


APPENDIX

Operational criteria for substance-related impairments (complications) in various areas of life functioning.\[13\]

<table>
<thead>
<tr>
<th>Area of functioning</th>
<th>Severity of complications</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health</td>
<td>No complications</td>
<td>At least one health-related complication</td>
<td>Two health-related complication</td>
<td>≥2 health-related complication</td>
</tr>
<tr>
<td>Occupation</td>
<td>No problem</td>
<td>Irregularity/inefficiency/AIDS/absenteeism</td>
<td>Job changes/transfer/delayed or blocked promotion</td>
<td>Suspensions, dismissal, chronic joblessness</td>
</tr>
<tr>
<td>Finance</td>
<td>No problem</td>
<td>Spending up to 25% salary or income on alcohol-related expenses</td>
<td>Spending more than 25% income</td>
<td>Serious debts, losses, misappropriation, theft, embezzlement, robbery</td>
</tr>
<tr>
<td>Legal</td>
<td>No problem</td>
<td>Public intoxication</td>
<td>Caught for drunk driving or violent brawls</td>
<td>Imprisonment or public prosecution due to alcohol-related offence</td>
</tr>
<tr>
<td>Family</td>
<td>No problem</td>
<td>Strained interpersonal relation (IPR)</td>
<td>Disrupted family functioning</td>
<td>Family lives broken, disowned by family</td>
</tr>
<tr>
<td>Marital</td>
<td>No problem</td>
<td>Strained IPR</td>
<td>Episodes of separation</td>
<td>Divorce</td>
</tr>
<tr>
<td>Social</td>
<td>No problem</td>
<td>Impaired IPR</td>
<td>Restriction of social circle</td>
<td>Social ostracism</td>
</tr>
</tbody>
</table>

Aims To detect anti-neutrophil cytoplasmic antibodies (ANCA) and correlate it with serological, hematological parameters, and the Birmingham Vasculitis Activity Score (BVAS).

RESULTS

LRT involvement was seen in 91.3%, URT in 78.3%, and renal manifestations in 69.6% cases. The BVAS in CWG was significantly higher than BVAS in the LWG. Decreased hemoglobin, increased WBC counts, ESR, CRP and Creatinine were seen in CWG as compared to LWG. The C-ANCA was present in 65.2% patients and P-ANCA in 13% cases. Anti-PR3 was seen in 69.6% patients and anti-LF in 17.4% cases. Severity of disease and ANCA was higher in CWG than in LWG.

Conclusions Vasculitis syndromes are known to overlap and many go undetected; therefore ANCA testing, along with the clinical and histopathological observations may be helpful in early detection and management of WG cases.

KEY WORDS: anti-neutrophil cytoplasmic antibodies; anti-Proteinase3; cytoplasmic ANCA; enzyme linked immunosorbent assay; indirect immunofluorescence; Wegener’s granulomatosis.
cytoplasmic antibodies (ANCA) too, often correlated well with the activity of the disease.[3][4]

The methods used in the demonstration of ANCA are indirect immunofluorescence (IIF) test or enzyme-linked immunosorbent assays (ELISAs), either using a purified neutrophil cytoplasmic extract called as ‘α granules (G)’ or using purified neutrophil cytoplasmic granules like myeloperoxidase (MPO) or Proteinase3 (PR3). Immunofluorescence is still the method of choice for ANCA detection,[6][7] and is the most commonly used technique where perinuclear ANCA (P-ANCA), cytoplasmic ANCA (C-ANCA), and atypical ANCA (X-ANCA) patterns could be well distinguished. Cytoplasmic ANCA pattern is mostly associated with WG and is less often seen in other conditions, and its major target antigen is PR3.[8]

An increase in C-ANCA titer often precedes relapse of WG while a decrease in C-ANCA is seen with remission. Treatment, based on changes in serum C-ANCA titers, prevents disease relapses.[9] Monitoring of C-ANCA, in most cases, would therefore be of great value in distinguishing between changes due to WG and symptoms caused by other diseases. The present study was undertaken to identify the specificities and strength of ANCA and its subtypes in classical and limited WG cases by IIF and ELISA, and to correlate them with systemic or organ involvement and with other laboratory parameters.[10][11]

MATERIAL AND METHODS

Twenty-three patients with an established diagnosis of WG were studied for ANCA serology. The diagnosis of WG was established according to the clinical and histopathological observations.[3] All these patients were classified according to the American College of Rheumatologists (ACR) criteria.[12] The clinical and laboratory findings were carefully noted on the specially designed proforma. Systemic necrotizing vasculitis scores were noted in all cases on the basis of the Birmingham Vasculitis Activity Scores (BVAS).[13] This prospective study was carried out over a period of 4 years (2000–2004) after obtaining the requisite Ethics committee permission and informed consents from patients. Patients’ selection bias was avoided by selecting cases only after they were diagnosed by clinicians and the blinding was achieved, as they were unaware of the laboratory findings. The patients having the ages of above 18 years were included in the study and the exclusion criteria was HIV and HbsAg positivity, as well as the pregnant and lactating women. Confirmation of the diagnosis of renal vasculitis, necessitated renal biopsies and the assay was standardized.

