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Abstracts

1

Characterization of SARS-CoV-2 epidemic and transmission dynamics in children over the four COVID-19 waves

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Background: No definite data were published so far regarding SARS-CoV-2 epidemic in children and potential correlation with clinical presentation. Here, we aim to fill these gaps thanks to the characterization of viral diversity, transmission dynamic and clinical presentation of 1291 SARS-CoV-2 positive children over the four COVID-19 waves in Italy.

Material and Methods: This study included 1291 SARS-CoV-2 positive nasopharyngeal-swabs, obtained from patients aged ≤12 years referred for the diagnosis at Bambino Gesù Children Hospital IRCCS from March 2020, to February, 2022. Whole SARS-CoV-2 sequences, obtained by Multiplex PCR system (Illumina MiSeq) were analysed by Maximum Likelihood and Bayesian coalescent methods, to define the phylogenetic structure of the paediatric epidemic against time, and to define potential transmission clusters. To describe the relatedness of the paediatric sequences against SARS-CoV-2 diversity, 722 SARS-CoV-2 sequences belonged to adolescent and adult population (>12 years) living in the same area of paediatric population were also included.

Results: Among paediatric individuals, 722 (55.9%) patients were male, with a median age of 2 (Interquartile-range, IQR: 1-6) years. Mild infections were the most prevalent (82.8%), followed by moderate/severe (10.9%), and asymptomatic infections (6.3%). 184 (14.3%) patients were hospitalized and 108 (16.1%) had comorbidities.

At least five clades circulated widely in the paediatric population during the four COVID-19 waves. Most of SARS-CoV-2 infections (36.6%) belonged to delta clade (B.1.617.2 and AY sublineages), followed by omicron (26.6%), EU (19.6%), alpha (9.8%) and gamma (2.7%) clades. At SARS-CoV-2 diagnosis, delta and gamma clades were characterized by higher SARS-CoV-2 RNA load respect to omicron, alpha and EU clades (viral load: 8.3 [7.3-8.7] vs 8.0 [6.1-8.6] vs 7.8 [7.1-8.3] vs 7.7 [6.2-8.5] vs 7.2 [6.1-8.4] copies/mL, respectively, P<0.0001). No significant association was found between clades and COVID-19 presentation, even if a lower number of moderate/severe cases were found during alpha epidemic (4.0% vs 13.0%). 12.6% of pediatric SARS-CoV-2 sequences were involved in local clusters, 6 of them large (≥10 sequences) and involving mainly alpha and delta clades, and 5 small (5-7 sequences) clusters, involving only the omicron clade. No cluster was significantly associated with moderate/severe manifestations, and no cluster carried mutations able to increase pathogenicity, except for one delta chain, characterized by the Spike-Q677H mutation known to enhance viral infectivity. Adult population was present exclusively in the 6 large chains.

Multivariate logistic regression analysis showed that age <5, gamma and delta clades were positively associated with transmission clusters (adjusted odds ratio, AOR [95% CI]: 1.51 [1.31–1.84] P=0.008; 6.51 [2.71-15.660] P<0.0001; 2.72 [1.45-5.11], P=0.002). Differently, comorbidities and alpha clade resulted positively and negatively associated with a moderate/severe COVID-19 presentation (AOR: 4.59 [2.69-7.82] P<0.001; 0.33 [0.12-0.92] P=0.034).

Conclusions: This study provides an increased knowledge of SARS-CoV-2 dynamic in children over the four COVID-19 waves, showing definite correlations among community transmission, children's age, and specific variants of concern (also characterized by enhanced infectivity). These results also emphasise that the molecular surveillance in this partially vaccinated population will be essential to closely monitor SARS-CoV-2 evolution and to define potential correlations between SARS-CoV-2 variability and disease manifestations.

2

Circulation of sars-cov-2 variants in central Italy: spike variability characterization by deep-sequencing

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Background: This study aims at characterizing SARS-CoV-2 variants circulating in central Italy by Next Generation Sequencing (NGS).

Methods: Nasopharyngeal-swabs (NS) of SARS-CoV-2 infected individuals were collected between June 2020 to January 2022. NGS of spike (S) gene was obtained by home-made protocol for S full-length or by COVIDSeq Assay (Illumina-Inc), using MiSeq platform. Spike mutations were defined according to the frequency prevalence as major (>90%), intermediate (>20-80%), minor (2-20%).
Additional mutations were defined as those not present in the consensus sequence of each identified variant.
All individuals had NS RealTime-PCR for envelope(E), nucleocapsid(N) and RNA-dependent-RNA-polymerase(RdRp)/Spike (S) genes with Cycle-Threshold(Ct) values <35.

Results: Deep-sequences were obtained for 433 individuals, 233 (53.8%) by Spike home-made protocol and 200 (46.2%) by whole-genome-sequencing; 250 (57.7%) were males, with median(IQR) age of 64 (51-73) years, 405 (93.5%) were Italian; 166 (38.3%) were hospitalized (80.1% with pneumonia). Median(IQR) NS Ct of E-N-RdRp/S was 24(20-27) 23(19-27) 25(20-28), respectively.
Overall, 387(89.4%) individuals carried a variant of concern (VOC), with Alpha/Gamma/Beta/Delta/Omicron variants detected from January/February/April/June/December 2021, respectively.
Alpha-VOC was observed in 118(30.5%) individuals, 22.9% carrying also ≥1 additional-major spike-mutation. Gamma-VOC was detected in 55(14.2%) individuals, 41.8% with ≥1 additional-major mutation (in particular S640F and G1219V). Beta-VOC was observed in 7(1.8%) individuals,14.3% with ≥1 additional-major mutation. Delta-VOC (28 sublineages identified) was observed in 158(40.8%) individuals, 34.8% with ≥1 additional-major mutation. Finally, Omicron-VOC was detected in 49(12.7%) individuals, 46.9% carrying ≥1 additional-major mutation [31(63.3%) BA.1, 45.2% with ≥1 additional-major mutation; 18(36.7%) BA.1.1, 50.0% with ≥1 additional-major mutation].
Also minor mutations were observed in 90(20.8%) individuals median(IQR) number:2(1-3).
Stratifying individuals according to type of unknown additional mutations [only major-additional (Ma, N=157), minor+/-major (mMa, N=90), and without-additional mutations (Wa, N=186)], individuals with mMa mutations had higher Ct-values than other groups: [E/N/RdRp/S Ct median(IQR) mMa: 26(22-29)/25(21-28)/26(26-29) vs Ma: 22(20-25)/21(18-24)/23(21-26) vs Wa: 21(18-24)/20(17-24)/22(19-26), all p<0.001]. Interestingly, Δ days from first COVID-19 symptoms to NS sampling was significant longer in hospitalized patients with mMa than others [median (IQR) mMa: 8(5-10) days vs Ma: 5(4-7) days vs Wa: 5(4-7) days, p=0.001].
Among 166 hospitalized patients, 64.5% were males, median(IQR) age of 65(54-75) years; 126(75.9%) carried a VOC: Alpha/Gamma/Delta/Omicron 36.1% 15.9% 42.9% 3.1%, respectively; no Beta-VOC was observed. By evaluating the percentage of hospitalized patients within each specific VOC we

found: 48/118 (40.7%) individuals in Alpha, 20/55 (36.4%) in Gamma, 54/158 (34.2%) in Delta, 4/49 (8.2%) in Omicron, p=0.005.
Interestingly, 170 individuals were vaccinated: 82(48.2%) males, with a median(IQR) age higher than in unvaccinated individuals [67(54-77) vs 62(50-71), p=0.004]. Also in this case, within each VOC we observed a different prevalence of vaccinated individuals: 15/118 (12.7%; 5/15 hospitalized) in Alpha, 6/55 (10.9%; 3/6 hospitalized) in Gamma, 103/158(65.2%; 43/103 hospitalized) in Delta, 44/49 (89.8%; 4/44 hospitalized) in Omicron, p<0.0001.

Conclusion: Starting from January 2021, a large variety of SARS-CoV-2 variants and sublineages were detected. This study underlines how SARS-CoV-2 has changed over time and how vaccination strategy has contributed to making this infection clinically less severe and with less impact on hospitalization. A lower hospitalization in Omicron infected individuals respect to other VOCs was observed, this in line with a higher vaccination rate in Italy during its emergence.

3

SARS-CoV-2 mutations and variants may muddle the sensitivity of COVID-19 diagnostic assays

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Background: The world is still being ravaged by SARS-CoV-2 and its variants, despite effective measurement including extensive diagnostic testing. The performance of diagnostic polymerase chain reaction (PCR) assays can be impacted by SARS-Cov2 variability and is highly dependent on the full complementarity between PCR primers/probes and viral target templates. Here, we investigate the genetic variability of SARS-CoV-2 regions recognized by primers/probes utilized by PCR diagnostic assays based on nucleotide mismatching analysis.

Material and Methods: We evaluated the genetic variation in the binding regions of 73 primers/probes targeting the Nucleocapsid (N, N=36), Spike (S, N=22), and RNA-dependent RNA-polymerase/Helicase (RdRp/Hel, N=15) of the publicly available PCR-based assays. About 4.9 million high-quality SARS-CoV-2 genome sequences were retrieved from GISAID and were divided into group-A (all except Omicron, >4.2 million) and group-B (only Omicron, >558 thousand). The sequences were aligned against NC_045512.2 and the binding sites of each primer/probe were marked in the alignments to calculate mismatches prevalence. Only primers/probes mismatches observed in >1% of viral sequences (corresponding to >41000 group-A and >5500 group-B sequences) were considered.

Results: In group-A sequences, a large range of variability in primers/probes binding regions in most PCR assays was observed. Particularly, 87.7% (64/73) of primers/probes displayed ≥1 mismatch with their viral targets, while 8.2% (6/73) contained ≥2 mismatches, and 2.7% (2/73) ≥3 mismatches.
Notably, most primers/probes with ≥1 mismatch target the N gene (54.6%; 35/64), followed by the S (29.7%; 19/64), and the RdRp/Hel (15.6%; 10/64). The 6 primers/probes with ≥2 mismatches target mostly the N gene (66.7%; 4/6), then the S (16.7%; 1/6), and the RdRp/Hel (16.7%; 1/6). Importantly, the 2 primers displaying ≥3 mismatches, target the S and N genes. Notably, the highest numbers of mismatches in N primers/probes were due to the mutations R203K/G204R characterizing Alpha and Gamma, R203M characterizing Delta, and T205I characterizing Beta, while in the S gene the deletion H69del/V70del characterized several variants.
In group-B sequences, 32.9% (24/73) of primers/probes were characterized by ≥1 mismatch, 13.7% (10/73) by ≥2 mismatches, and 5.5% (4/73) by ≥3 mismatches. Remarkably, most primers/probes with ≥1 mismatch target N gene (45.8%; 11/24), followed by S gene (33.3%; 8/24), and RdRp/Hel (20.8%; 5/24). The 10 primers/probes with ≥2 mismatches target mostly the S (50.0%; 5/10), followed by N (30.0; 3/10), and RdRp/Hel (20.0%; 2/10). Importantly, ≥3 mismatches were found in 4 primers targeting the S and N genes. The highest numbers of mismatches in S primers/probes were due to H69del-V70del and Omicron BA.2 specific mutations T19R, L24S, and P25del, while in the N gene were due to the mutation R203K/G204R and the deletion E31del-R32del-S33del in all Omicron sublineages.

Conclusion: The high rate of single and multiple mismatches, found in the target regions of molecular assays, worldwide used for SARS-CoV-2 diagnosis, reinforces the need to optimize and constantly update these assays according to SARS-CoV-2 genetic evolution and the future emergence of novel variants. This will assure the full efficacy of diagnostic assays, thus contributing to the goal of limiting viral transmission chains and contrasting viral spread.

4

Seroprevalence of SARS-CoV-2 infection and evolution of humoral immune response in PLWHIV in the Ile-de-France area

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Background: As an immunodeficiency disease, HIV infection could be associated with a similar or a higher risk of acquiring COVID-19 and/or worse outcomes. Based on current knowledge, it is hence unclear if data about the prevalence of SARS-CoV-2 infection from general population can be extrapolated to patients living with HIV (PLWHIV). Our main objectives were to determine the seroprevalence of COVID-19 infection in PLWHIV followed at the Pitié-Salpêtrière hospital during the initial COVID-19 outbreak and to identify associated factors to be infected with SARS-CoV-2. Then, we studied the kinetics of anti-SARS-CoV-2 antibodies (anti-N and anti-RBD) in those patients with positive serology at inclusion.

Methods: In this longitudinal prospective cohort study, we included all PLWHIV seen at hospital between April 2020 and September 2021. Serum samples were tested for IgG targeting the nucleocapsid (N) and all positive samples were tested for IgG targeting the receptor-binding domain (RBD) of spike (S) protein and IgA targeting S. Factors associated with positive IgG anti-N were identified using a logistic regression model.

Results: A total of 1,901 PLWHIV were included: 64.4% male, median age: 53 years (44-60). At inclusion, 254 (13.4%, 95% CI 11.9, 15.0) had positive IgG anti-N and among them, 88.2% and 64.1% had positive IgG anti-S and IgA anti-S, respectively. The mean levels of IgG anti-N, IgG anti-S and IgA anti-S were 3.95 (standard error: 0.16), 199.4 BAU/mL (21.7) and 3.14 (0.21), respectively. Over one year, levels of IgG anti-N and anti-S decreased significantly (-2.83p<0.0001 and -94.9 BAU/mL p= 0.0010 respectively), while IgA anti-S level increased significantly (+2.97 p=0.0032). Multivariable analysis showed that Sub-Saharan African patients were more likely to have positive IgG anti-N in comparison with patients from other countries (OR:4.78 (95% CI 3.39, 6.73), p<0.0001), while active smoking was a protective factor (OR: 0.57 (95% CI 0.36, 0.90), p=0.0176).

Conclusion: In this population of PLWHIV, a high SARS-CoV-2 seroprevalence was observed. The higher seroprevalence observed in sub-Saharan Africa patients highlights the need of an implementation of health and prevention system taking care of vulnerable people especially PLWHIV. More investigations are needed to understand the association between smoking and COVID-19 and to study the effectiveness of antiretroviral drugs for the COVID-19 treatment and protection.

5

Impact of SARS-CoV-2 omicron and delta sub-lineage AY.4.2 variant on neutralization by sera of patients treated with different licensed monoclonal antibodies

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Objectives: Newly emerging SARS-CoV-2 variants have the potential to escape monoclonal antibodies (mAbs) which were designed to halt the original wild type strain. In this work we have assessed the ex vivo inhibition of omicron and delta variants by sera obtained from unvaccinated patients treated with one of the mAb preparations licensed for post exposure prophylaxis (PEP) among bamlanivimab/etesevimab, casirivimab/imdevimab and sotrovimab.

Methods: Of 30 patients studied (14 males, 59±18 years) one was asymptomatic while the others had mild symptoms such as cough (n=19), fever (n=17), headache (n=13), gastrointestinal symptoms (n=4) and dyspnea (n=2). Patients were randomly treated with bamlanivimab/etesevimab (n=10), casirivimab/imdevimab (n=10), or sotrovimab (n=10), 3.5±1.7 days from diagnosis. Paired sera were obtained before (as baseline control for seronegativity) and 1 hour post mAb infusion (as a source for mAbs) and used in a live virus neutralization assay in VERO E6 cells with automated cell viability readout. Challenge viral isolates included the wild type (EPI_ISL_2472896), delta (EPI_ISL_2840619), delta sub-lineage AY.4.2 (EPI_ISL_6943992) and omicron (EPI_ISL_6777160). Neutralizing antibody (NtAb) titers were defined as the reciprocal value of the sample dilution that showed a 50% protection of virus-induced cytopathic effect (ID50). Statistical analyses were performed using IBM SPSS Statistics, version 20. Sera with ID50 <10 were defined as negative and scored as 5 for statistical analysis and data were expressed as median [IQR] according to statistical distribution of data.

Results: None of the patients required hospitalization. All pre-infusion sera were negative for SARS-CoV-2 NtAb activity. In post-infusion sera, casirivimab/imdevimab, bamlanivimab/etesevimab and sotrovimab showed activity against the wild type variant (19,814 [17,459-23,471]; 6,792 [4,736-8328] and 456 [259-592] ID50), the delta variant (58,858 [41,585-79,971]; 12,145 [10840-18667] and 1023 [798-1134] ID50) and the delta AY.4.2 (58,602 [42,941-82960]; 11,067 [10757-12614] and 1,333 [708-1714] ID50). Notably, sotrovimab was the only active treatment against the omicron variant (216 [118-233] ID50). Within each individual treatment group, the NtAb titers to delta and delta AY.4.2 variants were significantly higher than those to wild type (p=0.008 for AY.4.2 vs. wild type with sotrovimab; p=0.005 for all other comparisons). NtAb titers to wild type, delta and delta AY.4.2 variants were higher than those to omicron within all the individual treatment groups (p=0.005 for all comparisons). Comparing treatments, casirivimab/imdevimab neutralizing titers were significantly higher than bamlanivimab/etesevimab and sotrovimab against the wild type, the delta and the delta AY.4.2 variants and bamlanivimab/etesevimab neutralizing titers were significantly higher than sotrovimab for the same variants (p<0.001 for all comparisons).

Conclusions: Currently mAbs licensed for PEP retain activity not only against delta variant, as previously showed, but also against the delta sublineage AY.4.2 recently circulated in Italy, implying that the additional Y145H and A222V mutations have no impact on neutralization by these mAbs preparations. Most importantly, our results support full escape of approved PEP mAbs cocktails by the omicron variant, while sotrovimab retains activity against all the variants tested, including omicron, although reduced by 2.7-fold. Since omicron has rapidly replaced the circulating variants, the mAbs arsenal should be updated accordingly.

6

Levels and durability of humoral and T-specific responses after the booster dose of mRNA BNT162b2 vaccine in residents of a Long Term Care Facility

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Background: Residents of long-term care facilities (LTCFs) have been dramatically affected by the COVID-19 pandemic at global scale as older age and comorbidities pose a high risk of severe disease and death. The aim of the study was to assess durability of specific antibody response to SARS-CoV-2 after three doses of BNT162b2 vaccine.

Methods: We analyzed 117 subjects of which 92 residents and 25 Health Care Workers (HCWs) of Pio Albergo Trivulzio, the largest Italian LTCF. SARS-CoV-2 Spike-specific IgG (S-IgG) antibodies were evaluated 2 and 6 months after first cycle (2 doses) and 2 months before and after booster dose. At first time point samples were concomitantly screened for SARS-CoV-2 nucleocapsid-specific IgG (N-IgG). Response to vaccination, estimated as anti-SARS-CoV-2 spike protein receptor binding domain IgG, was arbitrarily classified by stratifying levels of anti-S IgG values in 4 levels: >1,000, 101-1,000, 1-100 and <1 BAU/mL, defined as high, medium, low and null response, respectively. T cell responses were evaluated thought QuantiFERON SARS-CoV-2 interferon gamma (IFN-γ) before and after the administration of third dose.

Results: Among LTCF residents 85.9% (n=79) were female and the median age was 88.6 years (IQR: 83.1-93.1). Median levels of anti-S IgG have significantly decreased from 7,385 BAU/mL (IQR: 32.8-7,005) to 1,482 (IQR: 33.3-5,970) at 2 and 6 months after first cycle, respectively. Subsequently titers increased to 3,675 (IQR: 2,003-6,213) and 9,823 (IQR: 3,742-12,005) at baseline and 2 months after the booster dose.
Overall, a significant association was observed between null and suboptimal anti-S IgG response and age >80 years at first, second and third time point. SARS-CoV-2 anti-nucleocapsid antibodies resulted positive in 40.2% of cases with a significant different distribution among residents and HCWs (45.6% vs. 20%, p=0.03). Residents with positive nucleocapsid serology showed a significantly higher response considering all levels of serologic responses at all time points (p<0.001).
Residents with ischemic heart diseases showed a reduced degree of response to vaccination considering all the levels of specific antibodies at first, second and third time point (p=0.005; p=0.009; p=0.05), but not at fourth one. Chronic lung disease, diabetes, cancer, corticosteroid and anticoagulant therapies not influenced humoral response.
Proportion of patients showing IFN-γ positive response significantly increased from 22.8% (n=21) to 57.6% (n=53) in residents before and after booster dose. A higher increase was observed in HCWs (from 24%, n=6 to 72%, n=18). No significant differences were observed in IFN-γ response among subjects with or without previous exposure to COVID-19 at both time points.

Conclusions: Our data provide additional insights into the longitudinal dynamics of the immune response to BNT162b2 vaccination in elderly after the administration of first cycle and booster dose. Future studies will help to determine the longer-term effectiveness of the booster dose against current and emerging variants in elderly. Both humoral and cellular responses to SARS-CoV-2 need to be studied to understand the persistence of their protective effects.

7

Transmitted drug resistance to integrase based first-line treatment in Europe, 2018-2021

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Background: Integrase strand-transfer inhibitors (INSTIs) based regimens are recommended regimens for first-line antiretroviral therapy. Currently, there is a growing interest on rapid initiation of first-line therapy. Monitoring of transmitted drug resistance in real time is needed. Our objective has been to study the prevalence of transmitted drug resistance to the INSTIs and the NRTI backbone in newly diagnosed patients that are naïve to antiretroviral therapy (ART).

Methods: MeditRes HIV is a consortium that includes ART naïve people living with HIV that have been newly diagnosed in France, Greece, Italy, Portugal and Spain during the years 2018-2021. Reverse transcriptase (RT), protease (Pro) and Integrase were sequenced following standard methodologies in use at the participating centres. To evaluate the prevalence of surveillance drug resistance mutations (SDRM) we used the Calibrated Population Resistance (CPR) tools (integrase and RT-Pro) available at Stanford HIV website. To evaluate clinically relevant transmitted resistance, we used the Stanford v.9.0 HIVDB Algorithm.

Results: Overall, we included 2705 patients with integrase and RT data available. At diagnosis, 72% were men, median age was 37 (IQR, 30-48) and median viral load was 108.006 copies/mL (IQR, 25.350-420968); 43.7% of the patients were infected by non-B subtypes. The prevalence of INSTI SDRMs was 0.23% (T66I, n=1; T66A, n=1; E138T, n=1, E138K n=1, E92Q n=1 and R263K n=1). The prevalence of NRTI SDRMs was 3.73% (M184V n=23, 0.85%; M184I n=5. 0.18%; K65R n= 1, 0.04%; any TAMS n=72, 2.66%). Clinically relevant resistance, defined as any resistance level for Stanford interpretation >= 3, was 2.42% for INSTIs (0.18% to Dolutegravir and Bictegravir; 2.29% to Raltegravir; 2.33% to Elvitegravir), and 1.76% to the components of the NRTI backbones (0.89% to TDF/TAF; 1.81% to Abacavir; 1.15% to Lamivudine/Emtricitabine).

Conclusions: Here we describe the most recent data on transmitted drug resistance to integrase based first line regimens in Mediterranean Europe. Given the low prevalence of clinically relevant resistance to second generation integrase inhibitors and to first line NRTIs, in the years 2018-2021 it is very unlikely that a newly diagnosed patient in MeditRes countries would present with baseline resistance to a first line regimen based on second generation integrase inhibitors.

