

Impact of the war in Ukraine on HIV-1 subtype distribution and antiretroviral resistance in Slovakia, 2022

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ABSTRACT

The study analyses the prevalence of HIV-1 subtypes and antiretroviral resistance among newly registered HIV-positive individuals in Slovakia in 2022, with a particular focus on the impact of migration caused by the war in Ukraine. A total of 198 cases were confirmed or reconfirmed HIV-positive of whom 109 (55.1%) citizens were from Ukraine, 80 (40.4%) were from Slovakia, and 9 (4.5%) were from other countries. HIV subtyping and resistance testing were performed in 99 patients (50.0% of all newly registered cases). Among 99 analysed patients, HIV-1 subtype B was detected in 51 (51.5%) and non-B subtypes in 48 (48.5%), with subtype A6 being the most frequent non-B variant (38.4%). Non-B variants were more common in Ukrainians (28/31; 90.3%) than in Slovaks (17/66; 25.8%). The Ukrainian subgroup was not fully representative, as most cases were reconfirmations of previously diagnosed and treated patients. Minor resistance-associated mutations were observed in 9 (9.1%) of patients, while one additional previously treated Ukrainian patient harboured the major resistance mutation M184I.

The findings demonstrate changes in the molecular profile of newly registered HIV cases in Slovakia in 2022, mainly associated with migration from Ukraine. Genetic diversity of circulating HIV-1 strains has increased mainly by introduction of subtype A6 and other non-B variants. This fact emphasises the need for continuous molecular surveillance and tailored therapeutic approaches for HIV infection in Central Europe.

KEYWORDS

HIV-1 subtypes – antiretroviral resistance – Slovakia – Ukraine – migration – subtype A6 – molecular epidemiology

SÚHRN

Hábeková M., Jánošíková L., Kovářová A., Takáčová M., Massmann, R., Valkovičová Staneková D.: Vplyv vojny na Ukrajinu na distribúciu subtypov HIV-1 a antiretrovírusovú rezistenciu na Slovensku v roku 2022

Štúdia hodnotila vplyv migrácie spôsobenej vojnou na Ukrajinu na molekulárnu epidemiológiu HIV-1 na Slovensku. Molekulárna analýza HIV-1 subtypov a testovanie rezistencie na antiretrovírusové lieky bola realizované u 99 pacientov (50 % všetkých nových prípadov) zo 198 novoregistrovaných HIV pozitívnych osôb, z ktorých 109 (55,1 %) tvorili občania Ukrajiny, 80 (40,4 %) občania Slovenska a 9 (4,5 %) osoby z iných krajín.

Výsledky ukázali, že subtyp B zostáva na Slovensku najčastejším variantom HIV-1 (51,5 %), avšak v dôsledku migrácie výrazne vzrástol podiel non-B subtypov, najmä subtypu A6, ktorý predstavoval 38,4 % analyzovaných vzoriek. Zatiaľ čo u slovenských pacientov dominoval subtyp B, u ukrajinských pacientov prevažovali non-B varianty (90,3 %), predovšetkým A6. Ukrajinská skupina nebola úplne reprezentatívna, keďže väčšinu tvorili pacienti s už známou diagnózou HIV, a boli už pred príchodom na Slovensko liečení.

Analýza antiretrovírusovej rezistencie potvrdila nízky výskyt rezistentných variantov HIV. Mutácie asociované s rezistenciou boli identifikované u 9,1 % pacientov, pričom iba jedna významná rezistenčná mutácia bola zistená u predtým liečeného pacienta z Ukrajiny. Výsledky preto naznačujú, že riziko prenosu primárne rezistentných vírusov na Slovensku zostáva nízke.

Štúdia dokumentuje, že migračná vlna po vypuknutí vojny na Ukrajinu významne ovplyvnila genetickú diverzitu HIV-1 registrovaných prípadov na Slovensku, najmä zvýšením zastúpenia subtypu A6 a ďalších non-B variantov. Napriek týmto zmenám zostáva výskyt klinicky významnej antiretrovírusovej rezistencie nízky. Výsledky zdôrazňujú význam kontinuálneho molekulárno-epidemiologického monitorovania HIV a potrebu zohľadňovať meniacu sa genetickú diverzitu vírusu pri diagnostike, sledovaní epidemiologických trendov a plánovaní terapeutických stratégií na Slovensku.

