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Pediatric HIV Infection: What's New?
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FEMALE SPEAKER: - the next session, and it's my pleasure to introduce Philippe van de Perre, who is a longtime friend and colleague, and he is going to be talking about breast milk pathogenesis.

PHILIPPE VAN DE PERRE, M.D., Ph.D.: Thank you very much, Linda [misspelled?]. First of all, I would like to thank the organizers of this conference for having invited me to present an overview on the pathogenesis of HIV transmission by breastfeeding, and I think this is particularly timely and relevant. Since, as you all know, the saga of breast milk transmission of HIV started in this country, in Australia, with the first observation made by our chairman of today, John Ziegler, about 22 years ago. Today, transmission of HIV by breastfeeding is recognized as being the cause of approximately 300,000 new infections each year.

But the pathogenic mechanism of HIV transmission by breastfeeding are particularly difficult to study. Indeed, the host, it's just the young infant, a newborn, and constant evolution and may be exposed to the pathogen at the moment their mucosal defense and their capacities to mount highly efficacious immune response are still in development.

On the other hand, breast milk is a biologically active body fluid of evolving and very complex composition. For

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example, colostrum and transition milk are characterized by a high cellular content that decrease rapidly over time. And concentration of antibodies and of other soluble factors in milk evolve in different ways, and consequently, the composition of nature milk is not comparable at all to the composition of earlier milk samples.

One of the characteristics of HIV is to be distributed and to diversify according to the constraints of different anatomic sites. This is particularly true for the mammary gland and breast milk. By a very careful cloning and sequencing of cell free and cell associated HIV in blood and colostrum of three HIV infected women clear [inaudible] confirmed this compartmentalization. Indeed in this study, major and minor viral populations were differently distributed in blood versus colostrum and in cell free versus cell-associated proviruses. In some cases, the major population in one anatomic site were only minority in the other one like in this case, for example the majority in colostrum and the minority in blood. And in many other cases, the species of 7-1 were not detectable at all in the others like in this case and this one. Furthermore, different distribution of HIV quasi-species in the right versus left breast, and in some women in different fractions of breast milk have been recently demonstrated suggesting difference in local microenvironmental

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selection preference. This compartmentalization is responsible for different expression of resistance profile between plasma and breast milk being found in half of the women receiving nevirapine in the HPTN023 Study in Zimbabwe. The portal of entry of the virus on baby mucosa exposed to HIV by breastfeeding remains largely mysterious.

HIV from breast milk could cross babies epithelial surface through different potential ways and gain access to lamina propria by breaches in the integrity of the mucosa like here, by transport through epithelial cells through a very specific transport mechanism known as transcytosis by gaining access to digitation of dendritic cells infiltrated into the epithelium, and by transcytosis to a very specialized M cells, which is a cell of epithelial origin which represent about 2-percent of the [inaudible] epithelial cells. These very specialized cells do not express glycocalyxes [inaudible] and extremely effective in transcytosing antigen from the lumen to a very large intracytoplasmic pocket from the basal membrane which is in close vicinity with cells protruding from the submucosa.

This picture was taken by Mayan Nurtri [misspelled?] a few years ago and shows the surface by electromicrography the surface of M cell from a rabbit and the attachment of an HIV viral particle at the surface of the epithelial surface of the

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cell. The cell membrane receptor in this case for this viral particle is not CD4 but is galactosylceramide which is present on the surface of epithelial cells. These particles are internalized in endocytic vesicles and transported and released at the basal cytoplasmic pocket of the M cell in very direct vicinity through the T lymphocyte from the submucosa. This mechanism has been observed for rabbit M cells, but as far as I know, has never been reported for human beings. Using a very elegant ex vivo model of monolayer viable human anthracite, Morgan Bomsel [misspelled?] showed that free HIV particles were not able to cross human anthracite by transcytosis.

However, when an infected cell is presented to this epithelial surface, it gets polarized. It delivers viruses that are able to be transcytosed across the epithelial cells, and these viruses are delivered through the submucosal cells, and this transfer has been shown to be able to be blocked by dimeric IGM and IGA present in the lumen. By means of this model, the nature of the interaction between the infected cell and the anthracite surface has been carefully scrutinized. After a very complex cell-to-cell signaling, the true virus in that is indeed formed as a sequence of the recognition of a membrane A-ring with the viral GP41 that favors interactions between the infected cell membrane and the anthracite [inaudible] cell receptor and finally trigger transcytosis

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through an integrated associated mechanism. However, the infant intestinal mucosa is not the only candidate as a portal of entry for HIV. Indeed, in a [inaudible] model a non-traumatic inoculation of [inaudible] with cell free and cell associated HIV was successful in transmitting the virus.

Several epidemiologic studies have demonstrated an association between both cell free and cell associated viral load it makes and the risk of post-natal transmission to babies. The question is whether both viral reservoirs are really involved in transmission. A clue to this enigma has been obtained by a very careful study performed by Colleen Scott a few months ago. In babies with evidence of post-natal HIV transmission she compared envelope sequence, a signature of the quasi species population in infants' blood as well as in cell free and cell associated reservoir in the make. In early transmission events occurring before nine months of age, the infant virus clustered very closely to the DNA integrated virus observed in the maternal breast milk. And in the opposite in the transmission events occurring later on, the virus in the infant was much closer to the cell free RNA reservoir in breast milk. This study showed that multiple mechanisms of transmission may be involved. Cell associated virus being the source of infection in most of early events and free virus being more frequently involved later on.

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In order to understand the different functional response of HIV infected quiescent T cells in milk versus blood, my team used an ELISPOT model where CD4 T cells were purified from their blood and breast milk samples, [inaudible] activated ex vivo, and antigen secreting cells quantitated by an immunoenzymatic technique. By this technique, each spot we present a single antigen secreting cell, that means an infected cells that is able to enter viral cycle and produce variance. In this study, latently infected CD4 T cells in milk were ten times more frequently entering viral cycle and producing new viral particles than their blood counterparts after activation. This study strongly suggests that local microenvironment in milk may favor transcription of integrated viral DNA from latently affected cells, and this translation pertains to new variants. This model strongly favored transmission particularly during early stages of lactation when breast milk still contains many cells.

The human milk contains also macrophages and dendritic cells. Recent studies have shown that macrophages home and in human make are morphologically distant from the potential precursor and counterpart in blood. They are spontaneity producing GM-CSF and are frequently expressing DC gene and protein. They are also a likely source of most of the breast milk dendritic cells as they defer and shade into CD1 plus

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dendritic cells after incubation with IL4. These DC positive macrophages and dendritic cells have been shown recently by Suturi [misspelled?] and coworkers to be able to transport R5 viruses in breast milk. As already mentioned, human milk is extraordinarily rich in soluble factors, some of them with immunomodulating or anti-infectious properties. Lactoferrin low risk X factor [inaudible] and [inaudible] have all been suggested either in vitro or in vivo to modulate transmission by interfering with viral attachment and entry or interfering with local immune environment. Many of other component such as lysosome complementary factors, interleukins and other cytokines, [inaudible] and prostaglandin are still to be studied and are the subject of ongoing studies in many study groups.

