Abstract Book
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Abstracts
Oral Presentations
Abstract: 1

Treatment Strategies for HIV/ Hepatitis infected Patients

Viro-immunological response to treatment of naïve HIV-1 positive patients with viral load > 500,000 copies / ml: a personalized treatment is needed?

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Background: very little information is available about response to treatment of naïve patients with viral loads >500,000 copies/mL. Main aim of this study was to understand whether a four drug regimen that includes raltegravir (RAL) works better in this population than a triple-drug regimen to reach a HIV viral load below the limit of detection (BLD) and may result in a better immune recovery.

Materials & Methods: the data of 224 naïve HIV-1 patients from eight Infectious Diseases Centers in Italy, with viremia levels pre-therapy >500,000 copies/mL, receiving a three-drug regimen, or a four-drug regimen including RAL, were collected retrospectively from January 2008 and analysed. Clinical and virological data were locked after 12 months of follow-up. Statistical analysis was conducted with intention to treat methods. The time to reach a BLD, was calculated using Kaplan Mayer survival test. CD4 cell counts were evaluated at 3, 6, 9 and 12 months.

Results: out of 224 patients, 13.4% had an acute infection (PHI) and 42% were AIDS presenters. 194 patients without PHI were included: 70.6 % males, median age 42 years (IQR 35-41), median CD4 nadir and baseline median CD4 cell count 86 and 100 cells/µL respectively, median HIV-RNA 5.98 log10 copies/µL, with sexual transmission 85.6%. A standard triple drug regimen was started in all patients, boosted-protease inhibitors (PI/r) were used in 159 (82.0 %), non-nucleoside transcriptase-inhibitors (NNRTI) in 35 (18.0 %). Integrase-inhibitors (INI) was added in 33 subjects, all of them on a PI based regimen for a median time of 11.5 months (IQR 4-12). The choice of a standard regimen vs a four-drug regimen containing RAL was driven only by higher baseline viral load (OR. 6.00; 95%CI 1.75-20.6; p=0.004) as shown by multivariate regression analysis. Mean time to reach a HIV-RNA BLD was similar in the three group (10.7 months; p= 0.140 Log Rank test) (Figure 1). The recovery of CD4 was similar with all the regimens used. Only 68.3% of the patients reached a HIV RNA below 50 copies in the observed period regardless of the treatment used. Nine (8 for AIDS) patients died during follow-up.

Conclusions: our results show that in naïve patients with HIV RNA >500,000 copies/µL the regimens containing PI/r are preferred, but PI give no advantage compared to NNRTI nor in terms of recovery of CD4 lymphocytes nor with regard to achievement of undetectable viral load. Furthermore we show that the achievement of undetectable viremia occurs very slowly. The clinical implication of these data may be of some significance, suggesting that personalized regimens in treatment-naïve patients with high viral load are needed to improve virologic response. However, with a four-drug regimen with RAL compared to standard therapy with either PIs/r or NNRTIs even though a viremia below the limit of detection is reached faster in some patients, this advantage is lost if the observation is extended over time.

No conflict of interest
Abstract: 2

Viral Evolution & Genetic Diversity

Children age, antiretroviral treatment and CD4 count are major determinants of HIV-1B evolution in pediatric patients

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Background: Determining the factors that modulate the genetic diversity of HIV-1 populations is essential to understand viral responses to antiretroviral therapy (ART), disease progression and may help to predict the appearance of resistant variants. Most attention on this subject has been focused in adult patients, in whom HIV-1 genetic diversity has been sometimes (but not always) associated with clinical factors such as age, CD4 cells count, viral load (VL), and ART experience. However, in children little is known about which are the clinical parameters that affect viral evolution and how they do it. This is the first study analyzing in detail the role of five clinical factors in HIV-1 evolution in a large number of infected children, and in an adult population of the same geographical region.

Materials & Methods: To address this issue, 167 HIV-1 subtype B (HIV-1B) perinatally infected children and adolescents (pediatric cohort) of Madrid, Spain, were selected between 1994 and 2011. An additional group of 640 HIV-1B infected adults (adult cohort) from Madrid was used for comparison purposes. For each of these 807 patients, one HIV-1B pol sequence was obtained. Sequences were used to estimate nucleotide substitution rates (subs/site/year), genetic diversity (π), rates of synonymous (ds) and non-synonymous (dn) changes, selection pressures (dh/ds) and frequency of drug resistance mutations (DRMs) in the virus population. We analyzed the relative importance of children age, CD4+ T cells counts (as %CD4 and CD4/mm3), VL, and ART experience in shaping patterns of HIV-1B sequence variation. The effect of clinical factors in the inferred evolutionary variables was analyzed by principal component and correlation analyses.

Results: In the pediatric HIV-1B population, children age, ART experience and CD4 count were the primary predictors of evolutionary parameters, explaining half of the variation in these parameters. Nucleotide substitution rates, genetic diversity and non-synonymous mutations increased with children age and ART experience, and decreased with CD4 count. Interestingly, these clinical factors did not affect the evolution of HIV-1B in adults. Maximum values of nucleotide substitution rates, genetic diversity and rate of synonymous mutations in the pediatric HIV-1B population were smaller than in the corresponding virus population in adults, whereas the opposite trend was observed for the rate of non-synonymous mutations. Hence, HIV-1B pediatric populations increased their diversity by accumulating non-synonymous mutations, and HIV-1B in adults increase their genetic diversity by accumulating synonymous mutations. As a consequence, negative selection pressures were stronger in the adult that in the pediatric HIV-1B population. This relaxed selection pressures in the pediatric virus population were associated with higher frequency of DRMs.

Conclusions: Our results indicate that exposure to the virus (age), ART experience and reduced number of CD4 cells promote faster HIV-1B adaptive evolution in infected children. However, these factors do not affect the evolution of HIV-1B in adult patients that generally evolves at higher rates, and is more genetically diverse; than the pediatric HIV-1B population in the cohort of Madrid patients. These findings contribute to understand the factors that modulate HIV-1B evolution.

No conflict of interest
Abstract: 3

Spread of Drug Resistance

Evolution of HIV-1 drug resistance in patients failing a standard thymidine analogue-based first-line ART: implications for the activity of next-line regimens from a longitudinal study in Mozambique.


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Background: Virological monitoring of ART is now being implemented in LMIC as a more sensitive and specific tool to detect failure of currently recommended NNRTI-based first line ART and as guide to switch to boosted PI-based second-line ART. However, given the paucity of next treatment lines in LMIC, the correct switching time should be informed by the probability of accumulating resistance to the subsequent treatment lines at a given time of virological failure. Aim of this study was to describe the accumulation of drug resistance and its effect on the activity of next-line therapy components in patients with virological failure after 1 year of first-line ART not switching to second-line ART for one additional year.

Methods: We selected patients from the DREAM cohort from 3 sites in Maputo, Mozambique who a) were on a first-line ART b) had an HIV RNA>1,000 copies/mL after 1 year (t1) and after 2 years (t2) of ART without switching to a second-line ART and c) had a stored plasma sample at t1 and t2. Genotyping of RT and protease was performed at t1 and t2 by the Trugene assay. Proportion of subjects with major IAS-USA mutations to NRTI and NNRTI and with resistance to individual drugs as interpreted by Stanford’s HIVdb 7.0 system was calculated.

Results: 48 subjects were analysed: 50% females, 95.8% carried subtype C. Pre-ART median age was 35y, CD4 165/mL, HIV RNA 4.69 log10 cps/mL, calendar year 2010. First-line ART contained ZDV in 85.4%, d4T in 10.3%, nevirapine in 83.3%, efavirenz in 10.4%. The t1 sample was collected a median of 12.2 months after ART start with a median HIV RNA 3.78 log10 copies/ml. The t2 sample was collected a median of 12.2 months after t1, with a median HIV RNA 4.0 log10 copies/ml and CD4 236/mL. Mean adherence to on time pharmacy appointments from ART initiation to t1 was 94.7% and from t1 to t2 94.3%. Table 1 shows the proportion with resistance mutations and predicted resistance to individual drugs at t1 and t2 and the projection at 3 years, assuming linear accumulation of resistance.

<table>
<thead>
<tr>
<th>Pts with NRTI RAM</th>
<th>M184V</th>
<th>Any TAM</th>
<th>K65R/E</th>
<th>Pts with NNRTI RAM</th>
<th>Pts &gt; LLR to ZDV and d4T</th>
<th>Pts &gt; LLR to 3TC and FTC</th>
<th>Pts &gt; LLR to TDF</th>
<th>Pts &gt; LLR to NVP and EFV</th>
<th>Pts &gt; LLR to ETR</th>
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<td>t1 §</td>
<td>79.2%</td>
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<td>81.2%</td>
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<td>8.3%</td>
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<td>t2 °</td>
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<td>38.3%</td>
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<td>t2 °</td>
<td>87.2%</td>
<td>87.2%</td>
<td>46.8%</td>
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<td>91.5%</td>
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<td>t3 *</td>
<td>95.2%</td>
<td>100%</td>
<td>76.9%</td>
<td>10.8%</td>
<td>100%</td>
<td>100%</td>
<td>55.5%</td>
<td>99.4%</td>
<td>32.2%</td>
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</table>

RAM= major resistance associated mutations (IAS-USA list 2014); LLR=low level resistance by HIVdb v.7.0; ZDV, zidovudine; d4T, stavudine; 3TC, lamivudine; FTC, emtricitabine; TDF, tenofovir; NVP, nevirapine; EFV, efavirenz; ETR, etravirine.
§ t1: 1 year after ART initiation, first time point of documented virological failure
°t2: 2 years after ART initiation (1 year after first documented virological failure)
*A: 2 years after ART initiation (1 year after first documented virological failure) considering historical genotype (accumulating resistance of t1+t2)
*3: 3 years after ART initiation (2 years after first documented virological failure), projected results assuming linear accumulation and historical genotype at year 2 (cumulating resistance of t1+t2)
While the activity of NNRTIs is compromised early during failure, tenofovir and zidovudine activity begin to be more frequently reduced after 1 year of documented virological failure of thymidine analogue-based first-line ART, particularly when the historical genotype is considered.

**Conclusion:** In subjects failing first-line NNRTI-based ART, drug resistance accumulates over time, as expected, but the activity of different components of the first- and next-line ART is affected at different time points. The present observation may inform decisions on when to switch to a second line therapy in patients on virological failure in LMIC settings.

No conflict of interest

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**Abstract:**

**Treatment Strategies for HIV/ Hepatitis infected Patients**

**Integrated HIV-1 DNA load during stably suppressive antiretroviral therapy is associated with the frequency of CD8 cells expressing HLA-DR/DP/DQ**

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**Background:** The characterisation of correlates of HIV persistence during suppressive antiretroviral therapy (ART) informs understanding of disease pathogenesis and guides the design of curative strategies.

The aim of this study was to investigate factors associated with levels of integrated HIV-1 DNA during stably suppressive first-line ART with 2 NRTIs + efavirenz or nevirapine.

**Materials & Methods:** Integrated and total HIV-1 DNA (per 10⁶ PBMC) and residual (below 50 copies/ml) plasma HIV-1 RNA were measured by Alu-gag PCR and sensitive qPCR, respectively. Soluble CD14 and cellular markers of T-cell activation (CD4 plus CD45RA, CD26, CD69, or CD38; CD8 plus CD38 or HLA-DR/DP/DQ) were measured by ELISA and flow cytometry, respectively. The association between integrated HIV-1 DNA load and other variables was tested using univariate and multivariable linear regression models.

**Results:** The study population comprised 50 patients that started first-line ART with 2 NRTIs plus either efavirenz or nevirapine, achieved plasma HIV-1 RNA suppression <50 copies/ml within 6 months of starting ART, and during subsequent follow-up showed continuous viral load suppression <50 copies/ml (no blips allowed) while remaining on the same NNRTI. At study entry, duration of viral load suppression was median 6.4 years (IQR 3.1-8.8) and the median CD4 count was 572 cells/mm³ (IQR 478-734). Residual plasma HIV-1 RNA was detected in 29/50 (58%) patients at a median level of 4 cps/ml (IQR 2-8). Total and integrated HIV-1 DNA levels were median 2.6 (IQR 2.3-2.9) and 1.9 (IQR 1.7-2.2) log₁₀ copies/10⁶ PBMC, respectively. The cross-sectional analysis showed an increase in CD4 cell counts (p=0.05), a reduction in frequencies of CD8⁺CD38⁺ and CD4⁺CD38⁺ cells (p=0.01), and a marginal decline in residual plasma HIV-1 RNA levels (0.08) with longer duration of suppression; there was no significant change in other measured parameters including total (p=0.60) and integrated (p=0.28) HIV-1 DNA load, sCD14 levels (p=0.19), and frequencies of CD8⁺DR/DP/DQ⁺ cells (p=0.17). Integrated HIV-1 DNA load was positively correlated with total HIV-1 DNA load (p<0.0001) and with the frequency of CD8⁺HLA-DR/DP/DQ⁺ cells (p=0.04), but not with residual plasma HIV-1 RNA levels (p=0.33) or frequency of CD8⁺CD38⁺ cells (p=0.57). A multivariable model including duration of viral load suppression on ART, pre-ART viral load, residual plasma HIV-1 RNA levels, current CD4 cell count, sCD14 levels, and frequency of CD8⁺HLA-DR/DP/DQ⁺ cells showed that integrated HIV-1 DNA load was mean 0.51 log₁₀ copies/10⁶ PBMC.
copies higher for each 50% increase in the frequency of CD8⁺HLA-DR/DP/DQ⁺ cells (95% CI 0.15, 0.86; p = 0.01). The association was confirmed in a separate model including the nadir CD4 cell count instead of the pre-ART viral load.

**Conclusions:** During stably suppressive ART, a higher integrated HIV-1 DNA load was associated with the frequency of CD8⁺DR/DP/DQ⁺ cells but not with the frequency of CD8⁺CD38⁺ cells. The mechanisms underlying the selective association of levels of integrated HIV-1 DNA in PBMC with distinct CD8 activation profiles warrant further investigation.

No conflict of interest

**Abstract: 5**

**Novel Diagnostic Technologies & Approaches**

**Comprehensive longitudinal characterization of HIV-1 reservoir markers in patients on stable antiretroviral treatment**

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**Background:** There is an increasing interest in characterisation of the viral reservoir in patients on long-term antiretroviral therapy in the context of HIV cure studies. The main question remains which assay is most relevant to accurately predict the size of the replication competent viral reservoir. Although both PCR and viral outgrowth assays have been proposed, mainly PCR based assays have been validated in clinical trials. Conflicting data exists about the correlation of viral outgrowth assays and PCR based assays. In addition, within individual patients, the long term variability of PCR reservoir markers of total HIV DNA, 2LTR circles and full length cell-associated (CA) RNA is poorly addressed.

**Materials & Methods:** We set-up a study with a well-defined patient cohort (N=25) to characterize the longitudinal kinetics of the viral reservoir by PCR based methods and to assess the correlation of the viral reservoir markers with the viral outgrowth assay. Blood samples were drawn at three time points with median (IQR) of 2.5 years (IQR 2.4-2.6) between time point 1 and 2; and median of 31 days (28-36) between time point 2 and 3. Total HIV-1 DNA, unspliced (us-) and multiply spliced (ms-) HIV-1 RNA, and 2LTR circles were quantified in peripheral blood mononuclear cells (PBMCs) using droplet digital PCR. Parameters of HIV-1 persistence were quantified at 3 time points. Alu-PCR was used to quantify integrated HIV-1 DNA. Viral outgrowth assay and integrated HIV-1 DNA were performed at one time point (2nd time point).

**Results:** No significant change was found for long- and short-term dynamics of all markers (total HIV-1 DNA, unspliced and multiply spliced HIV-1 RNA, and 2LTR circles) of HIV-1 persistence in peripheral blood. Integrated HIV-1 DNA was detected in all patients with median (IQR) of 3.04 (2.65-3.37) log_{10} copies/10⁶ PBMCs; and it correlated well with total HIV-1 DNA (p=0.002, R²=0.54); unspliced HIV-1 RNA (p=0.001, R²=0.40); and viral outgrowth assay (p=0.014, R²=0.20). Replication competent virus was detected in 80% (20/25) of patients and it correlated well with total HIV-1 DNA (p=0.017, R²=0.54). The mean difference (bias) between the HIV copy numbers generated with Alu-PCR and viral outgrowth assay, assessed with Bland-Altman test, was 2.38 ± 0.83 log_{10} (95% Limits of Agreement). And a corresponding bias between total HIV-1 DNA and VOA was 0.8 ± 0.72 log_{10} (95% Limits of Agreement).

**Conclusion:** This study supports the finding that viral reservoir size and long- and short-term dynamics remain stable over time in patients receiving stable cART. Our study shows the presence of a very stable reservoir in terms of viral dynamics (2LTR circles and CA RNA) in patient under ART. Interestingly, we found a correlation between integrated HIV DNA and the viral outgrowth assay, indicating that a stable fraction of integrated HIV is replication competent.

No conflict of interest
Abstract: 6

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Resistance Post Week 48 in ART-Experienced, Integrase Inhibitor-Naïve Subjects with Dolutegravir (DTG) vs. Raltegravir (RAL) in SAILING (ING111762)

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Background: SAILING was a Phase3 clinical trial evaluating DTG 50mg once daily vs RAL 400mg twice daily in ART-experienced, integrase inhibitor (INI)-naïve, HIV-1 infected subjects. Previous Week 48 results reported fewer subjects failed with INI-genotypic or -phenotypic resistance on DTG (4/354, 1%) versus RAL (17/361, 5%), p=0.003. Two SAILING subjects receiving DTG were reported through Week 48 with emergent R263K integrase substitutions and DTG IC50 fold change (FC) <2 versus wildtype.

Materials & Methods: Protocol defined virologic failure (PDVF) required confirmed HIV-1 RNA >400 c/mL (Abbott RealTime assay). PDVF non-response was <1 log10 c/mL decrease by Week 16, unless <400 c/mL, OR ≥400 c/mL on or after Week 24. PDVF rebound was ≥400 c/mL after confirmed <400 c/mL, OR >1 log10 c/mL above nadir of ≥400 c/mL. Integrase population and clonal genotypes and phenotypes were assessed by Monogram BioSciences. Pre-dose PK samples were collected at Weeks 4, 24 and 48.

Results: Three subjects without baseline population or clonal RAL primary resistance and receiving DTG experienced PDVF with treatment-emergent integrase substitutions during post-Week 48 continuation phase. One subject (HIV-1 subtype B) had DTG trough level below limit of detection (LOD=0.02 ug/mL) at Week 24, and experienced PDVF at Week 108 with emergent N155H, and DTG FC=1.8 and RAL FC=12. Clonal analysis showed 1/8 had N155N (FCs: DTG=0.9, RAL=1.3, EVG=1.0), 1/8 had S230R/N155H (FCs: DTG=2.5, RAL=44, EVG=134), and 6/8 had N155H (median FCs:DTG=1.9, RAL=23.5, EVG=57). A second subject (HIV-1 subtype B) with documented non-compliance developed emergent I60L/T97A/N155H at Week 72 PDVF with DTG FC=2.4 and RAL FC=113. Clonal analyses showed 8/8 had I60L/T97A/N155H; median DTG FC=2.4. A third subject (HIV-1 subtype B) with Week 48 DTG trough level=0.03 ug/mL showed gradual viral load increase >50 c/mL starting Week 96 through Week 132 (from 129 c/mL-1054 c/mL). Population resistance testing for the Week 120 PDVF time point and at Week 132 confirmation showed emergent A49G/S230R/R263K at each time point; respective Week 120 and Week 132 DTG FCs were 3.8 and 5.8, and RAL FCs were 2.4 and 2.6. Clonal analyses showed that 8/8 clones at Week 120 were A49G/S230R/R263K; median DTG FC=2.4. The S230 to S230R and S230R to S230G substitutions required two independent single nucleotide changes.

Conclusions: For three subjects experiencing PDVF post- Week 48 with emergent INI resistance there was data suggesting sub-optimal adherence. The HIV-1 in two subjects with N155H pathway had low level DTG FC values similar to N155H pathway virus observed during RAL therapy. One subject with R263K IN pathway virus at Week 120 and at Week 132 had DTG FCs that were 2- to 3-fold higher than the DTG FCs of <2 associated with R263K isolates previously observed through Week 48. The on-study increasing DTG FC and minority species codon changes in this case were consistent with evolving resistance of an R263K pathway virus during continued DTG therapy with replicating HIV-1.

Conflict of interest: Employee of GlaxoSmithKline.
Abstract

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Viro-immunological efficacy and tropism evolution in treatment-experienced HIV-1 infected patients starting maraviroc in clinical practice

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Background: We evaluated the viro-immunological efficacy and tropism evolution in an Italian cohort of treatment-experienced HIV-1 R5-tropic infected patients starting for the first time a regimen containing maraviroc (MVC) in clinical practice.

Material & Methods: 107 patients were evaluated. Among them, 34 started MVC under virological suppression (suppressed-group) and 73 after virological failure (viremic-group). All patients had an available baseline V3 sequence. Survival analyses were used to estimate, after MVC start, the probability and the predictors of: i) virological success (VS, first viremia <50 copies/mL) in viremic patients; ii) virological rebound (VR, the first of 2 consecutive viremia >200 copies/mL) both in viremic (after VS) and suppressed patients. Linear regression mixed model was used to evaluate the change of CD4 count from MVC starting. Baseline geno2pheno false positive rate (FPR, %) values were stratified as follows: 10-20; 20-60; >60. In a subset of viremic patients with an available V3 sequence both at baseline and during MVC-treatment, tropism evolution was evaluated.

Results: Patients were pluri-treated (N. of previous regimens, median [IQR]: 6 (2-9) for suppressed-group; 7 (4-10) for viremic-group). Median (IQR) baseline CD4 count was 434 (198-616) and 337 (180-463) cells/µl in suppressed- and viremic-group, respectively. The 58% of suppressed patients received MVC in dual regimen (95% with PI/r), while the 68% of viremic patients received MVC with at least two other drugs. By 12 months of ART, the probability of VS in viremic-group was 82%. By Cox multivariable analysis (adjusting for age, gender, baseline FPR/viremia/CD4 count, number of drugs co-administer with MVC, first darunavir/etravirine/raltegravir usage), in these viremic patients a higher baseline viremia was associated with a lower probability of VS (relative hazard, RH [95% CI]), per 1 log10 increase: 0.65 [0.49-0.87], p=0.003). Moreover, viremic patients showed a significant CD4 count increase after MVC starting (mean±standard error: +61±5 cells/µl per 1 year longer of follow-up, p<0.001). In particular, patients with a baseline FPR >60% showed a higher CD4 change compared to those with FPR 10-20% (adjusted mean±standard error cells per 1 year longer of follow-up: +93±16 for FPR>60; +25±14 for FPR 10-20; p<0.001). The probability of VR after 24 months of follow-up was 40% and 7% in viremic and suppressed patients, respectively. Viremic-group showed a lower risk of experiencing VR by increasing baseline CD4 count (RH, per 100 cells/µl increase: 0.43 [0.25-0.76], p=0.003). A switch from R5- to X4-tropic virus was found in 10/27 (37%) viremic patients during MVC-treatment, and was associated with a lower baseline FPR (proportion of patients with X4-tropism at VR: FPR <20, 75%; FPR 20-60, 50%; FPR >60, 15%; p=0.016).

Conclusions: ART-experienced HIV-infected patients receiving MVC show a good viro-immunological response. During MVC-treatment, the proportion of patients switching to X4-tropic increase by decreasing baseline FPR levels. This result confirms our previous findings showing that FPR determined by V3 population sequencing can predict the burden of X4-tropic HIV quasispecies.

No conflict of interest
Abstract

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

The HIV1 reverse transcriptase E138A polymorphism decreases the genetic barrier to resistance to etravirine in vitro

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Background: Etravirine (ETR) is a second generation non-nucleoside reverse transcriptase inhibitor (NNRTI) which is active against most of viral mutants selected by first generation NNRTIs. While several mutations have been clearly shown to be implicated in resistance to ETR both in vitro and in vivo, conflicting results have been reported on the impact of the natural polymorphism E138A. In this study, we compared the development of resistance to ETR by in vitro selection experiments starting with the 138A variant vs. the 138E wild type.

Materials & Methods: Clinically derived viral strains carrying 138E or 138A reverse transcriptase (RT) were selected from drug-naive patients. A PCR fragment comprising the entire RT and RNAseH region was amplified to create recombinant viruses (RVs) by homologous recombination into 293 Lenti-X cells with a modified version of pNL4-3 vector. ETR susceptibility of each RV was calculated by infection of TZM-bl cells in the presence of serial dilutions of the drug. In vitro selection experiments were performed through infection of MT-2 cells with an initial concentration of 50 nM of ETR. Cultures were monitored for cytopathic effects every 48-72 hours. In the presence of cellular syncytia induced by viral replication, supernatants were collected for genotypic analysis and used for infecting MT-2 cells with a fivefold increase of ETR concentration.

Results: Three RVs with 138E and three with 138A were created. Viruses with 138E showed ETR fold change (FC) values of 0.49, 1.04 and 0.92 compared to the reference strain NL4-3, while 138A viruses displayed FC values of 1.81, 2.25 and 1.76. During in vitro selection experiments, after 45 days post infection, 2/3 138A RVs and none of 138E RVs replicated in presence of 50 nM ETR. Genotypic analysis revealed that one of 138A carrying RVs acquired R174Q, E177D, V179E and A371T mutations, while the other 138A RV selected mutations V108I, Q174R, Y181C, K385R.

Conclusions: The E138A variant of HIV1 RT confers a low reduction of ETR susceptibility compared to the 138E wild type virus but appears to be associated to a reduced genetic barrier to ETR. Confirmation of our results in a larger set of experiments could advise to a more cautious use of ETR in the context of the E138A RT polymorphism.

No conflict of interest
Abstract: 9

Mechanism of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Comparison of two-drug combination passage in vitro for drug resistance emergence among dolutegravir, rilpivirine, elvitegravir and 3TC

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Background: A two-drug regimen may provide an option for less adverse events and reduced administrative burden; however, durable virologic suppression and the risk for emergence of drug resistant virus is of concern. In vitro passage studies of two-drug combinations were conducted to simulate therapeutic administration.

Materials & Methods: An in vitro passage studies comprising dolutegravir (DTG) + rilpivirine (RPV), DTG+lamivudine (3TC), elvitegravir (EVG)+RPV, EVG+3TC, or RPV + 3TC were performed for NL432 in MT-2 cells. Passage-starting drug concentrations were determined from EC50 for single drug and fractional inhibitory concentration (FIC) for each drug combination; combination EC50 (cEC50) = FIC x EC50. Passage studies were conducted by two methods: a constant drug concentration method and a stepwise increasing method in which drug concentrations doubled for both drugs when a cytopathic effect was observed in order to facilitate the selection of resistant viruses. The viruses that emerged were analyzed for genotype and phenotype.

Results: All combinations were additive in efficacy and the cEC50 of each compound was ~1/2 of the EC50. In a constant concentration method, neither DTG nor RPV resistance emerged in DTG+RPV at any concentrations tested; no DTG resistance emerged while 3TC resistance emerged in DTG+3TC at some concentrations. In other combinations, viruses resistant against each compound were observed. The lowest concentration which prevented viral replication was DTG+RPV at 4-fold cEC50. In a stepwise increasing method, no DTG resistant virus emerged for up to 85 days with DTG+RPV. For DTG+3TC, IN/R263K emerged at day 85, although the fold change (FC) of the R263K molecular clone was not changed significantly in our HeLa-CD4 assay (FC=1.5). Clinically-relevant resistant viruses for 3TC, RPV or EVG emerged in various combinations among these. The lowest concentration with no viral replication was seen with DTG+RPV at 8-fold cEC50 in a stepwise increasing method which was nearly equal EC90 for each compound.

Conclusion: The combination of DTG + RPV demonstrated the highest genetic barrier to resistance and no emergent resistant virus in these in vitro studies. The combination of DTG+RPV prevented measurable viral replication at the lowest concentration among all combinations tested.

Conflict of interest: Employee of Shionogi.
Abstract: 10

Novel Diagnostic Technologies & Approaches

Analysis of genetic and viral determinants of HBsAg levels in patients with chronic HBV infection

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Background: Some single nucleotide polymorphisms (SNPs) in the IL28B gene seem to play a role in the natural history, clinical evolution and response to treatment of HBV infection. The quantity of HBsAg in plasma reflects the replication competent cccDNA in the liver. The aims of this study are: to analyze 6 IL28B SNPs, to verify their correlation with HBVDNA (VL) and HBsAg levels (qHBsAg) in HBsAg+ chronic carriers and to identify predictors of qHBsAg, VL and of their changes over 6 months.

Materials & Methods: Patients HBsAg+ for >6 months were included; qHBsAg and VL were quantified at baseline (BL) and after 6 months; viral genotype and SNPs of IL28B (rs12980275, rs8105790, rs8099917, rs7248668, rs12979860, rs10853728) were analysed at BL by sequencing. ANOVA test was used to examine correlation of SNPs and qHBsAg, linear regression to identify determinants of qHBsAg and VL, Student’s t-test to investigate changes of qHBsAg over 6 months.