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two patients (12.5%) were in remission, whereas seven patients in the LWG group had active disease. The male : female ratio varied in CWG being 2.2 : 1, whereas in LWG it was 1 : 6 [Table 2]. Some of the hematological findings of interest, as given in Table 3, were the low Hb values, increased WBC counts, and increased ESR values in CWG as compared to LWG. The CRP and creatinine levels, too, were found to be significantly increased in CWG group. This indicated that the CWG group of cases has more disease severity than the LWG group.

### Table 2: Demographic features and BVAS in CWG and LWG cases

<table>
<thead>
<tr>
<th>Features</th>
<th>CWG</th>
<th>LWG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number tested</td>
<td>16 (69.6%)</td>
<td>7 (30.4%)</td>
</tr>
<tr>
<td>Age range (Mean ± SD)</td>
<td>19–55 years (29.6 ± 10.9)</td>
<td>20–64 years (31.7 ± 15.5)</td>
</tr>
<tr>
<td>Disease status</td>
<td>Active 14</td>
<td>7</td>
</tr>
<tr>
<td>Remission</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>2.2 : 1</td>
<td>1 : 6</td>
</tr>
<tr>
<td>BVAS Mean</td>
<td>28.3</td>
<td>6.6</td>
</tr>
<tr>
<td>Range</td>
<td>16–48</td>
<td>6–8</td>
</tr>
</tbody>
</table>

### Table 3: Laboratory findings in cases of WG

<table>
<thead>
<tr>
<th>Type</th>
<th>Systemic</th>
<th>Cutaneous</th>
<th>Membranous</th>
<th>ENT</th>
<th>Chest</th>
<th>Cardiovascular</th>
<th>GI</th>
<th>Renal</th>
<th>CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWG (16)</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>12</td>
<td>14</td>
<td>1</td>
<td>5</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>LWG (7)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total (23)</td>
<td>3*</td>
<td>5</td>
<td>2</td>
<td>18</td>
<td>21</td>
<td>2</td>
<td>7</td>
<td>16</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4: ANCA serology in CWG and LWG cases

<table>
<thead>
<tr>
<th>WG type</th>
<th>Immunofluorescence</th>
<th>ELISA anti-PR3</th>
<th>Anti-MPO</th>
<th>Anti-LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWG (16)</td>
<td>RPGN with crescents (8)*</td>
<td>6</td>
<td>80–640</td>
<td>4</td>
</tr>
<tr>
<td>FPNG with crescents (2)</td>
<td>2</td>
<td>160</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>MPGN with crescents (4)</td>
<td>1</td>
<td>80</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>Gran. Nephritis (2)</td>
<td>2</td>
<td>80–160</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>LWG (7)</td>
<td>4</td>
<td>80–160</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>Total (23)</td>
<td>15</td>
<td>80–640</td>
<td>3</td>
<td>80–320</td>
</tr>
</tbody>
</table>

| Percentage of positivity | 18/23 (78.3%) | 6/21 | 13 | 78.3 | 6/25 | 4.3 | 17.4 |

ANCA, anti-neutrophil cytoplasmic antibodies; CWG, Classical Wegener's granulomatosis; RPGN, Rapidly progressive glomerulonephritis; FPGN, Focal proliferative glomerulonephritis; C-ANCA, Cytoplasmic ANCA; MPO, Myeloperoxidase; PR3, Proteinase3 antibodies; MPO, Anti-Myeloperoxidase antibodies; ANCA, Anti-Lactoferrin antibodies; α, α granules.

* One case underwent kidney transplant, and another, which was also Hepatitis B antigen (HbsAg) positive.

