Asymptomatic Autoantibodies Associate with Future Anti-glomerular Basement Membrane Disease

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ABSTRACT
The pathophysiology of anti-glomerular basement membrane (anti-GBM) disease before clinical presentation is unknown. The presence of anti-GBM, anti-proteinase 3 (PR3), and anti-myeloperoxidase (MPO) antibodies associate with the disease at the time of diagnosis, but little is known about the presence of these autoantibodies before diagnosis. We used serum samples from the Department of Defense Serum Repository to conduct a case-control study involving 30 patients diagnosed with anti-GBM disease and 30 healthy controls matched for the age, gender, race, and age of the serum samples. We analyzed a maximum of three samples from each subject: the most recent sample before diagnosis, the penultimate sample before diagnosis, and the oldest sample available; the average time between the most recent sample and diagnosis was 195 days (range, 4 to 1346 days). Elevated anti-GBM levels (≥3 U/ml) were present in four patients, all less than 1 year before diagnosis but in no controls. Detectable anti-GBM antibody levels (≥1 U/ml but <3 U/ml) in a single serum sample before diagnosis were more frequent in cases than controls (70% versus 17%, P < 0.001). Only study patients had detectable anti-GBM levels in multiple samples before diagnosis (50% versus 0%, P < 0.001). Almost all patients had detectable anti-PR3 and/or anti-MPO that preceded the onset of disease. Among patients with a clear antecedent antibody, anti-PR3 or anti-MPO always became detectable before the anti-GBM antibody. In summary, our data describe the subclinical formation of autoantibodies, which improves our understanding of the pathophysiology of anti-GBM disease.


Anti-glomerular basement membrane (anti-GBM) disease is a rare autoimmune disease that causes significant morbidity and mortality in an often young and otherwise healthy population. Complete disease remission is possible with prompt diagnosis and treatment. The subclinical pathophysiology of anti-GBM disease is not fully understood.1–3

The heterogeneous clinical presentation of anti-GBM disease supports a “multiple hit” disease mechanism. Renal and pulmonary involvement can occur independently or together.4,5 Pulmonary involvement is associated with smoking and other environmental toxins, but the vast majority of exposed subjects do not develop anti-GBM disease. Renal involvement is associated with other glomerular diseases, but the majority of glomerulonephropathy cases do not develop anti-GBM disease. Moreover, anti-GBM antibodies have been documented in the absence of disease.6,7

Past research supports the importance of both auto-antibodies and target antigen display in the...
pathogenesis of anti-GBM disease. Anti-GBM antibody production is strongly associated with disease.8 The NC1 domain of the α3 chain of type IV collagen is the target antigen for anti-GBM antibodies.9 The normal structural configuration of collagen hexamers in the GBM prevents antigen and antibody interaction. The “cryptic antigen” is only exposed in the setting of faulty construction or GBM damage caused by disease.1–3 The strong association between elevated antineutrophil cytoplasmic antibody (ANCA) titers and anti-GBM disease suggests smoldering vasculitis as one potential disease culprit.5

Anti-GBM, anti-proteinase 3 (anti-PR3), and anti-myeloperoxidase (anti-MPO) antibody levels before disease diagnosis have not been investigated. We used the Department of Defense Serum Repository (DoDSR) to evaluate these antibodies in subjects before the diagnosis of anti-GBM disease and compared them to age, gender, race, and age of serum-matched healthy controls. We hypothesized that disease subjects form anti-GBM, anti-PR3, and anti-MPO antibodies years before clinical diagnosis.

RESULTS

Anti-GBM Antibody

Thirty patients were identified from the DoDSR with the ICD-9 code for anti-GBM disease. These patients consisted of predominantly Caucasian men less than 30 years old with more frequent renal involvement than pulmonary involvement (Table 1).

Thirteen percent (4 of 30) of study subjects had an elevated anti-GBM antibody level (>3 U/ml) before diagnosis compared with zero control subjects. Three elevations occurred less than 2 months before diagnosis, and the fourth occurred less than 10 months before diagnosis. The majority of study subjects did not have a banked serum sample during this high-yield time period. The average time between last serum sample and diagnosis was 195 days (4 to 1346 days).

