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IMMUNE ACTIVATION IN THE PATHOGENESIS OF HIV INFECTION: CAUSES AND CONSEQUENCES

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Immune Activation in the Pathogenesis of HIV Infection: Causes and Consequences

[video transcript]

[00:00:00] Welcome to Physicians' Research Network. I'm Jim Braun the course director of the monthly meetings of PRN in New York City. Since our beginning in 1990, PRN has been committed to enhancing the skills of our members in the diagnosis management and prevention of HIV disease, as well as its coinfections and complications. We hope this recording of Netanya Utay's presentation Immune Activation in the Pathogenesis of HIV Infection: Causes and Consequences will be helpful to you in your daily practice and invite you to join us in New York City for our live meetings in the future. PRN is a not for profit organization dedicated to peer support and education for physicians, nurse practitioners, and physician assistants. The membership is open to all interested clinicians nationwide at our website PRN.org. Now allow me to introduce Netanya Sandler Utay, Assistant Professor of Internal Medicine at UT Health and McGovern Medical School in Houston, Texas.

[00:00:57] Thank you Jim, and thanks to the PRN for inviting me. So I'm going to talk about immune activation and HIV Pathogenesis, and this is something I spend a lot of time thinking about and and hopefully will interest you as well.

[00:01:13] So learning objectives. Essentially, the goal is that you'll appreciate the role of immune activation in acute and chronic HIV infection and how that perpetuates infection. Know the drivers of immune activation and essentially know what the consequences of having this chronic immune activation are over years and decades of HIV infection.

[00:01:33] So I am going to start by talking about how HIV establishes infection. I'm just going into a little bit of a history of HIV, I don't know how many of you are familiar with the history but essentially it's thought that SIV crossed over into humans not that long ago. But SIV actually evolved about over 30,000 years ago in monkeys. And monkeys, particularly non-human primates such as sooty mangabeys and African Green monkeys, evolved with SIV and learned to essentially become tolerant of the infection and not have any untoward defense, not progress to AIDS, not develop end organ disease. Essentially they co-evolved and it wasn't a problem. Most primate species actually do harbor a single strain of SIV. HIV-1 is thought to have originated from SIV in chimpanzees in West Central Africa and the prevalence of SIV in chimpanzees is thought to be at least 10 to 13 percent if not higher.

[00:02:29] And HIV group 1 which is the HIV strain that became epidemic, is thought to have originated from a chimpanzee in southeast Cameroon. And it actually was thought to have originated from southeast Cameroon and then disseminated by transmission to Kinshasa. And essentially at the early 20th century there was a bustling metropolis of Kinshasa, there is a lot of trade along the river here and people from Cameroon would go hunting and they would capture some bushmeat and the hunters would cut up the bushmeat and sometimes they would get some nicks, and then SIV could be

introduced into the hunters. And then with the traffic and the river boats and the bustling city, the people who were then acquiring SIV from bushmeat were then able to transmit it to other people. And so that's how it was originally thought to have transpired. And really I mean to think about it, now this is what 2018 this was about a hundred years ago that SIV really crossed over into HIV. We know this based on phylogenetic analysis, the first tissue and blood evidence of when SIV crossed over into HIV was available from 1959 and 1960. So we actually have physical evidence, not just phylogenetic analysis and computations, but physical evidence that HIV was present in 1959 and 1960. And it's thought that HIV crossed the US about 1970 via the Caribbean. It's thought the HIV went to the Caribbean around 1967 or so, and actually the ancestral US virus by and large has been traced to New York City and it's thought that the key virus that set off this US epidemic originated to some extent here, where most of the viral diversification occurred around 1970/1971. And of course it took about ten years for AIDS to be recognized largely in New York and to some extent in San Francisco, and about a couple of years later for HIV to be discovered.

[00:04:34] So to understand how immune activation plays into HIV persistence, you really have to understand the HIV lifecycle. And of course we know CCR5 and CXCR4 as well as CD4, they're receptors that GP120 binds to enter the cell. So HIV will then shed its RNA, the RNA will be converted into DNA, this proviral complex, and then this DNA will enter the cell. And the DNA becomes a double stranded DNA essentially at this point here it's a double stranded DNA strand. And this double strand DNA can either form a circle and be pre-integration latency complex or it can integrate into the host DNA. And by host I mean humans so then the human, the person that we're trying to take care of, has this HIV integrated into his or her own host DNA. That's fine, it can live there for years without doing anything but when the cell is activated to transcribe, that's when the HIV DNA is transcribed. That's when our messenger RNA is made and then you get translation of the viral proteins, you get RNA replication, and you get assembly of the virion. So you see here I pointed out the key point, the immune activation, to activate those cells resting CD4 cells they have the HIV DNA integrated into human DNA activated by some event and subsequently viral replication ensues.

