Diagnostic value of anti-neuronal antibodies for paraneoplastic disorders of the nervous system

J W B Moll, S C Henzen-Løgman, T A W Splinter, M E L van der Burg, Ch J Vecht

Abstract
The diagnostic value of the presence of anti-neuronal antibodies in serum was examined in 21 patients suspected of paraneoplastic disorders of the nervous system (NS) (group 1) and was compared to three control groups; group 2: 25 patients with a neurological disease, without cancer and no signs of paraneoplastic disorder; group 3: 27 patients with neurological disease and cancer and no signs of a paraneoplastic disorder; group 4: 94 patients with cancer and without neurological disease. In group 1, anti-neuronal nuclear antibodies were detected in eight patients (38%), in titres from 1:1000 to 1:32 000. A small cell lung cancer was present in six patients, ovarian cancer in one patient and in one patient no tumour could be detected. The neurological symptoms preceded a diagnosis of cancer in five out of eight patients. Anti-neuronal antibodies were found in the serum of two out of 94 patients (2%) from control group 3 but not in serum from any of the other control groups. These data indicate a moderate sensitivity of 38%, but a high specificity of 98-6% (95% confidence interval 95.5-99.8%) for the presence of anti-neuronal nuclear antibodies if a paraneoplastic NS disorder is suspected.

Paraneoplastic disorders of the nervous system (NS) can be divided mainly into limbic encephalitis, brainstem encephalitis, subacute cerebellar degeneration and a sensory or motor neuronopathy. In more than 50% of cases, they precede the finding of the underlying neoplasm. Diagnosis during life can be difficult because clinical symptoms and findings on ancillary investigations are not specific. In recent years, several anti-neuronal auto-antibodies have been identified in the serum of patients with a paraneoplastic disorder of the NS. The antineural nuclear protein antibody (anti-Hu) is mainly associated with small cell lung carcinoma (SCLC) and binds to nuclei of neurons. The anti-Purkinje cell antibody (APCA) binds to the cytoplasm of Purkinje cells and has primarily been identified in patients with breast and ovarian cancer. Apart from its interest in the aetiology of paraneoplastic NS syndromes, the finding of these auto-antibodies can be used for diagnostic purposes. We investigated the diagnostic value of the presence of anti-neuronal antibodies in patients with neurological signs suspected of paraneoplastic origin and compared it with three control groups.

Patients and methods
Indirect immunofluorescence technique
The presence of anti-neuronal auto-antibodies was investigated by the indirect immunofluorescence method (IIF). Snap frozen tissue blocks of human neuronal tissue (obtained at necropsy within six hours after death, from individuals without neurological disease) and with fresh neuronal tissue obtained from healthy Wistar rats, were used as test tissue. Unfixed frozen sections of human and rat cortex, cerebellum, spinal cord and vagal and optic nerve were incubated with serum diluted with phosphate-buffered saline (PBS, pH 7.2) 1:50 up to 1:6400, for one hour at room temperature. After rinsing three times in PBS for five minutes the sections were incubated with anti-human Ig and IgG (Fab)2, IgM (Fab)2 and IgA (Fab)2 monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) for 30 minutes (Dako, Denmark). The dilution for the second antibody was 1:100 for Ig, IgG (Fab)2 and 1:50 for IgM (Fab)2. Sections were rinsed three times in PBS for five minutes, overlaid with a coverglass using a 10% glycerin/PBS solution as mounting fluid, and evaluated under a Leitz fluorescence microscope with a FITC filter.

As negative controls, sections were also incubated with PBS alone. To differentiate anti-neuronal antibodies from non-neuronal specific anti-nuclear antibodies, sera were also tested against frozen sections of normal human and rat liver. Stained sections were reviewed by the investigator and technician without previous knowledge of the patient’s clinical status. Sera were considered positive for anti-neuronal antibodies only when distinct fluorescence staining was present in a titre of 1:500 or higher. Antibody-positive sera were also incubated with frozen sections of normal human and rat cerebral cortex, cerebellum, spinal cord, optical and vagal nerve.

Four different categories of patients were distinguished: Group 1: patients with or without a verified cancer, in whom a reasonable or strong suspcion of a paraneoplastic NS disorder was present. Three case reports from this group have been described in detail; Group 2: control group of 25 patients with
neurological disease not related to a paraneoplastic NS disorder and without cancer; Group 3: control group of 27 patients with neurological disease not related to a paraneoplastic NS disorder and with cancer; Group 4: control group of 94 patients with cancer and no known neurological disease. Control sera of group 4 were collected over a period of five years and stored at −70°C. All patients in this group were prospectively studied and followed as part of ongoing chemotherapy trials.

All sera of groups 1, 2, 3 and 4 were negative for anti-nuclear antibodies (ANA), when tested on normal human and rat liver or muscle tissue. In peripheral nerve or non-neuronal tissue, no reactivity could be observed using the same sera. No differences were seen in staining patterns on human or rat tissue.

