BV-Associated Bacteria Cause Molecular and Physiological Dysfunction of the Vaginal Epithelium

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Background: Vaginal microbial dysbiosis (bacterial vaginosis, BV) leads to increased female genital tract inflammation and higher HIV-1 acquisition. However, microbiome-mucosal interactions in the FGT are not well understood. Here we characterized molecular differences in vaginal mucosa with vaginal microbial dysbiosis and evaluated functional mechanisms in vitro.

Material & Methods: Proteomic (cervicovaginal swabs, n=68) and transcriptomic (cervical biopsies, n=40) analyses were performed on samples from Kenyan women to provide microbiome and biological pathway data. Using vaginal epithelial cell lines (VK2 or Hec1A) we assessed proteomic changes, and physiological effects by epithelial resistance, porosity, wound healing, and barrier development in the presence of different vaginal bacteria.

Results: Two major microbiome profiles were identified in clinical samples: Lactobacillus dominant (LD, 60%), and non-Lactobacillus dominant (nLD, 40%) showing presence of G. vaginalis, M. mulieris and P.amnii. There were no differences in age, sexual behaviors, contraceptive type, or STI's between the two groups. At the transcriptome and proteome level nLD women showed changes to adherens junction ($p=2E-7$), decrease in tissue development ($p=2E-10$) and cell differentiation ($p=5E-7$). In vitro culture of VK2 cells with supernatants from G. vaginalis, M. mulieris or P. amnii induced similar proteome pathway alterations compared to L. crispatus, including epidermis development ($p<3E-4$), cell-cell adhesion ($p<3E-4$) and keratinocyte differentiation ($p<0.001$). In physiological assays, M. mulieris caused decreased epithelial integrity ($p<0.003$) and increased porosity ($p<5E-4$) while G. vaginalis caused decreased wound healing ($p<0.05$) and barrier thickness ($p<0.005$).

Conclusions: These results suggest that BV-associated bacteria directly cause barrier pathway changes in vivo and physiological dysfunction of vaginal epithelial barriers in vitro. These mechanisms may be important for BV development and STI risk, including HIV-1.

2

Immune modulatory effects of vaginal microbiota organic acid metabolites on lower female reproductive tract epithelial cells

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Background: Women with diverse vaginal microbiota, as exemplified by bacterial vaginosis (BV), have inflammation in the lower female reproductive tract (FRT) and an increased risk of acquiring HIV in contrast to women with Lactobacillus spp (LB, non-iners) dominated microbiota. BV is characterised by a pH>4.5, an increase in the vaginal microbiota metabolites (VMB) succinic and short chain fatty acids (SCFAs) and a dramatic depletion of the VMB lactic acid (LA) produced by LB, which acidifies the vagina to pH<4.5. Here we assessed the immune modulatory effects of VMB under conditions of LB dominance and BV on epithelial cells from the FRT.

Material & Methods: Physiological levels of VMB at pH<4.5 or pH>4.5 were added apically to human vaginal or cervical epithelial cells in transwells. Cells were stimulated apically with bacterial or viral mimicking toll-like receptor (TLR) agonists, TNF or genital fluids. Cytokines and chemokines were quantified by luminex-based assays. Cell viability was determined using the MTS assay. Statistical analysis was performed using the Mann-Whitney U test.

Results: Treatment of epithelial cell lines with LA ± SCFAs (pH<4.5) elicited significant increases in the anti-inflammatory cytokine IL-1RA. When added simultaneously to stimulation, LA ± SCFAs (pH<4.5) significantly inhibited the TLR agonist-induced production of inflammatory mediators IL-6, IL-8, IP-10, TNFα, RANTES and MIP3α. The same LA ± SCFAs anti-inflammatory effects were not recapitulated with media acidified to the same pH with HCl, and was mediated by the protonated form of LA present at pH≤3.9. Both L- and D-isomers of LA elicited similar anti-inflammatory effects against measured immune mediators. LA treatment following 6 h of TLR agonist stimulation or LA pretreatment of cells for 1 h, followed by extensive cell washing and TLR agonist stimulation, inhibited pro-inflammatory mediator production indicating a direct effect on cells. A similar anti-inflammatory effect of LA was observed in primary cervicovaginal cells and in an organotypic epithelial tissue model, and when FRT epithelial cells were exposed to either cervicovaginal (from women with LB or BV) or seminal fluids. In contrast LA+SCFAs (pH >4.5) did not elicit production of the anti-inflammatory cytokine, IL-1RA, and neither decreased nor potentiated the production of pro-inflammatory cytokines and chemokines elicited by TLR-agonists. LA and SCFAs at physiological levels and pH had little impact on cell viability.