Results: 70 pts were recruited: 46 (66%) male, 56 (80%) Caucasian, 3 (4%) HIV+, 39 (56%) with HBV genotype D, 15 (21%) non D, 31 (44%) on treatment with nucleos(t)ide analogues (NUCs 74% ETV, 22.6% TDF, 3.2% 3TC) of which 21 (39%) with VL<12 IU/ml since a median time of 1.2 years (IQR 0.3-2), 8 (11%) pts were cirrhotics, 26 (37%) inactive carriers (IC). Median age was 45 yy (33-58), time from diagnosis 5 years (2-12), qHBsAg 3.2 log¹⁰ IU/ml (2.2-3.9), VL 2.2 IU/ml log¹⁰ (0-3.3). SNPs were: rs12980275:GG 18.6%, GA 45.7%, AA 35.7%; rs8105790: TT 58.6%, CT 38.6%, CC 2.9%; rs8099917: TT 61.4%, GT 35.7%, GG 2.9%; rs7248668: GG 62.9%, AG 34.2%, AA 2.9%; rs12979860: CC 37.1%, CT 42.9%, TT 20%; rs10853728: CC 10%, CG 41.4%, GG 48.6%. Lower levels of qHBsAg were associated with CC allele of rs12979860 vs CT (p=0.05), AA of rs12980275 vs GG/AG (p=0.05 and p=0.04 respectively) and TT of rs8105790 vs CT (p= 0.05); among these favorable TT of rs8105790 was present in 81% of IC group (as compared to 46% in the remaining pts). Overall at univariate analysis, haplotype CC/AA of rs12979860/rs12980275, CC/TT of rs12979860/rs8105790 and AA/TT of rs12980275/rs8105790 were associated with lower qHBsAg (-0.7log, p=0.06;CI-1.45;+0.02). In the IC group, haplotype CC/AA of rs12979860/rs12980275 (-0.99log;p=0.05;CI-1.99;+0.01), CC/TT of rs12979860/rs8105790 (-0.99log;p=0.05;CI-1.99;+0.01) and AA/TT of rs12980275/rs8105790 (-0.98log; p=0.05;CI-1.97;+0.005) were associated with lower qHBsAg. After 6 months, a higher increase of qHBsAg was observed in those who had lower baseline (<3.15 log) levels (p=0.001), without effect of NUCs.

Conclusions: Specific haplotypes of IL28 SNPs are associated with lower qHBsAg and with inactive HBV carrier status, qHBsAg tends to increase over time, independently from NUCs treatment, particularly in patients with lower levels. Mechanisms underlying to the observed phenomena deserve further investigation

No conflict of interest
Abstract: 11

Viral Evolution & Genetic Diversity

Genetic elements clustered in specific immune active HBsAg regions drive HBV reactivation under immunosuppression: an extensive analysis of HBV genome

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Background: To investigate genetic features along HBV genome underlying immunosuppression-driven HBV reactivation.

Material & Methods: This study includes 127 patients (all genotype D): 47 with HBV reactivation (defined by Hwang, 2013), and 80 with chronic HBV infection, drug-naïve, as control. Genetic distance (using genotype D as reference and expressed as mean±SE) is used to estimate the extent of genetic variability in sequences of Pre-S1/S2 (aa:1-163), HBsAg (aa:1-226), RT (aa:1-344), Basal Core Promoter (BCP) (nt:1-72), and PreCore/Core (aa:1-112) obtained by population-sequencing. HBsAg ultra-deep sequencing (UDPS) is performed for 30 HBV-reactivated patients and 23 controls.

Results: The pre-reactivation HBV status is: 63.7% anti-HBc pos + anti-HBs, 21.3% inactive carriers, 6.4% isolated anti-HBs, 4.3% active carriers, and 4.3% negative to all HBV markers. 53.2% of HBV-reactivated patients was treated with rituximab for hematologic malignancies, 14.9% with corticosteroids for chronic inflammatory diseases, 27.7% with other immunosuppressive-chemotherapeutics. In 51.1% of patients, HBV reactivation occurs after completing immunosuppressive-therapy (range: 1-48 months).

HBsAg genetic variability is significantly higher in HBV-reactivated patients than controls (0.025±0.006 vs 0.018±0.005, P<0.001). Such increase is not observed in pre-S1/S2, RT and BCP/PreCore/Core, suggesting that HBsAg variability gives a specific contribution to HBV reactivation.

In particular, 78.7% of HBV-reactivated patients (vs 6.3% of controls P<0.001) carries mutations localized in immune-active HBsAg regions. Among them, 14 (M103I/T-L109I-T118K-P120A-Q129H/R-Y134H-S143L-D144E-G145A/R-S154P-E164D) reside in major hydrophilic loop (target of antibodies). Most of them are known to act as immune-escape mutations. The others (C48G-V96A-L175S-G185E) reside in specific Class-I T-cell epitopes. By UDPS, these mutations occur with an intra-patient prevalence >50% in 81.8% of HBV-reactivated patients indicating their fixation as predominant species. In controls carrying such mutations, their intra-patient prevalence ranges from 0.4 to 21.3% (P=0.003).

Conclusions: HBV reactivation occurs in various clinical settings, often after completing immunosuppressive therapy, and correlates with a high genetic heterogeneity specifically clustered in immune active HBsAg regions. This supports the need to carefully monitor all patients at risk of HBV reactivation, and to assess the best time for the onset and duration of prophylaxis to prevent clinical complications.

No conflict of interest
Abstract: 12

Viral Evolution & Genetic Diversity

A hyper-glycosylation in HBsAg characterizes immunosuppression-driven HBV reactivation and affects HBsAg recognition and quantification in vitro

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Background: To investigate N-linked glycosylation patterns of HBsAg in immunosuppression-driven HBV-reactivation and to evaluate their impact on HBsAg-antigenicity.

Materials & Methods: Mutations associated with acquisition of N-glycosylation site were investigated in 127 HBsAg genotype-D sequences from 47 patients with immunosuppression-driven HBV-reactivation (defined as Hwang,2014), and 80 chronically HBV-infected drug-naïve patients as control. The impact of N-glycosylation sites on HBsAg-antigenicity was analyzed by transfecting HepG2-cells with a plasmid encoding wild-type and mutated HBsAg linked to a streptavidin-tag (strep-tag). The strep-tagged HBsAg amount in supernatants was quantified by a specifically-designed ELISA targeting the HBsAg (Architect-Abbott, Monolisa-Biorad).

Results: Additional N-glycosylation sites are found in 19.1% (9/47) of HBV-reactivated patients versus 0/80 controls (P<0.001). They localize in the major hydrophilic HBsAg-region (MHR), target of antibodies. In 7 patients, a single additional N-glycosylation site results from the mutations T115N (n=2), T123N (n=2), T131N (n=2), and from the insertion of an N between 114 and 115 position (ins114-115N) (n=1). In the remaining 2 patients, 2 additional glycosylation sites result from S113N+T131N and ins114-115N+T117N, respectively. Notably, 5/9 patients with ≥1 additional N-glycosylation sites remain HBsAg-negative by diagnostic-test at HBV-reactivation (P=0.002). In-vitro, all additional N-glycosylation sites decrease the strep-tagged HBsAg quantification by the 2 ELISAs targeting the HBsAg. In particular, T115N, T123N, ins114-115N determine a >90% decrease in HBsAg-quantification by both ELISAs. Similarly, ins114-115N+T117N cause a 90.2% and 75.4% reduction in HBsAg-quantification, respectively. No decrease of strep-tagged HBsAg is revealed by ELISA targeting the Strep-tag. This suggests that additional N-glycosylation sites hamper HBsAg-recognition by antibodies without affecting HBsAg-release.

Conclusions: Additional N-glycosylation sites in MHR correlate with false HBsAg-negativity at ELISA despite HBV-reactivation, and profoundly affect HBsAg-antigenicity in-vitro. This supports the role of immune-escape mutations in HBV-reactivation during immune-suppression and the importance of HBV-DNA (more than HBsAg) in HBV-reactivation diagnosis.

No conflict of interest
Abstract: 13

Viral Evolution & Genetic Diversity

Key genetic elements in HBsAg significantly correlate with liver cancer onset by hampering HBsAg secretion and promoting cell proliferation in vitro


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Background: The goal of this study is to investigate HBsAg genetic elements correlated with the onset of HBV-induced hepatocellular carcinoma (HCC) and their impact on cell proliferation in an in vitro model.

Methods: This study includes 153 HBV chronically infected patients: 15 with HCC (60% D; 33% A, 7% E HBV genotype), and 138 asymptomatic patients as control (58% D, 31% A, 11% E). Mutations were identified according to the reference sequence of each specific HBV genotype. Association of HBsAg mutations with HCC was assessed by Fisher test.

HBsAg mutations were introduced into a 1.3x genome-length HBV genotype D. WT and mutated clones were transfected into HepG2 cells. Lysates and supernatants were harvested in triplicate daily until day 5 post-transfection, and HBsAg (representing 24 hours of accumulation) quantified by Alexsis assay. Mutations were also introduced into a pIRES II plasmid encoding HBsAg and GFP. Cellular cycle was analysed by flow cytometry (DNA propidium iodide-staining) on transfected GFP+ cells at day 7 post transfection (4 experiments in triplicate).

Results: Patients with or without HCC had median(IQR) log serum HBV-DNA of 4.1(3.2-6.0) and 4.1(3.1-6.0)UI/ml, respectively. Two HBsAg mutations significantly correlated with HCC: P203Q (27%(4/15) in HCC vs 2%(3/138) in non-HCC, p=0.002); S210R (40%(6/15) in HCC vs 12%(17/138) in non-HCC, p=0.012; P203Q+S210R(27%(4/15) in HCC vs 1.4%(2/138) in non-HCC, p=0.001). Both P203Q and S210R reside in transmembrane C-terminal domain known to be important for HBsAg secretion.

In vitro, the presence of P203Q, S210R and P203Q+S210R reduced the ratio of secreted/intracellular HBsAg compared to wt at each time point. Indeed, this ratio varied as follows: at 3 days P203Q (1.73±0.2; p<0.005 versus wt), S210R (2.54±0.7), P203Q+S210R (1.83±0.1; p<0.005) vs wt (2.99±0.2); at 4 days P203Q (2.22±0.2; p<0.005) vs wt P203Q+S210R (3.52±0.2; p<0.005); P203Q+S210R (2.33±0.2; p<0.001) vs wt (4.42±0.3), and at 5 days P203Q (1.15±0.1; p<0.005), S210R (1.69±0.1; p<0.005), P203Q+S210R (1.25±0.1; p<0.005) vs wt (2.23±0.1).

By flow cytometry, P203Q and P203Q+S210R significantly correlated with an increased percentage of cells in the S phase, indicating cell cycle progression: P203Q (26±13%) and P203Q+S210R (29±14%) compared to wt (18±9) (p<0.01).

Conclusions: Key mutations, residing in C-terminal HBsAg domain, are highly correlated with HBV-induced HCC in vivo. They affect HBsAg secretion and stimulate cell proliferation in vitro, suggesting their potential involvement in HCC development.

No conflict of interest
Abstract: 14

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

How Genotypic Resistance Test in PBMC (Proviral DNA) May Help to Identify Hidden Resistance in patients with Low Level or Undetectable HIV-RNA?

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Background: Genotypic resistance test (GRT) in peripheral blood mononuclear cell (PBMC) (proviral DNA) is often executed to detect possible resistance mutations that are not generally found with the standard GRT performed in plasma. Moreover, the PBMC on GRT can be also performed at low or undetectable HIV-RNA.

Aim of the present study was to explore association of HIV resistance in PBMC with resistance detected in plasma, in order to assess amount of information about HIV resistance that routine execution of GRT in PBMC may add.

Materials & Methods: Patients attending four Italian reference centers and selected by the caring physician for treatment change despite low or undetectable HIV-RNA were tested for PBMC GRT. The number and type of major resistance mutations (MRM) (IAS-USA list 2014 and Stanford data base) detected in PBMC were compared to those detected in concomitant plasma GRT if available (±6 months from PBMC GRT) or in cumulative plasma (for patients with >1 plasma GRT in treatment history).

Results: Overall, 468 patients, tested for DNA in PBMC during therapy were included: 264 patients (62.8%) were tested for GRT in PBMC with HIV-RNA <50 copies ml. Among patients included, 303 had concomitant plasma GRT, and 149 had >1 plasma GRT performed before the GRT in PBMC.

GRT in PBMC for INSTI drug class was executed in 102 patients. Overall, 198 patients (42.3%) carried MRM in PBMC, mean MRM per patient 1.37 (±2.41). The highest proportion was observed in NRTI class (30.6%) followed by NNRTI (22.2%) and PI class (14.1). INSTI MRM in PBMC were found in 4.9% of patients. Moreover, MRM in PBMC were found in 45.9% of 303 patients with concomitant plasma and PBMC GRT, in 18.2% of patients MRN were found only in PBMC and in 36.6% in PBMC and plasma.

Finally, MRM in PBMC were found in 55.7% of 149 patients with ≥1 plasma GRT, in 20.1% of these patients MRN were found only in PBMC and in 51.0% both in PBMC and cumulative plasma (any of the plasma GRT available). The most frequent MRM found for NRTI class were: M184V (14.3% of patients), K70R (11.2%), D67N (9.6%); for NNRTI: K103N (10.5%), E138A (6.9%), V108I (2.8%) and Y181C (2.1%); for PI class L90M (6.4%), M46I (5.9%) and V82A (2.8%) and for INSTI: N155H (3.0%), G140S (2.0%) and Q148H (1.0%).

Multivariable logistic regression, was performed in order to assess factors associated with detection of MRM in PBMC. Detection of any MRM is associated with CD4 nadir <200 (OR: 1.7, 95% CI: 1.1-2.6, p=0.014). Undetectable HIV-RNA at PBMC GRT, non-B HIV subtype and CD4 count <200 were not found significantly associated.

Conclusions: Genotypic resistance test in PBMC (proviral DNA), is both quantitatively and qualitatively relevant in clinical practice. Indeed, almost half of patients harbored major resistance mutations in PBMC and about 20% harbored mutations never detected in cumulative genotypic resistance history, highly improving the result of simple resistance test in plasma.

No conflict of interest
Abstract: 15

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Improvement of HIV resistance testing by proviral DNA analysis and Next Generation Sequencing

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Background: The integration of the proviral DNA (pDNA) into the host genome enables the virus to establish resistant variants into the cellular reservoirs. Thus, resistance analysis of pDNA or total nucleic acid (tNA) could be helpful in cases of low level viremia or requested therapy switches in patients with suppressed viral load (VL), especially in cases where no useful historic resistance data are available or therapy interruptions cannot be excluded. To analyse the validity of pDNA or tNA for resistance analysis we compared the mutation patterns of pDNA and tNA with plasma RNA of the homologues blood samples, detected by Sanger sequencing and next generation sequencing (NGS).

Materials & Methods: This study included 97 EDTA-samples of therapy-naïve (TN) and -experienced (TE) patients of the RESINA cohort. The direct comparison of drug resistance mutations (DRMs) in viral RNA (vRNA) and pDNA included 69 samples of TN and TE patients with high (>10,000 copies/ml) and low (<1000 copies/ml) VL. 28 samples of previously untreated patients were used for the comparative analysis of DRMs in vRNA and tNA detected by Sanger sequencing and NGS (Illumina MiSeq technology).

Results: The comparative analysis of 69 corresponding vRNA and pDNA genotypes of TN and TE patients showed a total of 47 samples with DRMs. Regarding drug classes and DRM positions, 36 different resistance-associated positions in PR and RT were detected, whereof only nine positions presented substitutions more frequently in pDNA (RT: 41, 62, 75, 108, 138, 236; PR: 45, 53, 73). Thus, the largest numbers of samples with DRMs were detected in vRNA, predominantly NRTI mutations. While samples of TE patients with high VL presented more DRMs in pDNA (76% vs. 86%), samples of TE patients with low VL displayed a higher rate of DRMs in vRNA (92% vs. 69%). Thus, the frequency of DRMs in pDNA of TE patients can be correlated with VL. In contrast, the distribution of DRMs in samples of TN patients was comparable in vRNA and pDNA genotypes (85% vs. 85%).

The analysis of DRMs in vRNA and tNA genotypes provided corresponding genotypes of both materials and both sequencing techniques of 28/33 samples. tNA analyses presented overall a higher frequency of DRMs compared to vRNA genotypes independent of the sequencing technique. Furthermore, NGS analyses detected a higher rate of DRMs compared to Sanger analyses (19/38).

Conclusion: Analysis of pDNA for HIV resistance testing showed evidence to be useful for untreated patients and for TE patients, predominantly those with high VL. Overall, the use of tNA from whole blood revealed more DRMs compared to exclusive RNA analyses. The application of NGS analyses displayed a higher frequency of DRMs than Sanger sequencing. In line with these results, tNA and NGS analyses establish the basis for future resistance analyses.

No conflict of interest
Abstract: 16

Novel Diagnostic Technologies & Approaches

Impact of hypermutation on the results of deep sequencing analysis of proviral DNA for baseline HIV-1 drug resistance.

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Background: Detection of baseline resistance is mainly based on Sanger sequencing, a method that does not allow consistent detection of mutants representing <25% of the viral population. Minority resistant variants have been found in plasma of untreated individuals using deep sequencing or allele specific PCR. It is hypothesized that deep sequencing of proviral DNA may present an even more complete picture of the resistance potential.

Materials & Methods: Paired plasma and buffy coat samples from 16 newly diagnosed HIV-1 positive individuals with at least one drug resistance mutation detected by Sanger sequencing and from 4 control patients with wild type virus, were selected. Deep sequencing of the HIV protease (PR) and reverse transcriptase (RT) gene was performed on plasma RNA and cellular DNA using a prototype version of the Roche 454 GS junior HIV-1 PR-RT protocol. Data processing and quality filtering was done with the AVA software. The cut-off for considering minorities as relevant was set at 1%. Selection of resistance mutations was based on the Stanford score. Hypermut 2.0 was used to screen for APOBEC-induced hypermutation.

Results: RNA and DNA deep sequencing was successful for 17 of the 20 patients. Failures were due to insufficient read coverage. All mutations detected by RNA Sanger sequencing were found after deep sequencing, both in RNA and DNA (mean frequency of 90.79% in RNA and 88.4% in DNA). Six and 42 additional minority mutations were observed in RNA and DNA respectively. Amongst the 42 minority DNA mutations overrepresentation of 30N and 46I in PR and 138K, 184I and 190E in RT was noticed. All these mutations result from a guanine (G) to adenine (A) transition so the presence of defective G-to-A hypermutated viruses was suspected. Following this, all DNA as well as RNA reads with minority resistance mutations were screened for hypermutation and defective reads were removed before repeating resistance analysis. None of the 2593 screened RNA reads but 1919 (51.3%) of the 3744 screened DNA reads had evidence of hypermutation. Minority mutations detected in RNA were RT98G, PR88S, RT69D, RT179D, RT215S and RT69N. The polymorphic 179D was found in a control patient, the other mutations were detected in 3 patients in whom also majority NRTI and/or NNRTI mutations were present. The minority mutations remaining in DNA after clean-up for defective viruses were PR30N, PR46I (2x), RT43T, RT53L, RT67E, RT69D, RT98G, RT108I, RT138K, RT184I (3x), RT190E, RT219R, RT227L and RT238T. The resistant mutants 215Y/F were not detected in any of the RNA or DNA samples despite the presence of a 215 revertant in 9 of the 17 patients with successful deep sequencing.

Conclusions: Deep sequencing of plasma RNA did reveal a limited number of additional minority resistance mutations in patients diagnosed with a resistant variant by Sanger sequencing. Minority resistance was noticed more frequently after deep sequencing of proviral DNA but these results were highly biased by the presence of defective G-to-A hypermutated variants. Care must be taken when interpreting results of sensitive mutation detection on proviral DNA.

No conflict of interest
Abstract: 17

Treatment Strategies for HIV/ Hepatitis infected Patients

European guidance on clinical management of HIV low level viremia

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Background: In clinical practice in Europe the majority of patients on antiretroviral therapy achieve sustained virologic suppression, i.e. viremia below 50 copies/mL. However, after achieving virologic suppression, some patients develop low level viremia: persistently low but detectable viremia between 50 and 1000 copies/mL. Low level viremia generates uncertainty among patients and clinicians and treatment guidelines are not in consensus regarding its clinical management. The European Guidance Group on clinical management of HIV-1 low level viremia was established to make recommendations to clinicians.

Materials & Methods: The guidance is an initiative of the European Society for translational Antiviral Research (ESAR). Essential topics to be addressed in the guidance were selected and a core writing committee was established. A Medline and conference search was performed with (synonyms of) the term low level viremia. In addition, panelists from 30 European countries and Canada contributed to a questionnaire on low level viremia. Results were analyzed according to background of the 52 panelists (medical doctor and/or PhD involved in diagnostics, treatment and/or research). Results of the questionnaire were discussed at the ESAR guidelines meeting in November 2014.

Results: Despite its current clinical relevance, the medline search revealed that there were no randomized controlled trials to determine the optimal strategy for clinical management of patients with low level viremia. Previous observational studies show an increased risk of virologic failure in patients with low level viremia. Although the success rate of resistance testing goes down when viremia is below 200 copies/mL, studies have indicated that selection of resistance can occur at these low viral loads. The following items were selected to be included to facilitate the development of a guidance document: the potential etiology of low level viremia (such as the role of the used assay, ongoing viral replication or virus production, adherence), the potential effects of low level viremia (including virological failure, development of resistance and immune activation), additional diagnostic testing and clinical management. Panelists indicated that clinical management of low level viremia is dependent on the genetic barrier of the regimen used and the detection of resistance mutations.

Conclusions: In the absence of clinical trials, ESAR provides clinical guidance for optimal management of patients with low level viremia based on a review of current available literature and expert opinion.


No conflict of interest
Abstract: 18

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Detection of the NS3 Q80K polymorphism by Sanger and deep sequencing in hepatitis C virus (HCV) genotype 1a strains in the United Kingdom

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Background: The glutamine to lysine substitution at codon 80 (Q80K) occurs as a polymorphism in the NS3 gene of HCV-1a strains. Q80K reduces in vitro and in vivo susceptibility to the protease inhibitor simeprevir, and has possible effects on susceptibility to asunaprevir, faldaprevir, and paritaprevir. This study aimed to establish the prevalence of Q80K in HCV-1a carriers attending for care in two regions of England, investigate viral phylogeny, and determine the occurrence of Q80K as a low-frequency variant by deep sequencing.

Materials & Methods: The study population comprised 238 patients infected with HCV-1a and naïve to all HCV therapy that in 2010-2014 attended for care in the North-West (NW) or the South-East (SE) of England. A total of 61/238 (25.6%) subjects from the SE were co-infected with HIV. Stored plasma samples underwent Sanger and deep (Illumina) sequencing of the NS3 gene. Maximum-likelihood (ML) phylogenies were estimated (including and excluding codon 80) using NS3 sequences from the UK, North America, and Europe.

Results: By Sanger sequencing Q80K occurred in 43/238 subjects (18.1%, 95% CI 13.2%-23.0%), including 19/70 (27.1%) in the NW and 24/168 (14.3%) in the SE (p=0.026). Prevalence in the SE cohort was 17/107 (15.9%) in HCV mono-infected subjects vs. 7/61 (11.5%) in HCV/HIV co-infected subjects (p=0.498). The median HCV RNA load was 6.4 (IQR 5.8-6.7) vs. 6.3 (IQR 5.8-6.8) log10 IU/ml in samples with vs. samples without Q80K (p=0.83). Overall 178 samples underwent deep sequencing, comprising 27 with and 151 without Q80K by Sanger sequencing (median 53,413 reads per sample; IQR 40,740-70,255). The 27 samples showing Q80K by Sanger sequencing also showed the mutation by deep sequencing, with mutant frequencies ≥98% in 25 samples, and of 41% and 46% respectively in 2 samples. None of the 151 samples lacking Q80K by Sanger sequencing showed the mutation by deep sequencing when applying an interpretative cut-off of 1%. A further 3% of samples showed Q80K at a frequency <1% and >0.5%. Additional resistance mutations identified by Sanger and/or deep sequencing (frequency 1-6%) occurred at codons 80 (Q80L, n=4; Q80R, n=3), 36 (n=6), 54 (n=6), 55 (n=6), 168 (n=1), and 170 (n=4). By phylogenetic analysis, HCV-1a strains separated into the two recognised lineages with and without Q80K. The sequences showing Q80K at frequency <1% did not cluster within the Q80K lineage. In the UK, 33 regional HCV-1a clusters were identified consisting of ≥3 sequences (range 3-20). UK sequences were highly interspersed with sequences from elsewhere in Europe and North America, and the findings were consistent with multiple introductions from different geographic regions.

Conclusions: Although appearing to vary geographically, the prevalence of Q80K in the UK is among the highest in Europe. Most subjects carried the mutation as a dominant variant (>40%), allowing detection by Sanger sequencing. Detection of Q80K as a low-frequency (<1%) variant was of doubtful significance. While regional transmission networks may lead to differences in the prevalence of Q80K, there was evidence of a high degree of interspersing of UK sequences with sequences from elsewhere in Europe and North America.

No conflict of interest
Abstract: 19

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Retreatment with an interferon-free combination of simeprevir-sofosbuvir in patients who had previously failed on HCV NS5A inhibitor–based regimens

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Background: Failure of direct acting antivirals (DAAs) with anti-HCV activity is commonly associated with the emergence of resistant variants. While variants with resistance to PIs are generally overgrown by wild-type virus over time, variants with resistance to NS5A inhibitors, such as daclatasvir (DCV), have been seen to persist. Therefore, treatment with a PI seems to be a promising retreatment concept following failure of an NS5A inhibitor.

Materials & Methods: Patients who had failed a regimen consisting of DCV in combination with pegylated interferon (PegIFN) and ribavirin (RBV; n=13) or in combination with asunaprevir (ASV [an investigational PI] and PegIFN+RBV (n=3), were retreated for 12 weeks with simeprevir, a PI, in combination with sofosbuvir, a NS5B-inhibitor without ribavirin. HCV RNA was measured with Abbott RealTime Assay (limit of detection/lower limit of quantification [LLOQ/LOD]: 12 IU/mL). This retrospective analysis investigated both efficacy (as measured by on-treatment response at weeks 4, 8, and 12 [end of treatment, EOT], and post-treatment sustained virologic response [SVR]) and safety.

Results: Patients (n=16; mean 54 [range 43-73] years; 13 males) were chronically infected with GT1 (11 GT1a; 3 GT1b) or GT4 (n=2), and had compensated liver disease and advanced liver fibrosis (fibroscan: 9.6 – 70 kPa; cirrhosis n=9). Median baseline HCV RNA was 1.38 x 10^6 IU/mL (>800,000 IU/mL: n=14). No patient discontinued treatment due to an adverse event or for virologic failure. Amongst the 16 patients, 10 had a rapid virologic response (HCV RNA <LLOQ at week 4), 1 patient first reached HCV RNA <LLOQ at week 8 and the remaining 5 patients reached HCV RNA <LLOQ at EOT. At the time of analysis, 15 patients have reached post-treatment week 4; of these 13 (87%) achieved SVR4. Two patients relapsed at or before post-treatment week 4 (both were GT1a, cirrhotic, and had reached HCV RNA <LLOQ at EOT). One patient who relapsed had evidence of advanced cirrhosis (fibroscan: 33 kPa; serum albumin: 32 g/L; 76,000 platelets/µL). The other relapser had previously failed a 24-week regimen of DCV-ASV in combination with PegIFN+RBV.

Conclusions: Simeprevir plus sofosbuvir was highly effective in this DAA-pretreated population. The high SVR rates seen in this retrospective analysis support the concept of retreatment of prior NS5A failures with a PI-containing regimen. Some patients could benefit from treatment extension beyond 12 weeks, or from the addition of RBV.

No conflict of interest
Abstract: 20

Treatment Strategies for HIV/ Hepatitis infected Patients

Comparison of two HCV-RNA assays assessing early response to simeprevir+PegIFN/RBV to select patients suitable to shorten therapy to 12 weeks


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Materials & Methods: Treatment-naïve, chronic HCV G1-infected patients with no-to-moderate fibrosis (METAVIR F0–F2) were recruited. In patients with HCV-RNA <25IU/mL detectable/undetectable by Roche COBAS® Taqman® (RCT) assay (lower limit of quantification [LLOQ]: 25IU/mL, limit of detection [LOD]: 15IU/mL) at Week 2, and undetectable at Weeks 4 and 8, all treatments were stopped at Week 12. If these criteria were not met, Peg-IFN/RBV continued to Week 24. In this analysis, Week 2 and 4 samples were reanalysed using the Abbott RealTime (ART) assay to retrospectively determine if an assay with LOD/LLOQ 12IU/mL could better identify patients with a high chance of SVR on shortened therapy.

