



ORIGINAL ARTICLE

Seasonal variations in the onset of positive and negative renal ANCA-associated vasculitis in Spain

Juliana Draibe¹, Xavier Rodó^{2,3,4}, Xavier Fulladosa¹,
Laura Martínez-Valenzuela¹, Montserrat Diaz-Encarnación⁵, Lara Santos⁵,
Helena Marco⁶, Luis Quintana⁷, Eva Rodriguez⁸, Xoana Barros⁹, Rosa Garcia¹⁰,
Anna Balius¹¹, Josep M. Cruzado¹ and Joan Torras¹; Grupo de Malalties
Glomerulars de la Societat Catalana de Nefrologia (GLOMCAAT)

¹Hospital Universitari de Bellvitge, Barcelona, Spain, ²Institució Catalana de Recerca i estudis Avançats (ICREA), Barcelona, Spain, ³Institut Català de Ciències del Clima (IC3), Barcelona, Spain, ⁴ISGlobal, Barcelona, Spain, ⁵Fundación Puigvert, Barcelona, Spain, ⁶Hospital Can Ruti, Barcelona, Spain, ⁷Hospital Clinic, Barcelona, Spain, ⁸Hospital de Mar, Barcelona, Spain, ⁹Hospital Josep Trueta, Girona, Spain, ¹⁰Hospital de Palamos, Palamos, Spain and ¹¹Fundación Althaia, Manresa, Spain

Correspondence and offprint requests to: Juliana Draibe; E-mail: jbordignon@bellvitgehospital.cat

Abstract

Background: The closure of long-standing gaps in our knowledge of aetiological factors behind anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a major challenge. Descriptive and analytical epidemiological studies can improve our understanding of environmental influences. Reported seasonal variations in AAV, mainly related to Wegener's disease, have shown an increasing number of cases in the winter months, which could be related to an extrinsic factor underlying infection. The objective of this paper was to study seasonal variations in AAV with respect to renal affectation diagnosed in Catalonia, Spain.

Methods: Two hundred and thirty-four patients diagnosed for renal AAV between 2001 and 2014 in eight hospitals in Catalonia were included in the study. We used medical records to retrospectively analyse the date of the first symptoms attributed to the AAV, ANCA subtypes, the degree of renal impairment and renal histology.

Results: Of the 234 patients studied, 49.2% were male and 50.8% female. For ANCA status, 8.5% were positive, 15.9% were proteinase-3-positive and 75.6% were myeloperoxidase-positive. In relation to histological classification, 17.8% were sclerotic, 11.7% focal, 38.8% crescentic and 31.7% mixed. Regarding seasonal distribution, we observed a clear seasonal periodicity with a significantly higher incidence of cases in the winter. Applying an Eigen decomposition, we observed a periodic fluctuation of frequencies around the annual cycle with peaks every 10–12 months, and higher incidence of AAV cases in February.

Conclusions: Our results confirm, in Catalonia, the seasonal periodicity of AAV with a higher incidence in the winter, as formerly described in the literature for other regions. An environmental factor, likely one that is infectious, may explain this finding.

Key words: ANCA, epidemiology, immunology, seasonal, seasonality, vasculitis

Received: June 12, 2017. Editorial decision: September 25, 2017

© The Author 2017. Published by Oxford University Press on behalf of ERA-EDTA.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) comprises three different clinical entities—granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis—a group of systemic diseases that are characterized by pauci-immune necrotizing inflammation of small to medium vessels. Present in most patients, ANCAs against proteinase-3 (PR3) or myeloperoxidase (MPO) are often associated with GPA and MPA, respectively [1]. AAV affects multiple organ systems, with glomerulonephritis and pulmonary involvement being frequent manifestations. AAV is currently defined as a rare autoimmune disease with a current annual incidence of 20/million/year [2], with increasing incidence in recent years [3]. The incidence of PR3-AAV and MPO-AAV varies worldwide. In general, MPO-AAV is seen more frequently in southern Europe, Asia and the Pacific (except in New Zealand and Australia), while PR3-AAV is more common in the northern countries of the world [4–7]. In Spain, a study of 450 patients with AAV found the proportions of entities to be 40.9% for GPA, 37.1% for MPA and 22% for eosinophilic granulomatosis with polyangiitis [8]. In another study, which analysed 151 patients with AAV and renal involvement, proportions of 39.7% for MPA, 6.6% for GPA and 53.6% for renal limited vasculitis were found [9].