Conclusion: LA ± SCFAs under conditions of LB-dominance act on FRT epithelial cells to inhibit inflammation that might explain in part the HIV protective properties of LA-producing LB. In contrast LA + SCFAs do not alter the pro-inflammatory effects of TLR agonists under BV conditions. This study highlights the potential use of LA-containing agents or LA-producing probiotics as adjuncts to female-initiated HIV prevention strategies.

3

A penile model in rhesus macaques to assess pharmacokinetics and characterization of HIV target cells within anatomical compartments of penile tissue

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Background: Systemic antiretroviral prophylaxis for HIV prevention has benefited greatly from preclinical macaque models which have provided invaluable data on pharmacokinetics and efficacy against vaginal and rectal HIV transmission. There is increased interest in developing penile transmission macaque models to extend PK and efficacy assessments of promising interventions against this important route of infection. Here we describe drug distribution in HIV target cells in penile tissues following oral Truvada (FTC/TDF) in rhesus macaques and performed comprehensive characterizations target cell distribution within anatomical compartments of penile tissue.

Methods: Drug levels were measured in foreskin and urethral tissue harvested from rhesus macaques (n=7) 24h after oral FTC (20mg/kg)/TDF (22mg/kg) dosing. Foreskin and urethral tissues were processed into single cell suspensions using enzymatic digestion. Intracellular FTC-TP and TFV-DP levels were measured in lymphocytes isolated from urethral and foreskin tissues using mass spectrometry. Immune cell characterization and HIV susceptibility markers were measured using flow cytometry. Paired two-tailed t test was used for statistical analyses.

Results: Median TFV-DP concentrations in foreskin and urethra lymphocytes were 221 (range 1-1,249) and 161 (range 19-756) fmol/106 cells at 24 h in animals dosed with oral Truvada. Immuno-characterization of foreskin and urethral cells revealed that both sites contained high frequency of CD4 T cell populations expressing HIV susceptibility markers CCR5 (p=0.01 and p=0.009) and HLA-DR (p=0.0005 and p=0.001) compared to PBMC. Foreskin contained a greater frequency of T cells within the lymphocytes population (p=0.03) and with a higher CD4/CD8 T cell ratio (p=0.006). Consistent with immune restricted sites, effector memory T cells were the principal subtype detected in both foreskin and urethral tissues (p=0.01 and p=0.005). The proportion of conventional (CDC) and plasmacytoid (PDC) dendritic cells were greater in urethra compared to foreskin (p= 0.03 and 0.02, respectively). Likewise, HIV susceptibility markers HLA-DR (p=0.007) and CX3CR1 (p=0.05) were higher on cells in the urethra than those in foreskin.
Conclusion: Oral Truvada results in high drug exposures in urethral and foreskin lymphocytes, two relevant cell populations for HIV transmission. TFV-DP levels detected in urethral and foreskin lymphocytes were in range with those that were highly protective against vaginal and rectal SHIV transmission. CDC and PDC abundance and CD4 T cell expression of HIV susceptibility markers HLA-DR and CX3CR1 distinguish unique immune barrier compositions in urethral and foreskin target cells. As evidence shows that urethral tissues are more susceptible to HIV infection, our results provide mechanistic insight into HIV susceptibility following penile exposure. These data support using this model to evaluate PK of next-generation antiretroviral prophylaxis products.

4

In vivo analysis of penile HIV-1 acquisition

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Background: Heterosexual men are at risk of HIV acquisition through the penis. Prevention methods for this mode of transmission are condom usage and male circumcision. However, low adherence to condom usage and the fact that 40% of circumcised men are not protected indicate a need for additional prevention strategies. Limited availability of tissues of the male genital tract and a paucity of relevant models has resulted in a lack of information regarding the precise mechanism of penile HIV-1 acquisition. Here we demonstrate that the penis of Bone marrow-Liver-Thymus (BLT) humanized mice is reconstituted with HIV target cells and that HIV acquisition in this model occurs after penile exposure.

Methods: Human cell reconstitution in penis of BLT mice was assessed by flow cytometry and immunohistochemistry. Susceptibility of human cells in the penis to HIV infection was evaluated by in situ hybridization (RNAscope®) and by quantitative PCR. BLT mice were challenged with HIV-1CH040, a transmitted founder virus, on the meatus urethra. Acquisition of HIV infection after exposure was confirmed in plasma and multiple tissues by quantitative PCR.

