

# Tests for paraneoplastic syndromes in neurology

## Karen Titus

September 2023—Laboratory testing for paraneoplastic neurologic syndromes is neither commonplace nor cheap. It also comes with its own enigmatical math, as Michael Levy, MD, PhD, recently experienced.

As the director of the Neuroimmunology Clinic and Research Laboratory in Massachusetts General Hospital's Department of Neurology, Dr. Levy keeps an eye on PNS laboratory testing (which is performed at Mayo Clinic) at his institution. "Last week we had three tests in two days that were positive for the GFAP antibody," Dr. Levy recalls, speaking with CAP TODAY in mid-August. "I started scratching my head, thinking, *Either we're in an epidemic—what are the chances of that happening?—or there's a false-positive or something.*"

Dr. Levy, who is also research director, Division of Neuroimmunology and Neuro-infectious Diseases, MGH, and associate professor, Harvard Medical School, chose a low-tech but effective response. "I reached out to each of the clinicians," he says.

Each expressed surprise at the positive result, telling Dr. Levy that they'd found these cases deeply puzzling. The results would now pull them out of the wilderness and prompt them to look into GFAP as a possible cause of their patients' symptoms. Glial fibrillary acidic protein is associated with meningoencephalitis as the neurologic phenotype and can be linked to ovarian teratomas and adenocarcinomas.

With paraneoplastic neurologic syndrome testing, there is no glide path. These immune-mediated complications of systemic cancers are rare and serious. Timely response is critical. Every aspect of the nervous system can be implicated. With the growing use of immune checkpoint inhibitors, researchers are eyeing the likelihood that treatments might increase the frequency of PNSs. And the search for antibodies—already under rapid pace in recent decades—continues, with money flowing in from the pharmaceutical industry.

Little wonder PNS has become a busy and complicated field. It's also compelling, filled with perils (for patients) and predicaments (for physicians), all straining against testing that is both advanced but far from finished.

## **Labcorp's Ajay Grover, PhD, BCMAS, HCLD(ABB), has a front-row view of the field, having introduced autoimmune neurology testing at Labcorp.**

Interest is high, he says, because of the rapid growth in the diagnoses of autoimmune diseases. "We know more about autoimmune neurology markers now," including high-risk antibodies, intermediate-risk antibodies, and low-risk antibodies, all of them associated with cancer, says Dr. Grover, scientific director, Center for Esoteric Testing, and discipline director, immunology and flow cytometry.

An international group recently proposed updated diagnostic criteria for paraneoplastic neurologic syndromes to reflect this and other changes in the field since the previous criteria were published in 2004, including the identification of new antibodies and phenotypes (Graus F, et al. *Neurol Neuroimmunol Neuroinflamm*. 2021; 8[4]:e1014). "It is likely," the authors write, "that the expanding use of immune checkpoint inhibitors in oncologic practice will lead to an increased frequency of similar syndromes."

Before the year 2000, less than a handful of antibodies, such as P/Q VGCC, Ri, and Hu, dotted the testing landscape, Dr. Levy recalls. These were "one-in-a-million-type things. It was very rare that you would find one of those patients, and they would almost always have cancer." Blood tests for autoimmune syndromes of the central nervous system weren't a gleam in anyone's eye.

## **The discovery of aquaporin-4 (AQP4) antibodies about 20 years ago**

## **opened the door wider, says Dr. Levy, observing how the field has evolved to include testing for autoimmune diseases.**

Early in his career, he says, “The vast, vast majority of autoimmune encephalitis cases were undiagnosed—they were thought to be postviral, monophasic, but no known etiology. If it was a relapsing disease, it was all diagnosed as multiple sclerosis.”

The AQP4 antibody test changed things dramatically, Dr. Levy says. “The cell-based assay is very, very specific, so patients with spinal cord disease were being diagnosed correctly.” The work of Josep Dalmau, MD, PhD, and others pushed the field forward even further. The interest in neuronal targets specifically, says Dr. Levy, led to the discoveries of many more antibodies to CNS antigens, including NMDA receptor, CASPR2, LGI-1, and GFAP.

“There’s a lot more now that have been discovered because of that ability to take those patterns that were discovered on immunofluorescence assays, group them together into homogeneous patient populations, and determine the precise immunological target in these diseases,” Dr. Levy says.

## **Joseph Volpe, PhD, has also seen the growth of PNS testing firsthand, having headed up Labcorp’s neurology testing program from its inception.**

He says the emergence of new technologies, both within laboratories and in drug development, have helped spur interest in PNS testing. Roughly seven years ago, “It was clear to Labcorp that neurology was about to have its ‘oncology moment,’ so we began to invest,” says Dr. Volpe, business segment and scientific discipline director, neurology.