Results: Using RCT, 123/163 (76%) patients treated (53% male, 92% white, 40% G1a/60% G1b, 76% METAVIR F0–F2) were eligible to stop after 12 weeks. 120 of these 123 patients were reanalysed using ART: results discussed below. At Week 2, in the 48 samples <25IU/mL undetectable using RCT, using ART, 27% were <12IU/mL undetectable, 58% were <12IU/mL detectable and 15% were >12IU/mL. In the 71 samples with <25IU/mL detectable by RCT, 10% of samples were <12IU/mL undetectable, 17% were <12IU/mL detectable and 73% were >12IU/mL by ART. At Week 4, all 120 samples were <25IU/mL detectable by RCT, 10% of samples were <12IU/mL undetectable, 17% were <12IU/mL detectable and 73% were >12IU/mL by ART. In patients eligible to stop after 12 weeks using RCT, SVR12 was 65% (78/120) vs. 81% (38/47) in patients <LLOQ at Week 2, and undetectable at Weeks 4 and 8 using ART. ART showed consistently higher SVR rates by subgroup vs. RCT (SVR for ART is from a sub-population of RCT): IL28B CC: 100% [14/14] vs. 94% [30/32]; non-CC: 73% [24/33] vs. 56% [51/91]; F0–F1: 81% [30/37] vs. 74% (69/93); F2: 78% [7/9] vs. 38% [11/29]; G1a: 88% [14/16] vs. 63% [31/49]; G1b 77% [24/31] vs. 68% [50/74].

In the above subgroups with SVR rates <80% using ART, SVR rates improved when altering the Week 2 criteria to be HCV-RNA undetectable – with this criteria, SVR for non-CC, F2 and G1b subgroups was 83% (10/12), 100% (6/6) and 83% (10/12), respectively. The number of patients that would qualify for 12 weeks based on ART is lower than that based on RCT across all subgroups.

Background: HPC3014 is a Phase 3, open-label study to assess if on-treatment virologic response to simeprevir+Peg-IFN/RBV at Week 2 can allow shortening of treatment to 12 weeks, irrespective of baseline and on-treatment factors. In clinical practice, on-treatment response is determined by commercial HCV-RNA assays, which have different sensitivities of detection.
Conclusion: Although concordance was moderate at Week 2, and high at Week 4, RCT was less sensitive at detecting low level HCV RNA than ART. Additionally, patients fulfilling criteria for 12 weeks of treatment with RCT had lower SVR rates across all subgroups when 120/123 samples were reanalysed with ART 12-week criteria. This suggests assays with higher sensitivity may improve the ability to select patients with a high chance of SVR with only 12 weeks of simeprevir+PegIFN/RBV therapy.

Conflict of interest: C. Sarrazin has conflicts with: Abbott, Abbvie, BMS, Gilead, Janssen, Merck/MSD, Qiagen, Roche, and Siemens

Abstract: 21
Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Comparison of three commercial platforms for Hepatitis C Virus Genotyping and NS5B sequencing. GEHEP-007 study.

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Background: The availability of new antiviral drugs against HCV infection requires the highest precision for estimating HCV genotyping, including subtype differentiation specially in the case of genotype 1 infection. In the setting of the Spanish Group for Viral Hepatitis Study (GEHEP), we aimed to evaluate the correct assignement of HCV genotypes by three commercial methods, TrugeneHCV genotyping kit (Siemens), VERSANT HCV Genotype 2.0 assay (Siemens), and Real Time HCV genotype II (Abbott), compared to NS5B sequencing.

Materials & Methods. 241 clinical samples carrying HCV genotypes representing the most frequent geno/subtypes circulating in Spain were studied. Samples were stored at -80°C until sequencing of an internal fragment of 330 bp in the NS5B gene (nucleotide positions 7935 to 8266). Genotypes were assigned using Blast (http://hcv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html), geno2phenoHCV (hbv.bioinf.mpi-inf.mpg.de/) and phyML. Major discrepancies were defined as differences in the assigned genotype by one of the three methods and NS5b sequencing (including genotypes 1a and 1b misclassification). Minor discrepancies were considered when differences at subtype level were observed.

Results: The overall discordance with respect to the reference method was 34% for Trugene, and 17% for VERSANT HCV2.0. The Abbott assay correctly identified all 1a and 1b subtypes, and genotypes 2, 3, 4 and 5, but did not discriminate the subtype in these cases. For Trugene HCV, 54.3% of the discrepancies (25/46) were minor [1 (Trugene) to 1a (NS5b sequencing), n = 2; 1 to 1b, n = 4; 1b 1, n = 1; 2a to 2i, n = 1; 2c 2i, n = 1; 3 and 3a, n = 2; 3d 3a, n = 1; 4a to 4d, n = 2; 4c 4a, n = 1; 4c-4d, n = 10]; conversely 45.7% (21/46), corresponding to 15.6% of the tested samples, were major discrepancies [1a (Trugene) to 1b (NS5b sequencing), n = 5; 1b to 1a, n = 13; 1b to 3a, n = 1; 3a 1b, n = 1; 4c 1b, n = 1]. In VERSANT HCV 2.0, three minor discrepancies [1 (VERSANT) to 1b (sequencing NS5b) in one case, and 4 (VERSANT) to 4a (NS%b sequencing) in two cases] and four major discrepancies [1b (VERSANT) to 1a (sequencing NS5b) in four cases]. With NS5b sequencing, genotypes 2, 3, 4 and 5 by the Abbott HCV genotype Real Time II assay were resolved as 2a (n = 1), 2b (n = 2), 2c (n = 3), 3a (n = 9), 4d (n = 3) and 5a (n = 2).

Conclusions: When NS5b sequencing is used as reference, Trugene failed to correctly assign HCV genotype in up to a third of cases, with more than 15% of errors with a high impact on clinical practice. VERSANT HCV Genotype 2.0 assay did not assign correctly HCV subtype in 10% of cases. Abbott Real Time HCV genotype II assay properly assigned all the genotypes 1 analyzed, although it was not able to discriminate subtype genotypes 2, 3, 4 and 5.

No conflict of interest
Abstract: 22

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Relevance of Hepatitis C Virus Sequencing for Genotype and Subtype Determination in the Era of New Direct Antiviral Agents


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Background: The large majority of new Direct Antiviral Agents (DAAs) against HCV show a multi- or a limited-genotypic coverage. Moreover, the new DAAs can show differences in response rates and resistance patterns according also to HCV subtype (i.e: 1a/1b). Therefore, a correct genotype and subtype assignment prior to treatment initiation is mandatory for the selection of the most appropriate treatment regimen. This study aims to evaluate the concordance between commercial genotyping assays and HCV sequencing in the subtype/ genotype assignment.

Materials & Methods: HCV sequencing of NS3-protease and/or NS5A and/or NS5B was performed by home-made protocols, specific for each genotype. Phylogenetic analysis was performed to evaluate appropriate genotype allocation and concordance with previous genotype/subtype assignment by commercial genotyping assays.

Results: A total of 359 HCV-infected patients candidate to DAA-treatment who performed a genotypic resistance test between 2011 and 2014 were reanalyzed to confirm the appropriate genotype allocation. All patients were previously genotyped by different commercial genotyping-assays (showing these results: HCV-1a [N=135]; HCV-1b [N=185]; HCV-1 [N=7]; HCV-2 [N=2]; HCV-3 [N=15]; HCV-4 [N=7]; mixed HCV-genotype [N=8]). According to the genotypic resistant test requests, HCV-sequencing was performed on NS3-protease (93.1% of samples), together with/in alternative to NS5A (9.6%) and/or NS5B (18.7%). When more than one genomic-region per patient was analyzed (N=62), phylogenetic results were 100% each other concordant in all genes, thus confirming the specific genotype/subtype assignment. Conversely, HCV sequencing and commercial assays were discordant in 91.7% of cases analyzed. Indeed, HCV sequencing identified 4/359 genotypes discordant with the assignment given by commercial assays (commercial/sequencing: 1a/2c; 1b/2c; 1b/4d; 2a-2c/1b) and 11/359 discordant subtype cases (commercial/sequencing: 1a/1g[N=2]; 1a/1b[N=3]; 1b/1a[N=6]). Of interest, among two discordant patients, one, found infected with HCV-1g and carrying baseline NS3-
mutation T54S, rapidly failed a boceprevir-containing regimen, while the other one found infected with genotype 4d (previously defined as HCV-1b) failed with resistance to daclatasvir + asunaprevir + ribavirin. Furthermore, 15/359 patients (4.2%) with a previous result of ‘mixed’ (N=8) or HCV-1 without subtype information (N=7) by commercial assays, were instead precisely resolved by HCV-sequencing. As a whole, 30/359 (8.3%) patients achieved the correct genotyping assignment thanks to direct sequencing.

Conclusions: Our results emphasize the importance of dedicating time and effort for a proper genotype/subtype assignment before starting therapy. HCV-sequencing allows precise subtype/genotype assignment, along with evaluation of natural resistance, thus reducing the risk of failure especially in difficult to treat patients with advance disease. The relatively low cost of sequencing (compared to therapy) should encourage studies aimed at better defining the advantage of its use at therapy baseline in clinical practice.

No conflict of interest

Abstract: 23

Viral Evolution & Genetic Diversity

The global spread of HIV-1 subtype A epidemic: a phylogeographic analysis


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Background: Subtype A is encountered mainly in Eastern Africa, Russia, former Soviet Union (FSU) countries and Central Asia. In Western and Central Europe the prevalence is approximately 2%, however, in some Mediterranean countries such as Albania, Cyprus and Greece subtype A has established extensive epidemics. Here, we analyzed the global dispersal pattern of subtype A by conducting a statistical phylogeographic analysis.

Materials & Methods: We assembled a global dataset of 2,274 HIV-1 subtype A sequences. To do this we pooled sequences from online database repositories for the globally sampled sequences (N=2,134) and from patient-cohort studies, SPREAD (Strategy to Control SPREAD of HIV Drug Resistance) from ESAR (European society for translational antiviral research) collaborations for the European sequences.
Abstract

(N=141). We estimated the subtype A global phylogeographic tree using maximum likelihood (ML) as implemented in the RAxML program. We also identified statistically significant viral dispersal pathways between geographic regions using the parsimony criterion from a set of 350 bootstrap trees estimated by ML.

Results: The global phylogeographic tree suggested that HIV-1 subtype A strains cluster according to their geographic origin. Specifically, we observed three major clusters from: i) East Africa, ii) Central and West Africa and iii) Eastern Europe/FSU (A_ESU). Phylogeographic analysis showed that Africa has been the main source of subtype A to Western and Eastern Europe with the latter to have been a source for viral dispersal to Central and Western Asia. Within Europe subtype A epidemic, we found three major patterns of viral mobility: i) within Eastern Europe, ii) between Eastern and Western Europe, iii) within Western Europe and iv) from Western to Central Europe. With respect to individual countries Greece and Sweden have acted as sources for viral migration with Greece exporting viral strains to Central European countries and Sweden to West Europe. Within Central and Eastern Europe, Ukraine, Czech Republic and Russia were hubs of viral migration. Interestingly, for Romania, Ireland, Italy and UK were found to be isolated.

Conclusions: Global subtype A has strong geographic compartmentalization in three major clusters. Two of them in East and West/Central Africa have spread mostly sexually and whereas the third in Eastern Europe among intravenous drug users (IDUs). Dispersal routes of subtype A from Africa to Europe were probably associated with immigration from Africa while viral mobility within Eastern Europe is probably due to IDUs’ connections. In general, several subtype A migration pathways exist among European countries which mainly are associated with heterosexuals and IDUs in Eastern Europe, in contrast to subtype B mobility associated with men having sex with men (MSM). Subtype B dispersal patterns dominate in Western Europe and include major touristic destinations, whereas Eastern Europe plays a major role in the case of subtype A. Greece is a consistent exception due to the establishment of a large HIV-1 subtype A local epidemic.

No conflict of interest

Abstract: 24

Spread of Drug Resistance


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Background: The surveillance of HIV-1 Transmitted Drug Resistance (TDR) is recommended. It has been recently shown that TDR was more common among Men having Sex with Men (MSM) in the 2010/2011 French survey study conducted in antiretroviral-naive chronically HIV-1-infected population. Here, we compared the presence of resistance mutations, both in majority (>20%) and in minority (1-20%) proportions, between treatment-naive MSM and treatment-naive African heterosexual population recently diagnosed for HIV-1.

Materials & Methods: The study enrolled 70 treatment-naive MSM (60% B subtype) and 67 treatment-naive African heterosexual population (94% non-B subtype) recently

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screened positive for HIV-1. We performed standard Sanger Sequencing (n=140) and UltraDeep Sequencing (UDS, Roche 454® Life Sciences; n=70 Reverse Transcriptase (RT) and n=70 protease (PR) sequences among MSM samples; and n=54 RT and n=67 PR sequences among African heterosexual samples). PR and RT mutations were identified using the consensus statement of the list for the TDR genotypic surveillance. For UDS, mutations >1% were considered.

Results: Among the 70 MSM patients, median viral load (VL) was 4.9 log_{10} copies/mL (IQR=4.4-5.4) and median CD4 cell count was 498/mm^3 (IQR=347-585). Prevalence of virus with PR or RT-TDR was 18.6% (95%CI=9.4%-27.7%) with UDS versus 4.3% (95%CI=0.0%-9.1%) with Sanger Sequencing. There was a trend for a higher frequency of TDR in MSM infected with B subtype compared to MSM non-B subtype (26.2% versus 7.1% with UDS (p=0.06)).

Among the 67 treatment-naive African heterosexual population, median VL was 4.9 log_{10} copies/mm^3 (IQR=4.3-5.3) and median CD4 cell count was 348/mm^3 (IQR=208-497). Prevalence of virus with PR or RT-TDR mutation was 22.8% (95%CI=12.7%-33.0%) with UDS (n=30) versus 7.1% (95%CI=0.9%-13.4%) with Sanger Sequencing (n=67). With Sanger Sequencing, prevalence of at least one TDR mutation to Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non NRTI (NNRTIs) and protease inhibitors was 1.4%, 2.9% and 0% in MSM samples versus 4.3%, 4.3% and 1.4%, respectively in African heterosexual population samples.

With UDS, prevalence of at least one TDR mutations to NRTIs, NNRTIs and protease inhibitors was 4.3%, 10.0% and 7.1% respectively in MSM samples versus 14.8%, 7.4% and 10.4% respectively in African heterosexual population samples (with a higher frequency of NRTI-TDR mutations observed among African heterosexual population samples by UDS (p=0.04)).

Conclusions: Higher prevalence of TDR was observed in antiretroviral-naive chronically HIV-1-infected MSM and African heterosexual population when UDS was used compared to population sequencing. These results demonstrate the interest of UDS for the surveillance of HIV-1-TDR, with a potential impact on the choice of first-line treatment. The only difference observed by UDS between the two groups is on NRTI-TDR frequency.

Otherwise, we have retrieved by UDS a trend for a higher frequency of TDR in subtype B infected patients among MSM.

No conflict of interest
of mutations found with a higher prevalence compared to B subtype was also evaluated by Kaplan-Meyer estimates. In patients with an available GRT at failure, the prevalence of resistance was also evaluated.

**Results:** Among 1057 patients starting HAART from 2004 to 2013, B was the most common subtype (N=881, 83.3%), followed by CRF02_AG (N=85, 8.0%), F (N=51, 4.8%) and C (N=40, 3.8%) subtypes. The most used PI/r was lopinavir/r (51.0%), followed by darunavir/r (22.1%) and atazanavir/r (21.2%). The proportion of patients with a pre-HAART viremia >500,000 copies/mL was higher among those infected with non-B subtypes (B: 16.0%; CRF02_AG: 22.4%; F: 23.5%; C: 27.5%, P=0.077). By 12 months of PI/r treatment, the overall probability of VS was 91.0%. Patients CRF02_AG infected showed a lower rate of VS compared to others, with a trend toward significance (C: 93.7%; B: 90.1%; F: 88.9%; CRF02_AG: 75.4%; p=0.076). By 24 months after the achievement of VS, the overall probability of VR was 20.2%. Patients infected with CRF02_AG showed the highest probability of VR compared to other subtypes (34.2% vs. 15.0% in F, vs. 19.4% in B, vs. 20.8% in C, p=0.026). By multivariable Cox regression (adjusting for gender, age, pre-therapy CD4/HIV-RNA, PI/r and NRTI backbone used), CRF02_AG infected patients showed a higher hazard of experiencing VR (RH [95% CI]: 1.97 [1.20-3.24, p=0.007]) compared to those subtype B infected. Among 236 mutations detected, 23 of them were significantly associated to CRF02_AG, while 5 were associated with B subtype. Among these mutations, by 24 months after VS the presence of K20I, K70R and L89M was associated with a significantly higher rate of VR (mutation: present vs. absent; K20I: 31.7% vs. 19.4%, p=0.019; K70R: 43.1% vs. 19.0%, p=0.001; L89M: 26.7% vs. 19.2%, p=0.026). In 76 patients with an available GRT at PI/r failure, the 17.1% showed resistance (2.6% to PI; 13.5% to NRTI). Among patients treated with lamivudine/emtricitabine, those CRF02_AG infected showed at failure a significantly higher prevalence of M184V mutation compared to B subtype infected patients (B vs. CRF02_AG: 26.6% vs. 2.0%, p=0.017).

**Conclusions:** Even though a high proportion of PI/r treated patients achieve and maintain VS at first line regimen, patients infected with CRF02_AG result more likely to experience VR. The presence of specific mutations such as K20I, K70R and L89M may correlate with this phenomenon. Further investigations are needed to clarify these observations.

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Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

High levels of NNRTI drug resistance amongst pMTCT recipients in Durban, South Africa.

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Background: Although the rates of vertical transmission of HIV in the developing world have improved to around 3% in countries like South Africa, resistance to antiretrovirals (ARV) used in Prevention of Mother-to-Child transmission (pMTCT) strategies may thwart such outcomes and affect the efficacy of future ARV regimens in mothers and children. The 2010 South African pMTCT guidelines provided ARV prophylaxis to women with CD4 counts > 350 cells/mm3. The strategy included antenatal AZT from 14 weeks gestation, intrapartum sd NVP with 3 hourly AZT during labour and postpartum single dose of TDF/FTC. The aim of this study was to assess the rates of resistance in this group of patients since resistance to NVP, AZT or TDF/FTC will have clinical implications for women initiating Highly Active Antiretroviral Therapy (HAART) containing these ARVs.

Materials & Methods: This study was conducted at Lwazi clinic, Addington Hospital in Durban, South Africa. Full ethical permission was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BF 069-09). Permission from the Department of Health and management of Addington Hospital was obtained. Ninety seven patients were recruited. Patients with a CD4 count of >350 cells/mm3 who did not qualify for HAART were included in the study. Full informed consent was obtained. A blood sample for viral load was collected at baseline and at six weeks post-delivery. A sample for resistance testing was collected at six weeks post-delivery. Population sequencing was performed at Inqaba Biotechnical Industries (Pty) Ltd in Pretoria using the automated sequencer, ABI 3500XL genetic analyser. (Applied Biosystems, Foster City, CA, USA). Genotypes were interpreted using the Geneious software (Biomatters Ltd. Auckland, New Zealand). Drug resistant mutations were interpreted using the Stanford database (http://hivdb.stanford.edu).

Results: Of 44 patients, resistance mutations were detected in 19 (43%) patients. Sixteen (36%) patients had mutations associated with resistance to NNRTI’s. Fifteen (34%) patients had mutations (viz K103N, Y181C, Y188C, Y188HY and G190AG) associated with high level resistance to NNRTI, in particular NVP, whilst 1 (2%) patient had K101E associated with intermediate resistance to NNRTI. The mutation most frequently detected was K103N, seen in 66% (10/15) of patients in whom a mutation conferring high-level NNRTI resistance was detected. No Thymidine analogue mutations (TAMs) or M184V were detected.

Conclusions: In this resistance study conducted amongst pMTCT recipients in Durban, South Africa, high rates of NNRTI resistance were detected despite the use of ante-partum AZT, intrapartum sd NVP and post-partum single-dose TDF/FTC. Factors which may have contributed to this include poor adherence, higher levels of transmitted resistance in KwaZulu-Natal, previous exposure to sd NVP and the possible association of K103N with subtype C. This high rate of resistance may negatively impact the clinical response to NNRTI-containing first line ART in the women exposed to this pMTCT strategy in South Africa.

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Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

A pilot study to evaluate the use of proviral DNA to test for archived resistance mutations against HIV integrase inhibitors

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Background: HIV proviral DNA may reflect viral populations that have reverted to the wild type after discontinuation of therapy. Dolutegravir (DTG) is a new integrase inhibitor, with an excellent efficacy, safety and resistance profile. DTG once or twice daily dosing is conditioned, among others, by the presence of resistance mutations. The mutational profile in patients with prior failure to Raltegravir may not be always available. Our aim was to determine whether the information in proviral DNA after a time of suppressive therapy and prior RAL failures may reflect the mutations present at failure.

Materials & Methods: we have performed a pilot observational ambispective study, which included 7 patients with an available integrase genotypic study at the time of failure. Following a regimen change, and after achieving virologic suppression, mutations in proviral DNA were studied. For this purpose, DNA was extracted from whole blood and ficoll isolated PBMCs. Integrase amplification (nested-PCR) and sequencing was performed by an in house protocol. Sequences were interpreted using the Stanford HIV database algorithm and identity was checked by phylogenetic methods.

Results: mean age was 51 years (43-56), 42.8% of the patients were men, mean viral load (Log cp/ml) was 2.03 (1.17 to 2.20) and CD4 count was 764 cells/uL (436-1023). The average time between the study of resistance at the time of failure (plasma) and the current proviral DNA sample was 42 months (29-53). All HIV viruses were subtype B. For 6 patients (85.7%) we found identical mutations in the corresponding proviral DNA and those detected at the time of failure. N155H was detected in four patients in failure and remained on proviral DNA in three cases. Polymorphisms were more frequently detected in the plasma sample at the time of failure than in proviral DNA after suppression.

Conclusion: Our pilot study shows that the analysis of integrase resistance in proviral DNA can reflect archived integrase mutations present at failure. This information can be very useful for those patients lacking resistance information at the time of prior integrase inhibitor failures. As this is a pilot study, our results need to be confirmed by other investigators.

No conflict of interest

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Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Analysis of coreceptor tropism evolution in HIV-1 infected patients interrupting suppressive antiretroviral therapy

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Background: We evaluated the evolution of genotypically-inferred tropism in HIV-1 infected patients undergoing planned treatment interruptions for reasons other than virological failure.

Materials & Methods: Eight HIV-1 infected patients virologically suppressed for a median (IQR) time of 33 (23-47) months under continuous antiretroviral therapy (ART, excluding any CCR5-containing regimens), and who interrupted therapy, were selected for the study. All patients had available frozen peripheral blood mononuclear cells (PBMCs) samples at different time points: one immediately before starting therapy interruption (T0); two during therapy interruption (T1, T2). Moreover, for each patient at least one frozen HIV-RNA plasma sample was available during therapy interruption. HIV-1 genotypic tropism was assessed by both Sanger sequencing and 454 GS-Junior ultra-deep pyrosequencing (UDPS). HIV-1 co-receptor usage was inferred by using the geno2pheno algorithm. In particular, samples were considered X4-tropic when ≥2% of viral species had a false positive rate (FPR) ≤3.5% by UDPS, or when FPR was ≤10% by Sanger sequencing. For each patient, the change of tropism and FPR from therapy interruption start was evaluated. The correlation of FPR values at T0 and T1 with CD4 and viremia changes at 6 and 12 months of therapy interruptions was also evaluated.

Results: The median (IQR) time at T1 and T2 was 5 (4.5-5) and 21 (12-27) months, respectively. At T0, X4-tropic variants were detected by UDPS only in one patient (81.8% of intra-patient species), and were still relevant at T1 and T2, despite their prevalence decreased over time (T1, 15.4%; T2, 7%). In this patient, X4-tropism was confirmed by Sanger sequencing in PBMCs at T0 (FPR=1.7%) and T1 (FPR=6.0%), but not at T2 both in PBMCs (FPR=32.4%) and HIV-RNA (FPR=38.8%). Among the other seven patients, only R5 variants were detected by UDPS at all-time points. However, intra-patient FPR values changed over time, and these changes interested mainly minority variants. Analysis from plasma HIV-RNA samples during treatment interruption, when available, confirmed the results obtained in the PBMCs. At T0, median (IQR) CD4 cell count was 916 (796-954) cells/µl. As expected, after 6 months of therapy interruption, a significant median (IQR) change of CD4 (-333 [-448;-281] cells/µl, p=0.008) and viremia (+2.8 [+2.5;+3] log10 cps/mL, p=0.008) was found. Patients with higher FPR values in species detected by UDPS at T0 showed a lower CD4 decrease (rho=0.302, p=0.019) and a higher viremia increase (rho=-0.514, p=0.001) after 6 months of therapy interruption. From 6 to 12 months of therapy interruption, CD4 count still decreased (-123 [-187;-41] cells/µl, p=0.063), while viremia change was not relevant (-0.4 [-0.6;+0.1] log10 cps/mL, p=0.563). No correlation was found between FPR values at T1 and CD4/viremia changes from 6 to 12 months of therapy interruption.

Conclusions: Next generation sequencing allowed the detection of minority species that can persist or evolve during treatment interruption in both PBMCs and plasma HIV-RNA compartments. The relevance of this evolution with the clinical and virological outcome during and after treatment interruption needs further investigation.

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Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Evaluation of the False Positive Rate as a quantitative predictor of virological and immunological response to Maraviroc including therapy

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Background: To assess the role of false positive rate (FPR) to predict virological and immunological response to ART regimens containing maraviroc (MVC) in HIV-infected patients with viral tropism predicted by geno2pheno[coreceptor] (G2P).

Materials & Methods: From ARCA database, we selected patients initiating ART regimens containing MVC after viral tropism assays by G2P<120 days before. Virologic response (VR) was defined by achieving or maintaining VL<50 cp/ml after switch to MVC regimen (intent to treat ignoring treatment changes). Predictors of VR were analyzed using logistic regression and changes of CD4 by Student’s T test (SPSS v.18.0-SPSS Inc., Chicago, IL, USA). The prevalence of intermediate or high-level resistance to the concomitant drugs was computed by Stanford HIVdb v.7.0 algorithm, both considering the last and the cumulative past available genotypes.

Results: We included 182 treatments: 137(75%) B subtype, 124(68)% males, 97(53%) sexual risk factor, median age 47 years (IQR 43-52), years since HIV diagnosis 18(12-23), CD4 367 cell/µl (212-597), nadir CD4 144 cell/µl (46-267), past ARV lines 9(5-13), duration of ART 11yy (7-15). The ART regimens included 118(65%) bPI, 88(48%) INI, 9(5%) T20. Baseline VL was <50 cp/ml in 45(25%) and >50 cp/ml in 137(75%) with median value 3.7 log_{10} cp/ml (3-4.6). The median GSS of the accompanying regimen was 1 (1-1.75): 18(10%) had GSS<1, 89(49%) GSS =1 to<2, 60(34%) GSS ≥2, 15(7%) unknown. Overall median FPR was 34 (17-66), in 13 cases (7%) FPR was 0 to<5; 14(8%) 5 to<10; 28(16%) 10 to<20, 78(44%) 20 to<60, 46(26%)>60. Baseline tropism test were on RNA in 139 cases (76%), on DNA in 39(21%). Among cases with BL VL<50 cp/ml, 30/36(67%) and 31/34(69%) maintained VL<50 cp/ml after 24 weeks and 48 weeks, respectively. Of the cases with BL VL>50 cp/ml, 74/103(72%) and 71/107(66%) had VL<50 cp/ml at 24 and 48 weeks, respectively. Median changes of CD4 were +35 (-31;+148) after 24 weeks (p=0.003) and +31 (-45;+184) (p=0.007) after 48 weeks in cases with BL VL<50 cp/ml, +61 (-18;+154) (p=0.001) and +97 (+21;+201) (p=0.001) in cases with BL VL>50 cp/ml, respectively. At 48 weeks, VR was 5/13(38%) with FPR 0 to<5; 6/13(46%) with FPR 5 to<10; 8/15(53%) with FPR 10 to<20, 38/68(55%) with FPR 20 to<60, 19/34(55%) with FPR>60. Independent predictors of VR were past use of NRTI (OR 0.6, p=0.03, CI 95% 0.4-0.9), NNRTI (OR 0.2, p=0.006, CI 95% 0.06-0.64), nadir CD4 (+50 cell/mmc, p=0.05, OR 1.2 0.19; 1.0-1.5), FPR (OR 1.04, p=0.05, 1.00-1.08) in cases with BL VL<50 cp/ml and past use of INI (OR 0.4, p=0.01, CI 95% 0.2-0.8), BL VL (OR 0.7, p=0.03, CI 95% 0.5-0.9) in cases with BL VL>50 cp/ml. During follow-up, 42 tropism RNA test results showed median FPR change -4.3 (-22;+0.22) respect to baseline (p=ns). Of these 31/42(74%) had baseline FPR>10% and 7/31(22%) shifted to FPR<10%.

Conclusions: A significant proportion of experienced patients treated with MVC regimens achieves VL<50 cp/ml and gains significant numbers of CD4 cells. As expected, FPR>10% is associated with better VR than FPR<10% but there do not appear to be different response rates with increasing FPR values over 10%.

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Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Identification of minority resistance mutations in the HIV-1 integrase gene using Next Generation Sequencing.

