Long-term kidney outcome of patients with rheumatological diseases and antineutrophil cytoplasmic antibody-glomerulonephritis: comparison with a primitive ANCA-glomerulonephritis cohort

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Abstract

Objective

Antineutrophil cytoplasmic antibody (ANCA) may appear in the course of rheumatic diseases (RD) but the kidney involvement is very rare and the prognosis poorly defined.

Methods

We retrospectively identified patients with RD among 153 patients with ANCA glomerulonephritis (ANCA-GN). Their clinical/histological presentation and outcome were compared with that of primitive ANCA-GN patients (1:4) matched for sex, age, ANCA type and follow-up.

Results

Nine patients (5.9%) were included: three had rheumatoid arthritis, two systemic sclerosis, two psoriatic arthritis, one ankylosing spondylitis and one seronegative spondylarthritis. Seven patients were MPO positive, two PR3 positive. ANCA-GN developed 74 months after RD with microscopic haematuria and acute kidney dysfunction in all but two patients. After 68-month follow-up, four patients (44.4%) achieved response to therapy defined as eGFR >60/min/1,73 m² or stable, no microscopic haematuria and negative ANCA. At ANCA-GN diagnosis, serum creatinine and C-reactive protein were significantly lower in RD-ANCA-GN (2.38 vs. 3.34mg/dl, p=0.05 and 2.3mg/dl vs. 7.2mg/dl; p=0.05, respectively) while haemoglobin was higher (12.3g/dl vs. 9.3g/dl p<0.01) than in the 36 primitive ANCA-GN patients of control group. At kidney biopsy, focal forms were more frequent in RD patients (44.45% vs. 18.75%, p=0.11). The treatment between the two groups was not significantly different. At last observation, the percentage of patients with ESKD was lower in RD than in controls (11.1%vs. 30.5%; p=0.23).

Conclusion

Patients with RD seem to develop ANCA-GN with less severe clinical/histological kidney involvement, and better long-term kidney survival than primitive ANCA-GN. This is probably due to the strict monitoring of RD patients that allows a prompter ANCA-GN diagnosis and treatment.

Key words

ANCA, ANCA-associated vasculitis, glomerulonephritis, rheumatic disease, rapidly progressive glomerulonephritis

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Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are systemic autoimmune diseases characterised by endothelial injury, necrotising inflammation of small blood vessels, and tissue damage. ANCA are autoantibodies directed against two main antigens: proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA). The group of AAV includes granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) (1). In AAV, kidney involvement is frequent, ranging from 80 to 90% both in GPA and in MPA and is associated with a high rate of morbidity and mortality (4, 5). The most typical clinical renal presentation is with a rapidly progressive glomerulonephritis (RPGN), characterised by a rapid decrease of glomerular filtration rate (GFR) and microscopic haematuria (4, 6) and histologically characterised by crescentic and necrotising glomerulonephritis (ANCA-GN), in the absence of immune deposits. The treatment of these forms must be promptly and aggressive to avoid the development of end-stage-kidney-disease (ESKD) (4, 5).

Testing ANCA with indirect immunofluorescence (IIF) and with enzyme-linked immunosorbent assay (ELISA) is crucial in the diagnosis of AAV. Since 1990, the introduction of ANCA assay in the clinical practice determined an increase in AAV incidence (7).

Positive ANCA test can be found in other vasculitic and autoimmune conditions such as in IgA vasculitis, cryoglobulinaemia, giant cell arteritis, rheumatoid arthritis (RA), systemic sclerosis (SSc), anti-glomerular basement membrane disease (anti-GBM) and in systemic lupus erythematous (SLE) (8-13). However, in these diseases, the clinical and prognostic value of ANCA positivity is not well defined because rarely the presence of these antibodies is associated to overt clinical vasculitic manifestations (8, 12, 14-17). In doubtful cases, kidney biopsy allows the differential diagnosis between GN associated with ANCA (ANCA-GN) and kidney involvement due to the primitive disease. Giving the rarity of these forms, only case reports and few retrospective studies including different rheumatologic diseases are available in literature (17, 18) and the impact on patient and on renal survival of over-imposed ANCA-GN on rheumatic disease (RD) is not clarify. Notwithstanding, an early recognition of these overlap syndromes is important to differentiate the clinical manifestations of the two diseases, to decide the treatment and to establish the prognosis.

