The Double-Edged Sword:
Long-Term Complications of ART and HIV
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JOSE ARRIBAS: I work at La Paz Hospital in Madrid in Spain. On behalf of my co-chair, Joel Gallant, from John Hopkins University, let me welcome all of you to this non [inaudible] driven section entitled "Double-Edged Sword: Long-Term Complications of Antiretroviral Therapy and HIV." So, as I said, this is a non [inaudible] driven section. We have four different speakers for four different, very different topics. Each presentation will last around 15 minutes, and then we will take questions from the audience.

The first speaker is Dr. Patrick Mallon. Dr. Mallon is an infectious disease specialist at the [inaudible] University Hospital in Dublin, and a lecturer in medicine at the University College in Dublin. He heads the HIV molecular research group at UCD and his research areas include lipid metabolism, cardiovascular disease and bone disease in HIV. Treatment of HIV infected injecting drug users are models of care delivering a special population. The title of his talk is "Bones of Contention: HIV and Bone Disease." Paddy?

PADDY MALLON: Thanks very much. Thanks for the very kind – I'd like to thank the conference organizers for the very kind invitation to talk about HIV and bone disease. When I received the title of the presentation I was to give, "Bones of Contention," I quickly realized that it wouldn't be difficult to fill a 15-minute presentation as there are many contentious

issues within this research field. What I decided to do is to concentrate on five main questions over the next 15 minutes.

The first is low BMD, more common in HIV-infected patients. Are fractures more common in HIV-infected patients? What's the best measure when we look at measuring BMD in our patients, should we be using the T score or the Z score? What are the relevant contributions of antiretroviral therapy, body mass index and Vitamin D to the low BMD that we see in our patients, and which patients should be screened?

So to start off with the first question, is low BMD more common in HIV? I think the unequivocal answer is yes. We now have a wealth of data that is from various populations from around the world that consistently shows very high rates of low BMD in HIV-infected patients. These start as far back as 2006 and were actually summarized by Todd Brown in his AIDS paper. In this paper, he summarized the cohorts that are being presented today that examine BMD in both HIV positive and HIV negative groups.

What this data clearly shows is not only across all studies that the prevalence of low BMD is much higher in the HIV positive groups versus the HIV negative groups, but that the prevalence rates are quite consistent across studies with only – with the exception of one study prevalence rates of low BMD in excess of 50-percent within the population studied, which is striking when you consider the very, varied

demographics of the different populations and the variety of countries from which these populations have been derived.

And you will notice in addition to conferences such as this where there are further examples of these types of cross sectional studies presented, that the consistent rates of low BMD are prevalent even as time goes by and as antiretroviral treatment regimes change.

We're also starting to get some information in terms of progression of bone mineral density. At the Retrovirus conference earlier this year, a Spanish study of 391 patients demonstrated that over a follow-up period of 2.5 years, more than 12-percent of patients progressed to osteopenia, and 16-percent of patients progressed to osteoporosis. A poster by the Aquitaine Cohort being presented at this conference similarly shows rates of progression over 2.3 years of 7.8-percent of patients progressing to osteopenia, and more than 11-percent of patients progressing to osteoporosis.

So these very high prevalence rates coupled with strikingly high rates of progression really raise very valid concerns among physicians that we will be seeing an epidemic of fractures, and that leads me to the second question: are fractures more common in HIV-infected patients?

This is less clear. There has been accumulating data presented over the last year, 18 months that have examined fracture rates amongst various groups of people. One of the

studies that I've chosen to highlight which was published in *JCM* in 2008 by a group in Boston, was a healthcare registry study which was able to examine data on more than 2 million people, more than eight and one-half thousand of which were HIV positive, and what this data demonstrated – the data shown in this graph represents the data from women, but the data from men is very similar – what this study demonstrates is right across the age group range, that the prevalence of fractures in HIV-infected patients is higher than HIV negatives.

Of particular concern within this study is the fact that if you look at the prevalence rates of fractures in HIV negatives, the prevalence really kicks off when patients get into their 60's and 70's; while in the HIV positive population, you can see that the prevalence of fractures really starts to take off at a much earlier stage when people enter their 50's and 60's.

Couple this with similar data in cardiovascular disease, similar data in cancers, really what you get is an impression of what we're seeing is that our HIV-infected patients are experiencing age-related morbidities at a much earlier age than what we'd expect from the general population. So how do we [inaudible] by measuring bone mineral density in our patients? Well, the DEXA scan provides us with bone mineral density data in two formats: the T score and the Z score. These two scores differ in the way that the data is

represented in terms of the comparative group or the reference group that your data is presented against. So the T score takes a patient's BMD and compares it to the bone mineral density of a young population with big bone mass; while the Z score takes a bone mineral density and presents it using a reference population of people from the same age, race and gender.

Now, that [inaudible] T score is the best predictor in terms of the clinical utility of DEXA scan to predict who's going to get fractures. Much of the data in the general population has used the T score to derive fracture predictions. The problem with HIV, though, is that the data in the general population is predominantly drawn from post menopausal women and from men over the age of 60, so using the T score in our patient populations which comprise younger men and a lot of pre-menopausal women, some would argue that the T score is not the most appropriate measure and that we should use the Z score.

My personal feeling is that the contentious issue here really necessitates further research in large patient populations to determine which of these scores best predicts fracture rates within our younger patient population.

So we move onto the relative contributions of antiretrovirals, bone mineral density and Vitamin D. To start off with antiretrovirals, the initial cross sectional studies

were quite confusing with a lot of conflicting results, but over the last two years, one consistent finding that's come through from clinical trials is that patients initiating antiretroviral treatment experience bone loss. This is a consistent finding that's been demonstrated in nearly every randomized control study that's looked at BMD over the past two years, and to date, there is not a single antiretroviral regime that is being tested in this setting that hasn't resulted in bone loss upon antiretroviral initiation.

This raises the question, is this effect and antiviral effect or is it a consequence of initiating treatment, i.e., suppressing the viral load and getting immune reconstitution? It's probably, or most likely, going to be both. But certainly, as more studies are presented, we do see that the changes in bone mineral density upon antiretroviral initiation will change considerably depending on which antiretroviral medication you start.

The study from Peter Rice's group comparing [inaudible] based treatment to nucleoside sparing treatment clearly shows the early loss of patients' experienced with antiretroviral initiation which is much larger in patients who are on [inaudible] based treatment versus those on nucleoside sparing treatment.

Other studies have looked within the nucleoside class comparing abacavir containing regimes and Tenofovir containing

regimes. This is the ASSERT Study that was presented at Cologne last year, and once again, we see all groups experiencing early bone loss, but what we see is greater bone loss in those exposed to Tenofovir versus those exposed to a abacavir containing HAART.

Similarly, at the Retrovirus conference this year, the ACTG-5224 study results were presented and this was a four-arm study of antiretroviral naïve patients initiating either Tenofovir or abacavir containing regimes with an additional randomization to [inaudible]. And, once again, we see an early bone loss upon treatment initiation with significantly greater bone loss in those randomized to Tenofovir containing HAART

If we move away from the NRTIs and start looking at differences between proteus inhibitors and non-ukes [misspelled?], again, we see differences depending on which types of treatments patients initiate. In this French study of patients initiating either PI-based treatment or non-PI-based treatment, when you look at the lumbar spine, the first two columns are two different types of PI treatment; the third column is a PI sparing regime. You can see that those initiating PI-based treatment experienced greater bone loss than those not initiating PI-based treatment, but in contrast to the effect we see within the Nucleus I Class, the effect related to proteus inhibitors seems to be especially prevalent

in the cortical bone of the lumbar versus the travecular bone at the hip.