Results

The number of patients in each group and a listing of the neurological diagnoses are summarised in table 1. The individual clinical and IIF data in patients with positive titres can be found in table 2. Anti-neuronal antibodies were detected in eight out of 21 patients from group 1 (38%): in six patients with SCLC, in one patient with ovarian carcinoma and in one patient no tumour could be found. A bright speckled fluorescence of nuclei of cerebellar granular and Purkinje cells and cortical neurons with sparing of the nucleolus was observed in six patients (Ig 1 and 2). This anti-neuronal nuclear antibody was of the IgG type (titres from 1:1000 to 1:32 000), except one patient with an IgM antibody (titre 1:1000).

Neurological symptoms preceded the diagnosis of a neoplasm in five out of the eight positive patients from group 1 with a median time interval of four months. An organic brain syndrome, compatible with a diagnosis of limbic encephalitis was seen in three patients with SCLC.

Extensive investigations including CT scan of the brain, bacterial and viral cultures of the cerebrospinal fluid (CSF) were negative. Causes for metabolic encephalopathies could be excluded and there were no indications of toxic exposure or drug abuse. Psychiatric evaluation did not reveal a cause for the mental abnormalities. A subacute cerebellar degeneration consisting of a truncal and appendicular ataxia together with dysarthria was seen in two patients with SCLC. A sensory neuronopathy in association with abnormal plantar reflexes was found in one patient with SCLC and in one patient in whom no tumour could be detected. A brain stem encephalitis consisting of a peripheral facial palsy, vestibular vertigo, glossopharyngeal and vagal nerve dysfunction, dysarthria and pyramidal signs, was observed in a patient with ovarian carcinoma.

In the controls, only two patients of group 3 had increased titres of anti-neuronal nuclear antibodies, and both had a SCLC. One of these had recurrent headache with equivocal signs of a pyramidal tract lesion, but no other neurological signs. The other patient had brain metastases, treated by radiotherapy and was neurologically asymptomatic at the time serum was taken. When we consider group 2, 3 and 4 as one control group, the presence of anti-neuronal nuclear antibodies in patients suspected of a paraneoplastic NS disorder (group 1) indicate a sensitivity of 38% and a specificity of 98.6% (95% confidence interval 95.5–99.8%) see table 3.

Discussion

Anti-neuronal IgG antibodies in serum of patients with a paraneoplastic disorder of the NS have been identified, and can mainly be divided in anti-neuronal nuclear or cytoplasmic antibodies.24 Antibodies binding to neuronal nuclei have been observed in Purkinje, granular, basket and molecular cells of the cerebellum as well as

Table 1

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>Neurological disease with suspicion of paraneoplastic NS disease</th>
<th>Neurological disease without cancer and no suspicion of paraneoplastic NS disease</th>
<th>Cancer with neurological disease but no suspicion of paraneoplastic NS disease</th>
<th>Cancer without neurological disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21 Neurological disease with suspicion of paraneoplastic NS disease</td>
<td>25 Neurological disease without cancer and no suspicion of paraneoplastic NS disease</td>
<td>27 Cancer with neurological disease but no suspicion of paraneoplastic NS disease</td>
<td>94 Cancer without neurological disease</td>
</tr>
<tr>
<td>2</td>
<td>1 Neurological disease with suspicion of paraneoplastic NS disease</td>
<td>2 Neurological disease without cancer and no suspicion of paraneoplastic NS disease</td>
<td>3 Cancer with neurological disease but no suspicion of paraneoplastic NS disease</td>
<td>4 Cancer without neurological disease</td>
</tr>
<tr>
<td>3</td>
<td>3 Neurological disease with suspicion of paraneoplastic NS disease</td>
<td>4 Neurological disease without cancer and no suspicion of paraneoplastic NS disease</td>
<td>5 Cancer with neurological disease but no suspicion of paraneoplastic NS disease</td>
<td>6 Cancer without neurological disease</td>
</tr>
<tr>
<td>4</td>
<td>5 Neurological disease with suspicion of paraneoplastic NS disease</td>
<td>6 Neurological disease without cancer and no suspicion of paraneoplastic NS disease</td>
<td>7 Cancer with neurological disease but no suspicion of paraneoplastic NS disease</td>
<td>8 Cancer without neurological disease</td>
</tr>
</tbody>
</table>

Table 2 Clinical and IIF data of patients with anti-neuronal nuclear antibodies

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Onset (months)</th>
<th>Main clinical feature</th>
<th>Titre</th>
<th>Class</th>
<th>CN</th>
<th>PC</th>
<th>GC</th>
<th>BC</th>
<th>SC</th>
<th>MLC</th>
<th>AHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>SCLC</td>
<td>−0.5</td>
<td>Organic brain syndrome</td>
<td>1:1600</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>SCLC</td>
<td>−2.5</td>
<td>Organic brain syndrome, seizures</td>
<td>1:1000</td>
<td>IgM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>SCLC</td>
<td>24</td>
<td>Ataxia, dysarthria</td>
<td>1:32 000</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>SCLC</td>
<td>2</td>
<td>Ataxia, dysarthria, sensory neuropathy</td>
<td>1:32 000</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>SCLC</td>
<td>+4</td>
<td>Organic brain syndrome</td>
<td>1:2000</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Ovarian cancer</td>
<td>+9</td>
<td>Brain stem encephalitis</td>
<td>1:6400</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>No tumour</td>
<td></td>
<td>Sensory neuropathy</td>
<td>1:1600</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>SCLC</td>
<td>−6</td>
<td>Sensory neuropathy</td>
<td>1:25 000</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>SCLC</td>
<td></td>
<td>Brain metastasis</td>
<td>1:800</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>SCLC</td>
<td></td>
<td>Pyramidal tract lesion</td>
<td>1:5400</td>
<td>IgG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