A greater percentage of patients with disease had a single detectable anti-GBM level compared with matching controls at any time point before diagnosis (70% versus 17%, P < 0.001), greater than 1 year before diagnosis (67% versus 13%, P < 0.001), and greater than 3 years before diagnosis (54% versus 13%, P = 0.04; Table 2). Only patients with disease had multiple detectable anti-GBM levels over time (50% versus 0%, P < 0.001; Table 3). There was no statistically significant difference between the patients with disease and controls in the subgroup greater than 5 years before diagnosis.

Proteinase 3 Antibody

A greater percentage of patients with disease had a single detectable anti-PR3 antibody level (>1 U/ml) compared with matching controls at any time before diagnosis (80% versus 44%, P = 0.007), greater than 1 year before diagnosis (83% versus 30%, P < 0.001), greater than 3 years before diagnosis (92% versus 20%, P < 0.001), and greater than 5 years before diagnosis (100% versus 25%, P = 0.007; Table 2). Detectable anti-PR3 levels in multiple serum samples over time better distinguished patients with disease from healthy controls (82% versus 14%, P = 0.006; Table 3).

Myeloperoxidase Antibody

A greater percentage of disease subjects had a single detectable anti-MPO antibody level (>1 U/ml) compared with matching controls.

Table 2. A comparison of the percentage of study patients with detectable anti-GBM antibody (>3 U/ml), detectable anti-PR3 antibody (>1 U/ml), and detectable anti-MPO antibody (>1 U/ml) compared with matched healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (%)</th>
<th>Controls (%)</th>
<th>OR</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBM</td>
<td>70 (21/30)</td>
<td>17 (5/30)</td>
<td>12</td>
<td>3.3 to 40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;1 years</td>
<td>60 (12/20)</td>
<td>9 (2/22)</td>
<td>15</td>
<td>2.7 to 83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;1 years</td>
<td>67 (16/24)</td>
<td>13 (3/24)</td>
<td>14</td>
<td>3.2 to 61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;3 years</td>
<td>54 (7/13)</td>
<td>13 (2/15)</td>
<td>7.5</td>
<td>1.2 to 48</td>
<td>0.04</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>50 (4/8)</td>
<td>11 (1/8)</td>
<td>7</td>
<td>0.57 to 86</td>
<td>0.28</td>
</tr>
<tr>
<td>PR3</td>
<td>80 (24/30)</td>
<td>44 (13/30)</td>
<td>5.1</td>
<td>1.6 to 17</td>
<td>0.007</td>
</tr>
<tr>
<td>&lt;1 years</td>
<td>85 (17/20)</td>
<td>36 (8/22)</td>
<td>10</td>
<td>2.2 to 45</td>
<td>0.002</td>
</tr>
<tr>
<td>&gt;1 years</td>
<td>83 (20/24)</td>
<td>30 (7/23)</td>
<td>12</td>
<td>3.0 to 49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;3 years</td>
<td>92 (12/13)</td>
<td>20 (3/15)</td>
<td>48</td>
<td>4.3 to 529</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>100 (8/8)</td>
<td>25 (2/8)</td>
<td>49*</td>
<td>2.5 to 949*</td>
<td>0.007</td>
</tr>
<tr>
<td>MPO</td>
<td>87 (26/30)</td>
<td>60 (18/30)</td>
<td>4.3</td>
<td>1.2 to 16</td>
<td>0.04</td>
</tr>
<tr>
<td>&lt;1 years</td>
<td>90 (18/20)</td>
<td>45 (10/22)</td>
<td>11</td>
<td>2.0 to 58</td>
<td>0.003</td>
</tr>
<tr>
<td>&gt;1 years</td>
<td>83 (20/24)</td>
<td>54 (13/24)</td>
<td>4.2</td>
<td>1.1 to 16</td>
<td>0.05</td>
</tr>
<tr>
<td>&gt;3 years</td>
<td>77 (10/13)</td>
<td>67 (10/15)</td>
<td>1.6</td>
<td>0.3 to 8.9</td>
<td>0.69</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>75 (6/8)</td>
<td>78 (7/9)</td>
<td>0.86</td>
<td>0.09 to 8.1</td>
<td>1.00</td>
</tr>
</tbody>
</table>

GBM, glomerular basement membrane; PR3, proteinase 3; MPO, myeloperoxidase; OR, odds ratio; CI, confidence interval.

