Moderators:

Massimo Galli
Dipartimento di Scienze Cliniche
Ospedale Luigi Sacco – Milano

Marco Borderi
Unità Malattie Infettive Ospedale
Sant'Orsola – Bologna

Cristina Mussini
Unità Malattie Infettive Azienda
Ospedaliera Univesitaria - Modena

Discussants:

Joel Gallant
John Hopkins University,
School of Medicine

Todd T. Brown
Division of Endocrinology and
Metabolism
John Hopkins University

Michael Yin
Irving Assistant Professor of Medicine
Division of Infectous Diseases
Columbia University - Medical Center

Antonella D'Arminio Monforte
Clinica di Malattie Infettive e Tropicali
Azienda Ospedaliera San Paolo
Polo Universitario - Milano

Fabio Vescini
Dipartimento di
Medicina Interna Endocrinologia e
Malattie del Metabolismo
Azienda Ospedaliera Universitaria -
Udine

Giovanni Guaraldi
Clinica Metabolica di Modena Azienda
Ospedaliera Universitaria - Modena

Video Conference Bologna-Baltimore
April 5, 2013 - 14:30 - 18:00 C.E.T.

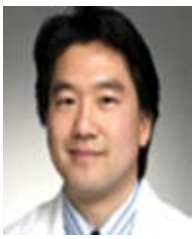
ROUND TABLE: Management of Bone Metabolism in HIV Infection: the role of the virus and HAART.

How can frailty, aging and co-morbidities affect treatment choice and guidelines.

John Hopkins Baltimora

Todd Brown

Joel Gallant



Michael Jin

M.Galli:
Good morning everybody. For us of course, it is the first hours of the afternoon. My job today is just to introduce this interesting initiative giving us the opportunity to discuss some hot aspects of a very important issue: everything around the problem of bone in HIV infection. This is a round table. We have a very important group of participants, and I have a new program with some changes due to the needs of the participants: so the first contribution will be from Dr Yin on fractures and the extent of the program. And so Dr Yin, I am in a strange situation because I have my shoulders to the screen. But you are now in the correct place and so if you would like to start this meeting. Thank you very much.

M. Yin:
And so if we bring up my first slide. (Will we be able to see the slides from here?)

M.Galli:
For the Italian participants, Dr Yin is a professor of medicine in the Division of Infectious Diseases at Columbia University Medical Center of New York City. Just to try to occupy some time, waiting for the slides…

M. Yin:
So, sorry about the technical difficulties. I will do a quick overview of the epidemiology of fractures, talk and little bit about screening and treatment, which are things that we are going to discuss later on in the cases. So, first this is a diagram of what we think happens with acquisition of bone. This is in the general population. The yellow line is for men and the green line is for women. And so, bone growth and density acquisition occurs until a peak at around your mid-20s, and then in the end, maybe in your mid-30s, it starts to decline. In then there's a steady decline of about 0.5 to 1% per year. In women, there is a similar decline in the beginning of about 0.5 to 1% per year, but during the years of the menopausal transition, it accelerates to about 1 to 2% per year, and thereafter, returns to a more stable decline.
Now what happens with HIV? In this figure I'm transposing some of the data that we have seen over the years from longitudinal studies of patients who are followed on established ART regimens, and also beginning antiretrovirals. What we think happens is that if you get infected, in your adulthood, after you've achieved peak bone mass that the rate of loss with your infection, without antiretrovirals, is relatively small. But then, with antiretroviral initiation, there is an acute drop over the first year or so of about 2 to 6% in your bone density. Thereafter, you sort of stabilize. You don't return to the original bone density before starting your antiretrovirals, but your rate of loss is mitigated at that point, and you kind of go on the trajectory similar to what you would have if you were uninfected with HIV. Now, towards the latter and the older ages, we really don't have enough data. And so I've left that as a dotted line, because we really don't know in older men whether or not the rate of loss is actually accelerated, even if you're on established antiretrovirals. In women, it's kind of the same thing, except that during the menopausal transition, there appears to be a greater loss of bone density in HIV-infected women even if they are on established antiretrovirals. We have some data in postmenopausal women as far as their rate of loss, and they seem to be higher than that of the unaffected postmenopausal women. But again, we don't have very much data in very old women.
So, given that the major risk of changing bone density occurs during that period of antiretroviral initiation, in most young patients, bone density is relatively stable after being on established antiretrovirals. The question is, does it contribute to increase fracture risk? The first look that we had of these data comes from a study of ICD-9 coded fractures from the Harvard cohort. And here in this database, they have a huge number of patient visits, and they looked at the prevalence of ICD-9 coded fractures in their HIV-infected individuals compared to the general population. And here they found that in both men and women who are infected with HIV, there is a higher prevalence of fractures, both at the hip, the spine, and the wrist. And this is the same whether or not you stratify for different races. And as you can see from this

nice diagram, the differences between the HIV-positive and the general population really begins to increase after the age of 50 in women, and maybe even a little bit younger in men. Over the last two years there have been a number of studies looking at incidence of fractures using large HIV cohorts. And here, I'd just displayed five of the larger ones. And the HIV-positive fracture incidence is in the black bar, and the HIV-negative fracture incidence is displayed in the grey bar. And you can see in four out of the five cohorts, the HOPS (HIV-out patient study) and the VACS (Veterans Aging Cohort Study) are both in the United States, and a large study from Denmark and Catalonia on the right, they all show that there is a higher incidence of all fractures – any fracture – in the HIV-positive compared to the general population. The one study that did not show that was from the Wise cohort, which is of women who are predominantly premenopausal. And the other distinction for this cohort is that the negative controls were actually prospectively enrolled women who had very similar risk factors to the HIV-positives. And so, I think that from these data we can say that compared to the general population, HIV-infected individuals do have a higher risk or, a higher incidence of fracture. In some of these studies they controlled for common causes or known traditional causes of fracture, such as age, weight, Caucasian race, smoking etc., and even controlling those variables, the HIV effect still remains significant.

This is another way to look at the composite data and some meta-analysis that we did looking at the available incidence studies. And here it gives you an incidence rate ratio for all fractures - HIV positive versus negative from these four studies that have data. And your incidence rate ratio is about 1.58. If you just look at the studies that have fragility fracture as their endpoint, the risk ratio is 1.35 for HIV-positive versus uninfected.

This is a really nice study that actually emphasizes another point, which is we may not be seeing in these risk ratios the true extent of the problem because it seems to diverge quite dramatically in the older years. And most of these cohorts have individuals that are still quite young. So, in this study that was recently published, you can see that the incidence of fractures in the HIV-positives really increased dramatically over the general population after age 70.

Drilling down on some of the other risk factors, if you look at the data for fractures in cohorts that just have HIV-infected individuals - so here, not comparing to HIV-negative - we can see highlighted on the right that there are risk factors that are traditionally known, such as smoking, co-morbidities, having older age, having a previous diagnosis of osteoporosis and alcohol use, and then others that have been highlighted such as Hepatitis C co-infection. And I think that's an important theme that has come out from some of these studies.

If you look specifically at the role of antiretrovirals, only the very large studies show some indication of their risk for fractures. So in this Hansen study, they had a large group of HIV-HAART unexposed compared to exposed, and they're able to see a significant association between HAART exposure and fracture. And in the Bedimo study, which is a very large study in the VA system, he was able to show that individuals enrolled into the cohort in the HAART era, the Tenofovir exposure was associated with increased fracture.

This is an analysis that I did looking at the observational cohort from the Aids Clinical Trials Group. And here, I was trying to figure out whether or not there was any effect of that first two years of initiation of antiretrovirals on increase in fracture risk, acute increase in fracture risk, similar to what you might see with glucocorticoid initiation. And, if you look at the fracture incidence in this cohort, fracture risk within the first year or two years after initiation of ART, you'll see that it's higher than all of these subsequent years. Now, these data suggest that maybe acute bone change with initiation of ART has an impact on bone strength and could increase fracture, but it's really compounded by the fact that maybe people just get better as far as the other fracture risks with continued antiretroviral therapy: that is, gaining weight, better nutritional status; and what we're seeing is that this

reflects actually an improvement of fracture risk with continuation of the antiretroviral therapy. We can't tease that out with these data, but it's certainly provocative.

So, given that, what is the best way for us to move as clinicians to identify patients at higher risk for fracture?

So the FRAX tool is one that is commonly used it in the general population. It gives you prediction of the ten-year probability of foot fracture, or major osteoporotic fracture, the spine, forearm, the hip or the shoulder. And it puts into the model risk factors that are known, and then you can also put in femoral neck BMD.

This has been looked at in a feud studies within the HIV population using, let's say, the European approach. The FRAX and the National Osteoporosis Guideline Group algorithm uses the FRAX clinical risk factors to classify as low risk, intermediate risk, and high risk. And different actions are assigned for those risks. For intermediate risk, the suggestion is to measure DEXA, and in a high risk to treat without a DEXA. So from this study by Calmy, published in 2009, FRAX using the classic risk factors alone seemed to underestimate the fracture risk that would have been determined if you used the FRAX and the bone density. And this is done in a cohort of patients where they had bone density and all the clinical risk factors.