Again, these results were confirmed in the ACTG-5224 study when you look at the differences between the fibrins [misspelled?] or [inaudible] based regimes, you see a much greater loss in bone mineral density in those randomized to [inaudible], but this difference is only occurring at the lumbar spine.

So what about body mass index and bone mineral density? From the cross sectional studies that have been presented, body mass index has consistently fallen out as being – having a close association with low BMD and HIV. In fact, in one meta analysis performed by colleagues in New Zealand, when they looked at HIV positive groups and HIV negative groups, even though they determined a difference in bone mineral density in those with HIV, much of this difference was actually explained by differences in body mass index. And it's clear, also from the general population, that as people get older, loss of weight is associated with a more rapid progression of bone mineral density, or a more rapid loss of bone mineral density, but this weight loss in the elderly population is usually associated with negative health implications, so a more unhealthy person is more likely to lose more weight as they get older; therefore, they lose more BMD.

That type of association really hasn't been [inaudible 12:03:00] out in the HIV positive setting where low BMI, especially in young gay men may not necessarily be associated with negative health. However, there are some prospective studies and, again, the French Acratang Cohort who are presenting their data this Wednesday, will also discuss the progression of BMD showing that patients with lower BMI who are HIV positive actually have a faster loss of BMD over time.

And lastly, there's the Vitamin D story. We have a lot of posters, there's a specific session devoted to this on Wednesday, devoted to the problem of low Vitamin D in HIV, and low Vitamin D levels and Vitamin D insufficiency and deficiency are very prevalent in HIV-infected populations. But when you look at the studies that have actually looked at HIV positive patients and matched HIV negative patients from the same socio demographic areas, what we tend to see is the low Vitamin D picture is really a picture that's spread throughout society.

Whether or not there's specifically a greater effect of low Vitamin D in HIV-infected patients in terms of bone health versus the general population really hasn't been teased out. There are associations between [inaudible] exposure and low levels of the inactive 25 hydroxy Vitamin D, and much is being made of this association, but the few studies that have actually looked at the active form of Vitamin D, the one 25 hydroxy, have failed to show this association, and, certainly,

it would put the clinical relevance of this finding in some [inaudible].

To go against Vitamin D being a major player in terms of what's happening to our patients' bones, a nice study, again by Peter Rice's group that's just recently been published looking at 33 patients with primary HIV infection, so in the very early stages of HIV infection, again, demonstrated very high prevalence rates of low bone mineral density, but none of the patients had a Vitamin D level that was in the deficient or insufficient range.

When we look at clinical studies where [inaudible] is a component, we don't see a greater loss of bone mineral density in those initiating a [inaudible] versus those initiating other types of antiretrovirals. And when you look closer at what's going on underneath, the data would suggest that what's happening in our patients, is that they are experiencing a high bone turnover state. Data from the Asearch study looking at markers of bone formation and bone resorption [inaudible] show that upon initiation of antiretroviral treatment, all of these markers increase quite dramatically. Vitamin D is not associated with high bone turnover; you need to be Vitamin D deficient for a very long time before you'll get clinically meaningful loss of bone mineral density, so all of these results really prove Vitamin D, in my view, on a much lower

perspective or the lower pecking order in terms of its potential to cause low BMD in our patients.

So to finish up, who should be screened? Well, clearly, everyone should be screened. It's how we screen and how we use our resources, I think, that are most important. There are – not everyone needs to have a DEXA scan – there are tools available that we can use at a clinic level to take patient demographics into account, such as the FRAX score, which is freely available online, and these tools can help us to predict which of our patients should be referred on for DEXA scanning.

The European AIDS Clinical Society in their recent guidelines on the Prevention and Management of Core Morbidity have also suggested a risk – or a list of risk factors that should prompt us to do a more formal assessment of bone health, and this should really be adopted in all of our patients while we wait for more data.

And, lastly, to finish off, when I thought about the double-edged sword and you look up about the double-edged sword, we're supposed to have favorable and unfavorable consequences, and everything that I've said up to now is probably quite negative, but there is an opportunity here. At the minute, we're really facing a patient population that is entering into that fifth decade of life, and now is the time that we should be introducing these tests, even – we should be

introducing the screening and we should be introducing treatment for these patients in order to maximize the reduction in fractures that our patients will experience over the next decade.

I'd like to thank both the Irish and European funders that fund our ongoing research program. I'd also like to thank a very supportive family and thank you for your attention. [Applause].

JOSE ARRIBAS: So thank you very much for an extremely clear and practical presentation. Are there questions from the floor? If not, may I ask you, Paddy, you know, is there any research of what we can do to avoid this initial drop in bone mineral density when we start any antiretroviral regimen?

PADDY MALLON: I know that there are studies ongoing that are looking at the use of bisphosphonates in the early parts of antiretroviral treatment to see if you can limit or eradicate that early bone loss. Certainly there are bisphosphonates on the market now that do have long half lives, some that can be given as, you know, six-month or yearly doses, so there would be the potential if this sort of strategy works in patients, particularly vulnerable patients, to perhaps give them a single dose of a bisphosphonate and limit the loss of bone mineral density. The one issue around bisphosphonate e use is we need to be clear what we want to do with bisphosphonates, and the reason to use bisphosphonates is to

reduce fractures, and treating low BMD alone even in that type of setting may not correspond to a reduction in fractures. So I think we still need data in terms – particularly in younger people – in terms of whether or not bisphosphonate use is both safe and effective in reducing fractures as it would be in the older HIV negative population.

JOSE ARRIBAS: Okay. I think Jules is on microphone six.

JULES LEVIN: Yes. Hi, Paddy. Jules Levin. So, I want to ask you to comment. On anecdotal research of my own, finding out that patients who were getting high dose Vitamin D supplementation from their doctors because they have low Vitamin D, that it doesn't seem to really be working. They take the supplementation and levels may go up briefly, and then they go right back down and so I know you talked about your dats about the association between Vitamin D and fracture, but still, it's disconcerting and I don't think there's been any research to see if supplementation works in HIV, as opposed to HIV negatives. Do you have comments?

PADDY MALLON: As usual, Jules, I think you're right on the button there. I think there has been a push amongst many clinicians to focus on Vitamin D and focus on Vitamin D replacement in terms of its – perhaps it's an easy option to reverse what's going on in the bone. The research hasn't been done. The research from the general population would suggest

that Vitamin D replacement and bone, although important, really isn't going to get you out of the woods in terms of reducing your fracture risks substantially. And, again, to go back to this – the whole point of any intervention is to reduce fracture risk.

So giving people Vitamin D to make their Vitamin D levels better is good because Vitamin D has a lot of health benefits, but widespread use of Vitamin D to try and solve the problem that we're seeing in HIV, I don't think the data's really there and I think that the focus needs to be on good quality research to answer the sorts of questions that you're raising.

JOSE ARRIBAS: Microphone three.

FEMALE SPEAKER: Yes. I'm curious whether the fractures are associated with impact, or are they spontaneous, or a combination of both?

PADDY MALLON: So the limitation of the Triann [misspelled?] study, although I think it's probably the best study to actually look at fracture rates, the limitation with it is that when you're dealing with health registry data, you can't really inform too much about what's causing the fractures and, certainly, when you look at the potential things that will cause fractures in our patients, we've got to look at things like fragility; we've got to look at things like drug use and a greater potential for falls; and then, we've got to look at

BMD. So I think the Triann data informs us that there is a clinical problem. What it doesn't do is inform us as to what's driving that problem, but I think if we start to put a lot of the studies together, a lot of this other ancillary data, we get an idea of the main issues within our patient population that need to be addressed if we're going to reduce the fracture risk.