Some of these soluble factors are, in fact, HIV antibodies that were shown to be detectable in all breast milk samples from HIV infected mothers. Surprisingly, many of these antibodies are IgG, some of these probably transudating from the vascular compartment and the secretory IGA and IGM appeared less frequently. When present, however, local secretory IGA and/or persistent of IGM were associated with an absence of transmission in this study in Rwanda many years ago. Although, two subsequent studies based in different population and different technique failed to demonstrate a protective role of

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these antibodies. It's remained plausible that transmission with HIV through transcytosis across mucosal cells or other mechanisms may be modulated by humoral local response.

The complexity and the multiplicity of pathogenic mechanism of HIV transmission by breastfeeding raised more questions than certainties. Breast milk transmission of HIV is clearly multifactorial, and recent studies have, however contributed to explore this. The exact picture is still not known, but certainly involves a very complex, intercompartmental cell trafficking, some being fueled by very complex viral populations modulated by an extremely rich and diversified microenvironment in milk. The more intriguing question is presently why is the majority of infant exposed to HIV through breastfeeding escape HIV infection, despite the fact that they ingest each day an estimate of 300,000 viral particles and 25,000 infected cells. [Inaudible] responses recently local humoral response and neutralizing antibodies might play a role. Also, as shown by studies from Stephanie Zapashan [misspelled?] the immune T cell response is also present with a specific anti-HIV MHC class I restricted CD8 positive CTLs in milk, but other known factors, especially are recognized in humoral factors may be acting as well.

With this respect, surprisingly welcome from very powerful and new technologies. For example, the protein of the

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human milk contains a large unexplored area. As an example, powerful liquid [inaudible] spectrometry tools have considerably improved the knowledge of proteomix, such as in recent study by Smulansky [misspelled?] that identify several thousand to peptides in [inaudible] none of them being immunologically active and others of totally unknown functions. At least four trials and was structured observational cohort tested the efficacy and [inaudible] of administering highly active antiretroviral treatment to HIV infected lactating women with the hope to reduce breast milk viral load and HIV transmission to the babies.

In the previous session in this conference, we learned very preliminary data from two of these cohorts with extremely promising results. It should be kept in mind, however, that the maternal antiretroviral are much likely to completely alleviate post-natal transmission of HIV, as antiretrovirals have livid short-term impact on the cell associated HIV in breast milk and eradication of potential cellular reservoir. Promising alternative interventions are also the subject of evaluation through clinical trials such as postexposure or periexposure prophylaxis with antiretrovirals given to exposed infants only or mucosal vaccine candidates or treatment of expressed milk or by microbicides.

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Finally, we should record that the very close presence of HIV with TLV-1 is a retrovirus that is transmitted from mother to child by breastfeeding almost exclusively through a cell-to-cell transfer of viruses. Breast milk transmission of HEIV-1 has been considerably reduced by freezing and thawing of expressed milk, an example that may be interesting to test in this occasion to prevent transmission of HIV. I thank you for your attention.

[Applause]

FEMALE SPEAKER: This paper is up for discussion. We can take one question. I see someone coming - no, she's not. She's leaving. [Laughter]

PHILIPPE VAN DE PERRE, M.D., Ph.D.: I was so boring.
[Laughter]

FEMALE SPEAKER: Hi. Barbara Abrams out of UC Berkeley showed pretty efficiently and effectively that boiling of breast milk will kill the HIV. I was wondering if you can speak to that because also you had mentioned freezing and thawing. Does the boiling process also [inaudible] proteins that would be good immunologically, or what's the deal?

PHILIPPE VAN DE PERRE, M.D., Ph.D.: That is completely right. There are some studies evaluating pasteurization or boiling of breast milk, and evidently it has a huge, major impact on infectivity of breast milk, most probably both on

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cell free and cell-associated viruses. But, as you mentioned, it could interfere also with the effectiveness of all those soluble factors, especially proteins who are not stable and may be affected by this, also, practically, it may be quite difficult in the field to use this alternative. But I know also that there is at least a couple of sites in Africa where this procedure is tested in real life and it will be quite interesting to have results from those trials.

FEMALE SPEAKER: Thank you very much.

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Okay, thanks, Philippe.

[Applause]

Our next speaker is Ruth Nduati. Ruth is a professor of pediatrics and child health in Nairobi, Kenya.

RUTH NDUATI, M.B.Ch.B., M.Med., M.P.H.: Let me begin by thanking the organizers of this meeting for inviting me to make this presentation. How do I -

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: The green button.

RUTH NDUATI, M.B.Ch.B., M.Med., M.P.H.: Now, during the first year of life of a child, a child develops rapidly moving from a milk only diet in the first six months to a mixed milk and weaning diet for the next six months, and finally actually being on a full adult diet by the age of two years.

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Now, breast milk meets all the nutritive needs for the child in the first six months of life, and continues to contribute significantly until the child reaches the age of two years.

Now, these buttons are not -

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: The green.

RUTH NDUATI, M.B.Ch.B., M.Med., M.P.H.: This one?

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Yes.

RUTH NDUATI, M.B.Ch.B., M.Med., M.P.H.: Okay. Now the question of whether breastfeeding is best for HIV exposed infants in resource-constrained settings is best addressed by considering magnitude, timing, and correlates of breast milk transmission, mortality associated with HIV infection, and with different modalities of feeding, morbidity associated with the different modes of feeding in the added newborn period, and following by the weaning, and how the HIV free survival associated with different feeding modalities, and the effect of the prophylaxis for PMCT.

There are four distinct patterns of feeding the very young infant, exclusive breastfeeding when the baby receives breast milk only plus medications and multivitamins, predominant breastfeeding where the baby is breastfed and in addition receives non-milk fluids but no solids, and then partial breastfeeding also called mixed feeding, where the baby takes other milks and liquids as well as solids in addition to

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breast milk, and then replacement feeding where the breast milk is not part of the infants diet. As we proceed, please consider the following scenario.

Mary is a 20-year-old woman expecting her first baby. She does not have access to HIV testing, but is concerned that she might be infected. What advice would you give her regarding infant feeding? With the slow rollout of PMCT programs, this is a common scenario facing pregnant women in sub-Saharan Africa. Let's note that even in the context of a generalized HIV epidemic, the majority of women are HIV negative, [inaudible] infant feeding should be based on the assumption that Mary is HIV-negative. The optimal feeding choice for Mary is exclusive breastfeeding for the first six months and then continue breastfeeding and complementary feeding into the second year of life. She will need skills on proper infant attachment in breast to prevent nipple and breast disease, conditions associated with very high breast milk transmission of HIV. She also needs counseling on HIV risk reduction using dual contraception, condoms and an effective planning method. And this was noted that to date the programs that have successfully promoted exclusive breastfeeding are characterized by weekly and four nightly home visits for breastfeeding mother for up to six months, and I think part of the discussion that we should come away from here is how that

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could be implemented in the typical public health setting that is in sub-Saharan Africa.

