Understanding MOG and AQP-4 Antibody Testing

You can view this presentation at: youtu.be/mAPwnT0_vSQ

[00:00:00] **Krissy Dilger:** Thanks everyone for coming to the RNDS. We are about halfway through now. We're about to hear from Dr. Elias Sotirchos about understanding MOG and aquaporin-4 antibody testing. Dr. Sotirchos is a neurologist and Director of the Johns Hopkins Neuromyelitis Optica, Neuromyelitis Optica Center. There are blood tests that can detect antibodies in some people with MOG antibody disease and neuromyelitis optica spectrum disorder. You can learn, this talk, during this talk you will learn about this testing and its importance in determining a correct diagnosis. And as always, you can submit questions in the Q and A portion for Dr. Sotirchos that we will be able to get to at the end of the presentation, and if you require closed captioning, you can find that in the CC section. Thanks so much and over to you Dr. Sotirchos.

[00:01:07] **Dr. Elias Sotirchos:** Great, thank you so much for that introduction, and thank you for the invitation to give this talk today. I'm going to talk today about MOG and aquaporin-4 antibody testing, which is a very important part of a work-up for patients presenting with inflammatory central nervous system conditions in order to make the diagnosis of MOG antibody disease and to characterize patients with neuromyelitis optica. So, there've been already talks today about MOG antibody disease and NMO, but I wanted to focus kind of for the purposes of this talk on the antibodies that characterize these conditions.

[00:01:47] And so this is a quite complex figure from a paper from Scott Zamvil, but what I would like to highlight here is that in both of these conditions, there are, they're characterized by the circulation in the bloodstream which we can see kind of at the top here. This is the bloodstream is being shown here and showing that there are cells in the blood, types of white blood cells, B cells, that can become what are called plasma cells which are the cells that are the factories that produce antibodies and then release the antibodies into the blood. What we see here is a plasma cell that is making an antibody that is directed against MOG, which is myelin oligodendrocyte glycoprotein.

[00:02:29] And here this picture shows that MOG antibody penetrating from the blood into the brain crossing what's called the blood brain barrier, shown here, and binding to the MOG which is a protein that's found in the, this is a nerve right here, kind of in this grey-ish color. And around it we can see in blue the myelin sheath there, and the MOG, if this can be made out, is this little yellow squares that are shown there on that surface and it shows here the MOG antibody going through, so the goal is to be able to test for these in the blood of

people and detect them with accuracy in order to avoid both false positive and false negative results which is important in order to make an accurate diagnosis.

[00:03:20] Now on the flip side we have aquaporin-4 where it's a similar kind of situation in terms of circulating autoantibodies are produced that are directed now against a different protein that's called aquaporin-4, that's a water channel that's shown in these kind of little red circles here. And here we see the antibody crossing through and binding to them. So, the antibody goes and sticks there and in both of these situations it seems like the antibody can then trigger additional events that can lead to an attack. Now, the whole point of testing here is to try and detect these antibodies in the blood and you'll hear, when you're hearing about tests, kind of different methods in order to approach this.

[00:03:57] This is a very nice figure from Patrick Waters and Sean Pittock and others who are describing kind of the most commonly used assays that are used in order to detect these antibodies in the blood of people. So, this is for the testing for aquaporin-4 that I'm showing here, and I want to really focus on these two that are highlighted in red. And I'll walk you through how this testing is done and what it means.

[00:04:21] Here we're seeing what's called a cell-based assay. And so, this yellow, yellow oval here is a cell that has been engineered in the lab to make aquaporin-4 which is the blue that we're seeing here. And then what happens is that's the starting material for the test and then we add a patient's serum, which is essentially what remains after blood has clotted, and trying to detect if there are antibodies like this. There are antibodies that can go and stick to that protein.

[00:04:53] And after that has happened, we add the blood and then we add another antibody that can go and stick to the patient's antibody and that's linked to this, what we see here, which is a florescent tag essentially that we can then detect and pick up, and so that can be picked up by various methods, either by using microscopy where we look under the microscope and if we see green we see that there is testing, that there's, the test is positive or by another method called flow cytometry, which the cells kind of are running through this tunnel essentially a laser stimulates them and then we can detect the fluorescence and we can quantify that and come up with what the level is, and if the test is positive first and then characterize how high that is.