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HIV transmission clusters in Europe: A perspective view of Late presenters and Non-Late presenters

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Background: In HIV epidemics, certain risk groups contribute to the spread of HIV disproportionately from others, whether by demographic, clinical or behavioral factors. One of those groups, late presenters (LP) can contribute to the onward spread of HIV-1 virus, by individuals that were not aware of their HIV status and could be spreading the virus without knowing.

Objective: In this study, we aim to describe the socio-demographic and clinical characteristics of HIV-1 infected individuals followed in Europe and to understand the factors associated with patients in cluster. We also aim to analyze the patterns of transmission clusters (TC) in LP vs non-late presenters (NLP) populations.

Methods: Our study includes clinical, socio-demographic and genotypic information from 38531 HIV-1 infected patients from the EuResist Integrated Database (EIDB) between 1981 and 2019. In this study, information from the ARCA, AREVIR, Luxembourg, IRISCAIXA, Portugal and Russia databases were used. For the analysis of TC, subtype B, A and G were analysed. Control sequences were collected from the Los Alamos database. Sequences were aligned using VIRULIGN. Maximum likelihood (ML) phylogenies were constructed in FastTree. Putative transmission clusters were identified using ClusterPicker v1.332 (genetic distance of 0.030 and a branch support ≥0.90 aLRT)..

Results: In this analysis, 2276 (5.9%) were from subtype A, 32652 (84.7%) were from subtype B and 3603 (9.4%) were from subtype G. The median age was 23.5 (IQR: 0.0-34.0) years old and 75.5% of HIV-1 infected patients were males. The main transmission route was through homosexual (MSM) contact (36.9%) and 86.4% were originated from Western Europe. Most patients included in this study were native (84.2%) and as having chronic infection (59.6%) based on the ambiguity rate of the first genomic sequence and 73.4% had acquired drug resistance (ADR). CD4 count and viral load at diagnosis (log10) presented a median of 341 cells/mm3 (IQR 170-540) and log10 4.3 copies/mL (IQR 3.4-5.0), respectively. 51.4% of patients were classified as LP and 21.6% of patients were in cluster. Most patients from subtype B (85.6%) were in cluster compared to subtypes A (5.2%) and G (9.2%). The phylogenetic tree analysis represents the consistent clustering of MSM individuals. In the final logistic regression model for subtype A the variables associated with subtype A were older age (>56yo), individuals with a heterosexual and IDU transmission routes, patients originated from Eastern Europe and Africa, migrants and patients not presenting TDR. The final logistic regression model for subtype B had the following variables associated, younger ages (19-30yo, 31-55yo), individuals with a MSM transmission route, patients originated from Western Europe and South America and native individuals. The final logistic regression model for subtype G was associated with individuals with a heterosexual transmission route, patients originated from Africa and migrant individuals. LP were mainly out-of-cluster and MSM transmission route had more proportion of individuals in cluster when compared to the other transmission routes. NLP were mainly out-of-cluster.

Conclusion: We conclude that late presentation is still a major threat to HIV-1 transmission, and LP individuals are more in small clusters.

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HIV-1 Transmitted drug resistance and transmission clusters in newly diagnosed patients in Portugal between 2014 and 2019

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Objective: To describe and analyze Transmitted Drug Resistance (TDR) between 2014 and 2019 in newly infected HIV-1 patients in Portugal and to characterize its transmission networks.

Methods: Clinical, socio-epidemiological and risk behavior data was collected from 820 newly diagnosed patients in Portugal between September 2014 and December 2019. Sequences obtained from drug resistance testing were used for subtyping, TDR determination and transmission clusters (TC) analyses.

Results: In Portugal, the overall prevalence of TDR between 2014 and 2019 was 11.0%. TDR presented a decreasing trend from 16.7% in 2014 to 9.2% in 2016 (pfor-trend = 0.114). Multivariate analysis indicated that TDR was significantly associated with transmission route (MSM presented lower probability of presenting TDR when compared to heterosexual contact) and with subtype (subtype C presented significantly more TDR when compared to subtype B). TC analysis corroborated that the heterosexual risk group presented a higher proportion of TDR in TCs when compared to MSMs. Among subtype A1, heterosexuals reached 16.6% of TDR, followed by 14.2% in patients infected with subtype B and 9.4% in patients infected with subtype G.

Conclusion: Our molecular epidemiology approach indicates that the HIV-1 epidemic in Portugal is changing among risk group populations, with heterosexuals showing increasing levels of HIV-1 transmission and of TDR. Prevention measures for this subpopulation should be reinforced.

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Reaching the 95-95-95 targets will not eliminate HIV transmission in 2030 among individuals born in sub-Saharan Africa residing in the Netherlands

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Background: The World Health Organization (WHO) defined the 95-95-95 goals (95% know their HIV status, 95% of those diagnosed receive antiretroviral therapy, of whom 95% are virally suppressed) as key targets for ending the HIV pandemic in 2030. In the Netherlands, achieving the 95-95-95 goals is challenging among individuals from sub-Saharan Africa (SSA) (15% of individuals in HIV clinical care) as they are frequently late presenters (65% with CD4<350 cells/µl at diagnosis) and experience poor retention in care (<90%). As the impact of achieving the 95-95-95 targets on reducing new HIV infections is unknown, we estimated the epidemiological impact on the epidemic that would correspond to achieving the 95-95-95 goals by 2030 among individuals from SSA residing in the Netherlands.

Methods: A deterministic mathematical model was used including heterosexual HIV transmission, the acute stage, three chronic stages (stratified by CD4 cell count >500 cells/µl, 350-500 cells/µl, and 200-350 cells/µl), and the AIDS stage. Each stage has a different duration and infectivity. Virally suppressed individuals cannot transmit HIV to others. The model was calibrated to the Dutch HIV epidemic in 2020 and includes the number of people born in SSA (100,000), new diagnosis from 2017 to 2020 (71 in women, 53 in men), late diagnosis (CD4<350 cells/µl in 50-70% of individuals), advanced diagnosis (CD4<200 20-40% of women, and 40-60% of men), retention in care (80-90%), viral suppression (90-95%), infections acquired in country of origin (40%) and the number of individuals from sub-Saharan Africa diagnosed with HIV (1535 women, 1110 men). The epidemiological impact was evaluated as the number of infections averted during 2020-2030.

Results: In our base-case scenario including the continuation of current rates of diagnosis, retention in care and viral suppression until 2030, a median of 490 (interquartile range- IQR- 411 to 574) and 483 (IQR 388 to 599) new infections will occur over the next ten years among women and men from SSA, respectively. Compared to this base-case scenario, reaching the first 95 target, which can be achieved by annually testing at least 95% of all individuals, will have the greatest epidemiological impact and will avert 50 (IQR 33 to 72) new infections in women and 57 (IQR 38 to 79) new infections in men. Achieving the second 95 target will avert 8 (IQR 0 to 17) and 27 (IQR 16 to 41) infections in women and men, respectively. Given the currently high suppression rates among those receiving antiretroviral therapy, achieving the third 95 will have a small impact and will avert at most three new infections in both women and men. If all three 95-95-95 targets are reached, then 56 (IQR 40 to 57) new infections in women and 63 (IQR 45 to 84) new infections in men are averted, or a relative reduction in infections of 11% in women and 12% in men compared to the base-case scenario.

Conclusion: Achieving the 95-95-95 goals in individuals born in SSA residing in the Netherlands will require universal annual testing and will have modest impact on preventing new HIV infections.

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HIV reservoir quantification by combining total HIV-1 DNA assay and intact proviral DNA assay (IPDA) in one triplex digital PCR reaction

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The major obstacle to reach an HIV-1 cure is the establishment of a persistent latent reservoir that is unaffected by current HIV-1 treatment. Hence, methods are needed to accurately measure this reservoir for evaluation of curative strategies. Digital PCR (dPCR) methods are gaining interest and are used more frequently for this purpose. Several commercial platforms enable (higher order) multiplex reactions, differentiating themselves based on partitioning method and number of fluorophore channels.

In this study, the performance of a total HIV-1 DNA assay and intact proviral DNA assay (IPDA) was compared on two dPCR platforms: Bio-Rad QX200 Droplet Digital PCR System (Bio-Rad, United States) and Qiacuity One, 5-plex device (Qiagen, Germany). Additionally, we combined both assays on the Qiacuity to generate a 3-plex assay measuring intact proviral DNA simultaneously with the total HIV-1 DNA readout on the same sample, saving valuable material and reducing costs and experimental setup.

To evaluate technical performance, a 2-fold limiting dilution curve was prepared containing J-Lat genomic DNA (gDNA) spiked into HIV-1 negative PBMC gDNA, ranging from 0.625 to 20 copies (cp) input, and measured in ten replicates. Subsequently, these assays were performed in duplicate on 500 ng gDNA, extracted from CD4+ T-cells from three people living with HIV (PLHIV) on ART. Specificity or limit of Blank (LoB) was measured in 34 negative template controls (NTCs), containing gDNA from HIV-1 negative donor PBMCs.

The LoB of the total HIV-1 DNA assay resulted in 3.0 cp/reaction for the Qiacuity and 2.6 cp/reaction on the QX200, whereas, for IPDA, both systems showed a LoB of <1 intact cp/reaction. The sensitivity or Limit of detection (LoD) for total HIV-1 DNA and IPDA was determined respectively at 5 copies and 10 copies input on both platforms, based on a 100% replicate positivity rate of the lowest dilution point from the 2-fold limiting dilution curve.

In three PLHIV, total HIV-1 DNA and intact proviral DNA was quantified on the Qiacuity and QX200 to determine the ratio of intact proviral DNA and total HIV-1 DNA. In the first patient, a ratio of 6.5% (5.4/83.9 cp/input) and 0.9% (1.5/65.5 cp/input) was found on the Qiacuity and QX200, respectively. In the second patient, 2.6% (0.5/20.7 cp/input) and 3.2% (1.1/20.4 cp/input) sequences were intact. For the third patient, there were 6.4% (3.0/46.9 cp/input) and 9.4% (3.2/31.3 cp/input) of intact sequences. Quantification of total HIV-1 DNA and intact proviral DNA in the combination 3-plex assay on the Qiacuity resulted in a similar ratio as the separate runs, respectively 7.6% (5.7/74.5 cp/input), 8.4% (1.6/19.0 cp/input) and 5.3% (2.2/41.9 cp/input).

In conclusion, total HIV-1 DNA quantification and IPDA performed comparable across these two dPCR platforms and showed an LoB lower than the LoD and at the single copy range for intact viruses. Finally, we show for the first time the merit to combine a total HIV DNA and IPDA readout on the same gDNA sample on the Qiacuity system which reduces costs with a simplifies readout and lowers sampling error improving the estimation of intact HIV-1 DNA.

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The evaluation of a 5-plex “rainbow” digital PCR assays to measure intact HIV-1 proviral DNA

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With the development of the intact proviral HIV DNA (IPDA) digital PCR assay, the evaluation of intact proviral HIV DNA is rapidly adopted as a standard outcome measure in most HIV (cure) clinical trials. Since the implementation of this IPDA which uses a duplex digital PCR (dPCR) assay to measure intact HIV DNA based on 2 HIV genomic regions, several attempts were made to increase the number of genomic regions to boost the discriminative power to more accurately estimate intact HIV DNA. These efforts were limited to the combination of 3 assays into a single readout but here we take advantage of the latest development in the field of dPCR and combine up to 5 HIV regions in a so-called “rainbow” digital PCR assay (Qiacuity dPCR platform, Qiagen).

We evaluated the technical performance of this “rainbow” assay on 10-fold standard dilution curves of intact J-Lat HIV DNA in triplicate (5000 to 0.5 copies) and tested the applicability on CD4 T cell samples from 15 people living with HIV (PLWH) which are virally suppressed and on treatment (mean 5y+/-0.3). Peripheral blood mononuclear cells were isolated from whole blood by Lymphoprep density gradient centrifugation, followed by CD4 T cell isolation via negative bead-based selection (STEMCELL Technologies) and genomic DNA extraction via the QIAamp column-based DNA kit (Qiagen). The performance of the 5-plex dPCR assay, designed in the genomic regions of the 5LTR, PSI, POL, GAG and ENV, was assessed in terms of linearity and sensitivity on the standard curves. dPCR was perfomed at 600 ng input DNA and data analysis and quantification was performed with the established ddpcRquant algorithm.

The technical performance of all 5 individual assays on the J-Lat standard curves showed significant correlation and high linearity down to 5 copies input (R2>0.99), which marks the limit of quantification for all these assays in this setup. Limit of blank (LOB) analysis based on the negative template controls showed a LOB <1 intact copy irrespective of considering 2, 3, 4 or 5 regions to define intact HIV DNA. Overall, for all patients at least 2 regions could be detected across the 5 regions and the number of patients with detectable intact HIV sequences dropped from 10/15, 8/15, 6/15 to 5/15 when the number of regions was increased from two to five regions, respectively.

Here, we present the combination of up to 5 assays into a single “rainbow” digital PCR reaction and evaluate its technical performance on cell line and patient-derived samples. These assays can increase the information retrieved from a single (digital) PCR readout over the current existing assays at reduced cost/time and improves the estimation of potentially intact HIV DNA in patient samples. Therefore, these assays show a benefit to be used in HIV clinical trials which are aimed to determine HIV reservoir dynamics or changes upon treatment challenges

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Temporal trend of drug-resistance and APOBEC editing in PBMC genotypic resistance tests from HIV-1 infected virologically suppressed individuals.

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Background: The evaluation of HIV-1 drug-resistance in PBMCs has been increased in clinical practice in the last years, especially in subjects with poor information about previous resistance. Genotypic resistance testing (GRT) in PBMCs is also useful to explore APOBEC editing in HIV-DNA. We aimed at evaluating the temporal trend of drug-resistance and APOBEC editing in a context of suppressed viremia.

Material and methods: We included virologically suppressed individuals for whom protease (PR)/reverse-transcriptase (RT) and integrase (IN, when available) Sanger GRTs in PBMCs were available over the period 2010-2021. In case of more than one PBMC GRT per individual, the last one was retained. Major resistance mutations (MRM) to PI, NRTI, NNRTI and INI and APOBEC-related mutations (APO-M) were evaluated through Stanford HIVdb algorithm (version 9.0). Among APO-M were considered also those substitutions related to stop codons (APO-stop) and to drug-resistance (APO-DRM). Potential changes in trends of MRM and APO-M over-time were evaluated by Chi-Squared test for trend; P-values <0.05 were considered statistically significant.

Results: Overall, 1126 individuals with a PBMC GRT were included (724 for PR/RT/IN; 402 for PR/RT). At GRT, patient's characteristics [median (IQR)] were: age: 50 (43-56) years; time of virological suppression: 44 (4-98) months; number of regimens experienced: 4 (2-7); nadir CD4 count: 180 (60-307) cells/mm^3; zenith viremia: 5.3 (4.7-5.7) $\log_{10}$ copies/mL. Around half of individuals (45.2%) were previously exposed to INIs (raltegravir: 24.2%; dolutegravir: 22.4%; elvitegravir: 6.7%; bictegravir: 2.4%) and were highly treatment experienced (46.5%).
Concerning drug-resistance, 35.2% of individuals harboured at least one MRM (23.4% to NRTI, 18.8% to NNRTI, 7.7% to PI and 1.4% to INI). APO-M were observed in 11.4% and 15.4% of individuals in PR/RT and IN, respectively, while APO-stop were observed in 6.2% and 5.2% in PR/RT and IN, respectively. APO-DRMs related to PI, NRTI, NNRTI and INI were observed in 3.7%, 9.7%, 3.7% and 4.4% of individuals, respectively. From 2010 to 2021 no significant changes were found in the proportion of individuals harbouring MRM to any drug-class (37.5% to 30.8%), to PI (5.0% to 6.7%), NRTI (17.5% to 17.9%), NNRTI (25.0% to 16.5%) and to INI (0.0% to 1.4%). No significant changes in the prevalence of specific MRMs were found over-time. Concerning APOBEC editing in PR/RT, no significant changes in trends of APO-M (7.5% to 7.6%), APO-Stop (7.5% to 4.0%), APO-DRM related to PI (5.0% to 2.2%), NRTI (7.5% to 8.9%) and NNRTI (7.5% to 1.8%) were observed from 2010 to 2021. No significant changes in the prevalence of specific APO-DRMs were found over-time. Concerning APOBEC editing in integrase, a slight increase of APO-M (11.1% to 16.1%), APO-Stop (3.7% to 5.7%) and APO-DRMs (3.7% to 6.8%) was observed, however without statistical significance. Among specific INI APO-DRMs, only G163R significantly increased from 2012 to 2021 (from 0% to 4.7%, P=0.008), while G140R significantly decreased (3.7% to 0%, P=0.016). Individuals who were previously exposed to INI

showed a similar prevalence of INI APO-DRMs compared to others (4.4% vs. 4.5%).

Conclusions: In virologically suppressed individuals, resistance in PBMC and the extent of APOBEC editing were generally stable in the last decade. Interestingly, a slight increase of APOBEC editing in integrase was observed, particular with a G163R significative increase. The low and stable prevalence of APO-Stop underlines that Sanger HIV-DNA GRT provides reliable information to manage treatment switch in individuals under virological control.

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Comparison of Markers of Inflammation and Associated Baseline Variables in Virologically Suppressed Participants in the TANGO Study at Week 144

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Background: Chronic inflammation is associated with increased risk of age-related diseases. PLHIV have multiple etiologies of acute and chronic inflammation. Highly effective ART-induced HIV suppression reduces some measures of HIV-related inflammation and immune activation, but not necessarily to levels observed in people without HIV. The TANGO Phase III, randomized, open-label study demonstrated non-inferior virologic efficacy (HIV-1 RNA ≥50 c/mL by Snapshot algorithm) of switching to a 2-drug regimen (2DR) of DTG/3TC vs continuing a 3- or 4-drug TAF-based regimen (TBR) in virologically suppressed adults at 144 weeks. In addition, using the more stringent measurement of HIV-1 RNA <40 c/mL and Target Not Detected (TND), at Week 144 (WK144) a high and similar proportion of participants had TND in the DTG/3TC and TBR arms (76% [279/369] vs 72% [267/372], respectively). As previously reported, changes in inflammatory biomarkers from baseline to WK144 were small and similar with no consistent trend between arms. In this post-hoc analysis, we present the adjusted comparison of WK144 inflammatory biomarker levels between treatment arms in the TANGO study.

Material and Methods: Inflammatory biomarkers including D-dimer (nmol/L), IL-6 (ng/L), hsCRP (mg/L), soluble CD163 (sCD163, µg/L) and soluble CD14 (sCD14, ng/L) were measured at baseline, and WK144 with testing conducted at Q2 Solutions. Using a multivariate ANCOVA model adjusting for baseline variables, loge-transformed WK144 biomarker levels were compared between treatment arms and associations with baseline variables were evaluated as fixed effects.

Results: WK144 geometric means (95% CI) were: 1.59(1.49,1.69) vs 1.56(1.47,1.66) for D-dimer, 1.73(1.58,1.89) vs 1.58(1.46,1.72) for IL-6, 1.11(0.98,1.26) vs 1.13(1.00,1.28) for hsCRP, 1.18(1.11,1.25) vs 1.28(1.21,1.35) for sCD14x106, and 559.11(534.93,584.38) vs 533.64(510.80,557.50) for sCD163 in the DTG/3TC and TBR arms, respectively. Multivariate analyses demonstrated that WK144 levels of D-dimer, hsCRP and sCD163 were similar between treatment groups: ratios (95% CI) of biomarker WK144 levels with DTG/3TC vs TBR were 1.04(0.96,1.12), 0.98(0.84,1.15) and 1.04(0.99,1.10) respectively. sCD14 level was lower 0.92 [(0.85,1.00), p=0.040] and IL-6 appeared higher 1.11 [(1.00,1.24), p=0.053] in the DTG/3TC vs TBR group. Across biomarkers, higher baseline biomarker values were strongly associated with higher WK144 levels. Increasing age appeared to be associated with higher D-dimer, sCD163 and IL-6 levels but not hsCRP or sCD14. Obese BMI at baseline was associated with higher IL-6, hsCRP and D-dimer at WK144. Other factors such as smoking were associated with a higher level of IL-6; elevated triglycerides and female gender appeared to be associated with higher hsCRP level at WK144; sex at birth and race were associated with sCD14; and baseline viral load was associated with D-dimer and sCD14.

Conclusions: Various baseline factors were independently associated with each inflammatory biomarker, indicating the multifactorial aspect of the inflammatory response. WK144 biomarker levels were low and comparable between 2DR and 3DR treatment groups, reflecting the similar, robust viral suppression observed using the stringent TND endpoint. These results continue to support the high potency and durability of 2DR and do not indicate increased inflammation after switching to DTG/3TC versus continuing a TBR over 144 weeks.

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HIV-1 Integrase Resistance Associated Mutations and the Use of Dolutegravir in Sub-Saharan Africa: A Systematic Review and Meta-Analysis

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Background: As sub-Saharan Africa (SSA) countries are transitioning to dolutegravir (DTG)-containing antiretroviral therapies, baseline data are required for optimal monitoring of therapeutic response. In this frame, we sought to generate up-to-date evidence on the use of integrase-strand transfer inhibitors (INSTIs) and associated drug resistance mutations (DRMs) within SSA.

Methods: In this systematic review and meta-analysis, we included randomized and non-randomized trials, cohort-studies, cross-sectional studies, and case-reports published on INSTI or integrase DRMs in SSA. We included studies of patients exposed to DTG, raltegravir (RAL) or elvitegravir (EVG) and excluded studies of naïve patients as well as those of patients under INSTI-sparing regimens. Primary outcomes were "the rate of virological control (VC: <50 copies/ml)" and "the presence of DRMs" on INSTI-based regimens among patients in SSA. We synthesised extracted data using subgroup analysis, and random effects models were used where appropriate. Additional analyses were conducted to assess study heterogeneity.

Findings: We identified 1,916 articles/citations through database searches, of which 26 were included in the analysis pertaining to 5,444 patients (mean age: 37±13 years), with 67.62% (3681/5444) female. Specifically, 46.15% (12/26) studies focused on DTG, 26.92% (7/26) on RAL, 23.08% (6/26) on both DTG and RAL, and 3.85% (1/26) on EVG. We found an increasing use of DTG overtime (0% before 2018 to 100% in 2021); and the median treatment duration under INSTI-based regimens was 12 [9–36] months. Overall, the rate of VC was 88.51% [95%CI: 73.83–97.80] with DTG vs. 82.49% [95%CI: 55.76–99.45] and 96.55% [95%CI: 85.7–100.00] with RAL and EVG respectively. In univariate analysis, VC with DTG-containing vs. other INSTI-regimens was significantly higher (OR=1.44 [95%CI: 1.15–1.79], p=0.0014). EVG (1/12 study) was also associated with VC but without any statistical significance (OR=4.36, 95%CI:[0.59–32.17]; p=0.17); and RAL (4/12 studies) seemed to disfavor VC (OR=0.67, 95%CI:[0.53–0.83]; p=0.0004). Regarding INSTI-DRMs, 13/26 studies highlighted the emergence of major mutations (T66A, T66I, T66V, G118R, E138A, E138K, E138Q, G140A, G140S, Y143C, Y143H, Y143R, Y143S, S147G, Q148R, Q148K, N155H, N155D, G163R and R263K) associated to varying levels of INSTI-resistance. Among these DRMs at failure, DTG resistance-signature (specifically G118R and R263K) were reported in East Africa, were there is almost no HIV-1 subtype B infection but rather prevailing HIV-1 subtype D.

