Review Article



# Detection of paraneoplastic antibodies and their significance in paraneoplastic neurologic syndromes: a narrative review

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**Background and Objective:** Paraneoplastic neurological syndromes (PNS) are a group of rare syndromes associated with immunopathological process and tumors. Paraneoplastic autoantibodies are important for the diagnosis of PNS and for searching for underlying tumors. With the development of detection methods and discovery of new autoantibodies, the 2004 guidelines on PNS have recently been updated by a worldwide PNS-Care expert group. For clinicians, proper testing methods and testing results explanation are important for the diagnosis and treatment of PNS. This review aims to review the detection of paraneoplastic autoantibodies and the significance of testing results.

**Methods:** We summarize the studies on detection methods, association of autoantibodies and PNS or tumors, particularly the guidelines of PNS.

**Key Content and Findings:** Antibodies are divided into 3 groups in the context of PNS according to the frequency of cancer association regardless of their eventual pathogenic effect. Instead of well-characterized antibodies and partially-characterized antibodies, high-risk antibodies, intermediate risk antibodies and lower risk antibodies were applied. According to the location of recognized antigens, these autoantibodies are divided as anti-intracellular antigen antibodies and neuronal surface antibodies (NSAbs). Tissue-based assays is recommended as screening method for paraneoplastic antibodies. Moreover, this method is helpful to discover new autoantibodies. A combination of a screening method [tissue-based assays (TBA)] and a confirmatory test [immunoblot and cell-based assay (CBA)] can improve sensitivity and specificity of the tests. Many PNSs are associated with specific antineuronal antibodies, but there is considerable diversity. Some autoantibodies are markers of specific neurological syndromes. Paraneoplastic antibodies are often specific for the PNS-associated tumor rather than for a particular neurological syndrome.

**Conclusions:** Diagnosis of PNS depends on integrated analysis of clinical manifestations and auxiliary examinations. During diagnosis, selection of candidate antibodies for testing is challenging due to the varying clinical phenotypes and tumors associated with a given antibody. Broad antibody panels are more likely to capture causative antibodies and should be considered. According to different subtypes of autoantibodies, specific tumors or PNS should be considered. However, antibody titers, including cerebrospinal fluid (CSF) titers, should not be the primary driver of treatment decisions.

**Keywords:** Paraneoplastic neurological syndromes (PNS); paraneoplastic autoantibody; testing methods

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## Introduction

Paraneoplastic neurological syndromes (PNS) generally refer to a group of syndromes mediated by immune responses that are triggered by underlying tumor. For some PNS, the immune pathogenesis has been confirmed, whereas in others there may be underlying immune mechanisms. In the majority of cases, autoantibodies against onconeural, intracellular antigens can be detected in serum or cerebrospinal fluid (CSF).

As early as 1888, Oppenheim described the first case of malignant tumor complicated with peripheral neuropathy, followed by another case of lymphoma sarcoma complicated with bulbar paralysis the next year. In 1948, Derek Denny-Brown documented two patients suffering from rapidly progressive sensory neuropathy with lung cancer. The term 'paraneoplastic' was put forward in 1949 for the first time, it was used to discuss the differential diagnosis of a patient with multiple cranial and radicular neuropathies caused by metastases of a neoplasm of the uterus (1). Subsequently, the authors found that there was no neoplastic cell in the spinal cords and nerve roots in three patients suspected of having similar metastatic neuropathies (2). In the 1980s, researchers discovered antibodies in the sera of patients with cancers such as ovarian carcinoma and lung cancer, successively (3,4). In 1985, Graus and colleagues revealed that some of these cases harbor a distinct antibody in their serum that labels neuronal nuclei. This antibody became known as anti-Hu antibody (also called antineuronal nuclear antibody 1, ANNA-1). Subsequently, additional antibodies, such as anti-Yo (also called Purkinje cell antibody, PCA-1), anti-Ri (also known as ANNA-2), were discovered.