[00:06:05] So much of this actually happens early on in the gut, and HIV is picked up by dendritic cells and cross the mucosal barrier. It can then go on to infect either activated CD4 T cells that are actively transcribing so piece of cake HIV enters, it enters the DNA, it's transcribed, makes more virus, the end. Or it can infect cells that then become resting and these cells will just hang out until they're activated to produce virions. But in the setting of acute HIV infection, of course it's a very inflammatory state, so many virions are produced and much of this happens in the lamina propria in the first few days. And that's because 60 percent or so of our body's CD4 cells are in the gut, and that's why we see so much of viral replication in the gut and why there's been so much emphasis on gut health and of course HIV enteropathy that the back in the olden days, but so much of an emphasis of the role that the gut plays ultimately in HIV Pathogenesis.

[00:07:04] So ultimately HIV will then go on to disseminate into the lymphoid tissues, but the lymph nodes and the lymphatic tissue in the gut. And then there's this homeostasis that's eventually reached where you have this ongoing immune activation from the virus and other factors that I'll talk about. You have this activation of regulatory T cells that try to kind of control that immune activation from getting out of hand, and ultimately you have these cytotoxic T lymphocytes who come in and try to control virus replication. So you have this balance of immune activation on the one hand and viral control and immunoregulatory factors on the other hand. And when they all reach a kind of plateau, then you end up seeing essentially the HIV setpoint. So as you can imagine with HIV causing such havoc on the gut it doesn't happen without any adverse consequences.

[00:07:59] And you can see here in the normal gut, you see a healthy mucosa here, you can see mucous level, you can see some antibodies here, you see some bacteria. These blue things here are the CD4 cells and this is someone without HIV. So this is the healthy gut here on the left. On the right here is what happens when the gut is exposed to HIV, and essentially you get depletion of course of CD4 cells, but as the HIV infects the CD4 T cells creates a local inflammatory process including production of TNF which can be cytotoxic to enterocytes and also damage enterocyte tight junctions. And you also get, as a result, translocation of bacterial products from where they should be isolated in the lumen of the gut, crossing into the gut parenchyma, and then into the portal circulation, and subsequently the systemic circulation.

[00:08:51] So ultimately what you get is this increase in translocation of bacterial proteins. It's not bacteria, people don't have gram negative sepsis or anything, but it's the bacterial proteins that are getting into the liver and into the systemic circulation and activating macrophages and monocytes to produce essentially inflammatory cytokines and perpetuate the systemic immune activation.

[00:09:16] So Jake Estes recently, I think last year, published this great paper looking at the SIV model. So these are rhesus macaques who grew up in India and didn't evolve with SIV, so then when they're infected with SIV they react much like humans do in that they progress to AIDS and die because they don't coevolve. So what he did with this SIV model is he evaluated at different stages of infection, both before antiretroviral therapy and on antiretroviral therapy, where in the tissues the virus was. And what he saw was before antiretroviral therapy about a third or so of the virus was in the lymph nodes and about two thirds was in the gut, actually a little bit in the lung here too. And if you look here, we will just focus on the black bars, we are going to come back to this figure a little later in the talk. But if you focus on the black bars, you can see that HIV is distributed throughout the gut, it is in the duodenum, it is in the jejunum, it is in the ileum, it is all the way down to the rectum. It is not in one specific area, it's just located throughout the gut. It really has a diffuse impact there.