with a diagnosis of limbic encephalitis, in two
patients with cerebellar ataxia and in one
patient with a sensory neuropathy, all with
SCLC. An isolated IgM antibody in a patient
with SCLC and suspected of limbic enceph-
alis is a new observation. An anti-neuronal
cellular antibody in a patient with brain stem
encephalitis and ovarian cancer has also not
been reported before. The cause of this varia-
tion in clinical syndromes is unknown, but it
can relate to differences in anti-nuclear
antibodies or variability in binding to one
group of neurons or another.

In contrast, anti-neuronal cytoplasmic
antibodies have primarily been associated with
binding to Purkinje cells together with the
occurrence of cerebellar ataxia and have been
designated as APCA (anti-Purkinje cell
antibodies).146 The clinical picture is usually
designated as a paraneoplastic subacute
cerebellar degeneration and has been encoun-
tered in gynaecological cancer, mainly ovarian,
in breast and small cell lung cancer or in
lymphoma.14 In more than 50% of cases the
paraneoplastic neurological disorder precedes
the finding of a malignancy,1 we which we also
observed here. Until recently a final diagnosis
of paraneoplastic disorder of the NS could only
be confirmed at necropsy since the results of
ancillary investigations are often negative or
aspecific. Viral and post-viral inflammatory
disease, Sjögren’s syndrome or an idiopathic
syndrome may give rise to similar clinical
pictures.89 The detection therefore of anti-
nuclear auto-antibodies in serum of patients
suspected of a paraneoplastic NS syndrome
could be of great help.56 For this purpose
information is needed about the sensitivity and
specificity of the presence of these auto-
antibodies.

For anti-Hu antibodies, Anderson et al
found a specificity of almost 100% in a series of
18 patients with paraneoplastic NS disorder
together with various control groups, up to a
total of 303 patients with or without cancer or
neurological abnormalities.2 They found false
positive antibodies in two patients with a possible
paraneoplastic neurological syn-
drome, but without the presence of a malign-
ancy.7 We found positive auto-antibodies in
two control patients of group 3. Two of these
had a small cell lung cancer of the lung and
positive titres of anti-neuronal nuclear
antibodies. Neither of them had signs which are
usually compatible with a paraneoplastic
disorder of the NS. When we restrict ourselves
to patients with positive titres of anti-neuronal

Table 3  Diagnostic value of anti-neuronal antibodies for the presence of a paraneoplastic NS disorder*

<table>
<thead>
<tr>
<th>Anti-neuronal nuclear antibodies</th>
<th>Number of positive tests in suspected patients</th>
<th>Number of negative tests in control patients</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al 1988</td>
<td>14/87</td>
<td>303/303</td>
<td>14-5%</td>
<td>100%</td>
</tr>
<tr>
<td>This series</td>
<td>8/21</td>
<td>144/146</td>
<td>38%</td>
<td>98%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-neuronal cytoplasmic antibodies</th>
<th>Number of positive tests in suspected patients</th>
<th>Number of negative tests in control patients</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaekle et al 1985</td>
<td>6/12</td>
<td>167/167</td>
<td>50%</td>
<td>100%</td>
</tr>
<tr>
<td>Anderson et al 1988</td>
<td>10/42</td>
<td>317/319</td>
<td>43%</td>
<td>99%</td>
</tr>
<tr>
<td>Smith et al 1986</td>
<td>6/26</td>
<td>125/125</td>
<td>23%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*In this table only series in which a titre of 1:500 or higher was considered to be positive have been included.
nuclear antibodies in serum, we found a specificity of 98%, for the association of these antibodies with a suspected paraneoplastic disorder of the NS.

The presence of APCA has also been shown to be highly specific for a diagnosis of a paraneoplastic cerebellar degeneration. In contrast to its specificity, the diagnostic sensitivity for the presence of either anti-Hu or APCA-antibodies was rather low in the few reported series, but this may be partly due to differences in opinion on a sufficiently high titre in serum. A cut-off point of a titre of 1:500 or higher prevents a false-positive interpretation of non-specific background stain.

Despite this precaution, the sensitivity may further differ from one series to another as the degree of clinical suspicion and the interpretation of subtle neurological findings is open to variation. Nevertheless, its high specificity makes the indirect immunofluorescence test a clinically useful screening test for the detection of anti-neuronal antibodies in serum of patients suspected of paraneoplastic disorder of the NS.

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