The number of clinically relevant antibody reactivities as markers of paraneoplastic disorders have grown at the rate of about one per year (5). Discovery of autoantibodies was a milestone in the research of PNS. With the results of autoantibody tests, PNS can be diagnosed faster and earlier than the past. However, with the increasing clinical case reports, we have known that paraneoplastic autoantibodies can exist in patients without PNS. Furthermore, these autoantibodies can be negative among part of the patients with PNS. Additionally, test methods and autoantibody panels differ among laboratories. In this review, we focus on the detection methods and the significance of paraneoplastic autoantibody test results. We present the following article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-2434/rc)

## Methods

We performed a series of PubMed searches to find relevant articles. The keys words 'paraneoplastic syndrome', 'autoantibody', 'onconeural antibody', 'detection methods' are used for searching. Search results were screened based on title and/or abstract. Article types included randomized controlled trials, observational studies, systematic reviews, guidelines, case series, case reports, commentaries, editorials, expert opinions (*Table 1*).

## Classification of paraneoplastic antibodies

In recent years, an increasing number of autoantibodies

**Table 1** The search strategy summary

| Items | Specification |
|---|---|
| Date of search | 2020/3/1 |
| Databases and other sources searched | PubMed |
| Search terms used | Paraneoplastic syndrome, onconeural antibody, detection methods |
| Timeframe | 1949–2022 |
| Inclusion and exclusion criteria | Inclusion criteria: (I) English language articles; (II) randomized controlled trials, observational studies, systematic reviews, guidelines, commentaries, case reports, case series, narrative reviews; (III) relative validity assessed |
| | Exclusion criteria: non-English language articles |
| Selection process | Author Lin Li conducted the selection, and it was conducted independently. The consensus was drawn out of the available literature and based on the experience of the assigned members |

were discovered. These autoantibodies are useful in clinical practice to confirm the immune-mediated origin of the neurological diseases and aid in tumor search. In a narrow sense, paraneoplastic antibodies include only classical anti-intracellular antigen antibodies, however, broadly speaking, paraneoplastic antibodies also include anti-neuronal surface antigen antibodies. They can be classified according to different standards.

According to the association of autoantibodies with tumors, autoantibodies can be divided into two groups: well-characterized onconeural antibodies with a high probability of an underlying malignancy and partially characterized onconeural antibodies with an unknown specificity for a tumor. Both in the diagnostic criteria for PNS by Bataller (6) and by Graus (7) in 2004, well-characterized antineuronal antibodies are referred to antibodies with high probability of an underlying malignancy, and partially characterized onconeural antibodies have an unknown specificity for a tumor due to the small number of reported patients (6,7). In the updated diagnostic criteria for paraneoplastic neurologic syndromes published in 2021, antibodies are divided into 3 groups in the context of PNS according to the frequency of cancer association regardless of their eventual pathogenic effect. That is, the high-risk antibodies (>70% associated with cancer), intermediate-risk antibodies (30–70% associated with cancer), lower-risk antibodies (<30% associated with tumors) (*Table 2*).The panel proposes substituting the term high risk for the term onconeural, for some of the antigens of classical onconeural antibodies (such as Tr/DNER) are not expressed in the associated tumor (Hodgkin lymphoma) (8).

According to the location of recognized antigens, these autoantibodies are divided as anti-intracellular antigen antibodies and neuronal surface antibodies (NSAbs) (*Table 2*) (9). The former antibodies are directed against intracellular antigens. Because intracellular autoantibodies are generally sequestered from autoantigens, therefore, they do not directly contribute to pathogenesis (10). However, they are associated with specific tumors and can identify particular PNS. However, researchers have confirmed that anti-Yo antibody can be taken up by Purkinje cells, bound to intracellular 62 kDa Yo antigen within these cells and caused progressive cell death (11). Moreover, the killing of Purkinje cell did not require the presence of immune cell including sensitized T lymphocytes, and death of Purkinje cell was not initiated by monocytes present in brain (12). Other research demonstrated that in diseases associated with antibodies to intracellular antigens, T-cells drive cytotoxic mechanism and significant neuronal loss (13). The latter antibodies directly target against cell surface antigens, such as synaptic receptors or components of trans-synaptic proteins complexes and are often directly pathogenic. B cell/plasma cell-related mechanism plays an important role in this group (14). Autoantibody can bind to the autoantigen and disrupt the normal function of a critical protein or pathway, and/or trigger antibody-dependent complement injuries of cell surface structures. Autoantibodies that are discovered in recent years target antigens on the surface of neurons, and they are also associated with tumors. For example, earlier study indicated that 58% of patients (>18 years) with positive anti-NMDA receptor antibody have ovarian teratoma, however, as the number of cases increases, the overall frequency of cancer decreases to 38%. Fifty percent of female aged between 12 and 45 with positive anti-NMDA receptor antibody have tumor (mostly ovarian teratomas), about 50% of patients with CASPR2 antibody have cancer, usually are malignant thymoma, more than 50% of patients with AMPAR antibody have small cell lung cancer (SCLC), breast tumor or thymoma (8). In a broad sense, these autoantibodies are included in paraneoplastic autoantibodies. Associated clinical symptoms often include different forms of autoimmune encephalitis and epilepsy and their association with tumor varies.

