DOI: 10.1111/hiv.12535
HIV Medicine (2018), **19**, 33–41

ORIGINAL RESEARCH

HIV incidence in the Estonian population in 2013 determined using the HIV-1 limiting antigen avidity assay

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Objectives

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 epidemic. Using a recent HIV infection testing algorithm (RITA) assay, we aimed to estimate HIV incidence in 2013.

Methods

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.

Results

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 heterosexual contact (n = 157, 53%) 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.

Conclusions

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

Keywords: Eastern Europe, epidemiology, HIV, HIV serological assay, injecting drug users, recent HIV infection, recent infection testing algorithm

Accepted 2 June 2017

Introduction

HIV infection 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 from the year 2000 among young people who inject drugs (PWID), who are being infected with a rare HIV subtype, CRF06_cpx, and the highest diagnosis rate in the European Union of 105.3 per 100 000 population was reached 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 programmes, and offering free testing for all pregnant women, prisoners, and those with behavioural risk factors, 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 infection is well established across Estonia and, since the first diagnosis of HIV

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[a]See Appendix.

infection in 1988, 9263 persons had been registered with the Estonian Health Board by the end of 2015. While injecting drug use was instrumental in 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 an important point as the ability to estimate the 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 instead of time consuming cohort studies [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 characterize the newly diagnosed HIV-positive population in Estonia using the recent HIV infection testing algorithm (RITA) 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 enzyme-linked immunosorbent assay (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, CA, USA) and NEW LAV BLOT II (Bio-Rad Laboratories Inc.) tests, and reported to the Estonian Health Board. Screening for HIV antibodies is mandatory for blood and organ donors and 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 being obtained as detailed elsewhere [8].

Study population and design

We conducted a prospective study using existing country-wide HIV testing services. The study population consisted of all individuals aged ≥ 18 years with newly diagnosed HIV infection between 1 January 2013 and 31 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) for all study subjects. Testing reasons were clinical signs suggestive of HIV infection, screening (antenatal or blood donors), indicator diagnosis (sexually transmitted disease or tuberculosis), indicator risk (injecting drug use, imprisonment or asymptomatic patient with known contact), other (pre-operation or patient will) or not known. Additional data on possible date of seroconversion, self-reported risk factors, 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 from the 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. All individuals with serum samples and available HIV viral load measurements were included in the final study population.

Laboratory methods

Left-over serum samples from newly diagnosed individuals in 2013 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 enzyme immunoassay (EIA) (Sedia tm HIV-1 LAg Avidity EIA; Sedia Biosciences Corporation, Portland, OR), according to the manufacturer's protocol [20]. The LAg avidity EIA is a single-well antibody avidity-based incidence assay, presenting multi-subtype recombinant HIV-1 glycoprotein 41 (gp41) antigen. The antibody avidity, the bond between antigen and antibody after using M citrate buffer as a dissociation agent, was measured as an optical density (OD) value. The initial test requires samples to be screened, with samples with an

OD ≤ 2.0 tested in triplicate for confirmation and the median of three results being the final result. According to the 2013 protocol, an OD value < 1.5 is classified as recent infection, and the respective mean duration of an infection (recency period) is 130 days [95% confidence interval (CI) 118–142 days] [12,20].

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]. According to RITA, samples with a viral load < 1000 HIV-1 RNA copies/mL and individuals presenting with AIDS were reclassified as showing longstanding infection.

Statistical methods

An extrapolation method initially proposed by Karon *et al*. [18] and modified by Prejean *et al*. [14] was used to estimate HIV incidence. This method estimates the true number of recent infections within the population from the observed number of recent infections using the probability that a person will present for an HIV test and be classified as having recent infection using RITA. The true number of recent infections is estimated by dividing the observed number by the probability of testing and being classified as having a recent infection.

The observed number of recent infections equates to the number of HIV-positive individuals testing for HIV and being classified as having recent infection by RITA, after reclassifying LAg assay 'recent' samples with a viral load < 1000 copies/mL and individuals with an AIDS-defining illness as showing longstanding infection.

