NEUROPSYCHIATRIC ASPECTS OF HIV INFECTION

Serena Spudich, MD, MA

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Neuropsychiatric Aspects of HIV Infection
[video transcript]

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Welcome everybody to This Month in HIV. Our November presentation is Neuropsychiatric Aspects of HIV infection and will be presented by Dr. Serena Spudich, Professor of Neurology and Chief of Division of Neurological Infections and Global Neurology at Yale. My name is Jessica Steinke. I'm a program coordinator for HIV/AIDS Education and Training Department at the Mount Sinai Institute for Advanced Medicine. Before I officially introduce our speaker, I would like to thank our funder the New York State Department of Health AIDS Institute Clinical Education Initiative. The Mt. Sinai Institute for Advanced Medicine serves as a cosponsor of This Month in HIV. For the duration of today's presentation, all lines will be muted to ensure that there will be no distractions during Dr. Spudich's presentation. If you have a question at any time, you can type into the chat box and direct it to all panelists. At the end of the presentation, I will read out your questions for Dr. Spudich. After today's presentation this afternoon, you will receive an e-mail with instructions on how to evaluate the presentation and claim your CME or CNE credits. Please do remember that This Month in HIV is supported via our New York State Department of Health CEI grant and your participation in the evaluation process helps to keep this program free of charge for all attendees. So at this point, I'd like to introduce our speaker, Dr. Serena Spudich. Dr. Spudich is the, like I said, Professor of Neurology and Chief in the Division of Neurological Infections and Global Neurology at Yale. She earned her medical degree from UCSF, pursued residency training in internal medicine at UW, neurology at Harvard, and completed fellowships at Harvard and UCSF. Her clinical and translational research explores HIV in the nervous system, focusing on effects of acute HIV infection, antiretroviral treatment, and HIV cure strategies on HIV pathogenesis and persistence in the central nervous system. She collaborates with colleagues of multiple disciplines in clinical studies in urban centers in the United States and in international settings exploring questions of CNS inflammation, injury, and compartmentalization of HIV. She was the first neuroscientist elected to the AIDS Clinical Trials Group, or ACTG, HIV Reservoirs and Eradication Transformative Science Group, its chair ex officio of the ACTG Neurology Collaborative Science Group, and co-leads multiple NIH funded projects addressing the pathobiology of neuro-HIV. She cares for patients living with HIV with neurological disorders in the Nathan Smith HIV clinic at Yale. So, Dr. Spudich, thanks so much for joining us and at this point I'm going to turn it over to you.

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Okay great. Well, thank you so much, Jessica and to Gail and to others who organized this call. I think this program is incredibly valuable, the idea that this is really accessible to people who want to join my webinar or these kinds of presentations and talks and I'm excited and honored that people are interested in learning more about the CNS and HIV. As you see in my title, and these are my disclosures. I receive I'm chair of a study through the ACTG that we receive medications from ViiV, study meds.

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And learning objectives are presented here. Basically, to learn about the clinical impact of HIV in the brain, identify the effects of HIV on behavioral cognitive function, and evaluate available effective treatment strategies.

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As you heard from Jessica, I’m a clinician and I’m a neurologist but my specialty clinic is 100 percent based in the HIV clinic at Yale and this has been throughout my career and different places I’ve been. And so, I continue to see individuals who have HIV infection and who have neurological complaints. Some of them turn out to have a standard migraine that’s unrelated to their HIV but some of the time I’m seeing people that have problems that are likely associated directly with their HIV infection. So, I’m going to start out actually with a case about an individual that I saw recently and I’m going to go through the case and sort of talk about what this made me think about and why I think this is relevant to start out this talk. This is an individual named Tom. He’s a 62-year-old man with progressive gait impairment and cognitive difficulties and he and his partner came and reported that he had been diagnosed with HIV in 1988. And the story that he and his partner reported was that he had weakness of his legs and difficulty with balance. He had had occasional falls and then over two years basically had proceeded to needing a walker because his legs were so weak and his gait was so unsteady. His partner also said that he had worsening cognitive impairment and that he really wasn’t able to do shopping or bookkeeping or take care of anything around the house but he was really fully reliant on his partner to take care of those things. He also didn’t report this but his partner reported severely reduced short term memory. On exam, he had what I call a flat affect which is he wasn’t very animated and he wasn’t very, he didn’t display a lot of emotions. He didn’t speak stuff spontaneously at all. He only answered questions when I asked him questions. He was disoriented, he didn’t know that he was in New Haven, he didn’t know the year. He cried easily. He had a snout reflex, which is basically a primitive what we call primitive reflex that’s a frontal release sign suggesting abnormality in the frontal lobes of the brain. And he was uncoordinated on walking and with his hands, his reflexes were quite brisk he had spasticity in his legs. This may be something that’s familiar to people who have taken care of people living with HIV for a long time especially in the 80s and 90s in terms of the clinical presentation.

