CSF biomarkers in ‘a new era’ for Alzheimer’s

Karen Titus

August 2021—The FDA’s approval in June of a new drug to treat Alzheimer’s disease has incited motley responses, a sort of Grand Tour of emotions and reactions. As with any stormy action, the aftermath becomes clear only over time, during the hard work of cleaning up. What comes next?

The arrival of aducanumab (Aduhelm) will likely speed the development of other therapeutics, as well as accompanying biomarkers. “Many of us feel we’re entering a new era of treating Alzheimer’s disease,” says William Hu, MD, PhD, associate professor and chief of cognitive neurology, Rutgers-Robert Wood Johnson Medical School and Institute for Health, Health Care Policy, and Aging Research—both part of Rutgers Biomedical and Health Sciences.

It has also turned up the volume on discussions about inequities in health care. As a recent *New England Journal of Medicine* editorial notes (Levey AI. 2021;384[18]:1762–1763), Black and Hispanic or Latino people are less likely than other groups to receive timely diagnoses and treatment despite the outsized impact of Alzheimer’s disease on these groups. Researchers are racing to recruit patients who are typically underrepresented in clinical trials, including Blacks and Asians. (And, as Abigail Adams once requested regarding another crucial matter, “Remember the Ladies.”)

Moreover, varying tau levels in different patient groups (as the editorial points out) raise worries of false-negative diagnoses and underestimated staging of the disease in Black patients. Then there’s the question of access to the basic diagnostic tools of CSF biomarkers and PET imaging.

All these bits are converging like the riot of cast members in the final pages of “Cymbeline.” But absent Shakespeare’s deft hand, no Jupiter will descend astride an eagle, helping to reunite the players and smooth over the plots. Instead, labs will have to make sense of all this themselves.

In ordinary times, more treatments and more patients would mean more testing.

Alicia Algeciras-Schimnich, PhD, and her colleagues at Mayo Clinical Laboratories are ready for both testing as well as questions about it. Her lab (she’s the director of the clinical immunoassay laboratory and medical director, BioPharma Diagnostics) has been offering Alzheimer’s disease biomarker testing for a little over a year and a half. Three CSF-based tests are packaged as an evaluation: Aβ42, T-tau (total tau), and P-tau (phosphorylated tau). They then use the P-tau/Aβ42 ratio to interpret whether changes are consistent with the presence of pathological changes associated with Alzheimer’s disease.

The neurology group at Mayo Clinic has been a regular user of the tests, says Dr. Algeciras-Schimnich, who is also a professor of laboratory medicine and pathology. While they’re early adopters, she expects aducanumab’s approval will spark more interest and inquiry from other groups of clinicians.

Will that translate into more CSF biomarker testing? “That is a core question,” says Leslie Shaw, PhD, director of the toxicology laboratory, director of the biomarker research laboratory, and co-director of the Alzheimer’s Disease Neuroimaging Initiative Biomarker Core laboratory, Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine.

“It’s a little too soon to tell,” says Dr. Algeciras-Schimnich. “We were expecting demand to go up for our tests, and it is going up some, but with the mixed reviews of the drug approval, perhaps some clinicians are a little more hesitant, or waiting a little longer, to start using the testing.”

The real demand, at least in the near term, may be for knowledge.

It’s worth stating, Dr. Shaw says, that Alzheimer’s is a complicated disease. Some patients have more

neurovascular disease than others; others have Lewy body dementia. Lewy bodies are the hallmarks of Parkinsonian diseases, but 40 percent of patients with Alzheimer's have some degree of Lewy body pathology. Perhaps 30 percent of the total sporadic cases of AD are "pure" amyloid plaque and tau tangle pathology. The bulk of cases, however, have varying degrees of neurovascular pathology, Lewy body pathology, TDP-43 pathology, and other less frequent findings, such as hippocampal sclerosis. For now, only a brain autopsy reveals the true picture of the mixed pathology, Dr. Shaw says.

The development of anti-amyloid agents will help clinicians treat Alzheimer's, Dr. Hu says, "but that piece is just one of many" and shouldn't be used in patients who have underlying frontotemporal lobar degeneration or Lewy body disease.

New drugs will launch more questions about testing context, Dr. Hu predicts. "When we're looking at spinal fluid changes, does the biological profile fit the clinical picture? And even if someone does have pathologic Alzheimer's disease, is it appropriate to treat the person with a drug, given what else might be going on?