Therefore, we searched among patients with biopsy-proven ANCA-GN followed in two tertiary nephrological Italian centres, those with a previous diagnosis of a systemic RD.

We compared the clinical presentation and the outcome of patients with overlap syndrome with that of a matched control group of patients with primitive ANCA-GN chosen based on the most important characteristics that predict AAV outcome.

Materials and methods

Study population and characteristics of control group

Among patients with AAV diagnosed in two nephrological Italian centres (San Gerardo Hospital, Monza and Humanitas Research Hospital, Milan) from 2005 to 2021, we selected patients who developed ANCA positivity during the course of a well-established rheumatic disease. Inclusion criteria were: i) kidney biopsy with diagnosis of ANCA-GN, ii) a previous diagnosis of a rheumatic disease, iii) age ≥18 years at ANCA diagnosis, and iv) a follow-up of at least 6 months.

The first patient identified had a former diagnosis of a seronegative spondylarthritis and developed ANCA-GN in 2005. Afterwards, other eight patients with an ANCA-GN-RD were recorded: three suffered of rheumatoid arthritis, (two seronegative, one juvenile), two had a systemic sclerosis, two complained of psoriatic arthritis and one had an ankylosing spondylitis. Both ANCA-GN and RD, were diagnosed according to the last validated classification criteria (19-26).

We recorded for all patients the data of diagnosis of the RD and that of ANCA development, the clinical features and

the therapy administered before and at time of ANCA. At kidney biopsy, in all patients, we collected clinical and laboratory data. The disease severity was evaluated with Birmingham Vasculitis Activity Score (BVAS) (27), and the renal biopsy was classified according to Berden *et al*. classification (28). We reported the therapy administered after kidney biopsy and the renal outcome. The patients were followed by a dedicated team.

We compared the clinical characteristics and the outcome of patients with overlap ANCA-GN-RD with that of a control group of primitive ANCA-GN patients. The control group included four controls for each patient with ANCA-GN-RD. Control group was chosen to have comparable year of diagnosis (± 5 years), sex, age (± 5 years), ANCA type and follow-up duration (± 5 years).

Data collection and definitions

We retrieved data retrospectively from the medical records of patients with attention to demographic data, clinical features (renal and extrarenal symptoms) and serological tests at both diagnoses.

The renal presentation and outcome of ANCA-GN-RD patients were evaluated based on serum creatinine, glomerular filtration rate (eGFR) estimated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) method, quantification of proteinuria (g/day), presence of microscopic haematuria (> than 5 red blood cells/ high power field (RBC/HPF) at urinary sediment (29).

In addition, erythrocyte sedimentation rate (ESR), C-reactive protein (PCR), complement levels (C3 and C4) and ANCA were evaluated at each visit. ANCA were tested by enzyme-linked immunosorbent assay (ELISA) for MPO and PR3.

At last observation:

a) The ANCA-GN-RD response was defined by the simultaneous presence of the three following features:

i) the recovery of the eGFR with a value of at least 60/ml/min/1,73 m^2 or a stable eGFR value compared to baseline;
ii) the absence of microhaematuria at urinary sediment;
iii) ANCA negative.

b) The renal outcome for both ANCA-GN-DR and primitive ANCA-GN was classified as:

- Chronic kidney disease (CKD): eGFR < 60ml/min/1,73 m^2.
- End stage kidney disease (ESKD): the need for renal replacement therapy.

Due to the retrospective nature of this study no informed consent was requested nor approval from local ethical committee was needed.

Statistical analysis

Demographic and clinical data were expressed as numbers (percentages, %) for categoric variables and as mean ± standard deviation (SD) or median and interquartile range (IQR) for continuous variables depending on their distribution. T-test was applied to continuous variables with normal distribution, Mann-Whitney U-test was applied to continuous variables without a normal distribution. Fisher's test was applied to categorical variables. The retrospective analysis was conducted in the respect of Helsinki Declaration.