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Furthermore, when they came in to see me in the clinic they had brain imaging. And so, here on the screen you’ll see two different brain images. These are axial MRIs, the T2 signal where the cerebrospinal fluid is bright. So, in the center of the images, and this is cut through sort of from the nose to the back of the head so cutting straight through the face. And basically what you’re seeing here is the ventricles, which are right in the middle of the brain. On the left side surrounding the ventricles, there is increased flare signal or increased T2 signal. So, especially posteriorly, there’s sort of hazy, bright white pattern in the white matter of the brain which should not be there. What they showed me was that actually he had a subsequent MRI after that initial one about a year and a half later which showed not only increased size of his ventricles suggesting progressive atrophy of the brain. That’s the bright signal in the middle. That's normal CSF signal. But also a severe progression of this hazy white matter change in terms of the white matter brightness now extending also anteriorly to the frontal lobes. So clearly, there is some kind of an inflammatory or damaging, demyelinating, some kind of a process that’s ongoing in the brain in
this gentleman during his HIV. Putting these things together, the imaging and the clinical presentation, I think that one who's taken care of patients for a long time living with HIV would say well this is a patient who probably has AIDS and has HIV-associated dementia. And both the clinical picture, the history, and the imaging is quite consistent with that.

However, this is a gentleman who has been on suppressive antiretroviral therapy for 20 years. So, basically this is a real patient that I saw about a year and a half ago and over the course of a few months trying to evaluate him, he ended up being removed from care by family members and he passed away. I don't know what the problem was. He didn't have an autopsy. And I don't know for sure that the problems in his brain and with his clinical presentation were 100 percent related to HIV. However, the presentation was very classic for an HIV encephalitis. So, I think what this is telling us, this is just one piece of evidence that adds to kind of growing signs that there can be persistence and even progression of CNS abnormalities in people despite ART.

Really what the rest of the talk is going to talk about is what is the long-term impact of HIV in the brain and is this something that we can try to understand through three major questions. So, one is when does the impact of HIV in the brain first begin? So, is this something that we need to be thinking about in individuals even early on in HIV infection before they are necessarily presenting to care and before hopefully they have significant signs of disease? And how much of that may be even happening before we typically start antiretroviral therapy? The next question is do abnormalities persist in the brain during ART? And I think this is something that the first case is maybe a very dramatic example of. Although again, I don't know for sure what was happening with that patient. But I think there is some evidence that there are abnormalities. I'm going to talk about those. And the final question then is how do we optimize brain health in our patients? If they do have, they are living with HIV infection, how do we make sure that they are really optimally healthy?

To answer all these questions there are just a couple of things that I think are worth trying to understand and this slide looks complicated but hopefully it's not too complicated. It's my schematic of how we think HIV affects the brain. And what I have here is on the bottom the pink section is the blood. The white section above is supposed to be the brain compartment. Basically what we know is that HIV infection is likely carried into the brain through infection of cells in the blood. And here those are denoted as monocytes which are the starry shaped cells in the blood and lymphocytes which are the circles. We know those cross the blood brain barrier. There's normal monitoring sort of and surveillance of the brain by white cells even without any kind of CNS infection. There's an normal immune monitoring, immune maintenance. And in this case, we know that the cells that are activated tend to be trafficking more into tissues. So, infected cells, and that's supposed to be denoted by the symbols with the specks on them in these cells. Infected cells tend to traffic into the CNS probably more than even regular cells do. Once those cells go in, those cells actually release signals that invite in more cells. These are called chemokines and they attract more inflammatory cells. So, what you have is a cascade where infected
cells and activated cells basically increase or have a vicious cycle of increasing inflammation. There's also breakdown of the blood brain barrier that happens due to certain chemokines and other substances that are released by these cells, again allowing for influx of more cells and maybe even influx of free virus. So, what happens when the virus, virally infected cells enter the CNS? This is really the part of the infection that's still quite a black box. But we do know that in later infection in people who have advanced HIV or who have encephalitis in the brain, infected cells, the local CNS cells, so the cells that are resident living in the brain for a long time, themselves become infected. And that is shown here by two types of cells. I have one type of cell—can you see my mouse? This is supposed to be a perivascular macrophage and this is probably a cell that came in as a monocyte and then turned into a long live macrophage in the brain the other cell type is A microglia cell. These are another type of immune cell that are actually present in the brain from embryonic development on and may also become infected by HIV. So, what we have is local brain infection probably initiated initially by trafficking of cells but eventually establishing a long-term infection in the brain. We don't believe that the neurons, which have a long silver cells here, become productively infected. But it's possible that astrocytes which maintain the blood brain barrier and keep the brain really intact and free of other infections may become infected or at least disrupted during HIV. Eventually we have damage to the neurons and that happens because the infected cells are producing products, free radicals, cytokines, HIV proteins such as Tat that actually do damage the neurons. So eventually, the neurons become damaged and one of the markers that we use to monitor this inflammation and neurotoxic environment in the brain is a marker that I'll mention later in the talk called neopterin. Neopterin is released from activated macrophages and microglial cells and so elevation of neopterin that can be detected in the cerebrospinal fluid is likely indicating an environment which is toxic in the brain due to this activation and release of these products. The final thing that you see after the neurons have been injured is that as the viruses are replicating in the brain, this is similar to what we see in the blood, as viruses replicate they mutate. But one thing that has been well understood is that as the viruses are replicating in a CNS cell, they actually can eventually respond to the fact that there are different immune pressures in different environments in the brain and the viruses themselves change. So, what we have here is a situation where viruses go from being similar to those in the blood to being unique viruses that are only found in the CNS. And this is called compartmentalization where if you sample brain tissue or sample spinal fluid, you'll find viruses that have different sequences than viruses in the blood. So, this is all to sort of paint a picture of how we think that the brain is infected and then affected by HIV, both through infection of certain local cells and then through damage to the neurons from this toxic environment.