"For those of us who've been dealing with these scenarios, we are seeing very exciting developments," says Dr. Hu, who, in addition to being a practicing cognitive neurologist (focused on mild and more severe dementing illnesses), leads research to establish novel, validated fluid biomarkers to reflect the underlying pathology. "And as we do more diagnostic work, we will probably also find more caveats, in terms of what medical and personal factors may influence measurement. That will really, hopefully, advance the understanding of CSF biomarkers."

Here, too, there is much to learn, though testing methods are, compared with other aspects of Alzheimer's disease, more straightforward (more "Macbeth," less ridiculous romance), thanks to the experience of European institutions and the network of National Institute on Aging-sponsored Alzheimer's research centers in the United States.

Aducanumab's label doesn't address methods for detecting plaque burden, Dr. Shaw says. Currently there are two highly automated methods for reliably testing CSF Aβ-tau and P-tau, he says, adding, "They work very, very well." The companies have been engaged with the FDA regarding approval—Dr. Shaw says it's "always an ongoing negotiation"—while the European Medicines Agency has already approved them.

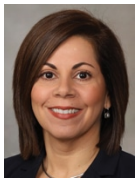
Meanwhile, a tremendous amount of work is being done at the dozens of NIA research centers. "That's where a lot of the focus tends to be in terms of biomarker studies," Dr. Shaw says.

Put another way, methodology and automation are the lowest speed bumps when it comes to using CSF biomarkers. "That's solved for CSF AD biomarkers," Dr. Shaw says.

Dr. Algeciras-Schimnich's experience with CSF biomarkers offers a peek at how they might be used should demand for testing rise. First, she says, "It's important to know these are not diagnostic tools." Rather, they're used in combination with other parameters to determine whether a patient's mild cognitive impairment is due to Alzheimer's disease.

In terms of test interpretation, she says, "We are looking for a decrease in the Aβ42 levels and an increase in phosphorylated tau."

Before that, however, they're looking for the right collection tube. Dr. Algeciras-Schimnich calls it the "critical preanalytical component of this testing," especially related to Aβ42. The sample needs to be collected in so-called low-bind tubes, made of polypropylene, which prevents further absorption of Aβ42. Low Aβ42 is consistent with Alzheimer's disease pathological changes, indicating presence of Aβ plaque in the brain. "We want to avoid a false-positive result due to sample handling.



Dr. Algeciras-Schimnich

“When we first implemented it,” she continues, “our compliance for getting the right tube to us was quite low—around 60 percent. We’re now about 95 percent. But we had to do a lot of education” with clinical colleagues and within the lab, to make sure everyone “understood why it was so important, why we were being so restrictive on the type of tube.”

Dr. Hu says solving the problem of “sticky proteins” took effort in his lab as well. The standard LP tray contains collection tubes made of polystyrene, itself a sticky substance (hence its use in ELISA plates). “If you put a sticky protein in a sticky tube,” he says, “you start to have a lot of issues in terms of the eventual analytic results.”

“We’ve known for some time that polypropylene tubes have to be used when collecting spinal fluid,” Dr. Hu adds, though here difficulties arise—most commercially available trays contain polystyrene tubes. “It’s a logistics issue. We actually engineer and customize our own trays to bypass that issue, but that has not been pushed at a national level.”

The engineering solutions, coupled with current automated analyzers, have meant superior precision, says Dr. Hu, who has published on the topic. “I was pleasantly floored.” Though he says he worries about the impact of automation on trainees’ jobs, “from a clinical proficiency perspective, it is a dramatic step forward” from manual methods. “One of the challenges of this testing has been the reproducibility across lots and assays.”

Mayo relies heavily on the P-tau/Aβ42 ratio, says Dr. Algeciras-Schimnich, since it’s the more reliable of the tests the lab offers. “In some instances there might be discrepancies in terms of the individual analytes.” When clinical colleagues call her to ask questions about results, that’s usually the reason why. Differences can occur because of changes in CSF dynamic that dilute all the analytes. “So you might see a low Aβ42, but at the same time the T-tau and P-tau will be decreased, indicating some other process might be happening in the patient.” Normal pressure hydrocephalus may be a cause of a decreased Aβ42 but a normal P-tau/Aβ42 ratio.