Results

Rheumatic disease and ANCA-associated vasculitis overlap patients

In our cohort of 153 patients with a diagnosis of AAV performed from 2005 to 2021, nine (5.9%) had a previous diagnosed RD (Table I). The median age at diagnosis of RD was 42 years old (range 17–70). The renal function at the time of RD diagnosis was normal in all but one patient who had serum creatinine 1.29mg/dl and eGFR 56ml/min/1.73 m^2. Only two patients had detectable proteinuria with a maximum level of 0.450g/day. All patients were treated with a course of steroids and variable combination of disease-modifying anti-rheumatic drugs, non-steroidal anti-inflammatory drugs and monoclonal antibodies (Table I). In four patients (number 2, 3, 4 and 5 in Table I) multiple lines of therapy were needed to achieve remission or to control the reactivations of the RD. Of note two patients were on treatment with an anti-TNF-alpha when ANCA positivity was diagnosed.

ANCA positivity was recorded in median 74 months after RD onset (range 10 months to 10 years) (Table II). At the diagnosis of ANCA-GN, all but two patients showed significant worsening of renal function (serum creatinine from 1.5 to 8.2 mg/dl, eGFR from 5.5 to 37.2 ml/min/1.73 m^2), active urinary sediment (mean 35 RBC/HPF, range 16–100), nephrotic proteinuria in two patients and proteinuria ranging between 0.33 mg/day and 2 g/day in the other patients. The BVAS score ranged between 10 and 19 (median 13; IQR 12–16). The extra renal manifestations at time of ANCA diagnosis were legs purpura in two patients, pulmonary involvement in two patients, weight loss > 9kg in two patients, nasal crusts, and fever in one patient, and newly reported arthralgia in two patients. Six patients (67%) were ANCA-MPO positive and 3 (33%) were PR3-ANCA positive. The AAV diagnosis was of MPA in seven patients and of GPA in the other 2 patients. The kidney biopsy revealed most frequently a focal pattern (4 patients); a crescentic pattern in two cases while other two had mixed form and the last patient had a sclerotic form (Table II). No signs of renal disease potentially associate with RD were present at kidney biopsies. In patient number 1 moderate arteriosclerosis of interlobular arteries was present as expression of the long duration of arterial hypertension.

As induction therapy for ANCA-GN, 8 patients were treated with three methylprednisolone pulses, followed by oral prednisone (from 30 to 75 mg/day) and the last patient received 75 mg/day oral prednisone. The immunosuppressive therapies included Rituximab in four patients, intravenous cyclophosphamide in two patients, mycophenolate mofetil in one and azathioprine in the last patient, in one case plasma exchange was needed (Table II).

After the start of induction therapy for ANCA-GN, RD patients were followed for a median of 68 months (range 6 to 198). No flare of AAV or of RD were documented during this observation. Maintenance therapy consisted of low dose prednisone associated to either methotrexate or rituximab. Four patients achieved response

Table I. Clinical characteristics at presentation of the rheumatic diseases and therapy.

Patient n.	Sex	Rheumatic disease (RD)	Erosive arthritis	Age at diagnosis of RD	Serum creatinine (mg/dl)	eGFR ml/min/1.73/m^2	Proteinuria (g/day)	Arterial hypertension	Therapy
1	M	SS		70	1.29	56	0.360	Yes	CS, MMF
2	M	AS	No	42	0.73	114	0.450	No	CS, MTX, INF, NSAIDs
3	M	RA	No	52	0.80	103	0	No	CS, MTX, HCQ, ET
4	F	RA	Yes	17	0.80	108	0	No	CS, ET, MTX,
5	F	PA	No	41	0.84	86	0	No	CS, MTX, ET
6	M	SS		49	1.00	88	0	No	CS
7	F	SA	No	39	0.90	81	0	No	CS, NSAIDs
8	M	PA, UC	Yes	39	0.90	107	0.262	No	CS, NSAIDs
9	F	RA	No	50	0.88	77	0	Yes	CS, HCQ
Median (min. max, IQR)	/	/		42 (17-70, 39-47)	0.88 (0.73-1.29, 0.80-0.90)	88 (56-114, 81-107)	0 (0-0.450, 0-0)		/

Table II. Clinical characteristics and treatment of patients with ANCA associated renal vasculitis and rheumatic disease at ANCA-GN diagnosis.