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Going to the next slide I'm just going to show you one example of the compartmentalization that I just demonstrated in the sort of schematic version. And this is called a phylogenetic tree where sequences derived from HIV from the blood are shown in reds and oranges and pinks and from the cerebrospinal fluid are shown in blue. And this was actually a series of sequences that were obtained over time in an individual who eventually, while he was being followed in a research study, developed HIV dementia. So, he was evaluated at negative 21 as negative 21 months before dementia, negative 16 months before dementia, and then presented with dementia. And the key feature here is that the phylogenetic tree which shows the relationship between the blood and CSF viruses for the most part shows viruses that are fully mixed where blood sequences and CSF sequences are found all around the tree. However,
starting two years before he had dementia, there were some viruses that actually were found only in the CSF compartment and evolved over time to be detected in the CSF compartment and related to each other over multiple visits where there were no similar plasma viruses found. And when these viruses were studied, they were found to be produced by a macrophage as compared to all of these viruses which appear to be produced by a lymphocyte. So, essentially what this is saying is that at some point in this individual's history, some viruses began to be developed locally in the brain and evolved in the brain and in fact it seems to be reflecting the fact that there was local macrophage infection. We think that this compartmentalization is not just a feature of HIV dementia but actually is a feature of HIV locally infecting brain cells and I'm going to show you how early in infection we think this may occur.

Really what I said is I wanted to look at three different questions and I think the first question here is if we say that we are looking to see whether there's persistent abnormality in people on treatment and what's really the long-term impact during ART, we really need to understand how early in infection there is an impact in the brain. And this is another clinical patient. This is a real case. He was a man who was diagnosed in 2013 and had on retrospect, on history and based on lab testing, probable acute HIV about six months before. And so, when he came in he said you know I'm feeling healthy. I'm on no medications. I'm an electrical engineer. I have a good support network. I don't want to go on treatment. I know the guidelines say start treatment but I don't want to be on a toxic medications. And so, this is really I think an unanswered question but it brings up the idea of are there concerns besides for example generally the idea of just reducing viral load, are there real concerns related to the brain that one may need to address by starting treatment early? And this gentleman says the most important thing to me is my job and being able to continue to work without cognitive impairment. So, I want to know whether I should start treatment for my brain. And you can see here that his CD4 count is pretty high and his viral load has been relatively low. So, what do we tell this gentleman and what do we know about early infection in the brain?

The way that I have addressed this question is by a couple of different research studies that I've been fortunate to work on where we actually identified people in very, very early infection and looked to see whether or not the brain seemed to be impacted at all by early HIV infection.

This is actually a combination of two different research opportunities. Jessica mentioned in my introduction that I'm fortunate to collaborate with many different people on many different kinds of projects and also to have opportunities to do studies in multiple places around the world. This is a study that I was able to join about seven years ago which is based in Bangkok, Thailand that is specifically focused on identifying and then enrolling in studies individuals during acute infection. So, this is a very, very large study that takes advantage of the fact that there is a anonymous HIV and STD testing center in the middle of Bangkok that is actually the largest and first anonymous testing center in Asia. So, it's really a place that many, many people go in for HIV testing. And a strategy was employed there about eight years ago to start looking at the samples where people were getting HIV tested by Nucleic Acid
Testing and not simply looking at HIV antibody. And over the years, many, many individuals have been screened and almost 500 people have now been identified who have acute HIV. They are asked if they want to participate in a research study and many people have enrolled. And then within that research study there’s clinical characterization phlebotomy in everyone and then some people consent to optional procedures which include lumbar puncture, MRI, MR spectroscopy, and also every individual gets neuropsych testing. This study is unique in the sense that everyone is also offered co-enrollment in an ART treatment study and essentially everyone except for just three people have enrolled for ART and started treatment within two days of identification of their acute infection. So they started treatment very, very early. So, this is a very, very early cohort. They’re typically identified a couple of weeks after infection and they’re all starting treatment.

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We’ve also had— I’m sorry— opportunity to study individuals with early infection in more of a sort of natural history study. This is a study that I participated in in San Francisco where we identified 109 people who were community identified so they were people who had, for example, recent negative testing and then recent positive testing at an STD testing center and sent to us. We also had samples from colleagues in Gothenburg, Sweden, Milan, Italy, and Sydney, Australia who also had identified similar individuals who had recent infection. We followed these individuals up in our San Francisco cohort every six weeks and then six months thereafter. And this was an observational study and much of the study was before test and treat. So, about half of the visits in the study were on ART but half of the visits in the study were also before people started ART because many people had high CD4 counts and didn’t want to start treatment.

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So in fact, from this study we have different information than the Thailand study. However, for a few of these figures and some of these analyses I’m going to show you, I’m combining data from both cohorts and so I want to show you the snapshot of how these cohorts are similar and different. The acute cohort in Bangkok is younger although almost all individuals in both studies are MSM, 94 percent male in both studies. Both studies have people with pretty high levels of education. The Bangkok study estimated duration infection at enrollment was 19 days versus about three months for the other study. And also the clades, the HIV subtype, were different in the two cohorts. The other lab differences that are here on this table are really reflective of simply the expected differences based on Fiebeg stage or how early in acute HIV individuals were in Bangkok versus in our primary infection study.

