CROATIAN MEDICAL JOURNAL
CMJ

44(5):545-549,2003

PUBLIC HEALTH

Unlinked Anonymous Monitoring of Human Immunodeficiency Virus Prevalence in High- and Low-risk Groups in Slovenia, 1993-2002

Irena Klavs, Mario Poljak[1]

Institute of Public Health of the Republic of Slovenia; and [1]Institute of Microbiology and Immunology, Ljubljana University School of Medicine, Ljubljana, Slovenia

Aim. To determine the prevalence of human immunodeficiency virus (HIV) infection in high-risk groups, ie, injecting drug users, patients with sexually transmitted infections, and men who have sex with men, and compare it with the prevalence determined in low-risk group, ie, pregnant women.

Methods. Residual sera from specimens obtained from patients with sexually transmitted infections and pregnant women were sampled in syphilis serology laboratories. Saliva specimens were voluntarily obtained from injecting drug users entering methadone maintenance program and once per year from men who have sex with men, at one of their meeting sites. Specimens were labeled only with the type of sentinel population, sampling year, sentinel site code, sex, and age group. Specimens were frozen and stored at -20° C and tested annually for anti-HIV antibodies.

Results. A cumulative total of 1,172 saliva specimens were collected from injecting drug users during 1995-2002, 774 saliva specimens from men who have sex with men during 1996-2002, 6,612 serum specimens from patients with sexually transmitted infections during 1993-2002, and 49,652 serum specimens from pregnant women during 1993-2002. The national annual HIV prevalence estimates for injecting drug users varied between 0% and 0.7% in 2000, for men who have sex with men between 0% in 2002 and 3.4% in 1998, and for patients with sexually transmitted infections from 0% (most calendar years) to 0.5% in 1995. Among specimens obtained from pregnant women, only one tested anti-HIV positive (in 1999), so the prevalence estimate was 0.01% for the particular year.

Conclusion. The prevalence of HIV infection in low-risk heterosexual population is very low and has consistently remained below 5% in all groups with high-risk behavior, including men who have sex with men, who are the most affected population group in Slovenia.

Key words: *confidentiality; HIV-1; HIV infections; HIV seroprevalence; population surveillance; risk factors; Slovenia*

Human immunodeficiency virus (HIV) surveillance should provide information of sufficient accuracy and completeness regarding the distribution, spread, and incidence rates of HIV infection in different population groups according to their demographic and behavioral characteristics, and geographic area (1,2). Such information is essential for planning, implementing, and monitoring the impact of HIV prevention activities and control.

Since 1986, HIV infection surveillance in Slovenia has been based on mandatory reporting of newly diagnosed cases of HIV infection and acquired immunodeficiency syndrome (AIDS). In addition, we collate the information on voluntary confidential testing in some population groups (e.g. clients at the national HIV testing and counseling outpatient clinics), mandatory testing of blood donations, and the extent of HIV testing in public health and other laboratories (3,4).

Diagnostic HIV testing in Slovenia is performed in a much smaller extent than in European Union (EU) member states, save Ireland, and to a rather moderate extent in comparison with other European countries (5). A total of 10.5 HIV tests per 1,000 population were performed in Slovenia in 2002. The majority of HIV infections in Slovenia are diagnosed in later stages of infection. Also, with routine laboratory diagnosis tests, we usually do not discriminate between recent and old HIV infections. Thus, the calculated HIV incidence rates based on newly diagnosed infections can not accurately reflect the real HIV infection incidence rate. To augment available HIV surveillance information, Slovenia introduced a national system for HIV infection prevalence monitoring with unlinked anonymous testing in several easily accessible sentinel population groups.