Results: All parts of penis of BLT mice (n=6) including urethra, and glans are reconstituted with human cells. T cells and macrophages were localized both in the epithelial and stromal compartments. Human cells in the penis of intravenously infected BLT mice were susceptible to HIV infection (n=5). Exposure of BLT mice (n=9 in 3 independent experiments) with a single dose of HIV-1CH040, resulted in HIV acquisition in 100% of inoculated animals that was detectable in plasma 2 weeks after HIV exposure.

Conclusions: The penis of BLT mice is reconstituted with human that are susceptible to HIV infection. A single penile HIV exposure to a transmitted founder virus resulted in 100% of HIV acquisition in BLT mice. This represents a new model for the investigation of the mechanism of HIV penile infection and for the assessment of HIV prevention strategies of female-to-male HIV sexual transmission.

5

Post-exposure prophylaxis with single doses of combination EVG/COBI/FTC/TAF protect macaques against Rectal SHIV Infection

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Background: On-demand post exposure prophylaxis (PEP) regimens that do not require anticipation of sex may be a preferred and cost-effective prevention option against sexual HIV acquisition. Integrase inhibitors act in a late stage of the HIV replicative cycle and are attractive candidates for PEP.
We investigated if a short and potent PEP regimen containing 1-2 oral doses of combination elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (EVG/COBI/FTC/TAF) can prevent rectal SHIV infection in macaques.

Methods: The PK profile of EVG/COBI/FTC/TAF (30/30/20/1.5 mg/kg) was evaluated at first dose. Efficacy of PEP was investigated in macaques exposed rectally to SHIV162p3 and treated with 1-2 oral doses of EVG/COBI/FTC/TAF at different times after exposure: +2h/+24h (n=6 macaques), +2h (n=5), or +24h/+48h (n=6). Virus exposures (up to 8) were done 2 weeks apart to minimize residual drug from previous doses. A Kaplan-Meier survival analysis was conducted and a log-rank test was used to compare time to infection relative to 10 untreated controls.

Results: COBI effectively boosted EVG in plasma (Cmax =1,936 ng/ml, AUC0-24h =23,336 ng*hr/ml) to human therapeutic levels. In PBMCs, peak FTC-TP (1737 fmols/106 cells) and TFV-DP (746 fmols/106 cells) levels were observed at 24h. EVG, FTC and TFV levels in rectal biopsies at 24h were 11.3, 1.1, and 0.2 ng/mg of tissue, respectively. After 8 virus challenges, EVG/COBI/FTC/TAF protected 5/6 animals in the +2h/+24h group (89% efficacy), 5/5 in the +2h group, and 4/6 in the +24/+48h group (71% efficacy) (p=0.0008, p=0.0002, and p=0.0065 respectively compared to untreated controls [9/10 infected]). Median residual TFV-DP prior to each virus challenge was undetectable except for the +24/+48 group (13.4 fmols/106 cells).
Conclusions. One or two doses of EVG/COBI/FTC/TAF initiated up to 24h after rectal virus exposure protected macaques from SHIV infection. These results identify novel on-demand PEP regimens with a wide dosing window and support efficacy studies in humans.

6

Transmitted/Founder Virus-Like Variants are Archived in the Reservoir

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The majority of heterosexually transmitted HIV infections are initiated by a single viral variant, the transmitted/founder virus (TFV). TFV replication has a persistent effect on disease progression, with individuals infected by low replicative capacity TFVs exhibiting a delayed time to <200 CD4+ T cells. However, the subject of persistence of the TFV in chronic ART-naive infection and the reservoir of latently infected CD4+ T cells is understudied. In a group of 13 HIV+ individuals, we have identified the TFV and investigated its evolution through amplification and sequencing of the env gene during chronic, ART-naïve infection at least two years from the estimated date of infection. Additionally, to investigate the potential persistence of the TFV in the reservoir, we amplified and sequenced env genes from peripheral white blood cells of six individuals in the group who were treated with ART for at least six months, and who demonstrated virologic suppression to <50 copies/mL. In all individuals, chronic, ART-naive viral variants demonstrated evolution from the TFV, with the time since infection significantly correlated to an increased median diversity from the TFV ($p=0.0005$). In assessing the phylogenetic relationship of the reservoir to the chronic viral quasispecies, we found that reservoir sequences included variants more closely related to the TFV than chronic viral variants. In all six individuals, the single closest genetic variant to the TFV was amplified from the reservoir, with an exact match observed in one individual. These variants may represent archiving of viruses that were present early in infection and remained in latently infected cells through treatment. Archiving of variants in the reservoir indicates that earlier initiation of ART, known to improve treatment outcomes, may additionally limit viral diversity of the reservoir and lead to a more homogenous population for targeting with reservoir elimination or HIV cure strategies.

