Research articles

SETTINGS FOR IDENTIFYING RECENT HIV INFECTIONS: THE PORTUGUESE EXPERIENCE

H Cortes Martins (helena.cortes-martins@insa.min-saude.pt)¹, M T Paixão²

1. Laboratório de Referência da SIDA, Departamento de Doenças Infecciosas, Instituto Nacional de Saúde (AIDS Reference Laboratory, Infectious Diseases Department, National Institute of Health), Lisbon, Portugal

2. “Centro de Vigilância Epidemiológica das Doenças Transmissíveis”, Departamento de Doenças Infecciosas, Instituto Nacional de Saúde (Communicable Diseases Surveillance Centre, Infectious Diseases Department, National Institute of Health), Lisbon, Portugal

Portugal has been the western European country with the highest rate of notified acquired* immunodeficiency syndrome (AIDS) cases since 1999 and human immunodeficiency virus (HIV) infection cases since 2000. Nonetheless, exact information on the magnitude and trends of recently acquired infections is missing. In a cross-sectional study we aimed to determine HIV prevalence, the proportion of recently acquired infections and the incidence among patients attending a Sexually Transmitted Infections (STI) clinic and among HIV positive cases tested at the AIDS Reference Laboratory (ARL), by using the Avidity Index (AI) of antibodies to identify recent HIV-1 seroconversions. Demographic and behavioural data were collected. At the STI clinic 253 patients were enrolled, 16 were found to be HIV infected (14 HIV-1, 2 HIV-2) and a prevalence of 6.3% was obtained. Four recent HIV-1 infections were identified and the HIV-1 incidence was 3.3% per year. At the ARL, 332 newly diagnosed cases of HIV-1 infection were studied, 59 (17.8%) were recent infections and an annual incidence of 4.1% was estimated. These findings support STI clinics as key sentinel sites for recently acquired HIV infections and illustrate the viability of testing for recent HIV infections in these settings and reinforce the value of this method in the surveillance for better monitoring current trends of the HIV/AIDS epidemic in Portugal.

Introduction

Portugal is the western European country with the highest rate of reported acquired immunodeficiency syndrome (AIDS) and newly diagnosed cases of human immunodeficiency virus (HIV) infection according to the EuroHIV end-year report for 2006 [1]. However, the proportion of recently acquired infections is unknown and information on trends is missing. After a decade (1990 to 2003) during which the epidemic was led by cases of HIV infections linked to intravenous drug use (IDU), the proportion of reported cases associated with sexual transmission (homosexual, bisexual and heterosexual) has progressively grown, accounting for over 60% since 2003, with heterosexual transmission currently being the driving force of the epidemic [2].

Similar to other countries, prevalence studies are used in Portugal for the epidemiological assessment of HIV infection as they provide relevant information necessary for the planning of health and social support services. Nevertheless, prevalence does not distinguish between infections acquired recently, i.e. in the

past six months and established infections acquired more than six months ago, and the picture of recent trends of the epidemic is less clear compared to that obtained by incidence studies. The fact that longitudinal studies to calculate incidence are expensive, time-consuming and difficult to perform is well known [3-5]. Alternative approaches to estimate the incidence of HIV infections have been used worldwide [4, 5] and the interest in cross-sectional laboratory based studies has grown in the past decade. Different methods, based on various properties of maturing antibodies for HIV-1, that allow the identification of recent seroconversion have been described and were used for this purpose [5-10]. These laboratory methods, applied to a single serum sample for each HIV-1 infected case, allow the distinction between recently acquired and established HIV-1 infections. The avidity index (AI) of HIV-1 antibodies has been used for this purpose based on the principle that antibodies produced in the early phase of infection show a low avidity for the antigen [7]. Therefore, a low avidity is likely to indicate a recent infection.

In Portugal, the first cross-sectional study in which recent HIV-1 seroconversions were identified was performed in a group of IDUs entering a low threshold methadone programme for the period of one year [11]. Of those 24.5% were found to be HIV-1 infected and among them 18.4% were infected recently. HIV-1 incidence was estimated 7.2 % per year. These results encouraged us to explore other settings where we expected to identify recent HIV-1 infections and to collect useful epidemiological information.