The significance of the above two classification methods is different. The former one emphasizes on the association of autoantibodies and tumors, which make it markers for tumor screening, while the later one describes these autoantibodies in a pathological view. The former is commonly used in diagnosis criteria of PNS.

## Antibody detection

There are various methods for detection of paraneoplastic antibodies. At the beginning, indirect immunofluorescence (IIF) on brain tissue of rodents or primates was the most commonly used technique for screening antibodies in the serum or cerebrospinal fluid (CSF) (4,15-17). Typically, IIF assay on tissue is performed first. The pattern of staining promotes further detection for reactivity to specific antigens. Methods using brain tissue is called tissue-based assays (TBA). This test is recommended as screening method for anti-intracellular antigen antibodies and NSAbs (9). Moreover, this method is helpful to discover new autoantibodies. The continuous discoveries of the new antibodies and new autoimmune encephalitis (AIE) syndromes associated with these antibodies (18) have led to

 

**Table 2** Classification of paraneoplastic antibodies and anti-neuronal antibodies

| Paraneoplastic antibodies | Anti-neuronal antibodies |
|---|---|
| High-risk antibodies (>70% associated with cancer) | Anti-intracellular antigens antibodies |
| Hu (ANNA-1) | Hu (ANNA-1) |
| CV2/CRMP5 | CV2/CRMP5 |
| SOX1 | SOX1 |
| PCA2 (MAP1B) | PCA2 (MAP1B) |
| Amphiphysin | Amphiphysin |
| Ri (ANNA-2) | Ri (ANNA-2) |
| Yo (PCA-1) | Yo (PCA-1) |
| Ma2 and/or Ma | Ma2 |
| Tr (DNER) | Tr (DNER) |
| KLHL11 | KLHL11 |
| | GAD65 |
| | Zic4 |
| Intermediate-risk antibodies (30–70%) associated with cancer | Anti-surface antigens antibodies |
| AMPAR | NMDAR |
| $GABA_BR$ | LGI1 |
| mGluR5 | $GABA_BR$ |
| P/Q VGCC | AMPAR |
| NMDAR | CASPR2 |
| CASPR2 | GlyR |
| | D2R |
| Lower-risk antibodies (<30% associated with cancer) | mGluR1 |
| mGluR1 | mGluR5 |
| $GABA_AR$ | VGCC |
| CASPR2 | DPPX |
| GFAP | Neurexin-3α |
| GAD65 | AQP4 |
| LGI1 | MOG |
| DPPX | |
| GlyR | |
| AQP4 | |
| MOG | |

ANNA, anti-neuronal nuclear antibody; CRMP, collapsin response mediator protein; PCA, purkinje cell autoantibody; GAD65, glutamic acid decarboxylase 65; DNER, delta/notch-like epidermal growth factor-related receptor; NMDAR, N-methyl-D-aspartate receptor; LGI1, leucine-rich glioma-inactivated 1; $GABA_BR$, gamma-aminobutyric acid-B receptor; $GABA_AR$, gamma-aminobutyric acid-A receptor; GFAP, glial fibrillary acidic protein; AMPAR, amino-3-hydroxy-5-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CASPR2, contactin-associated protein-like 2; GlyR, glycine receptor; mGluR1/5, metabotropic glutamate receptor type 1/5; VGCC, voltage-gated calcium channel; DPPX, dipeptidyl peptidase-like protein; MOG, myelin oligodendrocyte glycoprotein; Zic4, anti-Zic4 autoantibody.

