Clinical Chemistry

Early and Accurate Diagnosis of Parkinson Disease May Be Rooted in Seed Amplification Assays

Article:
Thomas F Tropea, George T Kannarkat, and Leslie M Shaw.
Early and Accurate Diagnosis of Parkinson Disease May Be Rooted in Seed Amplification Assays
Clin Chem 2023; 69(11): 1209–11. https://doi.org/10.1093/clinchem/hvad111

Guest: Dr. Thomas Tropea from the University of Pennsylvania and Pennsylvania Hospital.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Parkinson's disease is a common neurodegenerative disorder that can be diagnosed on the basis of clinical symptoms, typically slow muscle movements accompanied by tremor. Because symptoms must be present to fulfill the diagnostic criteria, early diagnosis remains a challenge. We know that Parkinson's disease is caused by aggregates of misfolded protein, but to this point, no assays have been available to test for this misfolded protein in living patients. The availability of such a test would not only allow for early and accurate diagnosis, but would facilitate development of therapeutics to help clear or prevent misfolded protein and identify patients most likely to benefit from these new therapies.

A new perspective article, appearing in the November 2023 issue of *Clinical Chemistry,* highlights a recent study evaluating a new diagnostic test for the early detection of Parkinson's disease and describes its potential impact on laboratory medicine and patient care. How was this new assay developed, and how well does it work? Most importantly, is it ready to transition to the clinical laboratory and what do laboratorians need to know? In this podcast, we are pleased to welcome the article's lead author, Dr. Thomas Tropea is an Assistant Professor in Neurology at the University of Pennsylvania, where his research focuses on genetics and biomarkers and neurodegenerative diseases. He is also a neurologist at Pennsylvania Hospital, where he sees patients with movement disorders. So Dr. Tropea, the α-synuclein seed amplification assay, and we'll just call it SAA, has gotten a lot of attention recently. What is the SAA and why is this test important?

Thomas Tropea: Sure. So α-synuclein, we should just define some of the terms first, right? α-synuclein itself is a protein that's found in cells around the body. It's highly expressed in neuronal tissue. And importantly, the accumulation of misfolded aggregates of α-synuclein in neurons or specific cells in the brain are the pathological hallmark for Parkinson's disease and for related disorder called dementia with Lewy bodies.

However, importantly, you can measure α-synuclein and detect it in other tissues outside of the brain.

So the seed amplification assay is a technique to detect very low levels of this pathological α-synuclein, either from brain tissue or from other tissues, and specifically the spinal fluid tissue. So essentially what it is, is you take a seed, which is basically a sample from a patient, and you mix it with the building blocks of this aggregated form of α-synuclein called monomers. And what you do is you take it and you perturb it in some way, and you perturb it over and over and over so that it induces aggregation and, like the name says, amplification. And what you do is you also attach a fluorescent dye to that and you detect that amplification by measuring the color change from the fluorescent dye. So you can take, essentially samples that amplify and reach a certain threshold on that test are considered to be abnormal and you can detect at the α-synuclein, while those that don't reach that threshold are considered normal. So that's the seed amplification assay.

And then why is this important? Well, typically, Parkinson's disease and dementia with Lewy bodies are diagnosed by an examination with a trained physician in the office. Most people with a clinical diagnosis of one of those disorders do have α-synuclein pathology in their brain, but not everyone does. The seed amplification test allows us to detect α-synuclein or pathological α-synuclein to confirm that diagnosis. And really what it also allows us to do by giving us the opportunity to measure that pathology during life, it allows us to move towards a biological definition of synucleinopathies rather than to rely on an imprecise clinical diagnosis.

Bob Barrett: Does this assay change how you diagnose and treat people in the clinic?

Thomas Tropea: Well, I think that the SAA test has the potential to change how we diagnose and treat people in our clinic. In practice, there's not been a dramatic uptake or at least in my practice, there's not been a dramatic uptake of this test. It is available to order as a clinical test. I think that people are wary of a lumbar puncture as a confirmatory test for Parkinson's disease or dementia with Lewy bodies. But outside of just how we practice in the clinic, I think that the work that's presented in our manuscripts, which is reporting on these important findings from the Siderowf et al. paper, report these key findings in differences in sensitivity of the SAA in different groups of people with Parkinson's disease. And I think this is where this becomes very important.

For one, all people with Parkinson's disease, if you measure this SAA test in all people with Parkinson's, the overall

sensitivity is close to 90%, which is really quite striking. However, people with Parkinson's disease that also experience a symptom called hyposmia which is decreased sense of smell, they have a higher frequency of an abnormal SAA test compared to those without hyposmia. And additionally, people carrying a variant in a gene associated with Parkinson's disease in particular, what's reported here is actually in a variant in either the *GBA* or *LRRK2* gene. The two most common genes associated with Parkinson's disease risk have different likelihoods of an abnormal SAA result.