Sexually transmitted infections (STI) are known to be associated with higher transmission or acquisition rates of HIV and patients referred to STI clinics are usually at increased risk for HIV infection [3]. STI clinics were identified as important settings for measuring the prevalence of HIV infection [12] and can also be important sites for estimating incidence. In Portugal STI clinics are rare. However, in Lisbon, a unique drop-in STI clinic exists at a primary healthcare facility, that provides free of charge same day appointments with specialists, laboratory diagnosis of STI (including HIV), treatment for diagnosed STI and counselling for risk reduction. Attending patients are mainly from the Lisbon area, self-presenting or referred by other primary healthcare services.

Laboratory based surveillance data on the number of new HIV diagnoses per year are available in some European countries such as France, allowing for studies to identify recent infections to be conducted [12,13]. However, such a system does not exist in Portugal where notification of HIV/AIDS cases has always been performed by clinicians, with limited laboratory information. In Lisbon, the AIDS Reference Laboratory (ARL) confirms over 300 new cases of HIV infection per year from various population groups, including IDUs, prison inmates and pregnant women.

The aim of our study was to identify population groups from specific settings where recent HIV infections are likely to be detected, to generate data on HIV prevalence and on the proportion of recent HIV-1 infections and to estimate the incidence of HIV-1 infection.

Methods

Study design and population

To achieve the aim of our study two settings, an STI clinic and the ARL, were selected as settings where recent HIV infections are likely to be detected.

STI clinic

A cross-sectional study was performed in a group of attendees at the Centro de Saúde da Lapa STI clinic, in Lisbon. Between February and August 2004, enrolment in the study was proposed by clinicians to all first time attendees and other patients eligible for HIV testing (i.e. patients reporting risk behaviour). Participation was voluntary and anonymous. Written informed consent was obtained from all participants and archived in the patient's clinical file. One blood sample was collected from each participant at the time of enrolment and data on demographics, behaviour and clinical condition were anonymously recorded by clinicians for each case.

AIDS Reference Laboratory

A cross-sectional study was also performed using anonymised serum samples from cases newly diagnosed with HIV-1 infection at the ARL during the year 2005. Demographical and behavioural data were collected of all cases selected for the study. Second time testers were excluded.

Laboratory methods

Prior testing for identification of HIV infection was necessary for samples from the STI clinic. Pools of five sera were prepared with an input of 100 µL for each sample and each pool was screened simultaneously for HIV-1 and HIV-2 antibodies using the third generation enzyme immunoassay AxSYM® HIV 1/2 gO (Abbott Diagnostics Division, Germany). Sera from pools with a positive result were individually tested using the same kit. Samples identified as reactive were further tested for confirmation using the HIV Blot 2.2 (Genelabs Diagnostics, Singapore), a Western blot assay for HIV-1 antibodies also harbouring a peptide for identifying HIV-2 antibodies. Samples reacting with this peptide were additionally tested with New LAV Blot II (Bio-Rad, France), a Western blot assay to confirm the presence of HIV-2 specific antibodies. All tests were performed according to the manufacturer's instructions.

Identification of recently infected HIV-1 cases

In HIV-1 positive sera the AI of the antibodies was determined following the method described by Suligoi et al [7] in order to identify recently acquired HIV-1 infections, i.e. seroconversions occurred six months or less prior to blood collection. For each

sample a pre-test dilution of 1:10 was prepared on two aliquots of 50 µL: one with phosphate-buffer saline (PBS - PBS aliquot) and a second with 1M guanidine solution (G aliquot). After incubation at room temperature, both aliquots were tested with the AxSYM® HIV 1/2 gO (Abbott Diagnostics Division, Germany) assay following the manufacturer's instructions. The sample's AI was determined by the ratio between the results obtained for guanidine aliquot and buffer aliquot using the formula: $AI = (S/CO \text{ of the G aliquot}) / (S/CO \text{ of the PBS aliquot})$. S/CO standing for sample/cut-off value obtained in the test. The 0.8 cut-off for the AI was used to differentiate between recent ($AI < 0.8$) and established ($AI \geq 0.8$) cases of infection [14].