But, again, it's a contentious area and an area that's definitely worthy of further study.

JOSE ARRIBAS: Okay. So let me thank you again for your presentation. [Applause]. Now, it's my pleasure to welcome to the podium Dr. George Behrens. Dr. Behrens is an assistant professor of T-cell immunology at Hanover Medical School in Germany. After he was in the division of clinical immunology at Hanover Medical School, he went as a first doctor of fellow to the immunology division at the Walter and Alisa Holland Institute in Melbourne, Australia. He's an internationally acknowledged expert in the metabolic side effects of HIV therapy, so he's going to talk about HAART to Heart: HIV and Cardiovascular Disease. Excellent.

GEORG BEHRENS: Thank you very much and also thank you to the organizers for inviting me to this conference and give this overview. I've split my talk into four different parts, and I would like to start with some data about the epidemiology on this issue.

If we look into different cohort studies, mostly retrospective, or also prospective HIV cohort studies or administrative databases, and compare the number of events for cardiovascular disease with seronegative people, we do see a continuous trend that there is an increase of the incidence rate of cardiovascular events in HIV-infected patients across different cohorts.

If we take this to another level and look for surrogate markets of cardiovascular disease, not only events, again, we do see in HIV-infected patients something very similar as compared to HIV negative individuals. As an example, for instance, age is an important factor as you see per year increase. We do see an increase rate of cardiovascular events of about six to nine-percent which is very similar to what we see in HIV non-infected individuals.

And that holds also true for other factors like diabetes maladies or smoking, for instance, where we know this is an important risk factor, as we do see in seronegative people. And also, HDL as a more beneficial factor, with every increase of this unit, we do see a decrease in HIV or non-HIV-related cardiovascular events, meaning a very similar pattern.

That's all well and good, but what does it really mean to other more serious events? If we look again in different cohorts, like the DAD or another multi-national, multi-cohort analysis, we do see the cardiovascular disease related events

are responsible for about 10-percent of all causes of death in the cohorts. That's still far below other reasons for liver-related death or AIDS-related death, so why is it important now to look at this and have some thoughts about it? I think it's for several reasons, and I want to illustrate this in the next slides.

First, it's traditional risk factors that we find very often in HIV-infected patients: smoking, obesity, hypertension, dyslipidemia is very prevalent in treated HIV patients, and also, the risk for Type II diabetes, for instance, is four to five times higher in HIV-infected patients as compared to others. And these risk factors also determine cardiovascular events as, again, being shown by the DAD study, we do see being male having previous cardiovascular disease; being a smoker or having diabetes really increases the risk for cardiovascular disease. And we do see on the very top also another factor, that's aging, also an important contributor to cardiovascular disease.

And Paddy already has shown some data on the aging population and HIV. I want to illustrate it in a slightly different way. I took this from a review of about the estimate [inaudible] of the total world population as you can see. And also, the percentage of the population older than the age of 60 for the next couple of years. If now with just a rough estimate calculate this for the German HIV population, for

instance, you do see the slope of this curve will be dramatically different because all the mid 40's infected – HIV-infected people will, within the next 15 years, turn 60 and older, and that's why this factor becomes more important.

That leads us to HIV therapy and its rules. Let's assume this is the lipid profile of any given HIV patient who is a not yet infected person with cholesterol triglycerides and HDL. What we, in general, assume is that HIV infection, at least in an advanced stage, if these values are rather normal, would lead to a slight decrease in cholesterol, a slight increase in triglycerides and, usually, HIV-infected patients have a low HDL.

Most HIV therapies will lead to a modest increase in cholesterol, to some increase in triglycerides, and have only little impact on HDL. And having said that, we all know that this is oversimplified because we know that certain drugs, for instance, like Nucleus I, NNRTIs have a more beneficial effect on HDL, that PIs defer their effects on triglycerides, and that more recently developed drugs like [inaudible] inhibitors and T-cell 5 inhibitors also have less effect at all.

But anyway, overall over the last year, it has become clear that factors in glucose metabolism and dyslipidemia and also associated factors with mainly central obesity induced by HIV therapy contributed to HIV for later cardiovascular disease. This all becomes more complicated because all these

factors are interrelated and, of course, there are other factors like age, genetics, diet and other underlying mechanisms that contribute to this.

Over the last years, it has also become clear that some specific drugs might directly interfere with the occurrence of cardiovascular disease. As you all know, many of these data, again, initially shown by the DAD study, among the PIs that had been looked at so far – not all of them have been studied – in [inaudible] with increased risk for [inaudible] infarction. There is no such signal in NNRTIs, but again, in the NRTIs, at least in this study for DDI and Abacavir, there was an increase risk for accumulative and also recent use for Abacavir.

But, of course, prompted a lot of other researchers to look in their databases, and I've summarized many of their studies. You see that many of them are very different in their design and for most – and very important – they're very different in the number of events that they can capture. Taken together, more often than not, most of these studies could confirm this signal and the cessation of Abacavir use in myocardial infraction, although not all clearly.

That raises the question which is critically the mechanism, and it was, I think, the Snarz [misspelled?] to do the first one to suggest that patients with Abacavir treatment, if when they compared to non Abacavir treated patients, signs

for systemic inflammation were increased and that COP levels in all six levels were significantly different.

More research is still ongoing on this. For instance, some researchers would suggest that Abacavir from in vitro studies might activate leukocytes to up regulate MAC 1 which then interacts with ikon [misspelled?], for instance, on endophilia cells. Other researchers would favor that Abacavir could interfere with the platelet function of HIV-infected patients leading to increased clotting risk.

But looking more into clinical data, again, at least four different studies did not find any association of Abacavir use with systemic markers of inflammation as I've listed them here. So there is still no real verdict, it's still unclear what the mechanism could be.

That's why put a little question mark behind this inflammation pathway, but with that we come to the next point because we have another candidate that could act through this informatory pathway, and that's HIV.

Again, the Snarz study was the first to show that patients that interrupted HIV therapy in this early phase had a higher risk for non AIDS defining events, namely, cardiovascular disease, again, as compared to those patients that continued therapy. It's very hard to dissect the rule of HIV in these conditions, so we really have to rely on surrogate markers and other settings. Again, this study, also looking at

treatment interruptions, could confirm that when patients went off therapy as the viral load went up, their activation of T-cells went also up and also [inaudible] markers for inflammation and the office then concluded like with a double-edged sword, lipids went down if you go off treatment. It's not shown in the slide, but immune activation went up. And what's the net benefit, then, for the patient?

Other surrogate studies like the Framm study which was recently presented, again, looking for surrogate markers for intermediate thickness as an equivalent to atherosclerosis. And they identified the known risk factors as we've seen in previous studies, but they also showed that HIV infection itself, even if rated, had an independent contribution to this surrogate marker suggesting that HIV directly does something in these patients.

Indeed, there is a whole bunch of further evidence. We know that HIV can induce [inaudible] and interfere with endophilia cells; that HIV can interfere with leukocyte activation and systemic markers of inflammation that we can measure; that HIV can interfere with chemokine access and loops that are very important also in ethrogeneticity [misspelled?] and that genetic risk factors are also important in these circumstances.

Some researchers even think, although I think there is limited evidence for that, that this is a distinct inflammatory

process going on. Coming to other co-infections, more viruses coming in, it's getting even worse in terms of surrogate markers and HCV. Co-infected people are also with an increased risk for myocardial infarction. We know that, at least in some, not in all studies, low CD4 cell counts can be a risk factor and that HIV therapy partially improves endothelia function, but cannot restore it to normal, and then, HIV is still an independent predictor of atherosclerosis and can also interfere with monocides.