Interpretation: Our systematic review and meta-analysis revealed increasing use of DTG in recent years in SSA. Furthermore, DTG presents a superiority effect in VC compared to other INSTIs. Nonetheless, the early detection of INSTI-DRMs calls for sentinel surveillance for a successful transition and a sustained efficacy of DTG in SSA.

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Predictors of integrase resistance in individuals who failed a regimen containing dolutegravir in French and Italian clinical settings

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Background: After a decade of a wide usage of dolutegravir (DTG) in clinical practice, virological failures under this integrase strand-transfer inhibitor (INSTI) have been rarely recorded and mostly without selection of new resistance mutations. This work aims at evaluating the potential selection of integrase resistance and its predictors in HIV-1 infected individuals who failed a DTG-based regimen.

Materials and Methods: We retrospectively analysed 467 HIV-1 infected c-ART experienced individuals who experienced a failure (two consecutive plasma HIV RNA values >50 copies/mL) under a DTG-based treatment among several French and Italian clinical centers. People for whom a plasma genotypic resistance test (GRT) was available at DTG failure were included. Major resistance mutations (MRM) and genotypic susceptibility score (GSS, used as a binary variable: ≥2 vs.<2) of DTG-companion drugs of the current regimen were evaluated at failure according to Stanford Drug resistance algorithm (version 9.0). Logistic regression analyses were used to evaluate factors associated to the risk of having at least one INSTI MRM under DTG pressure among demographical, therapeutical and viro-immunological variables.

Results: Individuals who failed DTG were mostly male (62%), with a median (IQR) age of 49 (39-55) years, and were HIV-infected since a median (IQR) time of 15 (5-22) years. At DTG start median (IQR) plasma HIV-RNA and CD4 count were 2.6 (1.6-4.6) log_{10} copies/mL and 358 (170-632) cell/mm^3, respectively. Around half of individuals were subtype B HIV-1 infected (53%) and were INSTI-naïve before receiving DTG (52%). Regarding the previous INSTI-experience, 32%, 19%, and 9% received raltegravir, DTG and elvitegravir, respectively. A small proportion (10.7%) of individuals failed DTG as first-line regimen. The majority of people (70.7%) received DTG in a regimen containing 3 drugs, while 15.4% received a dual drug regimen. At the moment of GRT at failure, individuals were under DTG since a median (IQR) time of 11 (5-20) months and showed a median (IQR) plasma HIV-RNA of 2.8 (2.2-4.1) log_{10} copies. Regarding resistance at DTG failure, 12.4% of individuals showed at least one INSTI MRM. N155H was the most prevalent MRM (5.4%), followed by G140S (4.5%), Q148H (4.3%), E138K (2.8%), S147G (2.4%) and R263K (1.7%). T66I/A, E92Q, G118R, E138A/T, G140A/C showed a prevalence <1%. GSS from 0 to 4 was observed in 17.1%, 21.9%, 56.5%, 4.2%, 0.3% of individuals, respectively. At univariable logistic regression, an older age (per 10 years higher, odd ratio [OR, 95% C.I]: 1.29 [1.01-1.60], P=0.040) and a longer history of HIV infection (per 5 years increase OR: 1.21 [1.04-1.40], P=0.010) were positively associated with having at least one INSTI MRM at failure. Whereas, to be INSTI-naïve before receiving DTG (OR: 0.22 [0.12-0.43], P<0.001) and having a GSS for the regimen ≥2 at failure (OR: 0.07 [0.03-0.17], P<0.001) were negatively associated with INSTI-resistance at failure. At multivariable analysis, only these two last variables were confirmed as independent negative predictors of INSTI-resistance at DTG failure.

Conclusions: In a large set of individuals failing DTG in real-life, INSTI-resistance was found in a low proportion of individuals (12%). People who never experienced first-generation INSTI and those who showed a susceptible GSS to companion drugs had a low risk of having INSTI resistance at DTG-failure.

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Evaluation of the virological efficacy of switching to dolutegravir-based tritherapy in patients followed under real-life conditions in Abidjan, Côte d'Ivoire

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Background: In Côte d'Ivoire, initiation of or switch to dolutegravir (DTG)-based triple therapy is done without taking into account the level of viral replication and the resistance developed by the virus against Nucleoside Reverse Transcriptase Inhibitors (NRTIs) which have a lower genetic barrier. The objective of our study was to evaluate the virological impact of switching to DTG-based triple therapy in people living with HIV-1 (PLHIV-1) followed at the Centre Intégré de Recherches Biocliniques d'Abidjan (CIRBA), Côte d'Ivoire.

Methods: This is a cross-sectional study conducted from February 2019 to January 2022. The study population consisted of two groups of treatment-experienced PLWH-1 followed at CIRBA who completed two measurements for viremia control. The first group had two measurements under constant DTG-based treatment and the second group had a first measurement under treatment without DTG and a second measurement under DTG-based treatment. The biological analyses concerned the quantification of plasma HIV-1 viral RNA. The quantification technique used was the one developed by Roche on the Cobas AmpliPrep/Cobas TaqMan HIV-Test, v2.0 (Roche Diagnostics, Mannheim, Germany) with a threshold of 1.3 Log10copies/mL.

Results: We received 6275 samples for quantification of plasma HIV-1 viral RNA. Patients for whom quantification was performed at two different time points represented 3.5% (220/6275) of all samples. Of these, 90% (198/220) had switched to DTG-based therapy. On the basis of the first viral load, there was 38% (74/198) detectable viremia, of which 17% (34/198) were virological failures (viral load > 3 Log10copies/mL). The distribution of patients according to the triple therapy used showed that 73% (145/198) were on the combination TDF+3TC+DTG (TLD) with 15% (22/145) of virological failure, 20% (40/198) on the combination TDF+3TC+EFV (TLE) with 15% (6/40) of virological failure and 7% (13/198) on the combination TDF+3TC+LPV/r (TLL) with 46% (6/13) of virological failure. On the basis of the second viral load, there was 27% (54/198) detectable viremia with 4% (9/198) virological failure. For patients who were consistently on TLD, 5% (7/145) were in virological failure, a decrease of 10% (15/145) from the first measure. For patients who were on TLE before switching to TLD, 5% (2/40) were in virologic failure, a decrease of 10% (4/40). No virologic failure was observed in patients who were on TLL before switching to TLD.

Conclusion: This study confirmed the efficacy of DTG-based triple therapy compared with triple therapy without DTG. However, some patients who switched to DTG-based therapy had detectable viremia and virological failures. It would be important to guide routine switching to DTG-based therapy by virological tests such as plasma viral RNA quantification and genotypic resistance tests.

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Major protease resistance in West African HIV-1 subtypes is associated with novel protease mutations and accelerated gag evolution during second-line ART

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Background: Protease inhibitors (PIs) are the class of choice for second-line HIV therapy. PI failure is associated with a low prevalence of major protease mutations, so there has been much interest in the contribution of other genes to PI resistance.

Material and methods: People with viral failure (VF, HIV-1 RNA >1000 copies/mL) during second-line PI therapy in Abuja, Nigeria, were included if they had residual plasma samples stored following viral load testing at two time points: first-line VF (before PI exposure) and second-line VF. Whole genome HIV-1 sequencing was performed on samples from both time points. Second-line VF samples were also tested for PI drug levels, if there was sufficient volume, to investigate whether the emergence of major protease mutations correlated with a therapeutic PI level. Intrahost longitudinal sequences from before and after PI exposure were examined to identify all emergent mutations, enabling the calculation of non-synonymous mutation rates across the genome. Mutation rates among participants with and without major protease resistance were compared using the Mann-Whitney test.

Results: Of 1,031 people who had received second-line PI therapy, 112 had experienced second-line VF, and 26 had longitudinal samples available from first-line and second-line VF: 62% (16/26) women, median (interquartile range [IQR]) age 36 years (31-42), CD4 count 174 cells/mm3 (70-270), and interval between samples 3.2 years (1.7-5.2). The HIV-1 subtypes were CRF02_AG (54%, 14/26) and G (46%, 12/26). Major protease resistance emerged in 9 participants (35%), of whom 8 had therapeutic PI levels (1 was insufficient to test). The 17 participants without major protease resistance were more likely to have undetectable PI drug levels (no PI detected in 10 of the 13 samples that were sufficient for testing).

The 9 participants with major protease mutations (including V32I, M46I, I47V, I54L/V, Q58E, T74P, L76V, V82A/S, I84V and L90M) all developed at least one of three other protease mutations: Q35H (n=3), K55R (n=4) and T91A/S (n=5). Major protease resistance was also associated with a higher mutation rate in the gag gene with a median of 3.1 (IQR 2.2-6.8) gag mutations per year, compared to 1.4 (IQR 0.7-2.0) among participants without major protease resistance, (p=0.01). There was no such difference in the mutation rates of regions outside of gag-protease. Among all participants with longitudinal data, a total of 186 gag mutations emerged at 112 positions. Most (82%) occurred outside the cleavage sites, in matrix (39%), capsid (16%), nucleocapsid (11%), p6 (10%), p1 (4%) and p2 (2%). The most common gag mutations were A431V (n=5), V128I, V362I and P453A/L (n=3 each).

Conclusions: PI failure without protease mutations was often due to poor adherence, as evidenced by undetectable PI levels. Major protease resistance and therapeutic PI levels were associated with potentially novel accessory protease mutations at non-polymorphic sites located in the hinge region (Q35H), active site (K55R), and dimerization region (T91A/S). These may be specific to West African subtypes and require further phenotypic evaluation. Major protease resistance was also associated with a doubling of the rate of gag mutations demonstrating that the gag gene was under selective pressure during PI therapy.

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Interpretation of the T66I and G118R mutations in integrase in HIV-2

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Background: HIV-2 infection is not so benign as once thought. Without treatment, most of the HIV-2 infected individuals eventually progress to AIDS and death [1]. However, treatment options for HIV-2 are limited compared to HIV-1: some drug classes like the non-nucleoside reverse transcriptase inhibitors (NNRTIs) have no activity against HIV-2 while other drug classes like integrase strand transfer inhibitors (INSTI) do have HIV-2 activity but IC50 are generally higher than for HIV-1. Here we describe an HIV-2 infected individual who failed INSTI containing cART resulting in emergence of the T66I and G118R mutations in integrase.

Materials and Methods: Genotypic resistance testing was performed by Sanger sequencing of HIV-2 reverse transcriptase, protease and integrase from plasma. Interpretation of the genotypic resistance test results was supported with three frequently-used HIV-2 drug resistance interpretation algorithms [2-4]. Clinical data were obtained from the electronic patient dossier.

Results: An adult female was diagnosed with AIDS (Pneumocystis jirovecii pneumonia, CMV retinitis and CD4+ T-cell count of 30 cells/µL) due to HIV-2 infection in 2002. She started cART containing TDF + ddI + 3TC, but was subsequently switched to other cART regimens because of virological failure: In 2003 to AZT + 3TC + LPV/r, in 2008 to DRV/r + ABC + 3TC + RAL, in 2010 to DRV/r + ABC + 3TC, in 2015 to DRV/r + ABC + 3TC + DTG (50 mg), in 2017 to DRV/r + ABC + 3TC + DTG (100 mg) and in 2019 to DRV/c + TAF + FTC + DTG (100 mg).
Over time she developed the D67N + N69K + K70Q + V111I + Y115F + M184V + K223R resistance-associated mutations in reverse transcriptase and the I50V + I54L + I82F resistance-associated mutations in protease. Interpretation of the HIV-2 genotypic resistance test results for integrase posed a challenge as scoring of mutations was not uniform between three frequently-used interpretation algorithms [2-4]. The T66I and G118R mutations in integrase were only scored by the Stanford Drug Resistance Database but not by the other two algorithms. Therefore, we isolated HIV-2 from the patient and are preparing HIV-2 molecular clones with and without T66I and G118R in integrase for phenotypic susceptibility testing and further characterization (in progress).

Conclusions: Treatment of HIV-2 poses additional challenges as treatment options are more limited and interpretation of genotypic resistance test results are less straightforward compared to HIV-1. Considering that the T66I and G118R mutations are well known INSTI resistance mutations for HIV-1, and that these mutations emerged in our HIV-2 patient during virological failure with INSTI containing cART, we hypothesize that T66I and G118R also result in INSTI resistance in HIV-2.

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Virological characterization of treatment failures and retreatment outcomes in patients infected with “unusual” HCV genotype 1 subtypes

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Background and Aims: Among so-called “unusual” HCV genotypes, genotype 1 (GT1) subtypes non-1a/1b are highly prevalent in regions of Sub-Saharan Africa. Some unusual GT1 subtypes have been shown to be less sensitive to NS5A inhibitor-containing regimens than GT-1a or GT-1b. In this study, we characterized the resistance-associated substitution (RAS) profiles in DAA-targeted regions in patients infected with unusual GT1 subtypes who failed to achieve SVR after NS5A inhibitor-based therapy and assessed their response to retreatment.

Method: This retrospective French national study included HCV-infected patients who experienced a virological failure after NS5A inhibitor-containing therapy referred to our National Reference Center between 2015 and 2022. RASs were initially identified by means of Sanger sequencing of the NS3, NS5A and NS5B regions of HCV genome. Shotgun metagenomics was then used for in-depth characterization of full-length genome sequences.

Results: Among 521 patients who failed to achieve SVR after NS5A inhibitor-containing therapy, 272 (52.2%) were infected with genotype 1, including 131/272 (48.2%) with subtype 1a, 93/272 (34,2%) with subtype 1b and 48/272 (17.6%) with GT1 subtype non-1a/1b. Among those infected with subtype non-1a/1b, 41/48 patients (85.4%) were born in Africa (1c, n=2; 1d, n=4; 1e, n=12; 1f, n=1; 1g, n=2; 1i, n=2; 1l, n=18), including 19/41 (46.3%) in Cameroun. The median age of the study population was 61 years (IQR: 55-68) and the male/female ratio was 1. Only 4/48 patients (8.3%) were HIV co-infected and 9/48 (18.8%) had cirrhosis. The received treatment regimens were SOF/LDV±RBV (n=33), SOF/VEL (n=2), G/P (n=3), and various others (n=10). NS5A sequences at treatment failure were available for 40/48 (83.3%) patients, showing ≥2 and ≥3 dominant NS5A RASs in 26/40 (65.0%) and 12/40 (30.0%) patients, respectively. The most frequent NS5A RASs were L31M (n=11; 27.5%), L28M (n=8; 20.0%), A92T (n=7; 17.5%) and Y93H (n=5; 12.5%). NS5B RASs (C316Y) were found in one patient (1/40; 25.0%). NS3 sequences at treatment failure were available for 24/48 (50.0%) patients. The most frequent NS3 RASs were Q80K (n=4; 16.7%), T54S (n=4; 16.7%), I132V (n=3; 12.5%). To date, 27 of the 48 patients have completed DAA retreatment (triple combination DAAs, n= 17; SOF + NS5A inhibitor; n=10) and follow-up. Among them, 25 achieved SVR, while 2 failed SOF + NS5A inhibitor retreatment, with an overall SVR rate of 92.6% (100% for triple therapy, 80% for SOF + NS5A inhibitor). Deep sequencing of full-length HCV genomes is ongoing and results will be presented.

Conclusion: We report the largest cohort of patients infected with unusual GT1 subtypes failing DAA therapy. These patients were mostly born and infected in Africa. Treatment failure was associated with at least 2 or 3 NS5A RASs, including RASs known to be pre-existent in these subtypes. Retreatment with a triple combination of sofosbuvir, an NS5A inhibitor and an NS3 protease inhibitor was successful in 100% of cases. Our results emphasize the need to identify these subtypes prior to therapy, especially in Africa where they are common, in order to guarantee access to first-line triple DAA therapy in patients who need .

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The acquisition of positively charged amino acids in HBsAg C-terminus impairs HBsAg secretion, affects its structural stability and is associated with HBV-induced liver cancer

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Background: Patients with chronic hepatitis B have a 100-fold increased risk of developing hepatocellular carcinoma (HCC). An impairment in HBsAg secretion is a mechanism mediating HBV related oncogenesis. HBsAg C-terminus is a hydrophobic transmembrane domain, crucial for HBsAg secretion. Gain of charged amino acids (aa) in this domain can alter its folding in the ER membrane, thus hampering HBsAg secretion.
The role of HBsAg C-terminus (aa189-226) mutations, associated with a gain of charged aa, on HBV-induced HCC onset needs more investigations.

Material and methods: We analyze 807 HBV chronically infected patients collected from routine clinical practice with an available HBsAg sequence: 28 with HCC (78.6% D; 21.4% A), and 779 patients without HCC (79.8% D; 20.2% A). Multivariable logistic regression model is used to assess the association of identified mutations with HCC.
The impact of identified mutations on HBsAg-secretion is analyzed in vitro by transfecting HepG2 cells with plasmids encoding wt- and mutated-HBsAg. Extracellular and intracellular HBsAg is quantified by an immunoassay (LiaisonXL, Diasorin) and used to define HBsAg secretion factor (ratio between extracellular and intracellular HBsAg). I-Tasser is used to assess HBsAg structures and its structural stability (ΔΔG[wt-mutated]<0 indicating decreased stability in presence of mutation based on Quan,2016).

Results: The acquisition of >1 positively charged amino acid at positions 204, 207, and 210 of HBsAg C-terminus strongly correlates with HCC (71.4% with HCC vs 30.2% without HCC, P<0.001). Multivariable analysis confirms this association stratifying for patients' demographics, HBV genotype, serum HBV-DNA and anti-HBV drugs use (OR[95%CI]:6.3[2.6-15.3], P<0.001). The acquisition of positively charged amino acids results from S204R, S207R and S210R mutations, found in 14.3%, 28.6% and 28.6% of HCC-patients, respectively.
By in vitro experiments, all these mutations determine a significant decrease in extracellular HBsAg amount compared to wt (42% for S204R, 39% for S207R and 32% S210R, P<0.0001 for all comparisons).
Moreover, S204R and S210R also cause a 58% and 28% reduction in HBsAg secretion factor compared to wt (P<0.0001 and P=0.009), further reinforcing their detrimental role in HBsAg release.
In silico, S204R, S207R and S210R decrease structural stability of HBsAg compared to wt (ΔΔG[S204R-wt]=-0.27; ΔΔG[S207R-wt]=-0.11; ΔΔG[S210R-wt]=-0.14) and determine a shortening of membrane-spanning alpha-helix motif (predicted alpha-helix length: aa209-224 for S204R, S207R and S210R vs 205-225 for wt), suggesting an impaired HBsAg C-terminus stability.

Conclusions: Gain of positively charged amino acids at specific HBsAg C-terminus positions tightly correlates with HBV-induced HCC, hampers HBsAg release in vitro and alters the proper folding of this domain. This could favour an intracellular HBsAg retention, posing the bases for HBV-driven hepatocarcinogenesis.
The detection of these mutations may help identifying patients at higher HCC-risk, deserving more intense liver monitoring.

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HBcrAg tightly correlates with elevated HDV replicative activity and with enhanced liver inflammation and damage: role of HBcrAg as a biomarker of liver disease progression in the setting of HDV co-infection

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Background and aims: HBcrAg is a non-invasive serum biomarker, proposed to reflect cccDNA transcriptional activity. Here, we evaluate HBcrAg levels and their correlation with virological and biochemical markers in the so far poorly investigated setting of HBV+HDV coinfection.

Methods: This study includes 64 HBeAg-negative patients: 32 co-infected with HDV and 32 HBV mono-infected, matched for patients' demographics. 37.5% of HBV+HDV infected patients is characterized by a highly-replicating HDV (median [IQR] HDV-RNA: 5.6 [5.1-5.8] log copies/ml) while 62.5% by a lowly-replicating HDV (detectable serum HDV-RNA below lower limit of quantification [LLOQ: 640 copies/ml]). HBcrAg is quantified by Lumipulse HBcrAg assay (Fujirebio; LLOQ: 3 logU/mL).

Results: HBV+HDV group has lower serum HBV-DNA and higher HBsAg levels than HBV-monoinfected group (median [IQR]: 22 [<20-136] vs 144 [63-430] IU/ml, P=0.007 for HBV-DNA and median [IQR]: 3.6 [3.1-3.9] vs 2.8 [1.7-3.8] log IU/ml, P=0.002 for HBsAg). HBV+HDV group is also characterized by higher ALT levels (median [IQR]: 42 [24-64] vs 21 [15-27] U/L, P= 0.0002).
Despite lower serum HBV-DNA, the % of patients with HBcrAg>3logU/mL is significantly higher in HBV+HDV-group than in HBV-monoinfected group (53.1% [17/32] vs 21.8% [7/32], P=0.02). Focusing on HBV+HDV group, HBcrAg>3logU/mL is observed more frequently in highly-replicating HDV than in lowly-replicating HDV patients (91.7% [11/12] vs 30% [6/20], P=0.001). Even more, a strong correlation is observed between HBcrAg and serum HDV-RNA levels (Rho=0.77, P=0.006), suggesting that HBcrAg parallels HDV replicative activity. By Auroc, HBcrAg>3logU/mL predicts highly-replicating HDV with a diagnostic accuracy of 78.1% (sensitivity:91.7%, specificity:70%).
Finally, in HBV+HDV group, patients with HBcrAg>3logU/mL are characterized by higher HBsAg levels (median IQR 3.7 [3.6-4.3] vs 3.2 [2.5-3.5] log IU/ml, P=0.02) and, notably, by more elevated ALT levels and liver stiffness (median [IQR]: 54 [47-96] vs 25 [15-37] U/L, P=0.001 for ALT levels and 10.8 [6.1-14.7] vs 7.2 [4.9-8.1] KPa, P=0.02 for liver stiffness).

Conclusions: HBcrAg, a valuable biomarker of cccDNA transcriptional activity, tightly correlates with enhanced HDV replicative activity and an increased liver inflammation. This suggests that an active transcription of cccDNA is required to support an effective HDV pathogenicity. This can have relevant clinical implications for the full effectiveness of novel therapeutic strategies targeting HBV/HDV co-infection.

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Delayed viral load decrease of Omicron variant compared to Delta variant in 2 nasopharyngeal samples from COVID-19 patients.

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Background: At the end of 2021, a new variant, B.1.1.529 (Omicron), classified variant of concern in 26th November 2021, was first described in South Africa and spread rapidly in Europe and the world. The Omicron variant has rapidly overtaken the Delta variant and was described as 3 to 6 times more infectious than other variants. In order to evaluate whether this high infectivity could be associated with a higher viral load (VL), we compared the relative VL of Omicron and Delta SARS-CoV-2 variants.