*Estimated because of actual infinite value.
controls at any time before diagnosis (87% versus 60%, P = 0.04) and greater than 1 year before diagnosis (83% versus 54%, P = 0.05; Table 2). Detectable anti-MPO levels in multiple serum samples over time better distinguished patients with disease from healthy controls (73% versus 27%, P = 0.006; Table 3). There was no statistically significant difference between the patients with disease and controls in both the subgroups greater than 3 years and greater than 5 years before diagnosis.

Time Course of Antibody Development

Within both the study group and the control group, there was no statistically significant evidence of a change in anti-GBM, anti-PR3, or anti-MPO antibody levels over time. The evaluation of rate of change of antibody level over time in each group is limited because, outside of the four study subjects with a substantially elevated anti-GBM level weeks before diagnosis, the majority of antibody levels fell in a very narrow range of either undetectable or detectable. We did demonstrate that there was no statistically significant difference between the percentage of patients with disease with detectable anti-GBM, anti-PR3, and anti-MPO antibody less than 1 year prior to diagnosis to greater than 1 year prior to diagnosis. GBM, glomerular basement membrane; PR3, proteinase 3; MPO, myeloperoxidase.

A comparison of change in autoantibody over time was limited by the binary nature of the majority of the data. GBM, glomerular basement membrane; PR3, proteinase 3; MPO, myeloperoxidase.

The summation of our data suggests that low level ANCA antibodies develop years to decades before diagnosis, followed later by the onset of low-level anti-GBM antibodies that can then persist for years before an acute rise weeks to months before the onset of clinical disease. Specifically, anti-PR3 antibodies were the only detectable antibodies associated with future anti-GBM disease greater than 5 years before diagnosis. Trends suggest that only a lack of power prevented the association from holding for additional years to decades. More importantly, anti-PR3 and/or anti-MPO antibodies were always detected before anti-GBM antibodies in disease subjects with a clear antecedent antibody. These findings suggest that ANCA antibodies play a direct role in the pathophysiology of anti-GBM disease, which confirms previous clinical observations and studies. There have been multiple case reports of elevated ANCA levels months to years before the diagnosis of anti-GBM disease.11–14 In addition, previous literature demonstrates that elevated ANCA levels are associated with anti-GBM disease.5,15 A single center study of 205 patients with anti-GBM disease reported 30.7% with elevated ANCA levels.16 A composite of 11

Table 3. A comparison between the percentage of disease subjects with detectable autoantibody in multiple serum samples over time versus matching healthy controls

<table>
<thead>
<tr>
<th>Detectable antibody in multiple serum samples per subject</th>
<th>Patients (%)</th>
<th>Controls (%)</th>
<th>Odds Ratio</th>
<th>Confidence Interval</th>
<th>P (Fisher’s Exact)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBM (≥1 U/ml)</td>
<td>50 (11/22)</td>
<td>0 (0/22)</td>
<td>30*</td>
<td>(3.4–268*)</td>
<td>0.0002</td>
</tr>
<tr>
<td>PR3 (≥1 U/ml)</td>
<td>82 (18/22)</td>
<td>14 (3/22)</td>
<td>29</td>
<td>(5.6–146)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MPO (≥1 U/ml)</td>
<td>73 (16/22)</td>
<td>27 (6/22)</td>
<td>7</td>
<td>(1.9–27)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Only 22 of 30 of the study subjects had multiple serum samples. GBM, glomerular basement membrane; PR3, proteinase 3; MPO, myeloperoxidase.

*Estimated because of actual infinite value.