The aetiology of AAV is currently unknown, and so too are the contributions of genetic and environmental factors to its development. Over the past few years, genetic factors contributing to AAV have been extensively studied, mostly by genome-wide association studies. These have demonstrated that patients with PR3-ANCA have significant association with human leukocyte antigen (HLA)-DP, and with genes encoding α 1-antitrypsin (Serpin Family A Member 1 (SERPINA1), the endogenous inhibitor of PR3) and PR3 (proteinase 3; PRTN3). On the other hand, patients with MPO-ANCA have significant association with HLA-DQ [8].

In relation to environmental factors, previous studies have demonstrated an increased incidence of AAV in patients exposed to a variety of air pollutants (e.g. silica) [9], after treatment with drugs such as penicillamine and hydralazine [10, 11], and in relation to infectious organisms like a nasal colonization with *Staphylococcus aureus* in GPA patients [12]. Supporting the idea of an underlying infectious factor, several studies have shown that the onset of AAV varies by season, with incidence peaking in the winter [13–17]. In clear contrast, a recent study suggested that AAV appears preferentially in the summer in GPA patients [18], supporting the idea of a possible allergic mechanism in its pathogenesis. In addition, in other primary systemic vasculitis conditions, such as Kawasaki disease, a seasonal pattern and possible environmental triggers have been shown [19, 20].

In the present study, we re-examined the hypothesis of seasonal variations in the onset of renal AAV in a Mediterranean area in Spain.

Materials and methods

This retrospective study included 234 patients diagnosed with AAV with renal involvement between January 2001 and December 2014 in eight different hospitals in Catalonia, Spain. Diagnosis of renal vasculitis was made by according to the criteria established at the Chapel Hill Conference [21], as determined by positive ANCA (MPO or PR3) antibodies and a renal biopsy with the presence of necrotizing pauci-immune glomerulonephritis.

Information regarding the following demographics were obtained from medical records: age, gender, disease features, the date of first symptoms attributed to the AAV, date of diagnosis, ANCA

subtype, the degree of renal impairment and renal histology classification. Renal pathology was classified according to the Berden classification as follows: focal, crescentic, mixed or sclerotic [22].

Related to the date of the first AAV symptoms, we included onset data for general symptoms such as fever, malaise and/or weight loss or specific organ involvement, and ear, nose and throat, pulmonary, renal, ophthalmological and cutaneous involvement. The disease onset–diagnosis interval was calculated as the difference between the onset of symptoms and the initiation of AAV treatment. For these calculations, onset data were arbitrarily set as the 15th day of the respective month, unless patients were able to specify the exact week or day of disease onset. Finally, we excluded 11 patients because a precise month of the onset of AAV could not be calculated.

Data analysis was performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). The distribution of symptom onset according to month and season was examined for uniformity using exact one-way goodness-of-fit chi-square tests as a means to identify significant deviations from expected frequencies. Seasons were divided into spring (April–June), summer (July–September), autumn (October–December) and winter (January–March).

An Eigen decomposition was applied to the ANCA time series with the covariance matrix equivalent of processing a forward–backward prediction data matrix by signal strength rather than by frequency. Due to the low signal-to-noise ratio in the epidemiological time series, it was possible in this way to isolate individual oscillatory components embedded in signals. In this decomposition, the first eigenvalue, corresponding to seasonality, accounts for 6.5% of the normalized variability. To further cross-validate this result, the series of cases was accumulated and then detrended by a linear least squares approximation.