So that's HIV directly, but also, we have to consider a new understanding of the pathogenesis of HIV. If this is the mucosa layer with immune cells underneath, what we've learned is that in the early phase of the infection, HIV infects the CD4 cells, also very important the TH17 cells and other cells, and this leads to a mucosal barrier disruption so that microbial products can translocate like [inaudible] into the circulation which then, in turn, activate microphages which release the other factors which contribute to immune activation and disruption of other problems.

And atherosclerosis is not only lipid driven, but also an inflammatory driven disease. As we can see LDL, for instance, is recruited, gets oxidized, then activates and Ophelia cells, which then recruit T cells and monocides and this is the first formation of an atherosclerotic lesion. And these monocides then turn into macrophages, which then by

scavenger receptor capture further lipids transforming to form cells and also become activated to further support inflammation, coagulation and apoptosis.

If we put all these together, we see that athro formation, plague instability and [inaudible] are all supported by an underlaying inflammation. We know that the traditional factors mainly support athro inflammation and growth and that HIV therapy through this mechanism can also facilitate this. Remembering the data about Abacavir, we now have to assume that at least some certain drug not also through inflammation or, specifically interfering with these pathways contribute to this. And then, a third player comes into the picture which is HIV, then through inflammation or direct interaction also with platelets, for instance, could facilitate atherosclerosis and the respective events.

With that, I come to the last point. Quickly, to summarize what could that mean in clinical care? I tried to illustrate it on this figure. Let's assume we've got a patient who's got a viral load and needs treatment. With the HIV therapy, we decrease the viral load to below limit of detection. With that, we also presumably improve the inflammation that's associated with HIV and this anyway low myocardial risk goes down further. But now, we've got some patients which might develop some side effects from the treatments that we use, like, Type II diabetes, disturbed

lipids, thereby the net effect might be gone so that there is a slight increase.

Most patients it wouldn't really matter because the modest underlying risk is very low, but let's consider some patients with an already underlying high risk because they have disturbed lipids or obesity. In those patients, it is particularly important to consider these factors and to choose the right therapy; not to do harm.

I selected just as a suggestion the European guidelines how to deal with these issues because they have been updated last year. Something they recommend would be to assess the cardiovascular disease risk for the next ten years using scores like the Frammingham [misspelled?] score, for instance, advise all patients on diet and lifestyle modifications and consider, especially in patients with a very high risk, to change therapy if that's possible and options are still available. And then, you continue to identify all the key modifiable risk factors including smoking, blood pressure and the others that I've listed; and then, if there is levels above the threshold, then decide really also to use drug treatment.

And then, also to consider certain drug targets. I don't want to end with one remark that HIV and the heart is not only about myocardio infarction because this is an international AIDS conference. There is also evidence for other problems, of course, diastolic dysfunction's very

prevalent, QT prolongation has a high prevalence in HIV and is cessated with several drugs and, of course, many of the infectious complications can also occur in these patients. Thank you very much. [Applause].

JOSE ARRIBAS: Thank you, George, for a great presentation. We can take questions from the floor now. So, George, when we have an HIV-infected patient and we achieve suppression and [inaudible] is below 50, there is still persistent information and immune activation, what is the research going about what can we do about that in those patients?

GEORG BEHRENS: And that's a very good point. Ongoing viral replication, of course, leads inflammation, but there is still some low level replication or inflammation apparently going on even in successfully treated patients, as we can see if we compare these to uninfected controls. It's very difficult to find specific interventions in that because you don't want to have an unspecific immune suppression in that situation.

I think there is some research now going on to look how HIV, for instance, interferes with platelets with endothelials in order to find potential targets, but I think for the next couple of years, we will rely on identifying patients with a high risk and try to treat them against HIV as good as possible.

JOSE ARRIBAS: So there are no more questions, and we thank you again for your presentation.

GEORG BEHRENS: Thank you. [Applause].

JOEL GALLANT: Alright, it's my pleasure to introduce my colleague, Dr. Mohamed Atta. Dr. Atta is an associate professor of medicine in the division of nephrology at the Johns Hopkins School of Medicine, and medical director of the Davida Dialysis Unit in East Baltimore. He's going to talk to us about Kidney Conundrums: HIV and Renal Disease.

MOHAMED ATTA: Thanks, Joel. Good afternoon. I would like thank the organizer for inviting me for this conference. Generally, my name actually gets a lot of recognition, usually in airport security, but I feel honored to be invited here.

My talk is going to be two-fold: one is to view the implications of kidney disease and HIV-infected individuals; and the second part is to discuss the pros and cons of the deferred versus early heart in this population from the renal standpoint. I'm going to start with a non-HIV population, and this is really one of my favorite studies, the HAART outcome study which included 9,000 patients. It was under my trial to look at the efficacy of albumin or Vitamin E on the primary outcome or ecology of what's called mortality.

When the investigators actually started looking at the best predictor of cardiovascular outcome, it was mycroalbuminuria. So mycroalbuminuria actually was the best

predictor better than actually history of coronary artery disease, diabetes, serum creatinine above 1.4, being a man or age, and obviously, your [inaudible] was protected in the study.

A more powerful analysis just came out in the *Lancet* in May that included more than one million individuals, non-HIV again, and then, the investigator, Joe Croatian and colleagues, looked at the interaction between GFR and proteinuria. They found 14 studies that actually looked at ASR plus GFR, so the upper panel is basically looking at ASR and GFR. As GFR goes down, below 60, you can see that there is definite increase in the hazard ratio for all cause mortality. Now, they've had seven studies that included only dip stick, so looking by the dip stick reaction, again, you can see as GFR goes down, mortality goes up, and you can see on the right panel here that the mortality is mostly driven by cardiovascular mortality.

What was interesting about this study, that even trace proteinuria by dip stick was associated with increased mortality. Can we apply this to the HIV population? [Inaudible] George from – a medical student from India, looked at and tested matching kits from [inaudible] study from the Hopkins cohort. She identified 63 cases who had cardiovascular events, and she had matched those with 315 HIV-infected individuals without cardiovascular events, and look at GFR identified by CKG [inaudible] formula and [inaudible] equation.

In the unadjusted analysis, GFR, or estimated GFR less than 60, was associated with 16-fold increase in cardiovascular events. Adjusted heart ratio showed that a decline in GFR by DML [misspelled?] was associated with 20-percent increase in cardiovascular events. The prevalence of proteinuria in cases was 51-percent versus 25-percent in control, and proteinuria in the adjusted analysis, again, was associated with two-fold increase in the cardiovascular events.

This is LOS, smoother curve. It's an aggression analysis looking at events here on the white axis versus no events. These are the control cases, the 300 plus cases, and these are – here on the top are the event cases, and you can see that the event cases, the GFR was – is spread all out. The axis, if you look at the cases, all the patients had GFR around 120, so the mean is submitted here for 68 in the cases versus 103 in the control.

The V.A. study is a cohort study by Andy Choi, looked at the same event trait in patients with HIV infections; 1,194 patients with [inaudible] GFR less than 60, and the outcome was, again, incident cardiovascular disease, or incident heart failure. He looked also at urine albumin by dip stick and, again, as the GFR goes down, you have increase in the risk for the hazards for cardiovascular events, or heart failure as well. Again, if you look at the proteinuria, from no proteinuria, 30 milligrams per this liter, or more than 100

milligrams for this liter, you can see a step wise increase in the cardiovascular event rate and also in the heart failure.

Christina Weiss from Mount Sinai in New York looked at Weiss cohort which are HIV-infected women, 1,500 women, and again, looking at survival by albumin dip stick. So those who had no proteinuria or albuminuria have much better survival compared to those with unconfirmed or confirmed mycroalbuminuria, meaning that they had one dip stick that was positive that was unconfirmed. Confirmed means that two dip sticks that were positive for albumin versus those with confirmed proteinuria had the worst survival.