Epidemiological methods and concepts

Prevalence

For both settings, the STI clinic and the ARL, the proportion of HIV infections overall and by type of virus, as well as the proportion of recently acquired HIV-1 infections was determined.

Incidence

A cross-sectional approach was applied and six months was the window period assumed for the AI test used to identify recently acquired HIV-1 infections. The concept for the estimation of incidence [5, 6, 10] using data obtained from cross-sectional study at both sites is based on the following assumptions:

- cases identified as negative were also negative six months before blood sampling;
- cases identified as recently infected were negative six months before blood sampling;
- cases identified as established infection were positive six months before blood sampling;

The rate of seroconversion in the six months before blood sampling is obtained by the ratio between the number of recent infections and the number of susceptibles multiplied by two to obtain the annual incidence.

The following formula was used to estimate incidence in both groups:

$$\text{HIV 1 incidence (\%)} = \frac{N_r}{N_{\text{neg}} + N_r} \times 2 \times 100$$

N_r Number of recently infected HIV cases

N_{neg} Number of cases with a negative HIV test result

Statistical methods and data analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) program for Windows, version 12.0. Descriptive analysis was performed for each variable, namely frequencies and proportions. Mean age values were compared using the t test for independent samples. Data from different subgroups were compared using two tailed Fisher's exact test or chi-square independence test. Results with a p value < 0.05 were considered as being statistically significant. Odds Ratios (OR) and their 95% confidence interval (CI) were calculated in order to measure the strength of the associations found.

Results

STI clinic group

A total of 253 participants, 143 men (56.5%) and 110 women (43.5%), were enrolled in the study. The age ranged from 16 to

70 years, with a mean of 31.5 years (95% CI: 30.3-32.8) and a median of 28 years.

The majority of participants (90.9%) stated having only heterosexual contacts, 35.5% had more than one sex partner in the previous six months and only 15.9% declared to always use condoms in sexual contacts with unknown partners. Additional risk behaviours for HIV infection such as illicit drug use (not specified) and prostitution were acknowledged by 13 participants. Of all participants 45.1% had never been tested for HIV. Clinical data showed that, at time of enrolment, an STI other than HIV was diagnosed in 87 cases, with a high proportion of viral infections (43.5%). The majority of cases (81.3%) had no previous STI history.

Sixteen cases were found to be HIV infected 14 with HIV-1 and two with HIV-2. An overall HIV prevalence of 6.3% (95%CI: 3.3-9.3) was obtained. Type-specific prevalence was 5.5% for HIV-1 infection and 0.8% for HIV-2 infection. Prevalence by sex and sexual orientation showed a higher value in men than in women, 7.0% and 5.4% respectively and a high prevalence of

18,2% for homo/bisexual men. The comparative analysis between characteristics of HIV-positive and -negative cases is shown in Table 1.

Increased risk for HIV infection was found for homosexual/bisexual clients (OR = 3.33; 95%CI: 1.17-9.49), for those who had five or more sex partners in the previous six months (OR = 4.69; 95%CI: 1.70-12.82) and for those with an STI history (OR=2.91; 95%CI: 1.09-7.77). Although difference was not statistically significant, mean age in the HIV-positive subgroup (34.8 years) was higher than in the HIV-negative subgroup (31.3 years).

The AI of antibodies determined for the 14 HIV-1 cases ranged between 0.33 and 1.06 with a mean value of 0.85. Using 0.80 as cut-off value we were able to identify four recent HIV-1 infections. HIV-1 incidence in this group, as defined for the purpose of the study, was estimated to be 3.3% per year. No independent statistical associations were found between recent infections and the study variables.

