AIRA BURKHART

Portable, head-mounted positron emission tomography scanners could make it easier to diagnose Alzheimer's disease.

BIOMARKERS

A tough spot

Confirming a suspected case of Alzheimer's disease used to be possible only after the patient's death. Fresh approaches mean it could soon be spotted even before symptoms appear.

BY ELIE DOLGIN

Frank Fiamingo was in medical limbo. In 2013, after struggling to find where he had parked his car and forgetting how to spell common words, the retired accountant underwent a brain scan to determine whether he might be suffering from mild cognitive impairment (MCI), a frequent precursor to dementia — but the test was inconclusive.

"I knew I had a problem," says Fiamingo, now 64 and living near his daughter and grandchildren in Concord, North Carolina. "But I couldn't convince my neurologists that I did."

Fiamingo endured this diagnostic uncertainty for years, until his brain was scanned again as part of the screening protocol for a drug trial that required all participants to show evidence of plaques comprising the peptide amyloid-β. In March 2018, doctors examined Fiamingo's brain using a specialized form of the imaging technique positron emission tomography (PET), known as amyloid PET. They injected Fiamingo with a radioactive dye that binds to the harmful clumps of amyloid-β that are associated with Alzheimer's disease. Inside a large, doughnut-shaped PET scanner, the dye lit up in four distinct regions of his brain.

This time, there was no ambiguity. The test, when combined with Fiamingo's diagnosis of MCI, indicated strongly that full-blown dementia lurked around the corner. Participating in the trial of the potential drug was Fiamingo's only realistic hope of forestalling the inevitable march of neurodegeneration — but he withdrew from the study after experiencing debilitating headaches. "Now," he says, "I've got these two to three years to get everything in order before I become incapacitated."

There's a growing consensus that the best way to fight the scourge of Alzheimer's disease is to prevent, rather than treat, the condition. But achieving that goal requires an ability to detect the disease's molecular hallmarks long before the onset of symptoms.

PET scans, analyses of the cerebrospinal fluid and other biomarker-based assays that can help to reveal the telltale presence of amyloid-β plaques or tangles of microtubule-associated protein tau (another protein implicated in Alzheimer's disease) in the brain are now making this possible. And such tests are rapidly changing the face of research on Alzheimer's disease by enabling investigators to diagnose the condition, demonstrate drug efficacy during clinical trials and monitor disease progression with unprecedented precision.

"We now have the tools to track this disease, from its beginning to its end, in living people," says Paul Aisen, a neurologist and director of the Alzheimer's Therapeutic Research Institute at the University of Southern California in San Diego. "That gives us enormously better odds of beating it."

DEVELOPMENT DIFFICULTIES

Biomarker testing for Alzheimer's disease entered the research arena in the late 1990s, but such tests have reached the clinic in only the past five years. Before this, neurologists could offer patients no more than a suspected diagnosis of Alzheimer's disease, which they made on the basis of cognitive assessments, neuropsychological evaluations and conventional brain imaging. It was only after those affected had died, enabling their brain

tissues to be examined, that doctors could say definitively whether the patient's condition had been Alzheimer's disease or another kind of dementia — although such post-mortem evaluations remain rare.

The diagnostic ambiguity had minimal consequences for clinical practice, because the only treatment options available were — and remain — drugs that temporarily help with memory and thinking difficulties, which are symptoms shared by other forms of dementia. But it posed a massive problem for the drug-development process because, to prove that a drug for Alzheimer's disease is working, researchers must test the potential agent on people who have the disease — and, with hindsight, it seems that most such trials were full of participants who did not.

The main clue that misdiagnosis of Alzheimer's disease might be rampant came from post-mortem-based studies showing that up to 30% of individuals who were clinically diagnosed with the condition did not have signature pathological changes in their brains[1]. Researchers from the pharmaceutical industry then revealed, using amyloid PET, that the same level of misdiagnosis existed among people participating in a drug trial[2].

