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Proteinase 3-and myeloperoxidase-serotype of anti-neutrophil cytoplasmic antibody (ANCA) in relation to demographical factors and geographical distribution in biopsy proven ANCA-associated glomerulonephritis

Authors and affiliations

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ABSTRACT

Background

In anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis, antigen specificity varies between myeloperoxidase (MPO) and proteinase 3 (PR3). This has been reported to vary in relation to age, gender, geography and extra-renal manifestations. However, studies are difficult to compare as criteria for inclusion varies.

The aim of this study was to investigate the relationship between ANCA serotype, latitude, ultraviolet (UV) radiation levels, age, gender and renal function at diagnosis in a large study with uniform inclusion criteria.

Methods

Patients with biopsy-proven ANCA-associated glomerulonephritis were identified from regional or nationwide registries in 14 centres in Norway, Sweden, the United Kingdom, the Czech Republic, Croatia, Italy, and the USA during the period 2000 to 2013. UV radiation levels for 2000 to 2013 in Europe were obtained from the Swedish Meteorological and Hydrological Institute.

Results

A total of 1408 patients (45.2% PR3-ANCA) were included in the study. In univariable analysis PR3-ANCA was significantly associated with male gender (OR 2.12; 95% CI 1.71-2.62), younger age (OR per year 0.97; 95% CI 0.96-0.98) and higher GFR (OR per ml/min 1.01; 95% CI 1.01-1.02) (p<0.001) at diagnosis, but not with latitude or UV radiation. In multivariable logistic regression analysis latitude and UV radiation also became significant, with higher odds for PR3-ANCA positivity at northern latitudes/lower UV radiation levels.
However, the latitudinal difference in MPO/PR3 ratio is smaller than differences previously reported concerning microscopic polyangiitis and granulomatosis with polyangiitis.

**Conclusions**

The ratio between PR3-ANCA and MPO-ANCA varies in glomerulonephritis with respect to age, gender, renal function and geographical latitude/UV radiation levels.

**Key words**

ANCA-associated vasculitis, MPO-ANCA, PR3-ANCA, glomerulonephritis, latitude
INTRODUCTION

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is often complicated by renal involvement and AAV constitute one of the most common causes of rapidly progressive glomerulonephritis and the nephritic syndrome(1, 2). Based on extra-renal features, AAV is classified as either microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA) or eosinophilic granulomatosis with polyangiitis(3). ANCA-associated nephritis (AAN) is seen in around 75 % of cases, with more frequent occurrence in MPA than in GPA and EGPA(4, 5). The etiology of AAV is still not known, but is believed to be dependent on both genetic and environmental factors with no single factor being sufficient to cause disease(6). Animal models provide strong evidence for a pathogenic role of ANCA in AAV, but it is less clear why the autoantibodies are formed(7).

In AAN the autoantibodies are directed against myeloperoxidase (MPO) or proteinase 3 (PR3)(8). Emerging data on the genetic association and clinical observations are likely to drive more changes in how ANCA-associated vasculitis is sub-grouped to guide management of these patients. Differences in the epidemiology between MPO-ANCA and PR3-ANCA AAN may reveal important clues concerning the etiology. MPO-ANCA is mainly associated with MPA and PR3-ANCA with GPA(9), and the ratio between MPO-ANCA and PR3-ANCA has also been reported to vary in relation to gender, age and geographical distribution(10, 11). Furthermore, the histological findings and renal outcome differ between PR3-ANCA and MPO-ANCA associated glomerulonephritis(12, 13).

The incidence of AAV is similar in the United Kingdom, Scandinavia and Japan(11, 14). However, in Asia there is a great predominance of MPO-ANCA and MPA compared with Europe and North America(15, 16). In Europe, studies have shown higher incidence of GPA in the north compared to the south(11, 17, 18). Data from New Zealand suggest a reciprocal gradient in the southern hemisphere(19). It has also been shown that the incidence of GPA
correlates inversely with ambient ultraviolet (UV) radiation (20) and a possible pathogenic explanation to the geographic gradients is the difference in UV radiation exposure at different latitudes. Latitudinal gradients and UV radiation have been studied extensively in other autoimmune diseases, such as multiple sclerosis and type 1 diabetes (21-23), but there are few studies of ANCA-associated vasculitis, and none focusing on serotype.