Patient n.	Rheumatic disease (RD)	RD active at AAV diagnosis	Age	Time from RD to AAV (months)	BVAS	Serum Creatinine (mg/dl)	eGFR (ml/min)	Proteinuria (g/day)	RBC/ HPF	Arterial hyper-tension	Histological classifi-cation	Type of ANCA	MPN pulses (mg)	Mainte-nance Predni-sone mg/day	IS
1	SS	No	71	10	12	2.47	25.1	0.33	20	YES	Focal	MPO	750 x3	40	/
2	AS	Yes	47	48	14	2.38	31.3	6.52	35	YES	Sclerotic	PR3	1000x3	50	RTX 1gx2
3	RA	No	64	132	19	1.90	36.4	0.73	60	YES	Focal	MPO	500 x3	37.5	RTX 1gx2
4	RA	Yes	20	36	13	0.86	97.2	0.90	100	NO	Focal	MPO	600 x3	30	RTX 1gx2
5	PA	No	50	98	12	2.48	21.9	0.80	16	NO	Mixed	MPO	1000 x3	37.5	RTX
6	SS	No	57	108	10	1.00	82.6	1.80	58	NO	Focal	MPO	PN 75*	75	AZA
7	SA	No	40	24	19	8.20	5.5	2.00	30	YES	Crescentic	PR3	1000 x3	75	PE + CPM
8	PA UC	Yes	46	74	12	2.50	29.7	5.50	35	NO	Mixed	MPO	1000 x3	75	MMF 1000
9	RA	No	60	120	16	1.50	37.2	1.70	40	YES	Crescentic	PR3	1000 x3	60	CPM
Median (min. max, IQR)			50 (20-71, 46-60)	74 (10-132, 26-108)	13 (10-19; 12-16)	2.38 (0.86-8.20, 1.50-2.48)	31.3 (5.5-97.2, 25.1-37.2)	1.70 (0.33-5.50, 0.80-2.00)	35 (16-100, 30-58)	5 patients: YES 4 patients: NO	/	/	/	/	/

n.: number; RD: rheumatic disease; AAV: antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis; eGFR: estimated glomerular filtration rate was calculated according to CKD-EPI equation (29); BVAS: Birmingham Vasculitis Activity Score calculated according to version 3[1] (27); RBC: red blood cells; HPF: high power field; IS: immunosuppressive treatment; MPN: methylprednisolone; PE: plasma exchange; PN: prednisone; RTX: rituximab; CPM: cyclophosphamide; AZA: azathioprine; Min: minimum value; Max: maximum value; IQR: interquartile range.

to therapy while the other five patients were non- responders. Of them, 4 were non-responsive because of incomplete recovery of renal function with eGFR <60 ml/min/1.73 m^2 (from 12.7 to 40.4 ml/min) and persistent ANCA positivity in three patients. In the last patient, the eGFR returned to the basal value (eGFR 51ml/min/1.73 m^2) but microscopic haematuria and ANCA positivity persisted (Table III).

Comparison between ANCA-GN-RD patients and control group

As requested by the established criteria for control group inclusion, there were no significant differences between the two groups in year at ANCA diagnosis, sex, age, ANCA positivity and duration of follow-up. At time of diagnosis, ANCA-GN-RD patients had lower levels of serum creatinine and eGFR (median serum creatinine 2.38 mg/dl and eGFR 31.3 ml/min in RD; *vs*. 3.34 mg/dl 16.5 ml/min in control group; p=0.05 and p=0.06 respectively), lower CRP (median 2.31 mg/dl in RD *vs*. 7.20 mg/dl in control group, p=0.05), lower median number of red blood cells in urinary sediment (35 red blood cells /HPF in RD group *vs*. 50 blood cells/HPF in the control group; p=0.43), and significantly higher median haemoglobin values (12.3 g/dl in RD group *vs*. 9.3 g/dl in control group, p<0.01) than in control group. There were no significant differences between the two groups in presence of arterial hypertension, in proteinuria, in ESR and in C3 levels. C4 complement fraction was found lower in ANCA-GN-RD patients: median 22 mg/dl in RD patients *versus* 31 mg/dl in control group (p=0.1) (Table IV).