Comparisons of seasonal distribution patterns for patient groups (sex, ANCA subtype, degree of renal impairment and renal histology classification) were performed using a chi-square test. Regarding renal impairment, we divided patients into two different groups based in their baseline serum creatinine: group 1, $<500 \mu\text{mol/L}$; and group 2, $\geq 500 \mu\text{mol/L}$. Regarding histology classification, we also divided the patients into two different groups: the first one included patients with crescentic, focal and mixed classification; and the second one included only patients with sclerotic classification. This decision was based on the fact that patients with sclerotic lesions usually have a more imprecise date of first symptoms of disease.

Results were considered significant when $P < 0.05$ and data are expressed as mean \pm standard error of the mean.

Written informed consent was considered not to be necessary for the study, as it was a retrospective analysis of our usual everyday work. The data of the patients were anonymized for the purposes of this analysis. The confidential information of the patients was protected according to national norms. This manuscript has been approved for publication by the Clinical Research Ethics Committee of Bellvitge University Hospital.

Results

Characteristics of the study population

The main characteristics of our patients at AAV diagnosis are summarized in Table 1. Most of the 234 patients (around 88%) lived in the city of Barcelona or in its metropolitan area, with a predominance of an urban and Caucasian population. Regarding the demographic characteristics, 49.2% were male

and 50.8% female; 15.9% were PR3-ANCA-positive, 75.6% MPO-ANCA-positive and 8.5% ANCA-negative. For renal impairment, the mean baseline serum creatinine at the diagnosis was 422.93 $\mu\text{mol/L}$ (range 62–1418). In respect of histological classification, 17.8% were sclerotic, 11.7% focal, 38.8% crescentic and 31.7% mixed. The mean onset–diagnosis interval calculated was 3.15 months (range 0.1–45.5).

Monthly and seasonal variations of AAV onset

Monthly and seasonal distributions of AAV onset are shown in Figure 1. Frequency of disease onset according to month showed a higher incidence in January, February and March, but there were no significant variations.

Regarding seasonal distribution, we observed a significantly higher incidence of cases in the winter and this variation was significant when compared with the seasonal distribution reported in ANCA MPO-positive patients (chi-square = 13.36, 3 degrees of freedom, $P = 0.003$) [17].

Applying an Eigen decomposition, we observed periodic fluctuation of the frequencies with peaks every 10–12 months around the annual cycle, and a greater incidence of AAV cases in February (Figure 2).

Using stratification of selected variables (sex, ANCA subtype, degree of renal impairment and renal histology classification), no significant differences were found in the analyses of seasonal patterns (Table 2).

Table 1. AAV patient characteristics

Variables	Baseline characteristics
Age, mean (SD), years	65.33 (16–89)
Sex (M/F), %	49.2/50.8
Baseline creatinine, mean (range), $\mu\text{mol/L}$	422.93 (51–1418)
<500 $\mu\text{mol/L}$, %	67.5
>500 $\mu\text{mol/L}$, %	32.5
ANCA subtype	
Negative, n (%)	20 (8.5)
Anti-MPO, n (%)	177 (75.6)
Anti-PR3, n (%)	37 (15.9)
Histology classification	
Focal, n (%)	26 (11.8)
Crescentic, n (%)	85 (38.6)
Mixed, n (%)	70 (31.8)
Sclerotic, n (%)	39 (17.8)

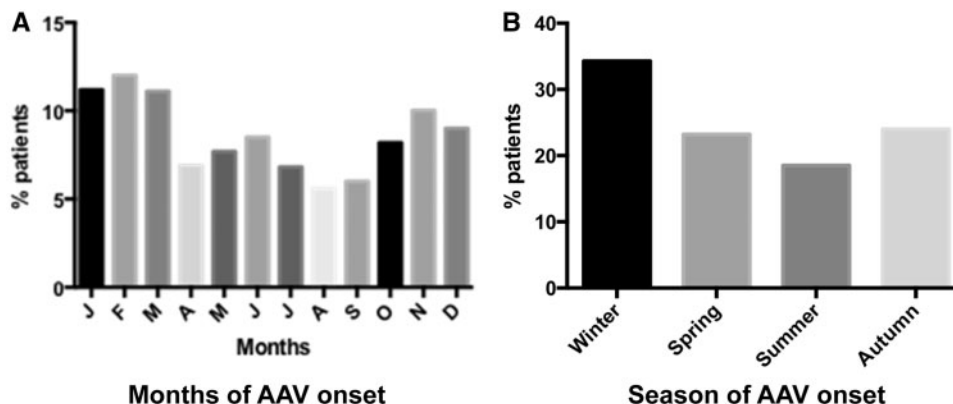


Fig. 1. Distribution of months (A) and season (B) of AAV onset for 234 patients.