There have not been any data to look at exactly what the underestimate of fracture incidence would be with FRAX in HIV-infected individuals. And as you know, DEXA is an important tool but it really is most useful and meaningful in the older ages. If you have a low bone density in your 40s with a DEXA, it really does not help to distinguish your fracture risk. And that's why mostly the guidelines that have been put out use a cut-off of age as the first criterion. The National Osteoporosis Guidelines in the U.S. recommend for the general population screening women over the age of 65, and men over the age of 70 with a DEXA, and possibly doing it for folks over the age of 50 if they have a high number of risk factors. The ID (Infectious Diseases) Society in the United States looked at these criteria and they truncated some of the lists of risk factors and they have pretty similar recommendations. Others have been more aggressive about this approach, and have looked at the data and said - well, almost all of our HIV patients have one of the risk factors, we should really move towards screening everybody over the age of 50. But this has not really been studied in terms of its cost effectiveness and uptake in the general population of practitioners.

Now, another way that has being looked at for screening is doing spine X-rays, and I think where going to talk more about this later on. The guidelines from the U.S. are to do this only in select populations. So not as a general screening criterion. And here they're suggesting that you do it if you know the T-score, if you know the DEXA, in really old folks over the age of 70 and 80, regardless of T-score, and in women and men with low–trauma fracture, and if there's height loss.

There have been 2 studies looking at this that have been published and presented in abstract at CROI, both from Italy, looking at this using chest X-rays as a way to pick up incidental vertebral fractures. In the first study, published by Torti in 2012, there's a comparison of 160 HIV-positives versus 163 controls, and they found that the incidental findings of vertebral fractures were much higher: 27% of the HIV-positives compared to the controls, which was only 13%. Gotti presented an abstract at CROI this year that had a very similar methodology, except they also had DEXA and they found a higher percentage of vertebral fractures in the HIV-positive versus controls: 30% versus 4%. Another interesting fact was that most of the fractures in the HIV-positives were part of multiple fractures, and they were picked up. And a comment about the use of DEXA: the HIV-positive patients had fractures similar types of fractures to patients that had osteoporosis versus osteopenia.

Just a quick word about who to treat. This is sort of the recommendation for the general population. If you have a vertebral or hip fracture, you treat. If you have a DEXA that's less than -2.5 at the femoral neck, total hip or lumbar spine, you treat. Otherwise, if you're T-score's between -1

and -2.5, then you kind of use the FRAX - in the U.S., this is the way that we do it - to prognosticate how high your risk is. And if your absolute risk of fracture is greater than 3% at the hip, and greater than 20% any osteoporosis-related site, then the recommendation would be to treat.
As far as what to treat, the only thing that's really been studied in the HIV-positive population is Bisphosphonate use, both in terms of weekly Alendronate, and Zoledronic Acid. These are six studies that have been published and they show the same short-term change in bone density as would be seen in the general population. As you know, there's emerging evidence that Bisphosphonates are not without adverse events: osteonecrosis of the jaw is really quite rare, but subtrochanteric fractures and atypical femoral shaft fractures have been getting a lot of notice. For the general recommendations, we look at this data and really target individuals that truly need to be treated. And then there's a consideration for not treating for more than 5 years without revisiting the issue.
Teriparatide, or Forteo - it's a PTH analog - that has been used and described in case studies of patients who have HIV and osteoporosis. It's difficult to use as a first-line therapy because its injectable, and it has not been studied widely in HIV infected individuals. Denosumab, which is monoclonal anti-RANKL antibody would be an interesting agent to study but there have been concerns about the infection issues given the Phase 2 data about increased skin and soft tissue infections with Denosumab.
So, in summary, the fracture incidence is clearly increased with HIV infected individuals, and especially in individuals who are older. We expect that we'll be seeing this more in our patient population as they age. The risk stratification and screening strategies, I think, are really interesting as an area to discuss and for future studies. There have been some data and it seems like clinical risk factor based FRAX really requires further validation for use in HIV-positives. Screening DEXAs for age over 50 has been recommended but cost and utilization I think require further study. The role of other imaging modalities, and the use of bone turnover markers are unclear, and also deserve some study. And lastly, prevention strategies with initiation ART: what to do about mitigating their bone loss within the first two years, and in patients with fractures and falls also require further studies.
And with that, I'll turn it over.

M. Borderi:
Thank you, I'm Marco. First of all, thanks a lot for coming on purpose from New York City for this meeting. Thanks a lot. We all have questions for you but before these, I would like to introduce Antonella D'Arminio Monforte who will give the next presentation. Antonella will speak on Subclinical Vertebral Fractures in HIV.
Antonella is head physician, Chief of the Department of Health Sciences at San Paolo Hospital and is founder and leader of our Icona Course in Italy. Thank you, Antonella.

A. d'Arminio Monforte:
Thank you, Marco. And first of all, I apologize for my voice but here in Italy it's still winter, so we have a cold.
I will present this study we have done on sub-clinical vertebral fractures in HIV. I would like to skip all the background because Dr Yin has already illustrated very well what is osteoporosis and how HIV-positive individuals have a higher risk of osteopenia and osteoporosis. And obviously also of fractures, increasing with age.
What I would like to underline is that we have used for this study FRAX, fracture risk assessment tools, a tool that can predict the 10 year risk of probability of fractures using the BMD, the DEXA data with other data relating to the patient. The objective of our study was to evaluate the clinical implication of the introduction of lateral spin X-ray in the screening program of bone metabolism in HIV-infected patients and also to assess the prevalence of sub-clinical vertebral fracture and the risk factors associated with it.
We enrolled 194 unselected HIV-positive patients who had a DEXA, lateral spine X–ray, bone biochemical markers and a FRAX algorithm - the estimated risk of fracture by FRAX. We also collected

all the demographic data, common risk factors for osteoporosis, and HIV-related parameters and co-morbidities, and we tried to look at risk factors associated with vertebral fractures by univariate and multivariable analysis.

First of all, the morphometric analysis of lateral spine X–Ray. It is done between the dorsal 4 and lumber 4 vertebrae, and the SDI is the sum of the grade of reduction of the body height of each vertebra and is classified in mild with an SDI of 1; moderate is with an SDI of 2; and severe with an SDI of 3, implicating more than 40% reduction of vertebra body height. In our 194 patients we found that 21% had at least one radiological identification of vertebral fractures, with 6% of SDI 1, 10% 2, 3 and 5% of an SDI score of more than 4. And this was well correlated with age, as you can see in the left part of this slide, and also with the female sex, particularly women over 50s. And if you look at the correlation between STI and BMD, you can see that STI of more than 1 is correlated with being osteoporotic or osteopenic. But looking at our population as a whole, we have defined with DEXA 29 osteoporotic patients, and of these 29 osteoporotic patients, 10 had an SDI of 1 or more than 1, and so had a fracture - a vertebral fracture - but also of the 165 non-osteoporotic patients there were 31 fractures. So, the indication of treatment is a standard not only for osteoporotic but also for non-osteoporotic fracture patients. This is an important implication in our opinion for treatment of these patients.

Looking at risk factors, as I said, with subclinical vertebral fractures, as you see, there are some factors that are related to having an SDI of 1 or more than 1, particularly in the CD4 count, and a history of smoking, alcohol and obviously of menopause in women. But in the multi-variable model, the risk factors associated with having an SDI of more than 1, so moderate to severe fractures, are age, with 9% of increasing risk with successive every year, steroid use, and intravenous drug use. In this model, sex was not one of the risk factors also because it was particularly adjusted for age and also none of the HIV-related parameters was associated with a higher risk of subclinical vertebral fracture.

What we have tried to do is look at the sensitivity of SDI in relation to BMI, to BMD, to osteoporotic BMD features. And as you can see, an SDI of more than 1 has a 30% sensitivity and an 87% specificity. This was also important in the case of FRAX. FRAX with an SDI of more than 1 has a 74% sensitivity and a 27% specificity. So we can conclude that there is a 21% prevalence of subclinical vertebral fractures, i.e. asymptomatic fractures in our cohort of patients, and this increases particularly in patients over 50 years of age. 76% of fractures occurred in non-osteoporotic patients, leading to the identification of an increased prevalence of osteoporosis, with important therapeutic implications. And age, previous or current steroid use and drug abuse were the risk factors independently associated with increased risk of vertebral fractures.

Furthermore, DEXA parameters and the FRAX score showed limited utility in screening of this population at increased risk. Therefore lateral spine X–Rays should be recommended.

Thanks to all my co-workers and the orthopedic and radiology units of the San Paolo Hospital, where I work.

M.Galli:

These two presentations are now open to discussion. Are there questions from the audience? Marco.

M. Borderi:

Mike, I'm Marco. Thank you for a very nice presentation. I have a question about one of your slides where you mention a paper by Roger Bedimo, will found a correlation between TDF use and increase risk of fracture. I remember another paper by Linda Mundy in AIDS, who in contrast found a correlation between TDF use and reduced risk of fracture. What is your opinion? Is there maybe a bias because it is not possible - it must be an increase risk or a lower risk of fracture with the use of TDF. What's your opinion Mike?

M. Yin:

Well, I think that's very hard to know at this point. Can you say again these study that you were quoting about the lower risk with TDF, what was that?

M. Borderi:
The study of Linda Mundy in AIDS. 2012. Last year.

M. Yin:
OK. So, I'm not familiar with that study, I'm sorry. I'll look at it. But you're hitting on a point which is that it's very tricky to define individual risks of antiretrovirals with these studies, especially when you need such a huge cohort to be able to have enough endpoints for fracture. And, you know, it depends a lot on how you code the variables, what other antiretrovirals you're including in the model, and so forth. And so, I think all of this is quite speculative, and I agree with you that you can't really use this evidence for thinking about what to do definitively with antiretrovirals. It's only in the context of other data.

M.Galli:
Cristina?