KLÚČOVÉ SLOVÁ

HIV-1 subtypy – antiretrovírusová rezistencia – Slovensko – Ukrajina – migrácia – subtyp A6 – molekulárna epidemiológia

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INTRODUCTION

Among the various HIV-1 subtypes circulating in Central and Eastern Europe, phylogenetic analysis based on partial HIV-1 *pol* gene sequences in Slovakia revealed a high prevalence of subtype B (93.0% and 81.6%), predominantly associated with men who have sex with men (MSM) during the periods 2004–2008 and 2009–2012, respectively [1, 2]. Migration from Ukraine and Eastern Europe to the post-Soviet region has caused an increase of the incidence of non-B subtypes, particularly subtype A6 [3]. Molecular surveillance in Central and Eastern European countries, including the Czech Republic and Bulgaria, showed an increase in the incidence of A6 and recombinant forms [4, 5].

Since the beginning of the war in Ukraine in 2022, many people, including HIV-positive individuals, have moved to Slovakia. Many Ukrainian patients included in this study had been previously diagnosed and were already receiving antiretroviral therapy (ART) before arriving in Slovakia; in most cases, their HIV infection was reconfirmed rather than newly diagnosed. They were most likely infected in Ukraine before arriving in Slovakia, and their reported mode of transmission refers to exposure outside Slovakia [6].

This study investigates the impact of this recent migration on the diversity of HIV-1 subtypes, the demographic composition of the HIV-infected population, routes of HIV transmission, and the occurrence of ART resistance-associated mutations in Slovakia in 2022.

MATERIALS AND METHODS

Study design and population

A total of 198 newly registered HIV-positive patients were in Slovakia in 2022 and were confirmed at the National Reference Centre for HIV/AIDS at the Slovak Medical University (NRC). Among them, 109 (55.1%) were from Ukraine, 80 (40.4%) from Slovakia, and 9 (4.5%) from other countries.

Virological analysis was performed in 99 patients (50.0% of study cohort) with sufficient biological material available.

This cohort was used for determination of antiretroviral drug resistance and HIV subtype classification. The analyses were performed on anonymised coded samples to ensure patient confidentiality.

Genetic subtyping, drug resistance testing and phylogenetic analysis

Partial sequencing of the *pol* gene was performed to determine HIV-1 subtypes and assess antiretroviral drug resistance [7, 8]. Viral RNA was extracted from plasma samples, and in selected cases with suppressed viraemia, proviral DNA was isolated from EDTA whole blood. Nested RT-PCR amplification was carried out ac-

cording to the ANRS consensus protocol for genotypic resistance testing [9], followed by Sanger sequencing. Sequence editing and quality control were performed using BioEdit [10], and sequences were aligned with reference strains from the Los Alamos HIV Sequence Database [11].

Drug resistance interpretation was conducted using the Stanford HIV Drug Resistance Database (HIVdb) algorithm [12]. Subtype classification was confirmed by phylogenetic analysis using the Neighbour-Joining method [13] implemented in MEGA X software [14] (Figure 1).

RESULTS

The analysed cohort of 99 patients included 74 (74.7%) men and 25 (25.3%) women. In the cohort 66 (66.7%) were from Slovakia, 31 (31.3%) from Ukraine and 2 (2.0%) from other countries. The lower proportion of analysed Ukrainian patients was mainly due to suppressed viral load in patients already receiving ART before arrival in Slovakia, which limited RNA-based sequencing.

Among Ukrainian patients, women predominated (20/31; 64.5%), while 11 (35.5%) were men. Of the two patients from other countries, one was male and one female.

Subtype analysis in 99 patients identified subtype B in 51 (51.5%) and subtype A6 in 38 (38.4%) cases. Other subtypes and recombinant forms were rare: A1 in 2 (2.0%), F in 1 (1.0%), C in 1 (1.0%), CRF07_BC in 1 (1.0%), CRF01_AE in 2 (2.0%) and CRF02_AG in 3 (3.0%) cases.

Subtype B was more common in men (49/74; 66.2%), while subtype A6 was more common in women (20/25; 80.0%). Subtype A6 was detected in 18 men (24.3%), and subtype B was detected in 2 women (8.0%).

According to country of origin, 66 (66.7%) patients were from Slovakia, 31 (31.3%) from Ukraine and 2 (2.0%) from other countries. Among Slovak patients, subtype B was dominant (49/66; 74.2%), and subtype A6 was detected in 9 (13.6%) cases. Among Ukrainian patients, subtype A6 was clearly dominant (28/31; 90.3%), while subtype B was found in 2 (6.5%) cases. One Ukrainian patient was infected with CRF01_AE. The two patients from other countries were infected with subtypes A6 and CRF01_AE.