an increasing need for a sensitive and brain-tissue-specific assay that can detect new or not yet commercially available antibodies. Gadoth *et al.* performed immunofluorescence assay (IFA) on 1949 patients' serum/CSF, 61 patients (3.1%) had specific IFA staining in serum, CSF, or both. Twenty-eight out of 42 patients who were positive only with IFA were designated as clinically relevant (67%), 8 inconclusive (19%), and 6 non-relevant (14%). In 13/28 (46%) cases, the initial diagnosis changed due to positive IFA results. IFA result led to the initiation or modification of treatment in 68% and 43% of all cases, respectively. Thus the assays which are not specific to known antigens, such as IFA, can identify antibodies not detected in commercially available kits and therefore are recommended in the evaluation (19). After the screening of autoantibodies, confirmation of the specific autoantibody should be carried out. Western blots, enzyme-linked immunosorbent assay (ELISA), and subsequent new methods of immunoblot and cell-based assay (CBA) are used commonly. As suggested by Hoftberger *et al.*, immunoblot is recommended as confirmatory test for anti-intracellular antibodies with the exception of anti-Tr-antibodies, whereas, CBA is recommended as to confirm neuronal surface antibodies and anti-Tr-antibodies (9). Both methods can be commercially available. For CBA, HEK293 cells and HEp-2 cells are most commonly used. Immunoblots and transfected cells for antibody detection from different manufacturers vary in sensitivity and specificity. ELISA only takes a small part in the routine diagnostic work-up of anti-neuronal antibodies and is mainly reserved for special purposes, such as determination of antibody titer (9).

Anti-neuron surface antigen antibodies can only recognize epitopes with natural conformation, and are detected mainly by IIF. However, because the epitopes of intracellular antigens are liner, anti-intracellular antigen antibodies can be detected by different methods, mostly blotting (20).

To assess the diagnostic accuracy of three methods available in clinical laboratories to detect anti-neuronal antibodies, Tampoia *et al.* tested anti-neuronal antibodies by Western blot (WB), Line-blot (LB) and IIF with primate cerebellum. They found that the diagnostic sensitivity of was 28.9% for IIF, was 26.3% for WB and was 36.8% for LB, and specificity of these three methods was 95.2%, 97.1% and 98.1%, respectively. The combined application of the three methods improves the sensitivity to 39.4% (21). The rat brain immunohistochemistry (IHC)/IFA is highly sensitive and specific for screening multiple autoantibodies, however, they are currently performed in only a few highly specialized neuroimmunology laboratories (8,22). Commercial fixed CBA kits for detecting AE autoantibodies are commonly used, however, the sensitivity and specificity of these commercial kits are low, with 12% positive cases being missed (22). Commercial kits that detect multiple antigens have shown false-negative results in a substantial number of patients, mainly those with anti-LG1, GABABR or AMPAR antibody-associated encephalitis (8,23). Commercial line blots which use the recombinant proteins are used in most clinical laboratories. The number of false positives are surprisingly high, particularly for well-established 'high-risk autoantibodies' including Yo, CV2, Ma2, ZIC-4, and SOX1 that can lead to over-diagnosis, unwarranted workups, and possibly unnecessary treatments (23-27). To improve sensitivity and specificity of the tests, researchers have proposed to use a combination of a screening method (TBA) and a confirmatory test (immunoblot and CBA) (9). Here we summarized the detection methods of each autoantibody in *Table 3*.

## Significance of paraneoplastic antibodies

It is important to explain the testing results of antineuronal antibodies. Many PNSs are associated with specific antineuronal antibodies, but the diversity is considerable such that no single antibody is associated with only one type of neurological presentation or underlying tumour (28). For example, anti-Hu antibody are found in patients presenting with a range of PNS such as encephalomyelitis, subacute sensory neuropathy (SSN), limbic encephalitis (LE), paraneoplastic cerebellar degeneration (PCD), but also in patients with LEMS where the presence of Hu antibodies may be a marker of an underlying small cell lung cancer (SCLC) (28). As for tumors, anti-Hu antibody are mainly associated with SCLC, however, there is also reports that it is present in neuroblastoma, prostate cancer and sarcoma, rarely no tumor is found (29,30).