C. Mussini:
I have a question about Bisphosphonate. The risk for harm to health happens after about five years. So for how long do we need to stop Bisphosphonate in order to decrease this risk? And what should we do? I mean, should we start up again after, say, one year?

M. Yin:
It's a great question. You know, I'm going to let Todd handled this one!

T. Brown:
Yes, there's a lot of controversy (this is Todd Brown). There's a lot of controversy about who to give a Bisphosphonate holiday to, and when you do give a holiday, how long do give it for. I think the first consideration is assessing the persons fracture risk, sort of retrospectively, to see first of all, whether or not they needed this Bisphosphonate in the first place. And if their absolute risk of fracture was low at the time that they started on the Bisphosphonate, it would be reasonable to take the Bisphosphonate off and give them an indefinite holiday, assuming that they haven't had previous fractures. For people who have a big history of fractures, who have very low T-scores, we don't know too much about holidays in that population. So, in the FLEX and FIT Trial, which is our only data to tell us that the 10-year risk of fractures on Bisphosphonates is no different than if you've been on Bisphosphonates for 10-years versus being on Bisphosphonates for 5 years and then stopping. So that trial excluded people who had a T-score of less than -3.5. So we don't really know too much about people who have very low T-scores. For people in this middle group, and these are patients that we see quite often, my clinical practice is to take them off it if they've had it for 5 years; assuming their absolute risk isn't all that high. I would take them off and I would follow their DEXAs, and if they have a drop in BMD of greater than 5% over the next year, I'd tend to restart. There's also some thought of whether or not you could use bone turnover markers to help you make the decision and to start Bisphosphonates when the measures of bone resorption like CTX go up above the premenopausal range for women. And that's another strategy that could potentially be used. But I think that it's a real black box, not only in HIV specialist care, but in the general population about what to do with Bisphosphonates. Clearly we are balancing the risks and benefits, and the risk of atypical fractures in the femur are definitely clearly there, but are really outweighed in a person at high risk by the 40% decreased risk in other fractures that Bisphosphonates will confer.

M.Galli:
Other questions? I have one. As you know, in Italy we have about 35 to 40% HIV-patients who are co-infected with Hepatitis C virus, and we have also a lot of former drug users, plus or minus 35 or 45% of all patients with HIV. So, there are many cohorts that identify HCV as a risk factor for fractures. On the other hand, Antonella's data identified drug users, and former drug users, as a population at increased risk for fractures. So what is your opinion, Dr Yin and Prof D'Arminio about the real role of HCV co-infection or about the possibility that there is a sort of interference in the cohort data between

HCV infection and the coincidence, the overlapping of many intravenous drug users as people with HCV infection?

A. D'Arminio:
If I can respond. This is a very intriguing story that occurs every time we look for HCV and drug addiction in cohort studies. So there is a co-linearity and in some cases we found that drug addicts are at higher risk, while in others HCV is the higher risk. Statisticians say that this is a question that is very difficult to solve from the statistical point of view. Looking at the possible pathogenesis, I think that both the events - co-infection with a virus that is a chronic viral infection supporting liver disease and liver failure - may result in higher risk for osteoporosis. On the other hand, I think that also long-term intravenous drug addiction may lead to a risk of osteoporosis.

M.Galli:
The fact is that Hepatitis C virus may contribute to inflammation… to a quantity of alterations, and probably there is some suggestion about a direct role against bone metabolism. But it's not so easy to distinguish between these two aspects. What's your opinion from America?

M. Yin:
From America. I think that Antonella said it beautifully. I think that it's very compelling to think about this mechanistically. And I think that both HIV and HCV chronic infection might impact upon the bone, and possibly both through this inflammatory, microbial translocation causing an inflammation pathway. And I think there are some studies that are ongoing that are trying to tease this out. Todd has some studies looking at bone geometry in patients who are co-infected with HCV and HIV, and certainly there's some evidence that there might be architectural differences in that population. So, I think it's an area that deserves further investigation. There are also data from HCV mono-infected individuals that if you treat their HCV, you actually improve their bone. So, I think there are data that both independent infections have effects on bone metabolism, and that together they may be additive.

C. Mussini:
Since there has been a change in the program, the next speaker will be Todd Brown talking about Osteoporosis or Osteomalacia. So please, Prof. Brown.

T. Brown:
Okay, let's get set up here; you guys can see my slides.
So, I'm going to take a little bit of a different approach, from a clinical angle. I have a couple of cases to present to try and get at this question of whether someone has osteoporosis or osteomalacia, and what's the difference.
So, this is a case from my clinic: a gentleman with Factor 9 deficiency, so hemophilia, who has a lot of problems, including hepatitis C with cirrhosis, and portal hypertension and esophageal varices. At the time when I first saw him, he was on AZT, 3TC and Efavirenz. He's had multiple complications, and his complications have had complications. He's had hyperlipidemia, severe lipoatrophy, lipohypertrophy and diabetes. Now, given his risk factors, including his age, I did a DEXA scan on him, and what you can see, if I can get my arrow up here, is that his T-scores, particularly at the femoral neck, are in the osteoporotic range. So, the clinician - me, in this case - was left to sort of wonder what to do next. And there are a few different approaches that one could take. You could treat him with a Bisphosphonate; treat him with calcium and vitamins D, thinking, well, he's a relatively young guy – 53 - and maybe he doesn't quite need a Bisphosphonate yet despite his T-scores. You could workup his secondary causes of low BMD, and you could do everything. You could treat him with a Bisphosphonate, you could give him calcium and vitamin D and you could do the secondary workup. And in this situation, I think it's absolutely critical to pause, not to jump into with treatment with a Bisphosphonate, but to evaluate the secondary causes of low BMD. And the workup that people do to evaluate secondary causes of low BMD really varies quite a bit depending on which textbook you read. I've listed

some of the causes here. The ones that I've highlighted in yellow are the ones that I do personally, and I think they have the highest yield. The other ones for idiopathic hypercalciuria, for celiac sprue, multiple myeloma, mastocytosis and Cushing's I do if there are other clinical symptoms suggestive of these disorders. So this is my secondary workup that I tend to do. And I want to zero-in on two specific items here, and those are severe vitamin D deficiency, and phosphate wasting. And mostly because in this setting with low BMD and severe vitamin D deficiency, and/or phosphate wasting, the low BMD may not be due to osteoporosis at all, but rather osteomalacia. And so, this is impaired bone mineralization. So the whole osteomalacic syndrome is one of weakness and pain and anorexia and weight loss and fracture. But this is sort of the extreme phenotype, and you can see much more mild phenotypes as well. Now, the critical thing here is that osteomalacia is not treated with Bisphosphonates. So, if the patient is severely vitamin D deficient, you need to treat with calcium and vitamin D at high doses. If the person has phosphate wasting, there need to be either interventions, including phosphate repletion, vitamin D and also withdrawal of Tenofovir if it's on board. And really this is the most important differential diagnosis for low BMD.

Now, the definitive way of making the diagnosis of osteomalacia is a bone biopsy. And this can be done, generally in the iliac crest, but typically in practice the easier way to do it is to identify a cause of osteomalacia and try to treat it. But I'll just show you what normal bone looks like. So all this stuff here is fat and the bone marrow, and the blood cells of the bone marrow. In green is mineralized bone, and now, on the edge of the green mineralized bone you see unmineralized bone, and that's osteoid. So this is new bone that has been laid down but has not been mineralized yet. In osteomalacia what you see is that you do have some mineralized bone, but look how wide this red part is: that's the osteoid scene. And so this is a big area of unmineralized bone.

So, I'm just going to briefly review calcium and phosphate metabolism because I think it's important in understanding some of the biochemical indices that are obtained in a patient who does have phosphate wasting, and I'm going to talk about the regulation of calcium and phosphate homoeostasis pretty briefly. So both of these ions are critical to functions of many cells and play many roles in the body. Therefore the body defends their levels in a very tight range. And this is done through the actions of multiple organs: through the gut, through the kidney, through the parathyroid glands, and then here's the major reservoir of calcium and phosphate, and that's the bone. So calcium and phosphate are both freely absorbed through the gut under the influence of 1,25 dihydroxy vitamin D. And then, the major reservoir of calcium phosphate is of course the bone. So let's talk about vitamin D deficiency first, and severe vitamin D deficiency, and what are the physiologic adaptations and what you might see on the biochemical profile. So, in severe vitamin D deficiency, you have relatively lower levels of calcium. This of course increases parathyroid hormone secretion. When parathyroid hormone levels go up, it has multiple effects. On the kidney it drives 1-alpha-hydroxylase activity, and this increases the conversion from 25 hydroxy vitamin D to 1,25 dihydroxy vitamin D. It also has the effect of reclaiming in calcium that is freely filtered in the glomerulus, and reclaims it in the proximal tubule so that more calcium is reclaimed from the kidney. The 1,25 that is made by the conversion from 25 hydroxy vitamin D has a couple of actions. One is to increase calcium and phosphate absorption across the gut; and the other is to liberate calcium and phosphate from the bone. And so that's what we see in severe vitamin D deficiency, and I'll be showing you the biochemical parameters in a second. With hypophosphatemia, or phosphate wasting, there are some slightly different adaptations. So, low phosphate levels have a few effects. One is to drive this 1-alpha-hydroxylase itself to increase 1,25 levels. And so the 1,25 levels go up, driving phosphate absorption across the gut, liberating calcium phosphate from the bone. Also the 1,25 feeds back to suppress parathyroid hormone secretion. So, in addition to reclaiming calcium from the proximal tubule, PTH decreases phosphate re-absorption;

so you dump phos in your urine, and that's the thing you don't want to be doing if you have a low phosphate level. So these systems work in concert to regulate calcium and phosphate metabolism. So, let's take a look at the biochemical profiles. So in severe vitamin D deficiency, if it's very severe, you might get frankly low calcium level and hypocalcemia. You get, of course, low levels of 25 D. Because PTH is stimulated, you get very high PTH levels, and this may drive the production of 1,25. And the increased PTH levels also have the effect of decreasing serum phosphate by inducing renal phosphate wasting. And in this situation alkaline phosphatase levels are really quite high. So we see mild vitamin D deficiency quite often. But here we're really talking about severe vitamin D deficiency that's marked by very high PTH levels, and increased alkaline phosphatase levels.