[00:10:22] So now if we step back to look at what's going on with the person, we've talked about the virus, we talked about the tissues. Now let's actually take a step back and look at the real life human being who's here before us in clinic. What is happening? Well let's say day zero is the day of exposure. So this is someone who's gone out, they haven't been on PrEP, they haven't yet sought out PEP. It's the day of exposure. So, fine they get exposed, they have a wonderful time and then the Eclipse phase happens. And the Eclipse phase essentially is when the person is feeling fine, they might have known they were exposed to HIV but they would have no way of knowing. And we as their clinician would have no way of detecting the HIV is there unless we decide to you know, I guess we could offer them a gut biopsy to see if there's any virus there. But generally speaking the E.R. doesn't usually do that. So this is the Eclipse phase, essentially asymptomatic, all tests are negative. It's before, here in the red line is the viral load, it's before the viral load reaches the level of detection. And then over the next week or so, days 10 to 17 or so you have detectable virus but no detectable p24 antigen. And then at about three weeks or so, on average is when symptoms begin. Now this is a big period, anywhere from you know perhaps seven days to 28 days after exposure people can be symptomatic. But on average it's about three weeks after exposure. And about this time their ELISA may be positive as well. So they might start to develop antibodies at this point. But subsequently, you can see what happens at the rest of the tests. But it's this period here the Eclipse phase, and this is Fiebig staging of acute HIV infection if anyone's interested, I can talk more about that later. But you can see the Eclipse phase and the early acute HIV phase may precede actually the symptomatic stage.

[00:12:22] So what makes people symptomatic? Well it is the cytokine storm. I mean HIV is just like any other virus, like the flu like Hepatitis B Hepatitis C, it induces a profound inflammatory response and these cytokines actually peak before the HIV viral load does. And you can see primarily one of the key drivers is interferon alpha and it's interferon stimulated proteins as well drive this inflammatory response that make people feel run down, feel like they have the flu, myalgia, fatigue, fevers, headaches, G.I. symptoms as well. So I've talked about inflammation does essentially in setting up infection and the acute phase. We start people on antiretroviral therapy we get them suppressed, so why doesn't the virus go away?

[00:13:12] Talk a little bit about that. Remember when I mentioned the HIV lifecycle, I talked about the resting CD4 T cells and then the activated replicating CD4 T cells. There's a biphasic decline after we start antiretroviral therapy and the first step, the steep decline, represents when those activated CD4 T cells are dying. So essentially the antiretrovirals are stopping the virus from replicating, so new cells aren't getting infected but these cells are dying off really really quickly, the activated CD4 T cells. They have half-life of about a day, they get infected they die. Now the resting CD4 T cells though, they've integrated the DNA, they are going to die more slowly. They have a half life of maybe 14 days, maybe even longer. And this can take time. This is the slower suppression of the virus. So eventually we get to this point that the viral load loads are underneath the limit of detection. But virus production has not disappeared and at this point when the viral load is under the limit of detection, the virus production is still 0.01% of what the virus reduction was before antiretroviral therapy. So 0.01% isn't much, but 0.01%

is still not zero and it is sufficient for the virus to persist. But what's interesting is that it's not like people who get to virologic suppression undetectable, and that's it. Undetectable, I don't know what the level is here, the test we use is 20 copies per ml, so undetectable is great but it's not like it's 'that's it. You're done.'.

[00:14:48] If we look at HIV RNA levels by single copy assay, the levels continue to decline with years of antiretroviral therapy. There's this nice study by Sharon Riddler showing that among people, this is four years after initiating the antiretroviral therapy, people who are undetectable by lab tests still have detectable HIV RNA when using the single copy assay. And if you zoom in here to the people, eliminating all these people who don't have detectable levels, but zooming into people with detectable levels at four years after infection, their levels continue to decline. So it's not like you reach the goal post and you're there, there's continued benefit to continuing the antiretroviral therapy.

[00:15:28] And this is paralleled by HIV DNA levels as well. The HIV DNA levels will go down early after starting antiretroviral therapy. But they continue to go down long after people are undetectable. So really it's this continued antiretroviral therapy that has an ongoing benefit. But what's interesting is that HIV DNA level on antiretroviral therapy was really only dependent on the pre-antiretroviral HIV DNA levels. I.

[00:15:59] 've talked a little bit about the reservoir, and both the reservoir as far as the cell and the reservoir as far as the tissue. Most CD4 T cells with integrated HIV die. And I talked about these resting CD4 T cells that do not produce the virus. The problem with these resting CD4 T cells that aren't producing virus is that they're also invisible. So for a cell to be recognized by the immune system as having a foreign agent in it, it has to present that foreign agent on its surface. When the HIV DNA is just integrated into the resting CD4 T cells, it's not expressing those proteins. That immune system is not going to recognize it and it's going to remain inert for years. But these cells when activated are the ones that make new viruses. So it's estimated, there have been some calculations that have been done estimating that maybe 60 years or so or more of suppressive antiretroviral therapy may be possible to eradicate the virus completely. But of course we'll probably have to wait for another 40 or so years to see that.