An alternative explanation for the geographical pattern could be genetic differences between populations. There is evidence of a genetic contribution to AAV with several genes and polymorphisms predisposing to disease (24, 25). The genetic composition seems to associate more strongly with ANCA serotype than with the phenotypic disease entities MPA and GPA (26).

Many of the epidemiological studies to date are small single center studies and difficult to compare directly due the heterogeneity in inclusion criteria and patient characteristics. Large, more homogeneous studies with greater geographical distribution are warranted.

The objective of the present study was to investigate the relationship between ANCA serotype, latitude, UV radiation levels, age, gender and renal function at diagnosis. This was done using uniform inclusion criteria in a large population of patients with biopsy-proven ANCA-associated glomerulonephritis identified from registries in Europe and North America.

**MATERIALS AND METHODS**

**Study population**

Patients with renal biopsy-proven ANCA-associated glomerulonephritis were identified from the Norwegian, Scottish, Croatian and Italian biopsy registries, the Czech vasculitis registry,
the regional vasculitis registries in Lund, Linköping and Cambridge, the Glomerular Disease Collaborative Network (GDCN) in North Carolina and Johns Hopkins Vasculitis Centre in Maryland.

The Norwegian renal biopsy registry is a national registry run by the Medical Department, Haukeland University Hospital. It started in 1988 and includes all native kidney biopsies except tumor biopsies performed in the country. For the present study, the patients were divided into groups based at four tertiary referral hospitals in the country.

The Scottish renal biopsy registry was established in 2005 (27). For this study, only data from Greater Glasgow, Clyde and Forth Valley regions were incorporated as they were known to include all cases of biopsy proven AAV along with relevant clinical data.

The Italian Registry of Renal Biopsies was established in 1987 and has been described in detail previously(28). For the present study the patients were divided into two groups according to region of residence.

The renal biopsy registry at Dubrava University Hospital in Zagreb is the largest renal biopsy registry in Croatia. Patients from all parts of Croatia referred to the Nephrology Unit for renal biopsy have been included in this study.

In the Czech Republic a single nation-wide vasculitis registry was formed in 2009, in which all patients with AAV diagnosed or followed-up in the participating centres were recorded.

The regional vasculitis registries in Linköping and Lund, Sweden both contain all patients diagnosed with AAV within defined geographic regions, and they have previously been described in detail(29, 30).

The vasculitis registry in Cambridge is based at a multi-disciplinary clinic at Addenbrooke’s hospital. It contains all patients with vasculitis referred to the hospital. For this study only
patients living in a defined geographical area surrounding the city of Cambridge were
included.

The registry of the Glomerular Disease Collaborative Network (GDCN) enrolls patients as
they are diagnosed at the University of North Carolina and in private practices throughout the
southeastern United States. Patients are primarily identified from renal biopsy diagnoses
evaluated through the University of North Carolina Nephropathology Service. The GDCN
has been described in detail in previous studies(31, 32). In this study only patients residing in
North Carolina were included.

The Johns Hopkins Vasculitis database enrolls subjects who are referred by practices within
the state of Maryland and surrounding states in North-East USA as they are diagnosed at
Johns Hopkins Hospital. For this study only patients residing in Maryland were included.

Only registries including consecutive patients from a defined geographic area were used for
this study. Tertiary referrals to Cambridge, Linköping and Lund from regions outside the
primary catchment area were excluded to reduce referral bias.

The inclusion criteria for the study was a clinical diagnosis of AAV verified by renal biopsy
during the period 2000 to 2013, ANCA-positivity verified by enzyme-linked immunosorbent
assay (ELISA) and age 18 years or above. Exclusion criteria were eosinophilic
granulomatosis with polyangiitis (EGPA) and polyarteritis nodosa (PAN) along with anti-
glomerular basement membrane (anti-GBM) disease, secondary vasculitis and drug-induced
vasculitis in accordance with the exclusion criteria in the European Medicines Agency
(EMA) algorithm(3). Patients were classified as either PR3-ANCA positive or MPO-ANCA
positive depending on the result of the ELISA test. Patients who were positive for both PR3-
ANCA and MPO-ANCA were classified to the serotype with the highest titre. Double-
positive patients were excluded if the ANCA titres were unknown. Glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) equation(33).