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Background: The current widely applied standard method to screen for HIV-1 genotypic resistance is based on Sanger population sequencing. This method does not allow for identification of low-level minority variants (MVs) (i.e. below the limit of detection for the Sanger-method) in patients already receiving integrase strand-transfer inhibitors (INSTI) as well as
patients about to start INSTI therapy. Next Generation Sequencing (NGS) has facilitated the detection of MVs present at a much lower proportion in the viral population than traditional Sanger population sequencing can detect. The aim of this study was to test six HIV-1 patients for the presence of INSTI resistance mutations with both Sanger and NGS and compare the results.

Materials & Methods: Samples from six HIV-1 infected patients were analyzed in this study. For three of the patients (INSTI Res+), INSTI resistance had previously been detected by population based Sanger sequencing, while no INSTI resistance were detected in the remaining three patients (INSTI Res-). We tested for the presence of MVs involved in resistance to the three commonly used INSTI: Raltegravir (RAL), Elvitegravir (EVG) and Dolutegravir (DTG). For each sample, the HIV-1 integrase gene was amplified by RT-PCR using in-house primers and prepared for Illumina Miseq sequencing using the Nextera XT kit. Sequence assembly and analysis of MVs were performed using the CLC Genomic workbench 7.5.1 and resistance levels were evaluated using the Stanford HIVDB 7.0 algorithm.

Results: NGS confirmed the Sanger population sequencing results but also led to the detection of three additional INSTI resistance mutations and three accessory mutations in sequences from the INSTI Res+ patients and one additional INSTI resistance mutation in one of the INSTI Res- patients. The additional resistance mutations N155HN, E92AE, N155HN and Q148QR were detected in 1.33 %, 17.18 %, 16.52 % and 4.48 % of reads respectively, while the accessory mutations S230RS, E138EK, G140EG and L74LV were detected in 31.59 %, 6.51 %, 6.77 % and 30.15 % of reads respectively. Each resistance or accessory mutation was supported by at least 1900 reads with an average quality score above 34.

Conclusions: Our observations suggested that NGS (by Illumina) provides a higher sensitivity in detection of MVs compared to Sanger sequencing and therefore has the potential to become a valuable tool for monitoring of antiretroviral resistance in both treatment experienced patients and naïve patients at baseline.

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Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Combination of two pathways involved in raltegravir resistance confers dolutegravir resistance

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Background: strand transfer inhibitors such as raltegravir (RAL), elvitegravir (EVG) or dolutegravir (DTG) can efficiently inhibit HIV-1 integration. Three main pathways conferring RAL/EVG resistance were identified, involving Y143, Q148 and N155 residues. DTG, a second-generation strand-transfer inhibitor used in clinical trials in drug-naïve patients, is now available. To date, no pathway leading to DTG resistance has been reported by in vitro studies. DTG displays limited cross-resistance with RAL/EVG and has been described to select for the R263K substitution in treatment-experienced patients and recently, several integrase mutations have been implicated in DTG resistance in a highly treatment-experienced patient having firstly selected a N155H mutation when treated by RAL.

Materials & methods: several mutations identified in one highly experienced patient failing to DTG were reported and introduced in the pNL43 backbone. Using recombinant IN assays, as well as virological experiments, we studied the impact of each mutation on viral activity as well as on DTG susceptibility.

Results: in this patient failing first RAL, the genotypic analysis showed the appearance of integrase mutations G140S and Q148H confirming RAL resistance. When the patient was then treated by DTG, we identified the appearance of several integrase mutations, first T97A and T112S, and secondly N155H, linked to a viral load rebound. We show that both pathways, involving the N155H or the G140S/Q148H mutations, respectively, do not
confer DTG resistance (FC of 1 and 4 for N155H and G140S/Q148H, respectively). Interestingly, the combination of these mutations led to a high DTG resistance (FC of 110).

Conclusions: in conclusion, our data show that combination of mutations at the level of the Q148 and N155 residues, both involved in RAL resistance, lead to a strong decrease of DTG susceptibility. Such profile needs to be monitored to evaluate the relevance to treat patients with DTG when viruses have firstly selected a Q148 mutation when the patient was treated by RAL.

No conflict of interest

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Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Clinical and epidemiological features in HIV-1 infected children and adolescents exposed to lopinavir-ritonavir in Madrid, Spain


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Background: The efficacy of the antiretroviral treatment (ART) in HIV-1 infected patients can be compromised due to the selection of resistant virus. According to international guidelines, first (ART) for HIV-1-infected children and adults is a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and a third agent, either a non-NRTI or protease inhibitor (PI). Lopinavir-ritonavir (LPV/r) is the most used PI in children today, usually combined with two NRTIs. Our study shows the virological and clinical follow up of a Spanish cohort of HIV-infected children with LPV/r experience.

Materials & Methods: Patients from the Madrid Cohort of HIV-1-infected children and adolescents LPV/r experienced were selected, recovering all available pol sequences or resistance profiles for each patient collected during 1996-2011. Epidemiological, clinical and virological data and drug-resistance mutations (DRM) according to the IAS-USA 2014 list were recorded before and during LPV/r treatment. Drug susceptibility was predicted using the Stanford HIVdb Algorithm. Changes in viral load (VL), lymphocyte CD4 and CD8 counts and in drug susceptibility were recorded until December 2013.

Results: A total of 199 (37.3%) of the 534 patients from the Madrid Cohort of HIV-infected children and adolescents were exposed to LPV/r within the period 2000-2013, being 40.2% transferred to adult units during the study period. Among these 199 children, 59.8% were HIV-diagnosed between 1990-1999, 81.9% were Spaniards and 96.5% perinatally infected, with subtype B infection in 65.3% cases. The mean age at LPV/r first exposure was 9.7 years (range: 1-19.3 years) and the mean time of LPV/r exposure 56.8 months (range: 0.03-155.6 months). LPV/r use decreased over time from 82.4% (2000-2007) to 17.6% (2008-2013) patients. LPV/r was included in first therapy within a high active-ART (HAART) regimen in 34 (17.1%) children, as rescue regimens in the remaining patients (82.9%). Among those 61 patients with available resistance information during LPV/r treatment, 42.6% reported any DRM to PI, being the most common M46IL (21.3%), L90M (19.7%), I54V (16.4%), V82A (14.7%) and D30N (13.1%) at protease. However, we observed a preserved susceptibility to several PIs: to darunavir in 51 children (83.6%), to tipranavir in 47 (77%), to atazanavir in 41 (67.2%), to LPV/r and saquinavir in 43 (70.5%), to fosamprenavir and
indinavir in 39 (63.9%) and to nelfinavir in 30 (49.2%). High resistance to nelfinavir was predicted in more than 30% patients and to LPV/r, atazanavir, fosamprenavir, indinavir and saquinavir in nearly 20%. During LPV/r treatment, 47.8% patients reached undetectable VL (<50 HIV-1-RNA copies/ml) at last clinical report and the median of CD4+ T cells was 911 (IQ: [608.15-1186.60]) and of T CD8 cells was 40.78% (IQ: [23.99-37.59]).

Conclusions: Despite the presence of DRM to PI in the study cohort LPV/r experienced, good clinical status at the last clinic visit was observed. Due to high genetic barrier of LPV/r, susceptibility to LPV/r as well as to other boosted PIs is preserved, despite LPV/r experience. This data will help to improve clinical management of HIV-infected children under virological failures to ART.

No conflict of interest

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Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

PI drug resistant HIV: Minor variants and cellular tropism

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Background: The evolution of HIV drug resistance resulting in treatment failure is most often a stepwise process. Drug resistance mutations and compensatory mutations gradually emerge one after the other compromising the inhibitor effect and facilitating viral replication in the presence of antiretroviral drugs. Little is known about the env diversification in PI resistant HIV and its diversification over time. Therefore we analyzed the amino acid sequences of HIV-1 Gag protein, protease, reverse transcriptase, integrase, gp120 and gp41 in PI resistant viruses.

Materials & Methods: Plasma samples from 20 patients containing PI drug resistant HIV-1 as assessed during routine Sanger sequencing in Cologne, Essen, Leuven, and Rome were selected. The viral loads in these samples ranged from 271 to 476,800 copies/ml (median: 15,126 copies/ml). Based upon Sanger-sequencing these viruses often carried drug resistance mutations (DRM) as listed by the International AIDS Society (IAS) affecting different antiretroviral drug classes: PI: n=2, PI+NRTI: n=8, PI+NRTI+NNRTI: n=7, PI+NRTI+NNRTI+INI: n=3). The genetic diversification in each sample was retrospectively analyzed using a more sensitive approach i.e. the gag, pol and env genes were amplified and sequenced using the Illumina MiSeq platform (NGS). DRM were scored as major variants when detected in more than 10% of the reads and as minor variants when detected in less than 10%. HIV tropism was predicted using the geno2pheno454 tool.

Results: Using the Illumina platform two extra DRM were detected in RT as major variants when compared to the initial Sanger sequencing results. Overall the number of DRM varied for PI from 1 to 7 (median: 4) in protease and from 0 to 6 (median: 3) in Gag, for RTI from 0 to 9 (median: 5), for INI from 0 to 4 (median: 0). Only one exception of the 20 PI resistant isolates did not harbor treatment-associated Gag mutations. In several patients DRM that were not detected using Sanger-sequencing were observed as minor variants in NGS derived sequences (protease (n=5), reverse transcriptase (n=4), Gag (n=9) and integrase (n=2)). In six samples HIV variants capable of using the CXCR4 coreceptor dominated the virus population (FPFR <5). Virus variants with FPR between 5 and 15 build 15%, 80% and 97%, respectively, of the quasispecies of three patients, whereas in the rest of patients R5-tropic variants clearly dominated. Furthermore, mutations previously associated with macrophage-tropic HIV (T158N, H278N) were detected in the gp120 sequences (n=5) and could also be found as minor variants (1-5%) in R5-tropic and X4-capable HIV.
Mutational patterns in gp41 were diverse and no specific mutations could be identified in this group of PI resistant HIV.

**Conclusions:** The NGS approach displayed an accurate detection of DRM in major variants and enabled the detection of HIV minority variants carrying drug resistance mutations that were not detected by Sanger sequencing. Gag and PR mutations were concomitantly detected as major as well as minority variants indicating the shared selective pressure for both proteins. However, the cellular tropism of PI resistant viruses was diverse.

No conflict of interest

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**Abstract: 34**

**Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)**

**In vitro phenotypes to integrate inhibitors of recombinant HIV-1 variants isolated from Raltegravir-treated patients**

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**Background:** Phenotypic resistance analysis is an essential method to determine HIV-1 resistance and cross-resistance to new drugs. To analyse developing resistances to new integrase inhibitors (INI) in vitro a recombinant integrase (IN) susceptibility assay was established. This assay enables the phenotypic resistance analysis of patient-derived HIV-1 IN. The correlation of the IN phenotype with its genotype supports the detection of resistance-associated mutations. This knowledge is used to improve rules-based and bioinformatics-driven tools for resistance prediction (HIV-Grade, geno2pheno [integrase]).

**Materials & Methods:** The IN gene region of nine patient-derived subtype B virus strains from raltegravir (RAL)-treated patients was analysed for phenotypic resistance and cross-resistance against RAL, elvitegravir (EVG) and dolutegravir (DTG). 4/9 viruses harboured RAL resistance mutations related to all three primary resistance pathways of RAL. The HIV-1 IN genes were cloned into a modified pNL4-3 vector. The analysis of phenotypic INI resistance and cross-resistance of the recombinant clones was adapted to the INIs RAL, EVG and DTG as described in Walter et al. 1999 for NRTIs, NNRTIs and PIs.

**Results:** The determined mean IC₅₀-values of the five HIV-1 clones without genotypic resistance to RAL (#1-5) were comparable to those of the reference strain NL4-3 depending on the used INI. The mean IC₅₀ for RAL was 7.4 nM (range 4.1-13.5), compared with 9.8 nM for EVG (range 4.0-23.8) and only 2.2 nM for DTG (range 1.7-2.9). Their calculated mean fold changes (FC) for RAL ranged from 0.8 to 2.1, for EVG from 0.8 to 3.6, and for DTG from 0.9-1.8. The IC₅₀-values and FCs of clone #6, presenting only a minor RAL resistance mutation (V151I), were comparable to the wild-type values in regard to all INIs. In contrast, the RAL-resistant clones revealed higher IC₅₀-values compared to NL4-3. The clone #7 (N155H+G163R) presented the lowest FCs for RAL with 7.0 and for EVG with 7.7. The mean FCs for clone #8 (Y143R+T97A+E138K) was 145.8 for RAL and 8.5 for EVG, proving the low impact of the Y143R on EVG susceptibility. The sensitivity to DTG was not influenced by both mutation patterns (mean FCs 1.1 and 0.6). The clone #9 (Q148R+G140S) with mean FCs for RAL and EVG >200 was the only variant reducing DTG sensitivity (mean FC 4.9).

**Conclusions:** This recombinant phenotypic resistance assay enables the analysis of the IN of patient-derived viruses to INI activities. While EVG presented a high cross-resistance to RAL, DTG maintained in vitro activity despite of the N155H and the Y143R resistance mutations, confirming the strong activity of DTG against RAL-resistant viruses. Just the Q148R+G140S variant presented a reduced susceptibility to DTG. This assay enables to determine the
impact of newly appearing INI mutation patterns and provides data for the improvement resistance interpretation and the update of genotypic interpretation systems.

No conflict of interest

Abstract: 35

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Prevalence of transmitted and acquired HIV drug resistance in a large single center cohort

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Background: The development and transmission of resistance inducing mutations may limit treatment options. Treatment acceptability, efficacy, easiness and forgiveness may influence the development of resistance as well as the transmission of resistant viruses to newly infected subjects. We assessed trends of resistance prevalence over a long period of time (14 years) in a large mono-center cohort.

Materials & Methods: All available viral sequences of tests performed between the years 2000 and 2014 were included. The effect of selected mutations was analyzed according to the Stanford algorithm. Transmitted resistance (TDR) was defined from viral sequences performed in treatment naïve patients, while acquired resistance (ADR) was defined from all viral sequences obtained after starting cART. Subjects with drug resistance to at least one of the currently available drugs were categorized as having resistance. Trends in the HIV drug resistance were calculated by logistic regression.

Results: The results of 6655 tests performed in 3805 subjects are presented. Patients were mostly men (72%) with a mean age of 40 years (SD 10 years). Overall, 19.4% of tests were performed in naïve subjects and the remaining 80.6% in pre-treated patients. The proportion of TDR over time was stable at 8.2% with a slight trend to reduction in more recent years and a range from 5.7% to 14.8% (OR 0.96 95% CI 0.89-1.04; P= 0.365 for trend). The proportion of ADR was high among patient tested (55.2%), however we observed a constant and marked reduction over time from 91.5% in the years 2000-2002 to 28.8% in the years 2013-2014 (OR 0.75 95% CI 0.74-0.77; P < 0.0001 for trend). The prevalence reduction was evident for all drug classes: NRTIs from 85.9% to 19.8% (OR 0.74 95% CI 0.73-0.76; P < 0.0001 for trend); NNRTIs from 63.8% to 18.1% (OR 0.81 95% CI 0.80-0.83; P < 0.0001 for trend) and PIs from 47.1% to 4.8% (OR 0.78 95% CI 0.76-0.80; P < 0.0001 for trend).

Conclusions: Over the years, we observed a dramatic reduction of ADR not paralleled by a reduction of TDR. The reduction of ADR is not therefore linked by a reduced TDR, but, more likely, to other factors. The availability of treatment alternatives may induce a more aggressive management of patients and praecox testing lowering the chance to accumulate mutations. New therapies may be more forgiving and the new drug combinations may have a greater treatment genetic barrier. Easier to take regimens with improved tolerability may increment adherence. Whether a reduced ADR could reduce TDR in next years is still to be demonstrated.

No conflict of interest

Abstract: 36

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Pretreatment NS3 variation of hepatitis C virus genotype 1a

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Background: With the advent of new DAAs for treatment of hepatitis C (HCV) it becomes important to investigate the relation between naturally occurring and previous treatment-induced viral mutations and treatment susceptibility. We investigated HCV variation in the NS3 region in relation with patient characteristics in HIV-positive and HIV-negative HCV genotype 1a patients.

Materials & Methods: Pretreatment samples were collected from 29 HIV-positive and 45 HIV-negative hepatitis C genotype 1a patients in whom treatment with simeprevir was considered. Drawing date from investigated samples ranged from 1999 until 2015. HCV RNA was isolated using the MagNA Pure 96 system (Roche). A reverse transcriptase PCR was then performed using the Superscript III One-Step Platinum Taq kit (Life Technologies). Subsequently, cDNA was amplified with a nested PCR using the Expand High Fidelity PCR System Kit (Roche). Product length was confirmed on an agarose gel (E-Gel iBase Power System, Invitrogen). Product was then purified using a QIAquick PCR purification kit (Qiagen). Following purification, a cycle sequence reaction was run using the BigDye Terminator v3.1 sequencing kit (Life Technologies) to prepare the samples for sequencing. After cycle sequence reactions, samples were sequenced by the Central Sequencer Facility using the 3730xl DNA analyzer (Applied Biosystems).

Results: Prevalence of mutations that are associated with resistance to telaprevir, boceprevir or simeprevir was investigated in the pretreatment samples and related to HIV-status and sampling date. In HIV-positive subjects (n=29), mutations associated with resistance to telaprevir (V36L/M, T54S, V55A, R117H, I132V, R155K, I170V, N174G), boceprevir (V36L/M, T54S, V55A, R117H, R155K, I170V, N174G) and simeprevir (Q80K, S122G, R155K) were present pretreatment in 14% of patients for telaprevir, in 7% of patients for boceprevir and in 66% of patients for simeprevir. Q80K mutation associated with resistance to simeprevir was present in 62% of HIV-positive, S122G occurred in 0% and R155K in 10%. In HIV-negative patients (n=45) resistance mutations occurred in 40% of patients for telaprevir, 40% of patients for boceprevir and 44% of patients for simeprevir. Q80K mutation was present in 22%, and S122G and R155K in respectively 18% and 2%. Significant differences in prevalence were found between HIV-positive and negative patients in the prevalence of Q80K (p=0.0012, more in HIV-positive) and S122G (p=0.0192, more in HIV-negative, Fisher’s exact test). No other significant differences were found in mutations that are currently known to be associated with resistance to simprevir. Mutations associated with resistance to telaprevir and boceprevir, like V36L/M and R155K, were also more prevalent in HIV-positive subjects whereas N174G was significantly less prevalent in HIV-positive than in HIV-negative subjects (p=0.02).

Conclusions: The Q80K mutation associated with resistance to simprevir was observed significantly more often in HCV patients with HIV compared to HIV-negative HCV patients, whereas S122G and N174G were found significantly more often in HIV-negative HCV subjects.

Conflict of interest: A financial grant from Janssen was received to start this project.

Abstract: 37

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Incidence of NS3 Q80K mutation in HCV genotype 1a mono- or HIV/HCV co-infected patients in a Berlin laboratory

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Background: Simeprevir, an oral NS3/4A protease inhibitor, was recently approved by the FDA and the EMA for the treatment of patients with chronic HCV genotype 1, 4, 5 and 6 infection.
It has been recommended in the 2014 UK Consensus Guidelines as a possible treatment of previously untreated genotype 1a-infected patients. The antiviral efficacy of simeprevir is adversely affected by the mutation at the Q80K loci.

There is controversial discussion that the incidence of Q80K in the European HCV 1a-infected community is very low and therefore testing of Q80K before starting a therapy including simeprevir is not necessary. We analyzed the appearance of Q80K in all sequenced HCV NS3A samples since 2014 in our laboratory.

**Materials & methods:** All received orders for HCV resistance tests before starting a (new) therapy were analysed with an in-house bulk sequencing/NGS method analyzing NS3A aminoacids 1-181. Sequence analysis was performed using geno2pheno HCV. The genotype 1a samples were selected, affiliation to clade I or II and Q80K status, protease-inhibitor relevant mutations and data of HIV-1 co-infection were collected.

**Results:** 138 HCV 1a samples were sent to us for resistance analyses from 18 different privat physicians in Berlin and Hannover, Germany. 55 (or 39.9%) of sequences showed a Q80K mutation. 31 extra clade I viruses had no Q80K mutation. Comparison between mono- and HIV-1 co-infected patients showed no significant difference in frequency of Q80K (mono-infected: 23 out of 63 patients (36.5%); co-infected: 30 out of 73 (41.1%)). Of the co-infected patients 69 were MSM of which 27 were positive for Q80K (39 %). For two Q80K-positive patients the HIV-status was not available. In 20.3% (n=28) of all patients protease-inhibitor relevant mutations (36L/M, 41H, 54ST, 55AI, 80LR, 122GN, 155K, 156S, 170V and/or 174SG) were detected.

**Conclusions:** The incidence for Q80K mutation in HCV genotype 1a with overall 40% is substantially high in our cohort and does not differ significantly between mono- and HIV-1 co-infected patients. Response to simeprevir is affected by the presence of viral Q80K. When treating HCV-infected patients with a simeprevir containing regimen, it is therefore important that HCV does not contain the Q80K mutation.

**Conflict of interest:** This work was supported with a grant from Janssen

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**Abstract: 38**

**Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)**

**Experience from using Next-Generation Sequencing (NGS) for routine HIV drug resistance tests**


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**Background:** HIV drug resistance tests are performed before the initiation of antiretroviral therapy (ART) to identify primary drug resistance and after ART failure to detect the evolution of drug resistance. In Germany transmitted HIV drug resistant variants are found in about 10 percent of treatment-naïve patients. Although the prevalence of drug resistance mutations (DRM) after treatment failure has declined in recent years, they still have major implications for treatment optimization. Therefore, DRM should be detected as early as possible and should have clinical implications even as minor variants.

**Materials & Methods:** Since August 2014 plasma samples obtained for routine HIV drug resistance tests (treatment-naïve (TN): n=62 and treatment-failure (TF): n=54) have been processed by extracting RNA, RT-PCR amplification and sequencing by using the NGS platform Illumina MiSeq (PRRT: n=102, IN: n=14). DRM according to the IAS list were scored as major variants when detected in more than 10% of the reads and as minority variants when detected in less than 10%. Here, the sensitivity cut-off at 10% is assumed to be comparable to the cut-off of the Sanger sequencing.

**Results:** DRM as major variants were detected in TN HIV (n=6, 10%) and TF HIV (n=19, 35%). The opposite trend was observed for DRM as minor variants (TN HIV (n=15, 24%) vs. TF HIV (n=4, 7%)). Through that, the overall number of
TN patients with HIV variants carrying DRM increased to n=18 (29%). TN HIV isolates from three patients carried DRM as major and minor variants. Interestingly, one TN HIV isolate carried a treatment-associated Gag mutation (I437V) as a majority variant and the PR mutation (V46I) as a minor variant. Minority variants of DRM were simultaneously detected in protease and reverse transcriptase of five TN HIV. In only five cases single DRM mutations were identified in TN HIV as minority variants. TF HIV carried mainly DRM in RT (n=12), and only few HIV isolates carried DRM in protease (n=3), protease and reverse transcriptase (n=3), and integrase (n=1). This trend held also true for DRM as minority variants, which were most often detected in RT (n=3) and only once in protease (n=1) but not in integrase.

Conclusions: Using the Illumina MiSeq platform with a sensitivity cut-off at 10% for majority and <10% for minority variants the detection sensitivity of DRM in HIV variants was higher when compared to Sanger sequencing. Due to the higher sensitivity primary drug resistant HIV in treatment-naïve patients could be detected by an incidence of 30%. In clinical samples that were obtained after treatment failure DRM, however, minority variant DRM were found less frequent. This probably indicates the rapid selection of viral variants carrying drug resistant HIV in clinical practice, which occurs after the emergence of DRM.

No conflict of interest

Abstract: 39

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Clinical implications of persistent viremia (PV) after the start of antiretroviral therapy in HIV-positive patients

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Background: After the start of antiretroviral therapy (ART) HIV RNA levels are expected to decline below the detection limit within 6 months. It was shown that a prolonged decline might be associated with the presence of drug resistance mutations (DRM). However, if baseline HIV RNA levels are extremely high, for instance, the decline might take longer without clinical significance. Furthermore, treatment regimens containing protease inhibitors (PI) tend to result in a slower decline of HIV RNA levels, which do not influence the long term efficiency.

Material and Methods: HIV-1 infected patients from routine diagnostics with persistent viremia (PV) after ART initiation were identified (n=26). Patients were treated with either boosted PIs (n=17, 65.4%), or INIs (n=5, 19.2%), or NNRTI (n=2, 7.7%) in combination with NRTIs. Only two patients (7.7%) received combined treatment-regimens without NNRTIs. Plasma-samples from these patients were tested for the amount of viral RNA (m2000 real time system, Abbott Molecular) and genotyped by analyzing protease, reverse transcriptase and gag sequences using Sanger sequencing following nested one-step RT-PCR amplification. In addition, clinical and immunological parameters (VL, cART, CD4+T-cells, CD3+HLA-DR+ T-cells and CD4/CD8 Ratio) were obtained from routine diagnostics. The statistical significance was tested using Fisher's exact test and t-test.

Results: HIV genotypes were obtained 369 (+/-498) days after starting or restarting ART (viral load: 204.1 (+/-189.6) copies/ml). Overall, HIV RNA levels decreased from approximately 630.000 copies/ml to 204.1 (+/- 189.6) cp/ml upon ART initiation. The percentage of HLA-DR+T-cells is reduced by 14.5% (145 cells/µl), whereas the CD4+ T-cell count increased by 170 cells/µl. HIV isolates carrying new DRM were detected in four patients (Y181C, M184V, Q58E, K70KR). These samples were collected and genotyped 238 days (median) after the start of ART, and HIV RNA levels were quantified (between 40 and 500 copies/ml). In contrast, in eight patients HIV RNA levels starting at 8.7x10⁵ cp/ml finally dropped below the detection limit after 380 (+/-165) days without...
any clinical intervention. These patients were dominantly treated with protease inhibitors (n=4). Only in six out of eight patients (23.1% of all) therapy switches succeeded in suppressed viral replication within 123 +/- 102 days. Interestingly, in two patients HIV RNA levels were still detectable after 552 days of treatment following therapy switch.

Conclusions: In some patients new DRM can be rapidly detected after the start of ART but the majority of patients finally showed suppressed viral replication. In this study there was no marker associated either with the detection of new DRM or a prologend decline of HIV RNA without any clinical significance.

No conflict of interest

Abstract: 40

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

The Evaluation of HIV/AIDS Patients in a Tertiary-Care Hospital


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Background: HIV infection has a recently increasing incidence in Turkey. HIV-positive patients have been followed in Yildirim Beyazit University, Ankara Ataturk Training and Research Hospital since 2013. The aim of the study is to evaluate demographical and clinical features and anti-retroviral resistance status of HIV/AIDS patients.

Materials & Methods: The naive HIV-positive patients were included in the study. The patients’ files and primary-resistance results from reference virology laboratory of Turkish Public Health Institute were used as data sources. Primary drug resistance mutations were identified according to the WHO 2009 drug resistance surveillance list.

Results: A total of 27 naive HIV-positive patients (5 women, 22 men) were included into the study. Seven patients and 20 patients were diagnosed in 2013 and 2014, respectively. The mean age of the patients was 37.4 ± 10.9 years. The most frequent route of transmission was sexual contact (58.3%). Transmission route could not be identified for the other patients. The median CD4 count was 371 (31-1184). CD4 count was lower than 350/µL and 500/µL for 6 patients (Stage 3, 22.2%) and 15 patients (Stage 2, 55.5%), respectively. Three patients have opportunistic infections as oral candidiasis, Pneumocystis jirovecii pneumonia and gastroenteritis. The primary drug resistance mutations was detected in 3 (11.1%) of the 27 patients. Non-nucleoside reverse transcriptase inhibitor resistance mutations were detected in 2 patients. Nucleoside reverse transcriptase inhibitor resistance mutation was recorded in one patient.

Conclusions: It is important to know epidemiological features and primary resistance rates to develop strategies for HIV infection. According to our results more than 75% of the patients have been diagnosed when they are Stage 2 or 3. The primary resistance rate as 11.1% implies the value of resistance test before deciding appropriate ART.

No conflict of interest

Abstract: 41

Therapeutic Challenges in Resource-limited settings

Correlation of opportunistic diseases with CD4+ T cell counts in HIV infected patients in Turkey
are shown in Table 1. Median CD4 counts of the patients with ODs were significantly lower (70 vs. 370cell/mm³) (p<0.05) and their median age was higher (45 y vs. 35 y, p< 0.05) than the asymptomatic group.

Conclusions: In Turkey, a small persantage of HIV -infected patients have ODs at presentation; their CD4 cell counts are significantly lower, and they are older than asymptomatic patients. Tuberculosi, candida esophagitis, and Pneumocystis jirovecii pneumonia are the most common opportunistic infections at presentation.