So, let's talk about phosphate wasting, and this would be proximal tubular phosphate wasting, and in our situation generally induced by Tenofovir. And so what we see is that the serum phosphate is low, but also could be on the low end of normal; there could be inappropriate phosphate wasting. The serum calcium levels are relatively normal; the 25 D level can be normal, or it can be low just like in the general population. Since the 1-alpha-hydroxylase activity is working, the low phosphate levels can drive 1-alpha-hydroxylase activity, increasing 1,25 levels, and you do get an increase in alkaline phosphatase, particularly if the phosphate wasting is severe. Now, one important clinical point is that if you have to concomitant vitamin D deficiency with Tenofovir-induced phosphate wasting, the low 25 levels - here I have put them as normal - but if this is low, this will drive increased parathyroid hormone secretion, and the increased PTH will cause even more hypophosphaturia and increase phosphate secretion, and compound the problem.

So, I want to illustrate these points with another case that I saw in clinic. And this was a 50-year-old Caucasian man who was diagnosed with HIV early on in '85; started on antiretroviral therapy in '95. His current CD4 cell count at the time I saw him was 236; doing well from a virologic standpoint on the regimen that you see here. He came to my endocrine clinic not with a bone problem but because he had fat accumulation in his dorsal cervical area, and also had moon facies - and he had a history of lipoatrophy, hyperlipidemia, schizophrenia and plantar fasciitis. The real reason why he came to me, which I won't go into, but is also interesting, is that he was getting injections of triamcinolone for his plantar fasciitis, and in the setting of Lopinavir/Ritonavir became Cushingoid. This was actually the first case I saw of this problem. As he was walking out the door, or getting to walk out of the door, I asked him, you know, 'is there anything else that's bothering you'? And I had noticed that he had trouble getting up on the examination table. And so this is always a tricky question to ask, as you never know how long you're in for after you ask it, but he said: 'yeah, my hip has been bothering me for the last two weeks, and I really have trouble walking'. And there wasn't any trauma that I could elicit, so thinking about his steroids exposure and hip pain, I was thinking that he might have avascular necrosis. So this is just a detour here, very common in HIV-infected patients, but the pathogenesis is really quite poorly understood. It's very tentatively linked to PIs but more recent studies really haven't shown any clear association, and the risk factors are steroid use, radiation, chemotherapy, sickle cell or trauma. And the best way to diagnose it is with an MRI. So I was thinking I would get an MRI and it would show, you know, collapse of the femoral head and that he'd have avascular necrosis. But no, he had a femoral neck fracture. So this is an expensive way of diagnosing a fracture! So he had a fracture right through here. He did have some osteoporosis risk factors: the steroid exposure; he had hypogonadism; he had a history of lactic acidosis in the past which at least in earlier studies that were associated with lower bone mineral density; some behaviors like smoking and past alcohol use that might have contributed. So he underwent surgical repair. And then he underwent a secondary DXA scan first. And here is his T-score. So spine: -2.6; femoral neck: -5.2; right hip T-score of -4.2. So very low T-scores. And here's his secondary workup: his vitamin D - he was obviously on supplementation here,

so this has been supplemented up; his PTH was normal; calcium was normal; TSH was normal. His testosterone even though he was on replacement was on the low side, so that gave something to tweak. But his serum phosphate was 0.8 mg/dl, so extremely low. And his fractional excretion of phosphate was 53%. And with this low level of phosphate, 1- alpha–hydroxylase activity was stimulated and his 1,25 dihydroxy vitamin D was elevated at 150.

So, treatment him: we switched him from Tenofovir to Abacavir, gave him oral phosphate supplementation, starting at 500 mg four times a day. And also, of course, he needs calcium as well as phosphate to mineralized his bone, so calcium was also instituted. And because his 1,25 level was also high, there was no need to give him any calcitriol. I generally follow these people 6 to 12 months without any Bisphosphonate, and look at their bones - you can look at their alkaline phosphatase levels or osteocalcium levels to see when those come down, to show that you've adequately re–mineralized the bone. But it does take some time. And so over the next few months he got a repeat DEXA and you can see this dramatic change in his bone mineral density; his hip going up 14%; femoral markup 5.5%; spine going up 8.9%. His bones are still a little on the low side but all the low bone mineral density that you saw back here, almost all of it was related to osteomalacia rather than osteoporosis . And the problem with giving him Bisphosponates before doing the workup is that Bisphosphonates can actually inhibit mineralization, and you might be doing the patient a disservice.

So, this is what it looks like in his case. But this is what resolution of osteomalacia looks like. So here are these thick osteoid seams, and they're in orange with mineralized bone in the blue. And then, once it is re-mineralized, it's pretty much all blue with a little bit of osteoid where bone has just been laid down.

So, I think the conclusions here are that the secondary workup of a patient with low BMD is absolutely critical, that severe vitamin D deficiency and renal phosphate wasting are the major causes of osteomalacia in the HIV-infected patient, and need to be evaluated and treated. You can make a definitive diagnosis with bone biopsy but oftentimes these are difficult to obtain. So treating for presumptive osteomalacia in the right clinical circumstances is probably the best way to go. Thanks.

M. Borderi:

Hi, Todd, I'm Marco. Thank you for your very nice presentation as usual. While you in Baltimore were probably still sleeping, we here in Italy had a morning meeting on managing bone metabolism, and one of the speakers was Fabio Vescini. So it's a great pleasure for me to introduce my "brother" Fabio Vescini, who is our endocrinologist referee here in Italy. And he will talk about Hypovitaminosis D in HIV.

Thanks.

F. Vescini:

Thank you, Marco. Good afternoon to everybody and good morning to the colleagues in the U.S. I'm going to talk about the case about Mr Domenico. He's an Italian Caucasian male, 52 years old, a librarian, and he got infected seven years ago by homosexual transmission, and he's on a stable HAART based on Abacavir, Lamivudine and Efavirenz. As you can see, his immunological status is satisfactory. Well, he is 65 kg and 1.75 m tall, but before Christmas he was 4 kg heavier, and we will see why I'm writing before Christmas. His BMI is 21.2kg/m^2. His risk factors for low BMI show just sedentary life style, and an intolerance to dairy products that cause him diarrhea when he eats them. Well, in December 2010 he felt pain in his right foot, and a month later, in January, this worsened. He began an anti-inflammatory drug, Piroxicam, on the advice of his family doctor. But still a month later in February he was forced to use crutches for walking. So his family doctor got alarmed and prescribed an X–ray of the foot. And here you can see his 5th metatarsus is that is clearly fractured with a particular type of fracture that is very painful and occurs sometimes, but quite frequently during osteomalacia. So, the first question is what is the next step? Are we going to treat him with a Bisphosphonate or with calcium and vitamin D? Or

would it be better to perform a DXA before any treatment? Or again, are we supposed to perform some lab exams? Obviously we chose the last points, and we performed a DXA that you can see here that showed a very low T–score or Z–score, as you prefer, in the lumbar spine and the femur, with -2.85, and a femoral neck of -3.34. His lab analyses were satisfactory with regard to in erithrocyte sedimentation rate, glucose, transaminase, creatinine, electrophoresis of proteins, and blood count. So you can rule out a large part of the signs of osteoporosis. But particularly interesting was testosterone, which was normal. On the contrary his alkaline phosphatase was elevated. His serum calcium and serum phosphate, but particularly his serum calcium was dramatically low, as well as urinary calcium excretion, as well as serum 25 (OH) vitamin D. Finally, his PTH values were very, very high. So it is quite easy to answer the question what is the diagnosis. He has got hyperparathyroidism secondary to hypovitaminosis D and an osteomalacia. But why did I put a question mark after osteomalacia? Well, Todd Brown told us but I'll repeat some of his points. We cannot be sure we are facing an osteomalacia when we pick up a low BMD if we don't perform a bone biopsy. That's the only way to distinguish osteoporosis from osteomalacia. Osteomalacia has low mineralization like osteoporosis, but it is caused by impaired bone mineralization. We have got a large amount of bone matrix that cannot be correctly or at all mineralized. And osteomalacia also has some clinical signs; we have already seen some of them: weakness, fracture - and we had a fracture in this patient -; pain and he had very intense pain in his right foot; and weight loss. Moreover, osteomalacia must be treated at least with calcium and vitamin D and/or phosphate, and should not be treated at least in the initial period of the disease with Bisphosphonate that can paradoxically increase the osteomalacic pattern. So, we confirm the first diagnosis. We had still a little doubt about the osteomalacia as a second diagnosis, but this can reasonably be ruled out, and we prescribed therapy that was based on a bolus of cholecalciferol: 300,000 IU each month for two consecutive months. And together with that he began maintenance with 7500 international units per week of cholecalciferol. We also added 1 g of calcium carbonate per day. And now, when and with what should we follow up with? Is it important to decide when to go see whether our therapy has improved the lab or other parameters. And what should we perform? Lab analysis in the short period, or lab and DXA in the short term? Or lab and X–ray in a fairly short period? Or lab and X–ray? So we chose the third option. As you can see four months later he showed a very impressive increase in serum calcium and phosphate together with a detectable urinary calcium excretion that was undetectable before, and with a satisfactory level of vitamin D. But especially his parathyroid hormone dramatically decreased from those very high values to 180 pg/ml, that's still very high but not as high as before. Okay we can see that, but how is he? He is a patient who came to our office; so how was he at the time? This was how we left him in February. In June he had no more pain. He could walk without crutches, and he was very happy, as you can see. So we succeeded in helping him, but does this improvement have a corresponding X–ray feature? Let's look at his X-rays. The fracture is still there as you can see, but it's less wide than before and particularly, especially in this projection, you can see the formation of a bone callus that's mineralizing. And he's improving; the pain is lessening as his bone mineralizes. You can also see in this projection that the fracture is still evident, but he is improving. So we confirm the therapy; we increased the cholecalciferol a little bit per week as 22 ng/ml are not so satisfactory. We would like to go over 30 mg/ml. And calcium. Okay. But the question is when are we going to repeat a DXA. Are we supposed to repeat it or not, and if yes, when?