Dr. Ajay Grover (left) and Dr. Joseph Volpe of Labcorp. “We know more about autoimmune neurology markers now,” including high-risk, intermediate-risk, and low-risk antibodies, all of them associated with cancer, Dr. Grover says. [Photo by Melville Nathanson © Kristine Dittmer Photographers]

Labcorp offers two basic paraneoplastic panels, he says. One has 12 markers, and typically is ordered by physicians who suspect a patient has an autoimmune

disorder and use this smaller panel as a cursory screen. The other offers 25 antibodies; Dr. Grover refers to it as a catchall panel.

Labcorp also has introduced panels broken out by phenotype, an approach Dr. Volpe suspects will gain traction in the field. This includes very specific autoimmune panels for encephalopathy, epilepsy, dementia, and myelopathy. Though many of the panels are similar, Dr. Volpe says, each contains markers that may be more appropriate for the given phenotype, and likewise excludes inappropriate markers. "It's a way for physicians to get a little more specific, based on what they're observing, rather than having to go on a fishing expedition with a catchall panel."

Laboratories that perform this testing typically use a two-step approach. The first is to perform immunofluorescence testing on mammalian tissue, on a slide/biochip. Each antibody marker exhibits a specific pattern, including intensity of the fluorescence and whether cells light up specific tissues, says Dr. Grover. Once that pattern is recognized, it's advisable to confirm with a reflex test, typically either a Western blot/line blot or a cell-based assay. The two-step approach is crucial for improving clinical specificity.

The significance of PNS testing lies primarily in its capacity for exclusion, Dr. Grover says. These are severe diseases. A negative test outcome can provide patients with relief and enable neurologists to redirect their focus on alternative possibilities.

Dr. Volpe agrees. "I think a lot of physicians run the paraneoplastic panels to rule out neoplasms, for which they would send a patient to an oncologist." Interestingly, he says, this parallels the discipline of neurology generally. "When you look across the neurology spectrum, and particularly with the neurodegenerative diseases, there are few rule-in tests. Most diagnoses are a clinical diagnosis that is based partly on lab testing that has ruled out other things."

Dr. Levy, for his part, welcomes the clarity of an obviously positive test. "If you're testing for something and that comes back positive, that's great—you found it and you can act on it." Rule-out testing is also valuable, he agrees, though he cautions that sensitivity is not perfect for ruling out these diseases.

## **As the number of markers increased, so too have questions about how to best test for them.**

Dr. Grover regularly receives inquiries from clinicians, including the common question about whether testing for newly identified markers is possible. "We often hear: 'We've learned about this novel marker linked to lung cancer—can you test for it?'" He chuckles. "We regretfully inform them that we don't yet have it in our repertoire. It's a recent discovery, and we're still unraveling its details."

Clinicians face their own challenges, Dr. Grover acknowledges. When a patient visits an autoimmune neurologist, the clinical presentation might not be evident immediately. "So they request testing for all available antibodies"—Labcorp's comprehensive approach.

Experience helps clinicians, of course. But there are instances in which the clinical manifestation can bewilder even the most well-informed experts, Dr. Grover says. "Deciphering the appropriate course of action becomes exceptionally challenging for them." Even autoimmune neurologists who have received top-tier training, "which isn't necessarily ubiquitous," he says, might have limited exposure because of the rarity of the cases. "Erring on the side of caution, they opt to conduct comprehensive testing."

Dr. Grover's counsel to physicians: "When encountering a patient with an unusual neurological condition and suspicions of cancer—particularly if there's a history of cancer—prioritize paraneoplastic neurological testing. Seek to understand the underlying cause of the occurrence."

"Paraneoplastic syndrome is not caused by the tumor itself, but it reflects the patient's humoral or cellular immune response to the tumor," he adds. The field is a wobbly Venn diagram. Some patients could be dealing with an autoimmune neurological disorder with no associated cancer. "A diagnostic hurdle arises in determining whether

the patient harbors an underlying oncological ailment,” he says, “or if it’s solely an autoimmune neurological condition without cancer.”

Another common question revolves around knowledge gaps. Though the pace of discovery has been undeniably quick, there may be markers that exist but have yet to be identified, prompting the lab to note in its reports that a negative test result doesn’t necessarily rule out a paraneoplastic condition, Dr. Grover says.

Neurologists also have questions about the presence of multiple markers. “The testing can get complicated,” he says. “It’s hard to correlate the clinical presentation sometimes with the results we get in the testing.”

Labcorp’s response to these complicated cases was to develop a UFO program, as Dr. Grover calls it with a laugh: unidentified fluorescent objects. As part of a discovery program, the lab works with academic partners already doing research in these areas to identify and learn more about new fluorescence patterns. “Then we keep a guide of the case studies,” which may help Labcorp suggest emerging antibodies to neurologists.