So, again, albuminoia in the non-HIV population and in the HIV population was associated with increased cardiovascular event rate and mortality. So, the second part is what about deferred treatment versus early treatment. In my mind, as a nephrocentric person, the deferred treatment carries the risk of heightened HIV-associated nephropathy; the early treatment is associated with heart toxicity and metabolic derangement.

[Inaudible] we did learn from the animal study the direct tool of HIV and the development of HIV as [inaudible] property through the products of accessory genes like the [inaudible] and the VBR. The disease is very aggressive.

The disease is very aggressive. If you look at the normal glomerulus, every one of us should have a million of those. You have the glomerulus encompassed by the Bowman's

capsule and then in green here is a basement membrane that separates the glomerulus two compartments. The inner compartment that has the blood and the mesangium, and the outer compartment that has the ovocytes [misspelled?] and the urine space.

Here by light micros could be a normal glomerulus. If you look at HIVAN patients, they have complete collapse of the capillary tuft and if you look at the tubules, they have microcystic dilatation. The tubule will actually increase in size by three fold.

None of the clinical features of diagnostics so biopsy is usually needed for the diagnosis. We know it is a progressive disease that is shaded with heavy proteinuria. Those patients have detectable viremia and detectable Proviral DNA. They have normal size kidney because the kidney does not have time to shrink. They have normal size kidneys and it progresses to renal failure within weeks to a month.

One unique feature about the disease is that it is exclusive of Africans or black patients. We didn't know why until Jeffrey Cup came in 2008 and did a genome-wide analysis and found chromosome 22 that looked promising as to be the site for association with HIV associated nephropathy. He compared black individuals with HIVAN and other kidney disease to black individuals without HIVAN and with Caucasians.

If you look at the chromosome 22, there were a lot of genes and the focus initially was on the MYH9. It is not seen until age nine. The reason the focus on this gene actually is because it is located or localized in the kidney. Fair enough, they found that actually variability in this gene was associated with fivefold increase in the incidents of HIVAN. If you have two risk allele of this variant, you have five fold risk for developing HIVAN.

Everybody was excited by the MYH9 SNPs until a group recently just published in *Science* a new finding. They focused on the APOL protein 1. The APOL protein 1 gene is a survival gene. It actually protects against the infection of Trypanosoma in Africa.

They found that if you have variation in this gene, you actually have a tenfold increase to develop kidney disease and end stage renal disease. This just came out three days ago.

We know that HIVAN can be prevented and treated with HAART. This is by my colleague Greg Lucas. He looked at a cohort at 12-year cohort in Hopkins and sorted the patients by having no AIDS versus hose with AIDS on the Y-axis cases per thousand per person per year. If you have no AIDS, the incidence of HIVAN is sero. If you have AIDS and you are not on HAART, incidence was 26.3 per thousand people per year, so about three-percent.

We showed in collaboration with the German Dr. Gallant that patients who were treated with antiretroviral therapy have a better renal survival versus those who were not treated with HAART. That is why now the guidelines recommend that HIVAN is an indication to start HAART regardless of the HIV status.

Now what about the risk of early HAART from the renal perspective? Again, you know that HAART is associated with increased in the incidents of diabetes. This is a study from the MAX where the Multicenter is cohort, man who is having sex with man. Those patients who were treated with HAART have a fourfold increase in the development of diabetes. Those especially who are treated with protease inhibitors have three fold increase and the risk of diabetes.

From the same cohort, those patients who were exposed to HAART were associated with increased incidents of systolic hypertension. Not too much diastolic hypertension.

What about specific renal disorders related to HAART therapy? We all know about Indinavir and its association with these crystals. Atazanavir is another drug that is associated with crystals. The incidents with the Atazanavir according to the French cohort is about one-percent.

What is unique about these crystals in both Indinavir and Atazanavir that they form actually in alkaline urine? Patients who are vegetarian for instance tend to have alkaline urine and they are at higher risk to develop these crystals.

Tenofovir has been associated with acute renal failure, Fanconi syndrome, nephrogenic diabetes insipidus and probably chronic kidney disease. The mechanism by which Tenofovir actually can cause kidney disease is not fully understood.

We know that the proximal tubular cells of the kidney are a target for a lot of toxicity. If you look at the proximal tubular cells, this is lumen; this is interstitium of the blood. The apical membrane, that is where they urine and a lot of drugs are filtered. A lot of drugs are also secreted from the blood through the urine.

The practical example for this was really during World War II when penicillin was discovered and it was in short supply and they tried to treat the tubes with penicillin. It was not until 1951 when probenecid was discovered and was found to actually delay the secretion of penicillin from the blood to the urine.

Also another story about probenecid in 1988 the Tour DE 'France, the winner was a Spanish guy who actually used probenecid because he was using an anabolic steroid and was able to win the crown despite the fact that he was using antibiotic steroid to prevent the secretion of the steroids and anabolic drugs in the urine.

You can protect the kidney actually by giving probenecid because you prevent actually the secretion into the proximate tubular cells. All NRTIs are secreted through these

transporters. The organic [inaudible] transporters go inside the cell and then it gets out in the urine through another transporter, the multidrug resistant transporters.

The idea is that if you actually over dose someone with a drug, you can actually overwhelm these transporters and you can increase the availability of these drugs inside the proximal tubular cells and you can cause mitochondria toxicity. If you give any drug that actually competes with the secretions of these drugs in the urine, again you can accumulate these drugs in the proximal tubular cells and develop toxicity.

This is a recent publication that came from Europe, the EuroSIDA Study that showed that in 6,000 plus patients, the incidents of chronic kidney disease were 3.3 over a median follow-up of 3.7. There was association between CKD and Tenofovir, Indinavir and Atazanavir and Lopinavir or Kaletra. The CKG incidence was between eight-percent and 21-percent using these drugs. The aging is another problem. This is a study from Hopkins 1,000 patients looking at the difference effect of Tenofovir on patients with above 45 versus those less than 30 versus those between age 30 and 45. Again, you see that those patients with age above 45, they had a much worse decline in the renal function. Usually at day 300 is the most effect.

These are my suggested recommendations. There is no evidence of benefit from the renal standpoint from early HIV

treatment except for HIVAN and this is a late manifestation of HIV. In treated or untreated patients, I think we should screen all patients with GFR/urine/albumin and urine protein.

For high-risk patients, monitor kidney disease on a regular basis. For those with non-HIVAN kidney disease, new studies are needed to determine benefits.

Thank you. [Applause]

JOEL GALLANT: Thank you Mohamed for that excellent presentation.

I wanted to ask you if you look at some large studies, and I was thinking specifically of the Dart study in Africa where Tenofovir was a component yet when you put people on antiretroviral therapy their kidney function tended to improve. Do you think that is entirely explainable by a reduction in HIVAN, or is there any other possible mechanism of benefit that could explain that kind of a finding?

MOHAMED ATTA: It is definitely important to realize that patients who are not treated with HAART and they have progressive HIV disease, they will develop kidney disease.

I am not sure. We do not have any studies from Africa about HIVAN and it is very premature to say that this is really a reduction in HIVAN. I will have to wait.

JOEL GALLANT: From the floor. Well since there aren't any; let me ask you another question. We typically hear that Tenofovir nephrotoxicity is approximal tubular toxicity of

phosphate wasting, and yet that seems like maybe we are not looking close enough. It seems like that the subset of the overall declines in GFR that we sometimes see. Can you just clarify, is there a real distinction between glomerular toxicity and tubular toxicity or are they ultimately all going to be the same entity?

