

Immune-Mediated Neuropathy and Autoantibody Testing

Background: Peripheral neuropathy is a generic phrase denoting functional and/or pathological changes in peripheral nerves. Neuropathy results in weakness, pain, loss of coordination, and/or sensory loss. The disorder varies in severity, but in some cases it can be crippling and, if vital organ function is affected, even fatal. Nerve damage that occurs in a single nerve is called **mononeuropathy**, in multiple nerves, **polyneuropathy**. It is often symmetric, but can be asymmetric. A neuropathy can be classified by the following (1):

- Course of onset (acute, subacute, or chronic)
- Target (myelin or axon)
- Affected fiber nerve function (motor, sensory, autonomic, or a combination)

Incidence and Prevalence: The exact incidence of neuropathy can not be determined precisely since it varies depending on the type of neuropathy, geographic region, and many other factors. Nevertheless, it's estimated that between 10 and 20 million Americans suffer from neuropathy. Although the condition may occur at any age it is more common among older adults; a 1999 survey of Medicare recipients found that 8-9% have neuropathy as either their primary or secondary diagnosis.

Causes and Risk Factors: Causes of peripheral neuropathy include nerve compression, entrapment, inherited disorders, deficiency states, exposure to toxins, remote effect of cancer or connective tissue disorder, and direct inflammation (Table 1).

Diabetes is a significant risk factor for peripheral neuropathy, and approximately 30% of adults with peripheral neuropathy have diabetes as the cause; however, 32-70% of all peripheral neuropathies are idiopathic (2). With the development of

autoimmune and genetic tests, the cause of these idiopathic neuropathies can be identified.

Table 1. Conditions associated with peripheral neuropathy

- *Alcoholism*
- *Amyloidosis (metabolic disorder)*
- *Bell's palsy*
- *Cancer*
- *Carpal tunnel syndrome*
- *Chronic kidney failure*
- *Connective tissue disease (e.g., rheumatoid arthritis, lupus, sarcoidosis)*
- *Diabetes mellitus*
- *Immune-based disorders (Guillain Barré)*
- *Infectious disease (e.g., Lyme disease, HIV/AIDS, hepatitis B, leprosy)*
- *Liver failure*
- *Medications*
- *Radiculopathy*
- *Vitamin deficiencies (e.g., pernicious anemia)*

Antibodies to Peripheral Nerve Antigens: Over the last 20 years, the contribution of autoimmune disease to peripheral neuropathy has been recognized and specific autoantibodies present in a range of neurologic diseases have been described (Table 2). Some occur in association with paraneoplastic syndromes, but most autoantibodies are present in neurologic diseases of unknown etiology. The following discussion focuses on the more common immune-mediated neuropathies.

Peripheral neuropathy with anti-glycolipid antibody specificity [Anti-MAG]

Myelin associated glycoprotein (MAG) is a glycoprotein component of peripheral and central

nervous system myelin. The carbohydrate epitope recognized by human anti-MAG autoantibodies reacts with the HNK1 adhesion molecule. This epitope is shared by a number of other neuropathy target antigens including the glycoprotein P₀, which is present in peripheral myelin, other adhesion molecules and several complex glycosphingolipids.

Table 2. Clinical syndromes associated with antibodies against peripheral nerve components

<i>Antibody</i>	<i>Syndrome</i>
<i>Anti-MAG</i>	Demyelinating neuropathy
<i>Anti-Sulfatide</i>	Chronic axonal sensory neuropathy
<i>Anti-GM1 (IgM)</i>	Multifocal motor neuropathy
<i>Anti-GM1 (IgG)</i>	Acute axonal motor neuropathy
<i>Anti-GQ1b</i>	Fisher syndrome
<i>Anti-GD1b GD1a, β tubulin</i>	Guillain Barré syndrome
<i>Anti-Neuronal Nuclear Type I (Hu)</i>	Paraneoplastic sensory neuropathy (e.g. small cell lung cancer, neuroblastoma, prostate cancer)
<i>Anti-Neuronal Nuclear Type II (Ri)</i>	Paraneoplastic sensory neuropathy (e.g. fallopian, breast and bladder cancer)
<i>Anti-Purkinje Cell Antibody I (Yo)</i>	Paraneoplastic cerebellar degeneration