Usually, as I told you this morning, the guidelines suggested repeating a DXA of the lumbar spine after 18 months from the first, or a femoral analysis after 2 years from the first one. But in this case were not facing osteoporosis, at least we're not sure we're looking at osteoporosis but osteomalacia. We suspect an osteomalacia. So in just one year, the following year, he showed a very high increase of

lumbar spine mineral density as well as the femoral neck and total hip T-scores. But he was still osteoporotic. Here you can see, particularly his femoral neck is -2.54. So the question was: were we facing perhaps a combination of the two diseases, osteoporosis and osteomalacia, and after correcting the second one, osteomalacia, the first remains as a feature of this patient. We must remember that he is HIV-infected; he is taking drugs even if correctly. So maybe this patient has both diseases. If this is the case, are there any further steps to take? I leave you with this question because we should, we could begin treatment with Bisphosphonates, and in this case which one should we choose? Or why not, should we switch him from Efavirenz to Raltegravir for example? Can we have any additional effect or should we just continue with calcium and vitamin D? Any other options or opinions you might have will be gratefully received by me but also by Mr Domenico, who thanks you very much for the kind suggestions you're going to give me now and to him. Thank you.

C. Mussini:
Thanks to both speakers for their very nice presentations, which I open up for discussion. While waiting for questions from the audience, I would like to start asking both of you how should we consider - since we have a lot of patients all are co-infected with HCV as we said before - how should we consider an isolated increase in alkaline phosphatase if the patient is completely asymptomatic? Do we have to go and search for osteomalacia?

F. Vescini:
Todd are you going to answer?

T. Brown:
Yes, sure. Yes, to the question, alkaline phosphatase, at least in the US, and I think it's the same in Italy, shows up on our chemistry panel, so it's easy to get. And a patient who has hepatitis C will have elevated liver alk phos, and so I think the important factor to look for would be the temporality of things, since we do get chemistry panels frequently on a patient, we can often look at the trajectory of alkaline phosphatase over time, and we can see whether or not there are elevations which correspond to the onset of symptoms for a given patient who's being followed longitudinally in the clinic. For a patient who doesn't have that kind of data, I think it's useful to do fractionation and look at the bone-specific alkaline phosphatase. And/or look at other markers, more specific markers of bone formation like osteocalcin or P1NP. But typically osteocalcin is a little bit easier to get.

V. Viscini:
I agree.

M.Borderi:
I have a question regarding vitamin D supplementation. Is there a difference between HIV-negatives and HIV-positive persons? Is there a difference between a non-NUCs-containing regimen and boosted PI-containing regimen? Is vitamin D supplementation different for a TDF-containing regimen and non-TDF-containing regimen? What is your opinion?

T. Brown:
Yes, so it is going to be just an opinion because there are no data to support the opinion, but it's a very interesting question. I think that the data do show that people are taking Efavirenz have a slightly lower 25-hydroxy vitamin D levels than people who aren't taking Efavirenz. And I tend to replace those people a little bit more aggressively than someone who's not on Efavirenz. I'll give numbers in a second. Regarding people on Tenofovir, I think the data that we do have, that I alluded to, would suggest that the impact on bone metabolism in a Tenofovir-treated patient is augmented in the setting of vitamin D deficiency. So, I would tend to be a little bit more aggressive in that patient population as well. So what I typically do for someone who doesn't have bone problems is take the universal supplementation approach and give them - I typically give people 1000 international units daily if they're not on Efavirenz, and then boost it up to 2000 if they are on Efavirenz, with the idea that they're going

to have increased catabolism of their 25-hydroxy vitamin D.
For people with bone problems, particularly with osteomalacia, I'm obviously much more aggressive and I would follow a similar regimen to what Fabio outlined here, giving them a high dose of ergocalciferol for a short period of time, usually 12 weeks in my case, and making sure that they have enough vitamin D to mineralize adequately, and also calcium.

M.Galli:
Have you seen at any time a coexistence of avascular necrosis of the bone and osteomalacia? Because it's quite rare, at least in my experience. We had probably no more than 30 cases of avascular necrosis, but in general completely distinct from a situation of osteoporosis or osteomalacia. Have you seen other patients with this particular characteristic?

T. Brown:
Yes, I have seen them our existence. It's interesting; we're doing a study in the Multicentre AIDS Cohort Study, a bone sub-study now and we're getting CT scans of their hips of HIV-infected and uninfected men, and reading them for incidental findings. We've scanned about 70 people so far and we've had multiple cases of asymptomatic osteonecrosis, AVN. I don't know what their siero status is, and I don't know what their bones look like yet, but it's going to be an interesting question. You know typically I do think of them, as you do, as completely separate entities, but whether or not there is a correlation, you know, it's an interesting area to study.

G. Guaraldi:
Thank you so much. I have one question. I would like to return to the question that Fabio left with us. I was wondering if in our clinical practice the approach that Fabio suggested that he first of all treats vitamin D deficiency and possibly osteomalacia and then, after one year of treatment, if you believe there is still a low BMD, then go ahead and treat for osteoporosis. Of course in this issue we know that our diagnosis of osteomalacia is presumptive. Nevertheless it's quite difficult to offer to all the patients we have with low vitamin D and low BMD a bone biopsy. So this is really what we currently are doing at the Metabolic Clinic, that is, just first of all treat vitamin D deficiency, try to see what happens to DEXA, and if after one year you are able to correct it, go ahead and treat with Bisphosphonate. Do you think this is clinically oriented or should we do something more?

T. Brown:
I think that's a generally a good approach, but the caveat being that there's vitamin D deficiency and there's the vitamin D deficiency! So there are patients that have, like the one Fabio presented, all have clear biochemical evidence of severe vitamin D deficiency, very elevated parathyroid hormone levels, increased Alk. Phos., evidence of insufficiency fracture - that patient on clinical grounds could be labeled as osteomalacia. But the more typical patient we see is the one where you get a DEXA and their T–score is -2.6, the lowest T–score. And you do the secondary workup, and their vitamin D level is low but not super-low, in the 18 or 19 range. In that situation, I think it's useful to - I don't think that the person's low bone density is due to the vitamin D efficiency - and my clinical practice would be to replace that person with vitamin D, mostly because we know that the effect of Bisphosphonates is augmented in someone who is vitamin D replete. So it's important that the person, before you start them on Bisphosphonates, have adequate vitamin D levels, particularly if you're going to be giving them an IV Bisphosphonate like Zoledronic Acid, which may induce hypocalcemia in a person who has vitamin D deficiency and is not going to be able to defend against low-ish calcium levels. And so I tend to agree to check the vitamin D, but it depends on what the vitamin D level is, and how bad their bones are in making the decision about when to put the Bisphosphonates on-board. Clearly in a person who has low absolute risk of, or low-ish absolute risk of fracture, it makes more sense to try to do what you can with vitamin D and wait. In a person who has higher risk of osteoporotic fracture in absolute terms, I think it makes sense to get the vitamin D level up over the next 4 to 8 weeks, and then treat.

M.Borderi:
In the first case you presented you mentioned calcium supplementation of 3 g daily. The question is, is a risk, if any, of too much calcium supplementation; is there a sort of threshold for calcium supplementation?

T. Brown:
Yeah, I mean, it is a worry about how much calcium to give, and certainly in the general population, the pendulum has been swinging against regular calcium supplementation for risk of coronary heart disease. In this case it was a temporary thing where the idea was to give the person calcium to re-mineralized as well, and once the person has re-mineralized, you back off obviously. But there is a risk, of course, when giving calcium, that much calcium. The immediate risk is a bother for the patient, and that's constipation, and the other risk is that some of this, especially with a high 1,25 level, it will be absorbed across the gut and you'll get hypercalciuria, and there's a risk of renal stones there. So like everything, it's a balancing act. If the person did have a history of renal stones, I would probably be a little bit more ginger about how I would replace their calcium.

M.Galli:
Thank you very much. I think that we have to move on. The next speaker is Dr Joel Gallant and the title of his presentation is: How can Frailty, Aging and Comorbidities Affect Treatment Choice and Guidelines. Please Dr Gallant.