Material and methods: Nasopharyngeal samples (NS) were collected at diagnosis from the Pitié-Salpêtrière hospital virology department in Paris during the epidemic peak of each variant (July-October 2021 for the Delta variant and December 2021-January 2022 for the Omicron variant). A total of 847 positive SARS-CoV-2 RT-PCR were screened to assess SARS-CoV-2 viral lineages. The TaqPath™ COVID-19 RT-PCR test (ThermoFisher) that amplified three target genes of the virus (ORF1ab, N, and S) was used to detect Omicron variant. The VirSNiP SARS-CoV-2 Spike L452R (TIB Molbiol) was used to detect the Delta variant. The correct screening assessment was confirmed by a full genome sequencing using the Artic V3 protocol on a Gridion device (Oxford Nanopore, Oxford, UK) or Sanger sequencing.

Result: We analyzed the results of 643 Omicron positive NS and 203 Delta positive NS. At time of first diagnostic test, no significant difference in VL was observed between the two variants. Consecutive CT values were available for patients who presented at least two SARS-CoV-2 RT-PCR tests (71 patients for the Delta variant and 84 patients for the Omicron variant). Similar median CT value was observed at the time of first diagnostic test: day 0 (Omicron variant median CT value 24.64 [20.22 – 30.62] vs. Delta variant median CT value 24.03 [19.75 –28.27]). However, a gap widening over time was evidenced. An increase of the relative VL (inversely proportional to the CT values) was observed for the Omicron variant at day 1-3 (Omicron variant median CT value 21.47 [19.02 – 32.11] vs. Delta variant median CT value 29.06 [20.50 – 34.46]) and stayed higher than the VL of Delta variant until day10~. Indeed, the slope of CT values decreased for the Omicron variant was significantly lower than the slope of the Delta variant (p=0.012).

Conclusion: Our results showed no significant difference between the Omicron and Delta variants relative VL at time of diagnosis. However, we observed that the VL of the Delta variant decreased more quickly than the Omicron variant VL through time. Of note, the VL of the Omicron variant increased one to three days after the first diagnostic test and remained below the CT of 33, defined as the threshold value for a moderate viral shedding, until day 10~ contrary to the Delta variant. These results suggested that the VL peak for the Omicron variant did not occur, as the Delta variant, at the beginning of the symptoms, but a few days later and are in favor of a longer phase of replication for the Omicron variant.

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A case of re-infection with a different SARS-CoV-2 lineage in an unvaccinated child

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Background: In the setting of SARS-CoV2 infections, the re-infection by the same or a different SARS-Co2 lineage were considered a rare event (about 1% of reported cases). Starting from the emergence of Omicron variants, this events was considered to occur in about 10 % of cases. The CDC underline the need of monitoring how soon re-infections take place after a previous infection and the severity of re-infections.
We describe the case of an un-vaccinated young adult, 11 years old, became re-infected 58 days after the first positive swab.

Methods: Nasopharingeal swabs were analyzed by means of commercial real-time RT-PCR assay and antigen test. The antigen test (Lumipulse G SARS-CoV-2 Ag, Fujirebio) detects and quantifies the nucleocapsid protein antigen, whereas Abbott-Alinyt m-SARS-CoV-2 assay targeted the RdRp and N-genes.
With the aim to identify the SARS-CoV-2 variant and to confirm the state of re-infected patient, a Sanger sequencing approach was employed. After manual RNA extraction with the QIAGen kit, an in-house RT-PCR protocol was applied, capable of fully or partially amplifying the Spike region with 5 pairs of primers. The amplification product was subjected to bi-directional sequencing using the same amplification primers and the BigDye terminator cycle sequencing kit vs 3.1 - Applied Biosystems - on ABI 3130.

Results: The first swab, collected January 4th 2022 resulted positive for the SARS-Cov2 antigen (pg/ml 13.45) as well as by real-time RT-PCR test (ct 27). Sanger sequencing approach identified a Delta variant.
The individual was asymptomatic and the young man was tested negative ten days later by means of an antigenic test performed with a point of care.
However, the young man become later symptomatic, with malaise and fever requiring the assumption of pyretics and a nasopharingeal swabs was carried out in March 3rd. The swabs resulted positive for nucleocapsid protein antigen (5,000 pg/ml) and the Sanger sequencing approach revealed the re-infection by a Omicron variant, lineage Ba.2. A following naso-pharingeal swab, collected eleven days later, resulted negative for search of nucleocapsid protein antigen (0.1 pg/ml).

Discussion: This case report points out the real risk of re-infection even in the paediatric settings. During the previous epidemic waves, characterized by diffusion of variants other than Omicron (Alpha, Gamma, Delta) most infected children were asymptomatic or with milder symptoms in comparison with adults, and the rate of re-infection was not clearly demonstrated. In our area the first case of infection by Omicron lineage Ba1 was identified December 10th, 2021, whereas the first case of Omicron Ba.2 in February 2022. It is possible that the rate of re-infections is rising rapidly, this points out the importance of implementation of vaccine administration and/or its improvement.

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Epidemiological distribution of SARS-CoV-2 lineages by spike region genomic analysis: January 2021-January 2022 variants distribution in Tuscany Nord-Ovest area

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Background: Despite the fact that SARS-CoV2 is an RNA virus characterized by a high replicative capacity, with proof-reading activity, novel variants continue to arise, with unexpected problems as regards the real effectiveness of therapeutic/prophylactic approach to infection control. The occurrence of a pandemic makes essential to track it through continuous monitoring of genomic changes

Materials and methods: The nasopharyngeal swabs were selected between those resulted positive for the presence of SARS-CoV2 genomes by real-time or TMA methods in the Pisa Virology Unit or in other Laboratories of the North-West Area, according to the ministerial and regional rules in force at the time. In the Virology Unit, the variant screening was carried out with two approaches: the TaqPath Covid-19 - ThermoFisher kit, which allows the identification of the Alpha variant through the evaluation of the drop-out of the amplification curve of the S gene, and, since their commercial availability (May and August 2021, respectively), the Allplex Variant I and II - Arrow-Seegene assays, which allow to identify SARS-CoV2 variants by identifying key mutations in the spike region.
688 samples collected since January 2021 to January 2022, not correctly characterized with the aforementioned kits and with a ct <25, were considered useful for the sequence analysis.

After manual RNA extraction with the QIAGen kit, an in-house RT-PCR protocol was applied, allowing the fully or partially amplifying the spike region with 5 pairs of primers. The amplification product was subjected to bi-directional sequencing using the same amplification primers and the BigDye terminator cycle sequencing kit vs 3.1 - Applied Biosystems - on ABI 3130.

Results: The Alpha variant was found in 149 samples, 142 collected in the period of February-April, and 7 in that of May-June; the Gamma variant in 86 samples of March-May and in 11 collected in June- July ; in April the Delta variant was observed in 1 sample and then in 60 samples of June-August, in 45 samples from Sep-Dec 2021, and only in 2 samples from January 2022. Omicron variants were detected in 13 samples since December 2021, and then in 155 January samples. Sporadic observations of other variants were: Beta (5), Zeta (5), Mu (3), Eta (2). 121 samples showed mutations that allowed their assignment to different Nextstrain clades (19 A, 20 A, 20 E, 20 C, 20 B). 90 of these variants were found in samples from January-May 2021, and 31 in samples from June-August.

Discussion: Although the reported data regard only a portion of the infections observed during January 2021-January 2022, they can describe the spread of the different variants in our region that reflect the trend described in other geographical regions of the world. Dominance of Alpha variant in the first trimester of 2021, occurrence of P1 (Gamma) variant in May, dominance of Delta variant starting from June, and then replacing by Omicron variant from December 2021 have followed. Interestingly, during the first waves characterized by prevalence of Alpha and Gamma variants, we observed the circulation of other minor variants, too, but these disappeared in the following months, when Delta and Omicron variants appeared.

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Study on the continuity of Tuberculosis (TB) care services in a COVID-19 context, Senegal, 2021

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Background: The COVID-19 response favours concentration on the latter to the detriment of routine services, including those related to tuberculosis, hence the risk of service discontinuity. The objective of the National Epidemic Management Committee was therefore to study the continuity of tuberculosis care services in the context of the pandemic in Senegal.

Materials and methods: The cross-sectional mixed-mode study (01/03/2020-28/02/2021) used a combination of stratified, elemental random and systematic sampling. Collection was by telephone and univariate, bivariate and multivariate analyses required Excel2010®, EPI InfoTM7.2.4.0®, StataSE/15.1® and R3.6.3® software. The approval of the Senegalese National Ethics Committee for Health Research and the free and informed consent of all respondents were obtained.

Results: There were 219 TB patients with a mean age of 36.47 ±13.58 years, predominantly male (70.18%). The average number of children aged 0-5 years in the households was 1.56 ±1.58 and 9.63% were on anti-tuberculosis drugs with 98.5% of sputum BAAR (70.83% negative). The search for extra-pulmonary tuberculosis was only 0.47% (15% positive), of which 33.33% were lymph node and osteoarticular localisations, compared to 16.66% of multifocal TB. TB-HIV co-infection was sought in 85.92% of cases (positive in 4.52%). The blood glucose test performed in 84.54% of cases showed a 9.15% TB-Hyperglycaemia association. The service continuity gap was 5.5% and the multivariate associations were with chronic disease (p=0.002; ORaj=16.79 [2.9221-96.4748]), cost of transport and consultation 500-2500FCFA respectively (p=0. 017; ORaj=0.1525 [0.0323-0.7190]) and (p=0.007; ORaj=0.0479 [0.0052-0.4365]) finally the search for TB-HIV co-infection (p=0.000; ORaj=4.28e+07 [2965906-6.16e+08]). The qualitative analysis showed the socio-economic barrier of COVID-19 on the continuity of health care services and the benefit of awareness and de-dramatisation.

Conclusion: COVID-19 has reduced the supply of and demand for follow-up services for TB patients. We recommend the development of context-specific continuity of services plans.

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Situation with COVID-19 among HIV-infected patients in Armenia

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Background: In the Republic of Armenia (RA) HIV services are centralized at the National Center for Infectious Diseases (NCID) of the Ministry of Health RA. The Government of the Republic of Armenia has recently merged the Nork Infectious Diseases Clinical Hospital and the National Centre for AIDS Prevention, establishing the NCID in 2019. According to 2021 data, an estimated 4,850 people are living with HIV (PLWHA) in the country. The prevalence rate of HIV-infection in Armenia is approximately 0.2% in the adult population, and it is concentrated among key population groups: migrant (seasonal) workers, people who inject drugs (PWID), men who have sex with men (MSM) and sex workers (SW) – with some of these categories overlapping and the largest reported mode of transmission being heterosexual contact. Last up-date version of RA MOH people living with advanced HIV disease have an increased risk of opportunistic infections (notably TB) and related complications in general. Facility-based HIV testing services should continue and those newly diagnosed should start antiretroviral therapy (ARV) as soon as possible. For PLWHA already on ARV, continuity of the treatment and prophylaxis for coinfections is essential.

During the 2019-2021 COVID-19 crisis, it was a major challenge to guarantee access to treatment to PLWHA country-wide. In response to the specific challenges presented by covid-19, transportation of ARV drugs by Civil Society Organizations (CSOs) was offered to beneficiaries who could not travel to the HIV Centre in the capital Yerevan. In addition, an extended service package is available to key population groups (PWID, MSM, TG, SWs) depending on client needs, such as provision of social assistance, food and shelter, and prevention materials (masks and alcogels).

Methods and Methods: According to MOH "Clinical management guidance and algorithms for COVID-19 patients" in RA risk groups patients, include PLWHA hospitalized in case of moderate (pneumonia without hypoxia), severe (pneumonia with hypoxia: SpO2 ≤93% room air or/and respiratory rate >30 in adults or/and >50% lung affection) and critically (ARDS, sepsis/septic shock, thromboembolism, and/or multiorgan failure, including acute kidney injury and cardiac injury) duration of COVID-19. Severe and critically ill patients hospitalized in 2 Medical Center with appropriate ICU beds.

We analyzed 113 SARS-CoV-2 PCR+ COVD-19 cases among HIV-infected patients, 57% male.

Results: Among HIV confirmed patients registered in NCID 113 SARS-CoV-2 PCR+ COVD-19 cases 54 patients (48%) had pneumonia. 10 HIV-infected patients (from 21 to 62 years old, average 44.6, 90% male) with COVID-19 died: 9 of them were diagnosed 4rd clinical stages of HIV-infection by WHO classification and in 1 patient 3rd clinical stages, all 10 with CD4 lymphocytes count less than 200 cells/mm3; 4 patients had also severe co-morbidities (TB, visceral leishmaniasis, lymphosarcoma, thrombosis) with associated role in lethal outcome of disease. In 6 cases HIV-infection was suspected and after that diagnosed in 6 COVID-19 patients, 5 of them died, 1 patient with persistent positive SARS-CoV-2 PCR+ test had also severe non-tuberculosis mycobacteriosis. In 3 patients, who need reaped hospitalization due to opportunistic infections enrolled persistent positive SARS-CoV-2 PCR+ test.

Conclusions: During the COVID-19 pandemic HIV-infection diagnosed in patients hospitalized with COVID-19. Advanced HIV disease worsening prognosis of COVID-19 with additional risk of opportunistic infections and related complications.

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Study on the continuity of HIV care services in a COVID-19 context, Senegal, 2021

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Background: The response to the COVID-19 pandemic favors concentration on the pandemic to the detriment of routine services, including the monitoring of people living with HIV (PLHIV), hence the major risk of discontinuity of services. This is why the objective of the National Epidemic Management Committee was to study the continuity of care services related to HIV infection in the context of the COVID-19 pandemic in Senegal.

Material and methods: The cross-sectional mixed-mode study, conducted from 01/03/2020 to 28/02/2021, used a combination of stratified, elemental random, and systematic sampling. The collection was by telephone and univariate, bivariate, and multivariate analyses required Excel2010®, EPI InfoTM7.2.4.0®, StataSE/15.1® and R3.6.3® software. The approval of the National Ethics Committee for Health Research of Senegal and the free and informed consent of all study participants were obtained.

Results: A total of 504 PLHIV were surveyed. The mean age was 43.2 ±13 years with a female predominance (67%) and 8.28% of women were pregnant or had recently given birth, 53.57% of whom had given birth in a hospital, 89.29% were on ART and 57.14% had PCR (87.5% negative). Stage I was more represented (90.28%) than Stage III (2.38%). Tuberculosis co-infection (TB) was detected in 76.79% of cases (positive in 2.33%). Viral load (VL) was found in 67.26% of cases, 71.68% of which were undetectable. The study found a gap in the continuity of follow-up services for PLHIV in a pandemic context of 2.38% and statistically significant associations in multivariate analysis with the existence of television (p=0.037; ORaj=3.475 [1.076-11.225]) and the cost of transport (p=0.026; ORaj=0.495 [0.267-0.919]). Finally, the qualitative analysis showed the barrier of the high cost of care services and the benefit of awareness.

Conclusion: COVID-19 has reduced the supply demand of follow-up services for PLHIV. We recommend the development of context-specific continuity of services plans.

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Prevalence of coranavirus disease-2019(COVID-19) in a testing centre in Snambra state, Nigeria

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Background: The COVID-19 pandemic is putting a severe strain on societies because of the increased morbidity, mortality, the economic and societal harm associated with the physical distancing measures. This State population-based study aims to estimate the prevalence of SARS-CoV-2 infection in Anambra State.

Methods: We analyzed Real-Time Polymerase Chain Reaction (RT-PCR) results of 1352 nasopharyngeal /Oropharyngeal samples from June 2020 to December 2021. The participants came from nine states of Nigeria at the Accunalysis diagnostics center LTD Nnewi, Anambra State. A questionnaire was used to collect participants' demographics and reasons for coming for the investigation. Statistical analysis was performed with a 5% significance level.

Results: A total of 1352 participants with a mean age of 42.20±15.50 years came for the COVID-19 test at the Accunalysis diagnostic center between July 2020 to December 2021. The majority of the participants were males, 70.0% (946/1352), with the majority (44.9%) within the age range of 36-55 years. The prevalence of COVID-19 was 9.02% among the participants. A more significant proportion of the female participants was significantly infected with the virus, 12.6%, than males with a 7.5% infection rate (X2=8.81; P<0.01; OR=0.57; CI=0.39-0.83). The participants 65 years and above were most likely to be infected 20.6% (28/108) (X2=34.95; P<0.01). Most of the participants were from Anambra State, 87.6% (1184/1352). The most typical reason for undertaking a COVID -19 testing within the study period was traveling (1057/1352 (79.0%). Participants with suspected cases were most likely to become positive for COVID-19 virus (80.0%), followed by those that had contact with known cases (28.6%), and least by participants that undertook the test because it was a pre-requisite for traveling 4.4% (P<0.01).

Conclusion: RT-PCR testing should target the population 65 years and above. Contact tracing and testing of individuals that have contact with suspected cases should be encouraged by countries.

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International Transmission Clusters of Acute Hepatitis C Virus Among Men Who Have Sex With Men in the Netherlands. A phylogenetic analysis.

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Introduction: In the Netherlands, infections with hepatitis C virus (HCV) predominantly occur among men who have sex with men (MSM) who are living with HIV with strong clustering of HCV sequences in this population (Popping et al. Clin Infect Dis 2020). Although, the unrestricted availability of direct-acting antivirals (DAA) therapy resulted in a 61% decrease of new HCV infections transmission continued to occur. More insight into the transmission dynamics of HIV-infected MSM on a larger European scale can help to establish HCV elimination goals. Therefore, we aimed to use phylogenetic analysis to determine if clusters are fueled via national or international transmission on the Dutch acute HCV epidemic among MSM

Method: Sequencing, demographic and clinical data was collected from the Netherlands, Belgium, France, and the United Kingdom. Male individuals were included that acquired HIV through sex with a man and who had an early HCV infection (less than 12 months old) after 2016. Plasma samples were sequenced using next generation sequencing or sanger sequencing, amplifying the NS5A and NS5B region of the HCV and yielding a concatenated sequence. Genotype was assessed using the Rega HCV genotyping tool. After removal of duplicates a maximum likelihood tree was constructed using 1,000 ultra-fast bootstrap replicates. The genetic distance was calculated using a Tamura and Nei model. A cluster was defined as having at least three sequences with a bootstrap support >90% and a genetic distance <3%. An international transmission included the HCV sequences from at least two different countries.

Results: We included a total of 155 individuals with an acute HCV infection, 82 (52%) from the Netherlands, 7 (6%) from Belgium, 35 from France (22%), and 31 (20%) from the United Kingdom. The most observed genotypes were 1 (118 sequences or 76.1% of the total) and 4 (28, 18.1%). Ten clusters were identified including 101 sequences (65.2% of the total) and clusters contained between 3 and 19 individual sequences. Interestingly, clustering was more commonly observed in genotype 1 (nine clusters including 97 individuals, 80% of sequences classified as genotype 1) as compared to genotype 4 (one cluster, 3/10.7% of sequences classified as genotype 4). All clusters included Dutch sequences. International clustering was common as highlighted by nine out of ten clusters which included sequences from at least two different countries. Sequences from Belgium were most frequently part of cluster with a Dutch sequence (4, 57.1%), followed by France (17, 48.5%) and the United Kingdom (13, 41.9%).

Discussion: In this study we found that the Dutch HCV epidemic amongst HIV infected MSM is phylogenetically related to HCV transmission in Belgium, France and the United Kingdom. This suggests that there are linkages between the MSM communities in these countries. The connections between these populations calls for the need of European cooperation to reach the WHO HCV elimination goals by 2030.

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Epidemiological and clinical features of SARS-CoV-2 variants circulating between April-December 2021 in Italy

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Background: Multiple SARS-CoV-2 variants have emerged worldwide since the end of 2020 potentially impacting the effectiveness of current vaccines. The present study reports data obtained in several Italian regions involved in the SARS-CoV-2 variant monitoring, spanning the period from April to December 2021.

Methods: Sequences, epidemiological and clinical data were collected at the centers participating to the collaborative group SCIRE. We analyzed 1,083 samples obtained by different techniques: RT-PCR variant screening assays (n=116), spike Next Generation Sequencing (n=43) and Whole Genome Sequencing (n=924). Sequences were characterized using the Pangolin COVID-19 Lineage Assigner and Nextclade.

Results: Samples were collected from centers located in Apulia (n=143), Liguria (n=375), Campania (n=57), Calabria (n=26), Lombardy (n=276), Basilicata (n=14) and Lazio (n=192). In the first three months Alpha variant (B.1.1.7/20I) was prevalent with a proportion of 68%, 66.7% and 55% in April, May and June, respectively. From June B.1.617.2/21A was firstly observed in Liguria and Lazio (16.3%). One (AY.122/21J) and two (AY.61/21I) cases of Delta descendant were reported in Apulia and Campania, respectively. The former was associated to return from Afghanistan. Delta variant and its descendents began prevalent from July. However, while in July clade 21A (65.1%) was predominant, it has been replaced by 21J in subsequently months reaching a highest proportion in August (93.9%). Gamma variant (P.1 and P.1.1, 20J) was observed only in April-June with a prevalence of 15.4%, 25% and 16.3%; in the same period only few cases of Beta variant (B.1.351/20H) were reported reaching 2% of total samples. A single case of Kappa (B.1.617.1/21B) and Mu (B.1.621/21H) variants was observed in May and July in Liguria and Lombardy, respectively; five cases of Iota (B.1.526/21F) were detected in June in Liguria. The first case of Omicron variant, was observed in November in Lombardy, overall increasing to 8.5% in December.
Globally, we observed an increasing number of vaccinated subjects starting from September.
Overall, 60.4% of included subjects were not vaccinated with a median age significantly different compared to vaccinated subjects (45 vs. 61, $p<0.001$).
Twelve patients reported previous exposure to COVID-19, 11 were unvaccinated subjects.
The majority of vaccinated subjects reported 2 doses (74.4%) of which 54.7% with BNT162b2 vaccine.
Stratifying patients according to viral variant, a largest proportion of symptomatic patients (58.7%) was observed among those carrying Delta variant. Nevertheless, considering vaccination and known clinical status, among non vaccinated subjects an equal proportion of dead was observed in those carrying Alpha and Delta variants (40% both). Omicron variant was present only in non-hospitalized vaccinated subjects (33.3% and 66.6% reporting asymptomatic and symptomatic status, respectively).

Conclusions: This study provides insights into the rapid change in the epidemiological landscape of SARS-CoV-2 variants in Italy reinforcing the need of continuous surveillance of viral variants. The association between genomic and clinical features allowed us to highlight the relevant role of vaccination with respect to severity of disease.

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HIV-1 Molecular Phylogenies Characterized by Near Full-Length Genome Sequencing

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Background: The HIV-1 has acquired a very broad genetic diversity, and hence it was subdivided into four groups (M, N, O and P), with ten distinct phylogenetic subtypes (A-D, F-H and J-L) within the major group M. Additionally, the high recombinogenic nature of HIV-1 has resulted in generation of 118 well-established CRFs, and many other recombinant strains. Therefore, the HIV-1 genotypic subtypes can differ based on the length of nucleotide sequences, the region of HIV-1 genome, and the HIV-1 genotypic subtyping tool used. As such, we have developed an in-house HIV-1 near full-length genome RT-PCR assay to test the accuracy of the genotypic subtypes based on HIV-1 PR/RT and pol regions, and performed comparative evaluation using the state-of-the-art phylogenetic analyses.