7

Comparison of genetic sequences from donor and recipient viruses from linked subtype A transmission pairs

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HIV-1 subtypes have distinct geographical distributions. Subtypes A, C, D, and recombinants are commonly found in sub-Saharan Africa, with subtype C being the most prevalent. Subtype differences in disease progression have been observed, with individuals infected with subtype A viruses exhibiting slower CD4 decline and progression to AIDS diagnosis, as well as lower viral loads observed for at least two years post infection. Our previous studies focusing on subtype C HIV-1 transmission have shown that a genetic bottleneck occurs during transmission with a single virus variant generally being transmitted. Moreover, the viruses, which initiate infection (transmitted founder viruses), were shown to encode a greater fraction of consensus amino acids are more closely related to the cohort consensus than those of non-transmitted founder viruses, consistent with selection. In general, most virological studies of transmission in Africa have focused on subtype C, with only limited information on subtype A viruses.
We describe here the amplification and sequencing of viruses from 10 epidemiologically-linked, subtype A transmission pairs with samples collected within 90 days of the estimated date of infection (13-82 days). Although transmission of multiple genetic variants is observed with a frequency of approximately 15% in this cohort, in this study, we excluded multiple transmissions, and transmissions from an acutely infected partner. In a preliminary study where we compared transmitted-founder and linked non-founder viral sequences to an A1 consensus sequence, we did not observe a significant selection bias for more consensus-like amino acids in Gag, Pol or Nef. While these results are from a very limited number of transmission pairs, it raises the possibility that subtype A transmitted founder viruses undergo less genetic selection during transmission than subtype C, and that this may have a role in the decreased pathogenicity found in subtype A infected individuals.

8

HIV, HBV and HCV infection and linkage to care in migrants: the Immigrant Take Care Advocacy (I.Ta.C.A.) experience in Palermo

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Background: The massive and persistent boost of migration from the Mediterranean coasts highlighted critical issues in the health care management chronic infectious diseases such HIV, hepatitis B and C in migrant population. Considering the prevalence of these infections in African countries associated with additional risk factors, connected to the ways those people travel and the time they spent in Libyan camps, before they resettled in Italy, migrants are a particularly vulnerable population. Thus it is necessary to perform a screening test for HIV,HBV and HCV, in order to start an eventual linkage to care.

Methods: Upon arrival the patients have been included in a not formal network, that connects the local healthcare institutions and the reception centres on the territory. Migrants, were screened for HIV,HBV and HCV 4 to 6 weeks after arrival. When the hospital took charge of every single patient, there was always an intercultural mediator. For those subjects with one or more diagnosis of infectious diseases, after a proper transcultural counselling intervention, a program of diagnosis and cure was offered, according to the national guidelines.

Results: During the triennium 2015-2017, 2.639 migrants were observed, 28% women and 72% men with a medium age of 24 years. The 74% of migrants came from seven countries: Gambia, Nigeria, Senegal, Ivory Coast, Ghana and Mali. HIV infection was diagnosed in 57 cases. All the patients followed the diagnostic procedures according to the national guidelines for diagnosis and treatment of HIV. Antiretroviral therapy was offered to all the patients, after a proper transcultural counselling intervention. The HIV Care Cascade observed in this population highlighted: 77% of patients retained in care, patients on HAART: 68%; HIV-RNA < 20 UI/ml: 60%. HBV infection was diagnosed in 257 cases. All the patients followed the diagnostic procedures according to the national guidelines. The therapy was offered according to the latest EASL guidelines, after proper transcultural counselling interventions. We highlight that chronic HBV infection without hepatic disease was observed in 185 patients. Coinfection HBV/HIV was found in 51 patients (20.2%). The 79 % of HBV infected patients were retained in care of 79%, 50 started the treatment, and 47 had an HBV-DNA < 20 UI/ml. 24 cases of HCV infection were diagnosed. 10 were treated in our hospital according to the national guidelines, all of them completed the therapy and had a sustained virological response at the 12 weeks follow-up visit. 4 patients moved to other European countries and four were lost to follow-up, HIV coinfection was found in one patient.