So, for instance, individuals carrying a variant in the *GBA* gene seem to have a higher likelihood of an abnormal result, while people carrying a variant in the *LRRK2* gene have a lower likelihood of having an abnormal SAA result. And that's important because it probably tells us something about the underlying pathology in individuals in those specific groups.

Bob Barrett: Well, it does seem that not everyone with Parkinson's disease has an abnormal SAA test. So what does this tell us about the test?

Thomas Tropea: Sure. And of course, with every test there are going to be false negatives. Right? And that can be related to issues with the test itself, the sample, sample quality. But outside of those technical challenges, a negative result may also tell us something about the underlying pathology for that individual. I was just mentioning that most people with clinical symptoms of Parkinson's disease and dementia with Lewy bodies have α-synuclein pathology in their brain, but not everybody. And so what we would be able to do is detect the pathology that somebody is experiencing while having those symptoms during life, whereas before we were not really able to do that until an autopsy.

`

Some people with a clinical diagnosis of Parkinson's disease or dementia with Lewy bodies do have α-synuclein brain pathology. And in those individuals, the likelihood is that the SAA test would tell us whether it is present or not. If they have symptoms of Parkinson's disease or dementia with Lewy bodies and the SAA is negative, those individuals, it's very possible that they have an underlying pathology that is non-α-synuclein. And this is a challenge, right? So we think of a single disease as having a single disease-causing pathology, but in truth that's really not the case. There are some people that have symptoms of Parkinson's disease that do not have α-synuclein pathology.

There's some evidence from the cohort that was studied in these manuscripts that in individuals with an abnormal SAA or a positive SAA who came to autopsy, the presence of α-synuclein was confirmed. And in individuals who had a negative or a normal SAA, suggesting that there was no α-

synuclein present in the spinal fluid, their pathology also did not show α-synuclein. So it was confirmatory by the SAA matched what we saw on the pathology or what was seen on the pathology.

Bob Barrett: Well, to close here, let's turn that around. Some people with an abnormal SAA test do not have a diagnosis of Parkinson's disease. Why is that and what does it mean for those people? What do they have to look forward to?

Thomas Tropea: Sure. Well, in people without Parkinson's symptoms they do not have Parkinson's disease, regardless of what the synuclein test or the seed amplification assay may say. But in individuals that were studied at least in this cohort who have prodromal symptoms or symptoms that are generally associated with Parkinson's disease but are not specifically Parkinson's disease, diagnostic of Parkinson's disease, things like hyposmia, REM behavior disorder, the seed amplification assay actually can detect α-synuclein in the spinal fluid. And in over 80% of those individuals actually did have an abnormal test. So while we can measure that α-synuclein in the spinal fluid and it is present, it does not actually at that time amount to clinical symptoms of Parkinson's disease.

Now, for many of those individuals, it may be a harbinger of things to come. It may be that they go on to develop symptoms of Parkinson's disease at some point later in their life. And there are now on ongoing studies that are very important to measure the rate of conversion of those individuals that have those prodromal symptoms who have an abnormal SAA to evaluate the rate of conversion into Parkinson's disease symptoms.

Bob Barrett: I imagine those people with the abnormal SAA and no symptoms every day that they even hiccup, they have to think, is this it?

Thomas Tropea: Yeah, I could imagine how that could be difficult for people. And we don't have a disease modifying therapy for Parkinson's disease. We don't have a therapy that can effectively reduce α-synuclein in the brain or the spinal fluid or anywhere else around the body. But those studies are ongoing, and I think that in the future, we may have novel therapies that can reduce α-synuclein. Measuring α-synuclein in individuals before they develop symptoms of Parkinson's disease would be the ideal cohort to target in a preventative trial where we might be able to remove pathological α-synuclein before it ever amounts into symptoms of Parkinson's disease. Now, I'm talking a little far down the line, but I think that we're all very eager and interested in working towards that goal.

Bob Barrett: Why would you give someone this test if they didn't have symptoms?

Thomas Tropea: So in many scenarios, we wouldn't. Of course, what we're reporting on is a very large research cohort.

Bob Barrett: Sure.

Thomas Tropea: And these are individuals that participate in a study so that we can learn more. But you can imagine in a scenario where if we were to have a therapy that could target α-synuclein, and say that that therapy was available to someone before they developed symptoms of Parkinson's disease, we might be able to measure it and screen people. So if you did develop a synuclein pathology that we can measure easily through the spinal fluid or other tissue, we might be able to administer a therapy to reduce your risk of developing symptoms of Parkinson's disease later on in life.

Bob Barrett: Yeah. Well, now we're getting way ahead of ourselves.

Thomas Tropea: Right.

Bob Barrett: Thank you so much. This is fascinating.

Thomas Tropea: Absolutely. And thank you for having me.

Bob Barrett: That was Dr. Thomas Tropea from the University of Pennsylvania. He served as the lead author of a perspective article describing the use of seed amplification assays for the early diagnosis of Parkinson disease in the November 2023 issue of *Clinical Chemistry*, and he's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.