Paraneoplastic antibodies are important in the diagnosis of PNS. Some autoantibodies are markers of specific neurological syndromes. These antibodies are associated with specific neurological syndromes and are found in patients with or without tumors. For example, Ri antibody is associated with brainstem/cerebellar syndrome, Yo antibody is often associated with rapidly progressive cerebellar syndrome. AMPAR and GABABR antibody are often associated with LE. Whereas P/Q VGCC antibody associates with Lambert-Eaton myasthenic

**Table 3** Paraneoplastic antibodies, associated tumors and neurological syndromes

| Paraneoplastic antibodies | Tumor present | Tumor types | Neurological syndrome | Common serological tests |
|---|---|---|---|---|
| Hu (ANNA-1) | 98 | 38.1% lung, breast 14%, ovarian 8%, Hodgkin's lymphoma 6%, Adenocarcinoma of unknown primary Miscellaneous 9.5% | Encephalomyelitis, limbic encephalitis, sensory neuropathy, cerebellar degeneration, autonomic neuropathy | TBA, immunoblot |
| Yo (PCA-1) | 98 | ovarian carcinoma, breast cancer | Cerebellar degeneration | TBA, immunoblot |
| CV2/CRMP5 | 96 | SCLC, thymoma | Cereberallar degeneration, sensory (motor) neuropathy, chorea, limbic encephalitis, encephalomyelitis, optic neuritis | TBA, immunoblot |
| Ma2 | 96 | Testicular tumor (males <50 years), lung cancer, breast cancer | Limbic encephalitis, brainstem encephalitis, cerebellar degeneration | TBA, immunoblot |
| Ri (ANNA-2) | 97 | Breast cancer, SCLC, gynaecological tumors | Opsoclonus-myoclonus, brainstem encephalitis, cerebellar degeneration | TBA, immunoblot |
| amphiphysin | 95 | Breast cancer, SCLC, ovarian cancer | Stiff-person syndrome, encephalomyelitis, sensory (motor) neuropathy | TBA, immunoblot |
| recoverin | 99 | SCLC, encometrium cancer, thymoma, prostate cancer | Cancer-associated retinopathy | TBA, immunoblot |
| Tr (DNER) | 89 | Hodgkin's lymphoma | Cerebellar degeneration | TBA, immunoblot |
| AMPAR | 70 | Thymoma, lung cancer, breast cancer | Limbic encephalitis | TBA, CBA |
| VGCC | 55 | SCLC | Lambert-Eaton myasthenic syndrome | TBA, CBA |
| VGKC | 25–31 | Thymoma, SCLC | Limbic encephalitis, neuromyotonia, Morvan's syndrome | TBA, CBA |
| $GABA_BR$ | 47 | SCLC, lung cancer | Limbic encephalitis | TBA, CBA |
| mGluR5 | ~50% | Hodgkin lymphoma | Encephalitis | TBA, CBA |
| AChR | 15 | Thymoma | Myasthenia gravis | CBA, RIA, ELISA |
| gAChR | 15 | SCLC, thymoma | Autonomic neuropathy | RIA |
| NMDAR | 9–56 | Ovarian teratoma, testicular teratoma | Encephalitis | TBA, CBA |
| LGI1 | <10% | Malignant thymoma and neuroendocrine | Limbic encephalitis | TBA, CBA |
| CASPR2 | <30% | Limbic encephalitis, acquired neuromyotonia (Isaac syndrome), and Morvan's syndrome | <30% | TBA, CBA |
| GlyR | <10% | Malignant thymoma and Hodgkin lymphoma | Limbic encephalitis and progressive encephalomyelitis with rigidity and myoclonus | CBA |
| GAD | 8 | SCLC, lung cancer, thymic cancer, pancreatic cancer, renal cell cancer | Cerebellar degeneration, limbic encephalitis, stiff-person syndrome | TBA, immunoblot, RIA, ELISA, CBA |
| mGluR1 | | Hodgkin's lymphoma | Cerebellar degeneration | TBA, CBA |
| ANNA-3 | | SCLC | Encephalitis, sensory neuropathy | TBA, immunoblot |
| PCA-2 | | SCLC | Encephalomyelitis, cerebellar degeneration | TBA, immunoblot |
| Zic4 | | SCLC | Cerebellar degeneration | TBA, immunoblot |
| IgLON5 | | – | Non-REM and REM-sleep disorder, brainstem and limbic dysfunction | TBA, CBA |
| Neurexin-3α | – | – | Seizures, orofacial dyskinesias | TBA, CBA |

