HIV incidence in the Estonian population in 2013 using the HIV-1 Limiting Antigen Avidity (LAg) assay

Pilleriin Soodla[1], Ruth Simmons[2], Kristi Huik[1], Merit Pauskar[1], Ene-Ly Jõgeda[1], Heli Rajasaar[1], Eveli Kallas[1], Matti Maimets[3], Radko Avi[1], Gary Murphy[4], Kholoud Porter[2], Irja Lutsar[1] for CASCADE Collaboration in EuroCoord*

1 University of Tartu, Faculty of Medicine, Department of Microbiology, Tartu, Estonia,

2 University College London, London, UK

3 Tartu University Hospital, Department of Infectious Diseases, Tartu, Estonia

4 Public Health England, London, UK

* See Appendix

Correspondence to: Pilleriin Soodla, email: pilleriin.soodla@gmail.com

Number of words: abstract (214), main text (3134)

Tables (3), Figures (1)

Keywords: epidemiology; human immunodeficiency virus – HIV; injecting drug users; Eastern Europe; HIV recent infection; HIV serological assay; Recent Infection Testing Algorithm (RITA)

**The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under EuroCoord grant agreement n° 260694*"

Abstract

Estonia has one the highest number of new HIV diagnoses in the European Union, mainly among injecting drug users and heterosexuals. Little is known of HIV incidence, which is crucial for limiting the spread of the epidemic. Using a Recent HIV Testing Algorithm (RITA) assay, we aimed to estimate HIV incidence in 2013.

All individuals aged ≥18 years newly-diagnosed with HIV in Estonia January- December 2013, except blood donors and those undergoing antenatal screening, were included. Demographic and clinical data were obtained from the Estonian Health Board and the Estonian HIV-positive patient database. Serum samples were tested for recent infection using the LAg-avidity EIA assay. HIV incidence was estimated based on previously published methods.

Of 69,115 tested subjects, 286 (0.41%) were newly-diagnosed with HIV with median age of 33 years (IQR 28-42) and 65% male. Self-reported routes of HIV transmission were mostly sex between men and women (n=157, 54%) and injecting drug use (n=62, 21%); 64 (22%) were with unknown risk group. Eighty two (36%) were assigned recent, resulting in estimated HIV incidence of 0.06%, corresponding to 642 new infections in 2013 among the non-screened population. Incidence was highest (1.48%) among people who inject drugs.

These high HIV incidence estimates in Estonia call for urgent action of renewed targeted public health promotion and HIV testing campaigns.

Introduction

Infection caused by human immunodeficiency virus (HIV) is a major public health issue in former Soviet Union countries (1), including Estonia. With a population of 1.312 million (2), Estonia has experienced a rapidly expanding HIV epidemic among young people who inject drugs (PWID) with a rare HIV subtype CRF06_cpx from the year 2000, reaching the highest diagnosis rate in the European Union of 105.3 per 100,000 population in 2001 (3-5). Several efforts have been undertaken by the Estonian government to contain the rapidly evolving epidemic, including a campaign to increase people's awareness of HIV infection, implementation of needle exchange programs, and offering free testing for all pregnant women, prisoners, as well as those with behavioural risks, together with the availability of free-of-charge antiretroviral treatment for everyone (6, 7). By 2013, the rate of reported new diagnoses had decreased and stabilized at 24.6/100,000 (4, 8).

Case reporting of HIV is well-established across Estonia and, since the first diagnosis of HIV in 1988, 9263 persons had been registered with the Estonian Health Board by the end of 2015. While injecting drug use was instrumental to the rise in new diagnoses, with 90% of cases in 2000 among PWID, the number of diagnoses attributable to heterosexual contact gradually increased, overtaking that of PWID in 2010, and representing 46% of all cases in 2014 (4).

Although these figures are important measures of the epidemic, they do not necessarily reflect current transmission patterns. This is important information as the ability to estimate current HIV incidence is an essential public health monitoring tool indicating the characteristics of individuals at greatest risk, and guiding prevention and intervention strategies.