In the HIV-unexposed infant, appropriate infant feeding is this exclusive breastfeeding for the first six months of life and then complimentary feeding following that. Exclusive breastfeeding, which is a gold standard for young infant feeding is not the norm. In fact, the norm is mixed feeding with a lot of pre-lacteal feeds being given to babies. This slide in a study by Bob Colete [misspelled?] presents some of the evidence supporting promotion of exclusive breastfeeding. In this study of children living in some of the most deprived environments in the world, the slums of New Deli, [inaudible] non-breastfed babies age six weeks to six months had a tenfold increased risk of dying compared to the babies who are predominantly breastfed, where partially breastfed babies had a twofold increase risk of death. These findings are very similar to the data that has been presented for HIV exposed children.

Today, 90-percent of HIV-infected children at identical centers children who are exposed to HIV live in sub-Saharan Africa, a region where 41-percent of the 10 million under-5 child deaths take place annually. We estimate that 13-percent of these deaths could be prevented by appropriate breastfeeding practice. Therefore, infant feeding counseling for HIV exposed

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children needs to take into account the risk of HIV infection as well as the environmental hazards that these children are exposed to.

Now, there are many studies that have looked at breast milk transmission of HIV, the early studies are largely observational where later studies are interventional in nature, often with women self-selecting method of feeding and sometimes being randomized to breastfeed or formula feed. Breast milk exposure variables that have been looked at have also varied, everything from comparing breastfeeding versus replacement feeding, as well as comparing the different categories of breastfeeding, exclusive breastfeeding, predominant breastfeeding and mixed feeding. Likewise, now comes of interest variable rates of HIV infection, mortality, diarrhea and pneumonia related morbidity and HIV free survival. Now this analysis in some studies are carried out for all the children while in other studies they are stratified by infection status, and in others they only present data for children who are not infected.

Now some of these study designs present challenges in addressing the question of breast milk transmission of HIV. An observational and interventional cohort studies one is not able to establish between late pregnancy, intrapartum, and adipose natal transmission, and therefore the rates that are reported

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underestimate the risk of breast milk transmission. Now the development of the antigen breast techniques for infant HIV diagnosis have opened up new opportunities for examining the timing of breast milk transmission of HIV, but to this then has come the ability in the period of observation of post-natal transmission.

Now, in other studies, sicker women often opt for replacement feeding or mixed feeding. Both factors are independent of child's HIV infection status associated with adverse infant outcomes. There are efforts in the studies to control for some of these problems, but very often the studies don't collect enough data. Sometimes you have CD4 counts, sometimes you have viral loads, sometimes you may have CD8 counts, and even when you control for these biological factors there are other factors associated with child survival which may differ with modalities of feeding such as [inaudible] literacy levels of the mother, and even access to district amenities like healthcare.

Now, randomization, which is justified when there is equipoise, is currently the best known method for controlling for measured and unmeasured potential confounders, and will help us in answering these questions. To date, there are two published studies of randomizing women to breastfeeding and formula, one in ARV-naïve women and the other in women who have

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been exposed to effective or antiretroviral drugs. Now an additional problem with the recent studies is that they lack uniformity in the duration of follow up for the various study points. Some of the studies you see 6 weeks, 6 months, 7 months, 18 months, 24 months, and this does introduce bias. An example of this bias is for example that babies who are on replacement feeding have a higher mortality compared to babies who are breastfed, so if your study only follows babies up to say six months, then you underestimate the adverse events that may be associated with breastfeeding and HIV infection as showed in this slide.

There are also other problems related to these studies that the timing of blood sample collection to determine when infants were infected, if babies miss their visits then their period of - the timing of infection would be assigned to a different time period than it really happened. Nevertheless, we have lots of data to show that there is breast milk transmission of HIV. The Annas [misspelled?] reports, as you heard, were from case reports and cohort studies. Another meta-analysis by Di Natal [misspelled?] has held true to date where the added risk of HIV transmission for women with established HIV was about 14-percent for women and 29-percent for newly infected women.

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Now the two studies that have evaluated breast milk transmission of HIV using a randomized trial design in some ways confirmed this data. The Nairobi study of the ARV-naïve women found a cumulative HIV infection rate of 20.5-percent in formula fed moms and 46.7-percent in breastfeeding moms, so the overall estimated transmission rate in these mothers who are ARV-naïve was 16.2-percent and 44-percent of the transmission in the breastfeeding arm was attributable to breastfeeding. The Marshall Study which was published more recently randomized where women exposed to efficacious ARV regimens and randomized babies to breastfeed or formula feed, breastfed babies had one month of [inaudible] in addition to the antenatal and peripartum ARVs, when we followed the babies to 18 months of life there was high HIV infection rates in babies who were breastfed 9.8-percent versus 6.0-percent, and overall, 37-percent of the HIV transmission in the breastfeeding arm could be attributed to breastfeeding.

Now, babies are at risk - this is not moving - okay. Babies at risk of HIV infection as long as they are breastfed. In ARV-naïve women most breast milk transmission takes place ante 63-percent by six weeks of life, 75-percent by six months, and 87-percent by one year. And the estimated risk of HIV infection per day of breastfeeding is very similar for estimated risk for exposure to unprotected sex in adults. Now,

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the quality of breastfeeding matters, and mixed feedings is dangerous and it increases the risk of HIV infection. Two large studies have determined the risk - I found now three large studies have been done and reported their data. This slide is from the South African Study by Dr. Kovaskovarti [misspelled?] and colleagues. These are babies whose median duration of exclusive breastfeeding was 159 days. The main findings from this study is that mixed feeding before and after 14 weeks was associated with significant increased risk of HIV transmission in breastfed infants. This study is particularly could because they are able to show the group that were exclusive with breastfeeding was very similar to the group that was having mixed breastfeeding in terms of their baseline characteristics, and this slide shows some of the CD4 counts in that group.

In further analysis, when they stratified who compared mothers who did mixed feeding and gave babies solids, there was an 11-fold increased risk of transmission. Now the lowest transmission rates in this study was the moms babies who are on replacement feeding, and the authors looked at - in fact there was two babies who were infected in that arm of the study had been breastfed. Now late post-natal transmission is important, and this slide shows that it changes from 6.9 to about 9.2 per 100 child years of follow-up, and overall post-natal

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transmission rates are pretty comparable across the different studies.

In the first two studies there the Ditram Plastadere [misspelled?] and the Vitombo [misspelled?] Study, they also show that even in late post-natal transmission, babies who are exclusively breastfed have no obvious transmission, and these are the blue bars. Now, there are other factors that contribute that need to be considered in the breast milk transmission. Mother's disease status is important. In the one from Nairobi we are able to show that mothers with high plasma viral load and low maternal CD4 counts were independently associated with breast milk transmission. In the same Study we demonstrated that HIV virus shedding in breast milk was associated with maternal shedding of HIV virus in both vaginal and [inaudible] shedding of virus, and it may be that these mothers who transmit are different from others. We also show that both cell associated and cell free virus was associated with transmission. Now in the Vitombo study, they show that mothers who have low CD4 counts that 33-percent of them transmit it, so there is 33-percent transmission in babies who are uninfected at six weeks and would continue to breastfeed for a prolonged period of time. In the Study by Katabi [misspelled?] and colleagues for babies who breastfed for the six months, either mothers had low CD4 counts, 34-

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percent of them also were infected, so mothers HIV status is an important driver for breast milk transmission.