Seasonal variations of AAV onset related to geographic location

In our study, we included patients from eight different hospitals and areas were as follows: one was urban, located in the city of Barcelona (Area 1, $n = 97$); two were located in the metropolitan area of Barcelona (Area 2, $n = 70$ and Area 3, $n = 39$; located west and east of Barcelona, respectively); two were located northeast of Catalonia (Area 4, $n = 22$); and one was located north-west of Catalonia (Area 5, $n = 6$).

When we analysed the seasonal variation of AAV related to geographic location, we found that patients from Barcelona and the western metropolitan area had similar patterns, with more homogenous distribution across the seasons. On the other hand, patients from the east or north-east of Barcelona had a higher incidence of AAV in the winter, whereas patients from the north-west had higher incidence in spring and summer (Figure 3).

Discussion

AAV needs to be understood as a multifactorial disease that results from environmental triggers affecting genetically predisposed individuals. Investigating the effects of seasonal climate and geographical variations on the onset of this disease could provide indirect information about its pathogenesis.

Our study re-examined the hypothesis of seasonal variation in the onset of AAV in a Mediterranean area in north-east Spain and demonstrated higher incidence of AAV patients with renal affection during the winter, supporting several previous studies that have found similar results [13–17]. This could be linked to a higher incidence of airway disease in this season, and subsequent respiratory infection factors may trigger the disease, as described previously [23]; however, our study could not provide support this proposed link.

In addition, we demonstrated a periodic seasonal fluctuation related to the incidence of AAV, with peaks every 10–12 months around the annual cycle. Tidman et al. [17] previously described periodic fluctuations in patients with AAV over a period of 21 years; however, these peaks occurred around every 3–4 years. This difference could be related to the shorter period of our study compared with that of Tidman et al.

Of note, most of the previous studies were performed in populations that included mostly GPA- or PR3-ANCA-positive patients that do not match with our cohort, which was predominantly composed of MPO-ANCA-positive patients. Only one study analysed seasonal variations in a population of MPO-ANCA patients [17] and found that very few of them presented

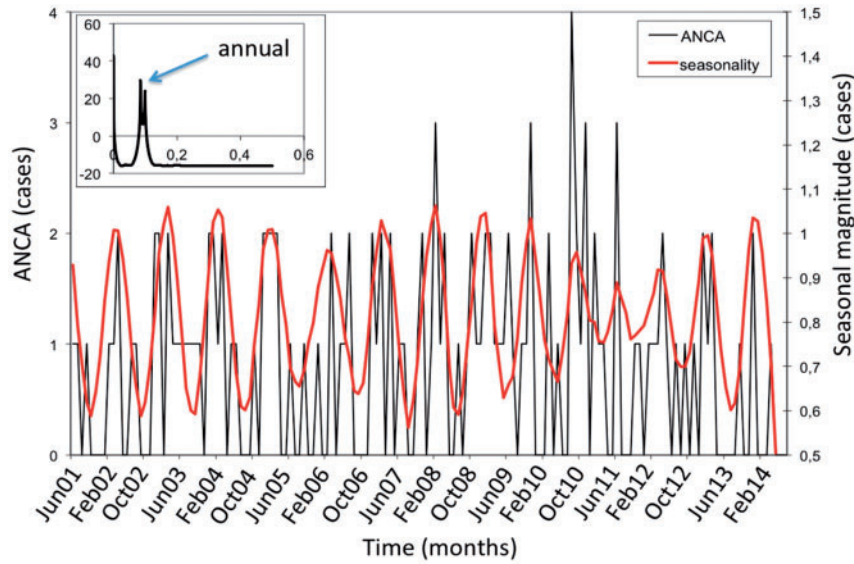


Fig. 2. Evolution of the number of ANCA cases (black) from 2001–14 together with their reconstructed seasonality (red). An Eigen decomposition of order $M = 30$ was applied to the raw ANCA time series to maximize the signal extraction process. The inset shows the maximum entropy spectrum derived for this component, showing two clear significant periods around the annual cycle (11.76 months and 10.12 months, $P < 0.05$).

