Detection of paraneoplastic anti-neuronal antibodies

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Presentation format

- Background
- Detection method
- Examples
- Conclusion
These are defined as **anti-neuronal antibodies** associated with paraneoplastic neurological syndrome and systemic malignancies.
Association of polyneuropathy & cancer

DES NÉVRITE S PÉRIPHÉRIQUES
CHEZ LES CANCÉREUX

(Laboratoire de clinique de M. le professeur Pitres.)

Par M. Auchê 1890

Médecin des hôpitaux de Marseille.

Rev. de méd., tome x. — octobre 1890.
• Relationship between neurological disorder and systemic tumours has been dates back to 18th century

• Immunological involvement was hypothesised by Russell Brain (JNNP 14:59-75, 1951)

• Breakthrough in 1980s by Posner & colleagues: detection of antibodies reacting with both nervous system and tumours
Paraneoplastic neurological syndromes involves:

- Immune system
- Systemic tumour
- Nervous system
# PNS + antibody (without cancer)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Ab without cancer (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu</td>
<td>2% (200)</td>
</tr>
<tr>
<td>Yo</td>
<td>2% (125)</td>
</tr>
<tr>
<td>CV2</td>
<td>4% (47)</td>
</tr>
<tr>
<td>Ri</td>
<td>3% (6)</td>
</tr>
<tr>
<td>Ma2/Ta</td>
<td>4% (55)</td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>5% (20)</td>
</tr>
<tr>
<td>Tr</td>
<td>11% (28)</td>
</tr>
</tbody>
</table>

# Cancer + antibody (without PNS)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cancer without PNS (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu</td>
<td>16% (196 SCLC)</td>
</tr>
<tr>
<td>Yo</td>
<td>1% (107 ovary, breast)</td>
</tr>
<tr>
<td>CV2</td>
<td>9% (74 SCLC)</td>
</tr>
<tr>
<td>Ri</td>
<td>4% (181 ovarian cancer)</td>
</tr>
<tr>
<td>Ma2/Ta</td>
<td>0% (350 testicular, lung)</td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>1% (146 SCLC)</td>
</tr>
<tr>
<td>Tr</td>
<td>0% (30 Hodgkins disease)</td>
</tr>
</tbody>
</table>

## Immunological and clinical diversity

<table>
<thead>
<tr>
<th>Immune response</th>
<th>Syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu</td>
<td>Paraneoplastic encephalomyelitis, Sensory neuropathy</td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>Paraneoplastic encephalomyelitis, Stiff persons syndrome</td>
</tr>
<tr>
<td>Hu, Yo, Tr</td>
<td>Paraneoplastic cerebellar degeneration</td>
</tr>
<tr>
<td>Hu, Ma2/Ta</td>
<td>Limbic encephalitis</td>
</tr>
</tbody>
</table>

Ref: Brain (1999), 9:275-284
Associated cancers:

• ~5 most commonly occurring tumours
• Small cell lung carcinoma (Hu, Ri, CV2, amphiphysin)
• Gynaecological (Yo, Ri)
• Breast (Yo, Ri, amphiphysin)
• Testicular (Ma2)
• Hodgkin’s lymphoma (Tr, GluR1)
Pathological features:

• Any part of CNS can be affected
• Single cell type e.g., Purkinje cells
• Single area e.g., limbic encephalitis
• Multiple level e.g., encephalomyeloradiculitis (inflammation of brain, spinal cord & spinal roots)
Neurological symptoms:

- Signs and symptoms are diverse
- Rapid onset
- Often precede the detection of the paraneoplastic tumours (>60%)
- Results from an autoimmune response against neuronal antigens
Neurological symptoms:

- Walking difficulties
- Swallowing difficulties
- Loss of muscle tone
- Loss of fine motor coordination
- Memory loss/dementia
- Visual problems
- Seizures
- Sensory loss in limbs
- Vertigo
- Slurred speech
Nomenclature

• Nomenclature is not internationally agreed
• Two systems exist
  ➢ One based on first two letters of the surname of the patient
  ➢ Second based on morphological distribution of the antibody
• To date, both systems are being used
Paraneoplastic CNS auto-antibodies:

- Divided into 3 groups based on immunocytochemical distribution of antibodies
  1. Binding to Purkinje cell cytoplasm
  2. Binding to neuronal nuclei
  3. Others
## Cytoplasmic antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>MW (kDa)</th>
<th>Staining pattern</th>
<th>PND</th>
<th>Associated tumour(s)</th>
<th>Target antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yo (PCA-1)</td>
<td>34, 52, 62</td>
<td>Purkinje cell cytoplasm &amp; axons</td>
<td>PCD</td>
<td>Ovary, breast</td>
<td>PCD17/CDR62 (58 kDa), cytoplasm, leucine zipper, zinc finger, transcription 34 kDa, 6 amino acid repeat</td>
</tr>
<tr>
<td>GluR1</td>
<td>~140</td>
<td>Purkinje cell cytoplasm, climbing fibre</td>
<td>PCD</td>
<td>Hodgkin’s lymphoma</td>
<td>mGluR1</td>
</tr>
<tr>
<td>PCA-2</td>
<td>280</td>
<td>Purkinje cell cytoplasm and other neurones</td>
<td>PEM, PCD, LEMS</td>
<td>SCLC</td>
<td>280 kDa protein</td>
</tr>
<tr>
<td>Tr</td>
<td>[??]</td>
<td>Purkinje cell cytoplasm with “dots” in molecular layer</td>
<td>PCD</td>
<td>Hodgkin’s lymphoma</td>
<td>Unknown function (found in the Purkinje cell cytoplasm)</td>
</tr>
</tbody>
</table>

**KEY**: PND = paraneoplastic neurological disorder, PCD = paraneoplastic cerebellar degeneration, PEM = paraneoplastic encephalomyelitis, SCLC = small cell lung carcinoma  
[??] = No common band has been identified by Western blot analysis
# Nuclear Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>MW (kDa)</th>
<th>Staining pattern</th>
<th>PND</th>
<th>Associated tumour(s)</th>
<th>Target antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zic4</td>
<td>~37</td>
<td>Nuclei of granular neurones, weaker on Purkinje cell nuclei</td>
<td>PCD</td>
<td>SCLC</td>
<td></td>
</tr>
<tr>
<td>Ma (Ma1)</td>
<td>37, 40</td>
<td>Neuronal nucleoli</td>
<td>PCD, BE</td>
<td>Various, lung cancer</td>
<td>Ma1 protein</td>
</tr>
<tr>
<td>Ta (Ma2)</td>
<td>41.5</td>
<td>Neuronal nucleoli, perikaryon</td>
<td>BE, LE</td>
<td>Testicular cancer</td>
<td>Ma2 protein, Unknown function</td>
</tr>
<tr>
<td>Hu (ANNA1)</td>
<td>34-40</td>
<td>Nuclei of both central and peripheral neurones</td>
<td>PCD, PEM, SN</td>
<td>SCLC</td>
<td>HuD, PLE21/HuC, Hel-N1, 35-40 kDa nucleus and slightly cytoplasm RNA recognition motifs, translation</td>
</tr>
<tr>
<td>Ri (ANNA2)</td>
<td>55, 80</td>
<td>Nuclei of central neurones</td>
<td>OM, PCD, BE</td>
<td>Breast, SCLC, gynaecological</td>
<td>Nova-1 (55 kDa) and 80 kDa, nucleus and slightly cytoplasm only in CNS, RNA recognition motifs, translation</td>
</tr>
<tr>
<td>ANNA-3</td>
<td>170</td>
<td>Purkinje cell cytoplasm &amp; nucleus + glomerular podocytes</td>
<td>PCD, PEM, SN</td>
<td>SCLC</td>
<td>170 kDa protein</td>
</tr>
<tr>
<td>AGNA</td>
<td>[??]</td>
<td>Nuclei of Bergmann glia of cerebellar Purkinje layer and glial in white matter</td>
<td>PND</td>
<td>SCLC</td>
<td>Nuclear antigen of Bergmann glia (cerebellar Purkinje layer) and glial in white matter</td>
</tr>
</tbody>
</table>