No conflict of interest

Abstract: 42

Therapeutic Challenges in Resource-limited settings

The virological response to antiviral therapy among HBV/ HIV co-infected patients

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Background:. Chronic hepatitis B (CHB) is associated with considerably morbidity and mortality, in HIV co-infected patients. The prevalence of HBV co-infection among HIV infected patients in Serbia is 5.6%. All HIV/ HBV co-infected patients in Serbia have been treated with HAART containing an anti-HBV drug, irrespective of CD4 cell count and HBV disease status in order to prevent more active liver disease. The main goals of antiviral therapy were to maintain durable suppression of viral replication and hepatitic inflammation, which response to therapy is assessed by achieving and maintaining undetectable viremia by a sensitive PCR assay, and normalisation of the of alanine aminotransferase serum activity. In addition, the successful therapy implicates the loss of HBs and HBe antigens and even seroconversion to anti HBe anti HBs antibodies,
which was shown to be possible. Guidelines recommend tenofovir as the first line treatment. This selection of first line treatment is based on the safety and efficacy of the drug and risk of resistance. However, lamivudine was the only antiviral drug available for the treatment of HBV infection, since 2010, in Serbia. Therefore all HBV/HIV co-infected patients initiated lamivudine containing HAART, which was switched to tenofovir based one, after HBV developed resistance to lamivudine.

Materials & Methods: A cross sectional cohort study was conducted to analyse the treatment response to lamivudine and tenofovir containing HAART after lamivudine failure, among subjects with CHB, from the Belgrade cohort of HI infected patients. The study included 62 HAART treated HIV infected patients with CHB, who initiated lamivudine containing HAART between 2000 and 2011.

Results: Patient population included 54 (87%) HBe Ag positive patients while severe liver fibrosis was present in only ten patients (16.1%). Patients were mostly yang mail with the mean age of 36.1±10.3 years. The mean duration of lamivudine therapy, within HAART, was 4.3±3.2 years (ranged 1-12 years). Lamivudine failure was recorded in 40 patients (64.5%). Out of twenty-two remaining subjects with favourable virologic response to lamivudine, all achieved HBs Ag loss, of which 2 patients developed anti-HBs antibodies, after 4.1±3.1 years (ranged 1-11years), and 9±2.8 years (7-11), respectively. The mean HBV viral load at the time of switching to tenofovir based HAART was 6.0±1.5 log_{10} IU/mL HBV DNA. After additional 1.7±1.0 years of tenofovir containing HAART, hepatitis B viral load was 1.5±1.1 log_{10} IU/mL HBV DNK. Undetectable viremia was recorded in seven patients, while additional ten patients achieved HBV DNK of less than 20 iu/L at the end of observed period, which gave the overall rate of optimal virologic response of 42.5%. However, none of the tenofovir treated patients achieved HBs seroconversion, while HBsAg loss occurred in one subject. The overall virologic response of CHB to HBV active drug containing HAART was rather good since after mean 5.3 years treatment over 40% achieved good virologic response, including either undetectable HBV viremia, or HBsAg loss.

Conclusions: The benefit of tenofovir containing HAART among HBV/HIV co-infected patients, who previously failed HBV therapy with lamivudine containing HAART, was rather modest after approximately a year and a half of treatment, which suggested that the prolonged TDF therapy is mandatory to achieve the favourable response.

Conflict of interest: sponsored lecture by Gilead

Abstract: 43

Treatment Strategies for HIV/ Hepatitis infected Patients

Glomerular and tubular impairment in HCV patients under treatment with directly acting antivirals with or without peg-interferon/ribavirin.

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Background: Decline of eGFR in HCV patients treated with peg-interferon/ribavirin (PEG-IFN/RBV) and directly acting antivirals (DAAs), such as telaprevir (TPR), was previously observed but it is uncertain whether or not a tubular damage exists aside glomerular impairment. Neutrophil gelatinase-associated lipocalin (NGAL) is a promising marker of acute tubular injury.

Materials & Methods: A prospective observational study was conducted, including 6 patients with TPR+PEG-IFN/RBV and 2 patients (liver transplant recipients) with simeprevir (SMV)+daclatasvir (DCV) without PEG-IFN/RBV. Blood samples were taken at baseline, weeks 4 and 12 for TPR and at baseline, weeks 4, 8 and 12 for SMV/DCV. Serum NGAL was measured by ELISA (normality range 41.2-118.1 ng/ml). GFR was estimated through CKD-Epi formula. Grade of renal impairment was assessed through KDIGO 2012 classification. Patients with heart, kidney or urogenital disease, kidney transplant and leukopenia or neutrophilia were excluded.

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Results: All patients prescribed TPR were males, Italians, infected by gt 1b HCV; 5 were relapsers and 1 was a partial responder to PEG-IFN/RBV. NGAL trends are illustrated in Figure 1. From baseline to week 12, median eGFR decreased from 95 ml/min (range: 81-109) to 82.5 ml/min (50-103). Worsening by a single lower KDIGO 2012 class was observed in 4/6 patients, with a decrease by two classes in 1 patient (from mild G2 to mild/severe G3b). Only 1 patient remained in the same class. All patients had normal serum NGAL at baseline, while it increased from a median of 67.5 ng/ml (36-92) at baseline to 87 ng/ml (56-186) at week 12, peaking to above upper limit of normality in 2/6 patients (131 and 186 ng/ml). In 1 patient serum NGAL decreased (from 92 to 77 ng/ml) with a paradoxical eGFR decrement (from 98 to 68 ml/min). Regarding liver transplant recipients, both were Italians (1M and 1F), infected with gt 1b HCV and relapsers to previous PEG-IFN/RBV. The woman had a decrease to a lower KDIGO 2012 class (from G2 to G3b) and a concomitant increase in serum NGAL (from 28 to 100 ng/ml), while the man had stable levels of all the parameters.

Conclusions: DAA combinations with TPR cause acute kidney damage through a glomerular effect, but also tubular function can be compromised as demonstrated by an increase of serum NGAL in most of the patients studied. Even combinations not including PEG-IFN/RBV or TPV could have the same effect. Further confirmatory analyses should be conducted and implications of our observations should be elucidated.

No conflict of interest

Abstract: 44

Treatment Strategies for HIV/ Hepatitis infected Patients

Effectiveness and Risk Factors for Virological Failure of Darunavir-Based Therapy for Treatment-Experienced HIV-Infected Patients

Background: Treatments in patients with multidrug resistance often involve the use of multiple agents with partial antiviral activity and overlapping metabolic toxicities. Darunavir (DRV) is a potent protease inhibitor (PI) with demonstrated activity and favorable safety in treatment-experienced patients. The aim of this study was to evaluate the effectiveness of DRV plus optimized background regimen (OBR) in real-life conditions in a cohort of highly HIV-1 ARV-experienced patients.

Materials & Methods: Retrospective cohort of treatment-experienced HIV-1-infected adults with virological failure who started therapy with a DRV-containing regimen. The effectiveness of DRV treatment was evaluated with percentages of undetectable HIV-1 RNA viral load (<200 copies/mL and <50 copies/mL) after 48 weeks of treatment, and changes in CD4+ cells count. Finally, we evaluated the risk factors associated to virological failure in these patients.

Results: One hundred twenty patients >16 years old were included, 83% were men; median age was 45 years old (IQR 40-51). Median years of experienced treatment were 13 years (IQR 9-17) and median of previous regimen was 6 (IQR 4-7), all of them had prior PI use. Baseline HIV-1 RNA viral load was 22,600 copies/mL (IQR 3590-77,797) and CD4+ cells count 245 cells/mL (IQR 129-400). After 48 weeks of treatment, 82% (IC95% 74%-88%) of patients had HIV-1 RNA viral load <200 copies/mL and 69% (IC95% 60%-76%) had <50 copies/mL. Increase in CD4+ cells count was 378 cells/mL (IQR 252-559) (P<0.001, with regard of basal count). Factors associated to virological failure were age >40 years [OR 0.15 (95%CI 0.10-0.78)]; P=0.015, use of RAL in regimen [OR 0.37 (95%CI 0.10-0.97)]; P=0.046,
and basal CD4+ cells count <200 cells/mL [OR 2.79 (95%CI 1.11-6.97)]; P=0.028.
For fasting lipid profiles (Total Cholesterol [TC], and Triglycerides [TG]), TC showed a light but significant increase ($p=0.03$) from baseline 167 mg/dL (136–195 mg/dL) to 185 mg/dL (150–214 mg/dL) at week 48; in contrast, TG showed no significant increase ($p <0.076$) from baseline 186 mg/dL (135–260 mg/dL) to 220 mg/dL (162–305 mg/dL) at week 48.

Conclusion: Our study provides clinically important evidence of the effectiveness and metabolic safety of DRV in highly ARV-experienced HIV-1-infected patients. These findings strengthen the results of previous randomized controlled trials with this agent.

No conflict of interest

Abstract: 45

Treatment Strategies for HIV/ Hepatitis infected Patients

Factors Associated With the Time to Achieve Undetectable HIV-1 RNA in Pregnant Women

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Background: The aim of highly active antiretroviral therapy (HAART) during pregnancy is to achieve an HIV-1 RNA < 50 copies/mL (c/mL) at delivery to reduce the risk of mother to child transmission, but concerns remain regarding the teratogenicity and tolerability of the treatment in the first trimester of pregnancy. Identification of characteristics which are associated with the time to achieve viral suppression could assist in the decision making concerning the timing of initiation of HAART during pregnancy in individual patients.

Materials & Methods: Pregnant women older than 18 years who started HAART during pregnancy between 1998 and 2013 were included in our analysis. Data were retrieved from the Netherlands ATHENA observational cohort database. Difference in time to viral load < 50 c/mL was compared between strata of baseline maternal viral load, CD4 cell count, gestational age at initiation of HAART, region of birth and HAART regimen using Student's t-test, Kaplan Meier plots and a Cox proportional hazards model.

Results: 227 pregnancies matched the inclusion criteria. Median age was 29 years (IQR 24-33). At start of therapy, median CD4 count was 346/mm$^3$ (IQR 209-500), HIV-1 RNA 4.2 log c/mL (IQR 3.7-4.6). In 200 pregnancies (88%) an undetectable viral load at the time of delivery was achieved. Median time to HIV-1 RNA < 50 c/mL after initiation of HAART was 60 days (IQR 42-83). A multivariate Cox proportional hazards analysis showed an independent association only with the baseline viral load ($p < 0.01$), with a shorter time to achieve a viral load < 50 c/mL in patients with HIV-1 RNA < 10,000 c/mL compared to ≥ 10,000 c/mL (mean time of 49 [95% CI: 44-53] vs. 75 [95% CI: 70-81] days, respectively). Specifically, no significant difference was found between PI- and NNRTI-containing antiretroviral regimens.

Conclusion: Our analysis shows that a baseline viral load of < 10,000 c/mL is associated with a shorter time to viral suppression when HAART is initiated during pregnancy. It seems therefore plausible in this group of pregnant women to postpone the initiation of treatment beyond the 12th week of pregnancy unless immediate start of treatment is required based on the clinical condition. In line with the recommendation of the current DHHS guidelines, earlier start of HAART should be considered in pregnant patients with a baseline viral load ≥ 10,000 c/mL to achieve an undetectable HIV-1 viral load at the time of delivery.

No conflict of interest
Abstract: 46

Treatment Strategies for HIV/ Hepatitis infected Patients

Effectiveness of Etravirine-Based Therapy for Treatment-Experienced HIV-Infected Patients


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Background: Treatment options are limited for HIV-1-infected individuals who have received extensive previous antiretroviral therapy. Etravirine, a non-nucleoside reverse transcriptase inhibitor, has shown significant clinical benefits in treatment-experienced HIV-1-infected patients with antiretroviral resistance in Phase III DUET-1 and DUET-2 trials. The aim of this study was to evaluate the effectiveness of ETV plus optimized background regimen in real-life conditions in a cohort of highly HIV-1 antiretroviral-experienced patients.

Materials & Methods: Retrospective cohort of treatment-experienced HIV-1-infected adults with virological failure who started therapy with an ETV-containing regimen. The effectiveness of etravirine treatment was evaluated using percentages of undetectable HIV-1 RNA viral load (< 200 copies/mL and < 50 copies/mL) and changes in CD4+ cell count after 48 weeks of treatment.

Results: Forty-two patients > 16 years old were included; 74% were men; median age was 45 years (IQR 41–53). Median years of treatment experience was 13 (IQR 10–17) and median years of previous regimen was six (IQR 4–7). All participants had prior NNRTI use (55% NVP, 83% EFV and 28% both). Baseline median HIV-1 RNA viral load was 15,598 copies/mL (IQR 2651–84,175) and CD4+ cell count was 276 cells/mL (IQR 155–436). After 48 weeks of treatment, 90.5% (95% CI 78–96) of patients had HIV-1 RNA viral load < 200 copies/mL and 76% (95% CI 61–86) had < 50 copies/mL. CD4+ cell counts increased from baseline to 48 weeks of treatment to a median of 407 cells/mL (IQR 242–579); p < 0.001. Virological outcome was associated with virological failure at baseline HIV-1 RNA viral load > 100,000 copies/mL (OR 7.6; 95% CI 1.2–44.80; p = 0.025).

Conclusion: Our study provides clinically important evidence of the effectiveness and safety of ETV in highly antiretroviral-experienced HIV-1-infected patients. These findings support the results of previous randomized controlled trials testing this agent.

No conflict of interest

Abstract: 47

Treatment Strategies for HIV/ Hepatitis infected Patients

Effectiveness of an Antiretroviral Regimen Based on Genotyping Data in HIV-1 Highly Experimented Children to Antiretroviral Therapy

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Background: Genotyping tests were developed to attenuate the impact of viral resistance. This test is able to detect mutations associated with phenotypic resistance of HIV drugs. Data on the use of ritonavir-boosted darunavir (DRV/r), tipranavir (TPV/r), etravirine (ETV) and raltegravir (RAL) for children are rare, and even more in low and middle-income countries.
Materials & methods: We conducted a retrospective multicenter cohort, that included 16 children with virological failure and triple-class drug resistant viruses (TD-DRM). All patients had protease and retrotranscriptase genotype and they were evaluated using the Stanford HIV database for resistance mutation interpretation. Switch of antiretroviral (ARV) regimen was based on genotyping data and an expert committee defined the better option for a salvage regimen. The primary end point was virologic suppression (<50 copies/mL [<1.6 Log10]) and immunologic improvement at week 48.

Results: A total of 16 children were enrolled. Median age was 14.5 years old (IQR 11-16.5). Baseline median CD4+ cell count was 382 cells/µL (IQR 281-687), and median HIV-1 RNA viral load was 4.2 Log10 (IQR 3.4-4.8). Drugs such as DRV (13/16), TPV (3/16), RAL (13/16) and ETV (3/16) were included in the new regimen. Primary end point was found in 11 children (68.8%), and 13 children (81.3%) had HIV-1 RNA viral load <200 copies/mL [2.3 log10]. Regarding the five patients with virological failure, one had virological rebound and four never achieved HIV-1 RNA <50 copies/mL [1.69 log10], tow of this four had<200 copies/mL. Median (IQR) for baseline CD4+ cells count were 382 cells/mL (281–686 cells/mL), whereas at weeks 48 the values were 640 cells/mL (361–936 cells/mL). (p< 0.001), Weight-for-age and height-for-age z-scores were not modified over the period of the study. Basal hypertriglyceridemia and hypercholesterolemia was present in 20% and 12% respectively and 12.5% (P=0.18) vs. 25% (P=0.5) one year after starting the new regimen.

Conclusions: DRV/r, RAL and ETV are well tolerated in our children population. These drugs provide potentially good options for highly experimented children to antiretroviral therapy. Regimens based on genotyping data were effective for children who have virological failure with multi-drug resistant HIV-1 resistant infection. Long-term follow-up is necessary to warrant the feasibility and sustainability of these regimens.

No conflict of interest

Abstract: 48

Treatment Strategies for HIV/ Hepatitis infected Patients

Therapeutic Drug Monitoring of HCV-Protease Inhibitors to Identify Patients at high risk of Viral Failure and Severe Adverse Reactions


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Background: The protease-inhibitors telaprevir (TPV) and boceprevir (BOC) were introduced in clinical settings in combination with pegylated-interferon-α and ribavirin (RBV) for treatment of HCV infection. Although the triple therapy showed a high sustained virologic response (SVR) rate, these treatment regimens commonly caused side effects and are no longer recommended by European guidelines. Nevertheless, protease inhibitors will continue to form a treatment option in many regions due to the high cost of second generation treatments. In a prospective cohort from Luxembourg we investigated the relationship between treatment response, severe adverse effects and drug concentrations.

Materials & Methods: HCV viral load, plasma and hair concentrations of TPV, BOC and RBV were determined on sequential samples. For drug measurement, 100 plasma samples were collected from 28 patients and 20 hair samples from 6 different patients. Drugs were extracted using methanol, purified using SPE C18 cartridges and then analyzed with liquid chromatography tandem mass spectrometry (LC-MS/MS). In plasma, limits of quantification (LOQ) were 10ng/mL for BOC and RBV and 25ng/mL for TPV. In hair, LOQ were 50pg/mg for BOC and RBV and 62.5pg/mg for TPV. HCV NS3 protease gene population sequencing was performed before treatment initiation and in case of treatment failure.

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Results: Fifteen patients received TPV and 13 received BOC including 4 HIV co-infected patients. Five patients interrupted treatment before 4 weeks of therapy for drug intolerance and 2 patients were lost-to follow-up. Four patients treated with TPV and one treated with BOC did not reach SVR and four acquired drug resistance mutations (V36M, N174S, V36M/Q80K). Overall median concentrations of TPV, BOC and RBV were 5050 ng/mL (IQR 3285-7180 ng/mL), 707 ng/mL (IQR 150-1605 ng/mL) and 1690 ng/mL (IQR 1280-3034 ng/mL), respectively. In patients in virological failure, drug plasma concentrations became undetectable in several samples at least two months before the onset of viral breakthrough strongly indicating non-compliance. Interestingly, two patients exhibiting a high median TPV concentration of 9175 ng/mL (IQR, 4580-9335) and 9510 ng/mL (IQR, 4705-9905) associated with a high median RBV concentration (3590 ng/ml, IQR 1525-3585 and 2554 ng/ml, IQR 805- 3600), had severe anemia and/or cutaneous toxicity, depression, atrial fibrillation (for one patient) but achieved a SVR after only 4 and 9 weeks of treatment. High concentrations of TPV and RBV were detected as soon as two weeks of therapy in these patients. High plasma concentrations of BOC and RBV correlated with high concentrations in the hair segment corresponding to the period of blood sampling suggesting the potential use of this biological matrix for therapeutic drug monitoring.

Conclusions: We reported here a high discontinuation rate of protease inhibitors, mainly due to drug toxicity, impairing the effectiveness of the triple therapy. Importantly, early high plasma concentrations of both TPV and RBV were associated with severe anemia. Therapeutic drug monitoring might be considered to predict viral failure and severe adverse reactions, at a much lower cost as compared to the triple therapy. In addition, we showed that hair might also represent a convenient matrix to determine drug compliance.

No conflict of interest

Abstract: 49
Treatment Strategies for HIV/ Hepatitis infected Patients

Genotype 3 HCV infection, lower CD4 cell count and higher liver stiffness are related with bone mineral reduction in HIV/HCV co-infected patients (MASTER cohort)

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Background: In HIV/HCV co-infected patients (pts), the bone mineral density (BMD) abnormality and risk of fracture are higher than in HIV or HCV mono-infected subjects (O’Neill JD et al.PlosOne,2104). To study the variables related with low BMD in HIV/HCV co-infected pts, a retrospective analysis of MASTER cohort was implemented

Materials & Methods: All sequential HIV and HCV Ab+ patients, enrolled in the observational Italian MASTER database, who performed at least one DEXA scan between 2010 and 2013, based on national and international recommendation, were enrolled.Baseline was defined when DEXA scan was performed. Socio-demographics variables, clinic and laboratory data in pts. with abnormal DEXA scan (T score < -1) or with normal DEXA scan are compared and analyzed. Osteopenia and osteoporosis were defined following the WHO classification (http://www.whqlibdoc.who.int/trs/WHO_TRS_921.pdf).
Elastometry was used to define liver fibrosis. Multivariate statistic analysis was performed to detect variables related with abnormal DEXA scan. Analysis was adjusted for the following covariates: age, sex, HCV genotype, presence of SVR after peg-IFN plus ribavirin, liver fibrosis stage, CD4 cell count, HIV viral load, time to HCV exposure, tenofovir use, use of boosted protease inhibitor (PI), calcium, phosphate and 25OH vitamin D plasma level, presence of proteinuria, menopause in women.

Results: The number of HIV/HCV co-infected pts enrolled was 86. In 34/86 (39,5%) the DEXA scan was abnormal: in 27/34 (79,4%) osteopenia was detected and in 7/34 (20,6%) osteoporosis was diagnosed. The median age was 53 and 54 years (IQR 51-54 in both groups) in pts with abnormal or normal examination respectively; 82 pts. were on cART. All subjects assumed tenofovir and 68% a boosted protease inhibitor. HIV-RNA was undetectable in 93%. CD4+ cell count tended to be lower when DEXA was abnormal (433 cell/mm$^3$ vs 514 cell/mm$^3$ p0.07). The exposure to HCV infection was 9.5 years (IQR 7-23) in pts with abnormal and 13 years (10-14) in pts with normal DEXA (p 0.02). Presence of SVR and menopause, among women, were not related with abnormal or normal DEXA. At multivariate analysis, variables related with abnormal DEXA were HCV genotype 3 infection [AOR3,3(1,0-10,9)0,04]; T CD4+ cell count < 500 cell/mm3 [AOR 3.6 (95%CI1,1-11,7)0,03] and a liver stiffness > 7,5Kpa[AOR 3,5(1,0-11,6)0,03]

Conclusion: In HIV/HCV co-infected pts, included in the MASTER cohort, who performed a DEXA scan, following the international and national guidelines, 40% had an abnormal examination. Genotype 3, lower CD4+ cell count and higher liver stiffness were related with abnormal DEXA despite having achieved SVR

No conflict of interest

Abstract: 50

Treatment Strategies for HIV/ Hepatitis infected Patients

Atazanavir/rit(atz/r), unboosted atazanavir (atz) or darunavir/rit(drv/r): metabolic safety and liver fibrosis in hiv/hcv coinfected patients (master cohort)

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Background: hyperglycaemia and diabetes are related with liver fibrosis progression and hccin hcv mono and hiv co-infected patients (pts). atz and drv based treatment seem to have less metabolic toxicity. A retrospective analysis of master cohort has be done to compare the safety and the impact on liver fibrosis of cART based on atz/r, unboosted atz and drv/r in HIV/HCV pts.

Materials & Methods: HIV/HCV Ab+ patients starting for the first time ATZ/r 300/100mg QD (group 1) or unboosted ATZ 200 mg BID (group 2), or DRV/r 800/100 mg QD or 600/100 BID (group3), enrolled in the observational Italian MASTER database, were included. Socio demographic variables and clinical and
laboratory data were collected during follow up. Liver fibrosis has estimated using FIB-4 formula. The mean difference between the final and the initial value of each variable along the therapeutic cycle was submitted to ANOVA using the therapeutic scheme as explanatory variable. The HIV-RNA was log_{10}-transformed, to ameliorate its distribution. The variables changes under the treatment were tested adjusting for confounding variables (baseline HIV-RNA, CD4 cell count, sex and age) using the AIPW estimator ‘augmented inverse-probability weighting’, probit model.

**Results:** Six hundred-eighty-nine pts were included: 466 in group 1, 89 in group 2 and 134 in group 3. The median years of follow up were 2.07 (1.0;3.7) in group 1, 2.8 (1.6;4.2) in group 2 and 1.7(0.1;2.5) in group 3. Baseline characteristics were similar in 3 groups; but LDL cholesterol plasma level (ANOVA p 0.023), HIV-RNA (p 0.006) and T CD4 cell count (p 0.002) differ among groups. ANOVA on the variation between final and initial value showed that triglycerides decreased more in group 1 [-15.4 mg/dl (-25.44;-5.38)p0.03] and 2 [-25.9 mg/dl (-48.8;-3.06)p0.02]; glycaemia [-4.15 mg/dl (-8.32;0.03)p0.05] and GGT [-3.3 mg/dl (IC95% -69.3;-5.32)p0.022] decreased significantly in group 2. The difference between paired treatments (1vs2; 3vs2; 3vs1) revealed that glycaemia was significantly increased with DRV/r vs unboosted ATZ [5.9(0.46;11.34)p0.03] and reduced with unboosted ATZ vs ATZ/r [-5.10 mg/dl (-9.59;-0.42)p0.03]. A mild increase in CD4 cell count (+47 cell/mm3) is evident with ATZ/r vs unboosted ATZ (p0.04). HIV-RNA decreased more in pts treated with ATZ/r or DRV/r vs unboosted ATZ (p0.01). Fib 4 lowered during treatment with ATZ/r [-0.34(-0.6;-0.03)p0.03]

**Conclusions:** In the real clinical setting DRV/r and ATZ based treatment are the most used among HIV/HCV co-infected pts. Boosted or unboosted ATZ based regimens could have more metabolic advantages and liver fibrosis seems to be reduced on unboosted ATZ. A stronger viral control is provided by boosted protease inhibitors than unboosted ATZ based regimen.

No conflict of interest

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**Abstract:** 51

**Treatment Strategies for HIV/ Hepatitis infected Patients**

**Comparison of efficacy among different antiretroviral drugs: an in vitro model of HIV infection with high and low viral load**

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**Background:** A consistent proportion of HIV-1 infected patients (about 53%) show a high viremia (>100,000 copies/ml) before starting first-line therapy. In particular, 15% of these patients have a viremia >500,000 copies/ml. We have recently shown that high pre-HAART viremia remains an independent factor associated with delayed and decreased virological success (Santoro et al Antiviral Therapy 2013). Little information regarding in vitro infection experiments simulating high level viremia condition is available. Therefore, the goal of this study was to evaluate the different responses to antiretroviral drugs in presence of standard and high viral input in an in vitro model of HIV infection of human primary macrophages (MDM) and T cells.

**Materials & Methods:** Primary MDM and C8166 T cells were infected by using a standard viral input (10^6pg/ml of HIV-1 gag-p24, corresponding to 3x10^6copies/ml HIV RNA; LVL) and a high viral input (10^9pg/ml of HIV-1 gag-p24, corresponding to 3x10^5copies/ml HIV RNA; HVL). Cells were treated with 5 different drugs including Darunavir [DRV], Efavirenz [EFV], Tenofovir [TDF], Maraviroc [MVC], Bicyclam [AMD3100] and Raltegravir [RAL] at 5 different concentrations (1,000nM, 10nM, 1nM, 0.1nM and 0.01nM). Experiments were performed at least in triplicate. Viral replication was measured as p24 release in supernatants at 7 and 14 days post infection for C8166 T cells (infected with pNL4-3, X4-virus) and for MDM (infected with p81A, R5-virus), respectively.

**Results:** In MDM culture, in absence of drugs, no statistical difference of viral replication was
observed between HVL and LVL input (HIV-1 p24 production mean±SD=814±278ng/ml versus 66±45ng/ml, p=ns). Notably, TDF showed the highest antiviral efficacy at both viral concentrations, even if a better response was found at low levels (LVL IC₅₀ =0.02nM±0.001 versus HVL IC₅₀=0.2nM±0.03 and LVL IC₉₀=0.5nM±0.1 versus HVL IC₉₀ =1.3nM±0.8). Both EFV and RAL exhibited a similar antiviral efficacy at LVL and HVL, without significant changes in IC₅₀ and IC₉₀ values (EFV: LVL IC₅₀=0.3nM±0.02, versus HVL IC₅₀ =0.3nM±0.2; LVL IC₉₀=2.4nM±0.4 versus HVL IC₉₀=2.2nM±1.8; RAL: LVL IC₅₀=0.1nM ±0.01 versus HVL IC₅₀=0.3nM±0.1; LVL IC₉₀ =2.4nM±0.7 versus HVL IC₉₀=2.3nM±0.1). The different initial viral input slightly affected the efficacy of entry-inhibitors (MVC: LVL IC₅₀ =0.7nM±0.01 versus HVL IC₅₀=2.3nM±1.3; LVL IC₉₀=3.5nM±0.1 versus HVL IC₉₀ =7.5nM±0.8). In contrast, by analyzing the efficacy of protease inhibitors, a remarkable change between LVL and HVL was observed particularly in the case of DRV (LVL IC₅₀=0.1nM±0.01 versus HVL IC₅₀ =0.9nM±0.1; LVL IC₉₀=3.5nM±1.3 versus HVL IC₉₀=224.4nM±136.4). In C8166 T cells, a statistical significant higher replication rate in HVL versus LVL input was observed (HIV-1 p24 production mean±SD=104,060±84,000ng/ml versus 223±118ng/ml, p=0.05). However, all drugs exhibited a similar antiviral efficacy at both viral inputs (data not shown), with the exception of DRV, in which the IC₉₀ value, as observed in primary MDM cell cultures, showed a high slope (>20 fold) between LVL and HVL (LVL IC₅₀=2.1±0.1nM versus HVL IC₅₀=9.1 ±1.5nM and LVL IC₉₀=1.1nM±0.1 versus HVL IC₉₀=230.1nM ±21.5).

Conclusions: Taken together, our in vitro results demonstrate an excellent efficacy for TDF, EFV and RAL also in presence of high viral load, in both human primary macrophages and T cells. The different dose response observed mainly with DRV in presence of high viremia condition is an interesting observation that needs further investigations regarding also other protease inhibitors.

