On the pathogenesis and clinical outcome of ANCA-associated vasculitis

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Chapter III

Renal function and ear, nose, throat involvement in anti-neutrophil cytoplasmic antibody-associated vasculitis: prospective data from the European Vasculitis Society clinical trials.

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Abstract

Objective

We investigated whether ENT involvement is associated with renal biopsy findings and renal function in patients with ANCA-associated vasculitis (AAV).

Methods

Newly diagnosed AAV patients derived from three international, multicentre trials were included. To investigate an association between ENT involvement and estimated glomerular filtration rate (eGFR) at diagnosis and 5-year follow-up, we performed multivariable regression analyses including clinical and histopathological parameters. To investigate whether our findings are specific to ENT involvement, we performed comparable analyses between eGFR and other early disease manifestations (arthralgia/arthritis, cutaneous and lung involvement).

Results

One hundred and eighty-five of the 414 patients had ENT involvement. The mean presenting eGFR of patients with and without ENT involvement was 39.16 and 23.88 ml/min/1.73m^2, respectively ($P<0.001$). Mean eGFR increased by 6.76 ml/min/1.73m^2 with each added ENT symptom ($P = 0.007$). Patients with ENT involvement had less interstitial fibrosis and tubular atrophy and a prognostically more favourable histopathological class on renal biopsy examination. Multivariable regression analyses correcting for clinical and histopathological parameters showed that ENT involvement is associated with both baseline and 5-year follow-up eGFR. There were no associations between baseline and 5-year follow-up eGFR and arthralgia/arthritis, cutaneous or lung involvement, suggesting that our findings are specific to ENT involvement.

Conclusion

The presence of ENT involvement in AAV patients is associated with prognostically favourable renal biopsy findings and better renal function. These results indicate that there may be different phenotypes of AAV defined by ENT involvement.

Introduction

Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are the major clinical subtypes of ANCA-associated vasculitis (AAV)[1]. Establishing an early diagnosis of AAV is complicated by the diversity and lack of specificity of symptoms AAV patients present with, introducing both patient and doctor delay[2]. To what extent clinical outcome of AAV depends on early recognition of the disease is largely unknown; however, it seems likely that early diagnosis and treatment can prevent progressive organ damage, particularly the occurrence of end-stage renal disease (ESRD).

The presence of ENT involvement may be an early sign of AAV[3]. ENT involvement occurs in ~53% of AAV patients [4] and is reflected in symptoms such as hearing loss, otalgia, (bloody) rhinorrhoea, otorrhoea, sinusitis, nasal crusting and recurrent otitis media[2]. The presence of ENT involvement is closely associated with the presence of PR3-ANCA and is observed much less often in MPO-ANCA-positive patients.

Approximately 80% of AAV patients will develop renal involvement during their disease course[5-7], which in a considerable proportion of patients will lead to ESRD and/or death[8-10]. Previously we found that patients with MPO-ANCA have more chronic lesions in their renal biopsy than patients with PR3-ANCA, which may reflect the association of a more smouldering disease with MPO-ANCA and a more active disease with PR3-ANCA[3].

Recent data from a genome-wide association study (GWAS) indicate that within AAV, distinct subtypes may be recognized[11]. Positivity for either MPO-ANCA or PR3-ANCA was found to be the most important indicator of these subtypes, supporting the hypothesis that PR3-ANCA vasculitis and MPO-ANCA vasculitis are distinct autoimmune syndromes.

The diagnosis of AAV is based on a combination of clinical symptoms, ANCA serology and histological findings. This combination of findings may also form the basis for a patient profile with respect to outcome and may be representative of certain distinct AAV phenotypes[12, 13]. One factor of interest is the presence or absence of ENT involvement, because the absence of ENT involvement has been shown to increase mortality risk in AAV patients[14-19]. In this study we focused on ENT involvement in AAV patients in relation to renal biopsy findings and renal outcome. In addition, we performed a subgroup analysis based on ANCA serotype, because of the well-known association of ENT disease and PR3-ANCA. We hypothesized that ENT involvement in AAV is associated with fewer chronic lesions in the renal biopsy and better renal outcome.