It's little wonder that every study so far that has involved a disease-modifying treatment has ended in failure, says David Holtzman, a neurologist at Washington University School of Medicine in St. Louis, Missouri. "If you're treating someone with cancer, you need to know they have cancer," he says, and the same goes for Alzheimer's disease.

Drug developers vowed not to make the same mistake again: beginning about six years ago, trials designed to evaluate the efficacy of drugs that target Alzheimer's disease began to require participants to show signs of a build-up of amyloid-β at the time of enrolment. Another important impetus for amyloid-β testing was the push to treat people earlier in the course of the condition, often before they show symptoms — a stage known as preclinical Alzheimer's disease.

Such trials also incorporated brain scans and cerebrospinal-fluid taps to provide a quick assessment of whether the potential treatments were working, because waiting to see if a drug affects the onset or severity of symptoms would necessitate running trials that are "too large, too long and too expensive", says Eric Reiman, a psychiatrist and specialist in brain imaging at the Banner Alzheimer's Institute in Phoenix, Arizona.

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Reiman would like drug regulators to be able to approve therapies for Alzheimer's disease on the basis of changes in the levels of amyloid-β or tau. Not everyone in the Alzheimer's disease research community has jumped on the biomarker bandwagon, however. For example, many clinicians who run observational studies — which track populations over the long term but don't involve active interventions — eschew tests for amyloid-β or tau and rely solely on neurological assessments.

That worries Clifford Jack, a brain-imaging specialist at Mayo Clinic in Rochester, Minnesota. "It makes no sense, scientifically, for a field to have this schism," Jack says. When research groups define Alzheimer's disease in different ways, he suggests, their findings are not directly comparable and progress as a whole suffers.

A DIVIDED FIELD

To unify the research community, Jack spearheaded an international effort to update standard guidelines for the diagnosis of Alzheimer's disease, in line with new technologies[3]. The amended framework groups biomarkers into three categories: those associated with amyloid-β plaques; those linked to tau tangles; and those related to more general signs of neurodegeneration. Only people who test positive for both amyloid-β and tau are considered to have Alzheimer's disease.

Because the accumulation of amyloid-β in the brain typically precedes that of tau by many years, the guidelines define a category known as Alzheimer's pathologic change for people who have amyloid-β plaques but no tau deposits. Such individuals might go on to develop tau tangles and progress to Alzheimer's disease, which would then be defined as either preclinical or clinical, depending on the degree of outward symptoms. However, those with no amyloid-β build-up whatsoever, regardless of tau status or overall symptoms, are not classified as having a condition related to Alzheimer's disease.

Despite two years of consensus building, the research framework, which was unveiled earlier this year, still has detractors. Some take issue with specific parts of the diagnostic criteria for evaluating the presence of amyloid-β and tau. For instance, the guidelines dictate that PET scans and cerebrospinal-fluid analyses are interchangeable. And indeed, there is ample evidence to show that the two measures are highly correlated for amyloid-β (ref. 4). But for tau, says Henrik Zetterberg, a neurochemist at the University of Gothenburg in Sweden, "it's a completely different story". He does not think that spikes in levels of phosphorylated tau in the cerebrospinal fluid serve as a proxy for tangle formation; according to Zetterberg, PET offers the only accurate picture of tau accumulation.

Others disagree with the basic premise of defining Alzheimer's disease strictly according to the presence of amyloid-β and tau. The amyloid cascade hypothesis, which posits that amyloid-β is the condition's causative agent, is being questioned from some quarters (see page S4). And there is a suggestion that using amyloid-β plaques and tau tangles to distinguish Alzheimer's disease from other forms of dementia is fundamentally flawed.

"If we're going to go through the exercise of diagnosing Alzheimer's based on the biology of the disease, then we have to understand that biology a lot better," says Adam Brickman, a neuropsychologist at Columbia University Medical Center in New York City.