Now when you look at outcomes, the single most important factor that becomes the outcome of these babies is HIV infection status. In the Nairobi Study we show that there is a ninefold increased risk of death if the baby is infected, and in the Ditram [inaudible] Study and the study from South Africa, infected babies had a 15-fold increase likelihood of dying if they were infected. Now the morbidity is also important because diarrhea, pneumonia and other such conditions affect the quality of life of the child and put them at risk of dying. In the Nairobi randomized trial, we found that the incidence of diarrhea and pneumonia in breastfed and formula fed babies was similar. The incidence of these conditions was high in HIV infected children, but was no difference between breastfed and formula fed babies.

In a different study were mother self-selected to breastfeed or formula feed, the found a significant increase in diarrhea and pneumonia among babies who are breastfed. In the Marshall study where again, mothers who are not selected in terms of criteria there was increased diarrhea morbidity. Now this slide is from the study in [inaudible] showing what happens when babies who are weaned early. This slide looks like the data also from Mogawi [misspelled?] showing that there

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is an increase in the incidence of diarrhea when babies are weaned at six months, and if the diarrhea is serious enough for the babies to be admitted. Now, this study did not support an increased mortality, but these are children who were in a clinical trial and accessing good clinical care.

Now, I think the best judge of what is safe for babies is really looking at the HIV free survival of infants, and this slide summarizes data from four studies and shows that when you look at the HIV free survival of infants who are breastfed or formula fed there is no difference. The difference you see is that in the studies where these mothers access antiretroviral drugs, the two studies the Marshall Study and the Ditram Klaus Study, the HIV free survival of breastfed and formula fed babies was much higher than in the other studies where mothers did not have access to antiretroviral drugs for [inaudible] prophylaxis.

So what does this mean in terms of counseling? It means that for mothers who meet office criteria for replacement feeding, if a mother truly wants to do that, I think we have reasonable confidence to say from the data that there is no added risk of dying because they are doing replacement. But more important is the fact that these babies who are breastfed do not have an added risk of death compared to babies who are

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on replacement feeding, in spite of having higher breast milk transmission rates.

Now I want to close off with this case scenario because I think the case scenarios illustrate really the problems we have. Akeni [misspelled?] is a 25-year-old primary school teacher expecting her third child. The two other older children are not alive having died of HIV-like illness. She has [inaudible] clinical stage II. Her CD4 count is 180 cells per millimeter cubed. HART is available at a district hospital which 80 kilometers away. She wants to delay ARV initiation until after delivery when she can move about much more easily. She lives in a region of the country where the only way to get to this faculty is to walk or on a bicycle. What advice would you give her regarding infant feeding?

This is a very common scenario for us who are practicing in sub-Saharan Africa. Akeni is at very high risk of infecting her infant through breastfeeding. As I said earlier in studies by Kovani [misspelled?] and others, she has the likelihood of 30-percent remission rate, even with six months of breastfeeding. Without ARVs she has a 14-fold increased risk of dying if she breastfeeds her baby for a prolonged period of time, and therefore every effort should be made to put her on HART. And she should also be counseled that maybe replacement feeding may be safer for her infant. If

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office criteria is not met, then she should be counseled to exclusively breastfeed, and we know that if she exclusively breastfeeds, her child has a threefold reduced chance of breastfeeding transmission as opposed to if she does mixed feedings. So the challenge is that for us to be able to make a difference for these mothers, we have to access the other package of care, the antiretroviral drugs which will protect breastfeeding.

This is my second to last slide which shows what progress has been made in the prevention of mother-to-child transmission of HIV, and I have highlighted the two studies that were presented in the session just before us.

If we are able to deliver effective antiretroviral drugs for PMTCT to women in sub-Saharan Africa, then we will be able to protect breastfeeding and their babies will benefit. Their infants will benefit from breastfeeding and they obviously will protect their mothers from HIV disease progression and transmission to their infants.

So in conclusion, breastfeeding transmits HIV and 38- to 44-percent of all transmission in breastfeeding babies is attributable to breastfeeding. Mixed feeding and especially additional solids in the first few months of life is dangerous, associated with a tenfold increased risk of transmission. Shortening duration of breastfeeding to six months will prevent

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about two-thirds of late post-natal transmission, and the data from observational studies show that both intervention and observational studies show that use of HART during breastfeeding period and shortening breastfeeding to six months offers babies the best option for HIV free survival. Thank you.

[Applause]

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Thank you, Ruth. We are pushed for time but we can allow one short question if someone would like to come to the microphone. Some people take that as a cue to leave.

RUTH NDUATI, M.B.Ch.B., M.Med., M.P.H.: I don't think there are any questions.

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Do we have any questions for Dr. Nduati? We may have time to come back for some further general discussion at the end. Thank you, Ruth.

FEMALE SPEAKER: I was not there at the [inaudible] neither I have read the abstracts from Botswana, but you haven't included the results from the Botswana where they did show the high mortality in breastfed infants over time - sorry in replacement feeding infants over time. Could you comment on that why you didn't include that in that study?

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RUTH NDUATI, M.B.Ch.B., M.Med., M.P.H.: Yes. I don't know if it's possible to put that. I would be happy to comment. The slide that I showed on HIV free survival takes into account mortality and HIV infection, and the Marshall Study from Botswana showed clearly that the HIV free survival breastfed and formula-fed infants was the same. There was no difference. The formula-fed babies had higher mortality and a lot of that mortality was in HIV uninfected children, but the breastfed babies had more HIV infection. And when you took HIV free survival as your outcome of interest there was no difference in the two arms of the study. Just like even in the South African Study of exclusive breastfeeding, when you compare HIV free survival, those who are replacement fed and exclusive breastfed there was no difference.

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Thank you very much.

FEMALE SPEAKER: Now we have Sam Walters, who is going to talk about the issues around diagnosis and management of kids who are co-infected infected with HIV and TB. Thanks, Sam.

SAM WALTERS, B.C.H.I.R., F.R.C.P.: I would like to start by thanking the organizers for asking me to speak here this afternoon. I am an infectious diseases pediatrician working in London, and I have spent much of the last 17 years

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looking after children with HIV infection and children with tuberculosis. I made this slide back in 1990 when we, in fact, knew very little about adult HIV infection and even less about this infection in children, and I used to use this slide to illustrate the point that what we do learn about adult HIV would not necessarily translate into pediatric practice. I think I can still use this slide today when talking about HIV/TB co-infection in children. We do not know enough about this co-infection in adults. There is not enough data and there is a woeful lack of data in pediatric practice, and I think we need to remind ourselves that what data is generated from adults will not necessarily translate into pediatric management.

It is a fact that the global society is unfair in that those who live in a resource-rich environment usually have good access to healthcare and the opposite is for those living in resource poor, and the HIV/TB co-infection is very common in many resource-poor environments, and this is perhaps best illustrated by sub-Saharan Africa where children living in this region probably have the greatest burden of this co-infection.

