Hot News

Bone Mineral Density Loss, Osteoporosis, and Fractures in HIV

The link between HIV and reduced bone mineral density, including osteopenia and the more severe osteoporosis, is well established. HIV infection has also been associated with an increased risk of fractures. It is so far unclear whether this is due to HIV itself, resulting inflammatory and metabolic changes, antiretroviral toxicity, or some combination of factors. This topic was the focus of major debate at the 7th IAS Conference held in Kuala Lumpur, Malaysia, in early July 2013.

Several studies have looked at bone changes after starting initial antiretroviral therapy (ART) or switching drugs while on suppressive treatment. In general, initial ART typically shows early bone loss soon after starting therapy, which stabilizes after a few months. Bone loss has generally been linked to the use of nucleoside reverse transcriptase inhibitors, especially tenofovir, and protease inhibitors.

It remains unclear if reduced bone mineral density in HIV patients may lead to clinical consequences such as fractures. Knobel, et al. from Barcelona (IAS 2013, Abstract WEAB0205) conducted a population-based cohort study to explore the association between HIV and major fractures, in particular of the hip. The researchers looked at patients in a Spanish database containing information on more than two million individuals receiving care within the public health system. Out of 1,118,587 patients aged 40 or older, the authors identified 2,489 (0.22%) who had a diagnosis of HIV or AIDS, with a median follow-up period of three years. The incidence of hip fractures and major osteoporotic clinical fractures involving the spine, wrist or forearm, pelvis, and humerus was examined during 2007-2009. People with HIV sustained a total of 49 clinical fractures during the study period, with 12 being hip breaks. In comparison, there were 24,408 total fractures, including 7,299 hip breaks, among HIV-negative individuals.

After adjusting for other factors that may influence bone risk (gender, age, body mass index, smoking, alcohol consumption, and other comorbidities), the hazard ratio for hip fracture among HIV patients was 4.7, or about five-times higher likelihood. For all clinical fractures, the hazard ratio was 1.8, or nearly twice the risk. Both were statistically significant.

Breaking the findings down by age, the researchers found that HIV-positive and HIV-negative people had similar fracture risk until about age 65, at which point HIV patients began to show an elevated risk. At around age 70, there was a dramatic jump in fractures in the HIV-positive group. However, there were few people with HIV in the oldest age group, suggesting these results should be interpreted with caution. Another limitation of the study was that the investigators were not able to consistently distinguish between fragility fractures (due to weak bones) and traumatic fractures (due to injury). Furthermore, there were no details about the patients' HIV disease status or antiretroviral treatment.

Like HIV, hepatitis C virus (HCV) has been independently linked to decreased bone mineral density. Both HIV and HCV cause persistent inflammation that may worsen bone loss, and liver damage related to hepatitis C may also play a role. Bedimo, et al. from the University of Texas (IAS 2013, Abstract WEAB0204) looked at mechanisms contributing to bone changes in people with HIV, hepatitis C, and HIV/HCV coinfection. The authors made a cross-sectional examination of 168 men, of whom 62 had HIV alone, 45 had HCV alone, 28 were coinfected, and 33 had neither virus. The average age was about 55 years. The majority were African American, followed by Caucasians. About half were smokers, with a higher proportion in the uninfected control group. The HIV-positive participants were on suppressive ART, with about 75% taking tenofovir and 55% using protease inhibitors. As expected, HIV and HCV were both significantly associated with lower bone density T-scores at the femoral neck and hip. Spine bone density was also lower. No interaction was seen between the two infections.

Both viruses increased the risk of osteopenia and osteoporosis. Rates of osteopenia were 58% in the coinfected group, 50% in the HIV only group, 57% in the HCV only group, and 37% among uninfected controls. For osteoporosis, the corresponding rates were 8, 13, 5, and 0%, respectively. HIV and HCV infection also affected levels of biomarkers associated with bone formation, resorption, and turnover. The researchers measured osteocalcin, osteoprotegerin, C-telopeptide, and receptor activator of nuclear factor kappa-B ligand (RANKL). While people with HIV showed evidence of both higher bone formation and resorption, there was no increased resorption among individuals with HCV alone. This could be due to differential effects on osteoprotegerin and RANKL, which influence bone destruction by osteoclasts.

The researchers concluded that HIV and HCV independently lower bone mineral density and T-scores. The impact of HIV could be explained by increased bone turnover, which does not appear to be driven by the RANKL/osteoprotegerin system. HCV, in contrast, does not seem to increase bone

resorption. Thus, bone loss in HIV patients is mostly driven by ART and not HIV itself. Immune reconstitution on ART may also play a role, as better viral suppression inversely correlates with bone mineral density.