Regarding route of transmission, MSM transmission was reported in 45 (45.5%) patients, heterosexual transmission in 40 (40.4%) and injecting drug use in 4 (4.0%); in 10 (10.1%) cases the transmission route was unknown. Heterosexual transmission included 25 of 25 women and 15 of 74 men. Subtype B was strongly associated with MSM transmission (36/45; 80.0%). Among heterosexual cases, subtype A6 was most frequent (28/40; 70.0%). Among people who inject drugs (PWID), subtype A6 was detected in 3 of 4 (75.0%) cases. All cases with unknown transmission were subtype B (10/10; 100%) – Figure 2.

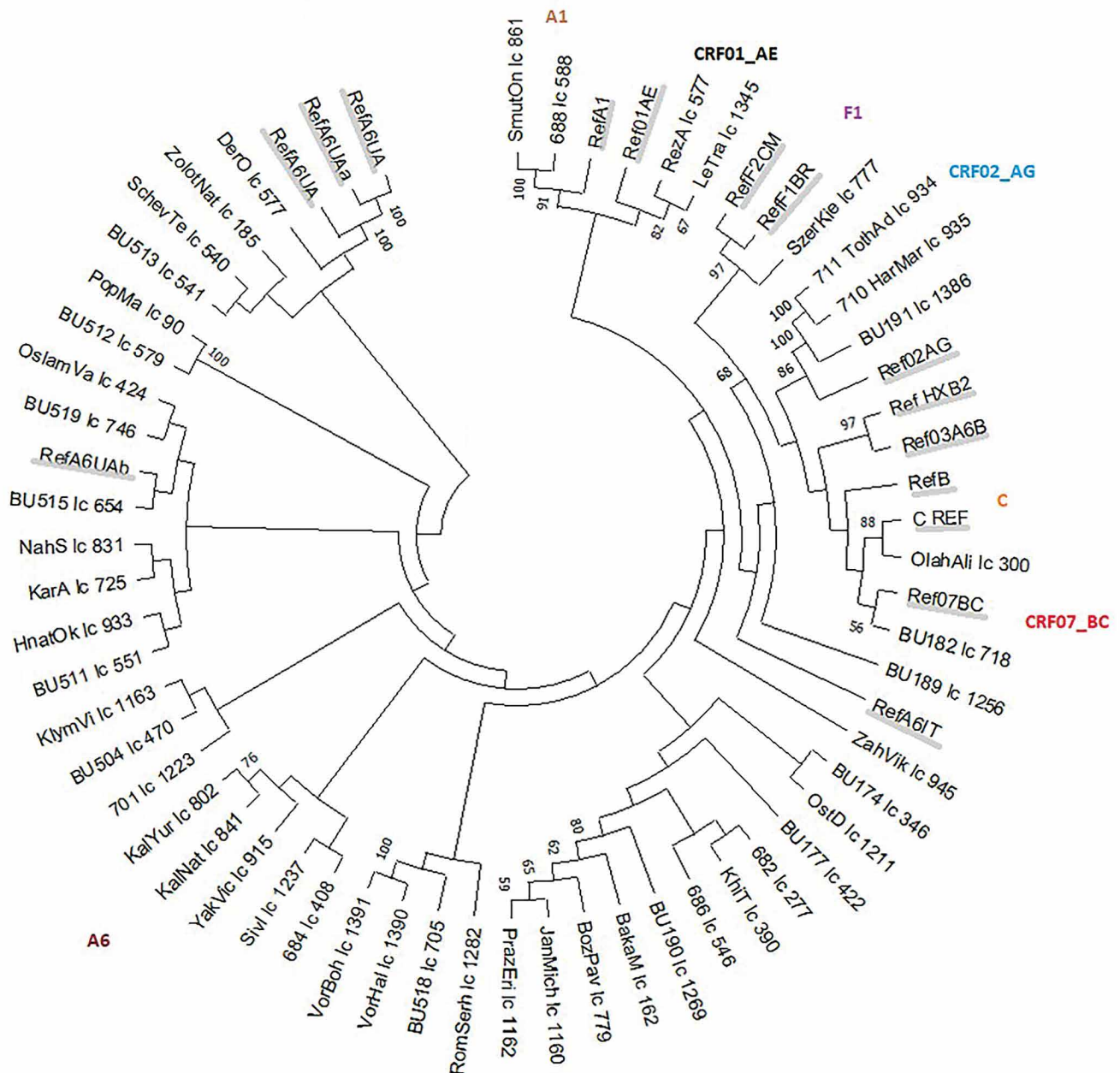


Figure 1. The phylogenetic tree illustrating non-B HIV subtypes distribution among analyzed patients. (Phylogenetic analysis was performed for subtype confirmation rather than for transmission cluster analysis.)