The summation of our data suggests that low level ANCA antibodies develop years to decades before diagnosis, followed later by the onset of low-level anti-GBM antibodies that can then persist for years before an acute rise weeks to months before the onset of clinical disease. Specifically, anti-PR3 antibodies were the only detectable antibodies associated with future anti-GBM disease greater than 5 years before diagnosis. Trends suggest that only a lack of power prevented the association from holding for additional years to decades. More importantly, anti-PR3 and/or anti-MPO antibodies were always detected before anti-GBM antibodies in disease subjects with a clear antecedent antibody. These findings suggest that ANCA antibodies play a direct role in the pathophysiology of anti-GBM disease, which confirms previous clinical observations and studies. There have been multiple case reports of elevated ANCA levels months to years before the development of anti-GBM disease.11–14 In addition, previous literature demonstrates that elevated ANCA levels are associated with anti-GBM disease.5,15 A single center study of 205 patients with anti-GBM disease reported 30.7% with elevated ANCA levels.16 A composite of 11

DISCUSSION

Our results describe the natural history of anti-GBM, anti-PR3, and anti-MPO antibodies before the diagnosis of anti-GBM disease for the first time. They parallel a previously published DoDSR study that found autoantibodies present months to years before the diagnosis of systemic lupus erythematosus.10

The summation of our data suggests that low level ANCA antibodies develop years to decades before diagnosis, followed later by the onset of low-level anti-GBM antibodies that can then persist for years before an acute rise weeks to months before the onset of clinical disease. Specifically, anti-PR3 antibodies were the only detectable antibodies associated with future anti-GBM disease greater than 5 years before diagnosis. Trends suggest that only a lack of power prevented the association from holding for additional years to decades. More importantly, anti-PR3 and/or anti-MPO antibodies were always detected before anti-GBM antibodies in disease subjects with a clear antecedent antibody. These findings suggest that ANCA antibodies play a direct role in the pathophysiology of anti-GBM disease, which confirms previous clinical observations and studies. There have been multiple case reports of elevated ANCA levels months to years before the development of anti-GBM disease.11–14 In addition, previous literature demonstrates that elevated ANCA levels are associated with anti-GBM disease.5,15 A single center study of 205 patients with anti-GBM disease reported 30.7% with elevated ANCA levels.16 A composite of 11
previous studies including a total of 727 patients found a similar 31.5% with elevated ANCA levels. The majority of these subjects had elevated anti-MPO antibody levels, but these studies did have a smaller subpopulation with elevated anti-PR3 antibody levels, and they did not comment on antibody levels in the detectable but not elevated range. Finally, there has been a case report of concurrent anti-GBM disease and Wegener’s disease confirmed by lung and kidney histopathology in addition to dual positive serology. The specific contribution of ANCA to the mechanism of anti-GBM disease is unknown. One hypothesis is that it disrupts the basement membrane to expose the previously cryptic α3(IV)NC1 antigen triggering anti-GBM antibody production. An additional hypothesis is that ANCA inhibits abnormally overactive intracellular proteolytic enzymes involved in disruptive processing to allow the surface membrane presentation of previously absent α3(IV)NC1 antigen triggering anti-GBM disease. Previous studies demonstrate the ability of autoantibodies to alter the level of antigen presentation. Autoantibodies specific to enzymes in the cathepsin family are both associated with vasculitis and directly implicated in the inhibition of disruptive processing.

Stable low-level anti-GBM antibodies followed ANCA production by an average of approximately 3 years and were associated with future anti-GBM disease greater than 3 years before diagnosis. Specifically, only disease subjects had two separate longitudinal serum samples with detectable anti-GBM antibody. This standard could provide 100% specificity for any future clinical application. Although there is no prior literature that describes detectable anti-GBM antibody levels before disease, low-level anti-GBM antibody titers specific to α3(IV)NC1 have been reported in healthy controls. This is consistent with our findings of a small percentage of healthy controls with sporadically detectable antibody. Cui et al. determined that antibodies found in healthy controls did not have the subclass (IgG1) nor the high avidity of those found in patients with disease.

After an extended period of time with detectable ANCA and anti-GBM antibody, there was an abrupt rise in anti-GBM levels weeks to months before disease in the four patients with documented elevated antibodies. The relatively high percentage of patients with disease without an elevated anti-GBM antibody level (>3 U/ml) also supports a late and abrupt rise in antibody titer because a majority of disease subjects did not have a banked serum sample in the weeks before diagnosis. The presence of elevated anti-GBM antibodies before clinical presentation supports the theory that anti-GBM antibodies play a causative role in anti-GBM disease. Previous evidence of direct anti-GBM antibody pathogenicity includes post-transplant recurrence in the setting of elevated serum autoantibody, correlation between antibody level and disease severity, and de novo disease in animals exposed to human anti-GBM antibody.