A number of serological tests have been developed which aim to differentiate recent from long-standing HIV infection (9-13) and allow the development of methods to estimate incidence using blood at a single time point from the diagnostic HIV test (14-16).

While data on HIV incidence in Western countries exist (14, 17), data for former Soviet Union countries, including Estonia, are limited (16). We aimed to characterise the newly-diagnosed HIV-positive population in Estonia using the Recent HIV infection Testing Algorithm (RITA) in order to determine the proportion recently-infected and estimate HIV incidence using previously published methodologies (14, 18).

Methods

HIV testing in Estonia

An HIV test can be requested by any physician, including general practitioners, the cost of which is covered by health insurance with an estimated 90% of the population being insured. Additionally, there are 23 counselling offices, where people can self-select for an HIV test free of charge, as well as occasional field-testing campaigns. Primary tests are performed in 24 local laboratories using ELISA tests of various generations. All positive results are confirmed in a single HIV Reference Laboratory at West Tallinn Central Hospital using INNO-LIA® HIV I/II Score (INNOGENETICS N.V., Gent, Belgium), Bio-Rad's NEW LAV BLOT I (Bio-Rad Laboratories Inc, Hercules, California, USA) and NEW LAV BLOT II (Bio-Rad Laboratories Inc, Hercules, California, USA) tests, and reported to the Estonian Health Board. Screening for HIV antibodies is mandatory for blood and organ donors, for pregnant women at two time points during pregnancy, and is strongly recommended for patients with tuberculosis, as well as prison inmates.

All HIV positive individuals linked to care are invited to participate in the Estonian HIV database (E-HIV, www.hiv.ut.ee) with signed informed consent as detailed elsewhere (8).

Study population and design

We conducted a prospective study using existing country-wide HIV testing services and included all individuals aged ≥18 years with newly-diagnosed HIV infection between 1st January 2013 and 31st December 2013. We linked test results from the Reference Laboratory to the Estonian Health Board database to obtain demographic data (sex, age, date of diagnosis, and reason for testing) using the unique Estonian national identification number (ID-code) Testing reasons were clinical signs, screening (pregnancy or blood donors), indicator diagnosis (STD, TB), indicator risk (intravenous drug usage, imprisonment, asymptomatic patient with known contact), other (pre-operation, patient will) or not known. Additional data on possible date of seroconversion, self-reported HIV transmission risk group, concomitant AIDS defining condition(s) (19), HIV-1 viral load and CD4+ cell count within 6 months of diagnosis, as well as information on the use of combination antiretroviral therapy (cART) were extracted form E-HIV database. Finally, the laboratory databases of four main hospitals - Tartu University Clinics, West-Tallinn Central Hospital, Narva Hospital, and Ida-Viru Central Hospital were searched in April 2015 to identify previous negative HIV-1 antibody tests within 2 years of HIV diagnosis date, as well as HIV-1 viral load measurements within 6 months of diagnosis.

Laboratory methods

All available left-over serum samples were obtained from the HIV Reference Laboratory of West Tallinn Central Hospital and tested for evidence of recent infection using the limiting antigen (LAg) avidity EIA assay (Sedia tm HIV-1 LAg Avidity EIA; Sedia Biosciences Corporation, Portland, OR, USA), according to the manufacturer's protocol (20). LAg-avidity EIA is a single well antibody avidity-based incidence assay, presenting multi-subtype recombinant HIV-1 gp41 antigen. The antibody avidity, the bond between antigen and antibody after using M citrate buffer as dissociation agent, was measured as an optical density (ODn) value. The initial test requires samples to be screened, with samples with an ODn $\leq$2.0 tested in triplicate for confirmation and the median of three results being the final result. According to the 2013 protocol, ODn value <1.5 is classified as recent, and the respective median duration of an infection (recency period) is 130 (95% CI: 118-142) days (12, 20).

As evaluations of the LAg avidity assay indicated that the assay performed poorly on specimens with a low or undetectable viral load and in persons receiving cART (13), samples with viral load <1000 copies/mL were reclassified as longstanding. All newly-diagnosed individuals were ART-naïve.