Although the presence of anti-MAG antibodies is not indicative of any specific disease state, 50% of peripheral neuropathy patients with IgM paraproteinemia possess anti-MAG antibodies. These autoantibodies may interfere with the process of myelination, with myelin maintenance or with axon-Schwann cell interactions. In such patients, a distal, symmetric, demyelinating neuropathy is usually present. The affected patients (men > women) usually have an increased IgM level and a monoclonal band on protein electrophoresis. This monoclonal IgM anti-MAG usually represents a monoclonal gammopathy of undetermined

significance, or MGUS. Anti-MAG antibodies can precede or occur without a monoclonal protein; therefore, patients with progressive sensorimotor neuropathy of the demyelinating type should be tested for anti-MAG antibodies even if a gammopathy is not detected.

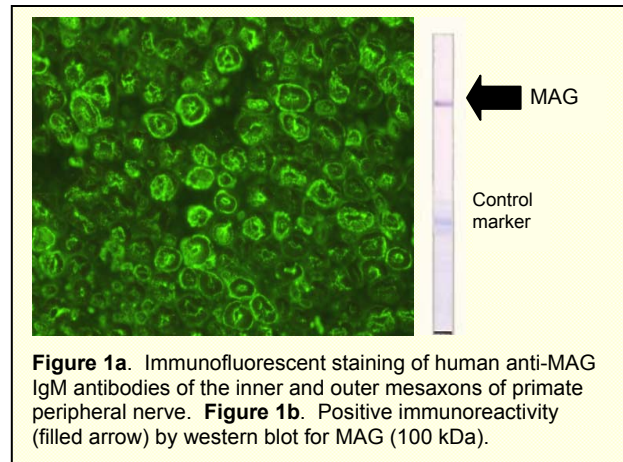


Figure 1a. Immunofluorescent staining of human anti-MAG IgM antibodies of the inner and outer mesaxons of primate peripheral nerve. **Figure 1b.** Positive immunoreactivity (filled arrow) by western blot for MAG (100 kDa).

Detection of anti-MAG antibodies is usually by indirect immunofluorescence (IIF) (Figure 1a). Since the predominant epitope recognized by MAG autoantibodies is also found on other glycoproteins, confirmation of positive sera by western blot is required (Figure 1b). Markedly increased titers are seen in demyelinating sensorimotor neuropathy whereas moderate titers have been reported in multiple sclerosis, inflammatory neuropathies and motor neuron disease.

Antiganglioside antibodies and neuropathy [Anti-GM1]

Gangliosides are members of the glycolipid family and are scattered widely throughout brain, nerve and myelin where they function in various cellular and membrane processes. Due to sugar moieties on the outer surface of the membrane, gangliosides can act as autoantigens, although they are usually hidden from immune attack due to a sialic acid residue attached to the sugar chain. Anti-GM1 antibodies have been shown to induce conduction block, considered by some to be the hallmark of multifocal motor neuropathy. The production of IgM anti-GM1 antibodies are seen in patients with multifocal motor neuropathy, whereas IgG anti-GM1 antibodies are associated with lower motor neuron syndromes including acute neuropathies such as Guillan-Barré Syndrome. IgM anti-GM1 antibodies

also coexist with other anti-ganglioside antibodies in several types of motor and sensory neuropathies. Approximately 10-15% of patients with elevated anti-GM1 titers have an IgM monoclonal gammopathy.

Anti-GM1 antibodies have also been reported in patients with multiple sclerosis, systemic lupus erythematosus, Alzheimer's disease and normal individuals (usually of low titer).

Paraneoplastic syndromes and anti-neuronal nuclear antibodies [Anti-Hu, Anti-Ri, Anti-Yo]

Clinical presentation of paraneoplastic syndromes include sensory and memory loss; cerebellar, brainstem, motor or autonomic dysfunction; involuntary eye movement, and ataxia. These syndromes result from multifaceted immune responses to underlying tumors. Tumors known to initiate paraneoplastic disorders are small cell lung cancer, neuroblastoma, breast, ovarian and testicular cancers. Detection of the tumors can be difficult and the identification of one of the anti-neuronal antibodies indicates that a focused search for the neoplasm is necessary. Evidence suggests that in patients positive for anti-neuronal antibodies, the neoplasms grow more slowly and are less likely to metastasize than those with the same cancer but antibody negative. Thus, detection of the anti-neuronal antibodies can be instrumental in the decision not to treat the neurological symptoms with immunosuppressive drugs since such management may accelerate tumor growth. Table 3 lists key features of the anti-neuronal nuclear antibodies.