TABLE 1

Comparative analysis of characteristics of HIV positive and HIV negative cases and characteristics of recent HIV-1 infection cases studied at the sexually transmitted infection (STI) clinic, Portugal, February to August 2004, (n=253)

Characteristics	HIV Antibodies			P ^b	Odds- Ratio (95%CI)	HIV-1 Recent Infection ^a		P ^c
	Tested*	HIV Positive				n [†]	%	
	N	n	%					
Sex								
Males	143	10	7.0	0.796	1.30 (0.46-3.70)	3	30.0	1.000
Females	110	6	5.4			1	16.7	
Age group								
<30 years old	144	6	4.2	0.122	0.43 (0.15-1.22)	0	--	0.221
>= 30 years old	109	10	9.2			4	40.0	
Sexual orientation								
Homo/bisexual	23	4	17.4	0.045	3.33 (1.17-9.49)	2	50.0	0.520
Heterosexual	230	12	5.2			2	16.7	
Number of sexual partners (prior 6 months) †								
>= 5 partners	16	4	25.0	0.015	4.69 (1.70-12.82)	1	25.0	1.000
< 5 partners	225	12	5.3			3	25.0	
Condom use								
Always	40	5	12.5	0.147	2.61 (0.86-7.97)	2	40.0	0.580
Occasional / Never	212	11	5.2			2	18.2	
Additional risks for HIV infection								
No	239	14	5.9	0.196	0.34 (0.07-1.70)	3	21.4	0.505
Yes	13	2	15.4			1	50.0	
Prior HIV test								
Yes	139	8	5.8	0.797	1.22 (0.47-3.14)	3	37.5	0.559
No	114	8	7.0			1	12.5	
Prior STI history								
Yes	47	6	12.8	0.040	2.91 (1.09-7.77)	1	16.7	1.000
No	205	9	4.4			2	22.2	
STI other than HIV diagnosed at enrolment								
No	166	12	7.2	0.588	1.57 (0.52-4.74)	3	25.0	1.000
Yes	87	4	4.6			1	25.0	

* Information displayed for those where available

Note: CI - confidence interval

a - Among HIV-1 antibody positive cases

b - Fisher's exact test for associations between characteristics and HIV antibodies status

c - Fisher's exact test for associations between characteristics and HIV-1 Recent Infection status

† Only cases with one or more partners

AIDS Reference Laboratory group

During 2005, 372 (11.8%) of the 3,159 individuals tested for HIV at the ARL had a positive test result. Western blot testing of these positive samples revealed 360 HIV-1 (11.4%) and 12 HIV-2 (0.4%) infections. We studied 332 HIV-1 infections from the 336 cases found to be first time diagnoses. In this group, whose main characteristics are summarised in Table 2, females accounted for 83 (25.0%) cases and males for 245 (73.8%) cases, for four cases information on sex was missing. The age ranged from 17 to 85 years, with a mean of 35.5 years (95%CI: 34.2-36.69) and a median of 33 years. The majority (75.6 %) of cases lived in the Lisbon district.

Data on behavioural risk towards HIV infection were missing in 145 (45.7%) cases. Available information showed that, of 187 cases, sexual risk was present for 29.9% (n=56), in 12 cases associated with homo/bisexual contacts and in 44 (78.6%) with heterosexual contact. Drug use was mentioned in 70.1% (n=131).

After testing 332 samples to determine the AI, values obtained ranged from 0.24 to 1.09, with a mean value of 0.89. Cut-off value of 0.80 was applied and 59 (17.8%) cases were identified as recent HIV-1 seroconversions. No statistical association was found between recent HIV-1 infections and study variables. Even though the difference is not statistically significant, the proportion of recent infections was higher in females than in males (24.1% versus 15.5%). Estimated annualised incidence of HIV-1 infection among cases tested at the ARL in 2005 was 4.1%.

Discussion

Our study illustrates the first application of a cross-sectional approach to identify recent HIV-1 infections and estimate HIV-1 seroconversion in a group of attendees at a Portuguese STI clinic and in a group of newly diagnosed HIV infected cases detected at the AIDS Reference Laboratory in 2005. Recent HIV-1 seroconverters (less than six months) were identified based on the AI of antibodies.

