A Blood Test for Alzheimer's Disease Draws Near

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New assays and biomarkers are bringing the decades-long quest to develop a blood test for Alzheimer's disease closer to the finish line.

Years ago, Sid O'Bryant learned that his grandmother was showing signs of a failing memory: she often got lost driving and needed notes posted around the house reminding herself to take medications. O'Bryant was a medical student then and had worked with people who had similar symptoms. But despite his experience and network of connections to medical experts, he found it tough to help his family get a firm diagnosis for his grandmother's illness. Eventually, doctors concluded it was Alzheimer's disease (AD), O'Bryant says, but the effort to reach that answer was a huge source of stress for the family.

Her situation is still frustratingly common. Approximately 5.5 million Americans over the age of 65 cope with these and similar indicators of AD. Symptoms include behavioral changes, cognitive difficulties, and mood swings: these could indicate a number of different illnesses, so many people don't know for sure what they are battling.

For the vast majority of these people, including O'Bryant's grandmother, no single test can provide a diagnosis. Physicians use a combination of cognitive tests, family medical history, and neurologic exams to evaluate a person's mental function. Blood tests and magnetic resonance imaging (MRI) scans to image the brain can only rule out other illnesses such as tumors or vitamin deficiencies. This means that in many cases, a diagnosis of AD is one of exclusion—there's nothing else left it could be. In the hands of specialists, these methods yield accurate results “75 to 85% of the time,” says neurologist Douglas Galasko of the University of California, San Diego, “but many patients with AD never see a specialist, so error rates may be much higher, mainly with the disease being underdetected.”

Better diagnostic tests do exist. Positron emission tomography (PET) scans can reveal the abnormal protein clusters in people's brains that are a hallmark of the disease, and

Researchers say a reliable blood test for Alzheimer's disease is in sight. Credit: Shutterstock/Romaset.

lumbar punctures—needles inserted into the spinal canal under local anesthesia—can detect these proteins in the cerebrospinal fluid (CSF). These tests can definitely diagnose AD, but they are also invasive, cost thousands of dollars, and are not covered by most health insurance plans in the US. So most people never get tested.

O'Bryant, driven in part by his grandmother's experience, is now a professor of pharmacology and neuroscience at the University of North Texas Health Science Center. He recalls submitting a grant seeking funds to develop a blood test for AD more than a decade ago. “I was told that one, we don't need a blood test, and two, it'll never work,” he says.

Yet without such a simple test, it's difficult to catch AD early—a critical step in managing the disease with drugs and lifestyle changes to cope with problematic symptoms. It's also tough to identify people who could potentially participate in clinical trials. Perhaps most importantly, a blood test, even an imperfect one, would be an important first screen for primary care doctors to figure out whether to refer someone like O'Bryant's grandmother to a specialist for more involved confirmatory tests.

After a hunt that's lasted nearly 20 years, a wave of new research now promises a sea change. Researchers have identified reliable, blood-based biomarkers of disease, thanks to more automated and sensitive detection techniques. “The improvement in methods makes it possible to learn

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much more about the biology” of the disease—and to develop a blood test to help patients, says neurochemist Henrik Zetterberg of the University of Gothenburg.

Better Biomarkers

When AD was first discovered, the only way to be certain the disease was present was by performing an autopsy. A diseased brain shows clumps of abnormal amyloid protein between neurons and misfolded tangles of a protein known as tau inside the cells. Amyloid clumps, or plaques, are made of a mix of peptides of varying lengths. These peptides form when enzymes chop up a particular glycoprotein in the brain known as amyloid precursor protein (APP).

Tangles of amyloid precursor protein (APP) (left, membrane-spanning domain shown in green) and tau protein (right) in the brain are characteristic of Alzheimer's disease (AD). Derivatives of these proteins may be part of a reliable blood test for AD. Credit: David S. Goodsell and the RCSB PDB under CC-BY-4.0 (left). Shutterstock/molekuul_be (right).