Methods

Study population and clinical parameters

Patients were recruited from 62 hospitals located in 15 countries. All patients were enrolled in three international, prospective multicentre trials conducted by the European Vasculitis Society (EUVAS): CYCAZAREM (Cyclophosphamide Versus Azathioprine During Remission For Generalised Vasculitis), CYCLOPS (Daily Oral Versus Pulse Cyclophosphamide During The Induction Phase For Generalised Vasculitis) and MEPEX (Plasma Exchange Versus Methylprednisolone For Severe Renal Vasculitis)[20-22]. Inclusion and exclusion criteria for all trials are described elsewhere[20-22]. Local research ethics committees approved the studies and all patients provided informed consent. All trials were performed in accordance with the Declaration of Helsinki. Disease definitions were adopted from the 1994 Chapel Hill Consensus Conference[23]and previous European Union studies[24, 25]. All patients had been newly diagnosed as having GPA or MPA as determined by local physicians. Only patients with generalized AAV and available BVAS data were included in this study[26]. The four-variable Modification of Diet in Renal Disease was used to determine the estimated glomerular filtration rate (eGFR) in all patients, including the patients with ESRD[27].

ENT involvement was assessed at trial entry and was defined as present if patients had at least one of the ENT items scored using the BVAS. These items included nasal obstruction, bloody nasal discharge, nasal crusts, sinus involvement, conductive hearing loss, sensorineural hearing loss, hoarseness/stridor, granulomatous sinusitis and subglottic inflammation[26]. When ENT involvement was suspected, this was confirmed by local ENT specialists.

To assess whether associations with renal function are related to ENT involvement or are confounded by reduced diagnostic delay in patients with ENT symptoms, we did comparative analyses investigating associations between eGFR and other organ manifestations that are likely to reduce diagnostic delay in a similar fashion, specifically looking at cutaneous involvement, lung involvement and arthralgia/arthritis as scored by the BVAS. Cutaneous involvement included cutaneous infarcts, purpura, other skin vasculitis, ulcers, gangrene and multidigit gangrene[26]. Lung involvement included persistent cough, dyspnoea or wheeze, haemoptysis/haemorrhage, massive haemoptysis, respiratory failure and nodules or cavities, pleural effusion/pleurosity or infiltrate on chest radiology[26].

Histopathological parameters

Paraffin sections of diagnostic renal biopsies stained with silver, periodic acid-Schiff, haematoxylin and eosin and trichrome were examined. Sections were reviewed by two of five participating pathologists (I.M.B., F.F., L.H.N., R.W. or J.A.B.). Both pathologists, blinded to patient data and the other observer's results, scored the biopsies separately and according to a previously standardized protocol

and the histopathological classification of ANCA-associated glomerulonephritis (AAGN)[28-30]. Discrepancies between observers were resolved during central reviews to achieve consensus for each biopsy.

A predefined set of histopathological parameters, including interstitial fibrosis and tubular atrophy (IFTA), interstitial infiltrate, tubulitis and histopathological class, were included in multivariable analyses. In analyses including the histopathological classification, only biopsies containing a minimum of seven whole glomeruli were included.

Statistical analysis

Student's *t*-test was used to investigate an association between baseline and 5-year follow-up eGFR and overall ENT involvement. Student's t-test was also used to investigate an association between baseline eGFR and individual BVAS ENT subitems. A chi-squared test was used to investigate the association between ANCA subtype and the distribution of ENT symptoms. A chi-squared test for trend (linear-by-linear association) was used to investigate the association between histological parameters and ENT involvement. Linear regression analysis was used to explore the relationship between the number of ENT symptoms and eGFR at presentation. A quadratic term analysis was not significant, therefore a linear model was considered appropriate for this analysis. Seven patients were positive for five ENT symptoms, but because including these patients violated the linearity of the model, these patients were excluded from the analysis.