Dr. Shaw also is a fan of the P-tau/Aβ42 ratio. Differences in opinion are to be expected, even among experts, he says. “But I don’t think anybody would disagree with the findings that are reported in many studies: that the P-tau/Aβ42 ratio is probably the best performer,” compared with Aβ42 alone, and is slightly better than the Aβ42/Aβ40 ratio.

As CSF biomarker use expands, labs will also need to figure out how to report results, something in which European labs have already been immersed. Should it be a black-and-white number? “Some do that,” says Dr. Shaw, “with a stated target range. But others are more detailed about it and will report not only the result but some degree of interpretation,” such as whether there is a gray zone around cut-point values.

His lab doesn’t routinely report results, of course, but “We are doing a lot of studies with our clinical colleagues and are always engaged in discussions around interpretation.” The lab and dozens of other centers were part of a recent study to look at their experiences “and lay it all out,” he says, noting the paper has been submitted for publication and provides an overview of how different centers report and interpret results.

There’s plenty of interpretation over what results mean—not the numerical value, but what biological processes they reflect, says Dr. Hu.

T-tau and P-tau reflect the neurofibrillary tangle component of Alzheimer’s disease, he says, whereas Aβ42 primarily reflects the neuritic plaque component. The role of Aβ40 is, in theory, to normalize Aβ42, Dr. Hu

continues, but at the same time it is highly correlated with tau and phosphorylated tau. So regardless of which ratio is used, “with Aβ42 we are very often using the same disease indicator—not just cerebral amyloidosis, but Alzheimer’s disease, consisting of plaques and tangles.”

“There are a number of different personal interpretations between investigators, in terms of whether you use the ratio or you use an arbitrary cutoff of tau, as well as an arbitrary cutoff for Aβ,” Dr. Hu says. The impact, on the whole, is focused on reclassifying a minority of patients. “But overall they are very consistent in the positive and negative cases. I understand that people have different preferences,” he says, “but these measures are giving very much of the same message.”

The basic clinical question is simple: Is it Alzheimer’s?

“My answer will be, ‘It depends,’” Dr. Hu says. Tests need to be ordered in the right context.

Alzheimer’s disease pathology can occur many years before symptoms appear. “If we start performing lumbar punctures on everybody at age 50, we’re going to find some people with Alzheimer’s biomarker changes,” Dr. Hu says. “The meaning of that right now is a little less clear, which is why we often say we want to reserve this test at least for people who have noticed a subjective memory complaint or a change from baseline.”

In addition, he says, “We need to be cautious when we are looking at other diseases, and then we find an incidental alteration in Alzheimer’s biomarkers.” A prime example: Patients with HIV, with or without dementia, may have decreases in amyloid similar to that seen in Alzheimer’s disease. “But when we look at the amyloid PET imaging, they don’t have plaque deposition in the brain.” That’s one area where there’s a dissociation between amyloid PET and spinal fluid Aβ42 levels, but there may be others, which is why Dr. Hu urges caution in using biomarkers. “We can’t just blindly apply these tools to other conditions.”

Another slowdown in the wider use of CSF biomarkers has been lack of standardization. Aβ42 has both a reference method and reference material, so when the tests become FDA approved, interpretation will be consistent across sites, says Dr. Algeciras-Schimnich. But P-tau is a work in progress.

That’s putting it mildly. With tau, there’s even more to untangle.

Dr. Shaw

In multiple studies, researchers have reported lower CSF T-tau and P-tau concentrations in Black participants versus white participants. And there is a push for data. The long-term Alzheimer’s Disease Neuroimaging Initiative (currently ADNI3) is ongoing, but last year researchers were charged with recruiting underserved populations for the study’s final two years, says Dr. Shaw, who has been involved with ADNI since 2004. The work won’t stop there. “In ADNI4 the recruitment effort will be like nothing we’ve ever done in the ADNI study, to [ensure] that we achieve that goal,” he says. “Alzheimer’s centers across the U.S. are recognizing that the population we are all studying has to be more representative of the [U.S.] population.”

“The whole art and science of reaching those folks requires a great deal of thought and effort,” Dr. Shaw continues. “You don’t just decide to do it and it happens. It’s not a piece of cake. There’s a lot of strategy and culture change at the level of the people doing the recruiting.”