I first will move on to challenges of diagnosis of tuberculosis. I am not going to talk about diagnosis of HIV in children, and it's important to say that we not only need to diagnose active tuberculosis in children but we need to know

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about latent MTB infection because there are issues of prophylaxis. The gold standard of diagnosis for tuberculosis has always been microbiological, but this has a very low sensitivity in pediatric practice, at best 10- to 15-percent. And this is due to a number of reasons, but one in particular is that most children with tuberculosis have poor superciliary disease. It's also very difficult to get sputum samples from young children. If we try and make the TB diagnosis from symptoms there is overlap with HIV disease and fevers, failure to thrive, and weight loss, along with persistent and chronic respiratory illness are common to both infections. Similarly, if we try and make the diagnosis with chest radiology there is considerable overlap. And before the HIV epidemic, I think all of us would have been happy to diagnose both of these children as having miliary TB, but one has LIP and to my eye there is no difference radiologically between these conditions.

The tuberculin skin test has been in medical practice for over 100 years, but it is of limited use in young children, and if these young children also have HIV infection it is even less useful. This is s study from South Africa confirming this, and disappointingly trying to factor in the child's CD4 count in order to improve the sensitivity of tuberculin testing did not make it more valuable. In many parts of the world, tuberculosis in children will be diagnosed using an algorithm

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or a score system contact history, symptoms, radiology, and skin testing, and this has proved to be very useful in many areas unless the child has HIV infection. And these are three studies one from South Africa and one from Zambia showing that these score systems do not work in the presence of HIV.

There are new diagnostic tests and the T cell base test for which there are two commercially available assays are useful in distinguishing between MTB infection and prior exposure to BCG and this is very useful. However, these tests are expensive. They require a laboratory infrastructure and you need trained personnel to perform them. In the current form, they do not distinguish between active TB and latent MTB infection, and they are not yet adequately validated in HIV infected children. Data are emerging, and this is a study from KwaZulu Natal showing the sensitivity of the ELISPOT which is just one of these commercially available tests was vastly superior to the tuberculin skin test. At this meeting there is a poster from a group in the western Cape showing the ELISPOT sensitivity of 80-percent in HIV infected children with active tuberculosis, so this is probably going to be useful to us but we need more data.

Moving on to the challenges of treatment, the first thing to say is that we cannot delay treatment of active tuberculosis. It must be started immediately, and the question

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arises is when should we start treatment for HIV? And one of the issues here is immune reconstitution disease, also termed IRIS. I will stick in my talk to referring to it as IRD. I think a good definition of this involves the restoration of pathogen-specific immune responses during antiretroviral therapy, and it presents as subclinical infections being unmasked or partially treated infections getting worse. In adults, it is more likely to occur if HIV therapy is started within two months of starting TB treatment if TB is extensive, if HIV is advanced, and if you get a good early response to your antiretroviral therapy. And it is of interest that in adults, when they repopulate their CD4s, this is predominately memory CD4 T cells, in children this will predominately be naïve CD4 cells, and a lot of this will have an influence on the passage of IRD that we see in children. We just don't know yet because there is precious little data. It does occur in children. There are case reports and there are a series of children starting on antiretroviral therapy in which IRD has occurred.

The management of IRD, we lack data for that of management in adults, but if mild, symptomatic treatment may be helpful but when severe steroids are used, and we don't know how long or what dose or how dangerous steroids are. There is as yet no control data. It is better to try and delay or

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prevent IRD by delaying HIV therapy and do that for two months if you can, or even better for the whole of time of TB treatment, however there is a significant mortality in adults from South African data in that first two months of TB treatment. This is a busy slide that, because of time, I will pass over but I have encapsulated the data here that what needs to be balanced is the early mortality. If you delay ART against the incidence of immune reconstitution disease if you start ART early, and we don't know how to weigh these issues in adults and where the balance should be pitched, and we certainly don't know where to put it in pediatric practice as yet.

Other treatment challenges would be drug interactions, and particularly we are talking about rifampicin which we know induces hepatic p450 cytokine complex. Mostly, rifampicin will not have an effect on NRTIs, but we know that triple NRTI regimens lack potency, and particularly as many children have very high viral loads, we would not promote this as the way forward for pediatrics. The non-nucleoside reverse transcriptase inhibitors. the doses show that rifampicin will reduce the area under the curve for neviraprine by about a third and for efavirenz by about a quarter. It is possibly all right to use a standard dose of efavirenz. There is adult data which was presented in this meeting yesterday by a group who

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previously reported good short-term results with rifampicin and standard dose efavirenz in adults. Now, they have presented long-term data showing that the HIV control is good.

Neviraprine well recommendations have been to increase neviraprine by anything between 20- and 50-percent. Again, yesterday some adult data from a group in Thailand showing that putting the nevirapine up to 600 milligrams instead of the standard 400 milligrams achieves equivalent virological control at 24 weeks. Now we don't like 24-week data in HIV practice but it's interesting, particularly as those adults on the higher dose of neviraprine have a significant increase in side effects. The protease inhibitors are mostly incompatible with rifampicin. You can use ritonavir with rifampicin but I don't think anyone should prescribe ritonavir liquid to children without first tasting it. It is disgusting and giving ritonavir to children is really quite difficult.

Kaletra, possibly we can use by increasing the amount of ritonavir so that we boost lopinovir levels, and this is some data from a Dutch group in adults showing that you can achieve adequate lopinovir levels by giving extra ritonavir.

I have seen unpublished data from the western Cape using a similar protocol in children that seems to get over this and maybe that will evolve. The problem with rifampicin is really worrying because if we overdose an antiretroviral for

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children we are likely to produce toxicities which short term is very uncomfortable for them, but if we underdose it, this will promote HIV resistance, and this is a long-term disaster for these children's HIV care. In a resource-poor country, treatments of HIV infected children has to go down the route of fixed dose antiretroviral combination tablets, but if we are going to use rifampicin we are going to need to augment individual components of these drugs, and this is extremely difficult. In a resource-rich country, we can increase the dose, do therapeutic drug monitoring and adjust the dose, and it sounds straightforward but it isn't. It is extremely difficult, and many of us have moved to using rifabutin instead of rifampicin to try and avoid the problem of incompatibility. Now I have no idea how difficult it is to manufacture this molecule but it may be that this is the direction that we have to go for simultaneous treatment of TB and HIV.

How long should we treat TB in an HIV infected child where we just don't have controlled trials? This is a study from South Africa showing that on six months of TB treatment recurrence was common, and of those who had recurrence, a third were thought to be due to relapse not through lack of adherence but six months treatment did not sterilize the lesions. Multi-drug resistant TB and extensive drug resistance TB is a real concern. And this map here, the coloring of the country

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shows the prevalence of MDR TB, but it's the hatched areas which are alarming. These are countries which have reported extensive drug resistant TB as of January of '07. In May of this year, 38 countries have been documented to report extensive drug resistant TB, and this widely publicized outbreak of XTR TB in KwaZulu Natal really is frightening. This particular strain had increased transmissibility and over 50-percent of patients were thought to have acquired this is primary infection. The mortality within 25 days of TB diagnosis was 98-percent, and every patient who had an HIV test was found to be HIV infected. And simply, children must have been infected with this strain, but of course, as we usually do not get the organism from a child with TB they weren't diagnosed, and the idea that what we know about MDR TB and extensive drug resistant TB is just the tip of an iceberg has to be correct. How much of this iceberg is due to resistant TB in children and how many of them have co-infection with HIV, we have no idea.