The probability that a person will present for an HIV test and be classified as recently infected using RITA is estimated for repeat testers and first-time testers separately. For repeat testers, the probability of testing and being classified as recently infected for an individual is the weighted sum of each possible date of seroconversion between the person's last negative and first positive test. For first-time testers, P is estimated by the weighted sum of the probability that a person tests within the assay's recency period and the probability that the test is performed before AIDS develops.

Incidence calculations have been thoroughly described elsewhere [16]. Rates were calculated by dividing the number of tests, the number of diagnoses or the estimated number of recent infections for the calendar year 2013 by the Estonian population denominator and multiplying by 100 000 [21]. Incidence rates were estimated for Estonia overall, and by age and sex using the general population estimates as denominators. The published estimates of the number of PWID were used 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 CIs are at the 95% significance level.

Ethics statement

The study was part of CASCADE within the EuroCoord Network of Excellence (www.EuroCoord.net) funded by the European Union Framework Programme 7. Additional funding was obtained from Institutional Research Funding (IUT34-24) provided by the Estonian Ministry of Education and Research, European Union through the European Regional Development Fund.

The study was approved by the Research Ethics Committee of the University of Tartu and the Ethics Committee of University College London. E-HIV is approved by the Estonian Data Protection Agency; all participants provided informed consent for participation in E-HIV.

Results

Over the study period, 212 506 HIV tests were performed across Estonia, with 151 641 adults (aged ≥ 18 years) being tested. Of these, 69 115 subjects were referred by a physician or self-selected for a test and 82 526 individuals were tested as part of screening (63 972 blood donors and 18 554 women undergoing antenatal screening). There were 322 new adult HIV diagnoses, 286 among the nonscreened population (study population) and 36 among the screened population; the latter were excluded from further analysis.

The crude rate of new HIV diagnosis was 29.7 per 100 000 population (40.5 for men and 20.7 for women). A study flow diagram is presented in Figure 1.

Characteristics of the study population

The median age of the study population at the time of HIV diagnosis was 33 years [interquartile range (IQR) 28–42 years] and men accounted for 65% of the sample. The majority of individuals lived in the capital Tallinn and were tested because of symptoms or high-risk behaviour (injecting drug use, imprisonment, or contact with a known HIV-positive individual). Two hundred and forty-two individuals of 286 (85%) had not undergone testing within the last 2 years and were thus classified as first-time testers (Table 1). The probability of testing and being

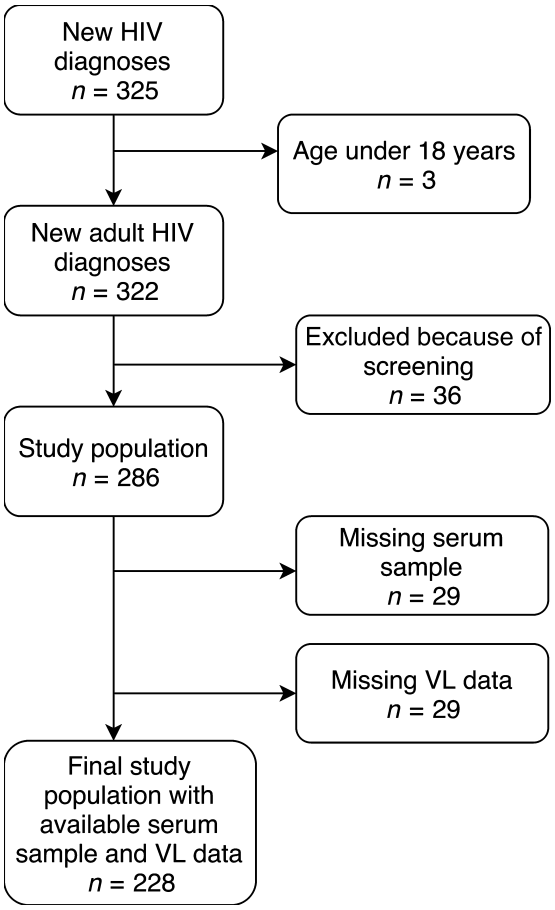

Fig. 1 Incidence study flow in Estonia in 2013. VL, viral load.

classified as recently infected by RITA overall for repeat and first-time testers and demographic characteristics are shown in Table 2.

The most common self-reported risk in the study population was sex between men and women (53%). Of the 63 individuals with unknown route of transmission, 46 (73%) were men. All individuals were ART-naïve.