Researchers have known for decades that amyloid breakdown products slip out of the brain into CSF and then into the bloodstream. But quantifying their levels in blood proved nearly impossible for several reasons. For starters, the peptides' concentrations are low in blood. These small proteins are also sticky, clustering with other blood proteins and clinging to the surface of plastic collection tubes or other containers. So even simple factors such as the number of times a sample is frozen, thawed, or pipetted can alter results.

“People kept trying to measure amyloid peptides,” even forming a global consortium to try to solve the problem, Zetterberg says. “But we couldn't reliably quantify and detect them, so the results were all over the place.”

The breakthroughs began with a better understanding of specific biomarkers in the blood that reliably correlate with AD. Neurology researcher Randall J. Bateman of Washington University School of Medicine in St. Louis and his colleagues used radiolabeled amino acids to observe when and where amyloid protein was produced and cleared away in the brains of study participants. They found that, compared with the control group, people with AD had

more amyloid- β 42 ($A\beta$ 42), an APP breakdown peptide that is strongly associated with AD, collecting between cells because it wasn't cleared away quickly enough.

But its levels in the blood are not a reliable marker of AD, because several studies have found that amyloid peptides, including $A\beta$ 42, accumulate in the brains and blood of even cognitively normal adults as they grow older—and because half of circulating amyloid comes from other parts of the body, like the liver.

So early tests that aimed to measure the total amount of amyloid proteins in blood circulation proved unhelpful. But the mix of peptide fragments formed when APP is broken down offered a clue: some peptides are more strongly linked to disease-causing plaques than others. Now, a growing quorum of research shows that one particular marker—the ratio of $A\beta$ 42 to another amyloid breakdown peptide, $A\beta$ 40—might be the ideal basis for a blood test because this ratio reflects how the key brain enzyme digests APP differently in AD than in the general elderly population. Katsuhiko Yanagisawa of the National Center for Geriatrics and Gerontology and colleagues have found that with new techniques, the $A\beta$ 42-to- $A\beta$ 40 biomarker in blood is almost as accurate a test as amyloid and tau PET imaging. In follow-up work, the team tracked people with an abnormal $A\beta$ 42-to- $A\beta$ 40 ratio and found that within one to five years, several went on to have positive PET scans, suggesting the test can detect low levels of amyloid build-up early on. If such a test were to reach the clinic, people who appear to be at risk for AD could be monitored over time for further symptoms.

“When that ratio is abnormal, it's indicative of the presence of amyloid plaques,” Bateman says. “The ratio really gives us the precision we need to individually identify people at risk.”

Another classic marker of AD, tangles of the protein tau within neurons in the brain, has also proved tricky to measure accurately in the bloodstream; correlating those measurements with AD is also difficult. However, recent studies hint that tau, too, may yield useful information. Certain phosphorylated forms of tau in the plasma appear to stably correlate with signs of AD.

Yet another promising marker is a protein named neurofilament light (NFL). NFL is released from neurons damaged by injury, concussions, multiple sclerosis, or neurodegenerative conditions, including AD. Increasing nerve damage of any kind causes its levels in the blood to increase, so it's not specific to AD, but it is useful as a relative marker to track how a person's disease is progressing, says Michelle M. Mielke of the Mayo Clinic.

NFL is an “excellent candidate” biomarker that’s further along in development for this purpose than the amyloid peptide ratio and tau markers that are specific to AD, Mielke says. But it’s difficult to pinpoint a single marker as the best candidate for a blood test: “It depends on the context of use—some may be better for diagnosis and others to track disease progression or response to a drug.”

In more of a shotgun approach, O’Bryant’s team and others have developed panels of blood protein biomarkers purely according to their correlation to AD, regardless of their function: collective changes in the biomarkers’ levels are linked to AD risk. Although this approach has yielded inconsistent results, particularly in trials with small numbers of patients, O’Bryant is trying to rectify this with larger studies and a validated method. He is currently testing the team’s panel with thousands of patients in primary care clinics.