All patient data in the present study are anonymous registry data. The project was approved by the Ethical Review Board in Lund, Sweden, the University of North Carolina Institutional Review Board and the Institutional Review Board in Maryland. For the American patients informed consent was provided by all patients for collection of demographic and medical information, while this was not required for the European patients.

**Data collection**

Data was collected from the time of biopsy and included gender, age, ANCA serotype, and estimated GFR (eGFR). No follow-up data was collected for the present study. Antigen-specific ELISA was used to detect ANCA. The mean monthly erythemally (CIE) weighted UV radiation level for 2000 to 2013 in the regions in Europe was obtained from the STRÅNG database provided by the Swedish Meteorological and Hydrological Institute (SMHI)(34). Latitude and longitude coordinates are given in the World Geodetic System 84 coordinate reference system. For Europe, the coordinates are given for the centers included in the study and for USA the coordinates are given for the capital city of every state (Raleigh, North Carolina; Annapolis, Maryland).

**Statistical methods**

Statistical analysis was performed using Statistical Package for the Social Sciences: SPSS Statistics for Windows, version 21.0 (IBM Corp, Armonk, New York, USA). P-values less than 0.05 were considered significant. Continuous variables were expressed as medians and
interquartile ranges. Categorical variables were expressed as percentages. Differences between groups were analyzed using the Mann-Whitney test for non-parametric data and the Chi-square test for categorical data. Univariable and multivariable binary logistic regression analysis was used to assess the association between ANCA serotype and the variables gender, age, eGFR, latitude, longitude, and UV radiation level. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Spearman rank correlation analysis was performed between the input variables to check for strong correlations between the variables entered in the multivariable logistic regression analysis. All analyses exclude missing data.

**RESULTS**

**Baseline patient characteristics**

A total of 1408 patients were included in the study. MPO-ANCA positivity was seen in 54.8% of the patients and PR3-ANCA positivity in 45.2% of the patients. Median age at diagnosis was 64 years (interquartile range; IQR 53-72). Median eGFR at time of biopsy was 19 ml/min/1.73 m² (IQR 10-36) (Table 1). Male gender was more common in PR3-positive patients compared to MPO-ANCA positive patients (61.5% vs 43.0%; p<0.001). Median age was 60 years (IQR 50-69) in PR3-positive patients compared to 67 years (IQR 57-74) in MPO-positive patients (p<0.001) and median eGFR was 21 ml/min/1.73 m² (IQR 11-44) compared to 18 ml/min/1.73 m² (IQR 10-31) (p<0.001). The longitude of the participating centers varied between 35.8 degrees in the south and 69.6 degrees in the north. UV radiation levels in Europe varied between 5246 mWh/m² in the northernmost center and 14565 mWh/m² in the southernmost center (Table 2).

There was no difference in the distribution of MPO-ANCA and PR3-ANCA (P=0.16), gender (P=0.86), or eGFR (P=0.32) between the European patients and the American patients. The
American patients were of younger age with a median age of 60 years (IQR 50-72) compared to 64 years (IQR 54-72) in the European patients (P=0.008).

**Comparison between biopsied and non-biopsied patients**

The Linköping, Lund, and Cambridge registries were used to identify all patients with AAV in the uptake area with renal involvement according to Birmingham Vasculitis Activity Score (BVAS). Patients with ANCA-associated glomerulonephritis verified by renal biopsy (patients included in this study) were compared to patients who did not have a renal biopsy-proven diagnosis (patients excluded from this study). In Linköping, 71.0% of the AAV-patients with renal involvement underwent a kidney biopsy, the corresponding figure for Lund was 63.2% and for Cambridge 76.0%. In the Cambridge center, male gender was significantly more common in biopsied patients, as compared to non-biopsied patients. In the Linköping center, MPO-ANCA was more common in biopsied patients. In the Lund center, biopsied patients were significantly younger. Overall, MPO-ANCA was more common and age was lower in the biopsied patients (Table 3).