Recent HIV infection

The final study population consisted of 228 individuals after the additional exclusion of 29 individuals with insufficient serum for LAg testing and another 29 who did not have a viral load measurement within 6 months of diagnosis, as presented in Figure 1. Of these, 86 (38%) were classified as recently infected according to the LAg assay. Four individuals out of 86 had a viral load < 1000 copies/mL and were therefore reclassified as having longstanding infection according to RITA, leaving 82 individuals (36%) classified as recently infected; 62 were first-time testers and 20 repeat testers (Table 1). The median age of recently infected individuals was 32 years (IQR: 26–41 years) and there were slightly more men than women among them (47 versus 35 individuals, respectively). Among 30 repeat testers in the final study population, 67% (n = 20) tested as recently infected with the LAg assay, together with all six individuals presenting with symptomatic seroconversion. The only independent predictor for being recently infected was being a repeat tester [odds ratio (OR) 3.96; 95% CI: 1.66–9.46] compared with being a first-time tester.

CD4 counts and HIV-1 viral loads in the final study population were measured within a median of 10 days (IQR: 5–38 days) of HIV diagnosis. The median CD4 cell counts among those recently infected and those with longstanding infection were similar [367 (IQR: 279–566) cells/μL versus 362 (IQR: 212–528) cells/μL, respectively]. Late presenters (CD4 count < 350 cells/μL) accounted for 32% of the sample (n = 73). Eleven individuals presented with AIDS at HIV diagnosis; they were all classified as long-term infected by RITA. Six individuals presented with symptomatic seroconversion; they all tested as recently infected with LAg. Median HIV-1 viral load was 4.94 (IQR: 4.26–5.65) $\log_{10}$ copies/mL with no differences between those who were recently infected and those with longstanding infection.

Most of the 224 successfully sequenced samples were HIV-1 subtype CRF06_cpx (n = 180; 80%) with only five individuals with subtype B strains (2%).

HIV incidence estimates for the Estonian population

We estimated HIV incidence at 0.06% in 2013, corresponding to an estimated 642 new infections in that year (Table 3). Of these, 12.8% (82 recent infections according to RITA among 642 estimated infections) were actually reported among the nonscreened population in the same year.

The HIV incidence was higher among younger age groups, being highest for age groups 18–29 (0.20%) and 30–39 years (0.22%). The incidence among men was higher than among women (0.08% and 0.05%, respectively) in the two above-mentioned age groups. 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 in a population infected predominantly with a recombinant HIV-1 subtype.

We estimated the HIV incidence for Estonia during 2013 to be 0.06%; higher than the 29.7 per 100 000 population new diagnosis rate, and considerably higher than

Table 1 Characteristics of study populations in Estonia, January–December 2013

Characteristics	Study population; new HIV diagnoses (n = 286)	Final study population; blood sample and viral load available (n = 228)	Classified as recently infected by RITA* (n = 82)	
Age [n (%)]				
18–29 years	99 (35)	76 (33)	35	46
30–39 years	97 (34)	76 (33)	25	33
40–49 years	50 (17)	43 (19)	15	35
≥ 50 years	39 (14)	32 (14)	7	22
Not reported	1 (< 1)	1 (< 1)		
Age (years) [median (IQR)]	33 (28–42)	34 (28–43)	32 (25–41)	
Sex [n (%)]				
Male	185 (65)	145 (64)	47	57
Female	100 (35)	82 (36)	35	43
Not reported	1 (< 1)	1 (< 1)		
Self-reported risk factor [n (%)]				
Heterosexual men	79 (28)	61 (27)	19	31
Heterosexual women	73 (26)	60 (26)	26	43
PWID	65 (23)	54 (24)	25	46
MSM	6 (2)	5 (2)	1	20
Not reported	63 (22)	48 (21)	11	23
Testing history [n (%)]				
Repeat testers	44 (15)	30 (13)	20	67
First-time testers	242 (85)	198 (87)	62	31
Reason for test [n (%)]				
Clinical indicators	95 (33)	76 (33)	29	38
High-risk behaviour	78 (25)	62 (27)	27	44
Other	22 (8)	17 (7)	3	18
Not reported	91 (32)	73 (32)	23	32

IQR, interquartile range; PWID, people who inject drugs; MSM, men who have sex with men; RITA, recent infection testing algorithm.
*Percentages in this column are percentages of the final study population.