Even as researchers have homed in on better biomarkers, however, many agree that the key to achieving a blood test for AD lies in the methods used to measure these proteins.

Upgraded Assays

Older tests to measure AD-related blood peptides lacked the sensitivity and precision required to distinguish a person with AD from someone without the disease. Since amyloid peptides also circulate in the blood of cognitively normal people as they age, the difference in the ratio of A β 42 to A β 40 between someone healthy and someone at risk of AD is small but significant, only about 15%, Bateman says.

New mass spectrometry instruments are now good enough to detect this difference. Just last year, three independent groups of researchers, including Bateman’s group, used mass spectrometry to quantify amyloid peptides pulled from plasma using antibodies. One of these studies, for example, predicted disease risk based on amyloid level with an accuracy of about 90% relative to those diagnosed by PET scan 12–60 months later.

Another new approach with groundbreaking sensitivity is a major upgrade to a classic technique known as enzyme-linked immunosorbent assay (ELISA). Massachusetts-based firm Quanterix has developed an automated version of ELISA that is 1000 times as sensitive as older systems. Instead of tethering antibodies to the floor of wells in a microtiter plate, which usually holds multiple antibodies in each well, the company’s assay uses antibodies tethered to individual beads, which come to rest in individual wells on a grid. Each well is only 50 fL—about half a billionth the volume of a microtiter plate well—and holds only a single bead. By diluting plasma samples and adjusting the ratio of beads to protein molecules, researchers can ensure that each bead carries only one bound protein. An automated digital

The Quanterix assay is 1000 times as sensitive as traditional enzyme-linked immunosorbent assay because it uses antibody-carrying magnetic beads that fit individually into femtoliter-volume wells. If the target is present (blue), a series of reporter molecules binds to it, generating a chemiluminescent signal that can be detected in the well. Credit: Quanterix.

detection system measures the glow that results from the reaction of a chemiluminescence-labeled reporter antibody with the protein-loaded beads, so the assay can detect single protein molecules. These adjustments to dilution, reaction volumes, and standardization—coupled with antibodies that are highly specific to the peptide biomarkers—overcome problems including amyloid stickiness and ensure that newer assays outperform older attempts.

Others are homing in on biomarkers using similar automated ELISA-based approaches, such as Roche’s Elecsys system, to detect amyloid peptides and phosphorylated tau proteins in CSF. Earlier this year, the US Food and Drug Administration granted the Elecsys assay a breakthrough device designation, which aims to expedite the approval process so people have quicker access to the test. Oskar Hansson of Lund University recently tested its performance on blood plasma samples instead of CSF. Somewhat to his surprise, it performed almost as well. “This could make it useful as a preliminary screen,” Hansson says.

Closing in on the Clinic

Ongoing studies aim to assess which blood tests measure up to the accuracy of PET scans and tests of CSF biomarkers. So far, the plethora of possible blood tests remain in preclinical and clinical studies; none are yet approved for commercial use.

Still, most researchers studying the area are optimistic that a blood test for AD will be on the market within approximately five years. A blood test is expected to cost only a few hundred dollars, much cheaper than the thousands for PET scans or CSF tests.

And it could help catch the disease early. Amyloid plaques start to build up and release peptides into the bloodstream long before symptoms such as memory loss appear. Irreversible and substantial damage to the brain has

usually already occurred by the time people with signs of the disease, like O'Bryant's grandmother, finally see a doctor and begin taking medicines, such as memantine or cholinesterase inhibitors, that can alleviate cognitive symptoms like memory loss, confusion, and other cognitive symptoms. Having a blood test would be "valuable to help these patients early on," says Richard Batrla-Utermann, medical director at Roche Diagnostics.

Ultimately, researchers hope that having a blood test will make it easier to develop better AD treatments. Lacking a way to cure or reverse AD has slowed the drive to develop such a test. But a blood test is crucial to a cure, since it will help identify participants for clinical trials to test candidate drugs. "Without a doubt, having one will help the other," Bateman says.

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