**Binary logistic regression analysis of ANCA serotype**

In univariable binary logistic regression analysis, age, gender, and eGFR were associated with ANCA serotype. Increasing age was associated with decreasing odds of being PR3-ANCA positive, while increasing eGFR and male gender were associated with higher odds for PR3-positivity. Latitude and longitude were not significantly associated with ANCA serotype in univariable analysis. In multivariable analysis age, eGFR and gender remained associated with ANCA serotype, and the association between latitude and ANCA serotype was significant. Increasing latitude was associated with higher odds for PR3-positivity (Table 3).
4). When analyzing the European patients separately, the results remained essentially the same (Table 5). Latitude and UV radiation were strongly correlated (Spearman’s rho -0.99), so were not entered in the multivariable analysis simultaneously. In multivariable analysis including UV radiation instead of latitude, UV radiation, age, gender and eGFR were significantly associated with ANCA serotype. Increasing UV radiation levels were associated with lower odds for PR3-positivity (Supplementary table 1). In an additional analysis excluding the centers in Southern Europe (Zagreb, Milan and Rome) and USA (North Carolina and Maryland), the association between ANCA serotype and both latitude and UV radiation level was lost, while it remained for age, gender and eGFR (Supplementary table 2).

**DISCUSSION**

The aim of this study was to describe geographic, demographic and clinical features in a large population of patients with biopsy-proven ANCA-associated glomerulonephritis using registry data from several countries.

We could confirm findings in previous studies showing that PR3-positive patients are of younger age and have higher eGFR than MPO-positive patients at diagnosis(10, 12, 35). The odds ratio for PR3-positivity was higher in men, which has also been reported before(35-38).

There was an association between ANCA serotype and both latitude and UV radiation levels in the multivariable analysis. The odds ratio of being PR3-positive increased from south to north and decreased with increasing UV radiation levels. These results are in line with previous studies by Watts(11, 39) showing that GPA is more common in the north of Europe and by Gatenby(20) showing a correlation between GPA and latitude and UV radiation levels. These previous studies of the geographical differences in AAV have been focused on disease phenotype and not on ANCA serotype. To our knowledge, this is the first study to
show an association for serotype specifically. The concept of PR3 ANCA-associated vasculitis and MPO ANCA-associated vasculitis in addition to the traditional division in the two disease entities GPA and MPA has been suggested to be preferred due to its superior predictive value in terms of relapses and renal survival(12, 40).

In our study the two centres in USA are located furthest south, and are the only centres with a westerly longitude. The population in South-eastern USA also has a mixed origin with large contributions from Latin America and Africa along with Europeans who are mainly of British descent. GPA/PR3-positivity has been shown to be less common in patients of non-European origin(44) and in African-Americans(45). It is difficult to exclude an effect of these genetical differences on the association between geographical location and serotype. We therefore conducted an analysis excluding these centres and the results remained essentially the same as in the entire study population. When analysing the centres in northern and central Europe separately, the significant association between latitude/UV-radiation levels and ANCA serotype was however not seen. The latitudinal difference between Tromsø and Prague is 20º and the UV-radiation exposure is more than twice as high in Prague compared to Tromsø. If UV radiation levels were of significant importance for ANCA serotype, an association between ANCA serotype and UV radiation levels could be expected in this sub analysis.

There are however, also genetic differences between populations in Europe along a north-south axis(41), and known genetic associations in AAV that affect MPO and PR3-ANCA differently(26). One gene variant whose global prevalence varies widely is the Pi*Z variant of alpha-1-antitrypsin which has been shown to influence the risk of PR3-ANCA but not MPO-ANCA. It is more common in Scandinavia, Western and Central Europe and countries colonized by Europeans(42). Watts and colleagues recently showed that GPA incidence was associated with latitude in univariable analysis, but multivariable analysis suggested that this was due to the distribution of HLA-DPB*0401 allele frequency(43). Hence, it is possible that
the observed latitudinal difference is caused by genetic differences affecting the distribution of ANCA serotypes, rather than an effect of UV radiation levels.

One proposed mechanism by which UV radiation could affect the immune system, and thus the occurrence of AAV, is by inducing vitamin D. Kemna et al. demonstrated that among AAV patients who have a rise in ANCA titre during fall, those with low vitamin D levels were more likely to experience a relapse compared to those who maintained normal vitamin D levels(46). However, vitamin D status is not only influenced by UV exposure of the skin, which is dependent on latitude and season, but also by diet, age, and skin pigmentation(47). An effect of differences in dietary intake of vitamin D between the participating countries cannot be ruled out since we did not have data on vitamin D levels.