J. Gallant:
Thank you very much. I'm going to be speaking to you today not as a bone expert but as an antiretroviral expert, and talking about the choices of when to treat and what to treat with, mostly in terms of bone issues but I'll also touch on other issues that occur in patients who are aging. So, we haven't really talked about frailty yet. The phenotype of frailty is defined as at least three of the following five characteristics: unintentional weight loss, self-reported exhaustion, no physical activity level, a slow ability to walk 15 feet, and weakness defined by grip strength. This has been very strongly associated with adverse health outcomes in the elderly. If you look at frailty in HIV, which Todd has been doing a lot of work with, compared with HIV-negative men of similar characteristics, HIV-positive men are more likely to be frail. Frailty problems increase with longer duration of infection, and if you look at frailty prevalence for 55-year-old men with HIV for at least four years, it's similar to that for HIV-negative men who are over 65 years. And this represents this graphically, just showing the associations of age and duration of HIV infection on frailty. So HIV-positive people who have been infected for 8 to 12 years, at age 55, 13% exhibit frailty, which is 9-fold higher than the risk for age-matched controls. So the first part of this talk, however, is about when to start therapy and how does that relate to these issues that we're talking about today. As you may know, now in the United States the DHHS Guidelines recommend HIV therapy for all patients with HIV for two reasons. One is to reduce the risk of disease progression; the second is to reduce transmission to others. And the strength on the recommendation varies by CD4 count and by risk group. And the IAS-USA Guidelines, which is another set of guidelines that we use, essentially say the same thing: offer treatment regardless of CD4 count, and then it lists some other conditions that should strongly be an indication for treatment, including older age, in part because of the fact that older people tend to have poorer CD4 responses to antiretroviral therapy, even though they tend to have better virologic responses. But I think we could also stretch that argument a little bit and say that it may have to do with the issues of frailty and some of the complications of aging that appear to be more comment in HIV-positive patients. Now, one of the things that have pushed the Guidelines to be more aggressive in the United States has been association of low CD4 nadir with a variety of clinical outcomes, and at every major HIV Congress we add new data to this list. But we know that the low CD4 nadir is now associated with higher rates of HIV–associated neuro-cognitive disorders, arterial stiffness, which is thought to be a marker of atherosclerosis and cardiovascular risk, overt coronary heart disease, decreased bone density, increased risk of fracture, and malignancies. And the important

thing about these studies is that most of them show that this is an independent risk, specifically independent of current CD4, and current viral loads, so that the idea is that the patients pay a price for waiting to start therapy that can't completely be undone, even with good responses to antiretroviral therapy later.

In terms of bone mineral density, this is a combined analysis of a variety of ACTG ART-initiation studies. And if you use a CD4 count of 500 as the reference range, you can see that starting with lower CD4 counts significantly increases your risk of decreased bone mineral density and that there's a sort of dose-response relationship, if you will: the lower your nadir CD4 count, the greater your risk of bone mineral density change. Now these are data from the Dutch ATHENA Cohort just showing that older patients tend to have poorer response to CD4 count. And if you look at the solid lines - this is looking at CD4 trajectory based on your pre-treatment CD4 count - obviously the lower you are when you start, the poorer your CD4 count is even after 7 years, so that the people with the best responses are those who start with fairly high CD4 counts to begin with. But the dotted lines show that this is perhaps even more true with older people who have blunted CD4 responses to therapy. And this is in part the reason why both the DHHS and IAS-USA guidelines single out older age as a potential reason to start therapy even earlier than you would in somebody else with the same CD4 count.

So, all of that sort of suggests that antiretroviral treatment is a good thing, that treating early is a good thing, and that there may be a benefit in terms of not just some of the other complications I talked about but also bone mineral density and fractures. On the other hand, we also know that initiating antiretroviral therapy causes bone mineral density loss. These are a variety of clinical trials comparing different antiretroviral regimens, and almost all of them show a loss of anywhere from 2 to 6% of bone mineral density over the first 48 to 96 weeks of therapy. I think the one exception on this study is down here of the Lopinavir/ritonavir plus Raltegravir versus Lopinavir/ritonavir plus Tenofovir/FTC. Todd, I think you were an author on this study that showed actually no change or even an increase in bone mineral density with the Lopinavir/ritonavir/raltegravir arm, but that's the one exception to the list where almost everything else reduces bone mineral density, at least early on. We also have data from the SMART Study, which, if you remember, was a study looking at treatment interruption versus continuous treatment. And almost all the news with treatment interruption was bad. It increased mortality; it increased a number of complications. The only exception was with bone mineral density: when you stopped antiretroviral therapy, you saw increased bone mineral density at the hip and the spine, whereas there was a steady decrease in those patients were on continuous antiretroviral therapy.

So, it's a little bit confusing in terms of what is the overall effect of antiretroviral therapy - I'll come back to that in my conclusions, and it may be a topic of debate with both sets of colleagues on both sides of the Atlantic.

Now, what about the question of what to start with. Well, if you look at the DHHS Guidelines here in the U.S., all the preferred regimens include Tenofovir. Alternative regimens also include Abacavir. The IAS-USA Guidelines have added Abacavir back as a preferred nucleoside component of preferred regimens, although they do have footnotes about being cautious with Abacavir in patients who are at high risk for cardiovascular disease, and in patients with high viral loads. So, Tenofovir is of course very widely used but has been more closely linked with bone problems than any of the other antiretroviral agents. And in choosing antiretroviral agents for older or frail patients we have a lot of considerations. So of course, Tenofovir can be a problem in patients with impaired renal function, and certainly our older patients by definition have poorer renal function than they did when they were young. And then of course they may also be at risk for osteopenia and osteoporosis, which could be a problem with Tenofovir.

Abacavir is still not completely innocent. There is still concern about whether it increases the risk of cardiovascular disease and myocardial infarction. There have been a lot of very conflicting studies, with some studies clearly showing a risk, others showing no risk. Although I think there's been less debate on this than there was a few years ago, there's still this outstanding question that may or may not

ever get answered about whether Abacavir increases the risk of MI. And, of course, in older patients, many of them have multiple risk factors for a cardiovascular disease and these are the very ones that we would generally try to avoid adding another potential risk factor. Lopinavir/ritonavir causes hyperlipidemia and has also been linked with increased risk for cardiovascular disease. Now based on a recent DID study, Atazanavir/ritonavir is being associated with impaired renal function as well as both nephrolithiasis and cholelithiasis, which is certainly an issue for older patients. And of course Efavirenz is associated with dizziness and cognitive effects, which can be more problematic in older people than in younger people; not to mention the vitamin D issue that Todd talked about already.

So early on, studies looking at Tenofovir looked fairly benign with respect to bone disease. This was the old GS 903 study comparing Tenofovir with d4T. It was interesting that the baseline prevalence of osteopenia and osteoporosis was fairly high in the population, but there was no real difference in those outcomes between the arms, with minimal progression of pre-existing disease and no difference really in bone fractures in the two arms, and most of the fractures, with the exception of one on the d4T arm, were considered to be traumatic fractures. So that looked pretty good in terms of Tenofovir, even though the effect on bone density was known at that time. But more recently we've seen a study – ACTG 5224s - which compared both Tenofovir /FTC and Abacavir/3TC as well as comparing Efavirenz and boosted Atazanavir. And this study better than many, I think, showed a pretty clear difference between Tenofovir and Abacavir in terms of its effect on bone density, especially early on. Now you see it appears to stabilize over time, but there was a significance difference favoring Abacavir. What is talked of less is the comparison between Efavirenz and boosted Atazanavir; not as big of a difference but still significant. When we choose between Efavirenz and Atazanavir, we don't tend to think too much about bone, but in this case there was a greater loss in bone density with Atazanavir. And that's something maybe we can discuss afterwards because it certainly isn't something I think about very much.

These are the data I think we have already talked about from the Veterans Group just showing that cumulative use of Tenofovir as well as boosted PIs was associated with increased risk of fractures that were felt to be osteoporotic. There are limitations to the data in the sense that they didn't have bone mineral density data, and they couldn't verify that these were osteoporotic fractures, but it was one of the early studies to suggest that the decrease in bone mineral density with Tenofovir might have clinical implications. And interestingly, the PI association was there as well. It was limited to Lopinavir/ritonavir. Cumulative use of Abacavir, thymidine analogs and NNRTIs was not associated with a higher risk of fractures.

Now we used to, or at least I used to say, well, once you're on Tenofovir, if you have osteopenia or osteoporosis, there's probably no point in making any changes because the damage is done and now you have stability of bone mineral density. But recent studies have suggested that switching both to and from Tenofovir can result in changes in density. This study looked at patients who switched from AZT to Tenofovir and found that there was loss of bone density as well as increased markers of bone formation and bone resorption. And then this study looking at switching from Tenofovir to Raltegravir actually showed improvement in bone density after the switch. Which I think was maybe the first study that had shown that discontinuing Tenofovir and switching to a different agent could possibly restore some bone density.

So implications of the Tenofovir data, I think, are that we should be considering non-Tenofovir regimens in patients who already have osteoporosis and osteopenia. And - and this is new I think - that we should consider switching to non-Tenofovir regimens in patients who are found to have osteoporosis and perhaps, even osteopenia - again that could be a topic for discussion.