Table 3. Characteristics of anti-neuronal antibodies

<i>Antibody</i>	<i>Immunohistochemistry</i>	<i>Western blot</i>	<i>Diagnostic value</i>
Anti-Hu	Neuronal nuclei of PNS and CNS	35 - 40 kDa	Sensitivity 30 - 40% Specificity >95%
Anti-Ri	Neuronal nuclei of CNS	55 and 80 kDa	Specificity >90%
Anti-Yo	Purkinje cell cytoplasm	34 and 62 kDa	Sensitivity 40% Specificity 70 - 100%

Anti-Neuronal Nuclear Antibody Type I (Anti-Hu). These antibodies are associated with sensory neuropathy, encephalomyelitis, and rarely autonomic neuropathies. Among patients with high titers of anti-Hu antibody in serum, small cell lung carcinoma was found in 80% of the cases (rarely neuroblastoma, prostate tumors and seminomas) (Chapter reference). Anti-Hu antibody is a polyclonal complement-fixing IgG that binds with the nuclei of all neurons in the peripheral and central nervous systems with sparing of the nucleoli (Figure 2). It also reacts weakly with the cytoplasm of neurons. Positive immunofluorescent studies should be confirmed by western blot, which generally shows antibodies against proteins in the range of 35-40 kDa.

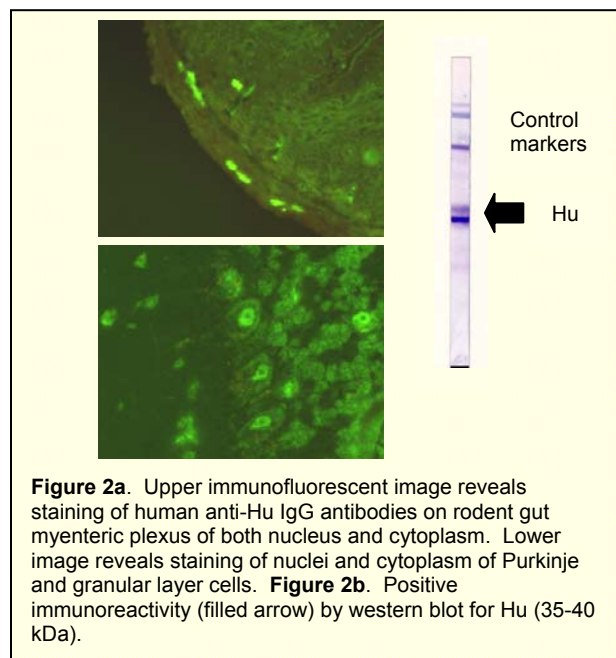
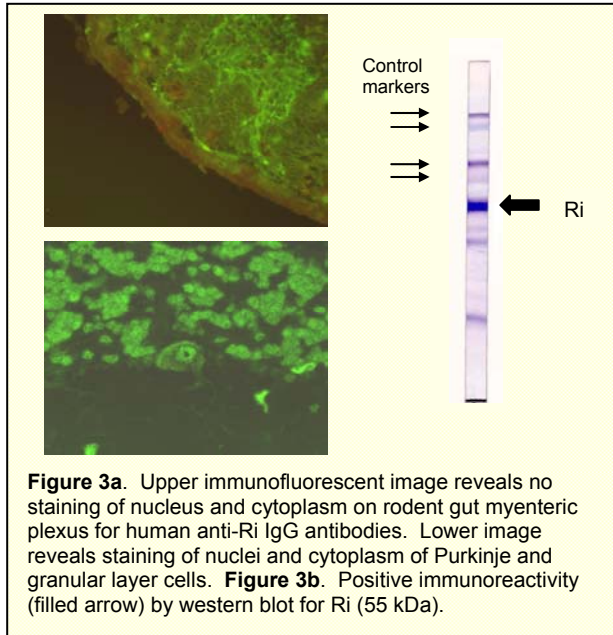


Figure 2a. Upper immunofluorescent image reveals staining of human anti-Hu IgG antibodies on rodent gut myenteric plexus of both nucleus and cytoplasm. Lower image reveals staining of nuclei and cytoplasm of Purkinje and granular layer cells. **Figure 2b.** Positive immunoreactivity (filled arrow) by western blot for Hu (35-40 kDa).