In non-renal AAV, there is a predominance of PR3-positivity and a clinical diagnosis of GPA(10, 35) while in renal AAV, the distribution is more equal(10, 32, 35, 48). In this study, the odds of being PR3-positive was higher at northern latitudes as compared to southern latitudes, but the association was not very strong. Previous studies have shown large differences in the incidence of GPA between northern and southern Europe. It is possible that this difference to a large extent consists of patients with GPA without renal involvement, and this patient group is not included in the present study. Whether environment is a major determinant for extra-renal disease manifestations and disease phenotypes such as limited GPA was not the scope of this study and remains to be determined.

There are limitations in this study that need to be taken into account. The use of the latitude and mean UV radiation level for one city in every region do not account for the latitudinal differences and variation in sun exposure within a region or for migration from other regions. We chose to limit the study to renal biopsy-proven glomerulonephritis in order to reduce sampling bias between different regions and different units (nephrology and rheumatology).
This makes the study population more homogenous, but firm conclusions can only be drawn regarding biopsy proven renal AAV and the results cannot be generalized to non-renal AAV or renal AAV that is not confirmed by renal biopsy. This approach could also introduce a risk of bias based on differences in indications to perform renal biopsy. In an effort to investigate this potential bias, we compared biopsied and non-biopsied patients with renal involvement in three of the participating centres. We found that MPO-ANCA positivity was more common in biopsied patients as compared to non-biopsied patients with renal involvement, which would lead to an underestimation of PR3-positive patients in our study. The reasons for this difference could be that PR3-positive disease more often is confirmed by other organ biopsies (e.g. nasal, skin, muscle or lung) or that MPO-positive patients have more severe renal disease which makes the physician more prone to perform biopsy. We cannot rule out the possibility that there are slight differences in biopsy policies among the centres, and it is important to bear this in mind when interpreting the results. However it is unlikely that this would have altered the main findings of this study.

In conclusion, we found differences in age, gender distribution and renal function at diagnosis between MPO-ANCA positive and PR3-ANCA positive glomerulonephritis. There was also a significant association between ANCA serotype and latitude/UV radiation levels, which was however not seen in northern and central Europe. The contribution of genetic differences to the observed latitudinal gradient needs to be elucidated in further studies.

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CONFLICTS OF INTEREST STATEMENT
None declared.

AUTHORS CONTRIBUTIONS
M.S. initiated the project. M.S. and M.W. were responsible for the final design of the project. M.W. conducted the data analysis and drafted the manuscript. All authors contributed to the acquisition and interpretation of data, revision of the manuscript and approved the final version.

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REFERENCES


### Table 1. Clinical and demographic characteristics at time of biopsy

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<tr>
<th>Center</th>
<th>No</th>
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<th>Gender</th>
<th>Age median (IQR)</th>
<th>eGFR ml/min/1.73 median (IQR)²</th>
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<tr>
<td>All</td>
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<td>45.2%/54.8%</td>
<td>51.3%/48.7%</td>
<td>64 (53-72)</td>
<td>19 (10-36)</td>
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<td>Europe</td>
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<td>51.2%/48.8%</td>
<td>64 (54-72)</td>
<td>19 (11-37)</td>
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<td>52.4%/47.6%</td>
<td>60 (51-69)</td>
<td>17 (7-25)</td>
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<td>53.2%/46.8%</td>
<td>45.2%/54.8%</td>
<td>68 (58-76)</td>
<td>20 (8-38)</td>
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<td>52.3%/47.7%</td>
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<td>65 (53-74)</td>
<td>19 (10-40)</td>
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<td>12 (7-20)</td>
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<td>15 (8-25)</td>
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<tr>
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<td>60.0%/40.0%</td>
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<td>8 (6-21)</td>
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<td>18 (10-33)</td>
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<td>63 (55-72)</td>
<td>18 (10-29)</td>
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<td>North Carolina</td>
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<td>55.3%/44.7%</td>
<td>59 (49-72)</td>
<td>18 (10-36)</td>
</tr>
</tbody>
</table>

Data missing in 84 patients

ANCA, anti-neutrophil cytoplasmic antibody; PR3, proteinase 3; MPO, myeloperoxidase; IQR, interquartile range; eGFR, estimated glomerular filtration rate
Table 2. Latitude, longitude, and ultraviolet radiation levels of the participating centers.