The cause of the abrupt rise in anti-GBM antibody titers is unknown, but there are likely multiple potential inciting agents. Disruption of the GBM by environmental exposure, trauma, medications, or glomerular disease (to include ANCA induced damage) could expose the target antigen to trigger disease. In addition, viral mimicry could potentiate the low level antibody production found in our disease population. Our data speak against an acute rise in ANCA as the inciting agent. Taken as a whole, our data support at least a “two hit” theory of disease, but the comprehensive mechanism of disease is likely much more complex to include invariant natural killer cells, TGF-β levels, IL-23 levels, T-cell-directed IgG subclass switching, GBM type IV collagen subsets, HLA subclass, and noncrosslinked type IV collagen hexamers or trimers. This level of complexity and an exceedingly small incidence of disease suggest that a “multiple hit” theory of disease will be more accurate, but confirmation will require further study.

Because a significant amount of the presented data includes anti-GBM antibodies in the detectable but not clinically elevated range, it is important that the laboratory technique is both transparent and widely accepted. The EIA used in our study (BINDAZYMETM anti-GBM enzyme immunoassay kit MK034; Version 5, February 2007; The Binding Site, Ltd., Birmingham, UK) uses human recombinant anti-GBM antigen (the α3 chain of collagen type IV) produced in a baculovirus expression system in insect cells (SF9/baculovirus). This method of anti-GBM antigen production is commonly used for clinical assay and has been shown to provide good performance with diagnostic sensitivity of 94.7% and specificities of 94.9 to 100% in selected clinical EIAs. An earlier investigation tested the performance an earlier version of the BINDAZYMETM EIA that used purified human anti-GBM antigen rather than recombinant human anti-GBM antigen and required a 1:50, rather than a 1:100, sample-serum dilution. Using a GBM indirect fluorescence antibody assay in human kidney tissue as the gold standard test, the BINDAZYMETM EIA had 100% sensitivity, but 72% specificity. However, indirect fluorescence antibody assay itself may have a high false-negative rate and lack the sensitivity of anti-GBM EIAs that use purified GBM antigen. Moreover, it has recently been shown that detectable but low-level anti-GBM antibodies may occur in normal healthy humans and that these antibodies are specific for
the same epitopes as antibodies from patients with anti-GBM disease. Thus, the BINDAZYME™ EIA assay used for anti-GBM antibody detection appears to be quite sensitive, and the antibodies detected are likely to be directed against the α3 chain of collagen type IV, even if they are below the “clinically positive” range.

Knowledge of subclinical autoantibody trends improves our understanding of anti-GBM disease, but the current clinical application of this data set is limited. It is not realistic to screen an entire population for a disease with a low pretest probability. However, increasingly robust and economic genetic analysis in the future may define a subpopulation at increased risk for disease. Unfortunately, the presence of culprit genes will not guarantee transcription, translation, post-translation modifications, and protein to protein interactions. Knowledge of serum biomarker presence and progression before clinical disease could provide diagnostic synergy with high-risk genomes or proteomes identified in the future. In this setting, our data suggest that a least two detectable samples over time should be the standard to maximize specificity. Early identification of people at risk for disease could allow for targeted patient education and closer follow-up, resulting in improved therapeutic outcomes.

The DoDSR has unique limitations. Because subjects were identified by ICD-9 code without the benefit of a comprehensive chart review, our study cohort may include a small number of normal subjects. Antibody levels may degrade in serum during prolonged storage. This limitation is attenuated by matching the controls by the age of serum but does not account for study cases with previously detectable antibody that degraded to undetectable. Some study subject samples may have actually been banked after diagnosis and treatment because of a delay in coding. All of these limitations would only reduce the statistical significance of our findings by underestimating study cases with detectable or elevated anti-GBM antibody. Because of the retrospective evaluation of the prospectively compiled serum samples, the time from last sample to diagnosis and the intervals between samples vary between study subjects. Although time-matched controls compensate for general comparisons, this makes the evaluation of specific time periods before diagnosis more challenging because of limited power in some subgroups. The 17% of study subjects without ICD-9 documentation of pulmonary or renal disease could be due to a primary ICD-9 misdiagnosis or, more likely, an accurate diagnosis with an incomplete ICD-9 documentation of associated comorbidities. Because the Department of Defense cannot bill Medicare, coding often focuses on the primary diagnosis, and ancillary diagnoses may not have been coded separately. Therefore, the lack of ancillary codes for renal or pulmonary disease in patients with anti-GBM disease does not necessarily mean that these patients did not have renal and/or pulmonary manifestations. The validity of the primary ICD-9 diagnoses in the DoDSR has previously been validated via chart review. The few healthy controls with detectable anti-GBM antibody could have a small but real increased risk but lack other required deficiencies for anti-GBM disease.