Conclusions: In the last three years, the migration phenomenon took on our territory a not negligible number of people with chronic viral infections that in the vast majority of the cases needed a pharmacological treatment. Thus, we consider essential the elaboration of working schemes that can make easier the use of the healthcare system for this population, with the objective to guarantee the right to health to every single migrant and to stop the spread of HBV, HCV and HIV.

9

Heightened Inflammatory Response to Acute HIV-1 Infection in Zambian Women Compared to Men

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Women infected with HIV-1 have been reported in several studies to have lower viral loads than men, despite similar progression of disease. The reason for this discrepancy is unclear. Furthermore, epidemiological and immunological parameters of HIV infection are rarely examined in both men and women of the same cohort.

We compared longitudinal HIV viral loads (n=158) and CD4+ T cell counts (n=116) between 30 and 900 days post-estimated date of infection (EDI) from men and women enrolled in the Zambia-Emory Research Project cohort. We analyzed cell markers of activation in PBMC by flow cytometry at 1, 9 and 30 months post-EDI in 47 individuals (22 female, 25 male).

We observed significantly lower HIV viral loads in women than men throughout infection. Women exhibited higher initial CD4+ T cell counts, but CD4 loss occurred at a higher rate than in men. Early in infection, total CD4+ T cells in women were more highly activated (CD38+), particularly in the effector memory compartment, than those in men. CD4+ T cell activation at 1 month post-EDI was associated with higher set point viral load in men, but not women. Total CD8+ T cells in women exhibited comparable levels of activation (CD38+HLA-DR+) to those in men but effector memory cells were more highly activated. CD8+ cell activation at 1 month post-EDI correlated with the time at which women, but not men, progressed to <300 CD4+ T cells. Further, CD8+ T cells from women expressed higher levels of LAMP1 at 1 and 9 months post-EDI.

We observed that immunological markers differed between men and women at 1 and 9, but not 30 months post-EDI, indicating that men and women exhibited a differential acute and early chronic immune response to infection with HIV. Collectively these observations suggest a more robust inflammatory response early in HIV infection in women, which may control viral load and yet exacerbate CD4+ T cell death.

10

Genotypic and initial phenotypic characterization of transmitted/founder viruses from heterosexual transmissions in Rwanda

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Background: Studies of HIV-1 transmission and early infection is crucial for the development of preventative interventions, since the early phase of virus and host interactions determine the course of disease progression. HIV-1 subtypes have distinct geographical distributions, with subtypes A, C, D, and inter-subtype recombinants circulating in sub-Saharan Africa. Subtype differences in disease progression have been observed. Individuals infected with subtype A viruses exhibit slower CD4 decline and progression to AIDS diagnosis, as well as lower viral loads observed at approximately two years post seroconversion compared to those infected with subtype C. Inter-subtype recombination has been observed in this cohort, but the biological properties of recombinant transmitted founder viruses are not well defined.

Material & Methods: In the current study, we amplified near full-length single genomes (NFLG) of viruses from the plasma of a total of 24 acutely HIV infected (~30 days post EDI) individuals from a Rwandan heterosexual transmission cohort. We sequenced individual NFLG amplicons using PacBio SMRT technology combined with the MDPseq work flow we have described previously. Infectious molecular clones (IMCs) derived from amplicons encoding the TF sequence were constructed using a unique high efficiency cloning strategy. In vitro replication was carried out by infecting single donor PBMC.

Results: In this study, we sequenced and amplified over 300 NFLG from 24 patients with acute HIV (median 11 amplicons/patient) from a Rwandan heterosexual transmission cohort. Phylogenetic analyses showed that 25% (6/24) of HIV infections were established by 2 or more T/F viruses. Two C clade variants as well as 5 A/C and 1 C/D inter-subtype recombinants (25% recombinant) were identified in this A1 subtype dominant (67%) cohort. We then generated infectious molecular clone (IMC) by using TF NFLG, to further characterize the A1, C and A/C, C/D recombinants in terms of replication capacity. Our preliminary data show that subtype C and C/D recombinant viruses replicate better than most of subtype A1 viruses, although one of the A/C recombinant derived from an HLA_B*5703 patient replicated poorly.

Conclusions: We found a relatively high rate of inter-subtype recombination between A1, C, and D subtypes in this acute heterosexual HIV transmission cohort. Because of the unique breakpoints for each HIV recombinant, full-length genome amplification and sequencing are essential to identify and fully characterize these HIV variants genetically and phenotypically. Preliminary data suggest that the subtype A TF viruses replicate less well in in vitro replication assays. These studies of transmitted founder viruses in Rwanda provide insight into the molecular epidemiology of the epidemic and have implications for HIV-1 vaccine design.