**KEY**: PND = paraneoplastic neurological disorder, PCD = paraneoplastic cerebellar degeneration, PEM = paraneoplastic encephalomyelitis, SN = sensory neuropathy, OM= opsoclonus/myclonus, BE = brainstem encephalomyelitis, LE = limbic encephalomyelitis, SCLC= small cell lung carcinoma. [??] = No common band has been identified by Western blot analysis.
## Others

<table>
<thead>
<tr>
<th>Antibody</th>
<th>MW (kDa)</th>
<th>Staining pattern</th>
<th>PND</th>
<th>Associated tumour(s)</th>
<th>Target antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recoverin</td>
<td>23, 65</td>
<td>Retinal photoreceptor</td>
<td>Retinopathy</td>
<td>SCLC</td>
<td>23 kDa, calcium binding protein</td>
</tr>
<tr>
<td>GAD</td>
<td>65, 67</td>
<td>Islet cells &amp; grey matter</td>
<td>SPS</td>
<td>Breast, colon, SCLC</td>
<td>GAD, 65 kDa, GABA-ergic neuron, synthesis of GABA</td>
</tr>
<tr>
<td>CV2/CRMP5</td>
<td>66</td>
<td>Oligodendrocytes cytoplasm</td>
<td>PEM/SN</td>
<td>SCLS, thymoma</td>
<td>POP66, 66 kDa, cytoplasmic in some oligodendrocytes</td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>128</td>
<td>Central presynaptic terminals</td>
<td>SPS, PEM</td>
<td>Breast cancer, SCLC</td>
<td>Amphiphysin, neurophil, cytoplasm doublet bands at 125-128 kDa, synaptic vesicle-associated protein</td>
</tr>
<tr>
<td>VGCC</td>
<td>64</td>
<td>Presynaptic neuromuscular junction</td>
<td>LEMS, PCD</td>
<td>SCLC</td>
<td>64 kDa P/Q voltage gated calcium channel, acetylcholine release</td>
</tr>
<tr>
<td>VGKC</td>
<td></td>
<td>Peripheral nerve</td>
<td>Neuromyotonia</td>
<td>SCLS, thymoma</td>
<td>Voltage gated potassium channel</td>
</tr>
<tr>
<td>ACh R</td>
<td></td>
<td>Post-synaptic neuromuscular junction</td>
<td>MG</td>
<td>Thymoma</td>
<td>ACh receptor</td>
</tr>
</tbody>
</table>

**KEY:** PCD = paraneoplastic cerebellar degeneration, PEM = paraneoplastic encephalomyelitis, SN = sensory neuropathy, LEMS = Lambert-Eaton myasthenic syndrome, SPS = Stiff person syndrome, SCLC= small cell lung carcinoma, MG = myasthenia gravis, ACh (R) = acetylcholine (receptor), VGCC = Voltage gated calcium channel, VGKC = Voltage gated potassium channel
## Characterised onconeuralonal antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Sex</th>
<th>Cases worldwide</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANNA1 (Hu)</td>
<td>M&gt;F (PCD)</td>
<td>&gt;600</td>
</tr>
<tr>
<td></td>
<td>F&gt;M (PEM,SN)</td>
<td></td>
</tr>
<tr>
<td>Yo (PCA1)</td>
<td>F&gt;M</td>
<td>&gt;200</td>
</tr>
<tr>
<td>CV2 (CRMP5)</td>
<td>F&gt;M</td>
<td>&gt;100</td>
</tr>
<tr>
<td>ANNA2 (Ri)</td>
<td>F&gt;M</td>
<td>61</td>
</tr>
<tr>
<td>Ma2 (Ta)</td>
<td>M&gt;F</td>
<td>55</td>
</tr>
<tr>
<td>Amphiphysin</td>
<td>F&gt;M</td>
<td>20</td>
</tr>
<tr>
<td>♦ Tr (PCA-Tr)</td>
<td>M&gt;F</td>
<td>28</td>
</tr>
</tbody>
</table>
Why are these important?