Statistical methods

HIV incidence was estimated using the stratified extrapolation method initially proposed by Karon *et al* and modified by Prejean *et al* (14, 18). Briefly, this modified method uses the observed number of recent infections, and the probability a person will present for an HIV test and be classified as recent using a RITA assay, to estimate the true number of infections within the population during the period of interest. The observed number of recent infections equates to the number of HIV-positive individuals testing for HIV and being assigned as recent by the LAg assay. To establish the true number of recent infections, the observed number is divided by the probability of testing and being classified as recent.

Incidence calculations are described elsewhere (16). Rates were calculated by dividing the number of tests, diagnoses or estimated number of recent infections for the calendar year 2013 by the Estonian population denominator and multiplied by 100,000 (21). Incidence rates were estimated for Estonia overall, and by age and sex using the general population estimates as denominators. We used published estimates of the number of PWID to derive incidence estimates for this subgroup (21).

Using a logistic regression model, we also examined factors associated with being recently infected with HIV. Variables included were: sex, age group, testing history, reason for test, and probable route of exposure. All confidence intervals (CI) are at the 95% significant level.

Ethics statement

The study was part of CASCADE within EuroCoord Network of Excellence (www.EuroCoord.net) funded by the European Union Framework Programme 7. The study was approved by the Research Ethics Committee of the University of Tartu and the Ethics Committee of University College London (UCL). E-HIV is approved by Estonian Data Protection Agency; all participants signed informed consent for participation in E-HIV.

Results

Over the study period, 212,506 HIV tests were performed across Estonia corresponding to 151,641 individuals aged ≥18 years. Of these, 82,526 were tested as part of screening (63,972 blood donors and 18,554 antenatal screening) with 36 (11%) being newly diagnosed HIV positive and they were excluded from further analysis. In addition, 286 (89%) newly diagnosed adults were referred by a physician or self-selecting.

Population characteristics

The 286 individuals testing HIV positive equated to a crude diagnosis rate of 29.7 per 100,000 population (40.5 for men and 20.7 for women). The median age at HIV diagnosis was 33 years (IQR: 28-42). Men accounted for 65% of new diagnoses, with the majority being tested within the capital Tallinn, and tested due to symptoms or high-risk behaviour (injecting drug use, imprisonment, or contact with a known HIV-positive individual). Self-reported risk factor information was available for 223 (78%), with the majority reporting sex between men and women (n=152; 68%). Of the 63 individuals without a recorded route of transmission, 46 (73%) were men. Two hundred and forty-two individuals (85%) had not undergone testing within last two years and were thus classified as first time testers (Table 1).

CD4+ counts and HIV-1 viral loads were measured within a median of 10 days (IQR 5 - 38) of HIV diagnosis. Median CD4+ cell count was 367 (IQR 279-566) cells/µl among those recently infected and 362 (IQR 212-528) cells/µl among those with long-standing infection. Late presenters (CD4 count < 350 cells/µl) accounted for 32% of all newly-diagnosed individuals. Eleven individuals presented with AIDS at HIV diagnosis, and six with symptomatic seroconversion. Median HIV-1 viral load was 4.94 (IQR 4.26-5.65) $\log_{10}$ copies/mL.

The majority of the 224 sequenced samples (n= 180, 80%) were HIV-1 subtype CRF06_cpx.

Recent HIV Infection

Of the 286 individuals newly-diagnosed with HIV, 29 had insufficient serum for LAg testing. Of the remaining 257, a viral load measurement was available within 6 months of diagnosis for 228 (80% of new diagnoses) as presented in Figure 1. Of these, 86 (38%) were classified as a recent infection according to the LAg assay, 4 of whom had a viral load <1000 copies/mL and were, therefore, reclassified as long-standing infection. Of 228 new diagnoses, 82 (36%) were,

thus, considered to have been recently-infected according to the LAg assay; 31% (n=62) among first-time testers and 67% (n=20) among repeat testers (Table 1).

The median age of recently-infected individuals was 32 years [IQR: 26-41], with slightly more men than women (57% vs 43% respectively). All 44 (15%) individuals with a negative HIV test within one year prior to HIV diagnosis and 6 (2%) individuals presenting with symptomatic seroconversion tested recent with the LAg assay.