11

Fc-gamma Receptor Affinity as a Host Factor for HIV Transmission

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Heterosexual HIV transmission is modulated by host and viral factors. Previous HIV vaccine trials have suggested that the Fc-gamma receptor (FcγR) is one such host factor. The human FcγRIIa and FcγRIIIa alleles are polymorphic, with SNPs resulting in different affinities for IgG antibodies. Changes in receptor affinity affect immune cell effector function and even HIV disease progression. This study aims to assess the potential role of FcγRs on HIV acquisition in a heterosexual transmission cohort. The 378 participants in this study, from the Zambia-Emory HIV Research Project (ZEHRP) study cohort, were categorized into: uninfected males (89), uninfected females (84), infected males (105), and infected females (100). Each was genotyped at both FcγRIIa and FcγRIIIa loci, using genomic DNA and a real-time PCR method, to define homozygous high affinity (Hhi), heterozygous (Het), or homozygous low affinity (Hlo) genotypes at each locus. In this subtype C population, no significant difference in allele frequency for either receptor was observed between infected and uninfected groups (p=0.97; 0.59). Men had a higher frequency of the Hhi genotype, and women a higher frequency of the Hlo genotype, regardless of infection status, however this finding was not statistically significant (p=0.07). The distribution of FcγRIIa alleles was 21% Hhi/49% Het/30% Hlo, while the FcγRIIIa allelic distribution was heavily skewed towards low affinity alleles with an 8%/37%/55% distribution. The genotypic distribution of FcγR alleles in the Zambian population was previously unknown, as is the role of FcγR affinities in HIV acquisition. Although no significant difference was observed based on HIV infection status in this small sample, further analyses on the impact of FcγR genotypes on the frequency and time to transmission, as well as disease progression, in the context of donor viral load, genital inflammation, HLA alleles, and replicative capacity of the transmitted virus are warranted.

12

Feeding practices of HIV-infected mothers for their infants within the first 6 months of life, Chantal Biya foundation, Cameroon

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Introduction: The risk mother-to-child transmission of HIV is increased by mixed feeding within the first 6 months of life. Thus, the WHO recommends exclusive breastfeeding for HIV-exposed infants within the first 6 months of life for resources-poor contexts such as sub-Saharan Africa. However, many studies have reported that some feeding practices are outside of the WHO recommendations in that region. This study aims to describe feeding practices of HIV-infected mothers for their infants within the first 6 months of life and determine factors associated with feeding choices in a Cameroonian urban area.

Methods: This cross-sectional study was conducted in the Mother and Child Centre, Chantal Biya Foundation, Cameroon. We recruited HIV-infected mothers who attended the first medical care of their infants within the first 6 years of life.

Results: In total, 83 HIV-infected mothers were recruited in this study at a median age of 29 years, of whom 58.7%, 33.7% and 8.7% were performing respectively exclusive breastfeeding, substitution feeding and mixed feeding. Mostly, breastfeeding was chosen due to health staff counselling (35.4%), free access (29.2%), and adaptation to nutritional needs of infants (27.1%). The choice of substitution feeding and mixed feeding were respectively related to fear of contamination (82.1%) and professional activities (49.2%). Being married (p=0,039), history of antiretroviral treatment (p=0,007) and disclosure of HIV status to a relative (p=0,007) were associated with exclusive breastfeeding choice.

Conclusion: This study suggests that breastfeeding is more performed than substitution feeding for HIV-exposed infants within the first 6 months of life in a Cameroonian urban area. Some mothers are still performing mixed feeding in this country.

13

PREVENGO: Prevention comes from you, an experience from Palermo, Italy

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Background: According to the latest annual report on new HIV diagnosis in Italy by the Italian National Institute of Health, a slight decrease in the number of new HIV diagnosis was registered; the majority of those were acquired by sexual transmission. PrevenGo "La Prevenzione vien da Te" is a project brought on by volunteers of Palermo ARCIGAY association in partnership with: Civico Hospital, with the advocacy of ANLAIDS Sicilia, "Ente Regionale per il Diritto allo Studio" and University of Palermo. Financed by "Fondazione con il Sud". The aim of the project is to test and detect HIV infection in general population.

Material & Methods: In this project a rapid test for the detection of HIV antibodies from a finger-stick sample of blood, was actively offered from Palermo ARCIGAY volunteers after the self administration of a questionnaire on the sexual habits that had the aim to understand any risky sexual behaviour. A counselling with a doctor was offered before testing. The questionnaire had "key questions" used to build up a risk score that ranges from a minimum of 4 to a maximum of 12 points, these include: condom use, number and type of partners, chemsex habit and previous STD. A statistical analysis was performed using SPSS software.