Materials and Methods: Blood samples were collected from 134 consenting, newly diagnosed and chronic HIV-1 infected patients in Cyprus (2017-2019). The HIV-1 pol RT-PCR assay developed by our laboratory was utilized to amplify HIV-1 pol (PR, RT and IN) region. DNA sequences obtained through Sanger-sequencing were analyzed for genotypic subtypes both for PR/RT (2253-3359 on HXB2 genome) and pol (2253-5250 on HXB2 genome) regions, separately. Six different HIV-1 subtyping tools were utilized; REGA 3.0, COMET 2.3, jpHMM, SCUEAL, HIVdb (Standord University), and Geno2pheno. The samples with disagreements among different subtyping tools in PR/RT and pol regions or pol region only were denoted as 'discrepant'. The total number of discrepant results generated by each subtyping tool was calculated to evaluate the most reliable subtyping tool. In addition, the respective total number of discrepancies in PR/RT and pol regions were calculated to exhibit the correlation between the length of nucleotide sequence and the consistency in subtypes. Subsequently, MEGAX software was employed for multiple sequence alignment (CLUSTALW algorithm) and maximum-likelihood tree construction (GTR model with a gamma distribution, 1000 bootstrap replicates) for both regions, to perform comparative evaluation between the subtypes and the clades formed based on genetic similarity. Then, the HIV-1 near-full length genome of the discrepant samples were amplified, using the HIV-1 near full-length genome RT-PCR assay developed by our laboratory. This assay comprised of three overlapping amplicons spanning the whole HIV-1 genome amplified through pol RT-PCR assay (2253-5250 on HXB2 genome), gag RT-PCR assay (790-2292 on HXB2 genome) and env RT-PCR assay (5041-8795 on HXB2 genome) utilizing HIV-1 specific primers, covering a various number of HIV-1 group M subtypes, CRFs and recombinants. Consequently, the HIV-1 genotypic subtypes based on the obtained HIV-1 near full-length genome nucleotide sequences were determined using the REGA 3.0, and the phylogenetic analyses were repeated against a reference dataset of all known HIV-1 subtypes and CRFs (RIP Alignment 2017) downloaded from Los Alamos HIV Sequence Database.

Results: Upon determination of the HIV-1 genotypic subtypes of the 134 samples, 38 discrepant samples were identified. The total number of discrepant results generated by each subtyping tool was calculated as; 17 for REGA 3.0; 18 for HIVdb (Stanford University); 24 for COMET 2.3; 26 for SCUEAL; 38 for jpHMM; and 40 for Geno2pheno. The respective total number of discrepancies in PR/RT and pol regions were calculated as 53 and 38, respectively. The phylogenetic analyses showed that the clades

formed both for PR/RT and pol regions mostly matched the subtypes determined by REGA 3.0 Following the determination of the HIV-1 genotypic subtypes based on the HIV-1 near full-length genome, it was revealed that six out of the 38 samples belonged to pure group M subtypes, eight were CRFs, while 24 were other recombinant strains. Comparing the subtypes based on pol region and near full-length genome, the subtypes of the 13 out of the 38 samples stayed the same, where four were pure subtypes, six were CRFs, and three were recombinants of two subtypes/CRFs. In contrast, the subtypes of the 25 out of the 38 samples changed, and 15 out of the 25 were complex recombinants, which are recombinants of three or more subtypes/CRFs. The clades observed on the final phylogenetic analyses of near full-length genome sequences, supported the genotypic subtypes determined by REGA 3.0.

Conclusions: It was evident from the results that REGA 3.0 was the most reliable HIV-1 subtyping tool among the tools tested. The results also revealed that the discrepancies among different subtyping tools were reduced when pol region sequences were used compared to PR/RT region, suggesting that the increased nucleotide sequence length is directly correlated with decreased number of discrepant results among different subtyping tools. While, the phylogenetic analyses mostly confirmed the subtypes both for PR/RT and pol regions, it was observed that subtypes determined by shorter nucleotide sequences were not representative of the HIV-1 near full-length genomes. It was also established that the discrepancies among different subtyping tools generally occurred due to the presence of recombinant strains, with some CRFs, rather than pure subtypes. It was concluded that, especially, in populations with polyphyletic HIV-1 epidemic resulting in high prevalence of complex recombinant strains, neither PR/RT nor pol region nucleotide sequences are reliable for determination of HIV-1 genotypic subtypes, but only HIV-1 near full-length genome sequences are sufficiently accurate.

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Genome characterization of HIV-2 biological clones isolated from long term non-progressors (LTNP) vs patients with progressive infection.

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Background: The rate of progression to AIDS in HIV-2-infected individuals is seemingly dichotomous. While some HIV-2-infected patients develop severe immunodeficiency and AIDS-related complications similar to HIV-1-infected patients, most people with HIV-2 have a better prognosis and may never develop AIDS in their lifetime (Long-term non progressors, LTNP). HIV-2 variants isolated from these LTNP have lower in vitro replication rates than variants from individuals who progress to AIDS. In this study, we have generated and characterized complete genome sequences of LTNP and progressor HIV-2 biological clones with divergent replication capacities to identify potential viral factors that may correlate to biological phenotype.

Materials and Methods: HIV-2-infected individuals with undetectable plasma viremia (LTNP) (n=3) or with detectable plasma viremia and progressive disease, progressors (n=4), were all attending the Rotterdam out-patient clinic [1]. Two-to-three HIV-2 biological clones per patient were isolated by co-cultivation of patient PBMC with healthy donor (CD8-depleted) PBMCs in limiting dilution series [2]. Genomic DNA was isolated from infected cell pellets for PCR amplification of 3 overlapping regions spanning the whole HIV-2 genome. Purified PCR products were sequenced using Sanger sequencing, using BigDye® Terminator on an ABI Genetic Analyzer 3130 (Applied Biosystems). Contiguous sequences were assembled and visually inspected for quality using SeqMan Pro in DNASTAR software 10.1. Manual sequence editing and alignment by Clustal W was implemented in MEGA 7.0. Maximum-likelihood phylogenetic trees were reconstructed using PhyML 3.0. (http://www.atgc-montpellier.fr/phyml/).

Results: At the time of virus isolation (year 2001), HIV-2 LTNP had spontaneous suppression of plasma viral load (pvl) <50 copies/ml and stable >550 CD4 T cells/µl, without cART. One LTNP experienced a sudden drop in CD4 T cells during follow up (2005) and cART was therefore started. Progressors had detectable pVL (>500 copies/ml) and 10-120 CD4 T cells/µl.
We reconstructed Maximum likelihood trees based on whole genome nucleotide sequences and reference sequence from the Los Alamos HIV Sequence Database, as well as based on the amino acids of the individual ORFs and the LTR. A similar distribution over the subtype A tree was observed. Our phylogenetic analysis showed clusters consisting of both LTNP and progressors, indicating that fast progression is not a viral characteristic
No distinct patterns were observed in terms of length or number of N-linked glycosylation sites within the V1-V2 region of Env sequences. The net charge of the V3 loop (# of basic amino acids) was higher in progressors, compared to LTNP but there were no distinctive differences in co-receptor usage.

Conclusions: All patients included in this study were from the same epidemiological region and infected with HIV-2 subtype A, which makes our dataset homogenous. The distinction between the HIV-2 variants isolated from LTNP and progressors is mostly attributed to differences in replication capacities. Results from a cross-sectional sequence analysis of biological clones did not reveal major differences in viral factors that may be associated with spontaneous viral suppression in LTNPs. Longitudinal analysis of biological clones isolated from individuals that lose control may provide additional insights, therefore we further investigate HIV-2 progressors and LTNP in the Rotterdam cohort by regular sampling and biobanking.

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Epidemiology of HIV -1 recombinant forms in Israel, 2010-2018.

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Background: Recombination has an enormous role in HIV-1 evolution. It acts as a damage repair mechanism for the virus and constitutes a major obstacle for the prevention, diagnosis, and treatment of HIV-1 infection. Data on the contribution of the circulating recombinant forms (CRFs) to the global epidemic are accumulating. Here we characterized the molecular and epidemiological HIV-1 inter-subtype diversity of newly diagnosed individuals in Israel in 2010-2018.

Materials and Methods: Israeli database containing partial pol gene sequences from treatment naïve HIV-1 patients diagnosed in 2010-2018 (n=1957) was searched for those suspected to be recombinants. Demographic and clinical characteristics were retrieved. Subtype was determined by recombinant identification program (RIP) and with recombinant detection program (RDP5). HIV-1 subtypes were also explored using the maximum-likelihood phylogenetic method and with 410 reference sequences obtained from the Los Alamos database.

Results: The recombinant analysis identified 213 of the 253 initially suspected cases to contain recombinant virus accounting for 10.9% (213/1957) of all sequenced cases. The median age at diagnosis was 38 (30-47) years. Most ~ 63% originated from Israel. Male sex comprised~ 82%. The most common circulating recombinant forms (CRFs) were CRF02_AG (30.5%), CRF01_AE (16.9%), BF1 (7%), A6B (3.8%), CA1 (2.8%), BD (2.3%) and BC (1.9%). Secondary recombinants comprised ~26% of all other recombinant forms and included CRF01_AE/CRF02_AG/A3(10.8%), CRF02_AG/A4 (6.6%), CRF56_CPX (2.8%), CRF01_AE/B (1.4%), CRF63_02A6 (1.4%), CRF06_CPX (1.4%) and CRF43¬_02G (1.4%). An increase in the overall proportion of CRFs was observed in recent years (from 6.6% in 2010-2012 to 17.7% in 2016-2018). Men who have sex with men (MSM) and those infected through heterosexual contacts comprised a similar fraction within the two largest CRFs groups (CRF02_AG, and CRF01_AE). MSM was the most common risk group in all other CRF groups except for those infected with the secondary recombinant CRF63_02A6 which were mainly injecting drug users. The most common mutations observed were M46L (2/213, 0.9%) and L10F (3/213, 1.4%) in the protease and K103N (5/213, 2.3%) and E138A (18/213, 8.5%) in the reverse transcriptase. Only E138A was significantly associated with a specific secondary CRF, CRF02_AG/A4.

Conclusions: HIV-1 recombinants formed ~11% of all circulating viruses in Israel. Most were observed in Israeli-born men infected through sexual contact. The most prominent resistance mutation was E138A characterizing the CRF02_AG/A4 infections. The increase in the proportion of local circulating CRFs in recent years may be an obstacle to containing the epidemic.
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Identification of a New HIV-1 Circulating Recombinant Form CRF91_cpx in Cyprus

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Background: The HIV-1 demonstrates a high degree of genetic variability with ten distinct pure subtypes within the major HIV-1 group M. The presence of multiple HIV-1 clades of the major HIV-1 group M subtypes in a population, accompanied by the high recombinogenic nature of HIV-1, results in generation of new HIV-1 recombinant strains. As such, the molecular studies of the HIV-1 nucleotide sequences from Cyprus revealed a transmission cluster of HIV-1 recombinants, which are not classified as previously established CRFs.

Materials and Methods: This transmission cluster, consisting of 11 HIV-1 recombinant sequences, was identified through phylogenetic analyses on HIV-1 pol (2253-5250 on HXB2 genome) region sequences, derived from consenting HIV-1 infected patients in Cyprus (2017-2021). Specifically, a multiple sequence alignment (CLUSTALW algorithm) and a maximum-likelihood tree of HIV-1 pol sequences were constructed (GTR model with gamma distribution, 1000 bootstrap replicates) on MEGAX. It was followed by phylogenetic clustering analyses using Cluster-Picker (genetic distance ≤0.045, bootstrap support value ≥70%), and subtype determination using REGA 3.0. Phylogenetic clusters were classified as transmission clusters on the condition of minimum of three patient samples clustering together. Then, the near full-length genome sequences (790-8795 on HXB2 genome) were obtained for the 11 recombinants, using the HIV-1 near full-length genome RT-PCR assay developed by our laboratory, which were later aligned against a reference dataset of all known HIV-1 subtypes and CRFs obtained from the Los Alamos HIV Sequence Database (RIP Alignment 2020). Afterwards, phylogenetic analyses were repeated and the subtypes were determined using REGA 3.0. Subsequently, a detailed bootscan and similarity plot analyses on HIV-1 near full-length genome sequences were carried out using SimPlot v3.5.1 to explore the putative inter-subtype recombination breakpoints. These analyses were performed against a reference dataset of HIV-1 group M subtypes (A-D, F-H, J and K) and CRF02_AG obtained from the Los Alamos HIV Sequence Database, by utilizing a sliding window of 400 nucleotides overlapped by 40 nucleotides. Later, sub-region confirmatory neighbor-joining tree analyses were performed using MEGAX to confirm the subtype origin of each fragment (Kimura two-parameter model, 1000 bootstrap replicates, ≥70% bootstrap-support value was considered as definitive).

Results: In the phylogenetic analyses of the HIV-1 pol sequences, 11 HIV-1 recombinant sequences clustered together. The subtypes of the pol sequences were either “Rec. of 02_AG, G” or “Rec. of 02_AG, G, A1”, while two samples were “CRF02_AG-like” and “Rec. of G, A1, B”. In the second phylogenetic analyses of the HIV-1 near full-length genome sequences, the 11 HIV-1 recombinant sequences did not cluster with any of the pure subtypes or CRFs but they exclusively clustered together, revealing their uniqueness. The subtypes of the near full-length genome sequences were either “Rec. of 02_AG, G, J” or “Rec. of 02_AG, G, J, A1”, while one sample was “Rec. of 02_AG, G, B, J, D”. The bootscan and similarity plot analyses illustrated the same mosaic pattern for the ten out of the 11 recombinants revealing six putative inter-subtype recombination breakpoints; 3059 ± 24, 3448 ± 37, 5422, 6623, 7554 and 8434 (on HXB2 genome). After dividing the HIV-1 genomes into seven fragments, the sub-region confirmatory analyses showed that the fragments clustered

with CRF02_AG (100%), G (72-78%), CRF02_AG (100%), G (100%), U (unknown subtype origin), G (99-100%) and J (59-84%), respectively. The sub-region confirmatory analyses were repeated for the last fragment, using reference sequences for CRF06_cpx based on the BLAST results, which demonstrated 98 to 100% bootstrap support value. One out of the 11 HIV-1 recombinants was later identified to have a slightly different mosaic pattern with eight putative inter-subtype recombination breakpoints; 3035, 3485, 3804, 4281, 5422, 6623, 7554 and 8434 (on HXB2 genome). This sample had two additional recombination sites with an extra fragment of subtype B origin (89%).

Conclusions: It was determined that the ten out of the 11 recombinants had the same and unique mosaic pattern, which consists of fragments of subtypes CRF02_AG, G and J, and a fragment of unknown subtype origin. The second fragment was derived from subtype G, however it was not strongly supported by the bootstrap support value (72-78%). The maximum-likelihood tree of this region with numerous BLAST hits showed substantial differences, which can be attributed to this virus diverging in human population for some time. Similarly, the last fragment is derived from subtype J, but it is also not strongly supported by the bootstrap support value (59-84%). Nonetheless, the sub-region confirmatory analyses using the reference sequences for CRF06_cpx, showed that this fragment was derived from CRF06_cpx with high bootstrap support value (98-100%). Consistently, this region of CRF06_cpx is also derived from subtype J. In conclusion, we have characterized the mosaic structure of the new HIV-1 circulating recombinant form in Cyprus, which was named in accordance with the standards of HIV nomenclature as CRF91_cpx. Additionally, we have also identified a URF of CRF91_cpx, with two additional recombination sites caused by recombination of subtype B into the genome of CRF91_cpx. Since the identification of the CRF91_cpx, 5 more patient samples have been introduced into the CRF91_cpx transmission cluster, demonstrating the active growth of this cluster. Furthermore, BLAST searches revealed two other partial HIV-1 sequences of CRF91_cpx, which were collected in Nigeria in 2014 and 2015, which also suggests that CRF91_cpx has been circulating for some time.

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Evolutionary dynamics of the recently identified HIV subtype a outbreak in Montenegro explored through advanced phylodynamic approach

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Backgorund: Montenegro is the country with one of the lowest prevalence of HIV infection in Europe (0.03%) with an epidemic lasting almost 40 years. HIV epidemic in Montenegro was originally introduced and disseminated through heterosexual transmission and later transferred to men who have sex with men (MSM). This epidemic is characterized by the predominance of the subtype B, with the growing prevalence of subtype A, identified with the high prevalence over the years of 2014 to 2018, that has not been detected in previous research. Therefore, we aimed this investigation to explore the evolutionary dynamics of the HIV subtype A epidemic through advanced phylodynamic research.

Material and Methods: The 58 samples from treatment naïve and experienced HIV infected patients monitored at the Clinic for Infectious Diseases, Clinical Center of Montenegro in 2014-2018. Subtyping was done using the REGA HIV-1 subtyping tool version 3.0 and confirmed by phylogenetic reconstruction performed through maximum-likelihood algorithm in MEGA software version 7.0. Birth-death approach (BDA), used to calculate the effective reproduction number (Re) over time, was performed in BEAST 2 software package, using the birth death serial model as selected prior. Re value denotes the average number of secondary infections caused by an infected person at a given time during the epidemic, where Re above 1 means that the number of cases are increasing. The log output files were plotted using the "bdskytools" package in the R studio software to visualize Re trends over time.

Results: This study has shown predominance of subtype B 42⁄58 (72.4%) while the subtype A was identified for the first time in Montenegro, found with a high prevalence 14⁄58 (24.1%). Subtype C was found in 3⁄58 (5.1%). Overall genetic distance among subtype A sequences varied between 0.3% to 8.5% with the mean pairwise genetic distance of 4.2%. The time of the most recent common (tMRCA) ancestor for HIV subtype A was dated 10 years ago, 2008 (mean 9.90, 95% HPD interval (8.02-11.48)). BDA showed Re value below 1 until 2013, followed by increase of Re value, reaching the high value of almost 1.9 in 2015, and decreasing thereafter.

Conclusions: Here we present the evolutionary dynamic of the recently identified outbreak of HIV subtype A analyzed through advanced phylogenetic tools. Several countries in Europe are characterized by the predominance of subtype A, such as Russia, where this subtype is predominant one from the beginning of the HIV epidemic, followed by Greece, Albania and Bulgaria. Furthermore, Serbia and Slovenia reported growing prevalence of subtype A. Considering the geographical position of Montenegro as well as strong tourism and commercial relations with over mentioned countries the emergence of subtype A in high prevalence in this country is not surprising, however it emphasizes the need for further continuous molecular and advanced phylogenetic investigation of the HIV epidemic. Even though the Re value of investigated subtype A clade was below 1 in the most recent period this still does not exclude the existence of other subtype A clade/clusters that could be identified by more dense sampling.

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Hepatitis B Virus Sub-genotype A1 Evolutionary Dynamics in Botswana

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Background: Hepatitis B virus (HBV) infection is a major global health problem. Botswana has an intermediate HBV prevalence of 3.1–10 %. The predominant genotypes are A, D and E with a prevalence of 80%, 18.6% and 1.4%, respectively. No studies have investigated the origins and evolutionary history of the HBV genotypes in Botswana. We sought to investigate the Time to Most Common Recent Ancestor (tMRCA) and spread of the predominant HBV sub-genotype, A1 (HBV/A1) in the population of Botswana. We also aimed to determine the diversity of HBV/A1 open reading frames (ORFs) in Botswana HBV sequences.

Method: A retrospective study was conducted utilising 24 near-full length HBV sequences sequenced in Botswana from 2009 and retrieved from NCBI sequence database. Additional 130 HBV near full-length sequences were included as references. Bayesian coalescent analyses were used to study the population dynamics of the 154 HBV/A1 sequences. The temporal signal was estimated through the root-to-tip method using node density in tempEST. Correlation coefficient was used to indicate the amount of variation in genetic distance explained by sampling time and used as a measure of the clockliness of the data. Skyline plots were used to estimate the effective HBV infections in Botswana population over time. Botswana sequences were partitioned into 7 HBV ORFs and used to calculate nucleotide diversity based on pairwise distances analysis implemented in MEGA.

Results: We estimated the tMRCA of HBV/A1 to be 1959 (1920–1980), 95% Highest Posterior Density (HPD) in Botswana. Skyline plot analysis showed an increase in the size of the HBV/A1 infected population around 1985 and 1990 which is over the last ~30–40 years. Pre-core region had highest median diversity of 1 (IQR, 0.0115–1) and the surface region was relatively conserved with median diversity of 0.0075 (IQR, 0.0029–0.0135) $p < 0.01$.

Conclusion: Study provides baseline subgenotype-based phylodynamic information by predicting the tMRCA of HBV/A1 sequences revealing the evolutionary dynamics of HBV/A1 thus aiding in theoretical, clinical prevention and treatment of HBV/A1 in Botswana. Statistically significant mean diversity was observed between the different HBV/A1 ORFs that should be taken into consideration in future treatments and vaccine designs of HBV/A1.

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Patterns of PrEP use among first-time users of oral HIV pre-exposure prophylaxis in the general population in Eswatini

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Background: High discontinuation rates within the first six months on PrEP have been frequently reported in studies around the world. Less is known about those who restart PrEP after discontinuation or engage intermittently with PrEP over a longer period. The aim of this study is to describe up to 18 months of PrEP use and factors associated with PrEP interruptions among first-time PrEP users in the general population in Eswatini.

Methods: Between August 2017 and January 2019, a demonstration project newly introduced the provision of oral pre-exposure prophylaxis (PrEP) to everyone at a high risk of HIV infection at six primary health care clinics in the Hhohho region of Eswatini. Using health facility records, we studied the PrEP usage patterns of 511 first-time PrEP users. For each PrEP user, a complete PrEP history from PrEP start until the end of the demonstration project was assembled. Retention on PrEP was calculated as the time between PrEP initiation and the day on which either the last received PrEP drug refill or the cumulatively received drug refills ran out, depending on which lasted longer. Time off PrEP was defined as time periods during which a user had no PrEP drugs left assuming that all PrEP drugs handed out were available for use. Multivariable linear and logit regression models, which controlled for the time in the study, the study site and the availability of a gradually introduced PrEP promotion package, were used to assess factors associated with PrEP retention and interruptions.

Results: For 287 of the 511 (56.2%) PrEP users, PrEP follow-up visits were observed. Among those who made follow-up visits, PrEP breaks were common (142 of 287, 49.5%) and frequently included periods longer than 7 days off PrEP (92 of 287, 32.1%). The median break duration was 18.5 days (IQR 4–61). Almost every fifth PrEP user (96 of 511, 18,8%) restarted PrEP, and up to four restarts occurred during the study period. In multivariate analyses, having multiple partners was associated with lower PrEP retention (odds ratio 0.4, 95% confidence interval 0.2 to 0.9) and more interruptions of PrEP (3.3, 1.2 to 9.2). Being in a relation with a HIV positive partner were associated with PrEP retention (2.2, 1.4 to 3.6). People aged 45 years and older were also more likely to be retained (5.6, 1.4 to 22.7, compared to people aged 16-25 years).