The probability of testing and being classified as recent overall for repeat and first time testers and demographic characteristics are shown in Table 2. The only independent predictor for being recently-infected was being a repeat tester [Odds Ratio 3.96, 95% CI 1.66-9.46] compared to being a first time tester.

HIV incidence estimates

We estimated HIV incidence at 0.06% in 2013, corresponding to 642 new infections in that year (Table 3) and 12.8% were diagnosed among the non-screened population in the same year.

Incidence was higher among younger age groups, and highest for age groups 18-29 (0.20%) and 30-39 years (0.22%). Incidence among men was substantially higher than among women in these two age groups (0.08% and 0.05%, respectively), and we estimated the highest incidence among PWID at 1.48%.

Discussion

To the best of our knowledge this is the first study to estimate HIV incidence covering the entire population of an Eastern European country and using a RITA assay in a population infected predominantly with a recombinant HIV-1 subtype.

We estimated HIV incidence for Estonia during 2013 to be 0.06%; higher than the 0.024% new diagnoses rate, and considerably higher than incidence estimates using the same methodology within Kiev, Ukraine (0.02%) (16); and the USA (0.019%) (14). The diagnosis rate for Estonia is in accordance with published estimates from previous years, and remains one of the highest rates of new HIV diagnoses in Europe (22, 23). The high incidence rate among PWID reported here of 1.48% is considerably higher than that reported previously by other investigators in the capital, Tallinn, of 0.02%, and likely reflects low success of preventive measures in this risk group (24).

Estonia has a very similar HIV epidemic to Ukraine; both are among the highest rates of new diagnoses rates in Europe, despite obvious economic differences with gross national incomes per capita in 2014 of 3,760 USD for Ukraine compared to 19,010 USD for Estonia (25). National surveillance for both countries suggest that individuals at high risk of HIV are predominately PWID or heterosexuals, and not MSM. Interestingly, however, when persons self-reported their risk in Ukraine, 24% identified as MSM, who were also disproportionately affected by HIV (16), in contrast to the proportion of 2% in our study in Estonia. In an epidemic which is predominantly PWID and heterosexuals, one would expect similar HIV diagnoses rates among men and women. In our study the HIV diagnosis rate among men is almost double that of women's (40.5 vs 20.7). Still, men may be more likely than women to be drug injectors as shown in Estonia in 2000 when more than 80% of HIV infections occurred in young men (4). It may also be that men who are injecting drug users or at risk of heterosexual transmission are more likely to seek HIV testing than their female counterparts. We also noted, however, that 73% of the unknown risk group, a substantial proportion of all testers at 22%, are male, who were also more likely to be repeated testers and previous studies have reported that MSM are more likely to be repeated testers (26, 27). Taken together, these data may indicate that a significant proportion of men reporting risk factors other than MSM, as well as those with no reported risk, had acquired infection through sex with other men.

Although several efforts have been made to increase the number of people testing for HIV, for example through the countrywide HIV testing guidelines which were introduced in 2012 (28),

we found that only 15% of those testing in 2013, and 25% of recent infections in that period, had previously tested for HIV, despite approximately 70,000 tests being performed that year, excluding screenings. We also found high percentage of recent infections (40%) and that a small percentage (12.8%) of estimated newly-infected individuals have been diagnosed, indicating that the risk population was being missed. Furthermore, we noted a high proportion of late presenters (32%) with median CD4 at diagnosis of 360 cells/µl, similar to late presentation rate reported by the investigators of western European cohorts of 38% and median CD4 count at diagnosis of 368 cells/µl (29). Given the high levels of viraemia which characterise undiagnosed HIV infection (30-32) and the risk of onward transmission (33, 34), as well as the higher risk of mortality and treatment failure for late presenters (35, 36), there is an urgent need to promote HIV testing in the at risk population to identify individuals close to the time of HIV infection and initiate cART.