Correlations of ENT involvement, age, PR3-ANCA, tubulitis, interstitial infiltrate, IFTA and histopathological class according to the AAGN classification with baseline and 5-year follow-up eGFR were assessed using Pearson's or Spearman's correlation test as appropriate. Along with ENT involvement, the following entry parameters were selected for inclusion in the multivariable model investigating baseline eGFR: age, PR3-ANCA, IFTA, tubulitis, interstitial infiltrate and histopathological class according to the AAGN classification. ENT involvement, age, PR3-ANCA, IFTA, tubulitis, interstitial infiltrate and protocolized treatment received during the trial period were included in a model investigating 5-year follow-up eGFR. Histopathological class was not included in this model, as including this parameter strongly reduced the number of patients that could be included in the analysis. Analysis of covariance (ANCOVA) investigating 5-year follow-up eGFR was performed by including baseline eGFR in the model. In prespecified sensitivity analyses, the same models were used to investigate the effect of ENT involvement on baseline and 5-year follow-up eGFR in patients only positive for PR3-ANCA. Multicollinearity statistics demonstrated no multicollinearity in the multivariable regression analyses performed, indicating that it was legitimate to perform these analyses. We did not include diagnosis (GPA or MPA) in the analyses, as diagnosis is often dependent on ENT involvement (patients with ENT involvement are often diagnosed as GPA

and patients without ENT involvement are often diagnosed as MPA). Correcting for diagnosis would mask the effect of ENT involvement.

Since renal biopsies were not available for all patients, including the predefined histopathological parameters limited the number of patients included in the analysis. This was particularly true for histopathological class since biopsies needed to contain at least seven whole glomeruli. To be able to include all patients we therefore created three models. The first model included only clinical data: ENT involvement, age and PR3-ANCA. In the second model we added the following histopathological parameters: tubulitis, interstitial infiltrate and IFTA. The third model included all of the above parameters as well as the histopathological class.

To investigate whether possible associations with renal function are specific to ENT involvement and not confounded by reduced diagnostic delay in patients with ENT involvement, we performed comparable analyses between baseline and 5-year follow-up eGFR and other early disease manifestations - specifically arthralgia/ arthritis, cutaneous involvement and lung involvement.

A *P*-value of <0.05 was considered significant. All analyses were performed using SPSS version 20.0 (IBM, Armonk, NY, USA).

Results

Patients

A total of 414 patients with generalized AAV recruited from the CYCAZAREM[20], CYCLOPS[22] and MEPEX[21] trials were included in this study. All 149 patients from the CYCLOPS trial were included. From the CYCAZAREM and MEPEX trials, 149 of the 155 and 116 of the 137 patients were included, respectively. Exclusion of 27 patients was based on incomplete data about the presence or absence of ENT involvement. Diagnostic renal biopsies were obtained from 199 patients, of which 152 contained a minimum of seven whole glomeruli. Two patients did not have renal involvement at trial entry. Both patients were included in the CYCAZAREM trial because of other disease manifestations that were considered to be life-threatening. Baseline eGFR of these patients was 102 and 118 ml/min/1.73m^2.

ENT involvement

Of the 414 patients included in the study, 185 (45%) presented with ENT involvement. Detailed clinical data at baseline of patients with and without ENT involvement are provided in Table 1. ENT involvement was associated with higher eGFR, younger age, diagnosis of GPA and PR3-ANCA positivity. ENT symptoms as scored using the BVAS are outlined in supplementary Table S1. Nasal obstruction, bloody nasal discharge and nasal crusting were the most common

symptoms. In patients with ENT involvement, the distribution of symptoms was similar in PR3-ANCAand MPO-ANCA-positive patients (supplementary Table S2).