In countries with low level HIV epidemic situation, like Slovenia, HIV prevalence monitoring should

be focused on higher-risk behavior groups (6). Ideally, monitoring of the chosen sentinel population groups should provide information on HIV prevalence and incidence with respect to all three major modes of HIV transmission: unprotected sexual intercourse with infected individuals, exposure to infected blood, and infected mother-to-child transmission. In Slovenia, we chose three sentinel population groups among individuals whose risk behavior makes them vulnerable to HIV infection: injecting drug users entering methadone maintenance program, men who have sex with men, and patients with sexually transmitted infections. In addition, we have monitored HIV prevalence in a group of pregnant women, which is a relatively low-risk or general-risk behavior population group. Pregnant women and patients with sexually transmitted infections have been monitored since 1993, injecting drug users monitoring was introduced in 1995, and monitoring of men who have sex with men in 1996. On the basis of serial measurements of HIV infection prevalence rates in such sentinel populations, we can determine the HIV incidence in these populations and infer the incidence rates in population groups comparable by their demographic and behavioral characteristics (2,7,8).

We decided to use unlinked anonymous testing because individuals with high-risk behavior might be more inclined than those with lower risk to refuse or avoid testing (9), and information on results from voluntary confidential testing might be biased. Such a testing is performed exclusively for surveillance purposes and not for the purpose of diagnosing HIV infection in individuals. Testing for HIV infection is performed only after all personal identifying information about the individual from whom the specimen was obtained has been irreversibly removed and impossible to link with the specimen. This practically means that we can not link the result of the test to the identity of the person from whom we obtained the specimen. Such unlinked anonymous testing is ethically acceptable only if those enrolled have access to voluntary confidential HIV testing and counseling. In the USA, clear legal authorization for unlinked anonymous testing was provided in the US Federal regulations, and in the UK a broad consensus concerning the legal and ethical basis was reached (10,11). There are no relevant legal provisions for unlinked anonymous testing in Slovenia.

Methods

Numerous public health and other laboratories, health care services, and a non-governmental organization have been participating in specimen collection from sentinel populations chosen for the study across Slovenia (Fig. 1). Residual serum specimens were used for monitoring patients with sexually transmitted infections and pregnant women, whereas saliva specimens were used for monitoring injecting drug users and men who have sex with men. Residual sera from specimens obtained from patients with sexually transmitted infections and pregnant women for routine syphilis serology were continuously and consecutively sampled in participating laboratories. The second inclusion of specimens obtained from the same individuals during the same calendar year was prevented by keeping a separate list of identifying information on individuals whose sera have already been included. Saliva specimens were voluntarily obtained with Omni-Sal collecting systems (Saliva Diagnostics Systems, Vancouver, WA, USA) from all consenting injecting drug users entering methadone maintenance program at two centers for prevention and treatment of illegal drug use. For injecting drug users who wanted to know their HIV infection status, confidential testing for HIV infection on a voluntary basis was also offered. From voluntary participants from the group of men who have sex with men, saliva specimens were obtained with Omni-Sal collecting systems at one of their meeting sites once per year. During the day of sampling period, information leaflets on safer sex, condoms, lubricants were offered as well as information on voluntary confidential testing and counseling for HIV infection. All specimens were labeled with the information on the type of sentinel population, sampling period, sentinel site, sex, and age group of the individual. All specimens were frozen and stored at -20 °C until testing.

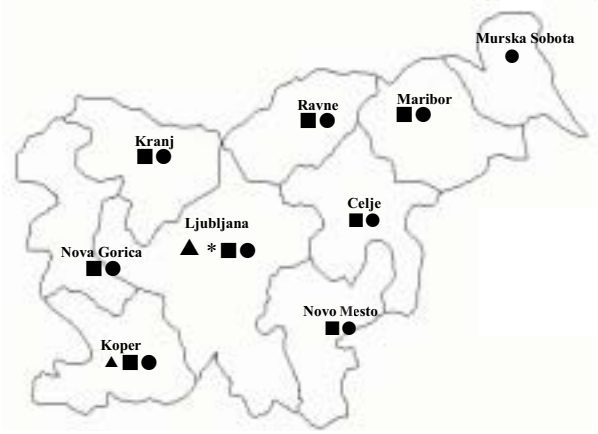

Figure 1. Unlinked anonymous HIV prevalence monitoring surveillance program in Slovenia. Sentinel sites and sentinel population involved in 1993-2002 monitoring. Closed triangle – injecting drug users; asterisk – men who have sex with men; closed square – sexually transmitted infections; closed circle – pregnancies.