[00:05:36] Now that's the one kind of test that's really cell-based assays you'll hear kind of the gold standards for testing, both for aquaporin-4 and MOG test because of the fact that they are the most accurate test that we use for testing for those conditions. The other tests that I'm showing here is a test that is still used and it's convenient because it's easy to do and it can be used also in a resource limited settings and it's called an ELISA in which essentially we start with a plate which has the aquaporin-4 protein kind of stuck to the bottom of it then we add a patient's serum which we're trying to detect antibodies and we see the antibody, here's this orange thing and then that can stick to the aquaporin-4 at the bottom and then we add aquaporin-4 that in any case is linked to something called biotin.

[00:06:24] And then we can add an enzyme and this kind of releases a florescent signal that can be detected and then we can quantify that, see how high it is, see if it reaches a threshold of what we've defined as being positive and see how much it is to see if the test is truly positive. Now similar principles apply to when we're testing for MOG, which we see that here. But I've X'ed out this ELISA test which called ELISA here because of the fact that in MOG really only the cell-based assays are useful. This test people had tried in the past to detect antibodies targeting MOG in MS and other conditions and really the results were inconsistent.

[00:07:03] They weren't really able to show that it was associated with a very specific kind of clinical syndrome and that's because this ELISA test, and I'll explain a little bit why afterwards, doesn't really work well for detecting the MOG, so it has to be really detected using a cell-based assay. So again, we add the antibody,

then we add another antibody that sticks to it and then we can detect this green, this fluorescence, in order to see if the test is positive or not. So, this is a picture of kind of how that looks in a positive test in a patient who has an NMO antibody.

[00:07:34] So what we're seeing here is these are actually each of these are cells actually and what happens is the green means that there, when we added the patient's serum onto this, that it bound to it and we're able to detect that by looking at the florescence there, that's, so this is an example of a photo from a microscope showing that that's positive. Now as I said, these cell-based assays seem to be, they are more sensitive, which means that there are less false negatives and more specific overall than the ELISA test for aquaporin-4 antibodies, but the ELISA test can still be useful and it is still actually rather sensitive and specific, just less so than the cell-based assays.

[00:08:10] And again, it's more convenient somewhat in terms of the ability of a laboratory to run it, whereas the cell-based assays are often run in more specialized laboratories. As I said, the ELISA is not useful for the detection of the MOG antibodies and then an additional kind of factor that may be important sometimes is that the cells that we use for what are called cell-based assays can either be live in which they're actually alive, they're dividing, they're active, or we can fix them with a chemical compound called formalin that kind of essentially just freezes the cell, and for aquaporin-4 antibody testing, the performance of the fixed and the live assays seems to be rather close overall.

[00:08:31] Although for MOG it seems per the latest study in which numerous centers around the world collaborated and compared assays that the fixed assay may potentially be associated with a risk of false negatives. And so, a negative in a patient with a high suspicion for MOG on a fixed assay generally should be followed up with a live test in order to confirm that in a patient who has otherwise a typical kind of clinical syndrome consistent with MOG. Now just kind of try to highlight kind of the difference between the assays I thought that I would show this which is kind of how just a basic protein structure.

[00:09:34] So proteins are essentially built out this building plugs called amino acids. And then they're folded in various ways, and this is really important because an antibody that binds to the protein when it's completely folded, it recognizes a specific part of it and it just sticks to it. But it might not recognize that when this is rolled out just with its building blocks like this. When it's not in this kind of 3D form after it's been folded. And so that is why sometimes that ELISA test can be, result in both false positives and false negatives.

[00:10:08] Whereas when we have these cell-based assays, this shows kind of how the antibodies kind of outside of the cell, there is a cell here and it has this aquaporin-4 sitting on its membrane. And so, this kind of has the protein in the way that it actually is in the body, the way that it sits in the membrane of the cells and so this allows us to be kind of more accurate in our ability to diagnose. Now, the utility of antibody levels of antibody testing is mainly for the diagnosis of this condition. So in NMO, we know that people who have had optic neuritis or transverse myelitis or other clinical syndromes that are consistent with NMO and are positive for the aquaporin-4 antibody, which using kind of the best tests available, the aquaporin-4 antibody is positive, in about 80, 85% of patients who have kind of a clinical diagnosis of NMO, that's very useful because we know that patients who have had even a single episode and are aquaporin-4 antibody positive, it's, we expect that they're at high risk for relapse in the future if they are not treated with an immune therapy. So that's very important to bear in mind.