<table>
<thead>
<tr>
<th>Center</th>
<th>Latitude</th>
<th>Longitude</th>
<th>UV radiation total $mWh/m^2$</th>
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<th>UV radiation June $mWh/m^2$</th>
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<td>60.4°N</td>
<td>5.3°E</td>
<td>7082</td>
<td>304</td>
<td>17724</td>
</tr>
<tr>
<td>Oslo</td>
<td>59.9°N</td>
<td>10.7°E</td>
<td>7623</td>
<td>400</td>
<td>18557</td>
</tr>
<tr>
<td>Linköping</td>
<td>58.4°N</td>
<td>15.6°E</td>
<td>9398</td>
<td>523</td>
<td>22730</td>
</tr>
<tr>
<td>Glasgow</td>
<td>55.9°N</td>
<td>-4.3°E</td>
<td>8086</td>
<td>736</td>
<td>18181</td>
</tr>
<tr>
<td>Lund</td>
<td>55.7°N</td>
<td>13.2°E</td>
<td>10087</td>
<td>726</td>
<td>23243</td>
</tr>
<tr>
<td>Cambridge</td>
<td>52.2°N</td>
<td>0.12°E</td>
<td>9851</td>
<td>1119</td>
<td>21613</td>
</tr>
<tr>
<td>Prague</td>
<td>50.1°N</td>
<td>14.4°E</td>
<td>12262</td>
<td>1588</td>
<td>26484</td>
</tr>
<tr>
<td>Zagreb</td>
<td>45.8°N</td>
<td>16.0°E</td>
<td>14395</td>
<td>2300</td>
<td>29484</td>
</tr>
<tr>
<td>Milan</td>
<td>45.5°N</td>
<td>9.2°E</td>
<td>13874</td>
<td>2807</td>
<td>26890</td>
</tr>
<tr>
<td>Rome</td>
<td>41.9°N</td>
<td>12.5°E</td>
<td>14565</td>
<td>2434</td>
<td>29562</td>
</tr>
<tr>
<td>Maryland</td>
<td>39.0°N</td>
<td>-76.5°W</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North Carolina</td>
<td>35.8°N</td>
<td>-78.6°W</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

UV, ultraviolet
Table 3. Biopsy versus no biopsy. Comparison between patients with renal ANCA-associated vasculitis who underwent renal biopsy and patients with renal ANCA-associated vasculitis who did not undergo renal biopsy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (N=175)</th>
<th>No biopsy (N=79)</th>
<th>P-value</th>
<th>Cambridge (N=54)</th>
<th>No biopsy (N=17)</th>
<th>P-value</th>
<th>Linköping (N=49)</th>
<th>No biopsy (N=20)</th>
<th>P-value</th>
<th>Lund (N=72)</th>
<th>No biopsy (N=42)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO</td>
<td>103 (58.9%)</td>
<td>34 (43.0%)</td>
<td>0.019</td>
<td>32 (59.3%)</td>
<td>6 (35.3%)</td>
<td>0.084</td>
<td>34 (69.4%)</td>
<td>7 (35.0%)</td>
<td>0.008</td>
<td>37 (51.4%)</td>
<td>21 (50.0%)</td>
<td>0.89</td>
</tr>
<tr>
<td>PR3</td>
<td>72 (41.1%)</td>
<td>45 (57.0%)</td>
<td>0.089</td>
<td>22 (40.7%)</td>
<td>11 (64.7%)</td>
<td>0.044</td>
<td>15 (30.6%)</td>
<td>13 (65.0%)</td>
<td>0.49</td>
<td>37 (48.6%)</td>
<td>21 (50.0%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Male</td>
<td>98 (56.0%)</td>
<td>45 (57.0%)</td>
<td>0.89</td>
<td>31 (57.4%)</td>
<td>5 (29.4%)</td>
<td>0.044</td>
<td>30 (61.2%)</td>
<td>14 (70.0%)</td>
<td>0.49</td>
<td>37 (51.4%)</td>
<td>26 (61.9%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Female</td>
<td>77 (44.0%)</td>
<td>34 (43.0%)</td>
<td>0.002</td>
<td>23 (42.6%)</td>
<td>12 (70.6%)</td>
<td>0.91</td>
<td>70 (61-75)</td>
<td>72 (60-77)</td>
<td>0.54</td>
<td>66 (54-75)</td>
<td>79 (64-84)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (median)</td>
<td>67 (58-74)</td>
<td>74 (60-81)</td>
<td>0.002</td>
<td>64 (58-73)</td>
<td>64 (51-72)</td>
<td>0.91</td>
<td>70 (61-75)</td>
<td>72 (60-77)</td>
<td>0.54</td>
<td>66 (54-75)</td>
<td>79 (64-84)</td>
<td>0.001</td>
</tr>
<tr>
<td>eGFR MDRD (median)</td>
<td>24 (12-43)</td>
<td>19 (11-56)</td>
<td>0.95</td>
<td>20 (10-43)</td>
<td>16 (8-58)</td>
<td>0.82</td>
<td>26 (18-37)</td>
<td>31 (10-59)</td>
<td>0.92</td>
<td>24 (13-44)</td>
<td>19 (11-47)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