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But the next couple of slides I think are going to start to ask this question about how early HIV actually affects the CNS. And this figure shows the plasma viral loads of each individual participant in the two studies at the time of enrollment, so before ART. Each of these symbols is a single individual. And these are the Thailand individuals because they are so early after HIV transmission and this is the rest of the cohort who were enrolled any time up to one year. This is the primary infection cohort. And this is really the typical viral load trajectory that we know about in plasma in acute HIV infection which is a big peak at about 21 days coming down to a set point by just about a month. What we now have superimposed
on this is the CSF viral loads in these cohorts and we don't have CSF in as many individuals in the Thailand study but we have CSF in many.

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And I'm going to show you here the CSF alone just to demonstrate I think what we see is the same sort of pattern in general that we see in the plasma. But again, we have sort of a peak trajectory and coming down with an individual here with four million copies in the CSF at about 2 after infection. So, what is this telling us? It doesn't necessarily tell us that we're setting up a reservoir where there's local infection of brain cells producing HIV but it is telling us that HIV is crossing the blood CSF barriers and becoming in contact with the brain very, very early in infection.

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I also mentioned that macrophage activation is sort of the substrate of neuronal injury and damage in HIV. And this is what we can see here is the CSF neopterin. This is a measure that I mentioned is the marker of macrophage activation or microglial activation. And the dotted line on this graph is the normal level in HIV-uninfected, healthy controls. And basically what you can see is in almost everybody between these two cohorts or in a very large proportion, neopterin is elevated in early infection. So, macrophage activation, microglial activation is being initiated very early in the CNS compartment during infection.

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We've also done some smaller studies looking at a marker that's specific for neuronal injury. So basically, Neurofilament Light Chain is released into the spinal fluid when neurons and axons are disrupted. So, when there's actually damage to the neurons, not just inflammation or viral presence, you have elevations in CSF NFL. And what you can see here is that I've shown the data for HIV-uninfected controls, individuals with acute HIV in Bangkok, and then individuals in our other cohort that I said was a median about three months after infection. And although the very, very acute people a couple of weeks after infection don't have elevations, you can see that even within this first year of infection which was the time course that we enrolled people in the primary infection study, there were quite a number of people who had elevations in CSF NFL. And I think this is important in thinking about where people are when we're starting them on treatment and actually in the first year of infection in primary HIV, there were really no differences in NFL levels than people with chronic HIV infection. So, sort of suggesting the cat is out of the bag in terms of starting an ongoing inflammatory process which is associated with CNS injury. On the right you can see there is a correlation between the neopterin, that macrophage activation, and the NFL levels and this is our primary infection group. Again, suggesting that the injury is really driven by inflammation.

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One question that we don't know is whether or not during that early period there's any compartmentalization, whether or not there's actually local differences in the viruses in the brain and the blood. You would expect that viruses entering in very acute infection in our Thailand cohort would
be identical to those in the blood of course because it's got to be coming from the blood. So, we've done some studies to try to sequence the virus in the spinal fluid and these very, very early people.

This next slide demonstrates that there are some differences in some individuals between the viruses detected in the spinal fluid and the blood. This is an individual that had deep sequencing, so sequencing that looks not only for sort of major virus populations but also minor variants because of course HIV is a swarm of viruses, looks for minor variants in blood which is shown in a left three columns and the spinal fluid which is shown in the right three columns. And what you can see here is that the overall the variants that were detected in the two compartments were the same, the same blue, green, and red. Those sequences are identical between the two but the proportions of those sequences were different in this individual. So, we've looked at about 17 people using this technique and in three of them there were different viruses enriched in the spinal fluid already-- this individual is 19 days after infection-- than in the blood. So, what exactly this means is not clear but it's possible that there is some sort of selection process in terms of certain viruses that might be neurotropic have a greater ability to cross into the blood brain barrier. The other possibility is that even as early as 19 days post infection, there are different selection pressures within the CNS that are allowing for enrichment of certain viruses. So, we're trying to identify this further and do more studies to try to understand how early this kind of compartmentalization actually has a longer term effect.

We have also done deep sequencing and other types and sequencing studies with viruses a little later in infection and this was with a different group. The first studies were done with collaborators through our Thailand work but with another group we looked at viruses in the CSF and the blood in people within the first year of infection. And what we found basically was that about 20 percent of people had compartmentalized viruses in the spinal fluid that were different from those in the blood within the first year of infection. So, typically this wasn't present in the first four months but we did find it later.

So, what we are interested in looking at that is how early not only is a compartmentalized virus is it detected but how early can we actually find evolution where over time it looks like there are different viruses that are evolving independently in the CNS. One compartmentalized virus at one time point might mean that a single cell crossed in and released a unique virus in the spinal fluid. But local evolution suggest that actually cells in the CNS compartment, maybe in the brain or in the the meninges, are producing HIV totally independently from the blood. And we found a couple of examples in our early infection cohort suggesting that within the first two years of infection there is local viral evolution. This is another phylogenetic tree that shows the differences between blood viruses, which are pink and red, with the CSF viruses, which are blue. And again, and you can see that at 165 days post estimated infection, there was a set of viruses that were detected in the spinal fluid. And then over time there was a series of related viruses that were detected only in the spinal fluid over the next two years that were totally independent from those in the plasma. So, what this suggests is that local replication in the brain is established during early infection at least in some individuals. So, it makes us worry that if we don't
intervene very, very early, as we are for example in the Thailand cohort, there may be time for a local brain infection.