[00:11:20] Now after the diagnosis has been made, monitoring aquaporin-4 antibody levels does not necessarily seem to correlate with treatment response or with the risk of relapse in patients with NMO. There are studies that have shown that the aquaporin-4 antibody levels may rise before an attack or may be higher at the time of an attack, but this is hardly a one-to-one relationship and the threshold of what that elevation is that may

lead to an attack is, seems to vary quite a bit from patient to patient. So, there is generally no recommendation currently for routine kind of longitudinal monitoring of aquaporin-4 antibody levels.

[00:11:58] And really, further studies are needed in order to understand how that can be helpful when, in the treatment of patients but generally the treatment is kind of defined, the treatment decisions are made by the clinical course of the patient rather than the aquaporin-4 antibody levels and serial monitoring. Now in MOG the testing is important in order to establish the diagnosis. Higher levels of MOG antibodies at the time of an initial attack have been associated with a higher risk of relapse. And persistently being positive is also, has been associated with a higher risk of future attacks in people who have had a single attack.

[00:12:41] So, sometimes patients can be positive at the time of their first attack. So, if somebody comes in for example with optic neuritis and their MOG antibody is positive at that time, but then they can have what we call transient seropositivity, which means that they're positive then, but if we test several months to years later, that antibody has disappeared, now is negative. And so, while this is not, this does not mean they will not have future attacks, it does seem to be associated with a lower risk. Although if somebody remains positive for MOG antibodies, it does appear that that may be associated with a higher risk of future attacks.

[00:13:21] Now after, in terms of serial testing apart from this kind of transient seropositivity or is it persistent, that really is not clear if that's useful to assess risk of future, of other relapses, additional relapses or response to therapy. So really our decision-making in terms of treatment in MOG is mainly guided by attacks, breakthrough attacks and the clinical course of a patient rather than necessarily treating the MOG antibody level itself. Although again, I think that this is also an area that's very important to understand further and that's why research studies in order to further understand this would be especially important moving forward.

[00:14:06] Now another thing I wanted to say also is that, I mean, there is, there is always a risk whenever every test has a risk for false positive tests as well in addition to false negative results. And so, it's important that the testing generally be performed in patients who indeed have kind of clinical pictures, symptoms, imaging characteristics that are consistent with either one of these diseases. Because we do sometimes see situations in which we have kind of a low positive test for example, in a patient who otherwise may not have a clinical picture consistent with MOG, and sometimes it's difficult to tease out whether that's a true positive or not and it really has to be guided by other findings like the spinal fluid findings and imaging, so while it, these tests are very important or very helpful in it, nothing is completely definitive.

[00:14:57] And often making a diagnosis is kind of putting a puzzle together of the, what is the imaging show, the MRIs, what does the spinal fluid show? And what has the clinical course and the clinical exam showed as well. So, the tests always have to be interpreted in that context when we're, when we have a patient in front of us. And so, these are the references for the talk, and I did leave some time for questions. It is a rather, it's a relatively short session, but we do have five minutes for questions. So, I thought it would be good to have a kind of interactive session to some extent.

[00:15:31] **Krissy Dilger:** Thanks so much Dr. Sotirchos. We did get a few here. So, the first question it looks like we got. In a MOG-AD patient who is stable on medicines, how frequently should antibody testing be done?

[00:15:50] **Dr. Elias Sotirchos:** Yeah, that's a great question. So, I think that we don't really know that answer and I have to say, I mean, I think that it's, I think that it's potentially useful to test people who have MOG and are stable in the context of especially kind of research studies in order to better understand how the antibody testing can inform us. But I haven't necessarily seen that there is a great relationship between the antibody level and the risk of relapse. So, I have patients for example who have MOGAD, are stable on treatment, and we test them for MOGAD, for MOG antibody, and it's still positive but they haven't had a relapse in years.

[00:16:27] So, while it's an interesting piece of information, and I think that it's useful to incorporate it into research studies, it's not clear to me that it's, it's useful at present with the data that we have right now for making clinical decisions. Like my clinical decisions are generally made based on how the patient in front of me is doing. Are they having attacks? Are they having disability? So that's really the most important thing to guide treatment decisions in MOG.