The serology of ANCA in WG showed interesting results. Totally 18 of 23 cases (78.3%) were ANCA positive by IIF and all of them were also detected by the broad spectrum PMN ag ELISA [Table 4]. In CWG, 13 cases (81.2%) showed ANCA positivity by IIF, of which 11 displayed the classical C-ANCA pattern and two had P-ANCA pattern. When these sera were further tested by ELISA for ANCA specificities, 12 in CWG (75%) had anti-PR3, one patient had anti-MPO and three had anti-LF antibodies. The two patients who were in remission after treatment did not show ANCA positivity, although earlier, during the active phase of disease, they were ANCA positive and had anti-PR3 antibodies. In the LWG group, five cases (71.4%) were ANCA positive by IIF, of which four cases showed C-ANCA pattern and one P-ANCA. When tested by ELISA, four cases in LWG (57.1%) had anti-PR3 and one patient had anti-LF. As shown in Table 4, serological specificity tests indicated a higher preponderance of anti-PR3 antibodies in both the WG groups.

**DISCUSSION**

Wegener's granulomatosis is an autoimmune disease of unknown aetiology where the initial phase is characterized by necrotizing granulomatous lesions, which usually affects the upper and lower airways. If undiagnosed and untreated, the kidneys may also be affected and the limited form would lead to a classical vasculitis form of WG and its accompanying necrotizing crescentic glomerulonephritis. [20][21] The involvement of other organs like skin, GI tract, ophthalmic and CNS as seen by us have also been noted by other workers.[22][23] An earlier clinical study of WG cases from India,[24] had reported that, among 25 patients studied, 84% had nose/paranasal, sinus involvement, 84% had lung involvement, 40% had ear manifestations, whereas kidney involvement was present in 72% patients and other symptoms such as joint and skin manifestations were present in 44 and 32%, respectively. Also the BVAS in...
the CWG group was higher than in LWG group. A male preponderance in CWG cases has been observed, which has also been reported by Kaushal et al., 1986 in an Indian study.[24]

Cytoplasmic ANCA is a serological marker for WG and C-ANCA titers mirror the disease activity, and relapses are often preceded by recurrence or increase in titer of C-ANCA.[27] Rarely, P-ANCA with anti-MPO specificity has been reported in WG.[28][29] Edgar et al.[30] had observed that titration of ANCA by IIF were clinically useful for monitoring disease activity in WG. van der Woude et al.[31] observed that 92.6% cases with active WG had high titer ANCA, while only 12.5% of patients in remission had ANCA in low titers and concluded that the detection of ANCA is a valuable tool for early diagnosis and estimation of disease activity. Bambery et al.,[32] has pointed out that a delay in diagnosis may lead to a higher mortality in patients with WG due to extensive vasculitis and renal syndromes. Cytoplasmic ANCA positivity was found in 88% of the cases and is considered as a sensitive marker of active WG.[30] Jennings et al.[33] noted that anti-PR3 antibodies were found in 77% of the active WG cases studied. Earlier, Haubitz et al., Ludemann and Gross too, had acknowledged the diagnostic value of ANCA in monitoring the clinical activity of WG.[31][32] Savige et al.[33] have reported that in patients with ‘limited’ WG about 60% are ANCA positive. Limited Wegener’s granulomatosis is recognized with increasing frequency, but probably most of the patients go on to develop renal involvement, indicating that LWG and CWG are part of a continuum and the long-term follow up of these cases is essential. In

the Indian scenario,[23][25] patients with LWG, have been mistakenly diagnosed to have tuberculosis, and drug therapies have been continued, despite the patients deteriorating. In such instances, ANCA testing would surely have helped. In the present study too, we have encountered three cases on anti-TB treatment since the last 6 months to 1 year, but have noticed no benefits.

This study shows a high incidence and strong titres of C-ANCA, along with anti-PR3 antibodies by ELISA in the active form of WG, whereas the two patients in remission after treatment, did not show ANCA positivity. We have also come across an unusual case of CWG with high titers of P-ANCA and having anti-MPO antibodies by ELISA, though P-ANCA is usually associated with a diverse disease spectrum, and is rarely seen in WG cases.[33] In a study, P-ANCA positivity with anti-MPO antibodies was reported in 20% of the WG cases studied.[28] Schonermarck et al.[44] however, had reported that fewer than 5% cases of WG have anti-MPO antibodies and also have a milder form of disease. The present study has also shown that the ANCA titers seen by IIF, correlated well with the ELISA values for anti-PR3 antibodies in WG cases, as has been reported in a larger study.[33]

Early detection of ANCA which shows a good correlation with disease activity is important because current treatment protocols can help to achieve remission in a large number of patients; however, it is difficult to establish a diagnosis of WG, especially in the early stages or in the limited forms of the disease where kidney involvement is not seen. Most of the vasculitic syndromes are known to overlap and many go undetected, and therefore the ANCA serology along with clinical and histopathological support may be helpful in early detection and management of WG cases. Also, from the immunopathological point of view, the CWG and LWG groups in WG may be a part of a disease spectrum with possible conversion of LYG to CWG, a more severe disease, which could be seen in follow-up studies.

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