BLOOD WILL TELL

For now, the biological definition of Alzheimer's disease, as outlined by Jack and his colleagues, is meant to guide clinical research, not medical practice. In part, that's because the associated tests are either expensive (costing more than US$3,000 for an amyloid PET scan) or invasive (requiring lumbar punctures for cerebrospinal-fluid analyses). Efforts are under way to develop alternatives.

Earlier this year, for instance, a team led by Katsuhiko Yanagisawa, a molecular biologist at the National Center for Geriatrics and Gerontology in Obu, Japan, described a blood test that uses the levels of various fragments of amyloid-β to ascertain, with roughly 90% the accuracy of amyloid PET, whether someone has plaques in their brain[5]. A test such as this could eventually serve as a broad screening tool for preclinical Alzheimer's disease, with the more expensive and invasive assays reserved for confirming preliminary blood-based diagnoses.

But that kind of routine biomarker assessment only makes sense, says Jack, after disease-modifying drugs have been developed. "Right now, there are no treatments," he notes, "and so there's really no compelling reason to do this sort of testing."

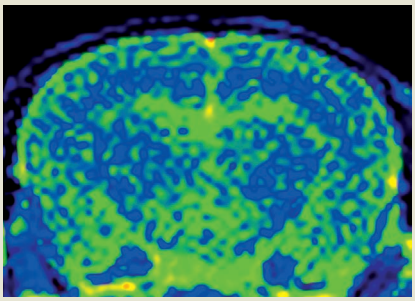
In the shorter term, biomarker assays might be more useful in the memory clinic, especially for people already experiencing cognitive issues that might or might not have been caused by Alzheimer's disease. In such an environment, a PET scan, a cerebrospinal-fluid tap (lumbar puncture) or a blood test might help specialists in dementia to improve the accuracy of diagnosis and treat patients.

Many clinics, especially those in Europe, already offer regular testing of cerebrospinal fluid. Last year, presence of the biomarkers amyloid-β and tau was shown to predict which people with MCI would go on to develop Alzheimer's disease, when used in conjunction with routine cognitive and neuropsychological assessments[6]. "This is a first step toward thinking about a future in which we can help patients prepare for what is going to come," says Wiesje van der Flier, a neuropsychologist at the VU University Medical Center in Amsterdam, who led the study.

Several other teams of researchers are also investigating the value of incorporating routine PET scans into the memory-clinic repertoire of tests. In the United States, for example, Gil Rabinovici, a neurologist at the University of California, San Francisco, led a study in which around 18,500 people aged 65 or older with either MCI or dementia of unknown cause underwent amyloid PET. Before each scan, the

HEADS UP

Next-generation brain imaging



High costs, exposure to radiation and lying still for long periods of time: these are three of the downsides for people who are examined using positron emission tomography (PET), the standard technique for visualizing plaques comprised of the peptide amyloid-β and tangles of the protein tau in the brain. A wearable PET scanner now aims to circumvent these shortcomings.

Developed by neuroscientist Julie Brefczynski-Lewis and her colleagues at West Virginia University in Morgantown, the 'PET helmet' is relatively cheap — US$250,000 compared with more than $1 million for a conventional PET scanner. The head-mounted design places the scanner's detectors so close to the brain that the PET helmet can produce an image using only about one-tenth of the radiation dose of scanners in use in the clinic. And the set-up enables a person to sit upright and move his or her head — a distinction that might not matter for most people, but that Brefczynski-Lewis describes as being "a big deal" for the elderly or those who are experiencing confusion.

So far, the PET helmet has been tested only on healthy people, in proof-of-concept studies using a probe that reports on tissue metabolism — and therefore general levels of activity — in brain cells. But soon, Brefczynski-Lewis, in collaboration with neuroradiologists, hopes to test the device with a probe that is specific to amyloid-β, on people with Alzheimer's disease.

Other researchers are working to adapt imaging methods that emit lower levels of radiation for the diagnosis of Alzheimer's disease. At Texas Children's Hospital in Houston, bioengineer Ananth Annapragada and his colleagues have developed a nanoparticle that targets amyloid-β, enabling plaques to be visualized using magnetic resonance imaging (MRI).