Conclusions: Half of the PrEP users did not continue PrEP after initiation, and among those who continued half interrupted PrEP use during the study period. Younger people and individuals reporting multiple partners had an increased probability of PrEP discontinuation and interrupted PrEP use. For effective PrEP use, periods on PrEP should be matched with periods of high potential exposure to HIV.

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Quality of life of injecting drug users living with HIV in Minsk, Belarus

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Background: The analysis of quality of life (QoL) indicators is an integral component of assessing the provision of medical care for various diseases and pathological conditions. HIV-infected drug users usually not only have a number of diseases, but are also subject to social pressure in society and stigmatization, which can also affect their QoL.

Methods: To assess the QoL of HIV-positive PWID, the method of anonymous questionnaire survey was used. The questionnaire was attended by HIV-infected people who inject drugs (PWID) taking opioid substitution therapy (OST) (study group, n1=83) and those who are not OST program participant (control group, n2=128). The questionnaire included a list of general questions and a standard questionnaire for assessing QoL SF-36. The analyzed groups were comparable in terms of age, gender, education level, marital status and employment ($p>0.05$).
For the compilation of databases and their statistical processing, standard packages of statistical programs Microsoft Excel 10, STATISTICA 10 were used. Descriptive-evaluative, analytical and statistical research methods were used in the data analysis.

Results: Role-physical functioning, general health significant differences in the study and control groups wasn't observed ($p>0.05$). However, significant differences were found in the values of the bodily pain indicator in the compared groups ($U=4326.50$, $p<0.05$). The median indices of bodily pain indicators in the study and control groups were 41 (31-62) and 51 (41-84), respectively.
The distribution of indicators of the physical component of health in the study and control groups indicated the presence of significant differences between the compared groups ($U=4452.50$, $p<0.05$). The median indicator of the physical component of health in the study group was 40.19 (34.76-46.35), and in the control group - 42.81 (35.53-51.53).
Analysis of the psychological component of the respondents' health showed the presence of significant differences in the compared groups in terms of vitality ($U=3983.50$, $p<0.01$). The median values of this indicator in the study group were 40 (30-55), and in the control group - 50 (40-65). The values of indicators of social functioning, role-emotional functioning and the indicator of mental health didn't differ significantly in the compared groups ($p>0.05$).
Despite the revealed differences in the indicators of vital activity in the study and control groups, the values of the psychological component of health in the compared groups did not differ significantly ($p>0.05$).

Conclusions: PWID living with HIV apply to OST programs with significantly more complex physical health problems, which affects their QoL.

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Using social media to improve follow-up and routine checks on newly diagnosed young people living with HIV and their adherence to Antiretroviral Therapy

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Background: This increasing incidence amongst young people has a female gender aged 20-24 years (1.3%) have over 3 times higher prevalence than males in the same age group (0.4%). This rising incidence amongst young people has been attributed to stigma, cultural barriers, and unfriendly approach of health workers to HIV positive young people. These have impeded access to HIV services and care, including testing, commencing treatment and counselling. As much as 60,000 people who tested positive to HIV in Lagos state, Nigeria did not return for treatment largely due to stigmatization and discrimination. Therefore, we assessed the use of social media as a tool for improving follow up of HIV patients since it reduces frequent physical contact with health workers.

Description: HIV Testing Services were conducted for young people between the ages of 18-35 years in Ifako Ijaiye, Lagos State through mobile outreach. A total of ninety-one subjects (60 female and 31 male) agreed to participate and were tested. Seven (7.78%; 5 female and 2 male) tested positive for HIV. They were counselled on the need to commence ART and were added to an existing WhatsApp Group for follow up and to schedule routine checks.

Lessons learnt: Using social media for follow up and routine checks seems to be effective, the platform makes it easier to reduce physical contact with health workers thus reducing the incidence of discrimination and stigmatization while providing routine counseling. Social media also helped to improve positive interaction amongst the participants as they found it easy to interact and share information about their health condition and experiences. This could also have positive influence on their mental health.

Conclusion/next step: This can be used to influence young people to access information on HIV and other important aspects of healthcare including sexual and reproductive health. This could improve access to care and reducing the rising incidence of the disease amongst young people.
Social media should be explored widely in improving access to HIV services as people can be referred to health facilities for screening, diagnosis, treatment, and counseling. The platform will curb discrimination and stigmatization of people living with HIV/AIDS.

Recommendations: The use of social media should be explored widely in improving access to HIV services as people can be referred to health facilities for screening, diagnosis, treatment, and counseling. Using social media platforms should also be integrated into the strategies to curb discrimination and stigmatization of people living with HIV/AIDS.

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Changes in the HIV-1 3'-polypurine tract in patients failing dolutegravir in Brazil

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Background: The 3'-polypurine tract (3'-PPT) is a conserved 15 nucleotide long region of the HIV genome. In vitro studies have shown that mutations in the 3'-PPT can cause high level resistance to dolutegravir (DTG) and other integrase strand-transfer inhibitors (INSTI). Whether mutations in the 3'-PPT also lead to INSTI resistance in HIV-1 infected patients is still under debate. Here we determined the 3'-PPT sequences in HIV-1 in patients failing DTG-containing cART in Brazil.

Material and Methods: The 3'-PPT sequences of HIV-1 from 51 patients failing DTG-containing cART were obtained by Sanger sequencing of total nucleic acid isolated from EDTA whole blood. Phylogenetic analysis was used to rule out cross-contamination in the 3'-PPT sequencing procedure. For all 3'-PPT sequences that deviated from the consensus 3'-PPT sequence, we calculated the frequency of the observed mutations in 3123 HIV-1 sequences from the Los Alamos database (2018, all subtypes). The binominal distribution was used to calculate the probability of obtaining a particular number of mutations given the frequency obtained from Los Alamos.

Results: From the 51 patients, 45 had the consensus 3'-PPT sequence (AAAAGAAAAGGGGGG) and in 6 patients we detected one or two nucleotide substitutions in the 3'-PPT (AAGRGAAAAGGGGGG, AAGAGAAAAGGGGGG (twice), AAAAGAACAGGGGGG, AAAAGAAACGGGGGG, AAAAGAAMAGGGGGG). In 3 patients, we observed an A --> G mutation at the 3rd position of the 3'-PPT, which was also found in 7% of the 3123 sequences (p=0.23) from the Los Alamos database. The A --> G mutation at the 4th position, the A --> C mutation at the 8th position, and the A --> C mutation at the 9th position of the 3'-PPT were found in 0.2% (p=0.08), 1.1% (p=0.07), and 0.2% (p=0.08) of the 3123 sequenced from the Los Alamos database, respectively.

Conclusions: In 6 out of the 51 patients failing DTG-containing cART, we detected mutations in the 3'-PPT. In 3 of these patients, a transition at the 3rd position of the 3'-PPT was detected, which is a polymorphic mutation based on comparison with HIV-1 sequenced from the Los Alamos database. In the remaining 3 patients, mutations were detected at the 4th, 8th or 9th position of the 3'-PPT (borderline significant due to low numbers), which are relatively conserved suggesting selection of these mutations by selective pressure of DTG. The phenotypic effect of the 3'-PPT mutations detected here on INSTI susceptibility and HIV-1 replication capacity are still unknown and will be investigated further.

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Analysis of mutational history of multi-drug resistant genotypes with a mutagenetic tree model

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Multi-drug resistance is a rare event in modern HIV treatment, but it can occur and be a life-threatening problem for HIV patients. To understand multi-drug resistance and evolutionary development we analyzed the EuResist database for patients with such multi-drug resistance. We performed a cross-sectional analysis of the data to uncover the accumulation of mutations over time. We hypothesize that accumulation of resistance mutations are not acquired randomly across genotypes, but seem to evolve in a predetermined order.

Uncovering the respective patterns of accumulation of resistance mutations will allow us to be aware of the existence of resistant variants in the reservoir. The currently visible variant might have an incomplete mutational pattern and might not be annotated “resistant” within the routinely used resistance interpretation systems. Our approach elucidates hidden information in such incomplete genotypes.

We analyzed 172 resistant and 7670 susceptible genotypes covering protease, reverse transcriptase and integrase. We used a subsampling routine to assess the stability of detected signals in the data over 1000 independent runs. In each run we sampled 90% of resistant and the same amount of susceptible genotypes and 25 mutations proportional to how often we observe them in the data. For each run we inferred an optimized mutagenetic tree and estimated the probability of resistance for each genotype represented by the mutations in the tree. We summarized the learned trees and probabilities in the form of a consensus mutational history and corresponding evolving probability of resistance.

Our evolutionary model shows an almost monotonic increase of resistance with each acquired mutation. This does not only hold true for resistance to single drugs, but we can also model paths accumulating mutations over all three analyzed proteins (PI,RT,IN) with mutations on the integrase indicating high resistance. Hence, these integrase mutations also imply mutations in the protease and reverse transcriptase. In summary we present a bioinformatics model for the analysis of multi-drug resistance.

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Resistance analysis of pre-treatment NS3 and NS5A variants in HCV genotype 1a, 1b and 3a infected patients in Croatia

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Background: The use of direct acting antivirals (DAA) resulted in significant improvement in efficacy of HCV treatment in the last decade with the rates of sustained virologic response that exceed 95%. However, the presence of resistance associated substitutions (RAS), especially in NS3 and NS5A region, has been associated with impaired response to DAA. The aim of this study was to characterize the differences in the prevalence of baseline NS3 and NS5A variants in Croatian patients infected with HCV prior to DAA treatment and assess clinical and virological characteristics associated with resistance to NS3- and NS5A- inhibitors.

Materials and methods: Direct Sanger sequencing of the NS3 and NS5A region was performed in 300 consecutive DAA-naïve patients chronically infected with HCV prior to DAA treatment at the University Hospital for Infectious Diseases Zagreb in Croatia. Resistance associated substitutions were identified using the geno2pheno [hcv] algorithm v.3.4. Phylogenetic analysis was done in MEGA v.10.2.6 using the maximum likelihood method under 1000 bootstrap replicates. Demographic, clinical and laboratory data (gender, age, HCV genotype, fibrosis stage, HCV RNA level) were collected for all patients. The association between the presence of RAS and selected variables was analysed by the Mann-Whitney test for continuous data and Pearson's chi squared test or Fisher's exact test for categorical data using Statistica v.13.5. Statistical significance was defined as $p < 0,05$.

Results: Among the 300 patients included in the analysis, 109 (36,3%) were infected with genotype 1a, 80 with genotype 1b (26,7%) and 111 with genotype 3a (37,0%). Overall NS3 and NS5A RAS prevalence was 33,0% (99/300) and 13,7% (41/300) respectively, with NS3 RAS being particularly more common in genotype 1a (75/109, 68,8%) compared with genotype 1b (21/80, 26,3%) and genotype 3a (2/111, 1,8%) ($p < 0,001$). All genotype 1a sequences segregated in 2 clearly distinct clades with NS3 variants being notably more prevalent in clade I (54/68, 79,4%) compared with clade II (21/41, 51,2%) ($p = 0,003$). Among NS3 RAS, Q80K was the most common overall (51/300, 17%) and found only in genotype 1a (51/109, 46,8%), predominantly in clade I (50/51, 98,0%). NS5A RAS were more frequent in both genotype 1a (17/109, 15,6 %) and genotype 1b (17/80, 21,3%) compared with genotype 3a (7/111, 6,3 %) ($p = 0,007$). The most common NS5A RAS across all genotypes were M28V in genotype 1a (11/109, 10,1%), Y93H in genotype 1b (9/80, 11,3%) and A62L in genotype 3a (4/111, 3,6%). No statistically significant associations between the presence of NS3/NS5A RAS and patients' gender, age, fibrosis stage or HCV RNA level were observed.

Conclusion: Baseline NS3 and NS5A RAS were frequently detected in DAA-naïve patients infected with HCV genotype 1a and 1b. Especially high prevalence of NS3 RAS was observed in patients infected with clade I of genotype 1a. This highlights the importance of combination regimens with a high genetic barrier to resistance and different target sites as the new standard of care for HCV treatment in order to limit potential risk of therapy failure, particularly for difficult-to-treat patients.

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Update on transmitted drug resistance in newly diagnosed HIV patients during the period from 2019 to 2020

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Introduction: In Spain, a program for the evaluation of transmitted drugs resistance (TDR) has been active since 2007 (CoRIS). This paper presents the results of the TDR update in newly diagnosed patients for the years 2019 and 2020.

Methods: New diagnostics with fasta sequences available from CoRIS centers were included. After fasta quality control, mutations in RT and Pro associated with TDR were investigated (CPR-Stanford tool, based on Bennett, 2009). For the integrase, we also used the CPR-Stanford tool. In addition, we evaluated clinically relevant resistances (Intermediate or Resistant level) to the drugs currently recommended as first-line treatment in the GESIDA guidelines, as well as to the NNRTIs EFV, DOR and RPV.

Results: A total of 1325 patients were analyzed, 758 in 2019 and 567 in 2020; of these, integrase data were available in 431 patients, 264 in 2019 and 167 in 2020. In 2019, resistance transmitted by families was: 3.8% for NRTIs, 5.9% for NNRTIs, 0.9% for PIs, and 1.1% for INIs. In 2020 it was 4.8% for NRTIs, 7.7% for NNRTIs, 1.6% for PIs, and 0% for INIs. Clinically relevant resistance to first-line drugs in 2019 was 1.2% for TDF (of which 0.96% were Intermediates), 0.68% for ABCs, 1.1% for 3TC/FTCs, 6.2% for EFVs, 2.6% for DORs (1.5% I), 6. 0% for RPV, 2.7% for RAL (all Intermediate), and 0 % for BIC, DTG and DRV; and in 2020, it was 1.9 % for TDF (all Intermediate), 2.6 % for ABC (all Intermediate), 0.4 % for 3TC/FTC, 7.9 % for EFV, 3.5 % for DOR (2.6 % Intermediate), 7 % for RPV (5.6 % Intermediate), 1.6 % for RAL (all Intermediate), 0.5 % for BIC and DTG (all Intermediate), and 0.22 % for DRV (all Intermediate).

Conclusions: As in previous years, the highest prevalence of transmitted resistance occurred for NNRTIs, with doravirine being the drug in this family with the lowest levels of TDR. Transmitted resistance to PIs, INIs, and 3TC/FTC continues at very low levels, with no patient presenting full resistance to 2nd generation integrase inhibitors or darunavir.

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Archived HIV-1 drug resistance is driven by NNRTI-mutations and associated with viral replication among adolescents in Cameroon

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Background: With the success of antiretroviral therapy (ART), children born with HIV are growing toward adolescence. However, frequent non-adherence in adolescents living with HIV (ALHIV) leads to viral replication (viremic infection). Of relevance, a viremic infection might prone archived drug resistance mutations (ADRMs), known as predictors of ART failure. Our objective was to compare the patterns of ADRMs in viremic versus non-viremic ALHIV.

Materials and Methods: A comparative study was conducted amongst ALHIV (10-19 years) receiving ART at the Chantal BIYA International Reference Centre (CIRCB) in Yaoundé-Cameroon, from October-November 2021. WHO-clinical stage was assessed; plasma viral load (PVL) was measured and the participants were classified as viremic and non-viremic; HIV-1 genotyping was performed on buffy coat (HIV-1 DNA) and interpreted using HIVdb.v9.0.1. Patterns of HIV-1 ADRMs were compared between the viremic and non-viremic participants.

Results: A total of 25 ALHIV were enrolled (median age 18 years); girls were most represented (64%, 16/25) of the study population; all were at WHO-clinical stage-1 and on ART for 11 [8-14] years since their diagnosis with a mean PVL of 1.39±1.69 Log10 HIV-1 RNA cp/mL); 68% (17/25) were non-viremic (<40 cp/mL) while 32% (8/25) were viremic (≥40 cp/mL). Overall rate of ADRMs was 64% (16/25); 44% (11/25) of ALHIV harbored NRTI+NNRTI resistance. Following PVL stratification, ADRMs were found in 87.5% (7/8) viremic vs. 52.9% (9/17) non-viremic ALHIV, (OR: 1.65[95%IC: 0.45-6.04], p=0.09); NNRTI ADRMs were found in 87.5% (7/8) viremic vs. 41.2% (7/17) non viremic ALHIV (p=0.04), while NRTI+NNRTI resistance was found in 62.5% (5/8) viremic vs. 35.3% (6/17) non-viremic ALHIV (OR: 1.77[95%IC:0.41-7.5], p=0.20). Twenty-two ALHIV were infected with CRF02_AG (88%), 2 F2 (8%) and 1 G (4%) subtypes. No significant effect of subtype on the presence of ADRMs was found (ADRMs in CRF02_AG ALHIV: 13/22 [59.1%]; ADRMs in non-CRF02_AG ALHIV: 3/3 [100%], p=0.28).

Conclusion: The majority of ALHIV receiving ART remains non-viremic, suggesting a good treatment response. However, among viremic populations there is a high burden of ADRMs, driven essentially by resistance to NNRTI. Thus, our findings underscore the use of NNRTI-sparing regimens would contribute in mitigating viral replication and ADRMs, thereby favoring a long-term success of ART in this difficult-to-treat-population.

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Increasing protease inhibitor resistance mutations among HIV-infected children and adolescents with virologic failure

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Background: The advancement of antiretroviral therapy (ART) in Tanzania has lead to increased ART resistance among children and adolescents living with HIV (CALHIV). However, few studies have examined ART resistance among ART users in Tanzania, especially in CALHIV.

Methodology: A retrospective chart review was conducted on genotypes taken from CALHIV between 2014 and 2019. Patient characteristics were obtained from the clinic electronic medical record. Genotyping results were analyzed using the Stanford HIV Drug Resistance Database.

Results: Thirty-seven clients with genotypes were included. Twenty-one (57%) were female and 32 (87%) were >15 years old at the time of genotype. Four (10%) CALHIV were severely malnourished and two (5%) had active TB. On WHO staging 36 (97%) were stage III/IV and at the time of genotype, 23 (62%) had CD4<200cells/mm3. For the most recent CD4, 14 (38%) had CD4 counts <200cells/mm3.
Examining chart status through 2021, 31 (84%) CALHIV were active in care and 6 (16%) were lost to follow-up. At baseline, all clients had unsuppressed viral loads (VL) (>1000copies/ml) and 35 (95%) were on PI-based ART. On follow up VL,19 (51%) successfully suppressed.
For genotype results, a total of 106 resistance mutations were found: 45 (42%) high-, 29 (27%) intermediate- and 32 (30%) low-level. Six (16%) clients had no resistance. PI mutations were 48% (51/106) of the total and of those, 43% were high-level. Among all clients, 11 (30%) had PI resistance only, 9 (24%) had NNRTI+PI, and 8 (22%) had three-class resistance.

Conclusions: There is a trend of increasing PI resistance mutations over time in CALHIV. Adherence to ART and timely VLs and genotyping for clients with failing VL will help to safeguard PIs from this progressive increase.

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Detection of mutations associated with antiretroviral drug resistance from HIV proviral DNA in patients with low-level viremias

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Background: Currently, it is necessary to evaluate people with HIV who, being on antiretroviral therapy and presenting a good level of adherence, do not achieve an undetectable viral load (<20 copies/mL). Our objective was carry out a genotypic resistance test from HIV proviral DNA and prove that the mutations detected are clinically relevant and consistent with the therapy in use.

MATERIAL AND METHODS: Between March and December 2019 a total of 212 ISP-confirmed HIV patients under antiretroviral treatment were recruited with complete epidemiological and clinical data. The samples were analyzed by PCR for reverse transcriptase (TR), protease (PRO) and integrase (IN). The obtained amplicons were sequenced and analyzed by RECall. For statistical analysis, SPSS v23 was used. A study of linear bivariate correlations was carried out through the Pearson method, using the value of the R statistic to evaluate the degree of connection. The connection between genotypes was performed using Cohen's kappa connection statistic, with interpretation according to Landis and Koch.34.

Results: Of a total of 212 recruited patients but only 203 patients (95.8%) could be studied for the reverse transcriptase and protease gene. For reverse transcriptase it was found that of the samples with valid reports 117 (71.3%) were susceptible and 44 samples had some degree of resistance (26.8%). In the viral protease, 163 susceptible samples (96.3%) and 1 resistant (0.6%) were found. For the integrase gene, 128 patients were studied and in 110 of them (86%) it was possible to obtain a report of genotypic resistance and 18 (14%) were Not Reportable. Of the valid genotypes 92 (83.6%) were susceptible and 18 (16.4%) were resistant. In resistant samples in the reverse transcriptase gene, the following mutations were detected: M184V/I (11.6%), T215F/Y (4.9%), K103N (6.7%), V106A/I/M (7.3%), E138K/A/G (3.0%), V179D/E/T (3.7%), Y181C/I/F (3.0%), Y188L (1.8%), G190A/E/K/S (4.3%). The following mutations were detected in the protease gene: M46I/L (1.8%), The following mutations were detected in integrase: L74I/F/M (18.2%), M50I/T (17.3%), G163E/K/Q/R/V (17.3%), S230N (12.7%), T97A (1.8 %), N155H (0.9%).
A high percentage of association was found between the mutations detected in the HIV proviral DNA of the patients who made resistance and the drugs that were part of the previous or recent antiretroviral therapy that the patients were taking. For nucleoside analog and non-analog reverse transcriptase inhibitors this percentage was 88.6%, for integrase inhibitors it was 88.8% and for protease inhibitors it was 100%.

Conclusions: An antiretroviral drug resistance profile can be obtained from HIV proviral DNA in patients with persistent low-level viremia. Therefore, proviral DNA is a suitable compartment for making HIV genotypes since it provides very useful information that is not available when the viral load is low. Given that a high percentage of association was found between the mutations detected and the patients' previous or recent ART, it is suggestive based on the information provided by the proviral DNA genotype, to change the therapy since this constitutes an earlier intervention with the consequent benefit to the patient.

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Clonal Case Assessments for Participants Failing with Emergent Integrase Resistance in the DAWNING Second Line Study

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Background: DAWNING is a Phase 3b non-inferiority study in HIV-1 infected adults failing first-line therapy of a non-nucleoside reverse transcriptase inhibitor (NNRTI)+2 NRTIs receiving DTG+2 NRTI regimen or a then WHO-recommended regimen of LPV/r+2 NRTIs; DTG+2 NRTI superiority was demonstrated at Week 48 by Snapshot <50c/ml HIV-1 RNA. We have previously published clonal analyses and integrase structural resistance modelling for the only two participants with emergent integrase resistance through Week 48 endpoint. To assess resistance development more comprehensively in this patient population, we assessed three additional participants within 110 weeks after the Week 48 analysis.

Material and Methods: Confirmed virologic withdrawal (CVW) criteria are defined as 1) a decrease in HIV-1 RNA viral load (VL) of <1 log10 c/mL by Week 16 with confirmation, unless <400 c/mL, or confirmed ≥400 c/mL on or after Week 24; 2) confirmed VL rebound to ≥400 c/mL after prior confirmed <400 c/mL. Genotypic and phenotypic resistance testing was carried out at Monogram for RT, PR and IN at Baseline and at CVW timepoint. Integrase clonal resistance testing was performed at Monogram. A maximum-likelihood tree was created using the IQ-TREE application with HIVB+G4 plus Gamma modeling, and a K clade sample used as the outgroup.