Results: A total of 1.007 tests were performed from April 2016 to May 2018, the population characteristics are the following: median age 25 years, 344 females and 663 males, 83 % were Italian and 18% were foreigners. As regards the sexual orientation the 52,5% of the subjects were heterosexuals, 41% homosexuals, 6% bisexuals and 0,5% queers. The 32,7% of the subject had performed a previous HIV test with a negative result and 5,9% had a previous history of a STD in the previous 12 months. 989 tests (98,2%) resulted negative and 18 (1,8%) resulted positive. In the test positive group 14 subjects were male and 4 female, 13 were Italian and 5 foreigners. One subject had had risk score of 4, five had a risk score of 6, two had a risk score of 7, three a risk score of 8, seven a risk score of 9 or more. In the test-negative group: 163 subjects had a risk score of 4, 73 had a risk score of 5, 214 had a risk score of 6, 190 had a risk score of 7, 179 had a risk score of 8, 89 had a risk score of 9 and 81 had a risk score of 10 or more.

Conclusions: The prevalence of new HIV diagnosis detected during the project is 1,8%. 12 subjects out of 18 with the new diagnosis had a risk score of 7 or more. Despite a low prevalence, it is crucial to raise awareness on the risk factors for sexual transmission of HIV with the implementation of primary prevention interventions, strengthen the role of NGOs in these activities, to study the possibility to offer the test to subjects that have high risky behaviours, and to treat all patient with HIV infection.

14

The experience in HIV perinatal transmission in Obstetric Gynaecology Hospital Ploiesti, Romania

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Background: In 2017, in Obstetric Gynaecology Hospital Ploiesti, Romania, 3580 new born babies were given birth.

Material and methods: All admitted pregnant women were tested for HIV infection with Artron HIV ½ Antibody Rapid Test, Catalog Number: A02-07-222 Specimen: Serum/Plasma produced by Artron Laboratories Inc, Canada. From the whole group only three women were positive.

Results: First HIV positive patient for the rapid test was a 38 years old woman, who was pregnant six times and delivered two children. The actual pregnancy was 38 weeks old. Her blood was sent for confirmation with a RT-PCR test and turn out negative for HIV infection. She gave birth to a female baby 2600 g, Apgar 8. The baby was also tested for HIV and turn out negative. The second HIV positive patient for the rapid test was 18 years old, first time pregnant and at her first delivery. The actual pregnancy was 39 weeks old. The patient was known as a HIV infected patient for 10 years and was in B2 HIV stage. At 34 weeks of pregnancy she presented CD3-864 cell/mm3, CD4-389 cell/ mm3, CD8 -443 cell/mm3, VL-277 copies ARN HIV/ml. The patient was on HIV treatment with Combivir 1cp and Kaletra 2 cp two times per day. She was recommended C-section and HIV prophylaxis for the new born. She gave birth of a male baby 3000 g, Apgar 10. She was not allowed to breastfeed and was treated with Bromocriptina 2 cp three times a day for three days. The new born was tested right after birth and turn out HIV positive for the rapid test (Artron HIV ½ Antibody Rapid Test) and started HIV prophylaxis with Retrovir 2 mg/kg at six hours and Epivir 2mg/kg twice a day. He was transferred in four days to a Infectious Disease Hospital. The third HIV positive patient for the rapid test was 26 years old, second time pregnant and at her first delivery. The actual pregnancy was 39 weeks old. The patient was known as a HIV infected patient for 5 years and was in B2 HIV stage. At 32 weeks of pregnancy she presented CD4-353 cell/mm3, CD3-823cell/mm3, no viral load was performed. The patient was on HIV treatment with Combivir 1cp and Kaletra 2 cp two times per day. She was recommended C-section and HIV prophylaxis for the new born. She gave birth of a a male baby 2800 g, Apgar 9. She was not allowed to breastfeed and was treated with Bromocriptina 2 cp three times a day for three days. The new born was tested right after birth and turn out HIV positive for the rapid test (Artron HIV ½ Antibody Rapid Test) and started HIV prophylaxis with Retrovir 2 mg/kg at six hours and Epivir 2mg/kg twice a day. He was transferred in four days to a Infectious Disease Hospital.

Conclusion: In Ploiesti Obstetric Gynaecology Hospital the incidence of HIV infection is low.