Table 1. Baseline characteristics of patients with and without ENT involvement

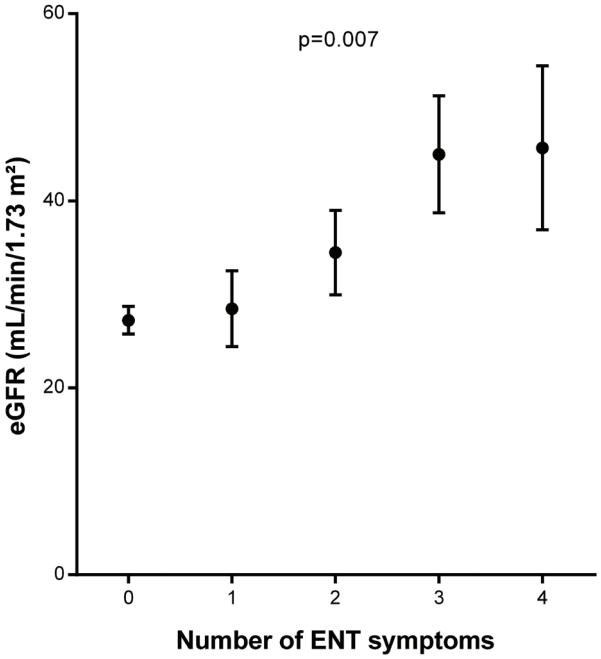
	ENT positive (n=185)	ENT negative (n=229)	P Value
eGFR, mL/min/1.73 m^2 (±SD)	39.16 (±33.11)	23.88 (±20.05)	<0.001
Gender, male / female	111 / 74	122 / 107	0.17
Age, years (SD)	56.49 (14.90)	60.89 (12.87)	0.001
Diagnosis, n (%)			<0.001
Granulomatosis with polyangiitis	142 (77)	41 (18)	
Microscopic polyangiitis	43 (23)	188 (82)	
ANCA-subtype, n (%)			<0.001
PR3-ANCA	124 (67)	77 (34)	
MPO-ANCA	46 (25)	138 (61)	
Double positive	9 (5)	3 (1)	
Negative	6 (3)	9 (4)	
Trial, n (%)			0.22
CYCAZAREM	75 (40)	74 (32)	
CYCLOPS	49 (27)	67 (29)	
MEPEX	61 (33)	88 (39)	

ANCA-serotype data was available for 412 of the 414 patients. Patients with and without ENT involvement are referred to as ENT positive and ENT negative, respectively. ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate; ANCA, anti-neutrophil cytoplasm antibody; PR3, proteinase 3; MPO, myeloperoxidase.

ENT involvement and baseline eGFR

The mean baseline eGFR of patients with and without ENT involvement was 39.16 (S.D. 33.11) and 23.88 (S.D. 20.05) ml/min/1.73m^2, respectively. The presence of overall ENT involvement was associated with higher eGFR [P<0.001 (95% CI 10.09, 20.46)]. Regarding specific ENT symptoms, the presence of nasal obstruction, bloody nasal discharge, nasal crusting, granulomatous sinusitis and conductive hearing loss was significantly associated with higher eGFR (supplementary Table S3). Moreover, the more ENT symptoms a patient had, the higher the eGFR was at presentation; average eGFR increased by 6.76 (95% CI 1.86-11.67) ml/min/1.73m^2 with each additional ENT symptom (P = 0.007; Fig. 1).

Figure 1. Increasing numbers of ENT symptoms are associated with increasing eGFR at diagnosis.



On average, eGFR increased 6.76 (95% CI 1.86 – 11.67) mL/min/1.73 m^2 with each additional ENT symptom (p=0.007). Data are presented as mean ± SEM. ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate.

ENT involvement and renal histology

The absence of IFTA in the renal biopsy was associated with ENT involvement (P<0.001; Table 2). Seventeen patients (20%) with ENT involvement had no IFTA in their renal biopsy, compared with four patients (4%) without ENT involvement. Nineteen patients (23%) with ENT involvement had severe IFTA in their renal biopsy, compared with 45 patients (39%) without ENT involvement. Furthermore, there was an association between histopathological class in the AAGN classification[30] and ENT involvement (P = 0.04; Table 2). Nineteen patients (30%) with ENT involvement had a focal class renal biopsy, compared with 16 patients (18%) without ENT involvement. Six patients (9%) with ENT involvement had a sclerotic class renal biopsy, compared with 13 patients (15%) without ENT involvement. Residuals analysis showed that the focal and sclerotic classes contributed most to the chi-square statistic.