All specimens collected during the preceding calendar year were tested at the beginning of the following year. Over a 10-year period, from 1993 to 2002, the laboratory testing approach varied depending on the improvement of the commercially available test kits. Since 1998, all sera specimens were initially tested in pools of 12 specimens for the presence of anti-HIV-1/0/2 antibodies by using third-generation enzyme immunoassay ICE HIV-1.0.2 Test (Murex Diagnostics, Dartford, UK). Individual sera from reactive pools were re-tested by using the same assay. Saliva specimens were tested individually for the presence of anti HIV-1/2 antibodies with enzyme immunoassay Wellcozyme HIV 1+2 GACELISA (Murex Diagnostics). Individual specimens repeatedly reactive on enzyme immunoassay were supplementary tested with HIV Western Blot 2.2 assay (Genelabs Diagnostics, Singapore). The Western blot results were interpreted according to the standards of Centers for Disease Control and Prevention (CDC) and Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) (*http://www.aphl.org/*). A particular specimen was defined as anti-HIV positive according to the positive result of supplementary Western blot test.

Ethical approval of the study protocols were obtained from the Medical Ethics Committee of the Ministry of Health of the Republic of Slovenia.

Results

The national annual HIV prevalence was determined for each of the four sentinel population groups at all sentinel sites for the 1993-2002 period, as well as the prevalence range between sentinel sites for a particular sentinel population group and calendar year (Table 1).

Table 1. HIV infection prevalence among injecting drug users, men who have sex with men, patients with sexually transmitted infections, and pregnant women in Slovenia, 1993-2002

Sentinel groups	Year	No. of sentinel sites	No. of individuals tested	No. of HIV-infected individuals*	Prevalence (%, range) of HIV infection†
Injecting drug users‡	1995	2	115	0	0
	1996	2	177	1	0.6 (0-0.8)
	1997	2	137	0	0
	1998	2	135	0	0
	1999	2	126	0	0
	2000	2	147	1	0.7 (0-1.1)
	2001	2	153	0	0
	2002	2	182	0	0
Men who have sex with men‡	1996	1	85	2	2.4
	1997	1	136	2	1.5
	1998	1	87	3	3.4
	1999	1	120	2	1.7
	2000	1	132	4	3.0
	2001	1	101	3	3.0
	2002	1	113	0	0
Patients with sexually transmitted infections§	1993	8	1,208	1	0.1 (0-1.0)
	1994	4	869	0	0
	1995	4	861	4	0.5 (0-1.4)
	1996	3	531	0	0
	1997	7	478	1	0.2 (0-0.4)
	1998	7	777	0	0
	1999	5	567	0	0
	2000	6	452	0	0
	2001	6	323	0	0
	2002	7	546	2	0.4 (0-1.0)
Pregnant women§	1993	11	9,875	0	0
	1994	9	10,369	0	0
	1995	9	8,528	0	0
	1997	7	5,834	0	0
	1999	8	6,900	1	0.01 (0-0.13)
	2001	9	8,146	0	0

*All infections are HIV-1 infections.
†The range within a category is the lowest to highest prevalence recorded at individual sentinel sites.
‡Saliva specimens.
§Serum specimens.

During the 1995-2002 period, a cumulative total of 1,172 saliva specimens were collected from injecting drug users entering methadone maintenance program. Two specimens were positive for anti HIV-1 antibodies, one collected in 1996 and one in 2000, which resulted in 0.6% and 0.7% overall estimated HIV prevalence among injecting drug users entering methadone maintenance programs in 1996 and 2000, respectively.