Working with Jason Eriksen, a neuroscientist at the University of Houston, the team has shown that, in mice, the nanoparticle — which contains a contrast dye used for diagnosing tumours of the brain and spinal cord — can cross the blood–brain barrier to reach the brain, where it binds to amyloid-β deposits (**pictured**, green) and emits a signal when scanned using the low-field strength MRI machines that are commonly found in clinics[8].

Alzeca Biosciences, a start-up based in Houston, is hoping to commercialize the nanotechnology. Clinical trials are slated to begin in 2019, and a dye that targets tau could follow soon after.

Carlo Medici, the company's chief executive, says that there are several advantages to using MRI. It's cheaper than PET, it has greater resolution and there are around ten times more MRI scanners than PET scanners worldwide. Most importantly, he notes, "MRI has no radiation for the patient. Zero." **E.D.**

doctor who referred each participant wrote a plan of intended treatment. Three months later, the doctor updated the plan on the basis of the PET findings.

Rabinovici and his colleagues thought that the scans might prompt about 30% of physicians to alter the treatment plan for their patient — by prescribing alternative drugs or recommending counselling, for example. However, according to interim results from almost 4,000 people enrolled in the study, the PET scans led to changes in care for more than two-thirds of patients. "It was more than double what we expected," says Rabinovici, who notes that the scans helped to clarify diagnoses and led to "more precise treatment plans". The final results from the $100-million study will help to determine whether Medicare, the US government-backed health-insurance programme, should cover the cost of amyloid PET in the future.

PET has drawbacks, however. Besides the expense, the technique exposes patients to a considerable amount of ionizing radiation. Each scan imparts up to 350 times more radiation than does a single chest X-ray. Patients also often require several scans, especially during trials to determine whether a potential treatment is working. That's why researchers from industry and academia are now working on less-radioactive substitutes for conventional amyloid PET probes (see 'Next-generation brain imaging').

Meanwhile, other protein targets for PET are being explored — not as alternatives to amyloid PET for use in diagnosis, but rather as the determiner of participants' inclusion in drug trials or as a way to monitor brain health in response to potential treatments.

TANGLED TESTING

Genentech, a biotechnology company in South San Francisco, California, is using PET with probes directed at both amyloid-β and tau to see whether two anti-amyloid-β therapies in late-stage trials alter tangle formation as well as the plaque burden. And Eli Lilly of Indianapolis, Indiana, has incorporated tau detection by PET into its enrolment protocols and evaluation metrics for ongoing trials of two treatment strategies. Imaging for tau "becomes just as important, if not a more important step forward than amyloid was", says Dan Skovronsky, president of Lilly's research and development wing.

Richard Carson, a biomedical-imaging researcher at Yale School of Medicine in New Haven, Connecticut, has developed a PET system that involves neither amyloid-β nor tau. Instead, it uses a radioactive probe that targets a protein found in the synapses of the brain to visualize the density of neural connections in the live brain[7]. "It's an *in vivo* marker of synaptic density," Carson says.

In a clinical first, Cognition Therapeutics, a biotechnology company based in Pittsburgh, Pennsylvania, is using Carson's test in a pilot study of an amyloid-β-targeting drug. "The more detailed brain-health biology we can measure over time, the more accurate our ability to target various changes with therapeutics will be," says Susan Catalano, co-founder and chief scientific officer at Cognition. The study enrolled its first participant in May.

An increase in the number of such biomarkers will bring more diagnostic certainty for both patients and clinicians. Fiamingo's amyloid PET scan has let him know what is coming, and made him accept the pressing need to legally designate someone to make decisions on his behalf. He wants that person to be his daughter, Danielle, who is aware of her father's diagnosis. But, he says, "I don't want to have that conversation just yet". Danielle had a third child in May, and "her life is pretty much full", he explains. "Having to take care of me would probably be a big concern."

In time, Fiamingo says, he will talk to Danielle about his wishes for end-of-life care. But for now, he just wants to revel in being a grandfather. ■

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