Table 2. Histological variables in relation to ENT involvement

	ENT positive	ENT negative	P Value
IFTA, n (%)			< 0.001
None	17 (20)	4 (4)	
Focal interstitial fibrosis / small foci of tubular atrophy	48 (57)	66 (57)	
Diffuse interstitial fibrosis / extensive foci of tubular atrophy	19 (23)	45 (39)	
Histopathological classification of AAGN*, n (%)			0.04
Focal	19 (30)	16 (18)	
Crescentic	32 (50)	42 (48)	
Mixed	7 (11)	17 (19)	
Sclerotic	6 (9)	13 (15)	
Interstitial infiltrates**, n (%)			0.18
None	9 (11)	4 (3)	
Mild	32 (39)	48 (42)	
Moderate	32 (39)	45 (39)	
Severe	9 (11)	18 (16)	
Tubulitis, n (%)			
Absent	29 (34)	36 (31)	0.63
Present	55 (66)	79 (69)	

*Only biopsies with a minimum of seven whole glomeruli were included in this analysis (n=152).
**Data available for 197 patients. Renal biopsy specimens were available for 199 patients. Patients with and without ENT involvement are referred to as ENT positive and ENT negative, respectively. ENT, ear-, nose-, and throat; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody-associated glomerulonephritis.

ENT involvement is associated with higher eGFR independently of PR3-ANCA

Univariate analyses showed a correlation between baseline eGFR and ENT involvement, age, PR3-ANCA, tubulitis, interstitial infiltrate, IFTA and histopathological class (supplementary Table S4). ENT involvement was independently associated with higher baseline renal function in all three multivariable linear regression models (Table 3; supplementary Table S5). In addition to ENT involvement, age, tubulitis, interstitial infiltrate, IFTA and histopathological class were independently associated with eGFR. In this model including ENT involvement, PR3-ANCA is not associated with eGFR. To investigate whether a strong association between ENT involvement and PR3-ANCA is the reason why PR3-ANCA is not associated with eGFR in this model, we conducted the multivariable model excluding ENT involvement. In this analysis, PR3-ANCA was not associated with eGFR (data not shown). Moreover,

we also performed a stratified analysis in which we started with ENT disease and then investigated the effect of ANCA specificity on eGFR in patients both with and without ENT involvement. In these analyses, PR3-ANCA was again not associated with eGFR (data not shown). Both findings contradict that an association between ENT involvement and PR3-ANCA is the reason why PR3-ANCA is not significantly associated with eGFR.

A prespecified subgroup analysis that included only PR3-ANCA-positive patients revealed that ENT involvement was also independently associated with baseline eGFR in this subgroup of patients. In addition to ENT involvement, age, tubulitis, interstitial infiltrate and histopathological class were independently associated with baseline eGFR (Table 3).

ENT involvement is associated with higher eGFR independently of GPA diagnosis

Because of the strong association between ENT involvement and GPA diagnosis, it was not possible to include both parameters in the same multivariable linear regression model. To investigate the possibility of ENT involvement being a surrogate for the GPA phenotype instead of being independently associated with eGFR, we therefore conducted the multivariable regression analysis including only GPA patients. ENT involvement was independently associated with baseline eGFR in this analysis (supplementary Table S6), indicating that ENT involvement is not a surrogate for the GPA phenotype but is associated with eGFR independently of GPA diagnosis. In addition to ENT involvement, age, interstitial infiltrate, IFTA and histopathological class were independently associated with eGFR. PR3-ANCA was not associated with eGFR.

ENT involvement and renal function at 5-year follow-up

Data on renal function at 5-year follow-up were available for 81 and 72 patients with and without ENT involvement, respectively. Univariate analyses showed a correlation between 5-year follow-up eGFR and ENT involvement, age, interstitial infiltrate, IFTA and histopathological class (supplementary Table S4). As shown in Fig. 2, eGFR increased in patients both with and without ENT involvement during follow-up. Patients with ENT involvement had a better eGFR at 5-year follow-up compared with patients without ENT involvement. Mean eGFR at 5-year follow-up was 54.58 (S.D. 25.43) and 44.02 (S.D. 20.01) ml/min/1.73m^2 in patients with and without ENT involvement, respectively ($P = 0.005$). Multivariate analysis showed that ENT involvement, age and IFTA were independently associated with eGFR at 5-year follow-up (Table 3). PR3-ANCA was not associated with 5-year follow-up eGFR. When adding baseline eGFR to the multivariate model, ENT involvement was no longer associated with 5-year follow-up eGFR (supplementary Table S7). This finding implies that ENT involvement is not associated with an accelerated increase in eGFR, but that