At kidney biopsy, focal class was more frequently diagnosed in ANCA-GN-RD patients (44.45% *vs*. 18.75% p=0.11). Corticosteroid treatment was similar; however, more patients in the control group received more frequently cyclophosphamide as induction immunosuppression (20 *vs*. 2 patients, p=0.073) and more patients in ANCA-GN-DR group received rituximab (33.3% *vs*. 22.2%; p=0.48).

At the end of observation, there was a similar rate of patients with CKD but a slightly higher number of patients reached the ESKD in the control group (30.5% *vs*. 11.1%, p=0.089).

Discussion

ANCA positivity can occur in patients with RD, but rarely overt clinical manifestations of AAV develop in these patients (16, 30). Braun *et al*. reported

Table III. Outcome at last observation of ANCA-associated renal vasculitis and rheumatic disease (AAV-RD).

Patient n.	Rheumatic Disease (RD)	Follow-up (months)	Serum creatinine (mg/dl)	eGFR (ml/min)	Proteinuria g/day	RBC/ HPF *	Type of ANCA	Response to therapy	ESR	CRP	BVAS	Maintenance therapy
1	SS	19	1.36	51.3	0.40	13	MPO +	No response	NA	3.53	4	PN 25 mg/die
2	AS	20	5.00	12.7	NA	3	PR3 -	No response	15	0.09	6	PN 15 mg/die
3	RA	25	1.11	68.8	0.09	0	MPO -	Response	24	6.60	0	PN 5 mg/die+ MTX 10 mg/ week
4	RA	134	0.76	104.5	0.28	0	MPO -	Response	10	0.32	2	PN 5 mg/die+ RTX 500 mg/6 months
5	PA	6	0.99	66.4	0.57	0	MPO -	Response	70	0.92	2	PN 10mg/die + MTX 7.5mg/ week
6	SS	68	1.00	79.7	0.16	4	MPO -	Response	9	0.18	0	AZA 50 mg/die
7	SA	198	1.49	38.9	0.29	0	PR3 +	No response	30	0.19	4	PN 5 mg/die+MMF 1000 mg/ die
8	PA, UC	73	2.80	24.8	0.60	3	MPO +	No response	45	0.55	5	PN 10 mg/die
9	RA	169	1.30	40.4	0.10	4	PR3 +	No response	47	0.42	4	MMF 1000 mg/die
Median (min. max, IQR)		68 (6-198, 20-134)	1.30 (0.76-5.00, 0.00-1.49)	51.3 (12.7-79.7, 38.9-68.8)	0.29 (0-0.60, 0.10-0.44) n.	3 (0-13, 0-4)	/	/	27 (9-70 14- 46)	0.42 (0.09- 6.60, 0.19-0.92)	4 (0-6, 2-4)	/

n.: number; eGFR: estimated glomerular filtration rate was calculated according to CKD-EPI equation (29); RBC: red blood cells; HPF: high power field; CKD: chronic kidney disease; ESKD: end stage kidney disease; ESR: erythrocyte sedimentation rate; CRP: C reactive protein; BVAS: Birmingham Vasculitis Activity Score calculated according to version 3[1] (27); ANCA: anti-neutrophil cytoplasmic antibodies; ELISA: enzyme-linked immunosorbent assay; MPO: myeloperoxidase; PR3: proteinase 3; PN: prednisone; MTX: methotrexate; RTX: rituximab; AZA: azathioprine; MMF: mycophenolate mofetil; NA: not available; NEG: negative; Min: minimum value; Max: maximum value; IQR: interquartile range.
*normal <21 RBC/high power field.

the presence of ANCA positivity (more frequently pANCA/MPO) in 16% (61/385) of patients with rheumatic arthritis but clinical manifestations of AAV developed infrequently (17, 31, 32). Akinomoto *et al*. reported seven (9%) ANCA positivity in a cohort of 77 patients with systemic sclerosis; however, among them, only one case was a true overlap syndrome (33). For these reasons, the occurrence of ANCA-GN in patients with a previous diagnosis of RD is even more uncommon and few data about the clinical picture, the therapy, and the outcome of these patients are available in the literature. Nevertheless, it is well known that AAV may cause organ dysfunction and life-threatening complications particularly when kidney involvement is present.