Table 2 Direct estimation of probabilities of testing and being classified as recently infected, with 95% confidence intervals (CIs), by subpopulations for Estonia, 2013

	Probability of testing and being classified as recently infected			
	Repeat testers (n = 44)		First-time testers (n = 242)	
Characteristics	P	95% CI	P	95% CI
All	0.360	0.306–0.414	0.136	0.111–0.163
Age				
18–29 years	0.357	0.292–0.418	0.163	0.015–0.252
30–39 years	0.333	0.227–0.431	0.106	0.077–0.156
40–49 years	0.384	0.188–0.580	0.218	0.136–0.452
≥ 50 years	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
Self-reported risk factor*				
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
Not reported	0.235	0.157–0.316	0.204	0.130–0.452

MSM, men who have sex with men; PWID, people who inject drugs.
*The total number of MSM was too small (six individuals) for calculations.

incidence estimates using the same methodology in 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 [23,24]. 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 probably reflects low success of preventive measures in this risk group [25].

Table 3 HIV incidence estimates for the Estonian population in 2013

	Estonian population*	Number of new HIV infections (95% CI)	HIV incidence [% (95% CI)]
Total	1 076 483	642 (559–761)	0.06 (0.05–0.07)
Age			
18–29 years	119 352	234 (172–311)	0.20 (0.14–0.26)
30–39 years	115 161	252 (189–333)	0.22 (0.16–0.29)
40–49 years	90 519	75 (46–115)	0.08 (0.05–0.13)
≥ 50 years	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)
Self-reported risk factor			
Heterosexual contact	NA	367 (308–454)	NA
PWID	14 000	207 (143–282)	1.48 (1.02–2.01)

CI, confidence interval; PWID, people who inject drugs; NA, not available.
*Source: Statistics Estonia 2013 [22].

Estonia has a very similar HIV epidemic to Ukraine; both have the highest rates of new diagnoses in Europe, despite obvious economic differences, with gross national incomes per capita in 2014 of 3760 USD for Ukraine compared with 19 010 USD for Estonia [26]. National surveillance for both countries suggests that individuals at high risk of HIV infection are predominately PWID or heterosexuals, and not men who have sex with men (MSM). Interestingly, however, when persons self-reported their risk in Ukraine, 24% identified as MSM, who were also disproportionately affected by HIV infection [16], in contrast to the proportion of 2% in our study in Estonia. In an epidemic predominantly in PWID and heterosexuals, one would expect similar HIV diagnosis rates among men and women. In our study, the HIV diagnosis rate among men was almost double that among women (40.5/100 000 versus 20.7/100 000, respectively). 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]. We also noted, however, that 73% of the 63 individuals in the unknown risk group were male, who were also more likely to be repeat testers compared to other risk group individuals, and previous studies have reported that MSM are more likely to be repeat testers [27,28]. 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 [29], we found that only 15% of those testing in 2013, and 25% of those recently infected in that period, had previously tested for HIV, despite approximately 70 000 tests being performed that year, excluding screenings. We also found a high percentage of recent infections (36%) and that a small percentage (12.8%) of the estimated number of newly infected individuals were diagnosed, indicating that the at-risk population was being missed. Furthermore, we noted a high proportion of late presenters together with a median CD4 count at diagnosis in the final study population of 367 cells/lL. These results are similar to the late presentation rate of 38% and the median CD4 count at diagnosis of 368 cells/lL reported by the investigators of several Western European cohorts [30]. Given the high levels of viraemia, which characterize undiagnosed HIV infection [31–33] and a higher risk of onward transmission [34,35], as well as a higher risk of mortality and treatment failure for late presenters [36,37], 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 repeat testers need to be addressed. Surveillance of new diagnoses suggests that the number among PWID in Estonia has decreased, with the number of diagnoses attributable to heterosexual contact overtaking the number of diagnoses in PWID [4]. However, our estimates suggest that PWID remain 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 the country and their previous negative tests may, therefore, not have been recorded; this would have resulted in an underestimate of the proportion of repeat testers. Such individuals are likely to be few in number, however, and, in any case, would have been assigned to the recent infection category by the LAg assay. Secondly, not all individuals who were newly diagnosed had a viral load quantified at diagnosis. We were, however, able to link their records to viral load where the measurements were undertaken within 6 months of the HIV test; the 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 [38], and the prevalent subtype in Estonia, CRF06_cpx, has not been included in assay development [39]. However, we found that most individuals with laboratory evidence of a negative HIV antibody test within 1 year prior to diagnosis were classified as recently infected, as were those diagnosed with seroconversion illness. Simulation of different assumptions in the incidence calculation was not conducted.