Anti-Neuronal Nuclear Antibody Type II (Anti-Ri). These antibodies are found in patients with ocular movement disorders (opsoclonus / myoclonus). Breast or small cell lung carcinomas are found in 75% of cases; fallopian tube tumors may also be associated with anti-Hu. Histochemically, anti-Ri is difficult to distinguish from anti-Hu as it reacts with all neuronal nuclei of the central nervous system except with dorsal root ganglia nuclei; therefore, western blot analysis is critical. Immunoblotting reveals antibodies binding

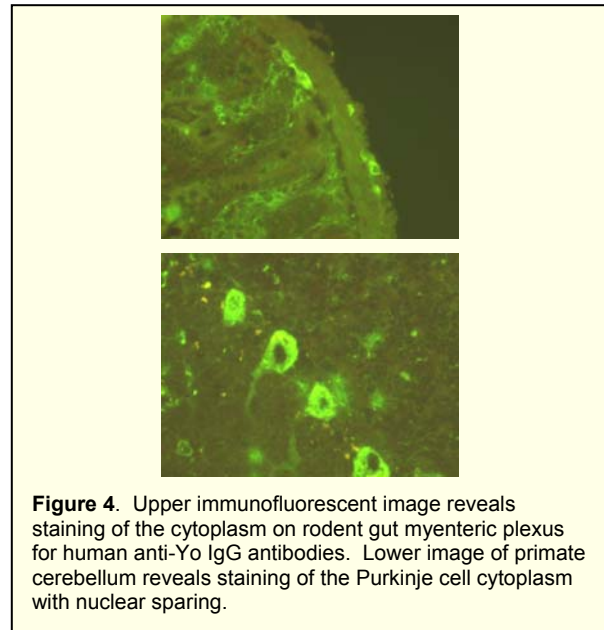
to specific proteins at 55 and/or 80 kDa (Figure 3b). The antibody binds to a gene product called Nova, a



highly conserved protein that appears to play a role in the post-migratory maturation of neurons. As indicated above, the detection of Anti-Ri should initiate a careful search for an underlying tumor, especially breast cancer.

Anti-Yo. These antibodies (also referred to as anti-Purkinje cell antibody I (PCA-1)) are associated with subacute cerebellar ataxia in patients with carcinomas of the ovary and breast (~90% of cases). Paraneoplastic cerebellar degeneration (PCD) with anti-Yo antibodies occur in females with breast and ovarian neoplasms and account for about 40% of PCD patients. These IgG autoantibodies react primarily with Purkinje cell cytoplasm, giving a characteristic granular staining pattern (Figure 4). The sera also react on western blot with extracts of Purkinje cell proteins or cerebellum to two bands at approximately 34 and 62 kDa, respectively (not shown).

In conjunction with the Washington University neuromuscular clinical laboratory in St. Louis, MO we have offered Maine's neurologists unique antibody panels since 1996. In 2004, we introduced a refined group of panels to these same physicians, and we are now expanding these services to all physicians statewide.



Methodology

Immunofluorescent studies (CPT code 86255 and 86256 (for specimens which require titring); enzyme-linked immunosorbent assay (CPT code 83520); and western blot for confirmatory testing of IIF positive studies (CPT code 84181).

Ordering Information

Check the appropriate box on the Neuroimmunology Requisition form. Testing is performed monthly.

Specimen: 2 mL, nonhemolyzed/nonlipemic serum
Storage and shipping: Room temperature (or 4°C if delayed more than 24 hours).

Price: Refer to current FBR Fee Schedule

Further Information

For additional clinical information, please contact Walter C. Allan, M.D.; for technical information, contact Thomas B. Ledue, Technical Supervisor, or Wendy Y. Craig, Ph.D.

References:

- 1) Cohen B, Mitsumoto H. Neuropathy syndromes associated with antibodies against the peripheral nerve. *Lab Med* 26; 7:459-63, 1995.
- 2) Dyck PJ, Oviatt KF, Lambert EH. Intensive evaluation of referred unclassified neuropathies yields improved diagnosis. *Annals of Neurology*; 10:222-6, 1981.