The STI clinic group consisted predominantly of young, sexually active, heterosexual individuals. The fact that a very low proportion (15.9%) of participants stated to use condoms consistently in sexual intercourse with unknown partners and 45.1% had never been tested for HIV may derive from a lack of awareness to recognise the risk of contracting an HIV infection through unprotected sex. In the STI group authors did not only identify cases of HIV-1 infections (5.5%) but also HIV-2 (0.8%) cases, which mirrors the pattern of HIV infection in Portugal [2]. The overall prevalence rate of 6.3% and the 18.2% prevalence rate for homo/bisexual men are among the highest figures published [12]. A higher prevalence of HIV infection in homo/bisexual individuals than in heterosexuals without additional risk behaviour has been described in most European countries [12, 15]. The increased risk for HIV infection found in homo/bisexual participants is therefore consistent with the literature. Our data also show that a high number of sex partners increase the risk of HIV infection. The presence of STI indicates a risky sex behaviour that can lead to HIV acquisition or transmission. Accordingly, for the cases included in this study, a history of STI was also found to be an increased risk factor for HIV infection.

The determination of the AI enabled the identification of recent HIV-1 infections among HIV-1 infected participants. The proportion of recent HIV-1 infections (28.6%) identified in this study and the estimated incidence (3.3% per year), are similar to the highest values observed in published studies [3,16,17]. Nevertheless, caution is needed when comparing results obtained with different laboratory methods used to study other population groups as the window period varies from test to test and consequently the proportion of cases classified as recent.

The HIV-1 seropositive group assessed at the ARL consisted mainly of young individuals (median age 33.0 years) who were predominantly male (73.8%) and the majority of those with available information on risks were drug users (70.0%).

The high prevalence of HIV-1 (11.8%) infection in the ARL group may be associated with the fact that reference laboratories are likely to confirm more infected cases than other clinical laboratories. The proportion of drug users in this group and a 12 to 19% prevalence of HIV-1 infection described in Portuguese IDUs [18] may as well influence the result.

The proportion of recent HIV-1 infections (17.8%) found is lower than the one recently described in France [19] for newly diagnosed cases of HIV infection (24.9%). Possible explanations for this are different testing policies or rates, awareness of HIV risk, sample size and the use of different laboratory tests. The assessment of our group focused on cases of HIV-1 infection cases nonetheless incidence could be estimated since denominator was known, the result being a high value (4.1%).

TABLE 2

Comparative analysis between characteristics of recent HIV (n=59) and established HIV-1 infection cases (n=273) identified at the AIDS Reference Laboratory, Portugal, 2005

Characteristics	HIV-1 Infections				p*
	Recent		Established		
	n	%	n	%	
Sex					
Males	38	15.5	207	84.5	0.195
Females	20	24.1	63	75.9	
unknown	1	25.0	3	75.0	
Age group (years old)					
≤25	7	19.4	29	80.6	0.506
26 - 30	17	27.9	44	72.1	
31 - 35	10	15.4	55	84.6	
36 - 40	7	15.2	39	84.8	
41 - 50	8	16.3	41	83.7	
51 - 60	1	7.7	12	92.3	
≥ 61	1	11.1	8	88.9	
unknown	8	15.1	45	84.9	
Origin of HIV test request					
Anonymous free test site	2	8.3	22	91.7	0.317
Prison clinical services	5	12.8	34	87.2	
External laboratories	7	13.2	46	86.8	
General practitioner	21	19.1	89	80.9	
Methadone programme	24	22.6	82	77.4	
Risk behavioural for HIV infection					
Sexual – Homo/bisexual	1	8.3	11	91.7	0.141
Sexual – Heterosexual	9	20.5	35	79.5	
Drug use	30	22.9	101	77.1	
unknown	19	13.1	126	86.9	

* χ^2 test

We used the AI described by Suligoi et al. [7, 14] for identification of recent infections taking into account our previous experience, the availability of reagents and equipment, as well as being aware that the results would not be affected by disease, clinical stage or antiretroviral therapy [7]. Even if not adequate for individual and clinical use, this method has been found suitable for epidemiological studies, based on its sensitivity and specificity when the 0.8 cut-off value is used [14]. Also, performance with non-B subtypes of HIV was recently assessed and similar results have been obtained [20]. This fact is of the utmost importance for using the method in Portugal where a high proportion of newly diagnosed patients carry non-B subtype viruses [21]. Although other methods for testing for recent HIV infections have been described, most of them are not available on the market and further constraints to their application have been clearly identified [10].