The next set of questions is do abnormalities persist in the brain during ART? And again, as I mentioned, the initial example of the case was a very dramatic example of somebody who seems to have abnormalities in the brain even progressive abnormalities that are clinically very severe and in all ways in terms of clinical presentation and imaging findings they seem to associated with HIV. So, how much do we see this on a more subtle level?

I think there are a number of different lines of evidence that this may be true. So, one thing that we use a lot to try to characterize abnormality versus normality in people in the CNS is looking at neuropsychological testing. There’s been a lot of attention in the last 10 years to work that has used neuropsych testing to evaluate individuals who are on treatment to see whether or not their performance on neuropsych testing is within the expected norm. So, neuropsych testing is basically a series of pen and paper and question and answer type of tests, some of the motor tests as well where individual performance is corrected for age, level of education, ethnicity, and gender. And we look to see whether people fall within what we call normal neurocognitive function or whether or not they fall within the definition of what's called categories of HAND. And HAND is HIV-associated neurocognitive disorder which has been defined basically based on whether or not performance is within one standard deviation below the mean or two standard deviations below the mean of the corrected norms. What you can see here is in the CHARTER cohort for example, where most individuals were on ART, although not every single one in this graph was on suppressive ART. Most individuals were on ART. Only 53 percent of the individuals were cognitively normal according to their neuropsychological testing. 33 percent had what’s called asymptomatic neurocognitive impairment and that is one standard deviation below the norm in two different cognitive domains of testing without significant symptoms. And another 12 percent had what's called mild neurocognitive disorder which is the same performance on testing but with symptoms or some kind of family reports or job changes that suggest that people are symptomatic. In this cohort a very small percentage had HIV dementia which was 2 percent shown in green, which is generally what we see now. That's very, very uncommon. But the fact that there's such a large prevalence of milder forms of cognitive impairment have raised the possibility that there is more abnormality during treatment than people would have expected.

We also know that there are other ways we can measure abnormality in the CNS and one is looking at markers in the spinal fluid. I mentioned Neurofilament Light Chain, which is this axonal injury marker and this is a very nice set of studies where individuals, many individuals living with HIV were evaluated. Those represented by the blue dots and the blue line are people who are not on a ART and this is the NFL levels in those individuals. The gold line represents individuals who are on suppressive therapy and undetectable plasma viral loads. The black indicates HIV-negative controls. And what this study indicated was that although people without ART have a much higher NFL, which is suggesting active
neuronal injury, so some people have signs very high active neuronal injury, the levels in people on suppressive ART were statistically elevated compared to HIV-uninfected individuals. And this figure shows the distribution of NFL levels over the age range because NFL naturally increases with age. Unfortunately, we have more neurodegeneration as we age over each decade. But in fact, the individuals living with HIV on ART had NFL levels that were about three years older than what would have been expected if they had not had HIV. Essentially suggesting that there’s a mild but significant elevation in active neuronal injury despite suppressive ART.

There's also been a plethora of neuro imaging data to suggest the same thing is true with brain measures of brain volumes, brain inflammation, microglial activation. And this is a figure that shows changes in brain volumes, so brain atrophy in individuals on suppressive ART related to their nadir CD4. But there's many, many lines of evidence that there is persistent abnormality and some new data is suggesting that there's progressive abnormality in the brains in individuals living on suppressive ART.

One other marker that I think is critical to understand sort of how active this abnormality may be is measures of brain inflammation. I mentioned that there are CSF measures of brain inflammation. There are also neuro imaging measures of brain inflammation. And one of the most exciting ones I think is using positron emission tomography. So, basically PET imaging allows us to attach radioactive ligands to molecules of interest to identify where those molecules are located in the brain and compare levels of those molecules between different groups of people. And in this case, a pet experiment was done where individuals living with HIV who had been on ART for at least three years and were completely suppressed in the plasma had PET imaging done using a ligand for activated microglial cells. And the bright images on these brain pictures are basically areas where the levels of uptake of this ligand were quite elevated compared to HIV-uninfected controls suggesting that despite suppressive therapy, there are significantly elevated levels of microglial activation in these specific brain regions in people with ART. This is also a brain autopsy study and one of the major limitations in studying pathology now in the brains of people living with HIV is that we don't have a lot of pathology specimens from people who are well suppressed on treatment. Many of the old brain banks and studies that have been done in the past have collected brains from individuals who died of complications of AIDS who had HIV dementia or opportunistic infections. And then now thank goodness we don't have very many people in the ART era who are dying and were healthy. We do, however, have some examples of individuals who have been on suppressive therapy and then died suddenly of other causes, sometimes something like a gunshot wound or a car accident unfortunately or occasional sudden death. And this is one study that looked at those individuals and some brain pathology specimens and what they found was that individuals who were on ART had very, very elevated levels of microglial cells and macrophages in the brains compared to those who do not have HIV. And on the left you can see all this brown staining. Those are CD68 stains for microglial cells and macrophages that are very, very much more rare in those individuals who did not have HIV suggesting brain inflammation even in people who seemed otherwise asymptomatic. And finally, I mentioned CSF neopterin and the fact that neopterin is quite elevated even in a very early infection in individuals who have HIV infection. We do know that neopterin responds very,
very well to treatment with ART and we think that's because when you are reducing replication, you reduce trafficking, you reduce inflammation. But what we have found is that neopterin does not completely resolve and a number of large studies have suggested that neopterin persists elevated in the CSF compartment despite resolution even of CSF neopterin in the blood in people on long-term treatment. And this was a study of people on more than 10 years of ART where they had been undetectable in blood for 10 years. They still have elevations in neopterin and interestingly in this study, neopterin was elevated in those who had a positive HIV RNA in the spinal fluid via single copy assay. So, the single copy assay is a very sensitive way to test for HIV that's for research purposes and those with the positive single copy assay had a much higher CSF neopterin, suggesting an association between ongoing inflammation and possibly release of virus.