MOHAMED ATTA: Well, my view is that really Tenofovir is a proximal tubular toxin, not a glomerular toxin. If you give the drug to someone who has underlying kidney disease, you actually put them at risk to develop also progressive glomerular disease. If you separate two patients, one was a normal GFR and one with low GFR. The one with normal GFR is at risk to develop proximal toxicity. Those with low GFR, they really, you have to be careful of using the drug because of the potential for glomerular toxicity as well.

One other aspect, which was shown in the Swiss cohort, it was in Croix last year that those on Tenofovir, they have a lot of phosphate wasting. You may ask what are the clinical implications of phosphate wasting. I think it has been presented before that if we are talking about bone disease; we have to actually think about osteomalacia from phosphate wasting as well.

JOEL GALLANT: Jules, microphone six.

MALE SPEAKER: Can you clarify? I think the guidelines, the U.S. guidelines recommend early therapy for

patients with HIVAN, but didn't you say that early therapy does not benefit patients with HIVAN. Is that correct?

MOHAMED ATTA: No, I said that yes the U.S. guidelines recommend starting the patients on HIVAN regardless of their HIV status. However, HIVAN is a late manifestation of HIV disease. It is uncommon to see actually a patient with CD4 count for instance of 500 or above developing HIVAN.

You have to have very high viral loads. You have to have very low CD4 count to actually develop HIVAN. Not to say that there are cases that has been aborted actually in the literature with early HIVAN. These are the rarity, not the usual cases.

MALE SPEAKER: Yet you said that HAART does not benefit HIVAN, right?

MOHAMED ATTA: No, I did not say that.

MALE SPEAKER: You didn't say that. Okay.

MOHAMED ATTA: No. HIVAN actually is treated by HAART. If you actually have a patient who is diagnosed with HIVAN and you give them antiretroviral therapy, they have a much better renal survival compared to those who are not treated.

JOEL GALLANT: Microphone three.

MATHEW DASHO: Hi. Thank you doctor for that presentation. My name is Mathew Dasho. I am a clinician with Botswana U. Penn. partnership in Habronema, Botswana. I appreciate you presenting this topic because we are seeing a

lot of renal disease in Sub-Saharan Africa. What I would like to ask you is what are your thoughts on how in a relatively resource limited setting, how we can proceed with screening for HIV associated nephropathies.

The only data that I am familiar with in Sub-Saharan Africa are small studies where it has been shown that the spectrum of renal disease in Sub-Saharan Africa associated with HIV is far more than just HIVAN. It is also immune complex disease. It is postinfectious glomerulonephritis.

In a resource limited setting, where you cannot biopsy everybody we are relying on urinalysis. With huge prevalence also of hypertensive kidney disease and increasing cardiovascular events. I wanted to see what your thoughts were in a resource-limited setting. How we can proceed and how to manage is becoming a huge problem.

MOHAMED ATTA: This is really a very good question. Again, I am going to focus on how to actually monitor patients. To monitor patients, we need to use tools that we have. I think everyone can get serum creatinine. Everyone can get a urine dipstick or urine albumin to creatinine ratio. If we can actually do that, we can monitor kidney disease very well in the resource-limited setting.

We are not going to look at the inflammatory markers like IL-6 and CRB and all of these. Even viral load is not measured on a regular basis in these areas. I think urine

dipstick, if we can monitor those patients every three months or every six months even by dipstick and serum creatinine that would be actually the best you can do.

JOEL GALLANT: Okay. One last very quick question and answer. Microphone nine.

DOUG FISH: Doug Fish, Albany New York. Even in resource available areas can you comment on the role of renal biopsy in the workup of HIVAN and is it necessary or not.

MOHAMED ATTA: Well again giving my view is I think HIVAN, the only way you can actually diagnose it is with kidney biopsy. The reason I am saying this is that there is a lot of disorder that actually can mimic HIVAN in terms of nephrotic range proteinuria and aggressive progression of kidney disease. A lot of these patients have hepatitis C coinfection. You have actually GN and you have immune complex lupus like GN in this population.

To rely on the just clinical markers may not actually be the best way to do it. The way I can actually look at it in a different way, how to exclude HIVAN. To exclude HIVAN, if you have a patient with no proteinuria or many proteinuria of the have high CD-4 count. If you have low viral load, those patients are unlikely to have HIVAN. Excluding HIVAN is much easier than confirming HIVAN with these resources.

JOEL GALLANT: Alright. Thank you very much again for that wonderful talk. [Applause] Our next and final speaker is

Dr. Victor Valcour who is an internist and geriatrician at the Memory and Aging Center at UCSF San Francisco, California where he is an Associate Professor of Geriatric Medicine and Urology.

He has completed fellowships in both geriatric medicine and neurobehavioral and his primary research interest is in brain health in aging HIV infected patients.

VICTOR VALCOUR: Great. Thank you very much for the introduction. Also thank you to the conference committee for inviting me to speak.

Over the past couple of years, I have enjoyed the ability to finally come out of the closet as a geriatrician in this audience. When I began this work 10 years ago, there was not a lot of thought about aging and HIV. When we started our first cohort of aged HIV patients who were over 50 in Honolulu back in 2001 it was not a great interest in people. Now there is a lot of interest. I will add some of the information regarding some of our aging work into the talk today.

I thought a little bit about the title as well. I did not choose this title. It was chosen for me, Out of Sight out of Mind. As I thought about it carefully, I thought about how appropriate it is. Even to this day cognitive impairment in the setting of HIV is a silent epidemic. In clinics, it is out of sight and it is out of mind. I want to point out to you today perhaps the most important point I can bring home is that it is quite frequent and needs more work.

I will start by telling you the main points I thought I would talk about today. First of all, the cognitive impairment is frequent. I will not be able to go through all of the mechanisms by which we think cognitive impairment may be continuing to be an active problem.

I wanted to point out just two examples. One from my research, which will point to you that there probably, is an active disease underlying some of the impairment. It is likely immunologically based. My bias is that it is probably monocyte based, a chronic immune activation T-cells maybe contributing as well.

I have another example where I would like to bring out the very important aspect of compounding factors with regard to brain health. I will not be able to go through all of these in detail. I will give you one example and leave you to think about that a little bit more.

I thought I would close the talk by speaking a little bit about aging in HIV and the concerns that have been raised and perhaps some cautiously good news regarding this.

First, I will start by presenting some data from 2008 that was published by Kevin Robertson and other using data from the ACTG. He identified that patients who were changing therapy or starting therapy, about 39-percent of them tested in an impaired range when they began treatment.

Unfortunately, after these patients were on treatment for 48 weeks, of the people who were healthy, a good quarter, 20-percent developed impairment over that period of time. These data are probably quite consistent with what we are finding in many other studies. Perhaps the largest one funded by the NIMH called the CHARTER study is just starting to point out some of the data.

This is an important slide that I borrowed from Igor Grant. I will talk you through it carefully. He had three cohorts that he had studied in San Diego and then the most recent cohort, of course the CHARTER, which are five centers over 1,000 patients. He compared rates of impairment on cognitive testing during the cohort that was enrolled in the pre-HAART era, the pre-antiretroviral era, and the HAART era.

What he found here and you can perhaps concentrate on CDC stage A disease. There really is not a lot of change in the rate of impairment despite the use of HAART. Across the other stages, you can see as well, there has not been a lot of improvement.

Some people ask me if you give cognitive tests to a large enough population of people, some people are going to be impaired. In fact, you can see here on the left that is true. If you test HIV negative people presumably reasonably healthy, some of them will test in an impaired range. You can see that

the rate of impairment in patients with HIV is much, much higher. You can consider that perhaps some of the background.