ANNA, anti-neuronal nuclear antibody; CRMP, collapsin response mediator protein; PCA, purkinje cell autoantibody; GAD, glutamic acid decarboxylase; DNER, delta/notch-like epidermal growth factor-related receptor; NMDAR, N-methyl-D-aspartate receptor; LGI1, leucine-rich glioma-inactivated 1; $GABA_BR$, gamma-aminobutyric acid-B receptor; AMPAR, amino-3-hydroxy-5-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CASPR2, contactin-associated protein-like 2; GlyR, glycine receptor; mGluR1/5, metabotropic glutamate receptor type 1/5; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel; SCLC, small cell lung cancer; TBA, tissue-based assays; CBA, cell-based assay; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; REM, rapid eye movement..

syndrome (LEMS) or rapidly progressive cerebellar syndrome. Evaluation for these antibodies should include serum and CSF, because some antibodies in this group are preferentially found in CSF and may not be detectable in serum (31). However, some antibodies are often only detectable in serum (like LGI 1) and more important, some can be positive in the serum of the patients who are actually not suffering from anti-NMDA receptor encephalitis. To avoid over diagnosing of PNS, positive antibody tests should be explained on the background of clinical presentation. Here we summarize common autoantibodies, their associated neurological syndromes and tumors in *Table 3*.

Paraneoplastic antibodies are often specific for the PNS-associated tumors rather than for particular neurological syndromes. In a large screening study for paraneoplastic neurological autoantibodies, a positive correlation between paraneoplastic antibodies and the presence of tumors was found. Antibodies were tested by either Line immunoassay or by cell-based indirect immunofluorescent assay. Among the 44 patients with PNS autoantibodies, 18 (40.9%) patients had positive autoantibody titers, 24 (54.5%) patients had low autoantibody titers, 2 (4.5%) had very low autoantibody titers. Cancer diagnosis correlated with antibody titer: among the 18 patients who were tested 'positive' for autoantibodies, 14 (77.8%) were diagnosed with cancer; whereas 9 (37.5%) of 24 patients with 'weak positive' and neither of the two with 'very weak positive' results were diagnosed with cancer (32). Anti-Hu antibody is a marker of SCLC. More than 85% of patients with anti-Hu antibody, whether at high or low titers, harbor a SCLC; some patients with anti-Hu antibody may have other tumors, including neuroblastoma, prostate cancer, and sarcoma; rarely no tumor is found (33). However, among anti-Hu antibody positive patients, LE only accounts for 14%, cerebellar syndrome accounts for 18%, subacute sensory neuronopathy 40%, Opsoclonus-myoclonus syndrome 1%, and Lambert Eaton myasthenic syndrome 6% (34-36). Moreover, Pittock and colleagues studied the presence of coexisting autoantibodies in sera from 553 patients with a neurological presentation, they found that 31% of sera had more than one of autoantibodies studied (ANNA-1, ANNA-2, ANNA-3, PCA-1, PCA-2, CRMP-5 and amphiphysin), except for PCA-1, which occurred alone. The authors concluded that the autoantibody profiles observed in patients with paraneoplastic disorders implied the targeting of multiple onconeural antigens and was predictive of the patient's neoplasm, but not a specific neurological syndrome. They highlighted that optimal serological evaluation for detecting autoantibody profiles predictive of cancer requires extensive screening for autoantibodies (37).

When the serological profile shows multiple autoantibodies predictive of a specific neoplasm, pathological diagnosis of potential neoplasm should be pursued. The initial search may focus on tumor types that are more commonly associated with the patient's syndrome or type of anti-neuronal antibody, but if no commonly associated tumor is found, the search should be expanded because unexpected association may occur between neoplasm and antibody. Similarly, if the tumor found is not a histological type that typically associates with the syndrome or antibody, a search for a second neoplasm should be undertaken (31). In summary, PNSs are associated with typical tumors. Paraneoplastic autoantibodies predict the patient's tumor more than the PNSs. When more than one autoantibody is detected in one patient, the search should begin with tumor types commonly associated with the patients' syndrome. Diagnosis of PNS is either based on the evidence of an underlying malignancy or the presence of circulating paraneoplastic autoantibodies. Either may be positive when the other is negative. If both are negative, this does still not rule out the diagnosis of PNS and investigations should be repeated every six months or so until an alternative diagnosis emerges.