Having a previous negative test was the only predictor for presenting with a recent infection, suggesting that these persons are aware of their risk factors resulting in frequent tests. The risks of repeated testers need to be addressed. Surveillance of new diagnoses suggest that the number among PWID in Estonia has decreased, with diagnoses attributable to heterosexual contact crossing that of the number of PWID (4). However our estimates suggest that PWID remains an important population; incidence estimates among them being 24 times higher than the overall estimates.

The main strength of our study is the overall design of the national surveillance in Estonia where individuals receive an ID-code which allows data from multiple sources to be linked successfully. As data are directly reported from the Reference Laboratory to the Health Board, we believe that all new diagnoses in Estonia in 2013 were captured. We also made every effort to use data on previous testing history by interrogating laboratory and clinical databases rather than relying on self-reporting which is a key element required to estimate incidence.

Some limitations should be noted. First, we acknowledge that some individuals may have been tested during testing campaigns or in 23 counselling offices across country and their previous negative tests may, therefore, have not been recorded and would have under estimated the proportion of repeat testers. Such individuals are likely to be few in number, however and, in any case, would have been assigned to recent infection status by the LAg assay. Second, not all individuals newly diagnosed had viral load quantified at diagnoses. We were, however, able to link their records to viral load where the measurements undertaken within 6 months of the

HIV test; median time from diagnosis was 10 days. Thirdly, we were unable to estimate incidence for any HIV risk group other than PWID as general population figures are not available and/or the number testing positive whose HIV risk group was identified as MSM was very low. Finally, some uncertainties have been raised relating to the performance of the LAg assay in non-B HIV subtypes (37), and the prevalent subtype in Estonia, CRF06_cpx, has not been included into assay development (38). However, we found that all individuals with laboratory evidence of a negative HIV antibody test within 1 year prior to diagnosis were classified as recent, as were those diagnosed with seroconversion illness.

In conclusion we demonstrate for the first time at a population level very high HIV-1 incidence in Estonia, especially among the young and PWID. There is clear indication of ongoing HIV transmission in all risk groups, with the possibility of undisclosed homosexuality in Estonia. All these findings need to be urgently addressed with improved implementation of preventive measures among appropriate risk groups and intensification of targeted HIV testing among the general population especially in the areas of concentrated HIV epidemic.