The good news about all of this is that HIV associated dementia; the worse kind of cognitive impairment is now quite rare. It probably constitutes only one to two-percent of the population as depicted in this slide.

There are two other kind of impairment diagnostically that constitute a large amount of impairment that occurs, HIV mild neurocognitive disorder and HIV asymptomatic neurocognitive impairment. Together about half of patients who have HIV will have abnormal testing and fall into one of these areas.

The flip side to that is about 50-percent of people will test perfectly fine on neurocognitive tests. That may speak a little bit to why it is somewhat of a silent epidemic. The asymptomatic portion I have to point out as well is quite concerning. Many people would never call a disease if it were asymptomatic.

The challenge is there is so much stigma attached to HIV and the populations who have HIV that many times we do not have good objective information. If you are asking a patient who is cognitive impairment have insight into how much impairment they have, I think you have to consider some of that suspect.

We are beginning to capture some information about that because in the study we are running now in San Francisco we insist on having proxy information and we will perhaps get more clarity on that.

It is a little bit unusual in a disease of dementia to have somebody come into a clinic and be alone. All of my Alzheimer's patients come in with somebody a caregiver, a loved one who assists them. It is not uncommon for me to see HIV patients who come in alone making it somewhat difficult.

I also want to remind you that the symptoms of cognitive impairment are not just my memory is bad. In fact, it has been known for many years based on where the virus gets into the brain that there probably is a triad of symptoms that constitute the neurobehavioral syndrome of cognitive impairment. If we focus only on cognition looking at memory loss, concentration, metal slowing we actually miss a lot of what probably is HIV related brain injury including symptoms that relate more to behavior and to motor.

I also want to point out that under the cognition I am not mentioning memory as the sole entity. In fact, many of the patients that I see who have cognitive impairment will tell me their symptoms are more complex than that. If they develop good compensatory measures, they can get through things. If you give them two or three tasks to do at the same time, they begin to have some challenges.

These compensatory measures as well as I think the energy of some people with HIV allows them to stay in employment many times even though they may take 10 hours to do an eight-hour job or they may use a lot more compensatory measures to get through the day. I think these are important aspect to remember with regard to the syndrome.

I also want to point out that from the San Diego group that even patients who have mild impairment do not function well when you give them tasks in a research setting and ask them to accomplish them. For example, if you ask somebody to do balancing of a checkbook, writing checks, doing medication compliance work, reading prescriptions in a manner that would allow them to be adherent even these more simple tasks there is a higher rate of impairment as depicted by this slide.

Just to bring this point home a little bit more with regard to this silent epidemic I will tell you a very interesting case of a patient I saw recently in my research study. He was 79-years of age. I am recruiting people who are over 60 living with HIV as a chronic illness. One of my colleges said this is a great patient for you. He would be a great control. He has no cognitive symptoms.

When I interviewed the patient, he agreed that he was generally asymptomatic. He told me that he liked opera and he used to be able to remember all of the names of the singers and a lot of it escapes him now but really minimized any symptoms.

He has likely been infected since the mid 1980s and fortunately for him had very few comorbid medical illnesses. He was asymptomatic for a long time, and started HAART when his CD4 cell count dropped below 200 in about 2001. He never had opportunistic infections. He had some neuropathy. His viral load is well controlled. It has been well controlled for a decade. His CD4 count never had a great rise but it has not been below 200 over the past decade.

As I mentioned to you, not a lot of medical problems, Hepatitis A. One challenge is he has intermittent drug use and I would caution anyone who sees older people not to assume that they do not use drugs over a certain age. He had used methamphetamine the last time when he was 75 years old.

His neurologic exam was clearly abnormal. He had increased reflexes, his finger tapping was slow, and his eye movements were abnormal. On neuropsychological testing, he was abnormal across the board on almost every test he tested well below.

A picture can paint a much better image for you. This is an image of his MRI. For those of you who are not familiar with reading these, the black area in the middle is the CSF and it should not be so large. It is likely large because of brain atrophy, shrinkage of the brain. The areas around it that have white matter are also abnormal. This is clearly an abnormal brain, an abnormal neurophysiological testing profile, and an

abnormal neurologic examination in a patient who is presumed to be asymptomatic.

Let's move on. Why is this happening? I am going to try to point out two things just to bring home two points to you. One point is that I think this is an active disease. I think if we begin to think this is burnt out disease of before our patients were on HAART, if we begin to think that these are all due to confounding factors I think we are missing the boat. In my research, I think there is an active disease and we are inadequately treating it with the existing regiments that we have.

I also want to point out to you that confounding factors, coexisting morbidity probably are going to be quite important in terms of cognitive impairment and in fact can be an addressable approach.

This is my big scientific slide. I apologize to people if this becomes too complex. I think in order to understand the brain in the HIV environment I have to point some of this out.

The blood brain barrier is depicted here in the top. The cells on the top are lymphocytes, monocytes particularly in this diagram. The brain is inside the blood brain barrier in the lower part of this screen. In fact, when people are infected with HIV, the HIV gets into the blood stream very quickly.

We have now enrolled I think 20 patients who have been infected with HIV for less than three weeks, patients who are in Fiebig stage two and three in Bangkok. We are beginning to see that in fact in these cases brain injury in some cases is present as early as the first month in viruses entering at least the CSF in some of those cases.

We know it likely gets into the brain quite early in infection and it is believed that these monocytes become infected and cross the blood brain barrier. This has been called the Trojan horse effect where the virus enters the brain through the cells, the monocytes.

Once they get into the cells and into the brain, they actually do not infect brain cells very much. They cause a lot of dysfunction, particularly neurons. There is not a lot of neuronal infection. There is damage to the neurons. The arborization, the trees of the nerves become cut back. The synaptic connections are not so good. These have been shown in nice work done by Eliezer Maslia. In fact, this probably has a rebound effect, affecting the blood brain barrier allowing more virus or cells to get through the blood stream.

Given this model what happens when you give somebody antiretrovirals HAART. You can clearly get rid of the virus in the blood stream. We cannot detect it in the blood stream. We are not measuring it in the cells that are in the blood stream.

In fact, in many cases it remains in the cells of the blood stream.

Moreover, we are not measuring it in the brain; we are not measuring it in the brain cells. There are several reservoirs, several important areas where virus remains. We think that this may be particularly important for the brain. I will tell you why.

We did a small study in Bangkok; we looked at people with and without dementia. We measured the amount of HIV DNA that was in their circulating monocytes. You can see on the right side of the screen that every case with dementia had a higher HIV DNA level. It almost completely separated our dementia and our non-dementia cases.

What I think is more intriguing is that when we treated these patients with antiretroviral therapy in the middle slide at six months and the far right slide at 12 months all of them had undetectable plasm viral loads. You can see that the cases that were enrolled with dementia many of them were not able to clear the reservoir of DNA in their monocytes.

We think this may be an active phenomenon. In work we did in Honolulu, we also looked at this HIV DNA in people who have been on antiretrovirals for four, five, or six years. On the right side patients with undetectable viral load, you can see that patients who are impaired are more likely to have HIV DNA in this circulating monocyte reservoir.

Based on information from SIV infected macaques, we think these cells are coming from the bone marrow. They are probably infected when they leave the bone marrow. They do not remain in the blood stream for a very long time. Staining of these cells indicates that they probably are the ones that are developing the perivascular inside the brain-infected cells causing inflammation.

Very intriguing work also from Doctor Bell where she looked at these macrophages. Monocytes become macrophages when they enter the brain. You can see that in the upper left hand corner the CD68 staining in HAART treated individuals. The point is really that you see a lot of brown staining. There are a lot of macrophages even in patients who are well controlled at the time they die. They had well controlled viral load in their blood.