The true overlap syndrome between ANCA-GN and RD is reported in the literature as case reports and in few case series, with rheumatoid arthritis being the most frequently RD involved (17, 34, 35). Thirty-five cases of overlap between rheumatoid arthritis and AAV were collected by Draibe and Salama; of them, 25 had kidney involvement. After immunosuppressive therapy, 16 of these patients improved, four developed ESKD and 5 died (17).

Martìn-Nares *et al*. described overlap syndromes of AAV in 28 (11.3%) out of 247 patients with systemic autoimmune disease. No comparison with a control group was performed. In 32% of these 28 patients, AAV and the autoimmune disease developed simultaneously, while in the other 68% the diagnosis of AAV was established 173 months after the onset of the autoimmune disease. In this study, kidney involvement secondary to AAV was present in 79% of cases. Rheumatoid arthritis was the most frequent RD being diagnosed in 13 of the 28 patients. Nine of these rheumatoid arthritis patients had kidney AAV involvement. Despite immunosuppressive therapy, four patients were non-responsive, two achieved partial response, one died and only two achieved complete response (35).

In our cohort of 153 patients with AAV diagnosed between 2005 and 2021, we have found nine patients (5.9%), who developed ANCA-GN during the course of a well-established systemic RD. We describe the clinical presentation the therapy and the long-term renal outcome of these patients compared with that of a control group of primitive ANCA-GN patients, matched for year of ANCA-GN diagnosis, sex, age, type of ANCA and duration of follow-up.

To the best of our knowledge, this is the first study comparing ANCA-GN-RD patients with a control group of primitive ANCA-GN matched for the most important characteristics that are associated with renal outcome (4, 36, 37). Only another important case-series compared the outcome of 16 AAV patients and connective tissue diseases with that of a control group of 106 AAV patients. However, the control group was not matched and included all AAV patients of the cohort (38). Females were significantly more frequent in AAV-connective disease group than in controls and it is well known that females AAV patients have a better prognosis than males (38). After a mean follow-up of 55.6 months, AAV-connective tissue disease patients had a higher rate of non-renal relapse and venous thrombotic events compared to the control group but had a comparable number of complications, renal relapses, and renal and patient survival. Despite the higher prevalence of female in the overlap patients, ESKD occurred in 30% of patients in each group (38).

In our study, the median age of patients at the onset of RD was 50 years and ANCA-GN was recorded in median 74 months after RD onset but with a wide range from 10 to 120 months. This wide range suggests that ANCA test should be checked during the whole course of the RD diseases. Matching ANCA-GN-RD and controls for sex, there is no gender bias in our cohort in contrast to the previous study (35, 38). In our, as in previous studies, the most common RD

Table IV. Comparison between the clinical/histological characteristics, therapy and outcome of ANCA-GN patients with a rheumatological disease *vs*. primitive ANCA-GN patients.