I did mention that there are very few studies that are available of people who have actually passed away on suppressive therapy again, for good reasons that we have people living for a long time. And then people who maybe go off treatment at the end of their lives if they're in hospice, for example. But there was one very nice study out of San Francisco that looked at people who had sudden death in the community usually from arrhythmia and those individuals had their tissues evaluated. And one of the questions that we have which is very important is is the level of inflammation or the level of ongoing injury in the brain in people living with HIV on ART is that related to actually HIV itself? Is that related to a reservoir in the brain of HIV DNA infected cells or even ongoing replication with HIV RNA? And what this group did is they looked at tissues across the board in individuals who died of sudden death and they actually were surprised I think to find that levels of HIV DNA per cell equivalent, so this is corrected for number of cells, was quite detectable in all of the individuals that they measured, that they looked at. In some it actually was comparable to other tissues which we think of as major HIV reservoirs such as the sigmoid colon or the lymph nodes. So, what they said is that there weren't very many cases where HIV RNA was measurable in the brain in these individuals so maybe there wasn't a lot of HIV replication but there certainly seemed to be HIV-infected cells that could serve as a sort of reservoir and may be associated with some of the CNS perturbation.

Finally, I'm going to talk about an example I think of where we think that HIV DNA may sometimes get reactivated in the CNS compartment for unknown reasons and cause a condition which has now been termed CSF "escape" where there's actually HIV replication and HIV RNA being produced in the CNS compartment despite suppression in the plasma. So, this is an example of an individual who's followed at Yale. And this is his plasma viral loads at each clinic visit over eight years of follow up. Undetectable every single time he came in for his visits. And then at almost eight years of suppressive therapy, he developed a hand tremor, ataxia, slurred speech, and difficulty finding words or aphasia. His CD4 count at that time was 308. He was on lamivudine/abacavir, and lopinavir/ritonavir and his nadir CD4 had been 60. He had an evaluation for this new neurologic condition including a lumbar puncture and his CSF viral load was found to be almost 500 copies. His CSF white blood cell count at the same time was 26 cells per microliter, which is quite high. Normal is less than three. And you can see that he still had an undetectable plasma overload at the time that his CSF viral load was detectable. This is why we call this
CSF escape because somehow the CNS virus seems to be escaping from antiretroviral therapy independent of the blood.

Fortunately, this gentleman's virus was at a high enough level in the spinal fluid that it could be sent for genotyping and basically he was found to have numerous resistance mutations. And in fact, when his Stanford database genotype was run and evaluated, it was found that he actually had resistance to every single drug in his current regimen in the CSF replicating virus. So, for some reason by some mechanism his CNS virus reactivated and somehow replicated and developed resistance and seemed to be having a clinical result. In this case, he had a couple of drugs changed and added to his regimen and he actually had complete resolution of his signs and symptoms. And actually has not had a further spinal tap because he's been following up clinic over the last 10 years without any other problems. So, do we think that this is happening in many, many of our patients living with HIV on suppressive ART? Absolutely not. I think it's a rare condition to have symptomatic CSF escape. But I think there is increasing recognition that this is something that happens in some individuals and should be at least looked for. There's also been increasing interest in trying to understand this condition to try to understand what it means in terms of understanding reservoirs for HIV in the brain and to try to understand how long-term ART could be associated with recrudescence of viral replication in different compartments. And this has implications not only for the neuro field and for our patients but also for the HIV cure field and thinking about what reservoirs need to be addressed in taking care of HIV completely.

I'm just going to show you on this next slide a table from a paper that we wrote focusing on this condition of CSF escape where a number of different individuals with cohorts all over the world are trying to bring together cases in a consortium thinking that a global CNS HIV escape consortium may be able to combine rare events and rare samples to larger studies that will have both clinical and biological significance. And actually there are two international meetings now. The last one about a month ago focused completely on this entity to try to understand it better both in terms of clinical relevance in clinical definitions and in terms of understanding its biology.

I'm just going to spend a couple more slides on persistent abnormality in the CNS and these are actually focused on something different than viral reservoirs. This is really looking at the possibility that some of the cognitive impairment that we're seeing in people living with HIV and on ART is related not to the same old pathology of HIV infection encephalitis that we used to see in the pre-ART era but in the current era may be more associated with vascular inflammation and cerebral small vessel disease which may be even associated with treatment. So, a couple of large cohort studies have identified that vascular risk factors including carotid intima media thickness and also vascular risk factors such as diabetes and hypertension are more associated with HIV-associated cognitive impairment than are typical HIV risk factors such as current CD4 nadir, CD4 viral load, et cetera in the current era. And another study that looked actually specifically at brain tissue specimens of individuals who were on ART.
at the time of death suggested that cerebral small vessel disease was more associated with cognitive impairment during life than any signs of encephalitis or any other type of typical HIV pathology. A very, very nice set of studies by Felicia Chow who's UCSF has started to explore ischemic stroke, increased ischemic stroke risk in people living with HIV infection on treatment, and particularly increased risk in women and in younger patients. So, I think there are some important investigations that need to be done in understanding vascular disease and neurologic dysfunction and maybe thinking about vascular disease either as a result treatment or long-term living with HIV and aging but also probably associated with vascular inflammation.