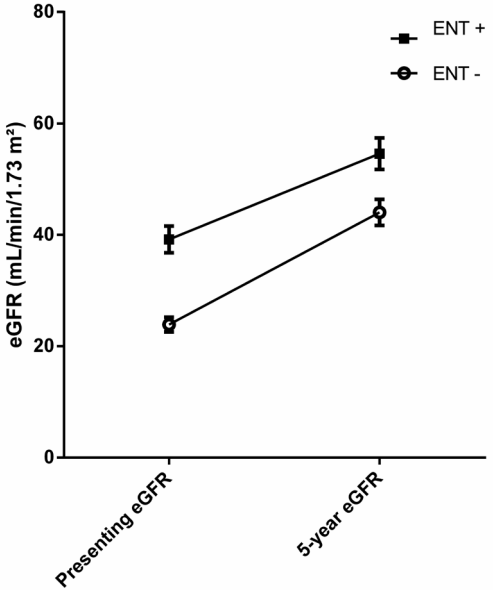
Table 3. Multivariable regression analyses investigating the relationship between ENT involvement and baseline and 5-year follow-up eGFR

	Model investigating baseline eGFR		**Model investigating 5-year follow-up eGFR[a]**		**Model investigating baseline eGFR in PR3-ANCA-positive patients**		**Model investigating 5-year follow-up eGFR in PR3-ANCA-positive patients[a]**	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
ENT involvement	9.14 (2.30 – 15.98)	0.009	9.86 (2.23 – 17.50)	0.01	12.92 (2.11 – 23.72)	0.02	16.40 (5.58 – 27.22)	0.004
Age	-0.60 (-0.83 – -0.36)	<0.001	-0.33 (-0.61 – -0.05)	0.02	-0.72 (-1.10 – -0.35)	<0.001	-0.17 (-0.54 – 0.21)	0.37
PR3-ANCA	-1.26 (-8.28 – 5.75)	0.72	-6.22 (-14.31 – 1.87)	0.13	N/A	N/A	N/A	N/A
Tubulitis	-17.82 (-25.78 – -9.86)	<0.001	-5.36 (-14.21 – 3.50)	0.23	-14.37 (-27.67 – -1.07)	0.04	-7.59 (-20.31 – 5.13)	0.24
Interstitial infiltrate	-6.86 (-11.20 – -2.52)	0.002	-3.54 (-10.15 – 3.07)	0.29	-8.91 (-16.52 – -1.31)	0.02	-5.14 (-14.44 – 4.17)	0.27
IFTA	-8.72 (-14.67 – -2.77)	0.004	-9.06 (-15.59 – -2.54)	0.007	-9.55 (-19.90 – 0.80)	0.07	-9.15 (-17.91 – -0.39)	0.04
AAGN classification	-6.20 (-9.84 – -2.57)	0.001	N/A	N/A	-8.41 (-14.97 – -1.85)	0.01	N/A	N/A

ENT involvement is associated with better eGFR at diagnosis and 5-year follow-up. A prespecified sensitivity analysis including only PR3-ANCA positive patients showed that this finding also held in this subgroup of patients. In all models, age is included per year unit. [a]Model is adjusted for within-trial therapy. 95% CI, 95% confidence interval; ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis; N/A, not applicable.

patients with ENT involvement start with a higher presenting eGFR and preserve this advantage during the 5-year follow-up. A prespecified subgroup analysis including only PR3-ANCA-positive patients revealed that ENT involvement is also associated with 5-year follow-up eGFR in this subgroup of patients (Table 3). Also in this subgroup of PR3-ANCA-positive patients, ENT involvement is no longer associated with 5-year follow-up eGFR when adding baseline eGFR to the model (supplementary Table S7).

Figure 2. Patients with ENT involvement have significantly better eGFR at diagnosis and 5-year follow-up.



Data are presented as mean ± SEM. ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate.