	AAV in Rheumatic diseases	Primitive AAV	*p*
n. of patients	9	36	
Female/male n. of patients	4/5	16/20	1
Data at ANCA diagnosis			
Age (years)	50.0 (20-72, IQR 46.0-61.0)	53.5 (19-76, IQR 42.0-61.5)	0.92
MPO/PR3 n. patients	6/3	24/12	1
BVAS	13.0 (10-19, IQR 12.0-16.0)	14.5 (8-31, IQR 12.5-18.5)	0.20
Serum creatinine mg/dl	2.38 (0.86- 8.20, IQR 1.50 – 2.48)	3.34 (0.72-19.00, IQR 2.10- 6.30)	**0.05**
eGFR ml/min	31.3 (5.5-97.2, IQR 25.1 -37.2)	16.5 (2.2 – 109.3, IQR 9.2- 33.5)	0.06
Proteinuria g/day	1.70 (0.30-6.50, IQR 0.80-2.00)	1.07 (0.16 – 25.00, IQR 0.50 – 2.60)	0.63
Red blood cells/HPF n.	35 (16-100, IQR 30-58)	50 (1-100, IQR 30-100)	0.43
Arterial hypertension n. pts (%)	5 (55)	23 (63.9)	0.64
Diabetes n. pts (%)	0 2 (5.5)	0.78	
Erythrocyte sedimentation rate mm/h	78 (14-120, IQR 69-88)	80 (32-140, IQR 50-110)	0.43
C-reactive protein mg/dl	2.31 (0.09-11.00, IQR 0.71- 6.20)	7.20 (0- 68.00, IQR 2.10-19.80)	**0.05**
Arterial hypertension N° patients (%)	3/9 (33.3%)	21/34 (61.8%)	0.13
C3 mg/dl	109 (89-144, IQR 96-129)	118 (64-156, IQR 90-133)	0.96
C4 mg/dl	22 (16-36, IQR 17-30)	31 (8-63, IQR 22-38)	0.1
White blood cells/ml	12.5 (7.2-14.9, IQR 9.0-14.5)	10.3 (5.5-22.8, IQR 7.2-13.2)	0.44
Haemoglobin g/dl	12.3 (9.3-14.6, IQR 10.7-13.5)	9.3 (6.7- 17.4, IQR 8.6-10.6)	**<0.01**
Platelets/10^3/ml	326 (118-485, IQR 292-455)	349 (152-722, IQR 244-413)	0.92
Histological classes n. of patients (%)			
Focal	4 (44.5%)	6 (18.8%)	0.11
Crescentic	2 (22.2%)	9 (28.1%)	0.72
Mixed	2 (22.2%)	12 (37.5%)	0.39
Sclerotic	1 (11.1%)	5 (15.6%)	0.74
Methylprednisolone pulses n. of patients	8/9 (88.9%)	36/36 100%	0.9
Immunosuppressive therapy n. of patients	8*	36**	0.9
Plasma exchange n. of patients	1/9	7/36	0.5
Data at last observation			
Follow-up months	68.0 (3-198, IQR 20.0-134.0)	51.0 (0 – 367, IQR 13.5 -122.0)	0.75
Proteinuria g/day	0.29 (0.09- 0.60, IQR 0.13 – 0.49)	0.25 (0-3.87, IQR 0.11- 0.71)	0.87
BVAS	4.0 (0-6, IQR 2.0-4.0)	2.5 (0-8, IQR 2.0- 4.5)	0.96
Response n. pts (%)	4 (44.4)	10 (27.7)	0.334
Renal outcome			
Chronic renal insufficiency	5/9 (55.6%)	21/36 (58.3%)	0.88
ESKD	1/9 (11.1%)	11/36 (30.5%)	0.24

If not differently specified, the numbers refer to median (ranges and interquartile ranges IQR).
n: number; AAV: antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis; MPO: myeloperoxidase; PR3: proteinase 3; HPF: high power field; ESKD: end stage kidney disease; BVAS: Birmingham Vasculitis Activity Score calculated according to version 3[1] (27); eGFR: estimated glomerular filtration rate was calculated according to CKD-EPI equation (29).
Immunosuppressive therapy: *Cyclophosphamide 3 patients, rituximab 3 patients, azathioprine 1 patient, mycophenolate 1 patient.
** Cyclophosphamide 20 patients, rituximab 8 patients, azathioprine 6 patients, mycophenolate 2 patients.

was rheumatoid arthritis. This could reflect the higher prevalence of RA in the general population compared to others RD (38). Prevalence of ANCA-GN-RD in our cohort seems to be lower than in other studies (5.9% *vs*. 11.3% *vs*. 15.1%) (35, 38). We reported a predominance of ANCA-MPO positivity, with 67% patients being ANCA-MPO positive and 33% PR3-ANCA positive, while MPO and PR3 were equally represented in the patients of French study (38).