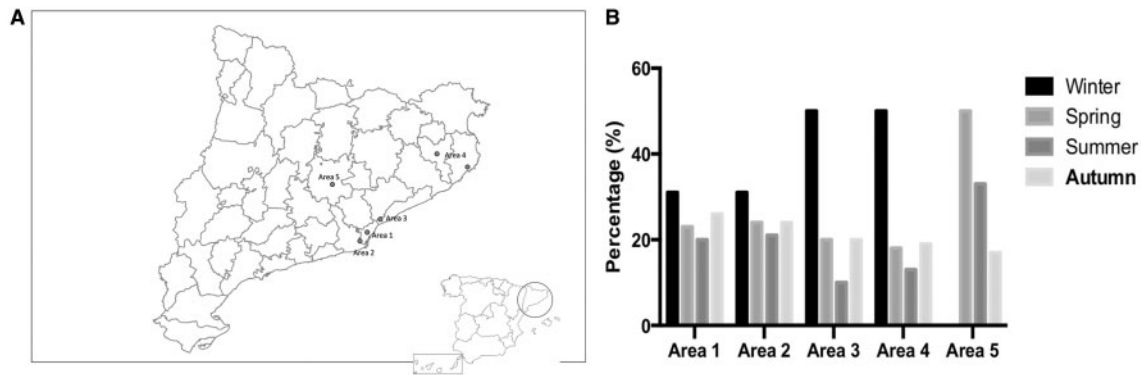


Fig. 3. Seasonal distribution of AAV patients in different hospitals included in the study. (A) Area 1 (Barcelona), Area 2 (L'Hospitalet de Llobregat), Area 3 (Badalona), Area 4 (Girona and Palamós) and Area 5 (Manresa). (B) The graph shows that patients from Barcelona (Area 1) and the south metropolitan area (Area 2) had similar patterns, with more homogenous distribution across the seasons. On the other hand, patients from the north metropolitan area or the north-east (Areas 3 and 4) had a greater incidence of AAV in the winter, while patients from the north-west (Area 5) had a greater incidence in the spring and summer.

with onset of the disease during the summer (14%), with a similar distribution across the other seasons. In our cohort, fewer patients presented with MPO-ANCA in the summer, but this association was not statistically significant.

With regard to ANCA subtypes, we found lower incidence in the spring for patients who were ANCA-negative, with only one patient's (5%) symptoms initiating during this season. Although ANCA positivity is noted in more than 90% of AAV patients, several studies have observed negative ANCA in 10–20% of cases [24–28]. Clinically, the majority of these cases are limited to kidney affection with a lower incidence of extra-renal symptoms. A functional effect of ANCA epitopes or different antibodies [28] (such as anti-moesin [29], anti-endothelial cells [30] and anti-human lysosome-associated membrane protein-2 [31]) has been implicated in the pathogenesis of this disease, but the exact mechanism remains unclear. The seasonal difference observed between ANCA-positive and -negative patients in our study supports the idea that these two entities may have different pathogenic triggers.

Interestingly, when we divided patients according to their geographic location, higher incidence of AAV was found in the winter for patients located in areas with similar characteristics (i.e. the east and north-east of Catalonia). It is possible that an environmental factor influences the higher incidence of the disease in these areas in the winter, a subject that deserves further investigation.