There are many other treatment challenges and each and every bullet point on this slide merits its own discussion, but I don't have time to address any of these and we'll move on to prevention challenges. And again the first two bullet points here are vitally important but I don't have time to talk about them. Isoniazid prophylaxis is topical. There is a study from

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south Africa showing a significant randomized placebo controlled study - good study - showing a significant decrease in tuberculosis and in mortality in children receiving isoniazid such that the study was stopped halfway through. What this study does not answer is, how long do we give isoniazid? Because the risk of re-infection of children in this environment remains. And also, if this is widely adopted, might this have an influence on background isoniazid resistance in a country. We don't know that. BCG, there's a lot we don't know about it but we do know that it's not good enough to protect HIV infected children, and we worried about it for many years, and there are many case reports of disseminated of BCG have come through.

This is a study from southern Africa showing that the risk for an HIV infected infant of getting disseminated BCG is several hundred times higher than a non-infected child. And world Health actually had modified their advice on BCG in January this year saying it should not be used in children who are known to be HIV infected.

For my final side, well how are we going to meet these challenges? We need new diagnostic tests for TB and there are many interesting and exciting ideas in the pipeline. We need new drugs to treat particular resistant TB and we need new vaccines to protect against TB, but we can't wait for this to

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come. We could do more now. We need more pediatric data. We need integrated care of tuberculosis and HIV for adults as well as children, and we need more rollout of antiretrovirals in pediatrics, and we need integrated family care because children and families with both these disease there is usually many are affected. And I think many in the audience will be saying same old list. We have seen this year in, year out, and we will continue to see this same old list year in, year out unless we can get more money into this area. We need massive investment. There are moves are there is some optimism because more money is coming but it's not enough, and it's up to every one of us to help keep pushing to better look after co-infection. Thank you.

[Applause]

FEMALE SPEAKER: This paper is open to discussion.

MALE SPEAKER: Thank you. I am Dr. Zunigal

[misspelled?]. I am from Cambodia. I have a specific question I want to ask you related to the efavirenz. Is there any study that we can use for children who are under three years of age that have co-infection HIV with TB or co-infection HIV with hepatitis, or the child who has reaction to neviraprine, is there any study that we can use a [inaudible] for children who are under 3 years?

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SAM WALTERS, B.C.H.I.R., F.R.C.P.: If I think I understood your question it's about safety of neviraprine use with co-infected children. In tuberculosis co-infection I have no concerns whatsoever using neviraprine in London, but I will measure therapeutic drug monitoring of neviraprine and make sure that I am in the right therapeutic range. With co-infection of hepatitis viruses I am not aware of data, but it would be something that potentially we might be worried about.

FEMALE SPEAKER: The next question. We will have the lady here.

DAWN TINIBER [misspelled?]: Yes, hi, Dawn Tiniber from Jamaica. I have a question about disseminated ECG disease. In Jamaica we use BCG at birth and it has not been our experience to have the extent of disseminated disease that was reported in that paper, and my question is now that the WHO has made this statement in '07, does it mean that we wait for the PCR results before we give the BCG? I mean, it's not really affecting us to that extent.

SAM WALTERS, B.C.H.I.R., F.R.C.P.: I don't think I am qualified to answer that question because TB is such a problem, but I think the reality is that BCG does not protect HIV infected children for long enough. I think it is sensible the way they have worded it that if you know the child is HIV infected to avoid giving it. Whether in some environments it

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is safe to wait for that time of knowing the child's HIV infection status or maybe you just missed the opportunity to protect those who are uninfected, so it is very difficult. We do need a better vaccine. It's as simple as that. In London, I have looked after several HIV infected children with disseminated BCG, so it's something which we have all seen, but the data from South Africa was - and you are probably familiar with this study - there were several scenarios looking at transmission rate. And even with the most conservative way of looking at it this was pretty worrying data.

FEMALE SPEAKER: Okay. We have a question from the lady here.

FEMALE SPEAKER: [Inaudible] from Saudi Arabia. If we have a patient, a child with HIV and they are discovered to have a [inaudible] TB and we have to shift the [inaudible] to efavirenz for example, but for the weight of the patient that we have to give the efavirenz as a half tablet which is 300, so how much of the bioavailability of the cut tablet instead of solution if we don't have the efavirenz solution, or a shortage of efavirenz solution?

SAM WALTERS, B.C.H.I.R., F.R.C.P.: Is there anyone from BMS in the audience for that? I don't know that. Do you not have efavirenz liquid?

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FEMALE SPEAKER: We have, but sometimes we have a shortage.

SAM WALTERS, B.C.H.I.R., F.R.C.P.: Okay. I don't have an answer to that and I have the luxury, in fact, of every child we start on antiretroviral therapy we will do therapeutic drug monitoring whether they have got TB or not because we simply do not have enough PK data across the board in pediatric practice, and many of the doses we use we are sort of guessing, and we quite often have to modify doses of several different antiretroviral agents. And yet, I suppose what I am saying is a plea, for we need more pediatric data across the board, pediatric HIV whether it's co-infection with TB, and I am ashamed of us as an international community that we still don't do enough pediatric research.

FEMALE SPEAKER: Thanks very much. I think we are going to have to move on now. We'll have some time at the end, hopefully, to do some of the other questions.

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: So our last speaker is Rita Jeremy. She is a developmental psychologist from the Department of Pediatrics at UCSF. She is going to talk to us about neurocognitive aspects of pediatric HIV infection.

RITA JEREMY, Ph.D.: When children become very sick soon after birth and die in infancy or toddlerhood, the newest

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cognitive performance is not of primary concern since they have to be taken care of anyway totally even if they were healthy, but when children grow older they are expected to become contributing members of their society, and then their neurocognitive functioning becomes critical to their daily life. Such skills as talking, understanding directions, learning new skills, remembering, using hands, making choices, planning, so as HIV positive children continue to live longer when they can have access to HART, their neurocognitive functioning will become increasingly important and even more so as they live into adulthood.

In real life, HIV infection in children occurs almost always with one or more other major risk factor each detrimental to children's development. Some of these risk factors are, perhaps, even more detrimental than HIV itself. Some of the other risk factors may be more common in certain areas or groups. Other co-occurring risk factors that can affect neurocognitive development are listed here. For example, prenatal alcohol exposure is a well-known risk factor for mental retardation as is, for example, iodine deficiency which is found among millions of people around the world.

So how do researchers estimate the effects of HIV infection on neurocognitive functioning of HIV-positive children when there are so many co-occurring risk factors?