On the right, you can see that patients on HAART actually they can identify these macrophages in the brain, even among patients who are treated with HAART.

I think our work is cut out for us to try to figure out ways in which we can attack these reservoirs and try to clear them perhaps causing some benefit for cognitive impairment.

I would be remiss if I did not mention the point that these antiretrovirals probably do not universally get into the brain. There is great work being done by Scott Letendre and others describing the impact of some of these antiretrovirals

on brain penetration. In fact, you can show that the higher the brain penetration effectiveness of your overall antiretroviral regiment the more likely you are to have undetectable virus in your CSF.

There is a lot of work that needs to be done to determine the cognitive impact of this. There have been some mixed reports on this. I think it is an area that requires a lot more work.

I thought I would switch now and talk a little bit about confounding factors. I am sure I am running a little bit late on time. We have a model of cognitive impairment that really considers the interactions of a lot of comorbid illnesses. We are worried a little bit about chronic immune activation that occurs even in a setting of well-controlled HIV. We are worried about long-term complications of antiretrovirals, particularly the cardiovascular and cerebrovascular effects and how they may affect the brain.

Some of the work that we have done in Honolulu shows nothing that is surprising to this audience. Hypertension is much more frequent. Smoking is much more frequent. In fact, during the time when we were evaluating the patients we had about 20 deaths and about 20-percent of them were cardiovascular deaths. We think this is a significant outcome with regards to these factors. It is likely that they are affecting the brain as well.

If you look at the number of these risk factors diabetes, smoking, high cholesterol and hypertension and just count the number that people have in this graph that performance on a neuropsychological test is worse with a greater number of these factors that you have. We think there is a clear correlation. In the Multicenter AIDS Cohort Study, the max they found that had carotid intimal thickness in the neck correlates to cognitive outcomes.

I do not have enough time to go into a lot of the other confounding and comorbid illnesses. I would also be remiss not to mention factors such as psychiatric conditions, co-infections, principally hepatitis C and illicit drug use. These are in some cases modifiable factors. So are the cerebrovascular risk factors. Things that you as patients can do and as you as doctors can recommend. Stop smoking, get exercise, treat diseases when they present, be very aggressive about treating depression and psychiatric illness. There are clearly some things that can be done.

Let me switch quickly to the aging point and then I will close up. We began studying HIV and aging a long time ago in Hawaii. On the right side of your screen, you can see me in my Aloha shirt back when I was a physician in Hawaii in 2003 on the front page of the Advertiser studying patients who were over 50 years of age living with HIV. I do not need to remind

this audience that we are dealing with aging in all organ disease systems, not necessarily just the brain.

If you look at the prevalence of dementia in a population that does not have HIV, it increased exponentially with increasing age. In younger ages, you do not see a lot of cognitive impairment. As you get over 90 years of age, about half of the people will have some form of dementia. This is an exponential increase.

There was an intriguing report that came out of St. Louis this year suggesting that the brain metabolic rate may be decreased rather as much as 15 years in HIV. If this is any indication that patients with HIV may be at higher risk for some of these non-HIV dementia syndromes. If you just increased this curve by 15 years, there is a grand possibility that we might see very high levels of cognitive impairment once we start studying people who are 70, 80, or older years of age.

I will concentrate on just one protein, but again Ellis and Masliah has pointed out in some nice work that he has done that we see increased deposition of a number of proteins. Oh, my time is up. I will have to go just quickly through these last points.

The number of protein ATPase that is associated with Parkinson's disease, TDP-43, and Amyloid. There is a wide range of evidence suggesting that there may be alterations in Amyloid accumulation and degradation in the brains of patients

with HIV. In fact, in limited studies that looked at it, we see CSF Amyloid levels that appear to pattern similar to Alzheimer's disease in HIV patients who are impaired.

The good news though is when we image for Amyloid in the brain using a new Pittsburgh compound there is no evidence that there is fibrillary amyloid at least in younger people with good control. I do not know where this will go in terms of older people and will there is Amyloid that is building up eventually, fibrillate and cause fibrillary plaques.

Also, the damage to the synapse I mentioned before is likely reversible. We have some good news. Moreover, in our cohort of people over 60 years of age you can look at our poster up on Wednesday this week. About half of the people test perfectly normal on cognitive tests. The rate does not seem to be terribly accelerated, but these are people between 60 to 70 years of age.

My closing slides. Where do we go from here? First, I think HIV cognitive impairment is a silent epidemic. Although dementia is rare, I think milder impairment is present. Many patients adopt compensatory means to get by. Work is needed to identify people with impairment and identify treatment options.

HIV appears to be inadequately treated using our existing regiments. Sequestered reservoirs appear to be active in some way and I am particularly concerned about monocytes. I think chronic immune activation even in T-cells may be

particularly concerning as well. I am not sure the data with regard to penetration needs to be clarified. The degree to which this is going to effect cognitive impairment needs to be assessed a little bit more.

Finally, confounding factors are very important to consider in HIV patients. Doctors should address these carefully. Patients should remain physically and cognitively stimulated. Physical exercise I think cannot be overstated. Although there is no direct evidence, the evidence in non-HIV cognitive impairment seems to be getting stronger and stronger and will help prevent some of the modifiable factors.

There are theoretical concerns for increased emergence of cognitive problems, disorders in older HIV patients. There is too much unknown at this time and a lot of research is needed.

I want to thank the people who funded all of this work. Particularly the NIH, NIMH, NIA, the Larry L. Hillblom Foundation, Cecilia Shikuma and Bruce Shirmizu who do a lot of the HIV DNA work, Jintanat Anaworanich in Bangkok and her team who have done a lot of the work there and Bruce Miller at the Memory and Aging Center.

Thank you. Sorry I went over.

JOEL GALLANT: Thank you very much. [Applause] Excellent talk. I think we can take one or two questions if we hurry. Any questions from the microphone?

Let me just ask you, you have made a good case for this being an active process yet there are some data suggesting that it is the nadir CD4 that is strongly predictive of dysfunction. If you start early enough you do not see this, which would argue the opposite. How do you put those two lines of evidence together?

VICTOR VALCOUR: The data on CD4 nadir have actually been shown by several different groups including our group. I think we published one of the first papers on CD4 nadir and cognition. I think it may be a little bit naive to just assume that means that if you treat early that you are going to protect from this. There are so many confounding issues related to CD4 nadir. Many of the people with very low nadirs were exposed to different medications. They may have had a prolonged period of time where they were not treated. Now in the current era we may be talking about treating it 355, 500 versus 200. I do not think there is sufficient data to draw those conclusions based on the nadir.

You are absolutely right. There is a very strong correlation reproducible correlation with the nadir count. I think it is a bit more complex.

MALE SPEAKER: What is your take on who should be tested by the neuropsychological test then?

VICTOR VALCOUR: Unfortunately, it is difficult to get neuropsychological testing. To do the types of batteries that

are done in research studies requires a lot of time. I was speaking about this last night. I really wish I had the answer with regard to what screening tool we could use. There is an HIV dementia scale. There is a Montreal Cognitive Assessment test. There are some bedside screens that can be done. Many doctors begin to think about the many mental status exams because they know it. It is really a lousy test for this disease. I would not recommend using it.

Who should be screened is going to be a feasibility issue. If 50-percent of the patients who come into your clinic could have a disease, wouldn't you want to screen everyone? It is a feasibility issue. I think we need to figure out a way to make this work.

JOEL GALLANT: Alright. Well I really want to thank the speakers for four excellent talks. From the audience we have certainly answered some questions, but I think left a lot of questions unanswered for future conferences. Thank you all for your attention. [Applause]

[END RECORDING]