

Incident risk factors as predictors of HIV seroconversion in the Lisbon cohort of men who have sex with men: first results, 2011–2014

P Meireles (paula.meireles@ispup.up.pt)¹, R Lucas^{1,2}, C Carvalho^{1,2}, R Fuertes³, J Brito³, M J Campos³, L Mendão³, H Barros^{1,3}

1. EPIUnit, Institute of Public Health, University of Porto, Porto, Portugal

2. Department of Clinical Epidemiology, Predictive Medicine and Public Health, University of Porto Medical School, Porto, Portugal

3. Grupo Português de Activistas sobre Tratamentos VIH/SIDA (GAT), Lisboa, Portugal

Citation style for this article:

Meireles P, Lucas R, Carvalho C, Fuertes R, Brito J, Campos MJ, Mendão L, Barros H. Incident risk factors as predictors of HIV seroconversion in the Lisbon cohort of men who have sex with men: first results, 2011–2014. *Euro Surveill.* 2015;20(14):pii=21091. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21091>

Article submitted on 31 August 2014 / published on 09 April 2015

HIV incidence in men who have sex with men (MSM) is increasing in western countries, including Portugal. We aimed to estimate HIV incidence and to assess how individual short-term changes in exposures over time predict seroconversion. We evaluated participants of an open cohort of HIV-negative MSM enrolled after testing at a community-based voluntary HIV counselling and testing centre in Lisbon. At each evaluation a structured questionnaire was completed and HIV status was ascertained using rapid followed by confirmatory testing. Between April 2011 and February 2014, 804 MSM were followed for a total of 893 person-years. Predictors of HIV seroconversion were identified using Poisson generalised linear regression. The overall seroincidence was 2.80/100 person-years (95% confidence interval: 1.89–4.14). Men who seroconverted had a higher mean number of tests per year. Seroconversions were significantly associated with partner disclosure of HIV status during follow-up, newly-adopted unprotected anal intercourse (UAI) with a steady partner and being newly-diagnosed with syphilis during follow-up. Likewise, sexual intercourse with HIV-positive men, having an HIV-positive steady partner at least once during follow-up and persistent UAI with occasional partners were predictors of seroconversion. High HIV incidence in this cohort is likely driven by short-term contextual and behavioural changes during follow-up.

Introduction

A well-established body of potential strategies for the primary prevention of HIV infection stems from increased understanding of disease pathogenesis and transmission [1,2]. Still, there is evidence of growing HIV incidence among men who have sex with men (MSM) in western Europe, North America and Australia [3–8]. These trends are unlikely to be explained by changes in surveillance or testing practices [3], rather

reflecting the fact that MSM remain at higher risk in most countries. This is apparent in the burden of newly-diagnosed infections in the European Union and European Economic Area: the largest fraction of HIV diagnoses reported in 2013 was attributable to sex between men (41.9%), followed by heterosexual transmission (32.4%), and finally by unsafe injection practices (5.0%) [9].

This is also the Portuguese pattern: after several years of an HIV epidemic driven by unsafe drug injection, sex between men has gained special relevance as a transmission mode making up 30.3% of all reported cases in 2013 [10]. Two pioneering cross-sectional studies [11,12] targeting MSM living in Portugal collected extensive self-reported information, leading to the first alarming estimates of the point prevalence of infection: 10.9% [13] and 10.3% (personal communication, A Gama, 2013).

Monitoring defined cohorts of MSM provides timely estimates of HIV incidence and predictors beyond the limited information produced by case reporting or cross-sectional surveys. In previous prospective cohorts, the occurrence of new infections has been modelled both as a function of factors that directly increase infection risk (frequency of unprotected anal intercourse (UAI), viral load of the index partner, presence of sexually transmitted infections (STI)), as well as potential markers of exposure, such as number of sex partners, substance use, and adverse childhood circumstances [14–19]. However, how individual exposures change over time and how those changes can predict HIV seroconversion remains to be clarified.

Innovative community-based HIV testing and counselling approaches have been developed that target specific population groups at higher risk and involve

community stakeholders as peer-counsellor and key informants [20]. As such, these are privileged settings for prospective research on the incidence and drivers of the HIV epidemic among MSM, with the ultimate goal of informing realistic preventive strategies.

The objectives of the present study were to estimate the incidence of HIV infection in a cohort of MSM and to assess how individual short-term changes in exposures predict seroconversion.

Methods

Cohort recruitment and follow-up

The Lisbon MSM cohort, established in April 2011, is an observational prospective study conducted at a community-based voluntary HIV counselling and testing centre in Lisbon, Portugal (CheckpointLX). It was designed as an open cohort, and inclusion criteria were: presenting for HIV testing at CheckpointLX, being a man aged 18 or more, reporting having sex with other men and having a negative HIV test result at recruitment. All eligible individuals were invited to enter the cohort by CheckpointLX peer counsellors at their first visit. Follow-up assessments were scheduled at intended intervals of 6 months, although the exact time between visits was adjusted according to the convenience of participants. Since follow-up visits occurred whenever clients decided to appear for testing, this does not strictly constitute an interval cohort and it is likely that a small proportion of MSM had very short or long periods between visits: e.g. in our sample, 6.3% of men had follow-ups shorter than three months. This is problematic for MSM who seroconvert between tests which are close in time (due to possible window period), which is why we opted to exclude five participants with seroconversions that occurred during follow-up periods of less than three months. At each visit a structured questionnaire was administered and a rapid HIV test was performed by a trained CheckpointLX peer counsellor. All participants gave their written informed consent and the study protocol was approved by the ethics committee of Hospital de São João and Medical School, University of Porto (ID 104/12).