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 areas where the HIV epidemic is concentrated.

Acknowledgements

Funding: The research leading to these results received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under EuroCoord grant agreement no 260694. Funding was also received from Institutional Research Funding (IUT34-24) provided by the Estonian Ministry of Education and Research, European Union through the European Regional Development Fund.

References

1 DeHovitz J, Uuskula A, El-Bassel N. The HIV epidemic in Eastern Europe and Central Asia. *Curr HIV/AIDS Rep* 2014; **11**: 168–176.
2 Statistics Estonia 2016. Available at http://www.stat.ee/en (accessed 10 December 2015).
3 Adojaan M, Kivisild T, Männik A *et al*. Predominance of a rare type of HIV-1 in Estonia. *J Acquir Immune Defic Syndr* 2005; **39**: 598–605.
4 Estonian Health Board [May 2016]. Available at www.terviseamet.ee (accessed 15 May 2016).
5 AIDS ECftEMo. HIV/AIDS Surveillance in Europe. End-year report 2001. Saint-Maurice:Institut de Veille Sanitaire, 2002. No. 662002.
6 Estonian National HIV and AIDS strategy for 2006–2015. Order No 771 of the Government of the Republic "Approval of the national HIV and AIDS strategy for 2006–2015 and establishment of the HIV and AIDS Committee of the Government of the Republic". 2005.
7 HIV epidemic in Estonia: analysis of strategic information. World Health Organization; 2011.
8 Soodla P, Rajasaar H, Avi R *et al*. Design and structure of the Estonian HIV Cohort Study (E-HIV). *Infect Dis (Lond)* 2015; **47**: 768–775.
9 Guy R, Gold J, Calleja JM *et al*. Accuracy of serological assays for detection of recent infection with HIV and estimation of population incidence: a systematic review. *Lancet Infect Dis* 2009; **9**: 747–759.
10 Duong YT, Qiu M, De AK *et al*. Detection of recent HIV-1 infection using a new limiting-antigen avidity assay: potential for HIV-1 incidence estimates and avidity maturation studies. *PLoS One* 2012; **7**: e33328.
11 Moyo S, Wilkinson E, Novitsky V *et al*. Identifying recent HIV infections: from serological assays to genomics. *Viruses* 2015; **7**: 5508–5524.
12 Duong YT, Kassanjee R, Welte A *et al*. Recalibration of the limiting antigen avidity EIA to determine mean duration of recent infection in divergent HIV-1 subtypes. *PLoS One* 2015; **10**: e0114947.
13 Kassanjee R, Pilcher CD, Keating SM *et al*. Independent assessment of candidate HIV incidence assays on specimens in the CEPHIA repository. *AIDS* 2014; **28**: 2439–2449.
14 Prejean J, Song R, Hernandez A *et al*. Estimated HIV incidence in the United States, 2006-2009. *PLoS One* 2011; **6**: e17502.
15 Aghaizu A, Murphy G, Tosswill J *et al*. Recent infection testing algorithm (RITA) applied to new HIV diagnoses in England, Wales and Northern Ireland, 2009 to 2011. *Euro Surveill* 2014; **19**: pii=20673.
16 Simmons R, Malyuta R, Chentsova N *et al*. HIV incidence estimates using the limiting antigen avidity EIA assay at testing sites in Kiev City, Ukraine: 2013–2014. *PLoS One* 2016; **11**: e0157179.
17 Mammone A, Pezzotti P, Angeletti C *et al*. HIV incidence estimate combining HIV/AIDS surveillance, testing history information and HIV test to identify recent infections in Lazio, Italy. *BMC Infect Dis* 2012; **12**: 65.
18 Karon JM, Song R, Brookmeyer R, Kaplan EH, Hall HI. Estimating HIV incidence in the United States from HIV/AIDS surveillance data and biomarker HIV test results. *Stat Med* 2008; **27**: 4617–4633.
19 Centers for Disease Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992; **41**: 1–19.
20 Sedia™ HIV-1 LAg-Avidity EIA Single Well Avidity Enzyme Immunoassay for Detection of Recent HIV-1 Infection Using Liquid Serum or Plasma Cat. No. 1002.: Sedia Biosciences Corporation, Portland, Oregon USA; Available at www.sediabio.com/LiteratureRetrieve.aspx?ID=127076.
21 Eurostat. Population and Social Conditions 2013. Available at http://epp.eurostat.ec.europa.eu/ (accessed 10 December 2015).
22 Statistics Estonia database. Available at http://pub.stat.ee/px-web.2001/dialog/statfile1.asp
23 Murray CJ, Ortblad KF, Guinovart C *et al*. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; **384**: 1005–1070.
24 Pharris A, Quinten C, Tavoschi L, Spiteri G, Amato-Gauci AJ, Network EHAS. Trends in HIV surveillance data in the EU/