[00:16:56] **Krissy Dilger:** Thank you. Another question we have is, so if someone was diagnosed with TM, for instance, say, more than five years ago, how important is it to get MOG antibody testing or NMOSD testing if they have never had it before? Is there a chance that they actually do have MOG or NMOSD and could relapse?

[00:17:26] **Dr. Elias Sotirchos:** That's a great question, so I think it's difficult to say. It depends on kind of a number of factors. So generally, the decision to do MOG or NMO testing depends on kind of the characteristics of a myelitis attack. In general, in transverse myelitis there are numerous features that we look at when seeing a patient with transverse myelitis. Like how long was the lesion in the spinal cord? So generally, with MOG and NMO, the lesions are more typically kind of what we call longitudinally extensive, which means that they're kind of more than what we call three vertebral segments long. Then it's kind of how big was the lesion?

[00:18:00] Did it involve the entire spinal cord when we're looking at it kind of cross-sectionally? Or part of it? And then we also, very important features are kind of what the brain MRI looked at, how they evolved with steroids, and how their clinical course was. Because there are features that could be consistent for example with a diagnosis of multiple sclerosis and so in that context, I mean I think that it's difficult and it would have to be kind of an individualized decision depending on what that transverse myelitis looked like on imaging, what the spinal fluid findings, what the brain MRI looked like in order to better, to better understand that.

[00:18:42] **Krissy Dilger:** Got it. Thank you. Looks like we just got another question. How likely is it for MOGAD to go away in an older adult after years of MOG positivity?

[00:18:57] **Dr. Elias Sotirchos:** So that's a great question. I think that, I don't think that we necessarily know the answer to that. I think that we, first of all, because MOG is kind of a more recently described condition and we just don't have I feel the long enough follow-up in order to know how what happens long-term. In the existing studies that we do have, it does seem to me that with the treatments that we have that we don't necessarily seroconvert our patients to negative.

[00:19:32] I have patients who for the most part do, if they've been persistently positive for years, it seems that it does remain positive after that. I think that it remains to be seen whether medications for example that deplete B cells for years could result potentially in patients converting to becoming negative and then we'd have to see what does that mean necessarily for the long-term risk.

[00:19:57] Does that mean that these patients will not experience future attacks? I don't think that we know that and it's really difficult to say. I think that right now what we know is that if patients have had more than one attack, that generally treating long-term is a practice that a lot of neurologists follow. But I do think that we need more long-term follow-up observational studies in order to better understand that in order to see moving forward, yeah.

[00:20:29] **Krissy Dilger:** Okay, I think we have only just a little bit of time left. Someone asked, is there, are there any other ways currently being developed to test for MOG and aquaporin-4?

[00:20:43] **Dr. Elias Sotirchos:** So generally, I mean the cell-based assays are really the gold standard right now. There's a, there's been a lot of work kind of over the years refining these assays and trying to

find better thresholds in order to find positivity, better detection methods like whether we should be using immunofluorescence, whether we should be using flow cytometry. That has been proposed and there are international collaborations looking at kind of comparing across centers, the ability of MOG and NMO testing.

[00:21:12] I mean generally there's not like a completely new assay or new approach that I'm aware of, but generally kind of working on refining the existing tests and improving. And that's kind of how the, what happened really with aquaporin-4 antibody testing, so the initial test was something called tissue-based immunofluorescence which uses kind of mouse or rat brain essentially to detect the antibodies and that assay was progressively refined over a period of about a decade actually until we got to the cell-based assays that we have today with the sensitivity, which is the percent of people with NMO and whom the test is positive kind of going from about 50 to 60% up to greater than 80% that it is now.

[00:21:56] So it's kind of these incremental stepwise refinements of the testing and of improving the testing and developing somewhat new tests that has kind of guided that. For MOG, I think that with the cell-based assay, we, it seems to be performing very well, especially the. live one but it remains to be seen if it can be refined further.

[00:22:17] **Krissy Dilger:** Okay, I think that's all the time we have today. But thank you so much for joining us and dedicating your time and expertise to us for this symposium. And we look forward to seeing you hopefully in the future events.

[00:22:31] **Dr. Elias Sotirchos:** Yeah of course. Thank you so much for the invitation. It was a pleasure.