Cutaneous involvement, arthralgia/arthritis and lung involvement are not associated with baseline and 5-year follow-up eGFR

To investigate whether the higher eGFR in patients with ENT involvement is specific for ENT involvement or is attributable to less diagnostic delay in these patients, we analysed the relationship between eGFR and cutaneous involvement, arthralgia/arthritis and lung involvement, as these symptoms are likewise expected to reduce diagnostic delay. Of the 414 included patients, 98 (24%) had cutaneous involvement, 162 (39%) had arthralgia/arthritis and 207 (50%) had lung involvement. Multivariable linear regression analyses demonstrated no association between cutaneous involvement, arthralgia/arthritis or lung involvement and baseline eGFR (Table 4; supplementary Table S8). In all three

Table 4. Multivariable regression analyses investigating the relationships between baseline eGFR and other early disease manifestations

	Cutaneous model		**Arthralgia/arthritis model**		**Lung model**	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
Cutaneous involvement	-2.98 (-11.58 – 5.62)	0.49	N/A	N/A	N/A	N/A
Arthralgia/arthritis	N/A	N/A	-4.85 (-11.40 – 1.71)	0.15	N/A	N/A
Lung involvement	N/A	N/A	N/A	N/A	-4.74 (-11.19 – 1.70)	0.15
Age	-0.63 (-0.87 – -0.38)	<0.001	-0.54 (-0.78 – -0.29)	<0.001	-0.61 (-0.85 – -0.37)	<0.001
PR3-ANCA	1.29 (-5.62 – 8.21)	0.71	0.41 (-6.52 – 7.33)	0.91	1.86 (-5.06 – 8.78)	0.60
Tubulitis	-16.54 (-24.62 – -8.47)	<0.001	-14.71 (-22.91 – -6.50)	0.01	-16.83 (-24.87 – -8.79)	<0.001
Interstitial infiltrate	-7.48 (-11.96 – -3.00)	0.001	-7.38 (-11.83 – -2.93)	0.001	-7.12 (-11.53 – -2.71)	0.002
IFTA	-9.57 (-15.62 – -3.51)	0.002	-9.92 (-16.01 – -3.83)	0.002	-9.97 (-15.97 – -3.97)	0.001
AAGN classification	-6.82 (-10.61 – -3.03)	0.001	-6.98 (-10.79 – -3.17)	<0.001	-6.79 (-10.49 – -3.09)	<0.001

To investigate whether our associations are specific to ENT involvement, analyses between baseline eGFR and other early disease manifestations were performed. There were no associations between baseline eGFR and cutaneous involvement, arthralgia/arthritis, or lung involvement. In all models, age is included per year unit. 95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis; N/A, not applicable.

models, age, tubulitis, interstitial infiltrate, IFTA and histopathological class were associated with eGFR. Multivariable linear regression analyses also demonstrated no association between cutaneous involvement, lung involvement or arthralgia/arthritis and eGFR at 5-year follow-up (supplementary Table S9).

Discussion

Our study demonstrates that AAV patients with ENT involvement have a higher eGFR at diagnosis and 5-year follow-up than AAV patients without ENT involvement. Specifically, nasal obstruction, bloody nasal discharge, nasal crusting, granulomatous sinusitis and conductive hearing loss were associated with higher eGFR. Moreover, the number of ENT symptoms was related to renal function, with eGFR increasing with each added ENT symptom. Patients with ENT involvement had less IFTA and less chronic damage in their renal biopsies. We also found that renal histology of AAV patients with ENT involvement had features associated with better renal prognosis compared with renal histology of AAV patients without ENT involvement. In the multivariable analysis, PR3-ANCA was no longer associated with eGFR when including histological parameters in the model. This finding indicates an association between ANCA serotype and renal histological findings, as previously described[3]. Interestingly, we found that ENT involvement is associated with renal function independently of GPA diagnosis and PR3-ANCA.

Our findings can be explained by two possible mechanisms. It is possible that through ENT involvement, patients are diagnosed earlier with AAV. Therefore, in patients with ENT involvement, renal function may still be relatively preserved at presentation. Alternatively, the presence or absence of ENT involvement may represent different phenotypes of AAV. We will discuss a number of features that are either in favour or against these two notions.