Results: Participants A, B, and C with respective baseline NRTI resistance: K65R,Y115F,M184V; K65R,M184V; and A62A/V,K65R,M184V; all received lamivudine+zidovudine as background regimen. Replication capacity (RC) data was available for population phenotypes but was not generated in the clonal phenotype data. Participant A with baseline integrase polymorphism L74I showed Week 48 CVW population sequence substitutions L74I,G118G/R,E138E/K,Q148Q/R,R263R/K (with Week 48 DTG fold change (FC)/RC = 20/27 versus 0.9/151 at Day 1) and showed concurrent mixed clonal pathways at Week 48; 4 clones with L74I,G118R (median DTG FC = 40), 4 clones with L74I,E138K,Q148R (median DTG FC = 6.8), and 8 clones had L74I,E138K,Q148R,R263K (median DTG FC = 17). Participant B showed Week 60 CVW population sequence integrase substitutions E138K,G140S,Q148H,N155H (median DTG FC/RC = >107/27 versus 0.6/53 at Day 1) and 16/16 clones with identical INSTI mutations at E138K,G140S,Q148H,N155H, and median DTG FC of 145. Participant C with baseline L74L/I showed Week 72 CVW population integrase substitutions T66T/I,L74I,G118R,E138E/K (median DTG FC/RC = 22/20 versus 0.6/117 at Day 1), and 3 clones with L74I,G118R (median DTG FC = 10), 2 clones with T66I,L74I,G118R (median DTG FC = 32), and 11 clones with T66I,L74I,G118R,E138K (median DTG FC = 35).

Conclusions: Baseline NRTI resistance with only zidovudine as active background agent, and diverse integrase resistance profiles at CVW, were observed. One participant unusually had both Q148R and N155H present together with other secondary integrase resistance substitutions, and highest median DTG FC. G118R and/or R263K were present as coexisting variant populations with other integrase substitutions, like prior observations for participants with pre-existing resistance and limited regimen support. Diverse integrase variant co-existence suggested potential inverse relationship of RC values with FC, and evolving resistance pathways. Overall, this data, along with previous assessments looking at the structural underpinnings of DTG resistance, is consistent with a challenging DTG resistance barrier pathway that HIV must traverse.

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K156N integrase substitution in combination with 3'PPT resistance mutations against dolutegravir

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Background: Most antiretroviral drug regimens recommended for treatment initiation are anchored with dolutegravir or bictegravir. These two integrase strand transfer inhibitors are safe and effective, and they have a high barrier against the development of drug resistance mutations in integrase. It is known that resistance against dolutegravir and bictegravir can occur outside the integrase coding sequence, namely in the 3'PPT. The 3'PPT is a short genomic region essential for reverse transcription. We were first to show mutations in the 3'PPT in a patient experiencing treatment failure with dolutegravir monotherapy. Here we further explored this alternative resistance pathway and characterized K156N, a natural integrase polymorphism found in vivo in combination with 3'PPT resistance mutations.

Methods: We created recombinant pNL4.3 proviral clones with the K156N natural polymorphism and clinically relevant 3'PPT mutations alone and in combination. We produced the corresponding viruses and characterized their infectivity, replicative capacity, and drug susceptibility.

Results: By itself, K156N had little impact on viral infectivity, fitness, or drug resistance. We confirmed that clinically relevant 3'PPT mutations conferred resistance against integrase inhibitors. The addition of K156N partially restored infectivity and fitness but was innocuous to resistance levels.

Conclusion: K156N is a new natural polymorphism of interest that can be found in association with 3'PPT resistance mutations against integrase inhibitors. Further investigation is needed to evaluate the clinical significance of our work.

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Long-term Efficacy, Safety, and Durability of Ibalizumab-based Regimens in Subgroup of TMB-202 Participants

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Background: Third line antiretroviral regimens have been associated with suboptimal virologic suppression, due to drug cross-resistance and regimen complexity. Yet, in treatment-experienced (TE) HIV patients, ART durability is essential for preventing further resistance and decreasing HIV-associated morbidity and mortality. Ibalizumab (IBA), the first long-acting, post-attachment inhibitor approved to treat multi-drug resistant (MDR) HIV, may support regimen durability given its directly observed administration. We analyzed the safety, efficacy, and durability of response in 12 patients who started IBA in a Phase 2b study.

Methods: In TMB-202, 113 patients with MDR HIV received either 2000 mg IBA every 4 weeks (n=54) or 800 mg IBA every 2 weeks (n=59) for 24 weeks with an optimized background regimen (OBR). Of 96 patients who completed TMB-202, 56 transferred into an investigator-sponsored investigational new drug protocol and 12 later moved onto an expanded access protocol, TMB-311, where efficacy and safety were monitored until IBA was commercially available (approval 2018).

Results: Baseline median viral load (VL) and CD4 count for the 12 patients were 4.4 log10 copies/mL (c/mL) and 135 cells/µL, respectively. The median duration of HIV infection was 22 years (range 18-25). At the completion of TMB-202 11/12 achieved virologic suppression (VL <200 c/mL) and 8/12 had VL <50 c/mL. All 12 patients were suppressed (VL < 50 c/mL) at their last TMB-311 visit. Patients gained an average of 99 CD4 cells/µL relative to baseline. There were no treatment-emergent adverse events (TEAE) or therapy discontinuations related to IBA during follow-up. Two patients died from unrelated causes. Overall, the 12 patients remained on IBA for an average of 8.9 years (range 8-9.5), during which 8/12 did not require addition of new ARVs to their OBR to maintain suppression.

Conclusions: Data from 12 patients who received IBA for an average of 9 years validate the long-term efficacy and safety of IBA in TE patients. Importantly, for most patients, the durability of virologic response was maintained with minimal adjustments to the OBR. Altogether, these data demonstrate the contribution of IBA towards durable viral suppression in TE HIV patients with limited therapeutic options.

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Residual HIV-1 RNA in 4d on/3d off (ANRS 170 QUATUOR) through W96

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Background: ANRS 170 QUATUOR study demonstrated the non-inferiority of a 4/7-day maintenance strategy vs a 7/7-day regimen in patients with controlled viral load (VL) under triple therapy with either PI, NNRTI, or InSTI based regimen at W48 and confirmed a great efficacy until W96. The aim of these virological sub-study was to assess residual viremia during the second year.

Methods: Ultra-sensitive plasma viral load (USpVL) was performed to assess plasma residual viremia at D0, W48 and W96. VL was determined using COBAS® HIV-1, v2.0 (Roche Molecular Systems, Branchburg, NJ, USA). For USpVL, the limit of detection (LOD) was defined as an undetected PCR signal. Patients from 7/7-day were switched to 4/7-day arm at W48 so we called them differed 4/7-day arm. Generalized estimating equation was used to compare the changes from baseline in detectable USpVL within and between the 2 groups. We also estimated the probability of occurrence of detectable USpVL up to week 48 and 96 among participants with undetectable USpVL at baseline, using Kaplan-Meier estimates.

Results: Plasma residual viremia was measured in 120 patients from D0 to W96. The proportions of patients with USpVL>1 copy/mL at D0 and W48 were 16.7% and 25.2% in the 4/7-day arm, and 22.4% and 30.5 % in the 7/7-day arm, respectively (+8.5% vs +8.1%, p=0.971). When we included all patients with available USpVL in the study we can compare 262 patients in 4/7-day arm versus 278 patients in 7/7-day arm with the same evolution between D0 and W48 (p=0.3023). The probability of having a detectable USpVL among those with an undetectable USpVL at baseline did not differ between the 4/7-day arm (48.9% [35.5-64.2]) and the 7/7-day arm (50.8% [37.8-65.4]) at W48 and between the immediate 4/7 days arm (76.2%[62.9-87.5]) and the differed 4/7-day arm (65.6% [52.3-78.5]) at W96 (p=0.472). The proportion of detectable USpVL from D0 to W48 for the immediate 4/7-day arm (48.9% [35.5-64.2]) and from W48 to W96 for the differed 4/7-day arm (47.0% [33.3-62.9]) were similar (p=0.695). During follow-up to W96, the percentage of detectability in the 4/7-day arm only was stable and similar at all visits (p= 0.7631).

Conclusions: No change was observed during the second year of 4/7-day maintenance therapy in the level of plasma residual viremia. The proportions of participants with undetectable USpVL were high over time and comparable between treatment arms. These long-term virology data continue to demonstrate the potency and durability of 4/7-day arm strategy, at the level of residual viremia.

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Switch to ART with BIC/FTC/TAF achieves virological control in patients with low level viremia

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Background: A subset of PLWH experience low-level viremia (LLV) while on ART. Currently, not much data or somewhat discordant are available on the impact of LLV on virological failure (VF), immune activation, microbial translocation and inflammation. The fourth 90 –measuring quality of life– is essential to face HIV-related health challenges and factors such as physical and mental wellbeing. Current assays can quantify HIV-RNA down to 20 copies/mL. However, achieving <50 HIV-RNA copies/ml is the reference definition of virologically successful ART, and patients experiencing this status are often referred to as "suppressed". The aim of this study was to analyze the effect of switching to BIC/FTC/TAF in patients with LLV in order to increase the probability of virological suppression.

Methods: Retrospective study of patients with LLV (two consecutive detectable VL between 21-400 copies/mL), with previously undetectable HIV-RNA (<20 copies/mL) for at least 12 months on the same ART, who switched to BIC/FTC/TAF. The primary outcome was undetectable VL on two consecutive samples separated at least 3 months. Statistical analyses: Categorical variables were represented by absolute value and percentage; continuous variables were represented by median and interquartile range. For statistical comparisons, the Mann-Whitney U test or the chi-square test was performed when necessary.

Results: We included 50 patients who were on the same ART before switching a mean of 18.5 months (sd-7.3), 4 had history of VF. Previous ART: EVG/COBI/FTC/TAF (60%), DTG/3TC/ABC (10%), EFV/FTC/TDF (4%), DRV/COBI/FTC/TAF (4%) and other. After switching to BIC/FTC/TAF, patients were followed a mean of 12 months and all of them remained on it. Data are presented as median (standard deviation). The characteristics of the patients were: Age in years 50.1 (9.8); Gender 82% male, 18% female; Polypharmacy 2 (2.9). HIV VL in copies/ml [and No. and % with VL<20 copies/ml at several time points]: at baseline 150.4 (85.5), at 4-6 months 21.9 35.6) [34/50 (68%)], at 8-12 months 39.4 (97.2) [28/50 (56%)], and at 12-18 months 27.4 (81.2) [24/34 (70%)]. As for the CD4 cells count/mm3: at Baseline 717.2 (334.8), at 4-6 months 720.5 (373.1), at 8-12 months 743.2 (325.6), and at 12-18 months 799.9 (376.7).

Conclusions: Switching to BIC/FTC/TAF among patients with sustained LLV resulted in a high proportion of viral suppression. in selected patients, an optimized treatment should be implemented to avoid health challenges and improve mental wellbeing.

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Implementation of HCV-infection treatment programm with DAA in armenia among HIV-infected patients in COVID-19 era

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Background: The National Center for Infectious Diseases (NCID) of the Ministry of Health the Republic of Armenia first time starts special program of HCV-infection treatment with directly acting antiviral agents (DAAs) among PLWHA in August 2020, despite difficulties associated with COVID-19 pandemic.

Methods and Methods: According to guidelines HIV-infected patients screened for HCV at time of HIV diagnosis and annually thereafter. Anti-HCV antibody positive patients subsequently should be checked on HCV-RNA by PCR and liver fibrosis stage with liver fibrosis stage determined by Shear-Wave-Elastography (SWE) or APRI calculation. Before starting the treatment with DAAs patients were performed CBC, biochemical analysis, HBsAg, US of abdomen and HCV genotyping. Drug-drug interaction checked by Liverpool HIV interaction charts.
158 HIV-HCV-coinfected patients from 25 to 65 years old (age 45.9±8.3, 88% male) treated in NCID with sofosbuvir (SOF) plus daclatasvir (DCV). Among involved in the study 34% from capital city Yerevan, 66% from rural areas. All patients antiretroviral regimen contains dolutegravir (DTG): 99% of patients receive TDF/3TC/DTG, only 3 patients AZT/3TC+DTG.

Results: Clinical stages of HIV-infection by WHO classification among HCV-coinfected treated with DAAs were following: stage 4 – 30%, stage 3 – 27%, stage 2 – 5% and stage 1 – 38%. CD4 lymphocytes count before starting the treatment with SOF+DCV – 526.6±333.0 cells/mm3 (minimum 5, maximum 1682 cells/mm3). 20 patients newly HIV diagnosed (13%).
Serious adverse events not reported. 5 patients (3%) interrupt therapy with SOF+DCV with loss of follow up. Two patients (1.3%) died during the treatment: one patient with F4 due to liver decompensation, second due to chronic obstructive pulmonary disease. Markers of HBV-infection (HBsAg) detected in 9 patients (5.7%), among them 1 with chronic hepatitis B. 12 HIV-HCV-coinfected patients have comorbidities: 3 of them CNS affection, 3 with benign tumors, by one case of diabetes mellitus, arterial hypertension, bronchial asthma, thyroiditis. Among HCV genotype checked 63% had genotype 3. SWE was performed in majority of patients (95%): 20 patients with F4 (13%), 13 (8%) with F3-4, 18 (11%) with F2-F3. Only in 7 patients Liver fibrosis stage calculated APRI. 20 patients with F4 (13%), 13 (8%) with F3-4, 18 (11%) with F2-F3. Duration of SOF+DCV treatment was 24-weeks in 17 patients (11%) with liver fibrosis 4 degree (12 patients of them with detected genotype 3), 89% take antiviral therapy for 12-weeks. Currently SVR 12 was checked in 124 patients (78%), in 6 patients HCV was detectable (4.8% among checked) despite adequate adherence. Two re-infected IVDU patients were previously treated with DAAs, reached SVR.

Conclusions: Despite the COVID-19 pandemic, the special program for the treatment of chronic viral hepatitis C with direct-acting antiviral drugs in HIV-HCV-coinfected patients has been successfully launched in the Republic Armenia.

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Impacts of Differentiated Service Delivery Model (DSDM) on Adherence (ADH) to clinical appointment Among Children and Adolescent Living with HIV (CALHIV) attending at Mbeya Center of Excellence (COE), Baylor-Tanzania.

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Background: Adherence to clinical appointment schedules by adolescents living with HIV (ALHIV) on antiretroviral therapy (ART) is necessary for the prevention of Opportunistic Infections (OIs), medication interruptions, HIV viral load (HVL) suppression failure, and the development of drug resistance. For this course, DSDM is employed as a strategical method to maintain clinical appointments. DSDM is a client-centred approach that simplifies and adapts HIV services across the treatment cascade aimed at reflecting the preferences and expectations of various groups of ALHIV while reducing unnecessary burdens on the health system and increasing number of clients on ART.

Method: Retrospective Cohort study design conducted for 2 years at Mbeya COE from 1st January 2019 to 31st December 2020. We abstracted data form Electronic Medical Record (EMR) whereby two group of CALHIV aged below 20 years of age attended at the clinic at 1-month and 3-month interval appointment respectively were involved. Inclusion criteria: 1st group included clients on ART not less than 6 months, with active opportunistic infections, under 5 years, and those above five years had Viral load more than 50 copies/ml. 2nd group included clients on ART not less than 6 months, without active opportunistic infections, below above 5 years but below 20 years of age, with Viral load less than 50 copies/ml. The assessment was based on Loss to follow-up (LTFU), Missed appointment (MISSAP) and On-appointment. LTFU were clients who missed appointment for more than 28 days, MISSAP were clients who missed appointments below 28 days and On-appointment were clients attended on their date of appointment.

Results: 65 adolescents who were on 1-month appointment made a total of 935 Appointment visits whereby 471 (50.4%) were On-Appointment visits, 222 MISSAP visits (23.7%) and 67 LTFU visits (7.2%) in contrast with 1792 appointments made by 225 adolescents who were on 3-months appointments with 856 On-Appointment visits, 375 MISSAP visit (20.9%) and 103 LTFU visits (5.7%). The study discovered there were adolescents who attended clinical appointments before their schedules due to different reasons include sick visits and travel visits.

Conclusion: Our study findings suggest that 3-month appointments aid to adhere to Clinical Appointment schedules. However, the study points that, there is almost equal chances for MISSAP and LTFU visits for both 1-month and 3-month appointments. Employing other intervention strategies in parallel with DSDM like call/Short-Message-Service (SMS) notifications before clinical appointment may help to rescue clinical appointment adherence.

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HIV and SARS-CoV-2: the interplay of two wicked problems

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The scientific community is increasingly concerned about the link between the AIDS and COVID-19 pandemics. COVID-19 outcomes are significantly worse in people living with HIV (PLHIV) because of their impaired immune system, even when vaccinated [Spinelli, Lancet HIV 2020, 19–21, 2020]. SARS-CoV-2 variants can evolve in immunosuppressed patients due to prolonged viral replication in the context of inadequate immune response. Accelerated intra-host evolution of SARS-CoV-2 was reported in a South African HIV patients with antiretroviral therapy (ART) failure [Cele, Cell Host Microbe, 30, 154-162.e5, 2022]. With 25 million PLHIV in sub-Saharan Africa (SSA) of whom an estimated 8 million are not virologically suppressed, this potentially creates a reservoir for future variants. Such variants, arising in PLHIV anywhere in the world, can spread to other continents [Dudas, MedRvix, 2021].

Conversely, the COVID-19 pandemic impacts HIV treatment programmes, due to supply chain issues, overburdening of healthcare systems, limiting access to testing, treatment and prevention programmes and increasing already existing inequalities [The Global Fund 2021]. Scientists advocate for AIDS and COVID-19 pandemics in Africa to be addressed simultaneously, by increasing African access to COVID-19 vaccines, prioritizing research on the interaction between HIV care and COVID-19, maintaining high quality HIV services and integrating health services for both viruses [Msomi, Nature, 600, 33–36, 2021].

Both the COVID-19 and AIDS pandemic, more specifically the issue of HIV drug resistance (HIVDR), have previously been described as wicked problems best studied as complex adaptive systems (CASs) [Klasche, Soc. Sci. Humanit. Open, 4, 100173, 2021; Kiekens, Pathogens, 10, 1535, 2021; Kiekens, BMC Public Health, 22, 2022]. Wicked problems consist of diverse interconnected factors and require complexity-informed and locally adapted solutions. We recently designed a qualitative model of all known factors influencing HIVDR in SSA and analysed its complexity [Kiekens 2022]. Our detailed systems map featured three main feedback loops driving HIVDR. As the literature indicates intersections between the HIV and COVID-19 pandemic, we visualized the interconnections between both pandemics. Our visualization shows how prolonged SARS-CoV-2 clearance in immunocompromised PLHIV facilitates the selection of immune escape SARS-CoV-2 variants, but also how this spread of new variants can increase the overall healthcare burden and threatens HIV care leading again to a higher risk of unsuppressed HIV viral load and disease progression in PLHIV. Moreover, at local level we also indicated the effects of both COVID-19 pandemic, and restriction measures on the system surrounding HIVDR in the Gongolamboto and Ukonga areas of Dar es Salaam, Tanzania.

Our visualisations confirm the urgency to integrate care for PLHIV and the current and future pandemic response measures, in order to mitigate a prolongation of the current COVID-19 pandemic and a rise in drug-resistant HIV.

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Virological suppression in HIV-infected Mexican individuals during the Covid-19 pandemic

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Background: The effects of the COVID-19 pandemic have been many, especially in developing countries. One of the important consequences in PLWH has been the continuing access to ARV therapy. Our hospital, a third-care facility with an AIDS Clinic with more than 2000 patients, was transformed in a COVID-only center, and the outpatient clinic was closed. Our objective was to analyze the virological control in our center during the COVID-19 pandemic.

Methods: we included all PLWH that attended our facility, and that have one or more viral load determinations in each of the two established study periods, the first covered all year 2019 and the first quarter of 2020, and the second the two last quarters of 2020 and all year 2021. Viral load was determined in our lab using the Abbott Real Time HIV-1 assay with a 40 copies/ml limit of detection. For this analysis, we present all cases that were undetectable in the first period with the behavior in the second period (during pandemic) as the endpoint.

Results: We included 1812 cases that fulfilled the entry criteria. Of those, 1448 (80%) had at least one undetectable viral load in the first period, being this the group studied. During the second period 1342 (92.7%) continued undetectable, and 11(0.76%) were detectable and were considered as failures. 95 cases (6.5%) during the follow-up period had at least one detectable viral load, being the last undetectable in 52 (3.6%), all considered as a blip. In 43 cases (2.9%), only the last viral load determination was detectable.

Conclusions: Despite the closure of the outpatient clinic and the limitations to retrieve their ARVs, our patients that were in virological control, persisted undetectable in their follow-up in more than 95% of the cases, thanks to a close remote contact with them for follow up, supported by the creation of an alternative sampling place in order to continue their lab monitoring.

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Prevalence and viral load suppression rate among children and adolescents with dual infections (any two of HIV, HBV, and HCV) in Nnewi, Nigeria

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Background: We analyzed the prevalence and HIV viral load suppression rate in children and adolescents with dual infection (any two of HIV, HBV, AND HCV) receiving Antiretroviral Therapy (ART) in Nnewi, Nigeria.

Method: A cross-sectional study was conducted among HIV-infected children and adolescents receiving ART at the Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi between January 2019 and December 2021.TheHBsAg and HCV antibody screening were done using the LabACON (Hangzhou Biotest Biotech C0, China). The plasma HIV viral load was determined using Roche COBAS AmpliPrep / COBAS TaqMan (CAP/CTM) HIV-1 test, version 2.0). Individuals with HIV viral load> 1000cp/ml were classified as unsuppressed, whereas those with < 1000 cp/ml were classified as suppressed. Data analysis was done with SPSS version 23.

Results: The prevalence of HIV/HBV and HIV/HCV dual-infected children and adolescents on ART was 15.9%. More male children/adolescents, 50.6% (156/308) than females, 49.4% (152/308), were recruited in the study. Up to 4.5% (14/308) tested positive for HBV antibody, while 11.0% (34/308) tested positive for HCV antibody. Up to 3.4% (4/116) and 5.2% (10/192) were positive for HBV among the children and adolescents, respectively, while 9, 9% (11/116) and 11.9% (23/192) of the children and adolescents tested positive for the HCV antibody, respectively (p =0.24 and p = 0.72). More male HIV-positive patients were infected with HBV, 6.4 %(10/156) than their female counterparts 2.6% (4/152). Of the 156 male HIV positive patients, 17 (10.9%) turned out positive whereas, 11.2% (17/152) females were positive to the HCV antibody (p = .0.11 and p = 0.94). Of the HIV/HCV dual infections in children and adolescents, 11.7% (26/223) were virally suppressed compared to HIV/HBV dual infected children and adolescents 5/223 [(2.2%) p =0.01]. The prevalence of triplex infections (combined HIV, HBV, and HCV) was 0% among our study participants.