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Well, two common approaches, one is comparison to own kind. One is to look at compared to test norms, and the comparison to own kind look at comparing children for HIV-positive moms to HIV-negative reverters of positive moms, for example. In other cases, researchers compare the children's performance to standardized norms on large sample tests that have been standardized on large samples of children with a very wide range of abilities. I have other few lectures here I will have to skip.

So what has been found to be typical neurocognitive functioning of HIV-positive children without antiretroviral therapy? The most documented evidence has been from the pre-ART period in western countries, and fortunately even in the area of HAART, the vast majority of HIV-positive children in developing countries do not have access to ART and they are untreated. And so the natural history of HIV effects on neurocognitive functioning is still relevant for this group. HIV-positive children without ART, their typical illness is developmental encephalopathy. For many, the symptoms onset is very early in infancy with rapid progression and often leading to death in the first few years of life, sometimes actually during infancy. This is typically the progressive encephalopathy. If children are examined on standardized tests, one of the definitions, characterizations of progressive

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encephalopathy is decline in test scores from more than one standard deviation from norms of the test, and even without tests you can see progressive encephalopathy from looking at milestones. Children are expected to gain milestones with age, and when they are not or if they are not gaining any milestones, or even worse losing milestones, there is a good definition that they have developed progressive encephalopathy. Also, their brain does not grow as fast and there are some other characteristics.

In contrast, static encephalopathy, in study, static encephalopathy children still acquire milestones but at a slower rate on a kind of a slower curve. This does not mean that they are eventually going to get sharp because they are just slower and eventually they will get to the same point of being normal, but it most likely means that they will lag behind norms and some of them also start losing skills later on. When you look at some of the children who have more pronounced HIV health symptomatology, they often are the older children who have had static encephalopathy. In terms of specificity, there is some evidence for certain areas being more specifically affected, but in general, you would say that the predominant effects of HIV infection in children are with the global deficits in neurocognitive performance in a variety of domains, so that was about performance.

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When you look at what can you observe by biological markers related to brain, there is calcification of basal ganglia, commonly observed lesions in the white matter seen on MRI, the calcification seen on CAT scans. And there are also changes that can be seen on MRS of cortical metabolites and it was an interesting one is lower N-acetylaspartate, the NIA, which seems to be related to cognitive performance, lower is worse. But a big puzzle still remains which is how come there are some children who have never had any HART, their mothers never had any HAART and they still continue to - they are still alive, they are still school age or maybe even older, and they show at most moderate effects on the neurocognitive functioning? Perhaps they have static encephalopathy.

Some of the discoveries about host genes, the genetics, may shed some light on it in the future. And, of course, by the school age these children are survivors of a cohort, of a birth cohort that by then a large percentage have died. In terms of timing of the initial infection that may affect neurocognitive functioning, it can happen during gestation, labor and delivery, infancy or later years, and generally the effects on neurocognitive functioning are most severe and probably appear earlier for children who are affected intrauterine and then probably not as fast in appearing or not maybe as severe for later infections, but there is a very wide

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range. Most of the initial infections actually happen during labor and delivery.

For children who are - an assumption of course as we heard are infected from breast milk during infancy, and for children who get infected later even like hemophilia children or children from sexual abuse or sexual activity or their own IV intravenous drug use, there of course it becomes increasingly more like that of adults.

So what is the picture of neurocognitive functioning of HIV-positive children who have been treated with antiretroviral medications? Results are quite limited because of very few large controlled studies with large numbers of participants. There are several small scale - most of the studies are very small studies with very small number of children - the small scale studies have shown some improvements in neurocognitive performance, but generally not recovery back to baseline after neurocognitive decline has been already observed. And then some people have reported change in the cortical metabolites that they have been able to increase some of the metabolites like this NAA, which is a big problem because it seems to be related to neurocognitive performance. Actually, some of them have suggested that you may be able to see some of the decline in brain by looking at the cerebral metabolites before you get anatomical changes in the brain as can be seen on the MRI.

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Of the large-scale control studies, there is a couple of big studies that looked at children pre and post availability of HART. In a big study that looked at when antiretrovirals were first introduced, there was a follow up of children who were drug-naïve to any ARVs and then were randomly assigned to either ACT, DDI, or a combination and in that particular very large study that took a lot of work, actually they did show improvement in neurocognitive scores of the children. And more recent, a very recent hot of the press a few months ago study, and analyzed big datasets looking at children from the era, the cohort of the era pre-HAART and the cohort of children in the HAART availability era, and they looked and what they found was, for instance, they showed that mental and motor functioning of the HIV-positive children remained even after the availability of protease inhibitors remained significantly lower than of HIV negative children, the zero reverters. They did put a little caveat saying that however, given the big amount of decline in scores of HIV-negative children, there was evidence of limited, very limited, improvement of HIV positive children relative to the uninfected peers. And this is after the introduction of PIs, and when they looked at infants who actually were the pre and post themselves, there was only a trend not a really significant improvement toward improvement in motor scores after the

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initiation of PI therapy. They actually were saying that they only saw evidence for very limited improvement with HART even though, of course, it was a very major biological change in terms of like viral load.

Another recent study that looked at actual report of which I am the first author compared neurocognitive functioning of a lot of children who were randomized to different regimens with a protease inhibitor and they were followed for at least a year. The children had been for at least 16 weeks on an NRTI and prior to entering the study, and they ranged all the way from four months to 17 years. At baseline, it was already evident that they were about one standard deviation below norms of tests, and defining after one year, at least after one year. We did 13 different measures and only one out of the 13 showed any improvement, and 12 out of the 13 showed no change really overall. I mean, there might have been individual differences, but overall, 12 out of the 13 measures there was no difference after one year, basically one year, with a protease inhibitor. And as reported in the previous study, the changes in the viral load were dramatic, great, great results.

So while ART and especially HAART can achieve major improvements in the physical functioning of children and their health as seen by the biological markers, HAART can do so without, of course, finding significant improvement in

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neurocognitive functioning of these children. Still, there is some evidence, hopeful evidence from some of the smaller studies so it's worth pursuing, and of course, questions why there is not equivalent improvements in neurocognitive functioning as there are in biologic and other things. Some say that maybe there is not enough penetration into the cerebral spinal fluid. Perhaps some of the first major effects are when you first get introduced when you are drug-naïve.

So recommendations - they are more like suggestions - we need to have more cognitive testing if neurocognitive skills are becoming critical to their daily life, we need to know more about what they are and include them as part of comprehensive HIV care. We need to start testing neurocognitive skills early to identify either early signs of decline, and if need be, either initiate therapy if it hasn't been done so already, maybe intensify it or change the regimen. We need usable neurocognitive tests that are appropriate and economical, both in time and cost for culture and language of the children. That's a very, very major issue, and some people actually are studying populations, large populations, in other settings renorming [misspelled?] western tests for the local populations. We need to deliver antiretroviral drugs for children, a very small percentage, of course, as you have heard many times are receiving them. And then a big behavioral issue

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is to help maintain adherence once they get the drugs, so that is a big issue whether it is some place in a developing country where the child is already an orphan and has like 10 different people coming with the child and they don't know what to do and so on, but it's also a big issue in countries where there is lots of medication and they don't want to take something, especially when you get to the teenagers, so adherence is a real major issue.