The bootstrap consensus tree was constructed using the Neighbor-Joining method [13] inferred from 100 replicates. Branches corresponding to partitions reproduced in less than 40% bootstrap replicates are collapsed. The evolutionary distances were computed using the Kimura 2-parameter method. The rate variation among sites was modelled using a gamma distribution. The phylogenetic analyses were conducted in MEGA X [14].

Drug resistance

HIV resistance testing performed in samples from 99 patients revealed a generally low prevalence of resistance-associated mutations. Most patients (89/99; 89.9%) showed full susceptibility to all analysed drug classes. Minor or accessory mutations associated with low or potential low-level resistance were identified in 9 (9.1%) patients.

One major resistance mutation, M184I, was detected in a previously treated patient. This mutation is known to confer high-level resistance to lamivudine (3TC) and emtricitabine (FTC).

The most frequently observed accessory or polymorphic mutations included A62V, M46L, D232N and E157Q, which are commonly reported as naturally occurring or treatment-associated minor variants.

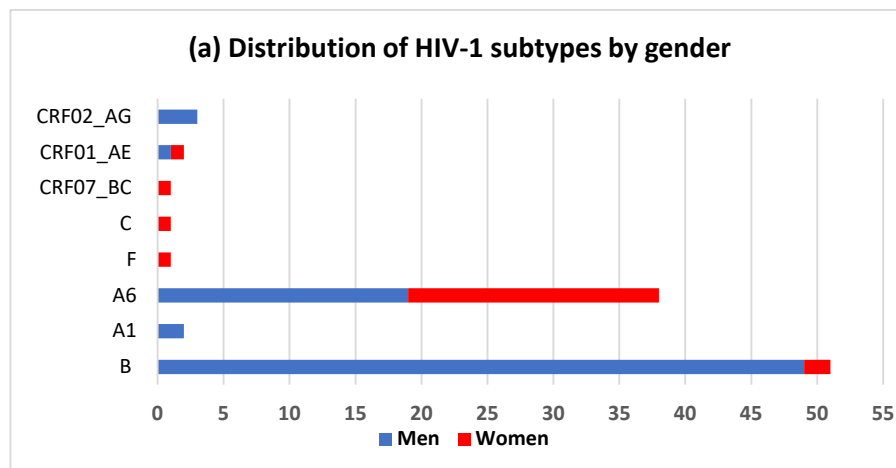
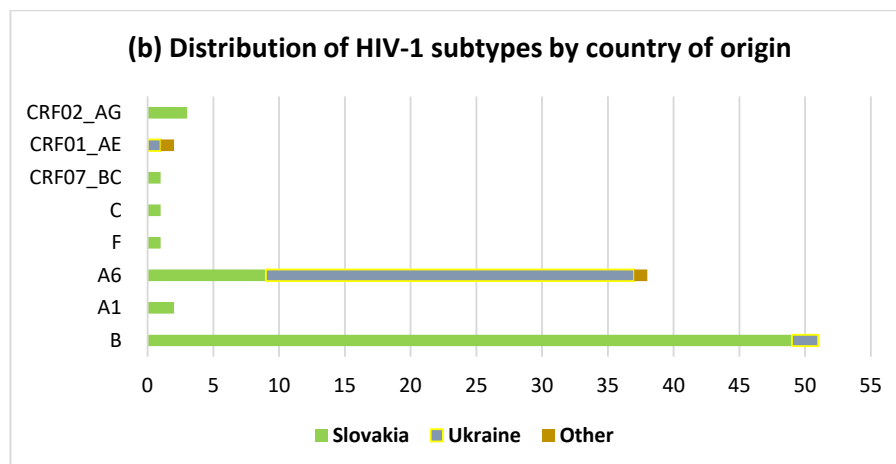
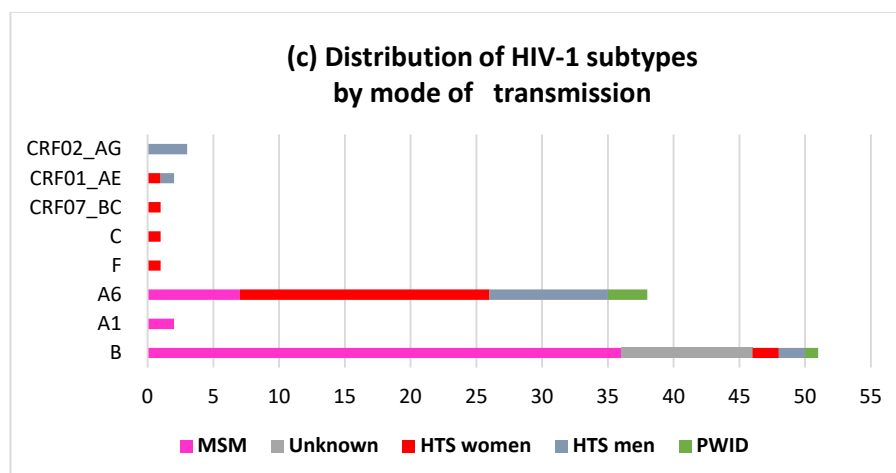
a**b****c**