a Data missing in 6 patients

MPO, myeloperoxidase; PR3, proteinase 3
Table 4. Analysis of ANCA serotype. Univariable and multivariable binary logistic regression analysis of ANCA serotype in all patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (per year)</strong></td>
<td>0.97 (0.96-0.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Gender (male)</strong></td>
<td>2.12 (1.71-2.62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>eGFR (per ml/min/1.73 m²)</strong></td>
<td>1.01 (1.01-1.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Latitude (per 10 units)</strong></td>
<td>1.12 (0.99-1.27)</td>
<td>0.071</td>
</tr>
<tr>
<td><strong>Longitude (per 10 units)</strong></td>
<td>1.03 (0.99-1.06)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*a The odds ratio refers to the probability of being PR3-ANCA positive

ANCA, anti-neutrophil cytoplasmic antibody; CI, confidence interval; PR3, proteinase 3; UV, ultraviolet
Table 5. Analysis of ANCA serotype in Europe. Univariable and multivariable binary logistic regression analysis of ANCA serotype in the European patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)(^a)</td>
<td>P value</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>0.97 (0.96-0.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>2.25 (1.78-2.85)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (per ml/min/1.73 m(^2))</td>
<td>1.01 (1.01-1.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Latitude (per 10 units)</td>
<td>1.12 (0.92-1.38)</td>
<td>0.26</td>
</tr>
<tr>
<td>Longitude (per 10 units)</td>
<td>1.09 (0.94-1.28)</td>
<td>0.26</td>
</tr>
<tr>
<td>UV radiation (per Wh/m(^2))</td>
<td>0.98 (0.94-1.03)</td>
<td>0.46</td>
</tr>
</tbody>
</table>

\(^a\)The odds ratio refers to the probability of being PR3-ANCA positive

ANCA, anti-neutrophil cytoplasmic antibody; CI, confidence interval; PR3, proteinase 3; UV, ultraviolet
**Supplementary table 1. Analysis of ANCA serotype in Europe.** Multivariable binary logistic regression analysis of ANCA specificity in the European patients including UV radiation levels as a variable.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year)</td>
<td>0.97 (0.97-0.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>2.08 (1.62-2.68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (per ml/min/1.73 m²)</td>
<td>1.01 (1.00-1.01)</td>
<td>0.016</td>
</tr>
<tr>
<td>Longitude (per 10 units)</td>
<td>1.03 (0.85-1.24)</td>
<td>0.77</td>
</tr>
<tr>
<td>UV radiation (per Wh/m³)</td>
<td>0.94 (0.89-0.99)</td>
<td>0.038</td>
</tr>
</tbody>
</table>

*a The odds ratio refers to the probability of being PR3-ANCA positive

ANCA, anti-neutrophil cytoplasmic antibody; CI, confidence interval; PR3, proteinase 3; UV, ultraviolet
**Supplementary table 2. Analysis of ANCA serotype in North and Central Europe.**

Multivariable binary logistic regression analysis of ANCA specificity excluding the patients in Southern Europe and USA.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio (95% CI)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year)</td>
<td>0.98 (0.97-0.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>2.01 (1.54-2.63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR (per ml/min/1.73 m²)</td>
<td>1.01 (1.00-1.01)</td>
<td>0.021</td>
</tr>
<tr>
<td>Latitude (per 10 units)</td>
<td>1.14 (0.85-1.52)</td>
<td>0.38</td>
</tr>
<tr>
<td>Longitude (per 10 units)</td>
<td>1.00 (0.84-1.19)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<sup>a</sup>The odds ratio refers to the probability of being PR3-ANCA positive

ANCA, anti-neutrophil cytoplasmic antibody; CI, confidence interval; PR3, proteinase 3; UV, ultraviolet