Pooling sera for HIV seroepidemiological surveys has been used before [22-24] and, due to economical reasons, this method was applied for the STI group. The amount of sera per pool is critical when looking for recent infections and our choice of using five was based on published data [22] where six samples per pool was the minimum format assessed. Even though the sensitivity of HIV tests has increased since 1993 we decided to pool five samples because STI patients are generally at higher risk towards contracting HIV infections and HIV-2 is also prevalent in our country.

There are several limitations and biases for this study: the voluntary participation in the STI clinic group and the fact that patients attending STI clinics are at high risk for HIV infection; the fact that reference laboratories are more likely to register a higher proportion of positive cases and detailed behavioural data are rarely collected in the laboratory setting and in our case were frequently missing in the ARL group. All these factors are likely to influence our results and need to be considered in the interpretation. However, it is the higher risk of the STI clinic patients that enables this population to serve as a sentinel for the wider community.

Conclusion

We were able to determine the prevalence of HIV infections and the proportion of recent HIV-1 infections and estimate an incidence for both groups. Determining the AI for identification of recent HIV-1 infections is possible and easy using a simple and automated method based on commercially available reagents. A high prevalence for HIV infection was found in both of our study groups at an STI clinic and the ARL. Detection of recent HIV-1 infections provides evidence of current transmission. The estimated incidences should represent a baseline for further assessments to enable temporal trends analyses in those settings. Due to the nature of our study which uses a convenience sample, the results can not be extrapolated to other similar health care settings or the general population in Portugal. Surveillance for recent HIV infections with serological methods is feasible and desirable for better monitoring current local trends of the HIV/AIDS epidemic.

* Erratum: The original phrase "Portugal has been the western European country with the highest rate of notified autoimmune deficiency syndrome (AIDS) cases..." was corrected on 12 December 2008 to read "Portugal has been the western European country with the highest rate of notified acquired immunodeficiency syndrome (AIDS) cases..."

Acknowledgements

We would like to express our gratitude to Dr. Jacinta Azevedo and Dr. Irene Santo, clinicians at Centro de Saúde da Lapa, for their collaboration on patient selection, data collection and expert advice. To Teresa Lourenço and Joana Carvalho for their laboratory work and to Paulo Nogueira for statistical analysis.