[00:17:08] What goes on with the tissue reservoir? Well what happens interestingly, if you remember before, your tissues before antiretroviral therapy, much of HIV was in the lymph nodes with some of it in the gut. Now with antiretroviral therapy, almost all the virus is in the gut you still have a little bit in the lung. But the vast majority of the virus is going to be in the gut. And again, even with chronic infection and late chronic infection it's distributed throughout the gut.

[00:17:40] So the anatomic reservoir, the lymph nodes in the gut, and the cellular reservoir, the resting CD4 T cells, are really the barrier to cure. And we talk about two concepts when it comes to cure, we talk about the sterilising cure which essentially is getting rid of all virus that's replication competent. And we talk about the functional cure, which is essentially permanent viral suppression without antiretroviral therapy to prevent immunodeficiency and transmission. But that's the problem, because for there to be permanent viral suppression you can't have any elements of immune activation to activate even one of those resting CD4 T cells. So this is really the big catch 22 when it comes to finding a cure.

[00:18:22] So what's driving this immune activation? So we talked a little bit about the virus but there is variety of other factors, and immune activation is also very general term and it manifests in a variety of ways. So we can talk about manifestations in the innate system, macrophage activation, monocyte activation, dendritic cells, production of pro-inflammatory cytokines such as TNF, IL-1, IL-6, serum Amyloid A, C-reactive protein, D-dimer, tissue factors their evidence of hyper-coagulation, it's all associated with immune activation. All consequences of immune activation. With chronic inflammation you get activation of fibrotic pathways, so you get increased markers of fibrosis, you get increased collagen deposition in a variety of tissues, you get increased responses to lipopolysaccharide and other microbial products, such as soluble CD14. And you also get increased T cell and B cell turnover.

[00:19:18] So with suppressive antiretroviral therapy, you wonder well can you get rid of all of the inflammation? Well the short answer is no. There have been a lot of a lot of studies and a variety of. And sometimes it's a little hard to interpret the studies, because there are different populations and of course as new classes of medications have come around and as our ways of detecting, as undetectable became less than 1000 then less than 400 then less than 50 then less than 20, our goalposts keep moving. But essentially all of these studies have shown that at least some of the inflammatory markers are not normalized with antiretroviral therapy. However, they do tend to decrease and you can see here IL-6 levels and soluble CD163 levels, which is a macrophage activation marker, do decrease with suppressive antiretroviral therapy.

[00:20:09] So to talk about these different drivers, I'm going to first talk a little bit about HIV production and replication and to what degree any of this residual resting reservoir has on inflammation. And essentially, according to this one paper, they make the argument that residual virus actually plays virtually no role in persistent inflammation in people who are on suppressive antiretroviral treatment. Rather, the pre-antiretroviral level of inflammation was the main determinant for continued inflammation on antiretroviral therapy. So if you look at IL-6 levels before antiretroviral therapy and year four, correlated pretty tightly. And they saw that for all of the inflammatory markers they measured.

[00:20:55] So if HIV is not doing it, what else? Well I'm sure that I'm not the only person who has observed that people can gain weight sometimes after starting antiretroviral therapy and we're starting to see more and more weight gain. There is this study from a few years ago, so kind of older days older antiretrovirals, but it holds true. Showing that before antiretroviral treatment, about 50 percent or so of people with HIV are underweight or normal weight, but after starting antiretroviral therapy close to 70 percent are overweight or obese.

[00:21:30] So really there's a shift towards weight gain after starting antiretroviral therapy. And it's not just muscle mass, it's not necessarily healthy weight. People are gaining fat and they're gaining fat everywhere. They're gaining fat and their trunk, they are gaining fat in their limb. It's not necessarily a healthy kind of weight gain.

[00:21:48] And in fact, because talking about the trunkal fat, that may end up being visceral adipose tissue and we're certainly seeing more and more imaging studies looking at the amount of fat deposition in the liver, and the heart, and so forth in HIV infection. And this visceral adipose tissue is important because when it increases in the setting of obesity, it becomes inflamed and actually there's recruitment of the macrophages to the adipose tissue, there's recruitment of T cells to the tissue. These inflammatory cells of course produce pro-inflammatory cytokines. The fat becomes less healthy, it's not capable of storing so much in the way of free fatty acid, so the amount of free fatty acid circulating increases. And then some of the more beneficial proteins that adipose tissue can make, like adiponectin, go down. And this results ultimately an increase in fat deposition in the liver, pancreatic B cell dysfunction, increased triacylglycerol deposition in the muscle which not only confers insulin resistance but also can increase the risk for frailty. And all of these converge along with endothelial cell dysfunction and increase in the risk of cardiovascular disease.