• Present in the serum and CSF, usually high titre
• Abs associated with rapid & devastating neurological symptoms leading to mortality
• Presence of the “characterised Abs” confirms diagnosis of PNS
• Early diagnostic marker of neoplasm, alerts the clinician to search for possible tumour
• Often aids prediction of cancer location
• Provides early treatment possibilities
Detection:

- Screened by indirect immunofluorescence
- Commercial cryo-sections of primate cerebellum
- Samples are tested at a dilutions of 1 in 50
- Further typing with appropriate substrates, *E.g.* Rodent Liver, stomach composite
- Suspected positives should be confirmed by western blot on primate cerebellum extract and recombinant proteins
Human IgG specific epitopes

Species specific anti-human IgG
FITC only binds human IgG

Human IgG specific epitopes

Monkey IgG

Monkey tissue
ANNA-1
(Hu)
ANNA-1 (Hu):

- Syndromes: Encephalomyelitis, sensory neuropathy, cerebellar degeneration, autonomic dysfunction

- Associated tumours: small cell lung cancer, neuroblastoma, prostate cancer

- Neuronal antigen – 35-40 kDa
ANNA-1 (Hu) on cerebellum:
ANNA1 on myenteric plexus:
Western Blot (Hu):

- Ri, 80 kDa
- Yo, 62 kDa
- Ri, 55 kDa
- Hu, 38 kDa
- Yo, 34 kDa

< 38 kDa Hu protein
< Recombinant Hu
Cerebellum (03.21460)
Ma/Ta
Ma1 and Ta (Ma2)

- **Ma1** syndromes: brain-stem encephalitis, cerebellar degeneration
- Associated tumours: Lung and other cancers
- Neuronal antigen – 40 kDa

- **Ma2** syndromes: limbic brain-stem encephalitis
- Associated tumours: testicular cancers
- Neuronal antigen – 41.5 kDa
- Negative on testis
Ma1/Ma2 on cerebellum:
Ma1 on testis:
The recombinant protein confirms the identity of the antibody as Ma2
Cerebellum (03.26930)
PCA-1 (Yo):

- Syndromes: paraneoplastic cerebellar degeneration

- Associated tumours: Ovarian, breast and lung cancer

- Neuronal antigen – 34 and 62 kDa
PCA-1 (Yo) on cerebellum:
Western blot (Yo):

- 62 kDa Yo protein
- Recombinant Yo
Cerebellum (03.31047)
Anti-Tr
Anti-Tr antibody:

- Tr syndromes: Cerebellar degeneration
- Associated tumours: Hodgkins disease
- Neuronal antigen: no common band identified on Western blot
- Identification is based on immunocytochemical distribution
Anti-Tr on cerebellum:

Molecular layer

Purkinje cell
Atypical Tr on cerebellum (05.38895(3))
Conclusions

• Rare abs but important early marker of PND
• IIF on monkey cerebellum is a good screening assay
• Can experience detection difficulties with some Abs.
• Confirmation by Western blot is necessary
• Must consider the possibility of PNA being masked.
• Expertise in detection maybe limited to few centre
Websites:

• http://www.ii.bham.ac.uk/clinicalimmunology/
  Neuroimmunology/

Publication:

Autoimmune involvement

- Frequently see intrathecal synthesis of Abs in PNS & in other degenerative CNS diseases
- Deposits of Hu Ab in the CNS & tumour found during autopsy of Hu subject.
- In PND, severe loss of Purkinje cells – no damage elsewhere in the brain
- Inflammatory infiltrates (lymphocytes and plasma cells) are present in the tumours
- Tumour antigen is identical to the neuronal antigen
- Tumours are small in size suggesting that immune attack controls the tumour growth
- Antigen specific T cells found in blood, CSF & brain
**Immune mediated mechanism (?)**

- Tumour expresses a “non-self” neuronal protein
- Tumour cell death → dendritic cells take up antigen & migrates to lymph nodes
- Antigen specific CD4+, CD8+ & B cells are activated in the lymph nodes
- B cell matures into plasma cells → produce antibodies against tumour antigen
- Antibodies and/or cytotoxic CD8+ T cells slow tumour growth (and attack the neuronal tissue)
Paraneoplastic neurological disorders are rare debilitating neurological illnesses associated with remote effects of malignant neoplasm and systemic malignancies.