During the 1996-2002 period, a cumulative total of 774 saliva specimens were collected from men who have sex with men. The annual HIV prevalence estimates ranged between 0% in 2002 and 3.4% in 1998. Since absolute numbers of specimens collected from men who have sex with men were low, these estimates were rather imprecise, but nevertheless remained consistently below 5%.

During the 1993-2002 period, a cumulative total of 6,612 serum specimens were collected from patients with sexually transmitted infections who were tested for syphilis. The annual number of specimens positive to anti-HIV antibodies varied between 0 and 4, resulting in national HIV prevalence estimates ranging between 0% in most calendar years and 0.5% in 1995. The highest sentinel site-specific annual HIV prevalence in patients with sexually transmitted infections was 1.4% and was measured in Ljubljana in 1995 (serum specimens for routine syphilis serology from the outpatient clinic for sexually transmitted infections in Ljubljana).

During the 1993-2002 period, a cumulative total of 49,652 serum specimens were collected from pregnant women routinely screened for syphilis. Only one specimen positive to anti-HIV antibodies was detected, in 1999, resulting in prevalence estimate of 0.01% for that year.

Discussion

Our study showed that HIV infection prevalence in Slovenia has been well below 5% in high-risk groups, and very low in low-risk heterosexual population. It is reassuring that such a low HIV prevalence has remained stable over the 10-year period. Fortunately, the rapid spread of HIV infection seems not to have started yet among injecting drug users in Slovenia. In comparison with some other European countries, the prevalence rates of other viruses transmissible through the exposure to infected blood (human T-cell leukemia/lymphoma virus type I; hepatitis B, C, and G viruses) in Slovenian injecting drug users are also among the lowest (12,13). Men who have sex with men are still the most affected group in our country.

Slovenia remains very low-level HIV epidemic country in comparison with most western European countries with mature concentrated epidemics and some Easter European countries, where the spread of HIV infection among injecting drug users has recently been rapid (5,14).

Unlinked anonymous HIV testing for surveillance purposes has also been conducted in many other European countries (15-23) and has been recommended for surveillance purposes by the World Health Organization (2). The strengths of such unlinked anonymous HIV prevalence monitoring are minimized participation bias, non-invasive specimen collection, and very cost-efficient approach to collecting substantial number of specimens and laboratory testing. Enzyme immunoassay testing for anti-HIV antibodies in pools of several sera proved to be a reliable and cost-efficient method for seroprevalence studies (24-27). The identification of all pools containing individual anti-HIV positive serum specimen is possible with 10-15 pooled sera and the cost of testing is substantially reduced without compromising sensitivity and specificity (24-26). The UK started unlinked anonymous testing of sera collected from pregnant women for HIV surveillance purposes in pools of 12 as early as 1991 (11). It was assumed that because of low HIV incidence and consequent rarity of specimens collected around seroconversion, little, if any, loss of sensitivity would result (28). Finally, with monitoring changes in HIV prevalence by repeating the surveys in these easily accessible sentinel population groups, we can draw conclusion about the distribution and spread of HIV infection in the corresponding population groups in the country. Such sentinel surveillance system cost-efficiently provides information accurate enough for developing public health policy and planning the allocation of available HIV prevention and control resources. It also provides early warning about where, when, and in which population groups HIV infection will start spreading fast.

There are also some limitations of unlinked anonymous HIV-prevalence monitoring. Additional risk behavioral information is not available, for example, we do not have information on possible history of sharing injecting equipment among men who have sex with men, patients with sexually transmitted infections, possible history of unprotected heterosexual and homosexual sex among injecting drug users, or possible history of having lived or traveled in high HIV prevalence countries. The number of specimens collected from injecting drug users, men who have sex with men and patients with sexually transmitted infections in our study was too low to monitor HIV prevalence changes precisely enough, since we still do not have a good national coverage of sentinel sites of injecting drug users and men who have sex with men. In addition, we have to be cautious when concluding about the distribution and spread of HIV infection in different population groups, as these easily accessible sentinel groups are not representative for all injecting drug users, men who have sex with men, and patients with sexually transmitted infections, or all women of reproductive age.