Another thing is to design drugs that actually work on brain. And I guess the end is that the most important thing which works the best for neurocognitive function is to prevent HIV in the first place. Thank you.

[Applause]

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Thank you, Rita. We have time for questions.

FEMALE SPEAKER: There is someone there.

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Is someone there - yes?

FEMALE SPEAKER: Yes, thanks for this excellent lecture.

RITA JEREMY, Ph.D.: Oh, is this for me?

FEMALE SPEAKER: Yes. It is. In fact, if you would allow me, it's two questions. First of all, I am a pediatrician but I can sometimes attend a clinic of adult HIV.

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From the clinical observation which I noticed that after the antiretroviral therapy is started for the adult HIV patients the neurological assessment is really improved dramatically within weeks, so my first question is that no improvement in neurocognitive function in the children because they have sort of developmental brain, and is children something different from the adults? Number two - or we can answer the first question.

RITA JEREMY, Ph.D.: Okay. I heard it partially.

FEMALE SPEAKER: The adult neurological CNS involvement in adult HIV patients, once they start the HART therapy they improve dramatically within weeks, so why -

RITA JEREMY, Ph.D.: The children you see?

FEMALE SPEAKER: No, this is adult, why that is not the case in pediatric? Is it because they have still developing neurons or what?

RITA JEREMY, Ph.D.: The story for children is very different than from adults and I don't know that much about adult's changes. Some of the changes that are seen in sort of a case-by-case basis that you see the difference often is a difference that the child if you saw them before that they were sick so they are not like doing things, they are tired, they are irritable, so sometimes you don't see sort of the optimal level of what the child dose and then you see it when they get

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to be healthier. Whether there is something permanently that was already happening that they had permanent damage - some damage - and then the damage was undone is not clear.

FEMALE SPEAKER: Okay. The second question is there any inductive therapy with the antiretroviral therapy - say, for example the immunoglobulin or something to enhance the blood brain barrier - to improve the HIV infection in the CNS?

RITA JEREMY, Ph.D.: I am a developmental psychologist so I have to defer to people who deal with the medical aspect of it. There was actually some scale that has been developed to see which medications and which combinations are most likely to cross the blood brain barrier, and I know that one of the ones that got a good rating was Kaletra, but it is still not clear that whether even if certain medications might be more likely to pass into the central nervous system whether that would make a difference, but that is the first step, and you have to have drugs that pass and then test them. It's not clear yet which ones would really work.

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Okay. Thank you. We have a question up here.

ANN FERRARA [misspelled?]: Hi, my name is Ann Ferrara. I have been working in the Netherlands and in South Africa, and I am very happy that you asked about this neurodevelopmental screening, actually neurocognition and neurodevelopmental

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exams. We have one which we use in the Netherlands which is called a Von Vekin [misspelled?] Scheme. It's a screening instrument used for children zero to 4 years old. I would be very happy to contribute and work with you further in developing this, especially in the African setting. I am sure there is other colleagues who are familiar with the earlier Gazelle Developmental Exam which I was first trained on in 1978 in the United States, and that was especially for infants that were premature and fragile infants, so I think we could start with the early childhood in any case, but that -

RITA JEREMY, Ph.D.: I would be very glad to see that.

ANN FERRARA: Yes.

RITA JEREMY, Ph.D.: One of the differences about developing scales for children to look at neurocognitive development of children as opposed to adults is that pretty much for adults they developed some scales that pretty much cover a wide range of ages whereas when you are working with children from zero to whatever you think is still childhood -

ANN FERRARA: Four.

RITA JEREMY, Ph.D.: - sometimes 17, 18, there is such dramatic changes in what is expected at these different ages very fast from one age to another that you need to practically develop like multi, multitasks for these different agents to be appropriate for their age and yet have it be available for

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people from different countries, different languages, different environments, so it's much more challenging to look at development of tests for children's neurocognitive development compared to adults - but not that developing tests for adults is easy, but it's even harder.

ANN FERRARA: But the other side -

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Can we allow this other question -

ANN FERRARA: - the other side of the screen is also to have therapy to go with it after that.

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: We have another question here.

FEMALE SPEAKER: Dr. Waldy [misspelled?] from South Africa, and just to comment on the neurodevelopmental side I have got about 500 children on ARVs and a lot of them are facing severe circumstances. I don't have any test that I can use to assess the neurocognitive function, but from just observing them in my practice they do exceptionally well [applause] considering what they are facing. I mean they are facing poverty, orphans, all kinds of things and these little kids are regaining milestones. They are doing exceptionally well, so if I could have something hard and concrete to measure them with I think it's needed, especially when you have 500 kids on ARVs - that was just a comment.

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But my other question I think is for professor Walters - or Dr. Walters, I am not sure. I am sorry, but this is very quick. I do have certainly children on ARVs so a lot of them - all of them - had a BCG. We have seen a few now with BCG immune reconstitution disease. Now I have been told that this is a new disease, a new entity. I have seen three children that have a bit of big glands under the arms on the site where they got the BCG. What organism do you isolate from this? Is it microbacteria bovi from the vaccine and how do you treat it? We just push through with the ARVs. I mean, that's all I can do. I am not a pediatrician. I am just a medical officer.

MALE SPEAKER: [Inaudible].

SAM WALTERS, B.C.H.I.R., F.R.C.P.: The short answer is that I don't know how to treat it because I don't think there are randomized controlled trials, but if I have problems with BCG and if it's just local, I do treat with rifampicine and isoniazid for about three months. But the reality is that dead microbacteria BCG bolus will still produce puss, so you don't always know. You have got live organisms there. If it's disseminated and causing organ dysfunction then we throw a lot more at it. Pyrazinamide will not have activity. Amycacin, you have to treat it very aggressively or it will kill the child.

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JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Okay. One last question then. We are over time.

FEMALE SPEAKER: I am from Nairobi, Kenya. I am a pediatrician, and my comment is about BCG in HIV infected children, and from my clinical observation we have routine BCG immunization of our children at birth, and we do have still some mother-to-child transmission. However, it is relatively rare to see disseminating BCG. We seen one patient in our country [inaudible] and one patient in the last six months out of thousands of HIV children who are exposed, so I think from our clinical observation it's not - [microphone falling]

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Oh, so very sorry.

FEMALE SPEAKER: We have a frequent disseminated BCG in HIV infected children at birth when they are usually not at the advanced disease stage.

SAM WALTERS, B.C.H.I.R., F.R.C.P.: I think that's right, but once HIV is advanced, the microbiological distinction of BCG and MTB requires culture and sophisticated laboratory backup, and I would predict that there are many children dying in Africa with a diagnosis of TB when, in fact, it could be disseminated BCG. I don't have any data to back that up, but certainly we know that it can act as a pathogen, and if your immune competence continues to decay, it is quite

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likely there are live BCG there which can act as a pathogen and kill you, but I don't have data for that.

JOHN ZIEGLER, M.B., B.S., M.D., F.R.A.C.: Okay. Well I think we have had a very interesting session here, and once again, I would like you to join me in thanking our four speakers.

[Applause]

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