One more very nice study along these lines was published by Shibani Mukerji and Donna Gabuzda from the MGH in Harvard looking at whether or not cholesterol on its own was a risk factor for cognitive decline. And this was looking at the MACS database. So, MACS study has a large cohort of individuals with HIV that are followed over time but also a large matched cohort of men without HIV but with similar risk factors followed over time. And what they found is summarized here in these two figures is that in HIV-infected people, people with high total cholesterol had a much, much higher risk of cognitive decline than those who had lower total cholesterol in those living with HIV. In fact, in those with low cholesterol the trajectory was really no different from those who did not have HIV. But in HIV a group only, cholesterol seemed to be associated with a faster rate of decline. So, it may be because those individuals have heightened vascular disease although I think the mechanisms of those seem to be obviously need to be elucidated.

I'm going to end by talking about optimizing brain health in our patients and I think that what is going to be clear is that we really don't have perfect answers in how to optimize brain health. And there has been a plethora of studies looking at whether or not certain treatments or certain types of interventions may be beneficial in the brain. But I'm going to follow on some of the information that I've presented to try to make some practical types of recommendations. So, I think the first thing that we know, and this was partly based on some of the data that I just showed you in terms of how early HIV infects the brain, one of the first things that we should really know is try to treat people early in the course of infection.

And I'll show you that in our Thailand cohort, we know that ART in acute infection really, really reduces HIV RNA. And I showed you that individuals had very, very high viral loads in the spinal fluid at their very first visit, so median 19 days after infection. But what I'm showing you here on the left is a figure that shows the viral loads at their initial time point and then the viral loads at six months post infection. So, their first follow up spinal fluid test. And then you can see that there is basically a trajectory where everyone has an undetectable viral load at six months. And on the right I'm showing you data so 62 people we have undetectable viral loads in the spinal fluid at six months and another 35 we actually have at 24 months so two-year follow-up treatment. Only one individual had a detectable CSF viral load and this was an individual who also had failure in the plasma. So, basically the individual either had resistance or had poor adherence. It seems that early ART does suppress HIV but we know that's true...
even in chronic HIV infection that people can be adherent and the vast majority of people, unless they have what I've termed CSF escape, have undetectable viral loads during treatment.

What's more important to see is that inflammation in the CNS seems to benefit greatly from very, very early treatment. And this is the CSF neopterin on the left in our Thailand cohort showing the levels in people who were HIV-negative and those are Thai controls compared to individuals at their baseline visit before they start treatment. And then you can see the six-month and 24-month values in individuals who started treatment very early in acute infection. And in fact what we found in this study, which is contrary to all the studies in chronic HIV infection, is that neopterin normalizes. We've also found this with YKL-40 which is another marker of microglial activation and probably astrocyte activation as well. And I think this is a very important takeaway message is that possibly if we start treatment early, we can prevent that cascade of persistent inflammation that's initiated quite early in infection. The question really is how early do we have to start because obviously we're almost recognizing no one in the 39 million people worldwide who are infected with HIV almost no one's identified in acute infection. But perhaps if we can identify people early and get them treatment early there'll be a relative improvement in the level of persistent inflammation in the brain.

We do know that early ART probably protects the brain and this is the CSF neurofilament light chain, that neuronal injury marker, suggesting that people who start treatment in acute HIV have normal levels of NFL.

The next figure here shows neurocognitive testing. And this is an early study from our Thailand cohort suggesting that people who start treatment early have better performance and people who start treatment early have sustained normal performance over time. This is complemented by a nice study from Teresa Evering at the Aaron Diamond in New York who looked at people who started treatment during primary infection and after a few years of follow-up, she found that all of those individuals had normal cognitive performance compared to HIV-negative controls.

The one question I asked earlier which is how early is early enough I think is brought to light by this particular figure. This is a figure looking at viral sequences in blood which are the squares and CSF which are the circles during ART. And what this is basically showing is a tree indicating that even when people started ART-- this gentleman started ART during the first three months of HIV infection-- he had persistent compartmentalization, meaning differences in the sequences of the viruses that could be detected in the spinal fluid and the blood despite really early therapy. So, even starting treatment within three months in this gentleman still led to a persistent, probably compartmentalized reservoir that could lead later to CSF escape. So, I think the early treatment is important but I don't know if early treatment will really be realistic or enough to completely prevent every problem in the brain.
We also know that people should stay adherent to ART and this is a quite dramatic example from some old studies that used treatment interruption as an experimental sort of approach to dealing with virological failure. Remember from the old days in the 90s we used to try ATI studies to see whether or not we could benefit people by stopping therapy or pausing therapy. On the top here you see plasma viral loads in people who interrupted therapy jumping up immediately within a week of stopping treatment. And then in the next figure you can see the CSF viral loads also jumping up very high after acute treatment interruption very, very quickly within two weeks or so of stopping therapy. And in fact, also showing a great degree of neuroinflammation. So, this is the CSF white blood cell count going up to over 60 cells per microliter in individuals who stopped therapy. So, we know that stopping therapy can be injurious to the brain.