Some limitations of this study have to be considered. Since this study was retrospective and data about the onset of the first symptoms were obtained from medical records, we have to consider the possibility of recall bias and the difficulty of accurately determining the moment that this subacute disease first becomes apparent. Mahr et al. [18] analysed this problem in their study, comparing data obtained from medical records and telephone interviews, and found an average discrepancy of about 2.6 months. However, they still found similar results using data collected from medical records. As a result, we discuss here the seasonal nature of symptom presentation rather than the exact

Table 2. Seasonal variation of AAV onset stratified for selected variables (P values refer to comparison distribution across data)

Variables	Patients (n)	Season of onset, n (%)				P value
		Winter	Spring	Summer	Autumn	
Sex	234					0.5
Male	116	39 (33.6)	34 (29.3)	19 (16.4)	24 (20.7)	
Female	118	43 (36.4)	25 (21.2)	20 (17)	30 (25.4)	
Serum creatinine	234	60 (38)	34 (21.5)	27 (17.1)	37 (23.4)	0.45
<500 µmol/L	158	22 (29)	22 (29)	15 (19.7)	17 (22.3)	
>500 µmol/L	76					
ANCA subtype	234					0.35
Negative	20	8 (40)	1 (5)	5 (25)	6 (30)	
PR3	37	16 (43.2)	10 (27)	5 (13.6)	6 (16.2)	
MPO	177	57 (32.2)	43 (24.3)	34 (19.2)	43 (24.3)	
Histology	220	18 (46.2)	9 (23)	6 (15.4)	6 (15.4)	0.5
Sclerotic	39	66 (36.5)	39 (21.5)	31 (17)	45 (25)	
Others	181					

month of occurrence. The other principal limitation of this study is that only patients with AAV and renal affection were included in this study; despite the large number of patients, this approach might not be fully representative of AAV in general.

Conclusion

In conclusion, our results in a cohort of AAV patients with renal affection in Spain confirm a periodic fluctuation and seasonal variation of AAV, as described in the literature, with higher incidence in the winter. These results make it plausible that environmental factors, most likely an infection trigger, act as activators for the development of the disease. The seasonal differences observed between ANCA-positive and -negative patients warrant further careful examination.

Acknowledgements

X.R. acknowledges the support from the Daniel Bravo Foundation through the grant Windbiome. The rest of the authors received no specific funding for this work.

Conflict of interest statement

None declared.

References

- Finkielman JD, Lee AS, Hummel AM et al. ANCA are detectable in nearly all patients with active severe Wegener's granulomatosis. *Am J Med* 2007; 120: 643.e9–e14
- Scott DG, Watts RA. Epidemiology and clinical features of systemic vasculitis. *Clin Exp Nephrol* 2013; 17: 607–610
- Knight A, Ekblom A, Brandt L et al. Increasing incidence of Wegener's granulomatosis in Sweden, 1975–2001. *J Rheumatol* 2006; 33: 2060–2063
- Ntatsaki E, Watts RA, Scott DG. Epidemiology of ANCA-associated vasculitis. *Rheum Dis Clin North Am* 2010; 36: 447–461
- Gibson A, Stamp LK, Chapman PT et al. The epidemiology of Wegener's granulomatosis and microscopic polyangiitis in a Southern Hemisphere region. *Rheumatology* 2006; 45: 624–628
- Ormerod AS, Cook MC. Epidemiology of primary systemic vasculitis in the Australian Capital Territory and south-eastern New South Wales. *Intern Med J* 2008; 38: 816–823
- O'Donnell JL, Stevanovic VR, Frampton C et al. Wegener's granulomatosis in New Zealand: evidence for a latitude-dependent incidence gradient. *Intern Med J* 2007; 37: 242–246
- Lyons PA, Rayner TF, Trivedi S et al. Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med* 2012; 367: 214–223
- Hogan SL, Cooper GS, Savitz DA et al. Association of silica exposure with anti-neutrophil cytoplasmic autoantibody small-vessel vasculitis: a population-based, case-control study. *Clin J Am Soc Nephrol* 2007; 2: 290–299
- Mathieson PW, Peat DS, Short A et al. Coexistent membranous nephropathy and ANCA-positive crescentic glomerulonephritis in association with penicillamine. *Nephrol Dial Transplant* 1996; 11: 863–866
- Short AK, Lockwood CM. Antigen specificity in hydralazine associated ANCA positive systemic vasculitis. *QJM* 1995; 88: 775–783
- Stegeman CA, Tervaert JW, Sluiter WJ et al. Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. *Ann Intern Med* 1994; 120: 12–17
- Falk RJ, Hogan S, Carey TS et al. Clinical course of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and systemic vasculitis. The Glomerular Disease Collaborative Network. *Ann Intern Med* 1990; 113: 656–663
- Raynauld JP, Bloch DA, Fries JF. Seasonal variation in the onset of Wegener's granulomatosis, polyarteritis nodosa and giant cell arteritis. *J Rheumatol* 1993; 20: 1524–1526
- Carruthers DM, Watts RA, Symmons DP et al. Wegener's granulomatosis—increased incidence or increased recognition? *Br J Rheumatol* 1996; 35: 142–145
- Blockmans D, Bley T, Schmidt W. Imaging for large-vessel vasculitis. *Curr Opin Rheumatol* 2009; 21: 19–28
- Tidman M, Olander R, Svalander C et al. Patients hospitalized because of small vessel vasculitides with renal involvement in the period 1975–95: organ involvement, anti-neutrophil cytoplasmic antibodies patterns, seasonal attack rates and fluctuation of annual frequencies. *J Intern Med* 1998; 244: 133–141
- Mahr A, Artigues N, Coste J et al. Seasonal variations in onset of Wegener's granulomatosis: increased in summer? *J Rheumatol* 2006; 33: 1615–1622
- Rodó X, Ballester J, Cayan D et al. Association of Kawasaki disease with tropospheric wind patterns. *Sci Rep* 2011; 1: 152