The DoDSR in conjunction with the large heterogeneous military population of young adults provides the only known resource to analyze the subclinical pathophysiology of anti-GBM disease. Our results establish a clear association between baseline detectable anti-GBM, anti-PR3, and anti-MPO antibodies and future anti-GBM disease.

**CONCISE METHODS**

We performed a matched-case control study of DoD beneficiaries to assess for the association between both detectable and elevated anti-GBM and ANCA titers and future anti-GBM disease. We identified patients from the military database with an International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) code for anti-GBM disease (446.0) between January 1990 and October 2008. Only current or previous active duty members were included because dependents do not have banked serum in the DoDSR. Subjects required one hospital-coded or multiple outpatient-coded visits for inclusion to increase study-subject disease specificity. Additional disease-relevant ICD-9 codes were queried for study subjects by the DoDSR because chart-review data collection was not possible for the whole cohort. The DoDSR indentified age, gender, race, and age of serum-matched healthy controls (as determined by lack of ICD-9-coded disease) and pulled the serum samples for both study subjects and matching healthy controls.

The DoDSR has been administered by the Army Medical Surveillance Activity since 1985. Serum samples from U.S Armed Forces personnel are stored at the time of enlistment after passing a full medical evaluation and again approximately every other year until discharge or retirement. Currently over 40 million serum samples are stored at −30°C.

A maximum of three 0.5-ml serum samples were pulled for each study subject to include the most recent sample before the date of anti-GBM disease diagnosis, the second to last sample before diagnosis, and the oldest sample. One healthy control subject, matched for age, race, gender, and age and interval of serum samples, was selected for matching to each study subject from the Army Medical Surveillance Activity database.

After the serum samples were identified and pulled, they were sent to Quest Diagnostics Nichols Institute (Chantilly, VA). Clinical assays for anti-GBM, anti-PR3 (ANCA), and anti-MPO (ANCA) antibody levels were performed on each sample using a commercial enzyme immunoassay, described below. Quest Diagnostics was blinded as to whether the samples were from cases or controls. Quest participates in the College of American Pathology proficiency-testing program for these analytes at least twice yearly.

**Measurement of Anti-GBM Antibody**

Quantitative measurement of anti-GBM antibody serum concentration was performed using the BINDAZYME™ anti-GBM enzyme immunoassay kit MK034; Version 5, February 2007 (The Binding Site, Ltd., Birmingham, UK). 10-µl serum aliquots from the subjects
were diluted 1:100 with sample diluent and run in duplicate. Micro-
were precoated with recombinant human GBM antigen pro-
duced in baculovirus-infected cells. 100 µl each of calibrators (100, 33.3, 11.1, 3.7, and 1.23 U/ml), controls, and diluted subject serum samples were dispensed into the wells, and the assay was performed as described in the instructions for use. The secondary antibody was a peroxidase-labeled rabbit anti-human IgG conjugate, and detection was achieved using 3,3’5,5’ tetramethylbenzidine (TMB) stopped with phosphoric acid solution. Absorbance was determined at 450 nm. The results are reported in U/ml, with a measuring range of 1.23 to 100 U/ml. A clinically positive result was indicated by ≥3.0 U/ml. The normal range (< 3.0 U/ml) was determined on serum from 205 normal blood donors, with the upper limit of normal expressed as the mean concentration ± 3 SD (equivalent to 3.0 U/ml). Intra-assay precision for an anti-GBM antibody sample concentration of 2.0 U/ml was 2.3% coefficients of variation (CV), and interassay precision was 3.7 to 4.7% CV for sample concentrations of 2.2, 11.1, and 23.8 U/ml. Analytical sensitivity of the assay was 0.1 U/ml.