No conflict of interest
control-arm (n=20) did not differ for the main characteristics except that study subjects significantly exceeded the controls for duration of HIV infection (median 17.7 yrs [IQR 14.9-22.7] vs 10.9 yrs [IQR 8.2-18.1], p=0.002), time on antiretroviral therapy (median 15.3 yrs [IQR 11.8-16.8] vs 10.3 yrs [IQR 7.5-15.9], p=0.042), proportion on PI-based therapy (66.7% vs 35.0%, p=0.042) and CDC stage C (33.3% vs 5%, p=0.026). At baseline the study arm showed markedly higher levels of HIV-DNA (median 440 copies/10^6 PBMCs [IQR 163-923]) compared to the control arm (median 125 copies/10^6 PBMCs IQR 57-218) (p=0.002). Noteworthy, at T48 no significant change occurred in HIV-DNA copies within both arms, whereas significant discrepancy between groups was maintained. No significant difference in HIV-1 RNA was observed during the study, albeit a virological failure was experienced by only one patient from both arms. By a linear regression analysis, duration of antiretroviral therapy (p=0.032), PI-based therapy (p=0.028) and CDC stage C (p<0.018) were independently associated with higher levels of proviral DNA at baseline. Decline of HIV-DNA values was inversely related to baseline HIV-DNA (p<0.025).

**Conclusion:** Despite a larger size of HIV reservoir observed in the study arm, due to both longer treatment history and type of therapy at baseline, the DRV/r+MVC dual regimen showed similar efficacy to standard triple therapy in terms of maintaining control on residual replication and on HIV provirus levels.

No conflict of interest

**Abstract: 53**

*Treatment Strategies for HIV/ Hepatitis infected Patients*

**Decrease of HIV-dna after simplification to atazanavir/ritonavir + lamivudine in virologically suppressed HIV-infected patients**

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**Background:** Preliminary 24-week results of the ATLAS trial suggest non-inferior efficacy of treatment simplification to a dual regimen with atazanavir/ritonavir (ATV/r) plus lamivudine (3TC) versus maintaining standard 3-drug ATV/r-based regimen in virologically-suppressed patients. In the setting of ATLAS-M, this substudy was designed to evaluate the impact of the treatment simplification strategy on HIV-DNA reservoir.

**Materials & Methods:** HIV-positive subjects were randomized to maintain ongoing triple therapy (ATV/r + 2NRTIs) (control arm) or switch to a dual regimen combining ATV/r+3TC (300/100/300mg once daily) (study arm). Eligibility criteria of the study were CD4 count >200 cell/μl, HIV1-RNA<50 copies/ml >3 months, no documented resistance mutation to 3TC, no previous virological failure. Total HIV-DNA copies were quantified in PBMCs by real-time polymerase chain reaction using 5’ nuclease assay targeting the long terminal repeat (LTR) region. The primary objective was to explore the evolution of total HIV-DNA level between baseline and 48 weeks (T48); the association between baseline proviral load and demographical, clinical or viro-immunological parameters was also evaluated. HIV-DNA copies changes from baseline were analyzed using Wilcoxon signed-rank test; nonparametric Mann-Whitney test was used to compare median between arms at two different time points.

**Results:** A total of 81 patients were recruited: 62 (76.5 %) males, 75 (92.5%) Caucasian, 28 (34.5%) heterosexuals, median age 43.9 years (IQR 37.1-50.1), CD4 count 646 cell/μl (IQR 533-761). Subjects randomized in study-arm (n=41) and control-arm (n=40) were well balanced for the main characteristics, albeit they significantly differed for nadir CD4 cell count (median 163 cell/μl [IQR 62-293] in study arm vs 300 cell/μl [IQR 202-355] in controls;
p=0.008). At baseline marked discrepancy was found in the median HIV-DNA levels: 406 copies/10^6 PBMCs (IQR 273-641) in the control arm vs 1043 copies/10^6 PBMCs (IQR 425-1522) in the study arm (p=0.003). At T48, patients in the control arm showed no difference in HIV-DNA copies versus baseline, conversely subjects in the dual therapy arm showed a significant reduction (-175.6 copies/10^6 PBMCs; p=0.003), with a simultaneous marked CD4 increase (73.7 cell/ml p<0.001). Most of the patients maintained undetectable viral load, albeit two failures and one blip occurred in the control arm and two study subjects experienced a single virological blip. By a linear regression analysis, previous blips (within 3 years from T0) (429.1 CI95% 53.5-804.6; p=0.026) were independently associated with higher levels of baseline HIV-DNA while higher nadir CD4 cell count (-1.3 CI95% -2.4- -0.2; p=0.025) was associated with lower values. Decline of HIV-DNA values was inversely related to baseline HIV-DNA (p<0.001).

**Conclusion:** when compared to continuing standard 3 drug ATV/r-based cART, simplification to ATV/r+3TC resulted in higher decline of HIV proviral level, with a favorable immune profile in terms of CD4 recovery. These data support the safety of this treatment simplification strategy on HIV reservoirs.

No conflict of interest

**Abstract: 54**

**Novel Diagnostic Technologies & Approaches**

**Evaluation of 4 virological tests using DBS for HIV-1 early infant diagnosis: interpretation of discrepant results**

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**Background:** An early diagnosis of the HIV-1 infection in infants born to infected mothers is critical for an early initiation of antiretroviral therapy and to reduce HIV-related mortality. Since HIV-1 antibodies from mother transferred across the placenta may persist in the child up to 18 months, it is required to detect HIV-1 genome (proviral DNA and/or viral RNA) or viral antigens for HIV-1 infant diagnosis. WHO recommends a second virological test on a separate specimen to confirm a positive result. We compared the sensitivity and specificity of four virological commercial assays (VCAs) for HIV-1 genome detection in dried blood specimens (DBS) from HIV-1-exposed infants.

**Materials & Methods:** Sixty eight infants born to HIV-1 infected mothers from Equatorial Guinea were selected. DBS were collected from November 2012 to December 2013 after spotting two drops of blood from each infant heel-prick onto each dot on a Whatman™ 903 Card by trained personnel and stored at -80ºC until use. Four VCAs were performed using one dot for each DBS following manufacturer’s instructions, determining their sensitivity and specificity. Two were quantitative viraemia assays: Siemens VERSANT HIV-1 RNA 1.0 kPCR assay (kPCR) and Roche CAP/CTM Quantitative test v2.0 (Roche-VL-v2). The others tests were qualitative: CAP/CTM Qualitative Tests v1.0 (Roche-dx-v1) and v2.0 (Roche-dx-v2). The limit of HIV-1 detection in DBS ranged from 300 to 1,090 HIV-1 RNA copies/ml, detecting viral integrase (kPCR), Gag (Roche-dx-v1) or Gag+LTR (Roche-VL and Roche-dx-v2). Longitudinal DBS were collected in some infants to confirm positive molecular results and the seroreversion of antibodies to HIV-1 using at least 2 serological tests (ELISA, Abbott; Geenius™ HIV ½ Confirmatory Assay and Western Blot, BioRad).

**Results:** The mean age at first DBS collection was 2.4 months (range 1.2-4.9), 53% were female, 78% were born by vaginal delivery and 98.5% were not breastfed. Two HIV-1 infected infants (2.9%) were detected by the four VCAs in first and confirmatory DBS. HIV-1 was not...
detected in 49 (72%) children by any virological assay. We observed discrepant results between VCAs in the first DBS in 17 (25.1%) infants, detecting HIV-1 by some assays but not for others. HIV-1 infection was excluded in 12 of 17 cases using serological and virological testing in additional DBS collected when infants were from 5 to 14 months of age. We observed false positive HIV-1 diagnosis in 9 (13.2%), 8 (11.8%), 2 (3.6%) infants using dx-v1, VL-v2 and dx-v2 Roche assays, respectively, but none using kPCR. No false negative results were found by any technique. Thus, although the 4 assays presented 100% sensitivity, only kPCR showed 100% specificity, followed by Roche-dx-v2 (96.2%), Roche-VL-v2 (87.9%) and Roche-dx-v1 (86.4%).

Conclusions: VCAs using DBS were useful for early infant HIV-1 diagnosis in settings with low HIV-1 mother-to-child transmission rates. More efforts are required to increase specificity of VCAs. We found a significant proportion of false positive results that might result in wrong diagnosis and unneeded treatment. We propose the use of a second different virological assay in DBS when first result is positive, especially for those with lower viral loads.


No conflict of interest

Abstract: 55

Evaluation of the new Aptima® HIV-1 Quant Dx assay for detection and quantification of HIV-1 RNA: a comparison with the Abbott RealTime HIV-1 assay.

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Background: The Aptima® HIV-1 Quant Dx assay (Hologic, Inc.) is a new method for detection and quantification of HIV-1 RNA in plasma samples from HIV-1 infected individuals. It is based on real-time Transcription-Mediated Amplification technology targeting two regions (pol and 5'LTR) of the HIV-1 genome. The performance of this assay was compared with the Abbott RealTime HIV-1, here used as reference procedure.

Materials & Methods: The assays were used according to manufacturer instructions. The evaluation was performed using 220 clinical plasma samples (with viral load range spanning from 'not-detected' to >1x10^6 cp/ml HIV-1 RNA), the WHO 3rd HIV-1 International Standard (WHO-IS; NIBSC code: 10/152), the WHO 2nd International Reference Panel Preparation for HIV-1 Subtypes for NAT (WHO-ST; NIBSC code: 12/224) and the QCMD HIV-1 RNA EQA panel (QCMD). Clinical samples were residual specimens obtained from HIV-1 infected individuals attending the out-patient care facility of the INMI for routine monitoring of HIV-1 viremia. Specimens were stored at –80° C and analysed with both assays in the same working day. All VL data were analysed as log_{10}-transformed values. Concordance on qualitative results between the Aptima and the reference assays was established by Cohen's kappa statistic. The correlation between quantitative results was evaluated by using linear regression analysis and Bland-Altman plot. Accuracy and reproducibility of quantification was evaluated using the WHO-IS. The ability to measure HIV-1 subtypes was assessed on the WHO-ST and QCMD panels.

Results: With clinical samples, agreement between two assays for qualitative results was high (91.8%) with a Cohen’s kappa statistic equal to 0.836. In comparison of samples with quantitative results in both assays (n=93), Pearson r correlation coefficient was 0.9868 (p<0.0001). Mean differences of measurement between assays, conducted according to Bland-Altman method, was low (0.11 log_{10} cp/ml). The WHO-IS (diluted from 2000 cp/ml to 15 cp/ml with HIV-1 negative human plasma) was quantified by Aptima assay at expected values, and showed excellent linearity (R^2 = 0.957) within this range. Reproducibility was very high, even at HIV RNA values in the lower part of the dynamic range. The Aptima assay was able to accurately quantify all the main HIV-1 subtypes.
in both the WHO-ST and QCMD reference panels.

Conclusions: A remarkable characteristic of the new assay is the elevated accuracy and reproducibility, even at low HIV-1 RNA values. Along with excellent performance, the automation and improved workflow of the Aptima assay on the Panther system make it a good choice for routine monitoring of HIV-1 viral load. The performance of the recently CE-IVD approved Aptima® HIV-1 Quant Dx assay can be considered equivalent to those of the reference diagnostic system.

No conflict of interest

Abstract: 56

Novel Diagnostic Technologies & Approaches

Comparison of the Aptima HCV Quant Dx Assay to the COBAS AmpliPrep/COBAS Taqman HCV Test v2.0 and the Abbott RealTime HCV Assay

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Background: The Aptima HCV Quant Dx Assay is a fully automated quantitative assay being developed on the Panther® system, and is based on real-time Transcription-Mediated Amplification (TMA) technology. This assay is intended for detecting and monitoring HCV in plasma and serum specimens.

Materials & Methods: A cohort of 490 clinical specimens from University of Athens Medical School was tested using the Aptima HCV Quant Dx Assay (Aptima assay), the COBAS AmpliPrep/COBAS TaqMan HCV Test, v2.0 (Roche assay) and the Abbott RealTime HCV Assay (Abbott assay). The specimens included genotypes 1, 2, 3 and 4 and ranged in concentration from 16 IU/mL (1.20 log IU/mL) to 26.4 million IU/mL (7.42 log IU/mL) as measured by the Roche assay. Analytical sensitivity of the Aptima assay was assessed using dilutions of the 2nd WHO Standard (NIBSC 96/798) run in replicates of at least 100 per dilution on multiple Panther systems. Linearity of the Aptima assay was tested by dilution HCV 1a armored RNA from 600,000,000 to 10 IU/mL and testing in replicates of 5 on a single Panther system.

Results: For the purposes of the clinical specimen correlation to the Roche assay, a lower limit of quantitation for the Aptima assay of 15 IU/mL (1.18 log IU/mL) was used. Four hundred and twenty-four specimens gave results quantifiable for both Aptima and Roche assays. The slope was 1.07 (Aptima vs. Roche) with an intercept of -0.35 and an R² of 0.97. For the purposes of the clinical specimen correlation to the Abbott assay, a lower limit of quantitation for the Aptima assay of 12 IU/mL (1.08 log IU/mL) was used. Due to specimen volume constraints only 412 specimens could be tested using the Abbott assay from which 409 gave results above 12 IU/mL (1.08 log IU/mL). The slope was 1.05 (Aptima vs. Abbott) with an intercept of 0.07 and an R² of 0.97.

The analytical sensitivity (limit of detection) of the Aptima assay as measured by dilutions of the 2nd WHO standard was 3.4 IU/mL for Plasma and 3.6 IU/mL for serum using probit analysis and a 95% positivity rate. The Aptima assay was shown to be linear over the range of 10 to 600,000,000 IU/mL.

Conclusion: The Aptima HCV Quant Dx Assay gave comparable viral load results when compared to the Roche COBAS AmpliPrep/COBAS TaqMan HCV Test, v2.0 and Abbott RealTime HCV Assay. The performance of the Aptima assay makes it an excellent candidate for the detection and monitoring of HCV.

No conflict of interest
Abstract: 57

Novel Diagnostic Technologies & Approaches

Serum prolidase activity as an indicator of inflammation in patients with chronic Hepatitis B

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Background: Hepatitis B virus (HBV) is a virus including DNA from Hepadnaviridae family. In the world there are approximately 400 million people that carry chronic HBV inflammation. HBV virus causes chronic liver disease, liver cirrhosis, and liver cancer. All these processes develop with common inflammation in liver. Prolidase hydrolyzes peptide bonds of immmiditeripeptides (X-Pro, X-Hydroxyprolin), immidodipeptides including carboxyl terminal position and hydroxyprolin. Prolidase is one of the manganese-dependent metalloproteinase. The level of prolidase is increasing with some disorders as melanoma in breast and lung cancer and peptic ulcer. In chronic hepatitis inflammation, fibrosis occurs due to necroinflammation and ongoing replications in liver cell. In the chronic inflammatory events, the activation of serum prolidase increases due to rising collagen synthesis. In this study, the diagnostic value of serum prolidase enzyme activity (SPEA) in chronic hepatitis B cases was aimed to evaluate.

Materials & Methods: In this study, 30 patients with chronic HBV inflammation who apply to Infectious Disease Clinic in Sakarya University Training and Research Hospital as a chronic HBV positive group and 30 people who are non-chronic hepatitis and are matched as age and sex are taken. Liver enzymes and parameter of inflammation are examined in patients' and controls' serum. Serum biochemical tests were studied using prepared commercial kits with the help of auto analyzer. In the statistical analysis, SPSS 20.0 software was used. p<0.05 was considered statistically significant.

Results: In this study, 60 cases including 30 patients with HBV and 30 controls were enrolled. In the study, Ages of subjects whit HBV+ and control groups are statistically stable (respectively, 38.53 ± 8.99 and 35.80 ± 9.31). AST (U/L),ALT (U/L),ALP (U/L),GGT (U/L),Total Bilirubin(mg/dl),PLT (unit/ml) and SPEA(U/L) levels in subjects whit HBV+ and control groups were found as 27,53±13,40 - 26,03±12,12, 38,10±38,68 - 23,27±11,75, 71,40±19,80 - 48,73±25,98, 27,57±20,07 - 30,20±19,18, 0,67±0,28 - 0,51±0,47, 213,20±68,13 - 269,20±73,52, 1263,80±151,26 - 784,60±144,88 (respectively subjects whit HBV+ - control). When viewed to these parameters, PLT level in HPV + patients reduced significantly according to control (p=0.003). But SPEA level in HPV + patients increased compared to controls (p=0.0001). SPEA levels were found significantly higher in ALT / AST ratio >1 compared to ALT / AST ratio <1 (p=0.0001), SPEA reduced in results of platelets >150.000 compared to platelets<150.000 (p=0.0001). SPEA levels were found higher in results AST>50 and ALT>50 compared to AST<50 and ALT<50 (respectively p=0.017 and 0.0001). The correlation between HBV-DNA level and SPEA levels were not found in chronic hepatitis cases (p=0.536).

Conclusions: SPEA level was significantly higher in the study group compared to the control group. SPEA levels of people with high ALT and AST levels in chronic HBV patients are also determined significantly higher. SPEA level was significantly higher in patients with thrombocytopenia. As a result, SPEA parameter may be an indicator of inflammation for patients with HBV.

No conflict of interest

Abstract: 58

Novel Diagnostic Technologies & Approaches

Longitudinal trends of recent HIV-1 infections in Slovenia (1986-2012) determined using baseline CD4 and HIV viral load measurements and THE BED assay
Background: Resolving dilemma whether the rise in the number of HIV diagnoses represents an actual increase in HIV transmissions or is a result of improved HIV surveillance is crucial before implementing national HIV prevention strategies.

Materials & Methods: Annual proportions of recent infections (RI) among newly diagnosed HIV-1 infected individuals in Slovenia during 27 years (1986-2012) were determined using an algorithm consisting of routine baseline CD4 and HIV viral load measurements and the Aware BED EIA HIV-1 Incidence Test (BED test). From the first confirmed HIV diagnosis in 1986 until the end of 2012, a total of 584 individuals were reported as persons infected with HIV in Slovenia. For the purpose of this study, nine children born to HIV-positive mothers were excluded. No CD4 baseline result and/or HIV viral load baseline results and/or baseline plasma samples for BED testing were available for 159 patients, making a total of 416 patients (71.2%) diagnosed in the 27-year period eligible for this study.

Results: Out of 416 patients, 170 (40.9%) had a baseline CD4 cell count less than 200 cells/mm$^3$ and/or HIV-1 viral load less than 400 copies/ml and were characterized as having long-standing infection (LSI) without further BED testing. The remaining 246 patients were tested additionally using the BED test. Overall, 23% (97/416) of the patients were labeled RI and 77% (319/416) LSI. The characteristics significantly associated with RI were: younger age at time of diagnosis (p=0.0004), acute retroviral syndrome (p<0.0001), CDC class A (p<0.0001), CDC class other than C (p<0.0001), no AIDS defining illnesses (p<0.0001), HIV test performed in the past (p=0.0008), a higher viral load (p=0.0108) and a higher CD4 cell count (p<0.0001). An interesting trend in the proportion of RI was observed over the years, with a peak in 2005 (47% of RI) and the lowest point in the 2008 (12%) in parallel with a rise in the numbers of new HIV diagnoses.

Conclusions: In this longitudinal study across almost three decades, in which by far the highest coverage of individuals diagnosed with HIV during an entire duration of an HIV epidemic in a given country/region was studied (71%), some interesting time trends in the annual proportions of RI among newly diagnosed HIV-1 infected individuals were identified and significant characteristics associated with RI determined, mostly in connection with earlier linkage to the care of individuals defined as RI. This study could help promote the idea of introducing organized regular periodic (e.g., annual) HIV incidence monitoring in Central/Eastern Europe using a reliable, simple and affordable test algorithm consisting of routine baseline CD4 and HIV viral load measurements and additional BED testing in the subset of newly diagnosed patients.

Abstract: 59

Novel Diagnostic Technologies & Approaches

The use of phylogenetic analysis in routine laboratory diagnostics

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Background: The ease of use of existing phylogenetic tools allows not only for sophisticated epidemiological queries but also for incorporating it into everyday laboratory practice. Here we present potential applications of phylogenetic analysis i.a. in quality control and assurance, implemented in our Laboratory.

Materials & methods: The sequencing of HIV, HBV and HCV for routine drug resistance testing was performed on Abi Prism 3100-Avant or 3130 Genetic Analyzer using either commercially available assays (HIV) or in-house tests (HBV and HCV). All obtained sequences were verified and, if needed, manually trimmed and corrected. Multiple sequence alignment, model testing for best fitting nucleotide substitution model and the phylogeny reconstruction was done using freely

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accessible MEGA6 software. The model with lowest BIC score among tested nucleotide substitution models was chosen for analysis. Maximum likelihood tree was constructed and tested by bootstrap method with 200 replicates.

Results: Phylogenetic analysis was used for HIV for subtype and risk groups analysis, quality assurance and searching for genetic correlations among tested samples. In case of HBV analysis we detected and confirmed presence and increase of prevalence of rare in Poland genotype H1. In case of HCV phylogenetic analysis was used for contamination control, searching for new subpopulations selected during long-time infection and for nosocomial infection verification.

Conclusions: Like in any assays based on PCR also in virus sequencing tests contamination with product of previous reactions or cross contamination between the samples can be an issue and checking for it should be mandatory. Running negative control and the use of uracil N-glycosylase system may not be sufficient. After each batch of analyzed samples a phylogenetic analysis was performed. Sequences clustering together with very little or no genetic distance between them should be further looked into. This result is expected for mother and child or couples in which one of the partners was the source of infection for the second one. If above mentioned situations could be excluded the samples should be rerun beginning from the extraction step, ideally using another aliquot of material. If a patient in the batch of samples was earlier tested for drug resistance and the sequence is accessible it should be included in the analysis. The sequences from one person should cluster together, even if drug resistance mutations emerged or disappeared. Lack of clustering may suggest either superinfection or mix up of the samples. Clustering of multiple sequences can also be observed in case of several infections from one source for example HCV infection of patients undergoing chronic dialysis. Adding viral reference sequences with known subtypes to post-run phylogenetic analysis allows the identification of subtype or genotype (or confirmation of previous results if any other web based tools were used).

Concluding, in modern molecular diagnostics the result obtained without proper controls is valueless. Due to high cost and time consumption of assays based on sequencing we suggest that additional post run phylogenetic analysis should be considered. It is a quick, easy and reliable method for quality assurance of routine diagnostics.

No conflict of interest
Additionally, correlations between the clinical parameters were analysed using the 2-sided Pearson analysis.

**Results:** In 30 of 58 patients the proviral load significantly increased \((p=0.0001)\), whereas 28 patients showed a significant decrease \((p=0.005)\) after 24 weeks. 28 patients displayed a significant increase in PBMCs \((p=0.0003)\), while there was a significant decrease in the number of PBMCs in 30 patients \((p<0.0001)\). Overall, CD4+ T cell counts were stable in all patients. A significant correlation of proviral load and PBMCs for data collected at the first \((p<0.0001)\) and the second time point \((p=0.0001)\) could be detected, while no relation was found between proviral load and CD4+ T cell counts. In general, patients with decreasing proviral DNA load had a longer time period since infection \((p=0.059)\) and under treatment \((p=0.135)\) than patients with an increase in DNA. With regard to the treatment history patients showing a decrease in proviral DNA load within the 24-week period of record had a higher number of ART regimens compared to patients with increasing proviral DNA load.

**Conclusions:** HIV-1 proviral DNA load seems to be an interesting marker in patients with undetectable VL and allows the assessment of replication under ART. Nevertheless, no correlation could be found with CD4 cell counts/µL, but with the number of the whole white blood cells/µL. Therefore, our data show that the presence of viral reservoirs in other cell types and not only CD4+ cells is most probable and white blood cells need to be investigated separately.

*No conflict of interest*

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**Abstract:** 61

**Novel Diagnostic Technologies & Approaches**

**Evaluation of RealTime HIV-1, Xpert HIV-1 and Aptima HIV-1 Quant Dx assays in comparison to the Nuclisens EasyQ v2.0 HIV-1 assay for quantification of HIV-1 viral load**

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**Background:** HIV-1 RNA levels measurement is an integral part of the carriers' management worldwide both before and during antiretroviral therapy. Measurements of HIV-1 viral load are expected to correctly assess the HIV copy numbers for all common HIV-1 subtypes, to be reproducible and comparable. Different commercial assays are already in the market new tests have recently been introduced. Here we have compared the performance of the bioMérieux Nuclisens v2.0 assay to the Abbott RealTime HIV-1 assay and to the newly developed Aptima HIV-1 Quant DX (Hologic) and the Xpert HIV-1 assay (Cepheid).

**Materials & Methods:** 404 plasma samples separated from whole-blood EDTA were frozen in aliquots in volumes required for the different assays. Nuclisens v2.0 viral load results and, if available, HIV-1 subtype (which was determined by sequencing the pol gene), defined sample selection. Viral load measurements were carried out according to each manufacturer's instructions. A cutoff of 1.59 log cp/ml was used in all assays to separate quantified from non-quantified results. Linear regression and Bland–Altman analysis were used to determine the relationship and the agreement between the assays.

**Results:** All 404 samples were tested by NucliSens v2.0 (72 subtype A/AE, 156 subtype B and 71 subtype C) of which 273 were tested by RealTime, 298 by Aptima and 255 by Xpert. Differences in viral load of more than 0.5 log cp/ml were observed in 33% (50/172) of samples with RealTime, 34.8% (48/138) with Xpert and 44.9% (75/167) with Aptima versus Nuclisens. The overall agreement between the assays varied between 90.9% (Xpert versus Aptima, best agreement >95% for subtypes B), 89.8% (RealTime versus Aptima, best agreement > 95% for subtype A/AE),91.5% (Nuclisens versus RealTime, best agreement >92% for subtype A/AE), 88.2% (RealTime versus Xpert, best agreement >94% for subtype B) , 85.1% (Nuclisens versus Xpert, best agreement >85% for subtype C),and 84.6% (Nuclisens versus Aptima, best agreement
Abstract

>87% subtype A/AE and C). Compared to Nuclisens, the correlation coefficient (Pearson’s R value) of RealTime, Xpert and Aptima was 0.91, 0.89 and 0.88, respectively. Correlation coefficients of both Aptima and Xpert were 0.98 compared to RealTime.

**Conclusions:** Each of the four viral load assays is characterized by unique technology and specific assay design. The degree of automation, physical size of equipment, turnaround time as well as the reported lower limits of quantitation and detection are different between these assays. While Nuclisens and the RealTime assays are in the market for several years, the Aptima and the Xpert HIV assays have recently been introduced. Here, Nuclisens best correlated to RealTime and also was in the highest degree of agreement with the RealTime assay, while the highest degree of agreement between any two assays was between Xpert and Aptima and both best correlated with RealTime. Although the correlation in samples with various subtypes was not identical between the different assays HIV-1 subtypes in Israel were quantified by all assays. Selection of an optimal assay should be based on quality of results, convenience and cost.

No conflict of interest

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**Abstract: 62**

**Novel Diagnostic Technologies & Approaches**

**An Allele-Specific RealTime PCR assay for the detection of Q80K polymorphism in NS3 protease of HCV genotype 1a.**

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**Background:** There is a need to test for Q80K NS3 protease polymorphism prior to Simeprevir-PegRiba regimens administration in patients infected by HCV genotype 1a. Currently, direct Sanger sequencing of NS3 protease is used to detect Q80K. In our study we have designed an Allele-Specific Real Time PCR assay for the detection of Q80K and compared it with Sanger sequencing.

**Materials & Methods:** We have conducted an observational, ambispective study of all genotype 1a samples from patients attended at Complejo Hospitalario Universitario Granada, Spain, from 2011 to 2015. These samples were used as training set. As a validation set, we have used a blinded panel of samples previously sequenced at the University of Cologne. HCV NS3 viral protease (codons 10 to 181) were Sanger sequenced and analysed using geno2pheno[ HCV] (http://hcv.bioinf.mpi-inf.mpg.de). Q80K polymorphism was also investigated by a homemade allele-specific real time PCR protocol. Briefly, the RT-PCR product of NS3 viral amplification was 1/25 diluted and subjected to AS-PCR reactions aiming to detect AAA/AAG (mutant) and CAA/CAG (wild type) alleles.

**Results:** The training set consisted of 132 HCV 1-infected patients [median age (years), 46 (IQR 41.25-53.75), 108 males (82%), median HCV viral load (Log IU/ml) 6.39 (IQR 6.12-6.76). Q80K mutation was detected in 10 patients making a prevalence of 7.6%; AS-RT PCR successfully detected all Q80K polymorphisms detected by Sanger (AAA allele in 90% of the Q80K positive cases and AAG in 10%). HCV 1a Clade II was the most prevalent (n=106; 80%). Two cases of Q80K were detected in the Clade II population and 8 in the Clade I (p<0.001). The validation set consisted of 48 samples, 26 carrying Q80K positive (22 AAA; 3 AAG; 1 AAR); our AS-PCR successfully detected all but one AAA positive case. Using Sanger as reference, sensitivity, specificity, positive predictive value and negative predictive values of the AS-PCR test were 97%, 99%, 97% and 99% respectively.

**Conclusions:** We have developed an allele-specific real time PCR for the detection of Q80K NS3 protease mutation in HCV 1a genotypes that can be easily performed in routine diagnostic laboratories without the need of DNA sequencing facilities. Q80K is low prevalent in Spain, mainly because of a major spread of HCV 1a Clade II in the Spanish population.