[00:23:01] So fat is bad. Co-pathogens have also received some attention and particularly CMV. Many of our patients are coinfecting with CMV. And what we see is that if we were to give people valganciclovir to treat CMV, we can actually see a decrease in T cell activation. This is a study Peter Hunt did years ago, showing the eight weeks of valganciclovir did decrease CD8 T cell activation, so one of those adaptive immune activation markers, but didn't have an impact on CD4 T cell recovery. Now certainly we'll probably see changes in immune activation and a lot more papers coming out with hepatitis C treatment and cure and so forth. But I can also say there hopefully will be more studies done on with regards to suppressing CMV in the future.

[00:23:50] So I'll go back to my favorite topic, the gut. Microbial translocations, so I mentioned that there are microbial products going into the gut. Well what are the data for that? You can actually see here from the SIV macaque model, early and acute HIV infection this brown spot here. The brown here indicates here E.coli. You can see E. coli throughout the gut and you also see the early destruction that

happens before AIDS in the SIV model, and destruction throughout the gut and more E.Coli throughout the gut once the animal has progressed to AIDS. Now what's interesting though, is it's not just the draining lymph nodes that collect LPS. LPS was actually identified in the axillary lymph nodes in these animals. That really shows evidence that these microbial products are disseminating throughout the circulation, not remaining local.

[00:24:43] And what we did, in a study of almost 700 people from the STARTT study, was look at markers of microbial translocation and gut inflammation. Lipopolysaccharide, which is part of gram negative bacteria, those levels were elevated regardless of whether people are treated or untreated compared to the gray bars which are HIV uninfected people. Soluble CD14, which is a marker that monocytes have been activated by LPS, was also elevated across groups whether they were on antiretroviral therapy or not. And endotoxin-poor antibody which is depleted as it binds LPS, was decreased in all people compared to HIV uninfected controls. And then there's this protein, intestinal fatty acid binding protein, which is released by enterocytes of the small intestine when the gut is damaged. And essentially we saw elevated levels in almost all of the people that we had in the study. Interestingly we found in the study as well, that soluble CD14 levels predicted mortality on average about two years after these levels were measured. Suggesting that this LPS-induced monocyte activation was potentially a contributor, if not at least predictive.

[00:26:05] One thing that has also gained recent traction is the idea of dysbiosis, an abnormal gut microbiome in people with HIV. There's been a lot of studies doing this, they study varied populations. Microbiome studies are complicated because the populations can vary, the diets can vary, that genetics can vary, the amount of exercise people get vary, other drugs they take vary, yogurt, you know whatever. It can make these studies complicated. But by and large, if I were to draw a consensus of all these microbiome studies, essentially people with HIV have less microbial diversity in their gut with more proteobacteria which are pro-inflammatory bacteria and less Firmicutes and Bacteroidetes which are more gut healing healthy bacteria. So certainly dysbiosis in addition to having this microbial translocation, may be a contributor.

[00:27:01] Antiretroviral drugs. Well this is something, of course we know like didanosine and stavudine we should stay away from. But what about some of less old drugs? I have a lot of people who are still on tenofovir, emtricitabine, efavirenz, and they are like 'it works great for me. I've been on it. I got suppressed, my CD4 count is 800. I don't want to switch.'. Are there any differences between these antiretrovirals? And there's this one study in which people were randomised to tenofovir, emtricitabine, and efavirenz versus tenofovir, emtricitabine, and boosted elvitegravir. And what they saw was that in the efavirenz group there is a tendency for this inflammatory marker, soluble CD14 monocyte activation marker, to increase after antiretroviral therapy and a tendency on the elvitegravir group for this marker to decrease. And there's a similar pattern seen with C-reactive protein with no difference in IL-6. This soluble CD14 pattern has also been seen in some other studies as well suggesting, not to put efavirenz

under the bus, and we don't know exactly what it means, but there may be some real differences in terms of how the body responds to these antiretrovirals and potential for some of these antiretrovirals to cause inflammation.

