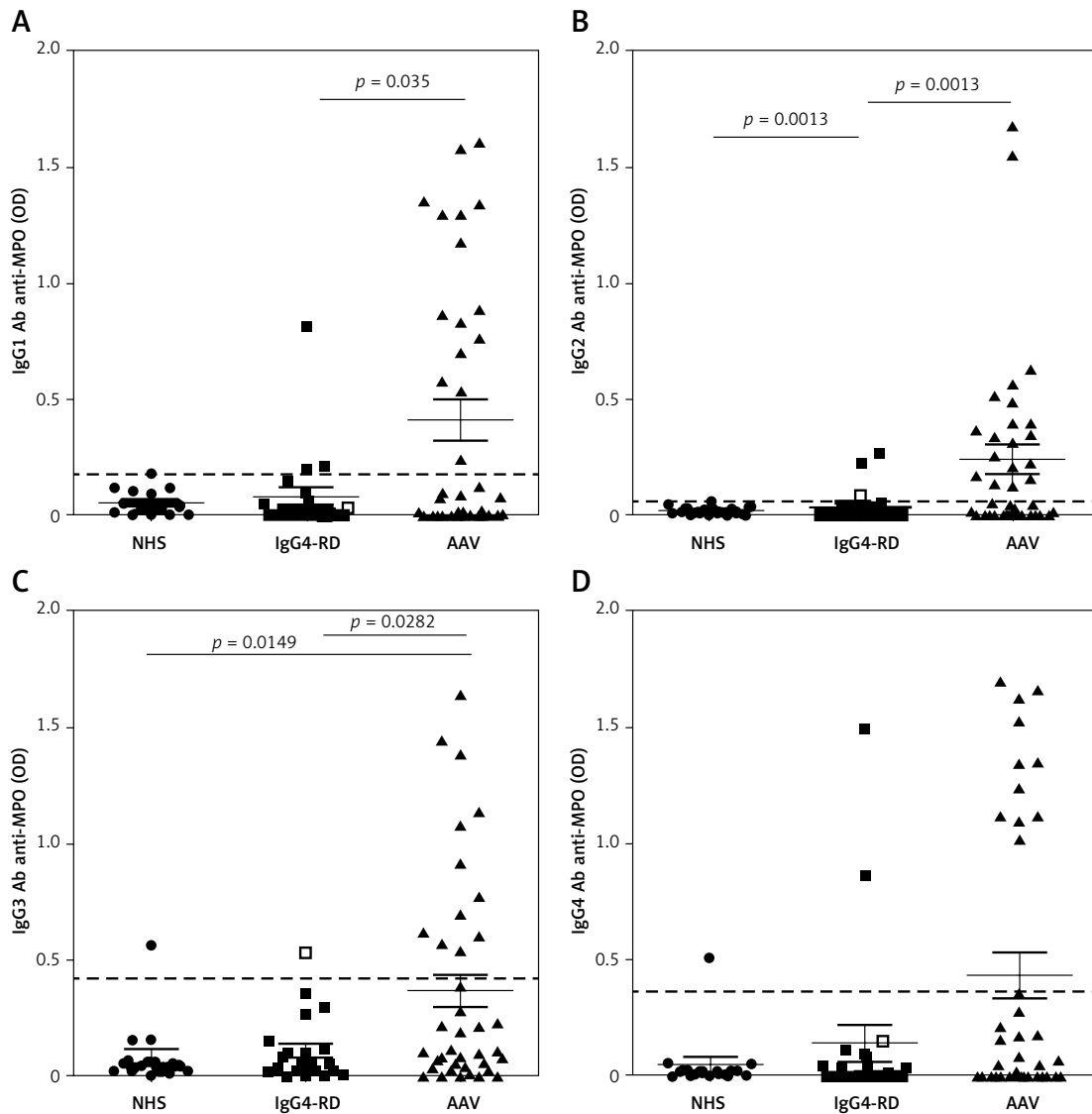
Figure 1. Anti-MPO IgG subclasses in IgG4-RD and AAV. Sera from normal controls (NHS), IgG4-RD, and AAV were incubated on myeloperoxidase-coated plates. Bound immunoglobulins were detected by subclass-specific antibodies. Results are given as OD obtained in the three groups of patients for IgG1 (**A**), IgG2 (**B**), IgG3 (**C**), and IgG4 (**D**). Mean and standard deviation are indicated for each group; the dotted line indicates the upper limit of normality. The open symbol in IgG4-RD represents the overlap patient

for at least one ANCA-MPO subclass, but antibody amounts were very low compared to AAV (Figure 1).

IgG1, IgG2, and IgG3 anti-MPO antibodies were detected in 15% (3/20), 20% (4/20), and 5% (1/20) of IgG4-RD and in 37% (13/35), 51% (18/35), and 34% (12/35) of AAV ($p = 0.1238$, $p = 0.026$, and $p = 0.0195$, respectively); mean levels were higher in AAV ($p = 0.03$, $p = 0.0008$, and $p = 0.0298$, respectively).

At variance with the other subclasses, the frequency of IgG4 anti-MPO (10% vs. 31%) and the mean levels were not statistically different in IgG4-RD and AAV ($p = 0.1$ and $p = 0.13$, respectively).

The seven ANCA-MPO subclass-positive IgG4-RD patients did not display higher disease activity (expressed by the IgG4-RD RI) [10].

Among AAV patients, IgG4 ANCA-MPO were higher in the EGPA group in comparison with GPA ($p = 0.018$) and normal controls ($p = 0.02$); higher amounts were detected in active patients (defined as BVAS > 8) ($p = 0.0393$), and the levels correlated with BVAS ($p = 0.0027$) [11].

We then evaluated the co-expression of anti-MPO of different subclasses. Among IgG4-RD patients, 1 patient was positive for anti-MPO of two different subclasses and one for three subclasses; these two patients were not characterised by a higher IgG4-RD RI. Among AAV patients, conversely, eight were positive for two subclasses, 4 patients for three subclasses, and three patients for four subclasses; AAV patients with anti-MPO belonging to different subclasses were characterised by a higher BVAS ($p = 0.0013$).

These results indicate that IgG4 ANCA-MPO antibodies can be detected in IgG4-RD sera, but other subclasses can also be present. Overall, 35% of IgG4-RD sera contain ANCA-MPO IgG of any subclass; however, the titre of these antibodies is very low in comparison with AAV. Anti-MPO in IgG4-RD are distributed among the four subclasses, and, despite the increase in serum IgG4, IgG4 is not the dominant subclass, not even in the AAV-IgG4-RD overlap.

Moreover, antibody titre and the number of ANCA subclasses are not correlated to clinical activity, at variance with the results obtained in AAV. In this disorder, the number of autoantibodies and the polyclonality of the response are related with disease activity index. Thus, our data support the pathogenic role of ANCA-MPO in AAV, but not in IgG4-RD.

The results reported in this study stress the relevance of ANCA titre more than subclass distribution for differential diagnosis, suggesting that the detection of ANCA-MPO at high titre, without analysing ANCA subclasses, is sufficient to corroborate serologically the clinical suspicion of an AAV-IgG4-RD overlap. On the other hand, the evaluation of ANCA subclasses does not help in phenotyping IgG4-RD patients.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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