It also requires—not to put too fine a point on it—money. “You have to spend money,” Dr. Shaw says. Lack of spending has inhibited efforts in the past, he concedes. But national efforts to advance Alzheimer’s disease

research, coupled with “the national recognition of our failure with underserved minorities, means we are galvanized. More money will be spent.”

“We’ll get to the bottom of that fairly quickly, once we get the data together,” he adds. “We can get something meaningful done in the next few years. I think ADNI will be out there, sooner rather than later, with systematically collected biofluid, imaging, and data across a more diverse and representative population.”

Dr. Hu and his colleagues were the first to report lower tau levels in groups of Black patients compared with groups of white patients, findings that have since been replicated in people as young as in their 20s, he says.

The first thing to note, he says, is that these are differences in averages, with a great degree of overlap between patient groups. “We’re not saying the spinal fluid levels of tau and phosphorylated tau in Black Americans are completely separate from the levels [in] white Americans. We’re just saying the averages are lower.”

In terms of disparities, he’d like to see more research consider inclusivity—as he defines it, being “inclusive of people with Alzheimer’s disease without sacrificing the accuracy of the test. If we just lower the T-tau and P-tau levels, we will gain more true positives at the cost of more false-positives. We need to better understand why, at the group level, the levels of tau and P-tau are lower in Blacks, and that means not excluding Black research participants when their tau levels are lower.”

Dr. Hu eyes individual factors to explain the difference. “That’s what many of us are trying to study—whether there are genetic factors that modulate tau levels or phosphorylated tau levels. This is a very active area of investigation.” Other proteins apart from the two taus also appear to be different between groups. “These are scientific observations that need to be resolved if we want to use them responsibly in the clinical setting.”

How confident is he that this work will be carried out responsibly? “I don’t think any of us has found a protocol yet on how to do this,” he says.

Arguably he and colleagues took a step in that direction when they first noted the differences. They contacted the National Center for Bioethics at Tuskegee University “and invited them to start looking at our work when we first came up with the differences between Blacks and whites.” It was not a hard decision. “We don’t know how this information will be used in the future,” he says. “And I think any discovery can be misused.”

Dr. Hu adds, “I want to make sure that we are scrutinized in a prospective way, so that we are not looking back—if some information that we learn today is abused in the future—and saying, *Oh, I wish we had thought more about those downstream implications.”*

“I don’t personally believe we should stop looking just because of the potential for misuse in the future,” he continues. Study participants of African ancestry are eager to know this information because it affects their health, he says. “But we need, at the same time, to put safeguards in place.” This work has been the focus of studies as well as a recent conference hosted by the Alzheimer’s Association, he says. He also heads a working group to develop common nomenclature to describe observed differences. “It’s easy to say ‘race-based norm.’ But that’s not what we’re saying. We’re saying there are caveats and we need to resolve those caveats.”

As Dr. Hu notes, there are also white patients with Alzheimer’s who have equally low levels of tau. “It’s a matter of proportion. It’s the group mean that’s different, but the spread is still somewhat similar.”

He and others are trying to extend the work of looking at biomarkers in patients of East Asian and South Asian descent. “Some of the work coming out of those countries show very different genetic risk profiles for Alzheimer’s disease.”

But equity should be seen with an even wider lens than U.S. racial categories, Dr. Hu insists. “I think it’s important to remember that spinal fluid is the most available test for the rest of the world.” The United States may lead the world in PET scanner availability, “but from an equity and justice perspective, we feel we need to advance this [CSF] methodology to accommodate the 40 million people outside of this country.”

Another equity issue, he says, has to do with body habitus. “A number of my colleagues don’t do spinal fluid in people who are over a certain weight limit.” Patients still have the right to that information, Dr. Hu says. “Excluding people with a certain BMI because it is more technically difficult [to do an LP] is not equitable.”

Nor is ignoring women. Research suggests tau levels tend to be higher in women than in men; it also appears women are at higher risk for Alzheimer’s disease than men, he says.

Putting all these observations together in a succinct story is going to be a challenge, Dr. Hu acknowledges. “But it’s a challenge that many of us are working on.” That includes looking at perimenopause in terms of whether changes in that phase can influence the trajectory of amyloid and tau.

“I even think sleep is going to be a big part of this,” he suggests. Data show that even acute sleep disruption can change spinal fluid protein levels. “Spinal fluid is not a static fluid,” Dr. Hu says. “It is a dynamic flow system that can be regulated and dysregulated.”