At ANCA-GN diagnosis, our patients with ANCA-GN-RD had less severe renal involvement (significantly lower serum creatinine and lower eGFR), lower activity of urinary sediment, significantly less systemic inflammatory index, and less severe anaemia than those of control group. In addition, histological picture was less severe in ANCA-GN-RD patients. At kidney biopsy, focal class was more frequent in ANCA-GN-RD (44.45%), whereas in the control group the more frequent histological classes were mixed (37.5 %) and crescentic (28.12%). BVAS was not different between the two groups. We have to point out that the better clinical renal presentation of ANCA-GN-DR in comparison to primitive ANCA-GN could have been influenced by the ongoing immunosuppressive therapy administered for the RD.

There were no significant differences in the treatment at ANCA diagnosis between the two groups. However, more patients in ANCA-GN-RD group received rituximab instead of cyclophosphamide confirming the increasingly importance of rituximab as alternative to cyclophosphamide as induction or as maintenance therapy in AAV patients (17, 38-40).

At the end of the follow-up of around 6.5 years, only four out of the nine ANCA-GN-RD patients achieved response to therapy defined as stable or recovery

renal function with eGFR>60ml/min, no microscopic haematuria and ANCA negative. However, in comparison to primitive ANCA-GN, ANCA-GN-RD had lower serum creatinine and developed ESKD in lower percentage compared to control group as well as in comparison to the French study (38). Although the exact pathogenic mechanism is not clarified, the occurrence of two autoimmune diseases in the same individual may be partially explained, by genetic predisposition and environmental factors (41). There are studies that described the genetic susceptibilities of the 620W allele of the PTPN22 gene as a risk for overlap between ANCA-GN and RA (42). Also, Menegatti *et al*. described an association between the development of vasculitis and RA and the polymorphisms in uteroglobin, a multifunctional immunomodulatory protein that interferes with INFγ and TNF alpha diminishing their biological activity, and NF-κB2 (43).

Among the environmental factors, several studies on exposure to drugs, such as D-penicillamine and anti-TNF-alpha treatment, are available in literature. Tumour necrosis factor (TNF)-α is a cytokine that plays a crucial role in causing inflammation by T-cell-mediated tissue damage. Ramos-Casals *et al*. described the clinical characteristics of more than one hundred patients who developed vasculitis after receiving anti-TNF agents. Renal vasculitis was rare accounting for 13% of cases only (44). However, we cannot exclude the role of anti-TNF-alpha in the development of ANCA-GN in two of our patients who were on treatment at time of ANCA-GN.

The main result of our study is that patients with a previous diagnosis of RD have a less severe renal involvement at diagnosis of ANCA-GN and a better renal outcome in the long term. These data were probably not due to different treatments because induction and maintenance therapies were not significantly different between the two groups. Indeed, the lower levels of serum creatinine and of eGFR at diagnosis together with less active urinary sediment and systemic inflammation could be related to the regular follow-up required for the RD, allowing an earlier detection of the declining renal function.

Because of the prognostic severity of renal involvement in primitive ANCA-GN, it is mandatory in all RD patients that develop ANCA positivity, a regular evaluation of urinary sediment and of renal function at diagnosis and during the whole follow-up.

In addition, when ANCA-GN is suspected, a kidney biopsy has a fundamental role, not only to differentiate ANCA-GN from a kidney disease secondary to the RD, but also for defining the prognosis and to guide treatment.

Our study has some limitations. The retrospective design and the small sample of AAV-RD overlap patients limited the statistical significance of study.

In conclusion, ANCA-GN can rarely occur in patients with a previous diagnosis of RD. Patients with RD and ANCA-GN who received a prompt diagnosis and a regular follow-up and laboratory tests monitoring, seem to present less severe renal clinical presentations and a better long term renal prognosis than patients with primitive ANCA-GN. This is probably due to an early diagnosis. Further studies are needed to confirm our results and to investigate the possible shared mechanisms for developing the overlap syndromes.

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