Figure 1. Incidence study flow chart in Estonia in 2013

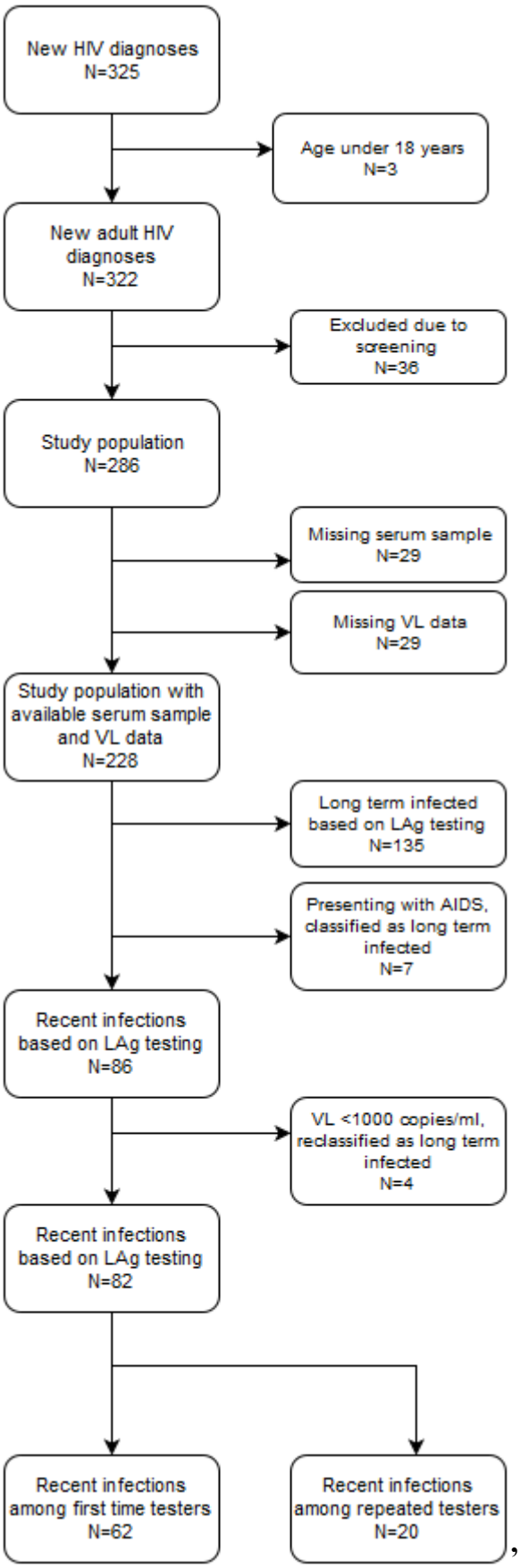

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Table 1: Characteristics of all newly-diagnosed* HIV-1 positive individuals in Estonia, January- December 2013

Characteristics	New HIV diagnoses	Blood sample and viral load available	Classified recent on LAg assay	
	n=286	n=228	n=82	%
Age (years)				
18-29	98	76	35	46
30-39	97	76	25	33
40-49	50	43	15	35
≥50	39	32	7	22
Not reported	1	1		
Median age (IQR)	33 (28-42)	34 (28-43)	32 (25-41)	
Sex				
Male	185	145	47	32
Female	100	82	35	43
Not reported	1	1		
Self-reported route of exposure to HIV				
Heterosexual men	79	61	19	31
Heterosexual women	73	60	26	43
PWID	65	54	25	46
MSM	6	5	1	20
Not reported	63	48	11	23
Testing history				
Repeat testers	44	30	20	67
First-time testers	242	198	62	31
Reason for test				
Clinical Indicators	95	76	29	38
High Risk Behaviour	78	62	27	44
Other	22	17	3	18
Not reported	91	73	23	32

* does not include individuals testing positive through screening

IQR= interquartile range, PWID= people who inject drugs, MSM= sex between men

Table 2. Direct estimation of probabilities of testing and being classified as recent by sub-populations for Estonia 2013

Characteristics	Probability of testing and being classified as recent			
	Repeat testers	**(95% CI)**	**First-time testers**	**(95% CI)**
All	**0.360**	**0.306-0.414**	**0.136**	**0.111-0.163**
Age (years)				
18-29	0.357	0.292-0.418	0.163	0.015-0.252
30-39	0.333	0.227-0.431	0.106	0.077-0.156
40-49	0.384	0.188-0.580	0.218	0.136-0.452
≥50	0.524	0.200-0.841	0.089	0.054-0.163
Sex				
Male	0.399	0.321-0.483	0.142	0.111-0.192
Female	0.313	0.243-0.383	0.115	0.086-0.172
Probable route of exposure				
Heterosexual Contact	0.355	0.283-0.425	0.12	0.092-0.156
PWID	0.418	0.344-0.484	0.125	0.089-0.192
MSM	-		-	
Not reported	0.235	0.157-0.316	0.204	0.130-0.452

CI= confidence intervals, PWID= people who inject drugs, MSM= Men who have sex with men

Table 3: HIV incidence estimates for Estonia, 2013

	Population*	Number new HIV infections (95% CI)		% HIV incidence (95% CI)	
Total	1,076,483	642	(559-761)	0.06	(0.05-0.07)
Age (years)					
18-29	119,352	234	(172-311)	0.20	(0.14-0.26)
30-39	115,161	252	(189-333)	0.22	(0.16-0.29)
40-49	90,519	75	(46-115)	0.08	(0.05-0.13)
≥50	751,451	73	(50-116)	0.01	(0.007-0.02)
Sex					
Male	490,847	376	(291-469)	0.08	(0.06-0.10)
Female	585,636	291	(224-364)	0.05	(0.04-0.06)
Risk category of transmission					
Heterosexual contact	NA	367	(308-454)	NA	
PWID	14,000	207	(143-282)	1.48	(1.02-2.01)

Source: (*) Statistics Estonia 2016

CI= confidence intervals, PWID= people who inject drugs

Appendix

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