These are complex tests, Dr. Grover says. When several antibodies are present, patterns can be overlapping, with one more visible than the other. It’s also difficult to find clinically validated specimens to validate the testing. “The markers are rare; rare are the specimens, too,” he says.

Dr. Volpe suggests—based on market research—that autoimmune neurology testing is an area in which many clinicians feel “less well informed than in others. One of the reasons is it’s a fast-moving discipline.” With new markers regularly being discovered and being added to panels or replacing current markers, “What we’ve learned is that physicians are looking for labs to keep panels up to date for them according to the clinical literature. Not because they’re not trained, but because the symptomatology of a lot of these conditions can be overlapping and hard to distinguish.”

In his experience, the most common question from neurologists who are less familiar with this testing tends to be, “How do I know which panel to order?”

Education and guidance, including from groups such as the American Academy of Neurology, could help physicians, he says. “The field itself is fairly nebulous,” Dr. Volpe says. “It needs to start clarifying itself around the markers that are most important and should be part of which phenotypic panels.”



Dr. Levy

Dr. Levy says the lingering questions revolve around sensitivity and specificity. “Who do you test? If you have a negative test, does that necessarily mean they don’t have it? How many false-positives are there? What does low positive mean? Should we retest if someone is low positive? If it’s positive twice, is that a confirmed positive? If we get a low titer, what does it mean? Does it mean the test is not as good as it should be, or does it mean the patient has the antibody but it’s at a really low level?”

How do he and his colleagues resolve such questions? “We call Mayo,” he says with a laugh. “They always pick up the phone.”

In that same vein, Dr. Grover sees interpretive comments as another must-have, “including any concerns regarding the clinical sensitivity or specificity, depending on the test methodology or the type of specimen. It is the duty, I would say, of the testing lab to provide as much interpretation as you can: defining your test methodology, defining potential clinical fall-out.” In providing such comments, he says, “I’ve noticed that I then open up more questions from neurologists. Just giving the result doesn’t mean anything, especially for patient management.”

**Perhaps not surprisingly, Dr. Grover says the most persistent question he encounters is, “Why is this testing so expensive?” It’s a common question in laboratory medicine, and Dr. Grover points to an unremarkable list of reasons: the number of antibody markers; the use of biochips/tissue cultures; automated platforms; highly trained technologists.**

Dr. Levy agrees that cost is an issue, given the limitations on reimbursement for hospital inpatient testing. When a patient is admitted for autoimmune encephalitis, for example, “If you blow \$2,000 on antibody tests, you’re already costing the hospital too much money.”

There’s also the issue of turnaround times. It’s not unusual, Dr. Levy says, for patients to be discharged by the time a positive test result is returned.

Cost and speed issues have led to suggestions to move this testing to the outpatient setting, right after discharge, he says. Would that approach work? “Yes, if we can get our act together—they have to get an outpatient appointment, and someone has to take responsibility for it, and send the test, et cetera.”

Phenotype-specific panels are less expensive, and Dr. Grover says he encourages their use when appropriate.

Dr. Levy agrees that panels are useful. “As smart as we think we are, we don’t really know 100 percent the full spectrum of any of these conditions. So we’re a little bit more modest about it. We recognize that we don’t know everything. So we usually send panels—we don’t usually send individual antibodies.” Moreover, he adds, the cost of ordering individual antibodies can quickly exceed that of a panel.

Still, the panels aren’t foolproof. The biggest misunderstanding, Dr. Levy says, is knowing which test to use for which condition. “That’s still a learning process for us.” Even in a room full of experts, he says, “We’ll still be scratching our heads saying, *Well I saw one case like this, and one case like that.* It’s not the fault of a particular lab. We’re all learning. You still have to think about it after the test result comes back.”

**Looking ahead, Dr. Volpe says that many physicians have made assumptions about how to test for certain markers—assumptions he suspects are based on papers published more than a decade ago.**

He compares it to using a decade-old iPhone. “So I think one of the things that needs to happen in the near-term future is establishing which technologies are best for which markers. There needs to be a gold standard or an acceptance across the clinical literature that perhaps multiple technologies are equally good.

“What complicates this is a lot of these types of tests aren’t as objective as you’d like them to be,” Dr. Volpe continues. “Where I think the whole field needs to go is establishment of guardrails” around panels—in other words, he says, what the key markers are that need to be in a panel for reimbursement.

Dr. Levy weighs in as well. “I think we’ve only scratched the surface of these antibodies that can be generated in these conditions. There’s a lot more to discover.” To wit: “We’ve only got three antibodies against astrocyte targets. Is that really all there are? We have none against microglial targets—I have a hard time believing that. We have two against oligodendrocytes,” he says.

“I just suspect there are many more,” he adds.

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