EEA, 2005 to 2014: new HIV diagnoses still increasing in men who have sex with men. *Euro Surveill* 2015; **20**.
25 Uusküla A, Kals M, Rajaleid K *et al*. High-prevalence and high-estimated incidence of HIV infection among new injecting drug users in Estonia: need for large scale prevention programs. *J Public Health (Oxf)* 2008; **30**: 119–125.
26 Bank W. World Bank Country Information [May 2016]. Available at data.worldbank.org.
27 Beyrer C, Baral SD, van Griensven F *et al*. Global epidemiology of HIV infection in men who have sex with men. *Lancet* 2012; **380**: 367–377.
28 Hightow LB, Miller WC, Leone PA, Wohl DA, Smurzynski M, Kaplan AH. Predictors of repeat testing and HIV seroconversion in a sexually transmitted disease clinic population. *Sex Transm Dis* 2004; **31**: 455–459.
29 Estonian Society for Infectious Diseases. Guideline for testing HIV and link to care for those testing positive for HIV in Estonia (in Estonian). Estonia 2012.
30 The late presenters working group in COHERE in EuroCoord. Late presentation for HIV care across Europe: update from the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study, 2010 to 2013. *Euro Surveill* 2015; **20**. https://doi.org/10.2807/1560-7917.ES.2015.20.47.30070
31 Simon V, Ho DD, Abdool Karim Q. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *Lancet* 2006; **368**: 489–504.
32 Ananworanich J, Sacdalan CP, Pinyakorn S *et al*. Virological and immunological characteristics of HIV-infected individuals at the earliest stage of infection. *J Virus Erad* 2016; **2**: 43–48.
33 Ammassari A, Abbate I, Orchi N *et al*. Acute HIV infection (AHI) in a specialized clinical setting: case-finding, description of virological, epidemiological and clinical characteristics. *J Int AIDS Soc* 2014; **17** (4 Suppl 3): 19676.
34 Quinn TC, Wawer MJ, Sewankambo N *et al*. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *N Engl J Med* 2000; **342**: 921–929.
35 Attia S, Egger M, Muller M, Zwahlen M, Low N. Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. *AIDS* 2009; **23**: 1397–1404.
36 Sobrino-Vegas P, Moreno S, Rubio R *et al*. Impact of late presentation of HIV infection on short-, mid- and long-term mortality and causes of death in a multicenter national cohort: 2004–2013. *J Infect* 2016; **72**: 587–596.
37 Platten M, Linnemann R, Kummerle T *et al*. Clinical course and quality of care in ART-naive patients newly presenting in a HIV outpatient clinic. *Infection* 2014; **42**: 849–57.
38 Rehle T, Johnson L, Hallett T *et al*. A comparison of South African national HIV incidence estimates: a critical appraisal of different methods. *PLoS One* 2015; **10**: e0133255.
39 Wei X, Liu X, Dobbs T *et al*. Development of two avidity-based assays to detect recent HIV type 1 seroconversion using a multisubtype gp41 recombinant protein. *AIDS Res Hum Retroviruses* 2010; **26**: 61–71.