We've already talked about this. These are recommendations that Todd Brown was a senior author on, which recommends bone density screening for postmenopausal women with HIV, men over 50 with HIV, and patients with fragility fractures. So this is typically how we're going to be identifying these people, either older people who are coming in to start antiretroviral therapy, or people who

have been on antiretroviral therapy and get older on therapy - that's typically how we find evidence of osteopenia and osteoporosis. Now what about avoiding nucleoside analogs altogether since a lot of the problems that I've discussed of treating patients who are older or who have fragility, or risk factors for fragility, have to do with the nucleoside analogs. Unfortunately we really have no perfect nucleotide–sparing regimen yet, at least that has adequate data. All of the studies of nucleotide-sparing regimens to date have suffering from either being too small to be definitive, showing too much toxicity, or showing poor biologic efficacy. Fortunately we have two fully powered comparative studies ongoing. One of them is the NEAT Study that I'm sure you're familiar with, being done in Europe, with Darunavir plus Raltegravir; another is Darunavir plus Maraviroc. Now our experience with Darunavir/raltegravir up to date has not been good, despite the fact that you would think this would be a great regimen. This was a single-arm study, unfortunately, looking at this as a first-line regimen for patients. And in patients with viral loads over 1000 at baseline, there was a 43% failure rate. This was very puzzling because you'd expect boosted Darunavir monotherapy to do better than that. And yet here by adding Raltegravir you get poor results. But I think we're all encouraged by the fact that in the NEAT study in Europe, the arm continues, the study continues, suggesting that these results from the ACTG study were a bit of a fluke, and that things seem to be going OK in this much larger clinical trial. So I am hoping that we will finally, within the next few years, have data on acceptable nucleotide–sparing regimens that might be good choices for people with some of the problems we've been discussing.

T. Brown:
Is there a bone sub-study?

J. Gallant:
I don't know. I bet some of the people here in Bologna know. We can talk about that.
Now the other bright news on the horizon is a nucleoside that may not have these issues, and that's a new pro-drug of Tenofovir that we call TAF. Of course Tenofovir dF it is also a pro–drug, but this is a different pro-drug that gives you higher intracellular Tenofovir diphosphate levels and lower circulating plasma Tenofovir levels. And as a result, you can give a much lower dose. You actually get better biological response, and the idea is that this could in fact reduce both nephrotoxicity and bone toxicity. And at CROI just last month, we saw results from the Phase II study from Gilead where they compared what we now call Stribild or Quad with a similar single-tablet regimen using TAF instead of TDF. So it was E/C/F/TAF versus E/C/F/TDF. And we see at 24 weeks the results biologically look very similar: good responses with both arms. But more importantly, in terms of bone density, you saw the usual expected loss of about 2.5% in bone density over 24 weeks, whereas with E/C/F/TAF the curve was virtually flat and the difference probably wasn't significantly different from zero.
So that was encouraging that there may be something to this idea of higher intracellular levels with lower plasma levels, at least in terms of bone. And I don't think I show here the renal data, but again, the renal data suggested that there may be also some advantages of TAF over TDF in terms of nephrotoxicity. So it may be that we will soon have a nucleoside analog where we don't have to be quite so concerned about some of these issues that I've talked about.
So to conclude, I think we can safely say that untreated HIV is bad for your bones, based on the data we have. I can also say that I think that's some ART is probably bad for your bones. And I'm guessing here that untreated HIV is worse for your bones than ART is but we can talk about that. I think that in elderly or frail patients we have to consider many factors, including effects on lipids, cardiovascular disease, kidneys and bone. And for patients who are not good candidates for either TDF or Abacavir, I think there is considerable promise for both nucleoside-sparing regimens in the future as well as the use of other nucleosides, and specifically TAF. So I'll stop there and thank you very much for your attention.

M. Borderi:
Thank you very much, Joel for your very nice pre-

sentation. It's a pleasure for me to introduce the last speaker of the day, who is my friend Giovanni Guaraldi. Giovanni is founder and in charge of a metabolic clinic in Modena, a town 25 miles from here, where the head physician is Professor Cristina Mussini. Giovanni's talk is Bone and Frailty in HIV.

G. Guaraldi:
Hello. Hello everybody and thank you Marco, for allowing me to participate in this very interesting discussion. I was asked to present a case and so here is a lady, a young lady 45 years old - anybody who is younger than me, or my age, is young, and anybody who's older than me is old. And I've got a disclosure that I'm getting old as well! Nevertheless I would like to tell you about the story of this lady who actually got HIV when she found out she was pregnant. Unluckily at that time she decided not to go on with the pregnancy and she discovered that her CD4 cell count was still low. And so she was a late presenter. The nadir CD4 was the CD4 at presentation, 280, and viral load was 12 000 copies. She was HCV and HBsAg negative, and at that time she was offered antiretroviral therapy immediately with Combivir and Nevirapine. Actually things changed over time, and as happens many times, there are some live events that make adherence poor. In fact she started to have a detectable viral load and decided to stop therapy because she developed a mood disorder associated with her life because she had separated from her partner. She didn't get any therapy for a while. In fact she had a screening for cervical cancer, and cervical dysplasia of high grade was diagnosed. After some time she decided to return to the HIV clinic, and in 2005 she was offered antiretroviral therapy again with the association of AZT + 3TC, that actually was the drug she had had the first time, and the Nevirapine was now switched to Lopinavir. A few years later, she started to complain of lipoatrophy, and we know this was a very common condition especially in patients on a thymidine analog. This is a picture of the patient when I first saw her. It was 2011. She was being followed in another centre. She came to the Metabolic Clinic for assessment, and you see at that time the CD4 cell count was good. She was 585 CD4 cell count, and viral load was undetectable. Actually she was normal body weight and her BMI was 22.9. Nevertheless she had quite a clear sign of mixed form of lipodystrophy. You can recognize that the leg fat percent was only 12%, that is quite low in a woman, but she had quite a remarkable increase in additional adipose tissue regardless of the fact that she was not fat. And if we move to the assessment of her metabolic condition, you can see that her HOMA was 6.3, just to demonstrate that she had insulin resistance, and definitely she had hypercholesterolemia. LDL was very high: 186 mg. No increase in blood pressure. With regard to her DEXA assessment, you can recognize that the Z-score was within the normal range with regard to the lumbar spine while she had osteopenia of the femoral neck because her Z-score was -2.4. And you can recognize that she had a low vitamin D level; this is quite common in our geographical area. Hemoglobin: she had quite significant anemia but this was somehow expected because of the AZT therapy she was taking at the time. But you see what generally we do is not just to look at the data but try to use these data to predict the risk; and so what we did was to estimate the risk for cardiovascular disease, and you can see that the Framingham risk score is 8%, somehow in the intermediate value, and the FRAX that was estimated - even if she was quite young, she was nevertheless older than 40 - is quite high: it's 4% for the femoral neck. And there was mild impairment in glomerular filtration rate. Dr Gallant before mentioned the VACS index – actually we're able to re-build the VACS index of all our patients, and here it was somehow in the intermediate range. But once again I would like to point out that maybe this was mainly driven by the anemia that may have been induced in this case by the antiretroviral regimen she was on. And second, we should also mention the fact that the FRAX index does not apparently correct for gender, and we know that for instance, normal value hemoglobin for women is definitely lower than in men. Actually I want to mention this because Professor Antonella d'Arminio Monforte recently

showed us some preliminary data from her cohort in which she was able to demonstrate that with regard to the VACS index, the female gender appeared to be somehow at higher risk just because of the hemoglobin value that is expectedly lower in women.

What happened at that point? Well, it was quite obvious for us to switch out from AZT that was mainly driven by the lipodystrophy story. We knew at that time that of course AZT produces lipoatrophy, which is entirely associated with the length of exposure to this risk. And what happened? Actually nothing happened from a virologic point of view because she continued to be well suppressed: CD4 was still very high. But something changed with regarded to BMI and to body weight, for in fact she gained weight this time. And this was no good because you can see clearly that actually the vast majority of this weight gain appeared to be in the abdomen with significant increase in the visceral adipose tissue value. Let us move to the metabolic parameters. You see that apparently glucose metabolism was good, was not worse: actually it was going better. We didn't have such a good value with regard to the metabolic risk profile and maybe this was not expected because our suggestion was that at this time therapy should have played a role in cholesterol levels. But at this time point this did not seem to be apparent. And also if you look at what was happening to her BMD values, we realized that there was a very severe decrease in the BMD value both at the lumbar spine and femoral level. Of course this was a two-year time period in which we observed the patient (this was January this year), and you can see that actually what had happened was that the Z-score moved from -0.5 to -1.5 and the femoral level from -2.5 to -3.1. We try to give some explanation to this, and we thought a possible explanation might have to do with the endocrine values. In fact what you can recognize here is that in this two-year period she passed the menopausal transition period. And so actually what's very clear here is that estradiol levels dropped almost to undetectable levels and FSH and LH increased. In fact this is an endocrinological diagnosis of menopause. Interestingly, the patient didn't tell us and in fact we had to ask her if she had had any changes in her menstruation periods, and she admitted that she had not had menstruation is for almost a year. She was reluctant somehow to tell us, and this psychological condition sometimes happens in women. They are shy to tell the physician about this situation. As to the kidney, there was no problem but in fact her hemoglobin levels were increased and this may be due to the fact that she had switched out of AZT. But you see once again that the VACS seemed to improve. Actually, once again, I believe this is driven by the hemoglobin change secondary to the change of antiretroviral therapy.

This diagram tells us and we must consider in the lifespan of women that there is a menopausal transition period, and this is a very important period to recognize because we know there is an early postmenopausal period in which they are quite profound changes in the metabolic condition of women. And we know in this period of time we can expect a much higher decrease in bone mineral density than in any other time of a woman's lifespan. But after two years this seems to stabilize.