20. Rodó X, Curcoll R, Robinson M et al. Tropospheric winds from northeastern China carry the etiologic agent of Kawasaki disease from its source to Japan. *Proc Natl Acad Sci U S A* 2014; 111: 7952–7957
21. Jennette JC, Falk RJ, Andrassy K et al. J Proposal of an international consensus conference. *Arthritis Rheum* 1994; 37: 187–192
22. Berden AE, Ferrario F, Hagen EC et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol* 2010; 21: 1628–1636
23. DeRemee RA, McDonald TJ, Weiland LH. Wegener's granulomatosis: observations on treatment with antimicrobial agents. *Mayo Clin Proc* 1985; 60: 27–32
24. Chen M, Yu F, Wang SX et al. Antineutrophil cytoplasmic autoantibody-negative Pauci-immune crescentic glomerulonephritis. *J Am Soc Nephrol* 2007; 18: 599–605
25. Hung PH, Chiu YL, Lin WC et al. Poor renal outcome of anti-neutrophil cytoplasmic antibody negative Pauci-immune glomerulonephritis in Taiwanese. *J Formos Med Assoc* 2006; 105: 804–812
26. Minz RW, Chhabra S, Joshi K et al. Renal histology in pauci-immune rapidly progressive glomerulonephritis: 8-year retrospective study. *Indian J Pathol Microbiol* 2012; 55: 28–32
27. Eisenberger U, Fakhouri F, Vanhille P et al. ANCA-negative pauci-immune renal vasculitis: histology and outcome. *Nephrol Dial Transplant* 2005; 20: 1392–1399
28. Furuta S, Jayne DR. Antineutrophil cytoplasm antibody-associated vasculitis: recent developments. *Kidney Int* 2013; 84: 244–249
29. Nagao T, Suzuki K, Utsunomiya K et al. Direct activation of glomerular endothelial cells by anti-moesin activity of anti-myeloperoxidase antibody. *Nephrol Dial Transplant* 2011; 26: 2752–2760
30. Cong M, Chen M, Zhang JJ et al. Anti-endothelial cell antibodies in antineutrophil cytoplasmic antibodies negative pauci-immune crescentic glomerulonephritis. *Nephrology* 2008; 13: 228–234
31. Kain R, Tadema H, McKinney EF et al. High prevalence of autoantibodies to hLAMP-2 in anti-neutrophil cytoplasmic antibody-associated vasculitis. *J Am Soc Nephrol* 2012; 23: 556–566