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A Multi-Step Pace Towards a Cure for HIV: Kick, Kill, and Contain

The 7th IAS Conference held in July 2013 in Kuala Lumpur, Malaysia, heard about a number of cases of "functional cure" in people who had started antiretroviral therapy soon after HIV infection, including a German case that can now be added to the "Mississippi baby" report presented at CROI 2012 and 14 individuals of the French VISCONTI cohort. All these persons maintained an undetectable viral load after coming off antiretrovirals.

The majority of subjects that do not start antiretroviral therapy until later on are very unlikely to achieve control of their HIV replication. Unless suppressed early, the HIV genome integrates into the chromosomes of some of the long-lived central memory cells in the immune system. As long as people are on antiretrovirals, the provirus is prevented from replicating, but as soon as treatment stops, these cells – only one in a million of which may contain the provirus – start producing HIV again, the virus reappears in the blood, and more cells are infected.

Several different approaches are being tried to halt ongoing HIV infection. The one that has received the most attention is the "kick and kill" strategy. Initially, gene-stimulating drugs are given that "kick" quiescent central memory cells, which then become activated and produce HIV. As long as virus remains suppressed at controllable levels with antiretrovirals, the hope is that by becoming activated, the cells will turn into "effector" cells with short lives, and the so-called reservoir of long-term infected cells would be drained. If the reservoir is sufficiently emptied, the body's own immune surveillance could keep HIV suppressed. However, a study where HIV reappeared in a subject with fewer than two in a billion HIV-infected reservoir cells casts doubt on this prospect. There may need to be further stages where drugs are given that actively target and kill off the activated reservoir cells, driving their number down still further, and then an immune therapy might be given that magnifies the body's natural immune response to HIV and contains the activation of the tiny number of remaining infected cells.

The class of drugs furthest along in investigations into reservoir-cell activators is a group called histone deacetylase (HDAC) inhibitors. Some are already in use as anticancer drugs as they activate genes that can kill cancer cells. They include panobinostat and vorinostat. The latter has already been shown to induce a 4.8-fold increase in HIV gene expression in reservoir cells after one dose. However, so far no production of the viral proteins that would stimulate an immune reaction to HIV has been seen. In contrast, panobinostat reaches levels 10-times higher in the cells than vorinostat. In the CLEAR trial (IAS 2013, LB-H589), Danish researchers gave 15 HIV-infected men 12 doses of panobinostat over eight weeks, three doses per week on a week-on, week-off basis. Patients had been diagnosed with HIV for between six and 28 years, and had spent between two and 16 years on antiretroviral therapy. After the first dose, 60% of participants expressed low but detectable HIV RNA in their blood, compared with only 28% before panobinostat, and only one of the 15 participants showed no detectable HIV RNA throughout the study period. The authors are currently testing groups of reservoir cells to find out how many remain with hidden HIV infection and how many can produce replication-competent HIV. The hope is that, if panobinostat can drain the reservoir sufficiently, it might be safe to take people off antiretroviral therapy for a monitored treatment interruption.

The HDAC inhibitors are not the only drugs capable of turning long-lived cells with occult HIV infection into short-lived cells visible to the immune system. Beta-catenin inhibitors can also do it, according to findings from Harvard researchers (IAS 2013, abstract TUAA0102). Beta-catenin is a body protein that stops stem cells from differentiating into memory cells. Drugs that inhibit it might be able to reach a small but important part of the HIV reservoir that appears to be most hidden and less susceptible to activation. A beta-catenin inhibitor turned, *in vitro,* 75% of former stem cells into effector-memory cells, namely stimulated cells that are actively producing HIV. Use in combination with panobinostat appeared to roughly double the cell-stimulation effect of beta-catenin.

As mentioned above, death of activated cells and immune surveillance may not be enough to deplete the HIV-infected reservoir sufficiently. Additional therapies that actively seek out and destroy the cells activated by HDAC or beta-catenin inhibitors may be needed. Researchers from North Carolina explored whether this cell-killing missile could be 3B3, a broadly neutralizing antibody that attaches exclusively to HIV surface proteins, and is joined to a toxin, PE38, derived from *Pseudomona* (IAS 2013, abstract TUAA0101). The antibody attaches itself to activated cells from which HIV is budding, and the toxin then enters the cells and kills them. This molecule was injected into mice that had been genetically altered so they could be infected with human HIV. Three weeks later they were started on antiretroviral

therapy. After four weeks they were given two weekly doses of 3B3-PE38. Antiretrovirals, as expected, produced a 2.1 log drop in HIV RNA inside cells, but the bacterial toxin produced a further 0.8 log. More importantly, the absolute number of cells expressing HIV RNA decreased from between 1,100 and 20,000 per gram of tissue to between 600 and 3,000 per gram, a sixfold drop in the presumed size of the reservoir.