The unlinked anonymous HIV prevalence monitoring in sentinel populations is a methodologically appropriate, logistically relatively feasible, and extremely cost-efficient HIV surveillance method. The challenge for the future remains to sustain such a national surveillance system and increase the monitoring coverage of population at highest risk of HIV infection. With such a surveillance system, we would be able to provide sufficiently accurate HIV surveillance information to public health policy decision makers and those who plan, implement, and monitor HIV prevention and control activities in Slovenia.

Acknowledgment

We thank the personnel from the syphilis serology laboratories in the health care institutions listed below, the personnel from the two centers for prevention and treatment of illegal drug use, and the personnel as well as volunteers from one gay non-governmental organization: General Hospital Izola, General Hospital Maribor, Institute of Microbiology and Immunology, Medical School, University of Ljubljana, Institute of Blood Transfusion of the Republic of Slovenia, Institute of Public Health Celje, Institute of Public Health Koper, Institute of Public Health Kranj, Institute of Public Health Maribor, Institute of Public Health Murska Sobota, Institute of Public Health Nova Gorica, Institute of Public Health Novo mesto, Institute of Public Health Ravne, Institute of Public Health of the Republic of Slovenia, Primary Health Care Centre Koper. We also thank Ms. Zdenka Kastelic for her dedicated support in co-ordination of the national unlinked anonymous HIV prevalence monitoring surveillance system and Robi Krošelj for the support in laboratory testing.

References

1 Sutkin G, Chin J, Tarantola D, Mann J. Use of HIV surveillance data in national AIDS control programmes. Geneva: Global Programme on AIDS, World Health Organization; 1990.

2 World Health Organization. Unlinked anonymous screening for the public health surveillance of HIV infections (proposed international guidelines). Geneva: WHO; 1989.

3 Klavs I, Vidmar L, Glonar L. Epidemiology and natural history of HIV infection and AIDS [in Slovenian]. Zdrav Vestn 1990;59:125-30.

4 Klavs I. HIV infection and AIDS: global pandemic and epidemic in Slovenia [in Slovenian]. Zdravstveno Varstvo 1993; 32:272-5.

5 EuroHIV. HIV/AIDS surveillance in Europe. End-year report 2002 (No. 68). Paris: Institute de Veille Sanitaire; 2003.

6 World Health Organization, Joint United Nations Programme on HIV/AIDS. Guidelines for second generation HIV surveillance. Geneva: UNAIDS/WHO; 2000.

7 Dondero TJ, Gill ON. Large-scale HIV serologic surveys: what has been learned? AIDS 1991;5 Suppl 2:S63-9.

8 PHLS AIDS Centre. The surveillance of HIV-1 infection and AIDS in England and Wales. CDR (Lond Engl Rev) 1991;1:R51-6.

9 Hull HF, Bettinger CJ, Gallaher MM, Keller NM, Wilson J, Mertz GJ. Comparison of HIV-antibody prevalence in patients consenting to and declining HIV-antibody testing in an STD clinic. JAMA 1988;260:935-8.

10 Wan L. The legality of unlinked anonymous screening for HIV infection: the U.S. approach. Health Policy 1990;14:29-35.

11 Bayer R, Lumey LH, Wan L. The American, British and Dutch responses to unlinked anonymous HIV seroprevalence studies: an international comparison. Law Med Health Care 1991;19:222-30.

12 Trišler Z, Seme K, Poljak M, Čelan-Lucu B, Sakoman S. Prevalence of hepatitis C and G virus infections among intravenous drug users in Slovenia and Croatia. Scand J Infect Dis 1999;31:33-5.