[00:28:20] So last I'll talk about inflammation itself. And why do we care? You know people are doing well, they're virologically suppressed, U=U, they're not spreading it. Well we care because people are still dying. We've seen that these markers of inflammation, specifically soluble CD14 I mentioned IL-6, D-dimer, C-reactive protein, predict mortality in cohort after cohort after cohort. I lost count of the number of studies that have now been done looking at these various inflammatory markers and trying to see which ones predict mortality. But what's interesting in the setting of antiretroviral therapy in the U.S. and Europe at least, T cell activation does not predict mortality. Incidentally neither does HIV by single copy assay. That level, yeah it's good to see it go down, but it's not predicting mortality. It's these inflammatory markers that are predicting mortality in people with treated HIV infection.

[00:29:28] So what are people dying of? Well I don't think I need to tell you guys what you're seeing in the clinic, but you can see here that hypertension of course is very common as far as comorbidities go. And it's much more common, black being the HIV infected population grey being the HIV uninfected people, it's much more common in people who have HIV. As is myocardial infarction, peripheral arterial disease is also a little more common, obstructive pulmonary disease appears more common although it's not statistically significant, and impaired renal function is also a little more common.

[00:30:08] Now that was from the age HIV cohort study out of Seattle. This other cohort study out of Denmark, showed not only the increased rates of these different comorbidities but also depending on age. And if you can see here at the bottom, the lowest age bracket being 16 to 44 it still seems like a pretty big age bracket but 16 or 44 years old, 45 to 54, 55 to 64, and then greater than 65 here. And you can see in the red here, is the rate the incidence rate in people with HIV and the blue is the incident rate in people without HIV. And you can see that the rates of myocardial infarction are higher at all age groups compared to people without HIV infection. The rates of stroke are significantly higher, the rates of chronic kidney disease are significantly higher, and the rate of fracture is also significantly higher in people with HIV compared to people without HIV.

[00:31:10] Not only that, people with HIV have not just one comorbidity and I think this is most obvious in the 50 to 60 year old group. If you look on the left the HIV infected people and on the right the HIV uninfected people. This black and grey area here is people with two or three comorbidities and you can see that there are a lot more people with two or three comorbidities in the HIV infected compared to the HIV uninfected group.

[00:31:37] So what are these comorbidities? Well coronary artery disease, there's about a two-fold increased risk of coronary artery disease. Overall if you look at most studies, 1.8 and 2.2, but overall if you average them all together is about a two-fold increased risk of heart attack and of coronary artery disease. And what's interesting is that people with HIV preferentially develop these non-calcified plaques that are more vulnerable, they're more prone to rupture, and to form thrombi. There's also a higher rate of recurrent ischemic events and the need for recurrent coronary vascularization. There's almost a two-fold increased risk of heart failure as well. People also present with heart failure younger, up to 20 years younger on average, and this can manifest as both systolic and diastolic left ventricular dysfunction. It's thought that this is in part due to again, that visceral adipose tissue deposition, as well as the activation of fibrotic pathways. Atrial fibrillation is also more common and sudden cardiac death was actually the leading cause of death in people from the SMARTT study. About four and a half fold increased the risk of sudden cardiac deaths, so that makes me nervous whenever I see prolonged QT.

[00:32:48] So cerebral vascular disease is also more common. It strikes me as odd, the number of people I've seen who've had strokes in their 40s. I mean it's just unbelievable. But preferentially, people who have lower nadir CD4 counts, who are African-American, drug and alcohol use may also be a risk factor, and of course intracranial lesions. Pulmonary artery hypertension it's rare overall, but 2500-fold increased in people with HIV and it's thought that this is because of local cytokine stimulation. And I don't know if you remember from this little dot plots, that bottom right corner that was yellow, that was HIV in the lungs. So there's the HIV in the lung there probably causing some local stimulation as well. Now according to this study, and I realize that contradicted the previous one according to this study which I think is a little more comprehensive, peripheral artery disease may not be more common.

[00:33:43] So risk factors for cardiovascular disease. Hypertension is the big one of course, inflammation can impair endothelial function, cigarette smoking. So going what Demetri said earlier, we've got to stop people from smoking. And dyslipidemia especially based on previous antiretroviral therapy. Some calculations have been done showing that if we can get rid of these top three risk factors, the hypertension, the dyslipidemia, and the cigarette smoking, we could decrease the rate of myocardial infarctions by 40 percent.