15

Analysis of impediments to the retention in care of people with HIV

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Background: In Italy, the phenomenon of non-retention in care among HIV+ people has been so far monitored by the Infectious diseases centers, but still little analyzed in its predictive elements.

Methods: This research focused on identifying eventual predictive elements of a potential lack of retention in care among HIV+ patients, analyzing some organizational aspects of Infectiology Centers and some others related to individual characteristics of HIV+ people in care. The data refer to 23.491 HIV+ patients in care in 18 Infectious diseases centers in 10 representative regions of North-Central and South Italy and the analysis covered the period between 2010 and 2017. In evaluating individual characteristics of people lost in follow up, the gender, age, position, location of the treatment centers were considered as assessments points.For investigating clinicians and nurses' opinions about predictive elements of lack of retention in care, they were asked to take two different kind of questionnaires, answered by 32 infectious diseases doctors and 55 nurses in centers that joined the project.

Results: The analysis highlighted how the issue of non-retention in care concerns particularly immigrants people, from European and Extra-European countries, whereas Italian people in care tend to rank low "lost in follow" rates. Comparing the percentage of HIV+ people in care lost in follow up migrating from European and non-European countries that stands at about 8.5%, the lack of retention in care in Italian patients is just over 4%. An important predictive element of a possible lack of retention in care is the age: patients under 30 years of age have ranked percentages of lost in follow up around 10%, a decidedly high value, also considering their greater mobility among treatment centers which could amplify the collection of data. On the contrary, gender was found not to affect retention in care in any way. Among others, two life situations of people in care were considered more predictive of a possible lack of retention in care: the use of substances of abuse and not having a permanent home. It also emerged how dimension of centers affects the retention of patients, with an advantage for larger centers.

Conclusions: The research underlined a cognitive element that so far has not been registered on the relationship between patients' young age and risk of failing the retention in care.
It also highlighted how new tools for tracing patients' mobility among centers, a significant phenomenon that makes quantitative data more difficult to interpret, should be considered for a further detailed analysis.
During the discussion of the research results, it was proposed to introduce the obligation, for the receiving center, of communicating the arrival of a new patient to the Center where the patient was previously treate

16

Vertical Transmission of HIV, a neverending story

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Background: According to UNAIDS data, 36.9 million people globally were living with HIV in 2017, 1.8 million of these were children (age <15 years). Great progress has been made in treating, prevention and screening of HIV, in fact new HIV infections among children declined by 35% since 2010 and by 16% in adults. Nowadays thanks to a wider access to antiretroviral therapy mother to child transmission rates could be reduced below 5 % if associated with artificial breastfeeding, despite this mother to child infection still exists, and it is important for clinicians to raise awareness towards this problem.

Case presentation: We present two cases of HIV positive migrant children that acquired the infection from their mothers. The first case is a three years old Ghanaian boy, whose mother was HIV negative at the moment of delivery. The boy was breastfed and had a normal growth curve. His HIV infection was diagnosed in May 2016 after his mother was discovered to be HIV positive. The mother acquired the infection after delivering the baby, from her husband and the kid is thought to have acquired the infection during the breastfeeding period. At diagnosis the CD4 cell count was 918 cellμ/L at the diagnosis with a viral load of 377400 copies/ml. The second case is a two years old Ghanaian girl that was born from an HIV infected woman. Her mother's infection was diagnosed after the arrival in Italy, the girl was subsequently tested after and resulted positive. The infection was acquired via vertical transmission since her mother was not diagnosed with HIV in her home country. At diagnosis the CD4 cell count was: 928 cell/μL with a viral load of 57650 copies/ml. Both children, had a negative serology for hepatitis B and C. They started ARV according to the current Italian guidelines with ABC+3TC+LPV/r oral suspension a few days after the definition of the immune virological situation. No side effect were reported during at the follow up visits, an optimal response to therapy was archived since the viral load is not detectable in the first case and is 94 copies/ml in the second child.

Discussion: Despite an encouraging decrease of new HIV diagnosis in children, we must keep in mind that vertical transmission of HIV still exists and that it is crucial to test pregnant women for HIV, above all the ones belonging to fragile population such as migrants. A prompt diagnosis will give the chance to treat the patient immediately significantly decreasing the risk of transmission to the new-born together with all the difficulties linked to the treatment of children such as different pharmacokinetics , compliance issues and the risk of developing resistances to the therapy. In conclusion all pregnant women should be tested for HIV in order to archive an early diagnosis and treatment, in this way we might put an end to this "neverending story".