No conflict of interest
Abstract: 63

**Novel Diagnostic Technologies & Approaches**

**Comparison of the HIV Quantification Assays Cepheid GeneXpert and Abbott RealTime in Clinical Routine**

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**Background:** Measurement of the viral load is the most important laboratory parameter for the evaluation of antiviral HIV therapy. A suppression of viral load below 50 copies/ml is defined as therapeutic success. New test systems need to deliver precise, reproducible, specific and particularly sensitive results. In Germany, the most widely used test systems for the quantification of viral load are the Abbott RealTime and Cobas TaqMan (Roche). An additional system and assay for HIV virus quantification (GeneXpert) has been released recently by Cepheid. The aim of this retrospective analysis was to compare viral load quantification results from GeneXpert and Abbott RealTime in patients receiving antiretroviral therapy over time.

**Materials & Methods:** The Cepheid GeneXpert system is a fully automated real-time PCR device, which combines steps of sample preparation, nucleic acid extraction, amplification and detection of target sequences in one cartridge. The limit of quantification is specified with a range of 40 - 10,000,000 copies/ml. In this retrospective evaluation 10 individual courses of therapy were re-measured anonymously with GeneXpert and Abbott RealTime using excess EDTA-plasma samples. Based on the preceding values (measured by Abbott RealTime), the time points during antiretroviral treatment were selected so that a large portion of the dynamic quantification range and HIV-1 subtypes was covered (subtype A, C, D, F, AG, AE once each and four subtype B). A course of therapy consisted of five serial measurements grouped in viral load values of >1,000 copies/ml (n = 20), 199-1000 copies/ml (N=15) and <200 copies/ml (N=15). In addition, 40 independent low viremic samples with predetermined values from 40 to <200 HIV-1 RNA copies/ml were also re-measured with GeneXpert. The sensitivity was assessed based on 30 samples, which were predetermined with Abbott RealTime as < 40 copies/ml (‘detected’) before analysis.

**Results:** The logarithmic mean of the GeneXpert results were 0.25 log higher in the group with viral load values above 1,000 copies/ml, and in the group 199-1000 copies/ml compared to Abbott RealTime. The highest logarithmic deviation was 0.64 log. In the sub-analysis of low viremic samples between 40-200 copies/ml, results varied with a mean of 0.08 log between both systems. A high level of accordance was additionally observed in sensitivity analysis: Of 30 samples predetermined with detected <40 copies/ml, 25 were also detected with GeneXpert (19 with detected <40 copies/ml and 6 with higher values ranging from 51-121 copies/ml). Five were not detected by GeneXpert.

**Conclusions:** In direct comparison, a good agreement of the results for the 10 courses of therapies (5 time points) were observed. The mean logarithmic deviation of both systems was independent from the amount of quantified HIV RNA copies. A high degree of sensitivity could also be confirmed for low viremic samples. Also in the range of the therapeutic target of <50 copies/ml GeneXpert showed a sensitivity comparable to Abbott RealTime results. Due to this concordance and a user-friendly handling the GeneXpert system represents a good alternative in routine diagnostics, also for smaller batches and individual samples.

No conflict of interest

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Abstract: 64

**Spread of Drug Resistance**

**HIV-1 Variants and Drug Resistance in Pregnant Women From Equatorial Guinea: 2012-2013**

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Background: The World Health Organization (WHO) recommends population-based surveys to detect whether the prevalence of resistance in antiretroviral (ART) naive people is reaching alerting levels. The HIV epidemic is increasing in Equatorial Guinea (GQ), but few studies have reported molecular epidemiology and resistance data. This study firstly describes the current circulating HIV-1 variants and the presence of drug resistance mutations (DRM) among pregnant women from GQ.

Materials & Methods: The study was conducted among 69 HIV-1 infected women who participated in a prevention of mother-to-child transmission program at Hospital of Bata in GQ. Dried blood spots were collected from November 2012 to December 2013. HIV-1 variants were identified by phylogenetic analyses, taken as reference GenBank sequences from all subtypes and 66 circulating recombinant forms (CRFs) available at study time. The transmitted antiretroviral drug resistance (TDR) prevalence was defined following the WHO mutation list using the Calibrated Population Resistance tool (http://cpr.stanford.edu), while for ART-exposed women the IAS-USA mutation list 2014 was considered.

Results: Among the 69 HIV-1 infected DBS collected in Bata from Equatoguinean women, 72.5% were under ART. A total of 38 specimens were successfully amplified and sequenced. Among these 38 sequences, 13 (34.2%) corresponded to ART-naive patients and 25 (65.8%) to treatment-experienced subjects, 11 of them receiving highly active antiretroviral therapy and 14 receiving zidovudine. HIV-1 group M non-B variants were carried in most (97.4%) infections, being mainly (78.9 %) recombinants: CRF02_AG (55.2%), CRF22_A101 (10.5%), subtype C (10.5%), unique recombinant forms (5.3%), and A3, D, F2, G, CRF06_cpx and CRF11_cpx (2.6% each). The overall TDR rate was 15.4%. The D30N major mutation-inducing resistance to the protease inhibitor (PI) nelfinavir was detected in a CRF02_AG strain from a naive woman in the absence of any other resistance-inducing mutation. The TDR mutation G190A, associated to non-nucleoside RT inhibitors (NNRTI), was identified in another woman infected with a subtype C virus. This is the first description of both TDR mutations in GQ. Global DRM prevalence among treated was 20%, being 16% for NNRTI (2V90I, 1V106I, 1M184V in RT), 4% for NRTI (M41L in RT) and absent for PI. No case of NNRTI+NRTI resistance was found.

Conclusions: Despite its high HIV prevalence (5%), GQ lacks of systematic surveillance studies on HIV-1 variants and DRM prevalence. The present study is the first one analyzing these parameters during 2012-2013 in GQ. This study shows an HIV-1 epidemiology in the country with predominance of group M recombinant variants, where CRF02_AG causes nearly 60% of infections. A high TDR rate (15.4%) during the study period was observed, 3 times higher than previously reported data, reflecting the increasing trend in transmitted resistance. Systematic DRM monitoring should be reinforced in GQ to reduce HIV-1 resistance transmission, preserving future ART options in infected patients.

No conflict of interest

Abstract: 65

Spread of Drug Resistance

Prevalence of transmitted HIV-1 drug resistance in Tel-Aviv, Israel, and antiretroviral strategy in a potential transmitter population from 2010-2014

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**Background:** The prevalence of HIV-1 transmitted drug resistance mutations (TDR) is assessed regularly in treatment-naïve patients in Tel-Aviv. All HIV patients from our clinic were considered potential transmitter's (PT). Antiretroviral (ARV) use and treatment failure (TF) were evaluated.

**Materials & Methods:** All blood samples obtained from treatment-naïve patients between 2010 and 2014 were analyzed for RT and PR resistance associated mutations. TDR was defined according to the 2009 criteria of Bennett et al. A phylogeny was inferred using pol sequences. The number of patients treated with ARV's was tallied. TF was defined as viral load >200 copies/ml. The c² test for trend was applied to compare the different parameters between each year.

**Results:** Sequences from 575 patients were tested. The resistance rate decreased from 13.45% in 2010 to 5.88% in 2013 (p<0.05) in 2014 but increased again to 11.65%. Phylogenetic analysis in subtype B viruses among MSM patients supports clustered transmission. The percentages of ARV treated patients among all patients followed in the clinic each year were 66.1% (n=1025), 68.1% (n=1067), 72.4% (n=1219), 75.2% (n=1340) and 85% (n=1429) for 2010, 2011, 2012, 2013 and 2014 respectively (p<0.05). The rate of patients treated by PIs among the ARV-treated patients decreased from 53.1% in 2010 to 33% in 2014 (p<0.05). The rate of patients treated by NNRTI decreased from 40.5% to 26.5%, and the rate of patients treated by InI increased from 12.2% to 44.6 in 2010 and 2014, respectively (p<0.05). Among the PT the rate of TF was 35.6%, 32.3% 22.3%, 13.2% and 8.9 % in 2010, 2011, 2012, 2013 and 2014 respectively (p<0.05).

**Conclusions:** There was a drop in TDR between 2010 and 2013 among patients followed in Tel-Aviv. The lowering of TDR could correspond to a change in antiretroviral strategy and lower TF rates among PT treated patients. However, an increase in resistance rate in 2014 supports the important role of clusters in TDR. Although Integrase region was not tested the rate of TDR for these drugs is reportedly low. Regular assessment of resistance in the ARV treated and naïve population is necessary to understand the potential epidemiological effect of new ARV strategies and drugs on TDR.

**No conflict of interest**

**Abstract: 66**

**Spread of Drug Resistance**

**HIV-1 Diversity and Resistance Mutations in Naïve and ARV Experienced Men who have Sex with Men in Bulgaria**

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**Background:** Since the beginning of HIV-1 epidemic in Bulgaria, for nineteen years (1986-2004) only 30 men who have sex with men (MSM) with HIV-1 were registered in Bulgaria. By 2012, the number of MSM with HIV-1 had risen to 277 reaching 17.2% of all 1606 registered HIV/AIDS cases. The aim of the present study was to determine HIV-1 diversity and resistance mutations in naïve and antiretroviral (ART) experienced MSM.

**Materials & Methods:** 145/277 (52.3%) HIV-1 pol gene sequences from MSM individuals were obtained with TruGene Sequencing System. HIV-1 subtypes were classified using REGA 3, COMET v0.5 and phylogenetic analysis with maximum likelihood implemented in FastTree 2 program. Transmitted drug resistance mutations (TDRMs) in naïve individuals were defined using the WHO HIV drug mutation list by the Calibrated Population Resistance tool v6.0 of the Stanford University. Resistance mutations in ARV-experienced patients were analyzed with HIVdb v7.0. Stanford University.
**Results:** Among the 145 MSM persons, 6 (4.1%) were intravenous drug users (IDUs) and 15 (10.3%) migrants, including 12 Bulgarian citizens infected abroad and 3 foreigners diagnosed with HIV-1 in Bulgaria. Most of MSM (89.6%) were 20-44 years of age and (58.6%) were from the capital city of Sofia. Our analysis showed that, the most prevalent HIV-1 subtype in MSM was found to be subtype B (75.2%), followed by URFs (13.1%), CRF01_AE (4.8%), subtype A1 (2.76%), CRF02_AG (2.1%), 12_BF (0.7%) and 14_BG (0.7%). In contrast, subtype B in non-MSM individuals was estimated to be only 28.1%.

TDRM were found in 5 out of 113 (4.4%) MSM, which was similar to that in non-MSM individuals (4.9%). Two of these individuals were infected abroad. Most TDRM were found to nucleoside reverse transcriptase inhibitors (NRTI) (M41L, F77L, T215C/D), followed by protease inhibitors (PI) (D30N, M46L, N88D). TDRM related to non-nucleoside reverse transcriptase inhibitors (NNRTI) (Y181C) were only found in one patient. Dual class resistance was identified in two individuals; one person with NRTI and PI, and one with NRTI and NNRTI TDRM.

In ART experienced MSM, resistance was detected in 13/32 (40.6%) patients with detectable VL who were offered resistance testing. In 7 patients, 4 major (M46L, G48V, I54V, V82A) and 8 minor (L10I/V/F, F53L, Q58E, A71T and T74A/S) PI resistance mutations were found, 7 individuals had 5 NNRTI resistance mutations (K103K/N, E138A/G, V179E) and 2 individuals had 5 NRTI (D67N, K70R, T215I, K219Q, V75LV) resistance mutations. Dual class resistance was identified in 3 individuals; one with NRTI and PI, one with NRTI and NNRTI and one with NNRTI and PI mutations.

**Conclusions:** Our analysis showed that subtype B is more common in MSM, compared to non-MSM transmission groups. The prevalence of TDRM in newly diagnosed MSM with HIV-1 in Bulgaria is low (4.4%) and is similar to that in heterosexuals and IDUs. We found resistance mutations in almost half of ART experienced MSM patients that could potentially be transmitted to new patients. Molecular epidemiological surveillance among MSM is of importance for public health measurements to regain better control of the HIV-1 epidemic.

No conflict of interest

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**Abstract: 67**

**Spread of Drug Resistance**

**The RESINA data support the individualized therapy based on primary resistance testing**

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**Background:** The RESINA study started in 2001 and was originally focused on the evaluation of primary resistance in patients at the time point of first therapy. Additionally, we could follow up these patients (RESINA cohort) since cART start by collecting the clinical, virological and immunological data.

**Materials & Methods:** The clinical, virological and immunological data were collected from 38 centers since 2001. Genotypic analysis of resistance-associated mutations (RAMs) was performed from viral RNA exclusively until 2012, since then additionally from proviral DNA and/or total NA. Resistance-associated mutations were detected by Sanger sequencing and recently by next-generation sequencing by the Illumina MiSeq technology. Additionally, we collect data from any therapy-experienced patient within the AREVIR project.

**Results:** Meanwhile the RESINA cohort consists of more than 3800 patients from almost 40 HIV-centers in North-Rhine-Westphalia. Furthermore, we performed a total number of more than 13000 resistance tests from therapy-naïve and -experienced patients (RESINA and AREVIR data). During this time we could
observe a decline in prevalence of resistance-associated mutations in treatment-experienced patients as documented in the AREVIR database. In contrast to the decline of RAMs in therapy-experienced patients the frequency of primary resistance-associated mutations at the beginning of cART remains relatively stable. The majority of the primary RAMs were NRTI resistance mutations throughout the whole time of observation. NNRTI resistance-associated mutations did not increase over time although the use of NNRTI increased in our cohort since 2001. We did not observe an increase in primary PI resistance-associated mutations and almost no primary INI-resistance mutations.

Conclusions: Despite the declining frequency of resistance-associated mutations in therapy-experienced patients the frequency of primary resistance mutations is still high and justifies routine primary resistance testing. We can further conclude from our data that the individualized therapies according to the DAIG therapy guidelines for therapy-naïve patients translate in a low number of NNRTI- and PI-resistance-associated mutations in therapy-naïve and -experienced patients.

No conflict of interest

Abstract: 68

Spread of Drug Resistance


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Background: CoRIS HIV-positive patients cohort provides a scenario to analyze the transmission of antiretroviral drug resistance at a national stage in Spain. CoRIS is updated annually. We present the data on transmission of drug resistance mutations and resistance to first-line drugs in antiretroviral therapy (ART) naïve patients in the latest update (June 2014).

Materials & Methods: We analyzed 607 Pro & RT fasta sequences of patients admitted to CoRIS from May 2013 to June 2014. For the evaluation of transmitted drug resistance mutations (TDR) we have followed the WHO list (Bennett, 2009). To estimate the prevalence of primary resistance to first-line drugs (NNRTIs - EFV, NVP, RPV-; NRTIs -TDF, ABC, FTC, 3TC- and PIs ATZr, DRVr, LPVr-) and to assign HIV subtype we used Stanford algorithm v 7.0.

Results: We found an overall 8.2% (3.5% NRTIs, 3.8% NNRTIs, 2.1% PIs) prevalence of TDR in the analyzed period. TDR to 2 drug classes was 1.2% and no TDR to all three-drug classes was detected. The prevalence of primary resistance to first-line drugs was 11.7% [1.5% NRTIs (1.1 Intermediate to ABC, and 0.7% to TDF), 9.7% NNRTIs (0.8% intermediate to EFV, 1.5% to NVP, 4.9% to RPV); 1.3% IPs (1.3% intermediate to ATV, 0.5% to DRV, and 1% to LPV)]. The E138AGQ mutation, which under current interpretations of Stanford is reported as intermediate resistance to RPV, was found in 4.4% of cases, prompting a rise in primary resistance to non-nucleoside compared to previous years. The most prevalent mutations were K103N/S (3.1%) to NNRTIs; T215 revertants (1.3%), M41L (0.3%), and D67NGE (1.6%) for NRTIs; and M46IL (0.8%) for PIs. The percentage of non-B subtypes was 19.4%, with CRF02_AG recombinant, being the most prevalent (6.6%), followed by subtype A (3.1%) and F (2.6%).

Conclusions: TDR in Spain is stable during the last update, when compared to previous calendar years. We found an increase in primary resistance to NNRTIs, which can be explained by the new interpretation of E138A mutation. The transmission of non-B subtypes in this cohort also remained stable compared to previous periods.

No conflict of interest
Abstract: 69

Spread of Drug Resistance

Transmitted Drug Resistance to Integrase Inhibitors in the Spanish AIDS Research Network Cohort (CoRIS) in 2012-2013.

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Background: In Spain there is no epidemiological surveillance program of transmitted antiretroviral drug resistance, and resistance to integrate in patients naïve to antiretrovirals is not determined routinely. The CoRIS cohort, associated with an HIV biobank, offers a unique opportunity for their evaluation. Our aim was to estimate the prevalence of transmitted drug resistance in the integrase gene in Spain in the period 2012- 2013.

Materials & methods: Stratified random sampling of CoRIS patients diagnosed in 2012-2013 and with an available plasma sample in the Spanish HIV Biobank drawn no more than 6 months from HIV diagnosis, and before the start of any antiretroviral treatment was performed. The sample size was calculated for an expected prevalence of 0.5%, with a 95% confidence and a ±1.0% precision. Patients were stratified by transmission category, using reference data from SINVIH 2012. The integrase gene was sequenced using an 'in house' test, assessing the presence of T66IAK, L74M, E92QG, T97A, F121Y, E138AK, G140SA, Y143RHC, S147G, Q148RHF, and N155H (IAS 2014 mutation list). Epidemiological, virological and immunological variables are analyzed.

Results: 192 patients were studied, with a median [IQR] age of 35 [30-43], 85.4% male, 65% MSM, 24.9% HTX and 3.4% IDU. Median viral load (log) and CD4 count were 4.7 [4.2-5.3] and 395 [248-638] respectively. 70.3% of the patients were Spanish, 10.4% from South America, 2.6% from Africa and 7.3% were European. 1.6% were primary infections, 79.7% had stage A, 10.6% B and 8.1% C. We detected three patients (1.6%) with mutations: a case with T66I (primary to elvitegravir) and two cases with T97A (secondary to elvitegravir and raltegravir).

Conclusions: transmitted drug resistance to integrase inhibitors is so far, a rare event in newly diagnosed patients in Spain over the period 2012-2013. These data support that, at present, the determination of baseline integrase resistance in routine clinical practice is not necessary. Efforts should be made to enable surveillance strategies that enable to monitor the prevalence of transmission annually.

No conflict of interest

Abstract: 70

Spread of Drug Resistance

Hepatitis C Virus Screening Project of Patients on current anti-HCV Therapy

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Background: Clinical outcome of HCV therapy combination of pegylated-interferon-alpha (IFN), ribavirin (RBV) and direct acting antivirals (DAAs) depends on host and viral factors. This non-interventional study collects data from DAA-resistance-associated-mutations (RAMs) within the viral NS3/protease, NS5A and NS5B genes, viral quasispecies distribution and host factors, in order to predict clinical outcome using the geno2pheno[HCV] tool.

Materials & Methods: NS3, NS5A and NS5B sequences from plasma samples were obtained. Subtyping (including GT 1a clade classification) and resistance against Boceprevir (BOC), Telaprevir (TVR), Simeprevir (SMV), Daclatasvir (DCV), Sofosbuvir (SOF) and Ledipasvir (LDV) was determined by sequencing of the corresponding viral gene and subsequent interpretation with geno2pheno[HCV] (http://hcv.bioinf.mpi-inf.mpg.de/) (FIG.3). Baseline samples from patients under following therapies were analysed: IFN/RBV (49); IFN/RBV/TVR (74); IFN/RBV/BOC (31); IFN/RBV/SOF (30); RBV/SOF (34); RBV/SMV (2); RBV/SOF/DCV (5); SOF/SMV (9); SOF/DCV (8); SOF/LDV (31).

Results: 647 HCV-infected patients have been enrolled until end of January 2015. The number of enrolled patients increased significantly during 2014, when the new DAAs were licensed. We produced baseline sequences of NS5B and/or NS3 regions from 537/647 patients. Therapy information and viral load values from 411/537 patients was obtained. Genotyping results were available for 353/537 baseline-samples: 104 GT1a (clade I), 71 GT1a (clade II), 143 GT1b, 15 GT2, 65 GT3a, 16 GT4.

251 NS3/protease baseline samples could be sequenced. We analyzed the presence of mutations in the amino acid positions 36, 41, 54, 55, 80, 87, 117, 122, 132, 155, 168, 170 and 174, which have been described to be involved in PI-resistance. In 165/251 (65.7%) samples RAMs were detected (most common ones: 132V 55/251 (21.9%); 80K 33/251 (13.1%); 122G 10/251 (4.0%).

353 NS5B baseline samples were successfully sequenced. We analyzed the presence of mutations in the amino acid positions 15, 96, 179, 289, 282, 293, 316, 414, 415, 423, 434, and 479, which have been described which have been described to be involved in SOF-resistance. In 113/353 (32.0%) samples RAMs were detected (most frequent ones: 415Y+479P 35/353 (9.9%); 293L+479P 21/353 (5.9%); 293L+415Y+479P 9/353 (2.5%); 293L+415Y+434L+479P 9/353 (2.5%). Furthermore, we could generate 26 NS5A baseline samples. We analyzed the presence of mutations in the amino acid positions 23; 28; 30; 31; 32; 58; 92; 93, which have been described to be related to DCV/LDV-resistance. In 9/26 samples (34.6%) RAMs were detected (58P 2/26 (7.7%); 30R+58P 4/26 (15.4%); 28L+30R+58P 3/26 (11.5%).

Conclusions: Analysis of NS5B and NS3 with geno2pheno[HCV] interpretation allows subtyping, clade classification, and prediction of DAAs susceptibility. PI resistance-mutations exist at baseline in higher extent as reported in previous studies. Incorporating additional viral and clinical data will continuously improve geno2pheno[HCV] for better therapy response prediction.

No conflict of interest

Abstract: 71

Spread of Drug Resistance

A High Genetic Heterogeneity in HBsAg Can Influence HBV Immunogenicity in the setting of Acute Infection

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Background: The goal of this study is to investigate HBV RT and HBsAg genetic-heterogeneity in acute HBV (AHB) infected patients and to define their clinical value.

Materials & Methods: 62 HBsAg+ and IgM/anti-HBc+ patients with clinical and
biochemical signs of AHB (44 genotype-D and 18 genotype-A) were enrolled from 2000 to 2010. Plasma-samples obtained at first-observation were analyzed by ultra-deep sequencing (UDPS) for drug-resistance and immune-escape mutations. Shannon-Entropy, weighted for intra-patient prevalence of each mutated-position, was used to measure the extent of HBsAg amino-acid variability.

Results: 75.8% of patients were male with median (IQR) age of 36 (29-46) years. Median (IQR) ALT and HBV-DNA were 2,544 (1,938-3,078) U/L and 5.9 (4.5-7.4) log 10 IU/ml. 61/62 (98.4%) patients became HBsAg-negative with 33/61 (54.1%) developing also anti-HBs. By UDPS, 8.1% (5/62) of patients carried >1 drug-resistance mutation (rtV173L/rtL180M/rtA181T/rtA194T/rtM204I). They were detected with an intra-patient prevalence ranging from 0.11% to 47.5% for primary mutations and from 10.5% to 99.98% for compensatory mutations.

Analysing HBsAg a-determinant, 48.4% (30/62) of patients carried >1 immune-escape mutation (intra-patient prevalence range: 0.2-100%). Vaccine-escape mutations were found in 11.4% of patients, all genotype D-infected. This is the case of sG145R, sM133L, and sP120S, detected with intra-patient prevalence ranging from 3.9% to 99.9% for sG145R, from 1.9% to 16.8% for sM133L, and always of 100% for sP120S.

Stop-codons were found in 19.3% patients (intra-patient prevalence range: 1.6-47.5%). They occurred at 11 HBsAg-positions including also 172 and 182, known to increase HBV oncogenic-potential.

Finally, by Shannon-Entropy, specific HBsAg-positions correlated with the lack of HBsAg seroconversion in genotype-D. In particular, positions 130 and 133 (localized in HLA-class-II epitope) were found mutated only in patients not developing anti-HBs (Shannon-Entropy mean ± SE: 1.98±0.01 vs 0, and 1.95±0.03 vs 0, respectively, P<0.05).

Conclusions: AHB is characterized by a complex coexistence of viral-quasispecies, some of them with reduced antigenicity/imunogenicity, enhanced oncogenic-potential and altered drug-susceptibility. These viral-variants may induce severe and/or difficult-to-treat forms of HBV-infection (ex. HBV-reactivation), and might affect the efficacy of current HBV-vaccination strategy.

No conflict of interest

Abstract: 72

Spread of Drug Resistance

Prevalence of transmitted drug resistance and late diagnosis in newly diagnosed HIV infected patients in Andalusia in 2014.

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Background: The Andalusian Network for Epidemiological Surveillance of Resistance to Antiretrovirals (RAVETRA) aims to characterize transmitted drug resistance (TDR) on all new diagnoses of HIV in Andalusia. In this study we analyzed TDR and late diagnosis in patients diagnosed in 2014.

Materials & Methods: We collected FASTA sequences of the protease and reverse transcriptase of HIV and clinical-epidemiological and immune-virological data of all the new diagnoses of Andalusia, South of Spain. To evaluate TDR, mutations included in the latest update of the WHO list (Bennet et al 2009) were considered. To estimate the prevalence of primary resistance to first-line drugs (NNRTIs: EFV, NVP, RPV; NRTIs: TDF, ABC, FTC, 3TC; PIs: ATZr, DRVr, LPVr) we used Stanford v7.0 interpretation algorithm, considering resistant any category other than 'susceptible' and 'potential low-level resistance'. Phylogenetic analysis of the sequences to characterize clusters of transmission was also performed. Finally, we linked the clinical and epidemiological variables analyzed in the cohort with the prevalence of TDR.

Results: We analyzed 495 fasta sequences from patients with a mean age of 36 years (27-44), mean viral load: 4.67log (4.22-5.16) copies/ml, CD4 count 380 cell/UL (163-554), 87.4% men. 22.4% had advanced disease (<200 CD4) and 41.6% late diagnosis (<350 CD4). The prevalence of primary resistance to
first-line drugs was 14.74% (3.03% NRTIs, NNRTIs 11.72%, 1.62% IPs). The prevalence of resistance mutations (WHO) was 11.4% (4.24% NRTIs, NNRTIs 6.06%, 2.42% IPs). The most prevalent mutations were K103N (4.04%), E138A (2.83%), E138G (1.62%) and G190A (1.01%) –NNRTIs-; T215 revertants (2.22%), D67N (2.02%), K219Q (1.82%) and M41L (1.21%) –NRTIs-; and M46I (0.81%), V82A (0.61%) and L90M (0.40%) for PIs. 395 patients were infected with subtype B (79.9%). Among the non-B subtypes, recombinant CRF02_AG was the most prevalent (7%), followed by subtype A (4.4%), D (2.8%), F (1.6%), G (1.6%), CRF01_AE (1.4%), C (1.2%) and K (0.2%). Phylogenetic analysis showed the existence of clusters of intra-provincial and interprovincial transmission. No relationship was found between the prevalence of resistance and none of the evaluated clinical and epidemiological variables.

Conclusions: transmitted drug resistance to first line NNRTIs in Andalusia is high, mainly due to the emergence of resistance mutations different from K103N. Late diagnosis remains high, but lower than in previous studies and than national estimates. The existence of clusters of transmission is common in newly diagnosed patients.

Other authors from the RAVETRA Study group: Viciana I, Llaves S, García-Rey S; Perez I; Fernández C; Mohamed O; del Arco, A; Parra, M; Fernández-Cuenca F; Delgado C; Fernández S; Palomares J; Castaño M; Galvez C; Téllez F; Hernández S; Palomares JC; Viciana P; Santos J

No conflict of interest

Abstract: 73

Spread of Drug Resistance

Recent transmission clusters of HIV-1 C and CRF17_BF strains with evidence of NNRTI-related mutations among newly diagnosed men in central Italy.

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Background: Increased evidence of relevant HIV-1 epidemic transmission in European countries is to date reported, with an increased circulation of non-B subtypes and circulating recombinant forms (CRFs). Here, we present two recent HIV-1 non-B subtype transmission clusters characterized by NNRTI-related mutations, involving newly diagnosed HIV-1 infected men, living in Rome (Central-Italy).

Materials & Methods: Pol and V3 sequences were available at diagnosis for all individuals. Maximum-Likelihood and Bayesian phylogenetic trees were performed to define the transmission clusters by using the bootstrap and Bayesian probability supports. HIV-1 drug resistance and V3 tropism were also evaluated according to IAS-USA/Stanford-DB/Bennett (2009), and Geno2Pheno algorithm (set at False Positive Rate of 10%), respectively. Atypical mutations at positions already associated with drug resistance were also investigated.