Autoantibody panels are recommended to solve the issue of poor sensitivity of individual autoantibodies in paraneoplastic neurologic syndromes. A retrospective research on the sensitivity of the testing panels found that 51 out of 321 patients tested were positive. Thirty-two patients met diagnostic criteria for paraneoplastic/paraneoplastic-like syndromes. The calculated collective sensitivity was 34% (95% CI: 17–53), specificity was 86% (95% CI: 81–90), the Younden's index was 0.2 and the positive clinical utility index was 0.07. This study suggested that although panel testing were recommended to improve detection of PNS, sensitivity remains low, and the utility for screening potential PNS patients is still poor. However, the high calculated specificity suggests a possible role in confirming the patients suspicious for PNS, when enough supportive evidence is lacking with other investigations (38).

The significance of antibody testing in managing PNS is controversial. Only a few researches on autoantibody titers and treatment effect are available. In an immunomodulatory treatment trial for classic PNS, serum paraneoplastic antibody titers were determined by serial titration in a standardized IFA at the Mayo Clinic Neuroimmunology

 

Laboratory. Among 8 of 12 patients who were initially seropositive (PCA-1 8 cases, ANNA-1 3 cases, CRMP-5 1 case), the serum antibody titer decreased after immunomodulatory therapy (5 were clinically improved, and 3 had worsened). Antibody titers remained stable in two patients (both had clinically improved). Two patients had an increase in antibody titer (both had deteriorated neurologically) (39). Because classic PNS associated with antibody against intracellular antigen are thought to involve a pathogenic T-cell response, therapies aimed at reducing antibody levels or limiting antibody effects might not be rational. After the initial diagnosis is established, following antibody titers is pointless (40). For anti-NSAbs, most information are from anti-NMDA receptor antibody encephalitis. Research found that in patients with high baseline anti-NMDA receptor antibody titers, limited or no decrease of CSF antibodies during the first 4 months of the disease are likely to have a worse outcome than those with low titers and/or significantly decreased CSF titers (41). However, it is notably that, most patients still have antibodies in serum and CSF after recovery; therefore, determination of "new baseline" serum and CSF titers after recovery may help to characterization of new onset symptoms as possible relapses (41). However, in practice, treatment decisions should be primarily based on clinical status (40,41).

Finally, it is important to emphasize that clinical association of paraneoplastic antibodies is always the discipline. Eric Lancaster has summarized the common pitfalls in autoantibody testing (40): (I) failure to address tumor risk by autoantibodies: Each autoantibody may be associated with a risk of specific tumor. This risk may exist even in the absence of the paraneoplastic syndrome. For instance, patients with anti-Hu antibody are at risk for lung cancer even if they do not have the neurologic syndromes (40). (II) Testing only serum is not enough: Positive incidence of autoantibody in CSF is higher in most of PNSs of central nervous system. (III) A presumed autoantibody finding must explain the diagnosis: paraneoplastic antibodies are usually associated with specific clinical syndromes, but some antibodies may appear in patients without these syndromes. If the antibody test results are inconsistent with the clinical presentation, other causes should be sought, and the diagnosis of paraneoplastic syndrome should be suspected. (IV) Taking treating the titer as a goal, but not the patient: The goal of treatment should be to provide the best possible clinical outcome, but not to reach any particular antibody level. (V) Take the detected antibody as the only existing immune mechanism: in clinic, some patients are predisposed to autoimmunity, and it is common that there may be a variety of autoantibodies or autoimmune diseases (40). Nine percent of anti-Hu antibody positive patients and 36% of anti-Hu antibody negative patients have P/Q type voltage gated calcium channel antibodies (32). Between 4% and 7.5% of patients with anti-NMDA receptor encephalitis develop concurrent immune responses that may target not only glial antigens (AQP4, MOG, GFAP) but also neuronal surface antigens or receptors (AMPAR, GABAaR, GABAbR) (42).

## Conclusions

PNS have various clinical manifestations. Diagnosis of PNS depends on integrated analysis of clinical manifestations and auxiliary examinations. Paraneoplastic antibodies play an important role in the diagnosis. Selection of candidate antibodies for testing is challenging due to the varying clinical phenotypes and tumors associated with a given antibody. Broad antibody panels are more likely to capture causative antibodies and should be considered. In the management of PNS, it is more important to focus on clinical status. Antibody titers, including CSF titers, should not be the primary driver of treatment decisions.

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## Footnote

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