Measurement of MPO-ANCA
Quantitative measurement of MPO-ANCA antibody serum concent-
tration was performed using the Varelisa™ MPO ANCA EIA kit (Ph-
adia GmbH, Freiburg, Germany). Serum aliquots from the subjects were diluted 1:101 with sample diluent and run in duplicate. Micro-
cells were precoated with purified human MPO. 100 µl each of cali-
brators (0, 3, 7, 16, 40, and 100 U/ml), controls, and diluted subject serum samples were dispensed into the wells, and the assay was per-
formed as described in the instructions for use. The secondary anti-
body was a horseradish peroxidase-labeled anti-human IgG conju-
gate, and detection was achieved using TMB stopped with phosphoric acid solution. Absorbance was determined at 450 nm. The results are reported in U/ml, with a measuring range of 1.0 to 100 U/ml, and a detection limit of 1.0 U/ml. A clinically negative result was indicated by <6.0 U/ml. Intra-assay variability was 4.8 to 9.5 CV, and interassay variability was 3.5 to 10.7 CV over the range of the standards. The frequency distribution in 432 healthy subjects was 1.5 U/ml (95th percentile = 3.4 U/ml).

Measurement of PR3-ANCA
Quantitative measurement of PR3-ANCA antibody serum concent-
tration was performed using the Varelisa™ PR3 ANCA EIA kit (Ph-
adia GmbH, Freiburg, Germany). Serum aliquots from the subjects were diluted 1:101 with sample diluent and run in duplicate. The microwells were precoated with purified human neutrophil PR3. 100 µl each of calibrators (0, 3, 7, 16, 40, and 100 U/ml), controls, and diluted subject serum samples were dispensed into the wells, and the assay was performed as described in the instructions for use. The secondary antibody was a horseradish peroxidase-labeled anti-hu-
man IgG conjugate, and detection was achieved using TMB stopped with phosphoric acid solution. Absorbance was determined at 450 nm. The results are reported in U/ml, with a measuring range of 0.5 to 100 U/ml and a detection limit of 0.5 U/ml. A clinically negative result was indicated by <6.0 U/ml. Intra-assay variability was 4.8 to 5.9% CV, and interassay variability was 2.4 to 9.3% CV over the range of the standards. The frequency distribution in 432 healthy subjects was 0.7 U/ml (95th percentile = 1.2 U/ml).

For all three assays, the results were rounded to and reported in whole numbers. Anti-GBM antibody levels ≥1 and <3 U/ml were considered detectable, and levels ≥3 U/ml were clinically elevated. Anti-PR3 and anti-MPO antibody levels ≥1 and <6 U/ml were detectable, and levels ≥6 U/ml were considered elevated.

The percentage of study subjects with both detectable and elevated levels of anti-GBM antibody, anti-PR3 antibody, and anti-MPO an-
tibody before diagnosis were compared with the percentage of matched controls with detectable or elevated levels using the Fisher’s exact test. Subgroup analysis of less than 1 year, greater than 1 year, greater than 3 years, and greater than 5 years before diagnosis utilized the same statistical analyses for only the serum samples in the designated time period. Not all subjects had samples available for each time period. If multiple serum samples were present for a subject in a specific subgroup-analysis time period, the highest antibody level dic-
ated group assignment. Calculations of elevated anti-GBM levels ex-
cluded samples 4 weeks or less before diagnosis to rule out mistaken post-treatment samples secondary to the potential delay between hospitalization and ICD-9 coding.

ACKNOWLEDGMENTS
The authors thank Dr. Tara Givens (Immunology/Serology Depart-
ment, Quest Diagnostics Nichols Institute, Chantilly, VA).

DISCLOSURES
None.

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CORRECTION
Olson SW, Arbogast CB, Baker TP, Owshalimpur D, Oliver DK, Abbott KC, Yuan CM: Asymptomatic Autoantibodies Associate with Future Anti-glomerular Basement Membrane Disease. *J Am Soc Nephrol* 22: 1946–1952, 2011. In the original published version of the article, the following disclaimer was mistakenly omitted: “The views expressed in this article are those of the authors and do not reflect the official policy of the U.S. Department of the Army, the U.S. Department of Defense, or the U.S. government.” A corrected version of the article has been posted online.

CORRECTION