Results: Among 534 new HIV-1 non-B cases diagnosed between May 2011 and September 2014, 35 individuals drew our attention, because a preliminary phylogenetic analysis by neighbor-joining method showed that their HIV-1 pol sequences (27 belonged to C subtype and 8 to CRF17_BF subtype) formed two distinct clusters (bootstrap >90%). These clusters were confirmed by Maximum Likelihood and Bayesian analyses (bootstrap support >90%; posterior probability=1). All patients in these clusters were men and Italians, with the exception of one patient, who belonged to CRF17_BF subtype, coming from Argentina. Regarding risk factors, while C cluster was...
entirely composed by men who have sex with men (MSM), the majority (5/8, 62.5%) of men involved in CRF17_BF cluster reported sexual contacts with men and women. Both clusters were centralized in Rome (Central-Italy), and their origins were estimated, by Bayesian cluster dating, around 2007 for C cluster and in the last months of 2010 for CRF17_BF cluster, confirming the recent appearance of this recombinant form in Italy. The phylogenetic clustering also highlighted that these viral lineages were characterised by NNRTI-related mutations. In particular, all HIV-1 strains in C cluster carried the atypical and rare RT-mutation K103Q, present at position critical for Nevirapine and Efavirenz efficacy. The CRF17_BF cluster has been characterized by HIV-1 strains resistant to both first and second generation NNRTIs, due to the presence of RT mutations K101E and E138K. The analysis of V3 sequences revealed that all patients were infected by R5 tropic viruses.

Conclusions: Our findings provide the first evidence of a strong and recent circulation in central Italy of non-B subtypes clusters carrying NNRTI-related mutations, among newly diagnosed Italian men engaging in high-risk behaviours. This implies that an improvement of HIV-1 prevention strategies and screening activities, especially in the setting of a population at high risk for HIV is needed, such as an earlier detection of HIV infection, and an earlier beginning of antiretroviral treatment, as recommended in the most recent treatment guidelines.

No conflict of interest

Abstract: 74

Viral Evolution & Genetic Diversity

Molecular epidemiology of HIV-1 in Scandinavia


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Background: Increased knowledge about transmission dynamics of human immunodeficiency virus type 1 (HIV-1) in different risk groups over time is fundamental for assessing and designing prevention efforts to limit the spread within and between countries.

Materials & Methods: HIV-1 pol sequences and clinical data of 51% of all newly diagnosed HIV-1 infections in from Sweden, Denmark and Finland 2000-2012 were analysed to determine subtype and transmission route dynamics over time in the Scandinavian region.

Results: The dominating forms of HIV-1 were subtypes B (54%) and C (11%); and the circulating recombinant form (CRF) 01_AE (14%) followed by subtypes A, D, G, CRF02_AG and CRF06_cpx. The prevalence of CRFs showed increasing trends over time. Of 4003 sequences, 56% fell into inferred clusters with distinct differences between transmission groups: the average number of sequences in heterosexual (HTX) clusters was 2.3 compared to 7.2 for men who have sex with men (MSM) and 20.8 for intravenous drug users (IDU). Sequences of MSM and IDU were more likely to form clusters than HTX sequences. The genetic diversity was lower among MSM and IDU clusters compared to HTX clusters.

Conclusions: The increasing trend seen for CRFs highlights the importance of further studies of these variants and their potential impact on transmission dynamics and spread of HIV-1 in different geographical regions and risk groups. The observed differences between risk groups may be linked to differences in transmission dynamics that may have implications for when and where public health interventions should be set in.

No conflict of interest
Abstract: 75

Viral Evolution & Genetic Diversity

Genetic Variation in IL28B in genotype 1 Chronic Hepatitis C infected patients studied in Centro Hospitalar Lisboa Norte (CHLN) between March 2012 and October 2014

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Background: Chronic hepatitis C is a major cause of cirrhosis and hepatocellular carcinoma worldwide. According with international studies, the analysis of rs12979860 polymorphism, located upstream of the IL28B gene, is a strong predictor of sustained virological response (SVR). So, this determination is important to improve the treatment in chronic HCV infected patients, particular in genotype 1.

Material & Methods: The study included 433 HCV genotype patients. The genotype determination was performed with Siemens technology (RNA extraction, amplification and LIPA). The polymorphism of IL28B was determined by LightCycler® System FastStart DNA Master HybProbe, using Magna Pure Compact (Roche) and LightMix® IL28B kit (TIB Molbiol).

Results: 60% of the hepatitis C studied patients are infected by genotype 1 virus. A total of 433 patients were evaluated (genotype and IL28B characterized), 289 males (67%) and 144 females (33%). According to the IL28B genotype the distribution was: CT genotype - 244 patients (56,4%), CC genotype – 128 patients (29,6%) and TT - 8 patients (14%). Particularly, in genotype 1a the distribution was: CT genotype – 163 patients (54,5%), CC genotype – 91 patients (30,4%) and TT – 45 patients (15,1%). In genotype 1b the distribution was: CT genotype – 81 patients (60,4%), CC genotype – 37 patients (27,6%) and TT – 16 patients (12%).

Conclusions: According to our study, patients with genotype 1 have higher prevalence of IL28B genotype CT (56,6%) and CC (29,3%), the latter being the most likely to achieve a sustained virological response. In genotype 1 infected patients, the subtype 1a is most prevalent than 1b (69,1% and 30,9% respectively). The percentage of male patients with subtype 1a (74,6%) is higher than female patients (25,4%), hile in subtype 1b the number of patients is quite similar in both gender (49,3%) male and 50,7% female.

No conflict of interest

Abstract: 76

Viral Evolution & Genetic Diversity

Comparing estimated time of HIV-1 infection obtained by Bayesian analysis and the BED assay

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Background: Different methods allow us to estimate the point in history when HIV was transmitted between individuals. The purpose of this study was to compare the estimates obtained in a Bayesian phylodynamic approach and the Aware™ BED™ EIA HIV-1 Incidence Test (BED test) (Calypte Biomedical Corporation, Portland, Oregon).

Materiasl & Methods: The BED assay, used to determine whether an infection was acquired recently, employs a 155-day time interval following infection for characterization of a recent infection. For this reason the time of infection for individuals defined as being
recently infected was thus estimated to be within a 155-day interval prior to the time of sampling. Times to the most recent common ancestor (tMRCAs) were determined by the Monte Carlo Markov chain method available in the BEAST package v1.7.1, using a relaxed clock model with uncorrelated lognormal distribution and the Bayesian skyline coalescent model. These methods were applied for 3 different groups of patients for whom their source of infection was known: 2 pairs of patients, among which at least one of the two patients was determined as recently infected; and one trio of patients, for which all were determined as recently infected.

Results: We found that the tMRCAs obtained using the Bayesian approach were estimated earlier in time compared to estimates obtained by the BED assay. The median tMRCA dates estimated by Bayesian analysis were 0.5, 1.1 and 1.1 years before the date of infection estimated by the BED assay for transmission pair 1, pair 2 and the trio, respectively. The estimated tMRCAs corresponds to the time of origin of the strain that gave origin to the infections of that cluster. This strain should therefore have been originated before the estimated time of infection of any of the patients in our cluster, somewhere along the branch that leads to that cluster and inside the body of the patient who transmitted that strain to one of the patients in the cluster. However, these results should be interpreted with caution, since the BED test used does not allow individual determination of the timing of infection, due to variations in immune system response, so the stated intervals of the supposed timing of infection based on this data have limitations.

Conclusions: The tMRCAs obtained using the Bayesian approach were estimated earlier in time compared to estimates obtained by the BED assay, as expected, since the term tMRCAs corresponds to the strain that gave origin to the infections of that cluster.

No conflict of interest

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Viral Evolution & Genetic Diversity

Frequency of HIV-1 non-B variants in Poland over time and across exposure groups.

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Background: Spread of HIV-1 subtypes varies considerably worldwide and in Europe, with non-B variants commonly found across various exposure groups. Distribution and temporal trends in the HIV-1 subtype variability has not been previously described for Poland at the country level and in this study we aim to bridge the gap in this knowledge.

Materials & Methods: For analysis of the subtype distribution 1219 partial pol sequences obtained from patients followed up in the 9 of 17 Polish HIV treatment centres were used. Sequencing was performed using standard methodology (ViroSeq 2.8). For subtyping the sequences in the 2013 version HIV sequence compendium (Los Alamos National Laboratory) were used with initial subtyping inferred using maximum likelihood (ML) with approximate
likelihood ratio test (aLRT) (online PHYLMLv.3.0-
Recombination was assessed using two
methods: simplot bootscan based on 2-
parameter Kimura model with a window size of
200 b.p. and 20 b.p. step and the jumping-
profile hidden Markov model (pHMM)
(http://jphmm.gobics.de/jphmm.html). For
statistics Chi2, Fisher's exact and U-Mann
Whitney tests were used as appropriate
(Statistica 8.0 PL). For analyses of subtype
distribution over time logistic regression was
implemented (R platform).

Results: Subtype B dominated in the studied
group (n=1059, 86.9%); in 160 (13.1%)
sequences non-B variants were present. The
following subtypes were found: A1 (n=63, 5.2%),
D (n=43, 3.5%), C (n=22, 1.8%), and F1 (n=2,
0.2%). In 25 (2.1%) cases CRFs were present
(CRF02_AG - 11 (0.9%) cases, CRF01_AE - 7
(0.6%), and one (0.1%) for each: CRF06_BG,
CRF07_BC, CRF11_cpx, CRF13_cpx,
CRF24_BG, CRF26_AF, CRF37_cpx. Five
viruses (0.4%) were unique AB recombinants
(URF) previously not identified in Poland. Non-
B clades were notably more common among
heterosexually infected individuals (n=103,
66.5%, p<0.001) and less frequent among men-
who-have-sex-with-men (MSM) (n=27,
17.42%, p<0.001) compared to the subtype B
with similar frequency for the injection drug use
(IDU) associated transmissions (n=24,
15.48%, p=0.07). Female individuals were more
commonly infected with non-B clades (n=70,
44.59%, p<0.001), including subtype A1
(p<0.001), D (p<0.01) and CRF02 AE (p=0.002)
compared to the subtype B. In overall
individuals infected with non-B clades were
significantly older [37(IQR:29-51) years] at the
time of diagnosis compared to the subtype B
[31(IQR:26-38) years], p<0.001. AIDS at HIV
diagnosis was notably more frequent (n=50,
32.5%) across non-B variants compared to the
subtype B (n=262, 25.5%, p=0.011) including
subtype C (n=10, 45.5%, p=0.03) and D
(20,48.8%, p<0.001). Additionally, HIV-1 viral
load at diagnosis was notably higher among
individuals infected with non-B clades
[5.0(IQR:4.4-5.6)] vs. [4.8(IQR:4.3-5.4)] log
copies/ml for subtype B (p<0.001), with lower
lymphocyte CD4 count at care entry
[248(IQR:75-503) for non-B vs 320(IQR:125-
497) cells/ul for B, respectively], p<0.001.
Frequency of the non-B subtypes proved stable
from 2008 (11.5%) to 2014 (8.0%) [OR: 0.95
(95%CI:0.84-1.07), p=0.409, with no temporal
differences found across exposure groups,
gender, age and AIDS.

Conclusions: Despite predomination of
subtype B, variability of HIV in Poland is notable;
both CRFs and URFs are present in the
analysed population. Non-B variants are
associated with heterosexual transmissions and
are diagnosed at a later age and more
advanced HIV disease, with stable temporal
frequency over time.

No conflict of interest

Abstract: 78

Viral Evolution & Genetic Diversity

Impact of European mitochondrial DNA
haplogroups in the outcome of HIV infection at a reference
medical care area in Northwest Spain

Abstract: 78

Viral Evolution & Genetic Diversity

Impact of European mitochondrial DNA
haplogroups in the outcome of HIV infection at a reference
medical care area in Northwest Spain

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Background: Mitochondrial DNA haplogroups
(mtDNA) have been associated with the clinical
outcome of several chronic diseases (i.e.
Alzheimer, Parkinson, type 2 diabetes mellitus,
osteoarthritis, and multiple sclerosis). In
addition, mtDNA haplogroups could also play a
role in HIV infection, such as AIDS progression
and CD4 recovery. Herein, we determine the
impact of European mtDNA haplogroups in the
outcome of HIV infection in a large cohort of HIV-infected patients in Northwest Spain.

Materials & Methods: This is a retrospective study in a large cohort of HIV, HCV and HIV/HCV co-infected patients in clinical follow-up at a reference hospital in Northwest Spain. Epidemiological, clinical and virological data were recorded. The European mtDNA haplogroups were determined using PCR-RFLP and Single Base Extension (SBE) techniques.

Results: A total of 537 patients were recorded during the study period. From these, we excluded 40 patients without Caucasian ethnicity. Overall, 49.1% were HIV-monoinfected, 17.5% were HCV-monoinfected and 33.4% were HIV/HCV co-infected patients. Most were male (76.2%), median age 47 years and 98.8% were Spanish citizenship. European mtDNA haplogroups were recognized in 98.2% of patients as follows: H (48.8%), U (12.1%), J (8.6%), K (7.4%), T (7.2%), V (2.5%), SHV (2.3%) and others (I, W, X, M) (11.4%). For further analysis, individuals were separated into the most common European clusters (HV, KU, JT and others). Overall, we did not find differences between mtDNA haplogroups and median age, gender, HIV or HCV infection, AIDS or HIV viral load at diagnosis time. A higher prevalence of cluster HV where observed among HIV-infected patients with lower nadir CD4 counts: 56.5% for those with <200 cel/uL vs. 47.2% in those with >200 cel/uL. However, the cluster HV was more prevalent among patients who reached undetectable viremia after at least one year under antiretroviral therapy compared with those who did not (53.3% vs 42.4%). Interestingly, mtDNA influence the likelihood of being co-infected with other viruses. The cluster HV was more prevalent among patients co-infected with HCV, HBV or HPV infections than in HIV-monoinfected (58.3% vs 47.6%). Multivariate analysis identified cluster HV with a higher likelihood to acquire co-infections compared with cluster KU (p=0.04; OR=0.61). Regarding comorbidities, cluster HV was more prevalent among patients with cardiovascular disease compared with those patients without it (72.2% vs. 52.6%).

Conclusions: Cluster HV was more prevalent among HIV patients co-infected with other viruses (HCV, HBV or HPV) being identified as a risk factor to acquire other viral infections. Moreover, the cluster HV was associated with lower nadir CD4 counts but with a higher likelihood of reach undetectable viremia after at least one year of therapy.

No conflict of interest

Abstract: 79

Viral Evolution & Genetic Diversity

European mitochondrial DNA haplogroups impact on liver fibrosis progression among HCV and HIV/HCV coinfected patients from Northwest Spain

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Background: Mitochondrial DNA haplogroups is associated with the clinical outcome of several chronic diseases (i.e. Alzheimer, Parkinson, osteoarthritis, multiple sclerosis). Moreover, mtDNA haplogroups might be also related with the outcome of liver disease among HCV-infected patients.

Materials & Methods: This is a retrospective study in a large cohort of HCV and HIV/HCV co-infected patients in clinical follow-up at two hospitals in Northwest Spain. Epidemiological, clinical and virological data were recorded. The European mtDNA haplogroups were determined using PCR-RFLP and Single Base Extension (SBE) techniques.
Results: A total of 259 HCV and HIV/HCV co-infected patients were included. From these, we excluded 5 patients without Caucasian ethnicity. Overall, 34.4% were HCV monoinfected patients and 65.6% were HIV/HCV co-infected. Most were male (73.9%), median age was 49 years and 98.8% were Spanish citizenship. The distribution of transmission routes was: 76% injection drug users (IDU), 3.3% sexual, 17.1% unknown and 3.6% others. European mtDNA haplogroups were recognized in 97.6% of patients as follows: H (52.6%), U (10.1%), J (6.1%), K (6.1%), T (8.9%), V (3.2%), SHV (2.8%) and others (I, W, X, M) (10.2%).

For further analysis, individuals were separated into the most common European clusters (HV, KU, JT and others). Overall, we did not find differences between mtDNA haplogroups and median age, gender, IL28B polymorphism, HCV G1-subtypes or HCV-RNA viral load levels at the diagnosis time. A higher prevalence of cluster HV was observed among HCV genotype 4 infected patients (G4 75%; G3 64%; and G1 54.4%). Moreover, the median of AST levels were higher in HV cluster compared with the rest of haplogroups (HV AST 30 UI/L vs. KU 25 vs. JT 29 vs. 26.5; p=0.038).

Interestingly, mtDNA could influence the likelihood of liver fibrosis progression. Fibrosis was scored as low fibrosis (F0-F2) and high fibrosis (F3-F4). In addition, patients with fibroscan values >9.4 Kpa was labeled as high level fibrosis. A higher prevalence of cluster HV was observed in patients with low fibrosis (F0-F2 61.6% vs. F3-F4 53.3%). Moreover, considering the median fibroscan values (Kpa), clusters HV and KU had lower values than clusters JT and others (8.8 and 8.5 vs. 10.15 and 12.49, respectively). Multivariate analysis showed a trend to higher level of fibrosis in clusters JT [OR= 1.8 (0.82-3.98)] and others [OR=2.41 (0.93-6.26)] in comparison with cluster HV. Indeed, multivariate analysis shows that cluster JT and others had increased the fibroscan values in 2.18 and 3.48 fold, respectively, compared with cluster HV. The multivariate analysis was adjusted by age, gender and the HCV-diagnosis time.

Conclusions: The mtDNA cluster HV was more prevalent among HCV genotype 4 infected patients. A higher prevalence of the cluster HV was observed among patients with lower fibrosis (61.2%). Moreover, clusters HV and KU had lower median fibroscan values than clusters JT and others. Interestingly, clusters JT and others have been recognized as risk factors for the development of liver fibrosis among HCV monoinfected and HIV/HCV co-infected. These results might be useful for prioritization of treatment strategies among HCV-infected patients.

No conflict of interest

Abstract: 80

Viral Evolution & Genetic Diversity

A CRF02_AG transmission cluster among men who have sex with men in Genoa, Italy

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Background: As known, in North America and in Europe, at the beginning of the HIV-1 epidemic, B subtype was predominant and the other subtypes and circulating recombinant forms (CRFs) were confined in the countries where the HIV infection started. However, in the past years, a progressive increase of non-B subtype and CRFs circulation, mainly due to the increasing number of migrants and travellers from endemic regions, has been observed and documented. Among non-B subtypes, the CRF02_AG is one of the most prevalent worldwide, it is responsible for at least 8% of total infections and its prevalence moved from 5% in 2000-2003 to 8% in 2004-2007. Moreover, whilst in many high income-setting the trend of new HIV-1 infections is in decline, an increasing incidence of new HIV-1 infections was reported in men who have sex with men (MSM). Although subtype B is the major responsible of the new infections, clusters of non-B subtypes have been reported. Here we report on a CRF02_AG transmission cluster in MSM.

Materials & Methods: From September 2014 to January 2015, 3 CRF02_AG sequences with the presence of 10I, 20I, 36I and 46I in the protease gene mutations were identified. To
establish the existence of a CRF02_AG transmission cluster, all the CRF02_AG sequences obtained in our laboratory from September 2014 to date were analyzed. On the whole 15 CRF02_AG sequences were studied. Sequences were obtained by Trugene HIV-1 Genotyping Kit (Siemens HealthCare Diagnostics) according to manufacturer’s instruction. HIV-1 subtypes were assessed by REGA HIV-1 & 2 Subtyping Tool - Version 3.0. Phylogenetic analysis to prove the existence of cluster was performed using the Mega 6.0 (Molecular Evolutionary Genetics Analysis) software. To better define the possible epidemiological link among patients, clinical data and personal information were revised.

Results: Among the 15 anti-HIV-1 positive patients harboring the CRF02_AG viruses, 3 were females and 6 were MSM. Sequencing analysis revealed the presence of the 20I and the 36I mutations in the protease gene in all sequences and of the M46I in 4/15. Identical mutations were detected in only 3/15 CRF02_AG sequences: 10I, 20I, 36I and 46I. Phylogenetic analysis demonstrated that these 3 CRF02_AG sequences belong to the same cluster. From data analysis about sexual behavior emerged that all three patients were MSM. Furthermore, two more heterosexual patients were classified in another cluster, the sequences belonging to a heterosexual couple.

Conclusions: Regarding the detected mutations, it should be underlined that in CRF02_AG strains the 20I is wild-type and the 36I is detected in the majority (99%) of them. Furthermore, although the real meaning of the 46I is unknown, caution should be used in protease inhibitor use. In conclusion, as reported by other authors, we identified a CRF02_AG transmission cluster among MSM and another one in a heterosexual couple. Considering the peculiarities of non-B viruses, especially in the diagnostic field, knowing the prevalence of non-B subtypes, CRFs and clusters can be crucial for a better epidemiological, diagnostic and clinical management of the infection.

No conflict of interest

Abstract: 81

Viral Evolution & Genetic Diversity

Patterns of HIV-1 subtype A1 epidemic dispersal in Spain

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Background: Based on sampled sequences from 26 cities across 12 Spanish states, the prevalence of subtype A1 was estimated to be 2.1% (95% CI: 1.76%-2.47%). The aim of the current study was to estimate the levels of regional clustering for HIV-1 subtype A1 by means of phylogenetic analysis using all available subtype A1 sequences on a global scale.

Materials & Methods: We studied 132 non-recombinant subtype A1 sequences in the protease and reverse transcriptase regions. Sequences were isolated from HIV-1 diagnosed patients during 2000-2014 across Spain. Sequences were merged from two datasets: a) CoRIS (2004-2013); b) Eastern Andalusia Resistance Cohort (2000-2014). Maximum-likelihood phylogeny reconstruction with bootstrap evaluation was conducted in RAxML, using GTR as nucleotide substitution model and gamma (Γ) distribution of rate variability among sites. Phylogenetic analysis was performed on the 132 subtype A1 sequences together with all globally sampled subtype A1 sequences available on the Los Alamos HIV-1 sequence database (N=5856) tested for recombination. Further analysis based on Bayesian inference method was performed on the monophyletic clusters with bootstrap evaluation lower than 75%. A multivariate binomial logistic regression model was fit to a subset of the original data, consisting of 83 complete observations. Presence in monophyletic groups was the binary outcome variable, while age, sampling
region, risk group and gender were chosen as possible explanatory variables.

**Results:** HIV-1 subtype A1 prevalence differed across the regions of Spain we sampled from (p=0.017). Catalonia (N=14, 4.1%, 95% CI: 2.2%-6.7%), Balearic Islands (N=3, 2.9%, 95% CI: 0.6%-8.2%), Andalusia (N=86, 2.5%, 95% CI: 2.0%-3.1%), Basque Country (N=5, 2.7%, 95% CI: 0.9%-6.3%) and Navarre (N=2, 2.5%, 95% CI: 0.3%-8.6%) were the regions where A1 appeared most prevalent. The phylogenetic analysis revealed that 69.4% of the sampled subtype A1 sequences, formed 25 monophyletic clusters. The size of the clusters ranged between 2 and 22 sequences. The largest monophyletic cluster consisted of 22 sequences, 91% of which originated from Andalusia. We also found a monophyletic cluster (N=8) consisting of sequences from men having sex with men (MSM). The majority of them (87.5%), had been sampled from Madrid. The phylogenetic analyses revealed Albania as the most possible source of this A1 subepidemic. Regional clustering was detected at different percentages across Spain. Madrid (82.6%) and Andalusia (67.9%) were characterized by high monophyly levels, while Catalonia (38.5%) was moderately monophyletic. Valencia (N=3), Galicia (N=2), Navarre (N=2) and Balearic Islands (N=3) provided less than 5 sequences. In multivariate analysis, risk group (MSM) was associated with regional clustering (p<0.05), having adjusted for the rest of the incorporated variables.

**Conclusions:** Our analysis suggests considerable differences in the patterns of HIV-1 subtype A1 regional clustering in Spain. Notably, for Madrid and Andalusia we found strong evidence for regional clustering associated mainly with transmissions among MSM. Notably, Madrid provides an area for considerable secondary non-B subtype transmissions; a finding with public health importance for Spain.

**No conflict of interest**

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**Abstract: 82**

**Viral Evolution & Genetic Diversity**

**HIV-1 CRF02_AG epidemic dispersal in Spain**

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**Background:** Circulating Recombinant Form 02_AG (CRF02_AG) was the most prevalent among the HIV-1 non-B clades with the highest prevalence in Spain. Previous studies estimated that the prevalence was 5.97% (95% CI: 5.41%-6.57%) across Spain. The aim of the current study was to estimate the levels of regional clustering for HIV-1 subtype CRF02_AG and to assess parameters associated with regional dispersal across Spain.

**Materials & Methods:** We studied 391 CRF02_AG sequences available in the protease and reverse transcriptase regions. Sequences were isolated from HIV-1 diagnosed patients during 2000-2014 from 10 states of Spain. Specifically, patients' samples were merged from two datasets: a) CoRIS (2004-2013); b) Eastern Andalusia Resistance Cohort (2000-2014). Maximum-likelihood phylogeny reconstruction with bootstrap evaluation was conducted in RAxML, using the GTR model and gamma (Γ) distribution. Phylogenetic analysis was performed on the 391 CRF02_AG sequences along with all globally sampled CRF02_AG sequences available on Los Alamos HIV-1 database (N=3302) as references. Further analysis based on Bayesian method was performed on the monophyletic clusters with bootstrap evaluation lower than 75%. A multivariate binomial logistic regression model was fit to a subset of the original data, consisting of 208 complete observations. Presence in monophyletic groups was the binary outcome variable, while age, sampling
region, risk group and gender were chosen as possible explanatory variables.

**Results:** HIV-1 subtype CRF02_AG prevalence differed across Spanish regions we sampled from \( p<0.001 \). La Rioja \( (N=11, 16.7\%, 95\% CI: 8.6\%-27.9\%) \), Navarre \( (N=11, 13.6\%, 95\% CI: 7\%-23\%) \), Valencia \( (N=22, 8.4\%, 95\% CI: 5.3\%-12.4\%) \) and Andalusia \( (N=240, 7\%, 95\% CI: 6.2\%-8.0\%) \) were the regions where CRF02_AG appeared most prevalent. Phylogenetic analysis revealed that the 61.7% of the subtype CRF02_AG sequences formed 81 monophyletic clusters, with a range of 2 to 79 sequences. For the largest monophyletic cluster \( (N=79) \), 49.4% of the clustered sequences originated from Madrid and most sequences \( (51\%) \) had been isolated from men having sex with men (MSM). Notably regional dispersal as suggested by monophyletic clustering differed across Spain. Specifically, 72.7% of sequences sampled in Basque area belonged to monophyletic clusters, followed by sequences from Madrid \( (72\%) \) and Valencia \( (68.4\%) \) and Andalusia \( (46\%) \), Navarre \( (45.5\%) \), Catalonia \( (44.4\%) \), La Rioja \( (36.4\%) \) and Balearic Islands \( (33.3\%) \) revealed the lowest monophly levels. For Galicia \( (N=3) \), Canary Islands \( (N=1) \) and Balearic Islands \( (N=3) \) less than 5 sequences were available. In multivariate analysis, MSM risk group was positively associated with regional clustering \( (p<0.001) \), having adjusted for the rest of the incorporated variables.

**Conclusions:** Our analysis suggests considerable variation in the patterns of HIV-1 subtype CRF02 AG regional clustering in Spain. We provide evidence that CRF02 AG has been introduced as a result of multiple introductions in Spain, following regional dispersal in several cases. Areas with the most extensive regional dispersal were Basque area followed by Madrid and Valencia. Our study suggests for the first time, that CRF02 AG transmissions are due to regional dispersal at a considerable rate in Spain. Due to the central geographic location of Spain and its popularity as a traveling destination, this raises concern regarding the risk of further dispersal of CRF02 AG to other European areas.

**No conflict of interest**

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**Abstract:**

**Viral Evolution & Genetic Diversity**

**A Population-Structured HIV Epidemic: Roles of Risk and Ethnicity**

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**Background:** A general aspect of the HIV epidemic in developed areas is that the infection spreads with different dynamics across the ethnically and culturally diverse populations, reflecting differences in risk behavior. HIV in Israel started with a subtype-B epidemic among men who have sex with men (MSM), followed in the 1980s and 1990s by introductions of subtype-C (largely acquired by heterosexual transmission) from Ethiopia and subtype-A from the former Soviet Union (FSU, largely acquired by intravenous drug use). The epidemic matured over the last 15 years without additional large influx of exogenous infections.
Between 2005 and 2013 the number of infected MSM increased 2.89-fold, compared to 1.63-fold and 1.27-fold for IVDU and Ethiopian-origin residents, respectively. Understanding the underlying dynamics is essential for effective public health planning.

Materials & Methods: We analyzed demographic and virologic data from 1,427 drug-naive HIV-infected individuals diagnosed with HIV-I during 1998–2012. HIV phylogenies were reconstructed with Maximum-likelihood and Bayesian methods.

Results: Subtype-B viruses, but not A or C, demonstrated a striking number of large clusters with common ancestors, including some suggesting presence of transmission networks. Transmitted drug resistance was highest in subtype B (13%). In cross-ethnic transmission, demonstrated by the presence of Israeli-born with non-B virus infections and FSU immigrants with non-A, MSM represented a frequent risk factor.

Conclusions: Reconstructed phylogenetic trees, demonstrating substantial grouping in subtype B but not in non-MSM subtype-A and not in subtype C, reflect differences in transmission dynamics linked to risk-behavior. Particular identifiable groups appear to fuel ongoing epidemic spread more than others. Cross-ethnic spread was due to multiple independent introductions, with a prevalent role of MSM in transmissions. Such data provide a baseline to track epidemic trends and will be useful in informing and quantifying efforts to reduce HIV transmission.

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