When it became clear that promising Alzheimer’s drugs were on their way, Dr. Shaw and research colleagues wrote a 2018 paper (Shaw LM, et al. *Alzheimers Dement.* 2018;14[11]:1505–1521) on appropriate use of lumbar puncture and CSF biomarkers, to help clinicians prepare for expanded use, including when to make referrals for more specialized testing. The authors finalized 14 indications, rating six appropriate and eight inappropriate. The criteria were designed partly with GPs in mind, Dr. Shaw says, though for now the general recommendation might be to send patients to a memory disorders clinic for testing. “They have the armamentarium and experience in the subtleties and variables and variations,” he says.

That same year, he says, the NIA and Alzheimer’s Association produced a paper that defined AD based on biomarkers, “recognizing that clinical diagnosis is only 80 percent reliable at best” (Jack CR Jr, et al. *Alzheimers Dement.* 2018;14[4]:535–562). That’s especially important earlier in the disease continuum, Dr. Shaw continues, with plaque buildup (and ultimately tau pathology) occurring in people who are cognitively unimpaired.

Based on this work, it’s now common to describe an ATN approach to characterizing the disease pathology: Aβ pathology, tau pathology, and neurodegeneration or neuronal injury, all of which can be measured reliably with CSF as well as imaging.

Amyloid PET imaging has three FDA-approved ligands; tau PET is a newer method. MRI has been around for years and is often the first step at memory disorders clinics to rule out a tumor or other space-occupying lesions, says Dr. Shaw. It’s also valuable for measuring brain volume shrinkage and particularly hippocampal volume.

Blood, primarily plasma-based biomarkers, will doubtless be important too. Much of the work is emerging from the aforementioned ADNI, a study of the natural history of the disease from a biomarker perspective, involving several thousand individuals. Dr. Shaw, who is currently helping to write the competitive renewal grant for ADNI4, says that from a biofluid biomarker perspective, efforts will continue for CSF. “But there will be major increased interest in plasma-based biomarkers in ADNI4. There are some outstanding ones—I could talk for hours.”

Indeed, interest in them has diverted attention from CSF at times. “CSF in certain ways is almost passé,” Dr. Shaw says with a laugh—not in terms of its value but in terms of patient/family preference for a blood stick rather than an LP. (Though he’s quick to add: “The LP should not be shortchanged.”)

Dr. Hu also likes spinal fluid because it can be used for multiple applications (it works especially well for ALS and other TDP-43–related disorders, he notes), while with PET, a separate session is needed for each ligand.

Apart from the core proteins found in spinal fluid, Dr. Hu says, “We are learning a lot about neuroinflammation.” Researchers have been examining how protein levels vary between individuals with the same diagnosis as well as between diagnoses. “We’re analyzing the increasingly complex data set.”

The other advance involves looking at cells. Traditionally, it’s been thought that spinal fluid is acellular. “And it can be, when you look at only 100 microliters under the microscope,” he says. But cells abound in larger volumes of

spinal fluid. “I think there will be exciting findings in the next two to three years.”

All biomarkers have been moving forward with the scientific community’s recognition that the most reliable detection method is based on biomarkers, says Dr. Shaw, along with the recognition that Alzheimer’s is indeed a disease and not an undodgeable aspect of aging.

Given his many years in the field, it shouldn’t be surprising that Dr. Shaw is also taking a long, wide view of the decision to approve aducanumab.

“From a purely scientific standpoint, it has its downsides,” he says. “There’s no way around that.” He has colleagues who say they plan to prescribe it to patients who request it and feel they can afford it. But they would like to see a further phase three trial.

When Dr. Shaw thinks about how cancer drugs have developed, he sees a helpful comparison, recalling the approval of early drugs that extended patients’ lives by several months. Repeated such approvals “kept the field going,” he says, offering hope against an equally challenging disease. “Look where we’re at now with breast cancer,” he says, which has moved into the curable stage. “And you can take other cancers now and start to say the same thing.”

Many hold a similar vision for Alzheimer’s disease, he says.

Aducanumab does knock out amyloid plaque burden to a significant degree, Dr. Shaw says, though the clinical impact is unclear. “But there is a measurable effect of removing one of the major pathologic things going on in the brain, despite all the controversy, despite all the questions—and there are plenty of them. It still looks like there is enough positive, pending experience—and that’s always going to be the bottom line—as clinicians begin prescribing this drug.”

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