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And finally, I’m going to end on what’s probably the most controversial area but I think this is a slide hopefully people can take a look at if they can download this and then think about if they want to look at more references, but there’s been a lot of investigation into whether or not exposure of actual ART regimens matters in the brain. And we know that the higher CNS penetration effectiveness leads to lower CSF viral loads but it does not necessarily seem to be associated with reduced risk of CNS disease either in large, very large retrospective cohort studies or in smaller studies. There have been a couple of studies that even showed worsened neuropsychological performance in people who are on high CPE drugs. There’s been a little bit of evidence that monotherapy or dual therapy, which is being used as simplification strategies, does lead to increased CSF escape and I think that needs to be studied in much greater detail. And finally, there’s been a lot of attention recently into neurotoxicity of therapies, including efavirenz and then more recently dolutegravir in particular and possibly the whole class and integrase inhibitors. The dolutegravir question is really quite confusing at this point but there’s a number of recent articles suggesting neuropsychiatric side effects and some peripheral neuropathy side effects of dolutegravir.

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One of the that one can investigate whether ART exposure matters is to do a randomized controlled study. And this is something that Kevin Robertson who’s a neuropsychology professor at University of North Carolina is spearheading and I’m helping him with through the AIDS clinical trials group. We’re looking at individuals with mild cognitive impairment who are on stable treatment and then randomizing them either to no changes in therapy or intensification with dolutegravir or with dolutegravir/maraviroc. And the idea here is we’ll be able to hopefully tell whether higher CNS penetrating regimens in cells are beneficial, dolutegravir/maraviroc, and it will also help us hopefully piece out some of these issues of dolutegravir since this is really a study aimed to look at neuropsychology effects.

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I’m going to end on this slide, which is just the clinical guidelines for HIV in the brain. There are a number of guidelines, including European guidelines, which for a long time have had really profound recommendations related to the CNS. But just recently the U.S. guidelines have started to integrate
those. So, of course the guideline is to treat all individuals when they're diagnosed and hopefully find people with early infection. But now due to CNS toxicities, efavirenz is no longer in the first line recommended regimen. There are treatment recommendations specifically for HIV-associated dementia. This isn't mild cognitive impairment or mild HAND but for dementia it's recommended to not use efavirenz and to favor regimens of higher CNS penetration. And finally, there's also new evaluation and treatment recommendations for symptomatic CNS escape. So, people who are presenting with new neurologic findings during suppressive therapy, including recommending LP for CSF viral load, CSF drug resistant testing, and consideration of CNS penetration.

I'm going to wrap up there and I want to thank the Clinical Education Initiative and also all of you for your attention and these are all my collaborators that I mentioned and the study participants in all of our studies that have been amazing. I don't think I left much time for questions but I'm happy to stay on the line to answer some.

Great. Thank you so much, Dr. Spudich. That was really excellent and really comprehensive. It was very interesting. So, we have a couple of minutes for questions and just as a reminder to everyone on the line that you can chat in your questions and direct them to all panelists and we do have a few questions that have been chatted in. So, we have a question about if the CNS penetrance score matters in asymptomatic patients?

That's a great question and I think that we don't really know the answer to that. I'm imagining that asymptomatic patients we're talking about people who may be absolutely healthy and have no issues at all. I think that if we take asymptomatic patients and give them neuropsych testing as we do often in research studies, we often find that there is about a quarter of people end up having what we would call asymptomatic neurocognitive impairment. So, that's this category of people who have testing abnormalities but don't have symptoms. We do not yet know whether those individuals have any benefit from better CNS penetrating drugs. That's part of the intention of the study that I mentioned with the ACTG. We are actually including individuals who are asymptomatic but have abnormal testing. And hopefully through that study we'll be able to find out whether the testing improves with increases in CNS penetration. However, even if the testing improves in those individuals with better CNS penetrating drugs, I think it's still not 100 percent clear that it's going to have clinical impact in their long-term trajectory, whether or not they're actually going to do better in the long-term because we improved their neuropsych testing. But I think this is a really important question and certainly if somebody is presenting and asymptomatic and is getting started on the ART regimen, I don't think there's any good data now to suggest that CNS penetration should be in the considerations for starting individuals on treatment. The number one consideration should be a regimen that is effective for rapid and complete sustained control of plasma viral replication. I think that's the best evidence that we have.
Okay great. Thank you. So, we are out of time. If you have questions, you can send them to me. Dr. Spudich, I believe you included your email address on the final slide if you want to advance to that. So, if you have questions, there is Dr. Spudich’s email. You can absolutely send them to her or if you don't see that or couldn't get to it, you can always help me and I can forward them along. So, I want to thank you again, Dr. Spudich for leading this presentation. It was very interesting. Thank you again to our funder, the New York State Department of Health AIDS Institute Clinical Education Initiative. As a reminder to everyone, you will receive an email later this afternoon with instructions on how to evaluate today’s presentation and claim your CME or CNE credits. Next month’s This Month in HIV webinar will be on December 20th with Dr. Jessica Robinson-Papp presenting Medical Marijuana: Exploring New Clinical Options in New York State. So, thank you again to Dr. Spudich. Thank you everyone for joining us and we hope you'll join us next month.

[Video End]