13 Poljak M, Bednarik J, Rednak K, Seme K, Kristančič L, Čelan-Lucu B. Seroprevalence of human T cell leukaemia/lymphoma virus type I (HTLV-I) in pregnant women, patients attending venereological outpatient services and intravenous drug users from Slovenia. Folia Biol (Praha) 1998;44:23-5.

14 Hamers F, Downs A. HIV in central and eastern Europe. Lancet 2003;361:1035-44.

15 Worm AM, Kvinesdal B. Human immunodeficiency virus surveillance at a sexually transmitted disease clinic in Copenhagen. Int J STD AIDS 1990;1:107-9.

16 Public Health Laboratory Service. Unlinked anonymous monitoring of HIV prevalence in England and Wales: 1990-92. Commun Dis Rep CDR Rev 1993;3: R1-11.

17 Tappin DM, Girdwood RW, Follett EA, Kennedy R, Brown AJ, Cockburn F. Prevalence of maternal HIV infection in Scotland based on unlinked anonymous testing of newborn babies. Update. Scott Med J 1993;38: 16-7.

18 Scott G, Goldberg D, Weir M, Cameron S, Peutherer J, Carrington D, et al. Prevalence of HIV-1 infection among heterosexual men and women attending genitourinary clinics in Scotland: unlinked anonymous testing. BMJ 1997;315:1281-2.

19 Simms I, Rogers P, Catchpole M, McGarrigle CA, Nicoll A. Trends in undiagnosed HIV-1 infection among attenders at genitourinary medicine clinics, England, Wales, and Northern Ireland: 1990-6. Sex Transm Infect 1999;75:332-6.

20 Johnston J, Cameron S, Stewart M, Goldberg D, Fletcher C, Smyth W, et al. Unlinked anonymous HIV study of hospital patients and general practice attenders in Glasgow, 1991-1997. J Clin Pathol 2000;53:117-21.

21 Castilla J, Pachon I, Gonzalez MP, Amela C, Munoz L, Tello O, et al. Seroprevalence of HIV and HTLV in a representative sample of the Spanish population. Epidemiol Infect 2000;125:159-62.

22 Kessel AS, Watts CJ. Evaluation of the unlinked anonymous prevalence monitoring programme for HIV in England and Wales: science, ethics and health policy. Med Sci Monit 2001;7:1052-63.

23 Hamouda O. HIV/AIDS surveillance in Germany. J Acquir Immune Defic Syndr 2003;32 Suppl 1:49-54.

24 Mortimer JY. Saving tests by pooling sera – how great are the benefits? J Clin Pathol 1980;33:1120-1.

25 Cahoon-Young B, Chandler A, Livermore T, Gaudino J, Benjamin R. Sensitivity and specificity of pooled versus individual sera in a human immunodeficiency virus antibody prevalence study. J Clin Microbiol 1989;27: 1893-5.

26 Behets F, Bertozzi S, Kasali M, Kashamuka M, Atikala L, Brown C, et al. Successful use of pooled sera to determine HIV-1 seroprevalence in Zaire with development of cost-efficiency models. AIDS 1990;4:737-41.

27 Kline RL, Brothers TA, Brookmeyer R, Zeger S, Quinn TC. Evaluation of human immunodeficiency virus seroprevalence in population surveys using pooled sera. J Clin Microbiol 1989;27:1449-52.

28 Parry JV. Mahoney A, Mortimer PP. Are seroepidemiological surveys for human immunodeficiency virus infection based on test on pools of serum specimens accurate and cost-effective? Clin Diagn Virol 1993;1: 167-78.

Received: September 4, 2003.
Accepted: September 17, 2003.

Correspondence to:
Irena Klavs
Institute of Public Health of the Republic of Slovenia
Trubarjeva 2
1000 Ljubljana, Slovenia
Irena.Klavs@ivz-rs.si