Conclusions: The prevalence of HIV/HBV and HIV/HCV dual-infected children and adolescents on ART was high in Nigeria. Viral suppression was significantly higher in HIV/HCV than in HIV/HBV dual-infected children and adolescents. Our finding supports increasing awareness of HBV and HCV infections and screening among the study population to guide future use of ART.

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Proviral DNA for detection of HIV-1 subtype A drug resistance mutations

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Background: There is growing interest of genotyping HIV proviral DNA in cases of low-level and suppressed viremia. Subtype A HIV-1 was introduced into Israel in the mid-1990s, predominantly by immigrants from Russia and Eastern Europe and subsequently spread to men who have sex with men (MSM) born in Israel. The objective of this study is to determine the contribution of proviral DNA and viral RNA testing for the detection of drug resistance mutations among subtype A paired samples from naïve and treated patients followed at the Tel Aviv Medical Center.

Methods: Genotyping of the viral RNA from naïve patients and proviral DNA paired samples from naïve and antiretroviral treated (ART) patients, (all treated patients had undetectable viral load) was performed by conventional Sanger sequencing and Next Generation Sequencing (NGS). The Stanford University HIV Drug Resistance Database was used for interpretation of drug resistance associated mutations (DRM) and subtype. A comparison of resistance profile from naïve viral RNA with proviral DNA was conducted.
Phylogenetic analysis was constructed by the neighbor-joining method, using the bootstrap approach with MEGA X.

Results: Among 49 samples tested, 44 paired samples have been analyzed using both RNA from naïve patients and proviral DNA in naïve and ART patients.
Among viruses from ART patients addition of 5 (2.9%) DRM was found in proviral DNA analyzed by SANGER compared to RNA genotyping in naïve patients. In RT no addition of DRM were found. In major PR and minor PR addition of 2 (4.6%) and 7 (16.2%) DRM were found, respectively while in integrase region four DRM were lost compared to samples from naïve patients. NGS revealed addition of 62 (36.6%) DRM, 24 (54.5%), 5 (11.6%), 20 (46.5%) and 13 (33.3%) DRM in RT, major PR, minor PR and integrase, respectively.
Phylogenetic analysis shows that DNA and RNA sequences from each patient are highly linked.

Conclusion: Proviral DNA can add valuable information about DRM, which may have clinical implications regarding therapy switch and dual therapy particularly using NGS for sequencing.
Studies of subtype A are important due to the expansion of this subtype in Israel and new developed drugs (Cabotegravir, Islatravir, Lenacapavir).

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Combined COVID-19 vaccination and hepatitis C virus screening intervention for high-risk populations at a centre for addiction services in Barcelona, Spain

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Background: The COVID-19 pandemic has hindered efforts to address the hepatitis C virus (HCV) by reducing testing, particularly in marginalised groups, such as people with substance use disorders (SUDs), who have some of the highest rates of HCV and lowest rates of COVID-19 vaccination. This study aimed to explore the acceptability of combining HCV testing with COVID-19 vaccination in a centre for addiction services in Barcelona, Spain.

Material and Methods: From 20 January to 10 March 2022, 51 individuals with SUDs were offered HCV antibody (Ab) screening along with a COVID-19 vaccine, as part of a pilot study on the acceptability of combining the two interventions. If HCV Ab positive, they were screened for HCV-RNA. If HCV-RNA positive, patients would be offered linkage to care.

Results: Of the 51 participants, 80.4% were male and 88.2% Spanish-born. The mean age was 47.5 (SD: 9.7); 23.5% reported being unemployed; 13.7% having a precarious living situation or being homeless; 29.4% having an incarceration history; 51.0% having multimorbidity; 13.7% having an HIV infection; and 7.8% having had a prior sexually transmitted infection other than HCV/HIV. Out of all the participants, 35.3% reported a prior HCV infection, of which all reported that the most likely route of transmission was injecting drug use. Of all participants, 11.8% reported a previous COVID-19 diagnosis and most (90.2%) had been vaccinated for COVID-19, of which 89.1% had received the full first round schedule, but none had received a booster. Everyone received a COVID-19 mRNA vaccine (Spikevax [Moderna]) without any identified adverse events. Within the total, 70.6% were tested for HCV Ab and 19.4% were positive. Of these, all were tested for HCV-RNA and none were positive.

Conclusions: The HCV testing intervention had an acceptability rate of 70.6% and was considered safe, as no adverse events were reported. It also optimised the use of participants' time, as HCV screening was done during the post-vaccination waiting period, and it prevented the need for multiple visits. This approach can serve as an example of a novel model of care to couple HCV screening and linkage to care with COVID-19 vaccination in high-risk populations. Next steps include continuing participant recruitment.

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Combined COVID-19 vaccination and hepatitis C virus and HIV screening intervention for high-risk populations at a mobile testing unit in Madrid, Spain

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Background: The COVID-19 pandemic has hindered efforts to address the hepatitis C virus (HCV) and HIV by reducing testing, particularly in marginalised groups, such as homeless people, those with substance use disorders and refugees, who have some of the highest rates of HCV and HIV and lowest rates of COVID-19 vaccination. This study aimed to explore the acceptability of combining HCV and HIV point of care testing (PoCT) with COVID-19 vaccination in a mobile testing unit in Madrid, Spain.

Material and Methods: From 28 September to 26 October 2021, 101 individuals from high-risk populations (i.e., homeless people, those with substance use and/or mental disorders, sex workers, refugees, undocumented migrants) were offered a COVID-19 vaccine along with HCV antibody (Ab) screening, as part of a pilot study on the acceptability of combining the two interventions. If HCV Ab positive, they were offered HCV-RNA PoCT. Everyone was screened for HIV, as per the standard of care. HCV-RNA and HIV-positive patients not on antiretroviral therapy (ART) were offered linkage to care.

Results: Of the 101 participants (mean age 35.9 [SD: 11.4]), 69.3% were male, 30.7% of Spanish origin, most reported having a precarious living situation or being homeless (59.4%), being unemployed (70.3%) and having a substance use disorder (59.4%), 28.7% having a history of incarceration and 17.9% having multimorbidity. Of all participants, 11.9% reported a previous COVID-19 diagnosis, none had been vaccinated for COVID-19 and all received the Janssen vaccine without any identified adverse events. All individuals were tested for HCV Ab and HIV and 14.9% (n=15) and 8.9% (n=9) were positive, respectively. Of those HCV Ab positive, all were tested for HCV-RNA and 60.0% (n=9) were positive, of which most (55.6%, n=5) reported that the most likely route of transmission was injecting drug use, 44.4% (n=4) were probable reinfection cases and 33.3% (n=3) were HIV co-infected. Of those HIV positive, none were new diagnoses and most (55.6%, n=5) had abandoned ART. To date, 66.7% (n=6) have started treatment for HCV and 1 person (20.0%) has re-started ART. The duration between positive HIV diagnosis and ART re-initiation for the latter was 25 days. The average duration between positive HCV-RNA diagnosis and treatment initiation was 41 days (minimum: 22, maximum: 54) and of the MTU intervention 20 minutes (minimum: 7; maximum: 60).

Conclusions: The combined intervention had an acceptability rate of 100% and was considered safe, as no adverse events to HCV testing were reported. It also optimised the use of participants' time as they were vaccinated during the time that they would have been waiting for HCV/HIV test results and it prevented the need for multiple visits. This approach can serve as an example of a novel model of care to couple HCV and HIV screening and linkage to care as well as COVID-19 vaccination in high-risk populations. Next steps include continuing treatment initiation and monitoring.

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Community perspectives on self-testing for HIV and HCV in the WHO European region

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Background: The landscape of HIV and HCV testing service delivery models continues to evolve alongside the introduction of novel biomedical technologies and evidence-based testing guidelines. At the same time, late diagnoses of HIV and HCV persist in areas of the European WHO region. As part of the range of diverse testing offers, self-testing diagnostics have demonstrated to provided great opportunity to reach and engage key populations according to their specific needs.

In 2021, EATG in partnership with the Foundation for Innovative New Diagnostics (FIND) examined country-specific policies, regulations, and practical factors enabling or hindering availability and integration of HIV and HCV self-testing diagnostics as one of the ways to advance early diagnosis and linkage to care in most affected populations, in addition to healthcare provider-initiated testing and testing by trained lay provider.

Materials and Methods: An online cross-sectional survey was distributed between July and September 2021 to HIV and HCV-related community organisations within the European region on the availability and cost of self-testing kits for HIV and HCV. Based on the self-reported data on varying levels of self-test kit availability, 7 countries were selected for further qualitative analysis. A socio-ecological approach was used to carry out qualitative semi-structured interviews (SSIs) in English and Russian with key informants to understand community perspectives on self-testing for HIV and HCV in Armenia, Bosnia and Herzegovina, Kazakhstan, Kyrgyzstan, Poland, Slovenia, and the Russian Federation.

Results: 70 individual responses from 37 countries in the region to the online survey. For the qualitative SSIs, a total of 18 online interviews (via Zoom, Skype, and Microsoft Teams) were analysed in four Eastern Europe/Central Asian and three Central/Southeastern European countries. Commonly reported barriers HIVST and HCVST included lack of comprehensive information relayed to community, high cost of kits, stigma, and discrimination.

Conclusions: To ensure HIVST and HCVST access and uptake by those who benefit the most, a three-prong approach is required. First, at policy-level: introduce a legal framework for HIV and HCV self-testing and monitor how it is implemented in the field. Second, funding for the implementation of self-testing with the full-service cycle and/or needed treatment and reducing the cost of self-testing kits. Third, improving understanding and awareness of the self-testing concept and advantages of self-testing among key populations, local authorities, and healthcare providers.

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Viral load suppression after three-year Ibalizumab treatment in a multidrug-resistant HIV-1-infected woman: Clinical experience.

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Background: Diarrhea is a common and diverse etiology problem in HIV infected patients that can cause deterioration in the quality of life, malnutrition, and failure of antiretroviral therapy (ART). Ibalizumab, a humanized monoclonal antibody that binds to domain 2 of CD4 T lymphocytes receptor, has been recently approved for treating adults infected with multidrug resistant HIV-1 infection. This case report describes the first case treated with ibalizumab in Spain.

Case report: Fifty-three years old woman diagnosed of HIV-1 in 1993, heavily treated with more than 25 antiretroviral agents and with resistance to 3 drug classes of ART. In August-2016 she started with liquid stools (more than 15/24h) without vomiting, fever and/or abdominal pain. At that time, she had a viral load (VL) of 3,940 copies/ml under treatment with emtricitabine+tenofovir/QD; darunavir/QD and ritonavir/QD. After an exhaustive medical study, she was diagnosed of HIV-associated diarrhea with malabsorption and severe mixed malnutrition syndrome with multivitamin deficiencies. ART was changed but after several months failed again. In December-2018, the patient had a stage C3 HIV infection and ART failure, high resistance to nevirapine; intermediate resistance to efavirenz, rilpivirine and etravirine; possible resistance to azidothymidine and stavudine. She was sensitive to integrase inhibitors and protease inhibitors, although in the past there was resistance to protease inhibitors. The patient underwent treatment with ibalizumab in combination with optimized ART from March-2019 until now. After gaining admission to an early access program, ibalizumab loading dose (2000 mg) was intravenously administered on March-2019; followed by maintenance dose of 800 mg of ibalizumab every 14 days until the present day. Viral load decreased rapidly after the initial infusion of ibalizumab from 204,000 to 33,000 copies/ml in April 2019 and maintained gradual reduction to 20,300 copies in December 2020. Remarkably, in October 2021, VL became undetectable (<50 copies/ml) with no ART modification and nutritional supplements until today. The total amount of lymphocytes increased from 0.24 x 103/ml in May-2019 to 0.45 x103/ml in September-2019, remaining stable throughout follow-up. CD4 did not significantly change over the follow-up (2 CD4/µl in December 2018 versus 6 and 4 CD4/ µl in October and December 2021, respectively). Additionally, administration of ibalizumab was associated with a significant decrease in the number of stools from 8-10/24 hours in March 2019 (before starting with ibalizumab) to 2-3/24 hours in December 2021, as well as with a weight gain from 35.1 kg in march 2019 to 40.0 kg in December-2021.

Conclusions: In this multidrug-resistant HIV-1-infected woman, intravenous ibalizumab in combination with optimized ART was able to suppress the VL and improved the patient clinical outcomes and quality of life over a period of three years, as well as the diarrhea associated with malabsorption, probably related to HIV.

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CASE - HIV+ End-Stage Renal Disease Patient with Immunosuppressive Drug-Drug Interactions Received Successful Transplantation after Ibalizumab Suppressed Switch

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Background: The patient is a 67-year-old black male diagnosed with HIV in 2003. Hemodialysis dependent for a number of years, the challenge was to find a regimen to maintain suppression while eliminating future drug-drug interactions (DDIs) between his antiretroviral (ARV) regimen where resistance was present and his immunosuppressive medications.

Physical exam was remarkable for gait disorder from past cerebrovascular disease and vision changes from diabetic retinopathy.

Resistance testing showed classwide NRTI resistance and significant NNRTI resistance from prior treatment failures. No protease inhibitor nor integrase mutations were known. Tropism test indicated an X4 virus.

Concomitant medications included carvedilol, atorvastatin, levetiracetam, tamsulosin, and others for T2DM including insulin. Boosted darunavir and etravirine had to be discontinued due to DDIs. Ibalizumab was selected for combination with raltegravir, tenofovir disoproxil fumarate, and emtricitabine due to its lack of expected DDIs.

Results: Successful kidney transplantation occurred in June 2019 at the University of Miami. His recovery was prolonged by anemia as he refused transfusions. Transient dysphagia required temporary PEG tube placement for enteral nutrition and medications. Ibalizumab infusion was important as other ARV absorption may have been impacted until return to PO intake.

Conclusions: The HOPE Act of 2015 allowed organ transplantation between HIV positive donors and recipients enabling life-saving transplants for our patients. While the current possibility of transplant is welcome, the complexity of managing ARV regimens that are limited due to resistance and DDIs poses significant clinical challenges.

Pre-transplant, the combination of ibalizumab, emtricitabine/tenofovir disoproxil fumarate and raltegravir maintained viral suppression successfully. Post-transplant, raltegravir was changed to dolutegravir and almost 2 years after transplantation, HIV suppression has been maintained and patient's quality of life has improved dramatically.

Ibalizumab is the first monoclonal antibody approved for the treatment of HIV-1 infection. With no expected DDIs or cross resistance with other ARVs, it became a key option for this patient. Importantly, this case represents the first known use of ibalizumab in an HIV+ person undergoing kidney transplantation.

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Clinical course of AIDS with COVID-19 coinfection with prolonged SARS CoV-2 replication despite remdesivir/molnupiravir treatment.

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Introduction: COVID-19 (Coronavirus disease 2019), a disease caused by the SARS-COV-2, affects the upper and lower respiratory tract, often resulting in acute respiratory failure. Patients with a properly functioning immune system eliminate the virus in approx 8-14 days, this period can be shortened by using anti-replication drugs such as remdesivir, molnupiravir or the latest nirmatrelvir / ritonavir. Immunodeficient patients are particularly at risk of severe COVID-19 infection and prolonged elimination of the virus from the respiratory tract.

Case presentation: A 41-year-old woman of Ukrainian nationality was referred to the local infectious diseases department due to SARS-COV2 pneumonia. On admission, the patient was in a moderate general condition, blood oxygen saturation of 83%, requiring non-invasive low-flow oxygen therapy from the moment of admission. The first CT of the chest showed massive pneumonia covering 80% of the lung volume (interstitial changes), relatively low inflammatory values were observed in laboratory parameters (CRP 61.13 mg/l; Interleukin-6 50.9 pg/ml; Procalcitonin 0.08 ng/ml). The HIV Ag/Ab combo screening test was positive and was later confirmed by Western Blot (positive for HIV-1). During diagnosis, HIV VL 990,000 copies/ml (6.0 log); (HIV-1 of Subtype A1 genotype, with no primary resistance mutations) was obtained.After a month of treatment with TDF/FTC/RAL VL was 417 copies (2,62 log). CD4 lymphocyte count was: 1 cell/ul. Due to the positive SARS-COV-2 antigen test and negative IgM and IgG antibodies (patient wasn't vaccinated against SARS-COV-2), remdesivir (100mg BID iv) was included in the treatment, which due to the lack of elimination of the virus and increasing respiratory failure was extended to a total of 23 days, additionally, the treatment was supplemented with molnupiravir (4 capsules BID for 5 days), without any therapeutic effect (repeatedly positive SARS-COV-2 antigen tests in 14th; 21st day and from 22nd-35th day in 2-3 days intervals). When an attempt was made to discontinue the anti-replication drug, the patient experienced a rapid clinical deterioration. Additionally, during hospitalization, 1 unit of plasma from COVID-19 convalescents was transfused.

During the stay, the patient was treated empirically for PJP (pneumocystis jiroveci pneumonia) with trimethoprim/sulfamethoxazole, urinary tract infection (Enterococcus faecalis) with ampicillin and ceftriaxone/metronidazole for sinus involvement. After obtaining a positive result for serum CMV DNA (23,217 IU/ml; 77,392 copies/ml, CMV-IgM negative, IgG positive) intravenous ganciclovir was added to the treatment. In addition, thromboprophylaxis and symptomatic treatment were applied as needed. Despite extensive treatment, no clinical improvement was observed, and progression of inflammatory changes to almost 100% of the lung volume was seen in the control CT of the chest. During the stay, the patient suffered two episodes of sudden deterioration of ventilation with confirmed left-sided pneumothorax, the first time improvement was obtained after suction drainage, the second time without improvement despite surgical treatment. Because of that the patient was transferred to the Thoracic Surgery Ward for further treatment.

The patient obtained the SARS-COV-2 negative antigen test for the first time on the 35th day of hospitalization. After 72 days of hospitalization the patient is still alive.

Conclusion: COVID-19 can lead to more severe outcomes in people with immunodeficiency, complicating the clinical picture especially among cases with multiple opportunistic infection and complications. Prolonged SARS CoV-2 replication may be of concern especially in late diagnosed HIV cases and clinical vigilance will be warranted especially among the currently expanding Ukrainian migrant population.

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A Case of IRIS Syphilis Due to Prozone Effect

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Background: HIV & Syphilis are both sexually transmitted infection which could be frequently found as single or coinfection in MSM population. Syphilis rapid diagnostic tests (RDTs) available have proven improving case finding with affordable price while maintaining patient's comfort. Despite its ease and comfort, antigen-antibody reaction based RDTs have potential to give false negative result if the titer of antigen greatly outnumber antibody and vice versa (this phenomenon known as prozone effect), which lead to misdiagnosis of the infection. This simple case illustrates the effect of the phenomenon in management of a patient with HIV-syphilis coinfection, leading to unpleasant cutaneous Immune Reconstitution Inflammatory Syndrome (IRIS) of syphilis.

Case: A 35 years old HIV positive homosexual male patient came without any syphilis pathognomonic skin lesion. Initial laboratory study showed advanced immunosuppression (CD4 3 cell/mm3) and non-reactive rapid diagnostic test (RDT) VDRL & TP-rapid test. He was given fixed dose combination ARV of tenofovir-lamivudin-efavirenz once daily after completion of acute opportunistic infections treatment. After 3 months taking ARV with good adherence, he started complaining skin patchy redness and return to the clinic with painless skin ulcers distributed equally all over body areas including palm and sole. He was retested for syphilis using semiquantitative with results VDRL titer 1:2 and TPHA 1:256. CD4 count had increased to 53 cells/mm3. Syphilis treatment given with weekly intramuscular injection of 2.4 million unit of benzatin-penicillin for 3 consecutive weeks. Following completion of penicillin treatment, the lesions started drying and finally fully recovered leaving anesthetic scars 12 months after.

Discussion: Patient with severe immunosuppression due to HIV infection potentially give false negative result when tested using antigen-antibody reaction based RDTs due to impaired body's ability to produce sufficient amount of antibody resulting in imbalanced antigen-antibody ratio. Semiquantitative tests, which include patient's serum dilution to balance the antigen-antibody ratio, could avoid false negative result, preventing underdiagnosis of chronic asymptomatic infections and IRIS after ARV started.

Conclusion: Diagnosis of latent syphilis infection in advanced HIV infected patient remains challenging. Semiquantitative syphilis test should be done as a standard routine screening in every newly diagnosed HIV patient.

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A clinical case of three times treatment of hepatitis C with repeated infection in HIV-positive patient

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Case: Male, 41 years old (born in 1980), HIV-infection and anti-HCV diagnosed in 2000, Hepatitis B in anamnesis (HBsAg – negative, anti-HBcor – positive, anti-HBsAg >1000 IU/ml). No clinically significant comorbidities. Route of infection – parenteral drug use.

The first course of hepatitis C treatment was in 2010, patient received Interferon Alfa-2b and ribavirin for 24 weeks (before treatment: HCV RNA – 3.5x10*5 copies/ml, genotype 3, ALT – 115 U/l, AST – 66 U/l). Sustained virologic response (SVR) was obtained (HCV RNA was not detected for 6 years). Patient was not eligible for ART according to current clinical guidelines at the time (CD4 – 647 cells/mcl, 35%; HIV RNA – 43,011 copies/ml).

In 2014 routine ultrasound scan revealed liver hemangioma 33x29x55 mm. Patient was consulted by surgeon, follow-up was recommended.

According to Russian recommendations for monitoring and treatment HIV-positive patients, screening for viral hepatitis and syphilis is carried out every 12 months or by symptoms. On scheduled examination in 2016 HCV RNA was detected again – 5.1x10*5 copies/ml, genotype 1. Patient denied intravenous drug use. Patient was recommended ART, but he refused. Ledipasvir/sofosbuvir during 12 weeks for HCV treatment was prescribed. And SVR was obtained.

In 2018 patient complained of weakness, heaviness in the right hypochondrium. During examination: liver enlargement +1 cm; there are no signs of hemangioma growth, cirrhosis (METAVIR F0-1, 5.7 kPa) or hepatocellular carcinoma; no clinical signs of HIV progression; ALT – 58 U/l, AST – 49 U/l, CD4 – 530 cells/mcl, 34%; HIV RNA – 60,624 copies/ml. Due to symptoms patient was unscheduled tested for hepatitis: HCV RNA – 8.2x10*5 copies/ml, genotype 3a. Patient reported occasional intravenous drug using and was referred for consultation with narcologist and psychotherapist. From September 2020 to January 2021 patient received daclatasvir/sofosbuvir.

ART started in February 2021 by phosphazide + lamivudine + elsulfavirine, patient had refused ART previously. At ART start CD4 – 507 cells/mcl, 36%; HIV RNA – 15,068 copies/ml.

At examination in February 2022 patient is clinically stable, receiving ART, denies drug using, CD4 – 989 cells/mcl, 45%; HIV RNA < 50 copies/ml, HCV RNA – not detected, normal CBC and biochemical tests.

Conclusion: the case describes the possibility of multiple effective treatment of hepatitis C with modern antiviral drugs in HIV-positive patient. However, it is necessary to intensify prevention of parenteral hepatitis and laboratory monitoring in risk groups.