Appendix : CASCADE Collaboration in EuroCoord

CASCADE Steering Committee: Julia Del Amo (Chair), Laurence Meyer (Vice Chair), Heiner C. Bucher, Geneviève Chêne, Osamah Hamouda, Deenan Pillay, Maria Prins, Magda Rosinska, Caroline Sabin and Giota Touloumi.

CASCADE Co-ordinating Centre: Kholoud Porter (Project Leader), Ashley Olson, Andrea Cartier, Lorraine Fradette, Sarah Walker and Abdel Babiker.

CASCADE Clinical Advisory Board: Heiner C. Bucher, Andrea De Luca, Martin Fisher and Roberto Muga.

CASCADE Collaborators: Australia PHAEDRA cohort (Tony Kelleher, David Cooper, Pat Grey, Robert Finlayson and Mark Bloch); Sydney AIDS Prospective Study and Sydney Primary HIV Infection cohort (Tony Kelleher, Tim Ramacciotti, Linda Gelgor, David Cooper and Don Smith); Austria Austrian HIV Cohort Study (Robert Zangerle); Canada South Alberta clinic (John Gill); Estonia Tartu Ülikool (Irja Lutsar); France ANRS CO3 Aquitaine cohort (Geneviève Chêne, Francois Dabis and Rodolphe Thiebaut), ANRS CO4 French Hospital Database (Dominique Costagliola and Marguerite Guiguet); Lyon Primary Infection cohort (Philippe Vanhems); French ANRS CO6 PRIMO cohort (Marie-Laure Chaix and Jade Ghosn); ANRS CO2 SEROCO cohort (Laurence Meyer and Faroudy Boufassa); Germany German HIV-1 seroconverter cohort (Osamah Hamouda, Karolin Meixenberger, Norbert Bannert and Barbara Bartmeyer); Greece AMACS (Anastasia Antoniadou, Georgios Chrysos and Georgios L. Daikos); Greek Haemophilia cohort (Giota Touloumi, Nikos Pantazis and Olga Katsarou); Italy Italian Seroconversion Study (Giovanni Rezza and Maria Dorrucci); ICONA cohort (Antonella d'Arminio Monforte and Andrea De Luca); Netherlands Amsterdam Cohort Studies among homosexual men and drug users (Maria Prins, Ronald Geskus, Jannie van der Helm and Hanneke Schuitemaker); Norway Oslo and Ulleval Hospital cohorts (Mette Sannes, Oddbjorn Brubakk and Anne-Marte Bakken Kran); Poland National Institute of Hygiene (Magdalena Rosinska); Spain Badalona IDU hospital cohort (Roberto Muga and Jordi Tor); Barcelona IDU Cohort (Patricia Garcia de Olalla and Joan Cayla); CoRIS-scv (Julia del Amo, Santiago Moreno and Susana Monge); Madrid cohort (Julia Del Amo and Jorge del Romero); Valencia IDU cohort (Santiago Pérez-Hoyos); Sweden Swedish InfCare HIV Cohort, Sweden (Anders Sönnerborg); Switzerland

Swiss HIV Cohort Study (Heiner C. Bucher, Huldrych Günthard and Alexandra Scherrer); Ukraine Perinatal Prevention of AIDS Initiative (Ruslan Malyuta); United Kingdom Public Health England (Gary Murphy), UK Register of HIV Seroconverters (Kholoud Porter, Anne Johnson, Andrew Phillips and Abdel Babiker); University College London (Deenan Pillay); African cohorts: Genital Shedding Study (USA: Charles Morrison, Family Health International, Robert Salata, Case Western Reserve University, Uganda: Roy Mugerwa, Makerere University, Zimbabwe: Tsungai Chipato, University of Zimbabwe); International AIDS Vaccine Initiative (IAVI) Early Infections Cohort (Kenya, Rwanda, South Africa, Uganda, Zambia) (Matt A. Price, IAVI, USA; Jill Gilmour, IAVI, UK; Anatoli Kamali, IAVI, Kenya; Etienne Karita, Projet San Francisco, Rwanda).

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