I was very curious to try to interpret all of these trajectories in the metabolic parameters I have described within the description of frailty. And I think that we have a very unique opportunity to describe aging in HIV-infected women in particular, because if we've got, lets us say, many arbitrary definitions of aging, defining an age cut-off, maybe 60, maybe 50, well, there's a physiological aging period of transition which is menopause in women. And so I think we should consider analyzing the different conceptual approaches of frailty with particular regard to women going into menopause. And here in this slide I would like to sum up the fact that so far there have been two major conceptual approaches to frailty. One is what Dr Gallant just showed us, that is the Linda Fried definition that is the phenotypic definition of frailty that has been described especially in the MACS cohort in the U.S. in the very many publications by this group. But there's also another approach that has been recently introduced, that is the group of Amy Justice that tried to give a

score. And this is the interesting part because having a quantitative assessment of the score it made tell us how things change across the period. And so, of course, these are the frailty–related phenotypes that have been described, and this is the VACS index that can be considered, let's say, a different approach to assess frailty at the same time, given that it does predict mortality very well, and not only the HIV–related mortality, but also the non-AIDS-related mortality. Nevertheless I believe that a lot of the information may be lost with regard to daily living conditions associated with frailty that actually are very important from the clinical management point of view of an aging patient.

And so in my view we are far away from having a good clinical definition of frailty in HIV-infected patients. As has been shown, we have a frailty phenotype that actually has been shown to depict quite well this proportion of frailty. This diagram tells us that we expect an increased prevalence of frailty in people will have low CD4 cell count, high viral load, and will of course may also have an AIDS-defining condition. Actually all the studies that use the Linda Fried definition depicted this part of the frailty condition, i.e. frailty associated with a condition of immune deficiency. What I think is still missing is a good definition of frailty in patients, like my patient here, who are doing well as regards CD4 cell count, are well suppressed for many years even if they may continue to have chronic inflammation that we can call an inflammation–inducing situation. In this situation we will not have any AIDS-defining condition but we have an increasing prevalence of non-infectious comorbidities. Of course, if we take this into account we need to ask ourselves which is the best drug regimen we should offer. And I think that Dr Gallant's presentation before was very, very informative for us, and told us what the best options are. But my impression is that really we have so far no good clinical comparative studies that tell us which is the best treatment to be given because we don't have frailty as an endpoint in clinical studies so far, while in the future, I believe, that as soon as we have a good definition of frailty, this may become an endpoint in clinical studies and allow us to address the different impact of different drugs with regard to the aging process.

This slide has already been shown. It tells as that we expect to have an increased risk associated with Tenofovir, and this may be an issue in my patient who is growing old.

So I would like in the next two or three slides to present a study that was inspired by Mike's work. We know that he published two interesting studies assessing prospectively the BMD changes both in pre-and postmenopausal women. And so what we did was to study the same process but across the menopause transition period. The aim of this study was to describe BMD changes in women entering menopause and to evaluate the predictors of BMD change in both the pre-, the early, and in the late menopausal period. The inclusion criteria were at least one DEXA assessment in the pre-menopause and one in the postmenopausal period, but you will see that most of our patients had multiple DEXAs, both before and after entering menopause. This is the number of DEXAs that were evaluated. You can see that we included the menopausal transition period in which we had 56 DEXAs - the cohort comprised around 50 women – and 125 in the early menopausal period, and 63 in the late menopausal period. These periods were classified according to hormone levels that told as the presumptive menopause date in all these women. You can see that the age of these women was still young: 46 years, and that most of these women had a low nadir CD4 - less than 400 - their median nadir value was 161. What I can tell you is that in the baseline evaluation, that is when women were in the pre-menopausal period, the prevalence of osteopenia was 60% while the prevalence of osteoporosis was only 3%. But of course entering menopause, this proportion changes and a significant number of women previously at an osteopenia threshold became osteoporotic on entering the menopause. So at the end of this study we had 45% of women who were osteopenic, and 21.8% in the osteoporosis class.

This table gives you the most frequently used antiretroviral regimens that have been used in this population. You see that nearly half our patients

were undergoing 2NRTI plus 1 NNRTI - this is the most frequently prescribed regimen in our country. And half our women were in fact taking Tenofovir and were entering menopause while still on Tenofovir therapy.

This is the lowest smoothing curve that actually depicted the median value of lumber BMD in our population. As expected, BMD values decrease across the menopause period, and this is totally physiologic and to be expected. But actually what we were able to show was that there was a different trajectory of BMD loss in 50% of the women who were entering menopause while on Tenofovir and who had a steeper slope in their BMD decrease compared to the other 50% entering menopause not under treatment with Tenofovir. And actually if we were to put a value on the expected change in lumbar BMD, what you see is that women entering menopause undergoing Tenofovir therapy at the time of entering menopause, there was a yearly change in lumbar BMD that is less than 4.7%, which is statistically different to what is expected, what is shown in women entering the menopause without Tenofovir. And I would like to point out that this change, this slope is very similar to the slope that has been demonstrated in women starting antiretroviral therapy. And so this tells us that it is possible that actually this is a second step of BMD lowering. The first step is when you start antiretroviral therapy. But the second step is if you enter menopause, and this will tell is why we see such an acceleration in the decrease of BMD levels. Apparently, however, this seems to be compensated for after two years after menopause, because after two years we were no longer able to find any difference in the yearly change in lumbar BMD values in women on Tenofovir compared to women not on Tenofovir as a backbone therapy. Of course we looked for traditional and HIV-specific risk factors with regard to the risk of BMD change in the transitional and in the early, and in the late menopausal period, and you can see that this was somehow expected because of the traditional risk factor, that was, for instance, years. Post-menopause was independently associated with lowering of BMD values, as well as higher BMI appeared to be protective. What was interesting to see was that apparently there is a higher risk factor associated with PI use. We also find an independent risk factor associated with Tenofovir. But in particular we find a higher risk factor associated with the two together, that is, boosted PI plus Tenofovir appeared to, let us say, explain quite a proportion of the effect of the increased loss of BMD in the early menopause period. And so I think that the study tells as that now we are facing a very particular condition because we know that the vast majority of our women in the HIV cohort are now in the transitional period. So we must ask ourselves if we need to adopt a different approach or intervene to avoid a greater loss of BMD values.

I would also like to point out that this lady had quite a high Framingham risk score, and for this reason we offered her a calcium assessment of the coronary. And you see that the total calcium score was not high, but was significant. This means that she didn't simply have higher risk but she had in fact a subclinical cardiovascular disease. And I say this because I would like to show you what we presented last year at the Washington meeting when we pointed out that there is somehow an association between an increased cardiovascular risk together with a low BMD value, and so some way there's crosstalk between the bone and the heart that must be explored, and any time we see a fall in BMD, we should think that this may be associated with an increase in the deposition of calcium in atherosclerotic plaque. Of course this is interesting because it may be associated with the new biomarkers, and actually there was an excellent presentation by Sharon Lewin at CROI this year. But I can tell you that the only biomarker that has been explored thus far is a CRP and an interleukin-6 as well as a T-cell activation marker. But I would like to point out that most recently there are other biomarkers that seem to tell us something about the association of bone, heart and kidney at the same time. And so these are some studies done in HIV-negative populations that are now going to be reproduced in HIV-infected patients and I think that FGF 23 is a promising biomarker that will tell us something about the association between the bone, the

heart, and the kidney in all these patients.
Now I will go back to my patient. The point is now to decide what the best treatment for this patient is. I would like to leave this open because I think that so far we do not have enough data to say that any of all these possible labeled treatments that have got have comparative studies so as to suggest which is the best treatment. I would like to mention the fact that more and more we are offering patients a nucleoside–sparing regimen to treat this condition. Nevertheless I think that all the new approaches need to be studied in the future so that we will have better management of these patients. Thank you so much.

M.Galli:
So we have just time for two short questions, and of course, short answers. Giustino.

G. Parruti:
Giustino Parruti, Pescara, Italy. I want to thank everybody and would like to ask two short questions. First of all I didn't hear a single word about the possible use of a strontium ranelate in the context of HIV infection, which has been episodically reported. It would be interesting to know from this panel if you think it could be of any use for bone remodeling and modulating the metabolic proteic part of the bone. This would be very interesting to know. And the thing is what about not taking into account for the moment osteopenia in patients with very low risk for bone fractures at present? I would be happy to hear a bit of speculation on this. Is this something that we can disregard at present, all would it be just better to consider in the whole story of this situation a bit more from the beginning?

G. Guaraldi:
I have no experience at all with strontium ranelate. I don't know if you have any…

T. Brown:
Strontium ranelate's an interesting compound that not only decreases bone resorption but increases bone formation. So it does all the right things to the bone. The mechanisms aren't clear but the problem with strontium in the US is that it is not approved. So we have absolutely no experience with it here. But I agree it's an interesting compound to study.

M.Galli:
Another short question–a very short one? Is that okay for everybody. So Marco, the final remarks are up to you of course because you are the organizer of this meeting.

M. Borderi:
Thank you, Massimo. My closing remarks – tempus fugit. It has been a pleasure for me to participate in this very interesting cross- Atlantic session videoconference between Bologna and Baltimore. First of all I would like to thank our special guests from the U.S.: Joel Gallant, and my friend Todd, and Mike. Thank you very much. And I would also like to thank my Italian colleagues for coming here to this meeting. I hope you will continue to discuss these interesting issues in the future. So thank you everybody.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