Figure 2. Distributions of HIV-1 subtypes in 2022 cohort by gender illustrated in graph (a), country of origin (b), and mode of transmission (c)

HTS = heterosexual; MSM = men having sex with men; PWID = people who inject drugs

DISCUSSION

Epidemiological shift and migration impact

The molecular epidemiology of HIV-1 infections diagnosed in Slovakia in 2022 reflects the impact of migration following the war in Ukraine. However, findings of our study should be interpreted with caution, as most Ukrainian patients had been previously diagnosed and treated before arrival and were only reconfirmed in Slovakia.

HIV-1 subtype B accounts for about 11% of infections globally [15] and has historically represented approximately 70% of newly registered cases in Slovakia [1, 16, 17]. In this study, subtype B remained dominant (51.5%) and was strongly associated with MSM transmission (36/45; 80.0%), whereas subtype A6 predominated among Ukrainian patients (28/31; 90.3%), reflecting the known epidemiology of Eastern Europe rather than ongoing local transmission in Slovakia [18–20].

Demographic differences were observed between Slovak and Ukrainian patients. In the analysed molecular cohort, Slovak patients were predominantly men (62/66; 93.9%), reflecting MSM transmission, whereas women predominated among Ukrainian patients (20/31; 64.5%) with mainly heterosexual transmission. These differences likely reflect the demographic structure of the refugee population and the epidemiology of HIV infection in Ukraine [19, 20], as also reported for Ukrainian HIV-positive refugees in Slovakia [6, 21].

Similar migration-associated changes in subtype distribution have been reported in other European countries, including the Czech Republic, Bulgaria, Poland and Germany [4, 5, 22, 23]. In Slovakia, subtype B still predominates among domestic infections, whereas the increase in subtype A6 is mainly linked to imported cases.

Drug resistance patterns

The resistance profile observed in this study is consistent with recent findings from Slovakia and neighbouring countries [16, 17]. The overall prevalence of resistance-associated mutations was 9.1%, in the studied population.

Most patients (89/99; 89.9%) were fully susceptible to all major antiretroviral drug classes. Minor or accessory mutations identified in this cohort included A62V, M46L and E157Q, which are commonly reported in subtype A variants circulating also in Eastern Europe and the post-Soviet region [24, 25].

One major resistance mutation, M184I, was detected in a previously treated patient. This mutation confers high-level resistance to lamivudine (3TC) and emtricitabine (FTC) and is usually associated with prior treatment exposure. Apart from this single case, suggesting that major resistance-associated mutations were uncommon in the analysed cohort. Continued effective-

ness of ART in Slovakia and suggesting only a minimal risk of widespread transmission of resistant strains [26].

Subtype diversity and its implications for public health policy

The presence of multiple non-B subtypes, including A6 and several recombinant forms, indicates increasing genetic diversity of HIV-1 in Slovakia. Similar diversification has been reported in Bulgaria and Hungary [5, 27], highlighting the impact of population migration. Although subtype B remains dominant among MSM, the occurrence of non-B variants in heterosexual transmission suggests evolving epidemiological dynamics and supports the need for continuous molecular surveillance [18].

The conflict in Ukraine has created large numbers of refugees from its inhabitants, caused significant population movements, which has had implications for epidemiology and molecular diversity of HIV1 in receiving countries. These data show that geopolitical events can influence the spread of infectious diseases. It is therefore essential to allocate funds with the aim to improve HIV resistance testing and targeted prevention initiatives for both MSM and heterosexuals. These strategies are critical for continuing effective ART and thus to monitor the spread of resistant or recombinant strains in Central Europe.

CONCLUSION

The findings of this study, such as the increased proportion of non-B HIV-1 subtypes, especially A6, the change in the demographic composition of the HIV-infected population, and the associated shift in the mode of HIV transmission suggest that migration related to the war in Ukraine influenced the structure of the HIV epidemic in Slovakia. Despite the observed changes in subtype distribution, major resistance-associated mutations were uncommon in the analysed cohort. Nevertheless, continuous surveillance and monitoring of resistance and the effectiveness of ART in Slovakia remains an important task in HIV prevention regardless of the origin of HIV-infected patients.

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Conflict of interest statement

The authors declare no conflict of interest.

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