[00:34:16] So what about non-alcoholic fatty liver disease? Well this is something that's really been gaining traction lately, it's been in the news a lot, it's thought among the general population that non-alcoholic fatty liver disease is going to emerge as the leading cause of liver transplantation, especially now that Hepatitis C is being cured. And there are a variety of reasons why NAFLD may be more common in people with HIV. As I mentioned earlier the dysbiosis and bacterial translocation, well all that stuff and the gut goes straight to the liver. And you know LPS is there in liver, Kupffer cells are responding to it and creating a pro-inflammatory situation. Aging of course, can also increase the risk. Visceral adiposity, which I've talked about before. HIV actually infects Kupffer cells and there's detectable HIV in Kupffer cells in the liver, so that may also perpetuate the liver disease. Metabolic

syndrome, we've talked about. And then there are factors that would preclude the strict diagnosis of NAFLD. And that NAFLD requires that people not be drinking excessively, they not be on drugs that can contribute to fatty liver disease, and they not have coinfections that affect the liver. But these entities still would induce steatosis and perhaps accelerate fatty liver disease in people with HIV, even though it strictly doesn't meet the definition of NAFLD.

[00:35:40] The prevalence of NAFLD is variable depending on the study, the older studies a lot of them looked at CAT scan which really can't detect less than 30 percent steatosis in the liver, so it's not very sensitive. Of course biopsy is the gold standard. But we can't do biopsies on everybody. With Fibroscan or transient elastography now, there have been a lot more studies and it looks like in many populations the rates about 50 percent. And I think many people that we see, who have elevated transaminases, we send them for a liver ultrasound they end up having hepatic steatosis. So it's getting to be more and more recognized. It's more common in males, and people with metabolic syndrome, and of course people with larger waists who are older and have higher BMI. And what's concerning about NAFLD, even if people don't develop steatohepatitis and don't develop cirrhosis, is that independently it's associated with an increased risk of cardiovascular events. So paying attention to it and figuring out ways to potentially reverse or prevent NAFLD may also have a morbidity benefit on our patients. Fibrosis and cirrhosis of course can be accelerated by alcohol and hepatitis B and C.

[00:36:47] Osteopenia. I think we've heard a lot about TDF and the role it plays in osteopenia, but other antiretrovirals can also contribute to decreased bone mineral density loss. There's about a 2 to 6 percent decrease in bone mineral density. Typically in the first 48 weeks after starting antiretroviral therapy. And this is associated with twice the risk of fractures. You can see here a study of age and sex matched HIV uninfected people and people with HIV, and you can see that the bone mineral density in the people with HIV declines over the subsequent seven or so years after starting antiretroviral therapy, whereas the bone mineral density is pretty stationary in the people without HIV.

[00:37:36] Cancer. I think we're starting to see more and more cancer as our patients are thankfully growing older. And lung, prostate, colorectal, and breast, the same major players in people without HIV are the main cancers that we see in people with HIV. And in fact lung, prostate, and colorectal cancer account for more than 50 percent of infection-unrelated cancers. I'm excluding the cervical cancer, the anal cancer, the Hodgkin's lymphoma, and cancers that are associated with underlying infectious etiologies. But what's interesting is if we look at the role the inflammation may be playing in these malignancies, lung cancer in particular is associated with a higher IL-6 and C-reactive protein levels are associated with an increased risk of lung cancer. So again going back to the whole thing about smoking cessation and people with not only the risk of heart attack, but the risk of stroke and the chronic inflammation has really been a way we can intervene.

[00:38:40] So to summarize, the HIV reservoir is established in the gut and lymphoid tissues early in infection, inflammation begins in acute infection and persists into chronic infection even with suppressive antiretroviral therapy, immune activation creates targets for HIV infection, and HIV, microbial products, metabolic issues, antiretrovirals and coinfections all drive this inflammation and chronic HIV infection. Cardiovascular disease, malignancy, non-alcoholic fatty liver disease, osteopenia are all likely sequelae of this chronic inflammation, and the chronic inflammation is ultimately a major barrier to achieving a functional cure.

[00:39:22] Whether we can attenuate this inflammation and thereby have any impact on the reservoir or on the risk of end-organs diseases really remains to be determined. Thus far many studies that have been tried to decrease inflammation, have not been successful. But there's an ample opportunity, there are many studies going on right now including a large, thousands of persons statin study and a variety of other immunomodulatory agents are currently in the works to see if we can really try to impact this inflammation and decrease morbidity in people with HIV. So thank you for your attention.

[End]