References

1. EuroHIV. HIV/AIDS Surveillance in Europe. End-year report 2006. Saint-Maurice: Institut de Veille Sanitaire, 2007. No. 75.
2. Portugal - CVEDT. Infecção VIH/SIDA: a situação em Portugal a 31 de Dezembro de 2006. Lisboa: Comissão Nacional de Luta Contra a SIDA (CNLCS) 2007; Doc. 137.
3. Schwarcz S, Kellogg T, McFarland W, Louie B, Kohn R, Busch M, et al. Differences in the temporal trends of HIV seroincidence and seroprevalence among sexually transmitted disease clinic patients, 1989-1998: application of the serologic testing algorithm for recent HIV seroconversion. *Am J Epidemiol*. 2001;153(10):925-34.
4. UNAIDS/WHO. AIDS epidemic update, December 2002. World Health Organization (WHO), UNAIDS/02.58E. 2002. Available from: http://data.unaids.org/publications/IRC-pub03/epiupdate2002_en.pdf
5. Janssen RS, Satten GA, Stramer SL, Rawal BD, O'Brien TR, Weiblen BJ, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA*. 1998;280(1):42-8.
6. Parekh BS, Kennedy MS, Dobbs T, Pau CP, Byers R, Green T, et al. Quantitative detection of increasing HIV type 1 antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. *AIDS Res Hum Retroviruses*. 2002;18(4):295-307.
7. Suligoi B, Galli C, Massi M, Di Sora F, Sciadra M, Pezzotti P, et al. Precision and accuracy of a procedure for detecting recent human immunodeficiency virus infections by calculating the antibody avidity index by an automated immunoassay-based method. *J Clin Microbiol*. 2002;40(11):4015-20.
8. Wilson KM, Johnson EI, Croom HA, Richards KM, Doughty L, Cunningham PH, et al. Incidence immunoassay for identification of recent HIV-1 infection in therapy-naïve populations. *AIDS*. 2004;18(17):2253-59.
9. Barin F, Meyer L, Lancar R, Deveau C, Gharib M, Laporte A, et al. Development and validation of an immunoassay for identification of recent human immunodeficiency virus type 1 infections and its use on dried serum spots. *J Clin Microbiol*. 2005;43(9):4441-7.
10. Parekh BS, McDougal JS. Application of laboratory methods for estimation of HIV-1 incidence. *Indian J Med Res*. 2005;121(4):510-8.
11. Fernandes SB, Martins HC, Trigo H, Leitão E, Coutinho R, Paixão MT. [Human Immunodeficiency Virus type 1 seroincidence estimate among a group of drug users: a new approach] [In Portuguese] *Acta Med Port*. 2005;18(1):37-44.
12. Hamers FF, Downs AM. The changing face of the HIV epidemic in Western Europe: what are the implications for public health policies? *Lancet*. 2004;364(9428):83-94.
13. Lot F, Semaille C, Cazein F, Barin F, Pinget R, Pillonel J, Desenclos JC. Preliminary results from the new HIV surveillance system in France. *Euro Surveill*. 2004;9(10):pii=481. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=481>
14. Suligoi B, Massi M, Galli C, Sciadra M, Di Sora F, Pezzotti P, et al. Identifying recent infections using the avidity index and an automated enzyme immunoassay. *JAIDS*. 2003;32(4):424-8.
15. Dougan S, Evans BG, Elford J. Sexually transmitted infections in Western Europe among HIV-positive men who have sex with men. *Sex Transm Dis*. 2007;34(10):783-90.
16. Schwarcz SK, Kellogg TA, McFarland W, Louie B, Klausner J, Withum DG, et al. Characterization of sexually transmitted disease clinic patients with recent human immunodeficiency virus infection. *J Infect Dis*. 2002;186(7):1019-22.
17. Weinstock H, Dale M, Gwinn M, Satten GA, Kothe D, Mei J, et al. HIV seroincidence among patients at clinics for sexually transmitted diseases in nine cities in the United States. *J Acquir Immune Defic Syndr*. 2002;29(5):478-83.
18. Wiessing L, Nardone A. Ongoing HIV and viral hepatitis infections in IDUs across the EU, 2001-2005. *Euro Surveill*. 2006;11(47):pii=3084. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3084>
19. Semaille C, Barin F, Cazein F, Pillonel J, Lot F, Brand D, et al. Monitoring the dynamics of the HIV epidemic using assays for recent infection and serotyping among new HIV diagnoses: experience after 2 years in France. *Infect Dis*. 2007;196(3):377-83.

20. Suligoi B, Buttò S, Galli C, Bernasconi D, Salata RA, Tavošči L, et al. Detection of recent HIV infections in African individuals infected by HIV-1 non-B subtypes using HIV antibody avidity. *J Clin Virol*. 2008;41(4):288-92.
21. Palma AC, Araújo F, Duque V, Borges F, Paixão MT, Camacho R; Portuguese SPREAD Network. Molecular epidemiology and prevalence of drug resistance-associated mutations in newly diagnosed HIV-1 patients in Portugal. *Infect Genet Evol*. 2007;7(3):391-8.
22. Parry JV, Mahoney A, Mortimer PP. Are seroepidemiological surveys for human immunodeficiency virus infection based on tests on pools of serum specimens accurate and cost-effective? *Clinical and Diagnostic Virology* 1993;1(3):167-78.
23. Verstraeten T, Farah B, Duchateau L, Matu R. Pooling sera to reduce the cost of HIV surveillance: a feasibility study in a rural Kenyan district. *Trop Med Int Health*. 1998;3(9):747-50.
24. Klavs I, Poljak M. Unlinked anonymous monitoring of human immunodeficiency virus prevalence in high- and low-risk groups in Slovenia, 1993-2002. *Croat Med J*. 2003;44(5):545-9.

This article was published on 4 September 2008.

Citation style for this article: Cortes Martins H, Paixão MT. Settings for identifying recent HIV infections: the Portuguese experience. *Euro Surveill*. 2008;13(36):pii=18974. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18974>