Clinically overt ENT symptoms may lead to an earlier diagnosis of AAV by reducing patient and possibly doctor delay, as previously proposed[3]. This may explain the relatively preserved renal function in patients with ENT involvement. However, in our study, manifestations of the skin, lung and arthralgia/arthritis that would supposedly reduce patient and/or doctor delay were not associated with baseline and 5-year follow-up eGFR. Generalized symptoms (such as fever) have been shown to occur earlier in the disease than manifestations of the skin, lung and arthralgia/arthritis[31]. However, these symptoms were not associated with a higher eGFR in this cohort (data not shown). This lack of distinctiveness is probably due to the large proportion of patients with general symptoms at diagnosis (91% in our cohort).

Even though a number of studies have investigated diagnostic delay in AAV[31-33], to our knowledge, very few studies have investigated diagnostic delay for specific

organ involvement. Recently Poulton *et al.*[34] showed that AAV patients with ENT involvement were more likely to experience a delay in diagnosis because both physicians and patients focused on the more common causes of the ENT symptoms instead of regarding them as signs of vasculitis. Even though there was a risk of recall bias in this study, the authors tried to minimize this by interviewing patients within 5 years after diagnosis. Bligny *et al.*[18] showed that patients with ENT involvement have better survival than patients without ENT involvement. They argued that the better prognosis was likely related to different pathogenic routes and not to earlier diagnosis and treatment, as 13 of the 15 patients without ENT involvement had lung involvement, which can lead to earlier diagnosis. Interestingly, in AAV patients with ENT involvement, ENT symptoms mostly start within 12 months after diagnosis[35]. Considering these findings, the argument that ENT involvement is an easily recognizable sign of vasculitis that can lead to early recognition and better eGFR does not appear to hold.

A recently published GWAS provided support for the concept that PR3-ANCA vasculitis and MPO-ANCA vasculitis are distinct autoimmune syndromes[11]. Single nucleotide polymorphisms were shown to be associated more strongly with PR3-ANCA and MPO-ANCA than with the clinical syndromes MPA and GPA. In general, AAV patients with ENT involvement are likely to be PR3-ANCA positive and AAV patients without ENT involvement are likely to be MPO-ANCA positive[2]. In our multivariable analyses, ENT involvement overruled ANCA subtype in relation to the preservation of baseline and 5-year follow-up renal function, and in a prespecified subgroup analysis consisting of only PR3-ANCA-positive patients, ENT involvement was still associated with higher baseline and 5-year follow-up eGFR. Both findings indicate that ENT involvement is associated with a favourable renal profile independent of ANCA serotype and may be an important factor in the determination of different phenotypes of AAV.

One limitation of our study is that no data about the time from onset of symptoms to diagnosis of AAV were available. However, low reliability of a patient's interpretation of symptoms that may or may not have been attributable to AAV makes it hard and perhaps not desirable to use these data in an analysis. One strength of our study is the large number of patients we were able to include due to a large European collaboration. Moreover, renal biopsies were available for a large proportion of patients. Because our patients were recruited from three randomized controlled trials, clinical data were accurately documented prospectively.

Our study demonstrates that ENT involvement itself is an important clinical parameter of AAV, first because it is closely related to renal histology and outcome, and second because it may be a determinant of different phenotypes in AAV. Taking the findings of the GWAS into consideration, it is possible that within the subdivision of PR3-ANCA vasculitis and MPO-ANCA vasculitis,

AAV patients can be further categorized as ENT positive or ENT negative. Just as in SLE, where presenting symptoms in relation to clinical outcome have been compared to a hand of cards, in AAV, PR3-ANCA positivity together with the presence of ENT involvement may be representative of a relatively good hand of cards. Regarding future diagnostic and classification criteria for AAV, we suggest that the presence or absence of ENT should be taken into account as an important clinical parameter.

Key messages

- ENT involvement in ANCA-associated vasculitis patients is associated with higher baseline and 5-year follow-up estimated glomerular filtration rate.
- ENT involvement in ANCA-associated vasculitis patients is associated with prognostically favourable findings on renal biopsy examination.
- The presence or absence of ENT involvement may define different phenotypes of ANCA-associated vasculitis.

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