Immune therapies and therapeutic vaccines that contain any onward infection of HIV from activated cells to others may also be a crucial part of the "kick and kill" strategy, both in order to encourage the body to kill or contain the tiny fraction of HIV-infected cells left after reservoir draining and to prevent onward infection of HIV into new cells during the "kick" phase. French researchers tested whether natural killer (NK) cells, which represent the body's first line of defense against viruses, could assist in this objective (IAS 2013, abstract TUAA0103). First, dendritic cells were incubated with a candidate vaccine, in which HIV proteins are wrapped up inside the shell of another harmless, modified *Vaccinia* virus. These dendritic cells were then mixed with NK cells. Once sensitized to HIV and able to recognize and kill cells expressing HIV proteins, they were mixed with CD4+ T-cells and other dendritic cells. HIV was then introduced. The presence of the sensitized NK cells reduced the proportion of dendritic cells infected with HIV from 45 to 25%, and of CD4+ T-cells from 35 to 20%. This is only one of a large number of therapeutic vaccines devised, but it stimulates an immune response in a different cell type, NK cells, that had been sensitized to HIV before, and one that, because its response to HIV is less specific, may be less prone to cells evading its immune control.

Altogether, these experiments are in very early clinical or preclinical stages. They are designed to drive ongoing HIV infection down to the absolute minimum, a level where it may, in the long run, be possible to take even chronically infected people off antiretroviral therapy for long periods without HIV reappearing. It may be a number of years before this is turned into an effective strategy, even if the "kick and kill" approach turns out to be the right one.

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Chronic Hepatitis E: Knowing What to Look For, So It Can Always Be Found

Hepatitis E virus (HEV) infection constitutes a leading cause of waterborne epidemics of acute hepatitis in developing countries, mostly in Southeast Asia and Africa. In these areas, the seroprevalence for HEV antibodies in the general population may be as high as 20-40%, and HEV genotypes 1 or 2 are responsible for an estimated 20 million incident infections every year. Most cases are non or mildly symptomatic, but mortality may rise to 30% in certain risk groups as the elderly, patients with chronic conditions, or pregnant women (Hoofnagle, et al. N Engl J Med. 2012;367:1237-44).

A growing number of cases of hepatitis E have been reported in Western countries since 1997. These cases were initially considered as imported infections from endemic countries, but in most instances no history of travelling was identified. It was also observed that HEV genotype 3, and in rare cases genotype 4, was responsible for these sporadic infections rather than genotype 1 or 2 as observed in endemic areas. The epidemiological picture was finally framed when HEV genotype 3 was found as a zoonosis affecting pigs and certain wild mammals such as wild boar or deer (Colson, et al. J Infect Dis. 2010;202:825-34). As for genotype 1 or 2 infection, hepatitis E in Western countries occurs as an acute disease, usually with full recovery except at-risk populations.

It was in 2005 when the first reports of chronic liver disease caused by HEV genotype 3 appeared in the literature. In all cases, patients receiving chemotherapy or immunosuppressants were affected; HIV infected patients with chronic hepatitis E were reported later (Renou, et al. AIDS 2010;24:1493-9). Rapid progression of liver damage to cirrhosis and end-stage liver disease seemed to be common characteristics.

Two cases of chronic hepatitis E genotype 3 in HIV patients with CD4 counts < 200 cells/ul have been recently reported (Neukam, et al. Clin Infect Dis. 2013;57:465-8). Both had progressed to cirrhosis in just three years, and one of them had developed esophageal varices. Interestingly enough, a cycle of oral ribavirin at 1,000-1,200 mg per day for 24 weeks was able to clear the virus in both cases, although longer follow-up is needed to confirm sustained HEV eradication. Other reports have shown therapeutic success with ribavirin and pegylated interferon, either alone or in combination (Alric, et al. Ann Intern Med. 2010;153:135-6).

Therefore, screening for HEV in HIV-infected individuals presenting unexplained liver enzyme elevations or with liver fibrosis of unknown origin should be included in the diagnostic evaluation in order to early detect chronic hepatitis E.

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