Participation and losses to follow-up

Data reported in this study refer to the period from April 2011 to February 2014, during which 3,301 potential eligible individuals presented for testing, 195 (5.9%) of whom had an HIV-reactive test at entry and therefore were not included in the cohort. The remaining 3,106 (94.1%) were eligible to the cohort. Among those, 2,183 (70.3%) were enrolled, of whom 804 (36.8%) had at least one follow-up evaluation (893.37 person-years of observation) and 923 (29.7%) choose not to participate. Those who choose not to participate were less self-identified as homosexual, less frequently born in Portugal, and less educated than those who chose to

participate, but had a similar proportion of HIV testing before cohort entry.

Operationally, participants were classified as lost to follow-up if they had chosen to participate but appeared for testing only once ($n=707$). However, MSM who had been recruited for the cohort recently (12 months or less before the end of the period considered in the present analysis, i.e. from February 2013 to February 2014) were not considered lost to follow-up ($n=672$). Therefore, we assumed an overall attrition rate of 52%. MSM who were not followed-up were older than those who were (31.2 vs 30.3 years old, $p=0.034$), but both groups were similar regarding the remaining background characteristics. Also, no significant differences were found between MSM who appeared for follow-up and those who did not regarding such behavioural characteristics as: sexual intercourse with HIV positive men (13.5% vs 12.9%, $p=0.955$), having an HIV-positive steady partner (5.8% vs 5.2%, $p=0.528$), and condom use with a steady partner (27.7% vs 27.9%, $p>0.999$) and with an occasional partner in the previous 12 months (57.1% vs 51.7%, $p=0.069$).

Rapid HIV testing

Rapid HIV-1 and HIV-2 testing was performed at each visit. From April 2011 to April 2012 two commercial kits were used, the Retrocheck HIV (QUALPRO DIAGNOSTICS, Goa, India) (manufacturer-described sensitivity=100.00% and specificity=99.75%) and Hexagon HIV (Human GmbH, Wiesbaden, Germany) (sensitivity=100.00% and specificity=99.50%) and since that time, only the Alere Determine HIV-1/2 (Alere Medical Co., Ltd. Chiba, Japan) (sensitivity=100.00% and specificity=99.75%) has been used. In case of a reactive test, an outpatient appointment was scheduled at Santo António dos Capuchos Hospital's HIV/Infectious diseases clinic in Lisbon where a confirmatory test was performed. Pre- and post-test counselling was offered at each visit.

Study instruments and variables

Structured questionnaires were administered at entry and at each follow-up visit collecting data on background and behavioural characteristics, according to European Centre for Disease Prevention and Control (ECDC) [21] and the Joint United Nations Programme on HIV/AIDS (UNAIDS) guidelines [22] for HIV surveillance. For time-varying information the recall period was the previous 12 months (cohort entry questionnaire) or the time since the previous assessment (follow-up visits). Background characteristics included age, sex, country of birth, educational level and sexual identity.

Behavioural indicators included information on the following topics:

- History of previous HIV testing and reasons for index test;
- Age at first anal intercourse, role at anal intercourse, characteristics of sexual partners (bisexual

men, men with different sexual partners, sex workers, HIV-positive men, people who inject drugs, women and trios/group sex), steady (number, sex and HIV status) and occasional partners, having been paid for sex and venues used to meet occasional partners;

- Frequency of condom use for anal intercourse with steady and occasional partners.
- Use of alcohol or recreational drugs (cannabis, lysergic acid diethylamide (LSD), poppers, heroin, ecstasy, amphetamines, mephedrone, gamma-hydroxybutyric acid (GHB), ketamine and cocaine) before or during intercourse;
- Knowledge and use of non-occupational post-exposure prophylaxis for HIV;
- History of other STI and hepatitis.

We were interested in assessing whether intraindividual changes over time in well-documented determinants of HIV incidence were predictive of seroconversion. Even though multiple changes in those determinants throughout follow-up were theoretically possible, we opted to use information collected at two time points for each participant: cohort entry and either the visit of the first HIV positive test (for MSM who seroconverted) or the most recent visit (for the remaining MSM). This choice was based on two main arguments: i) the majority (53.8%) of participants had only two visits, and ii) for participants with three or more visits, using multiple combinations of information from all visits did not change the direction of associations or the main conclusions, i.e. first and last visit were good surrogates for exposure changes during follow-up (data not shown). For this purpose we created new variables for time-varying information that compiled responses from the first and the most recent visit, categorised as 'Yes to No' or 'No to Yes' if the information had changed between those visits, and 'No and No' or 'Yes and Yes' if answers were persistent. In case of 24 participants with more than two visits who preferred not to disclose one or more of the behavioural items at the most recent visit, we used the information obtained in the preceding visit. This option did not alter substantially the magnitude of associations.

Statistical analysis

Characteristics of participants at cohort entry were described using absolute frequencies and proportions in the case of categorical variables. Means and standard deviation (SD) or median and percentiles 25 and 75 (P25-P75) were used, as appropriate, to describe continuous variables. In data analysis, the missing category was excluded from the denominator for each item. In time-varying information related to characteristics of sexual partners, the options 'I do not know' and the 'No' options were collapsed once the incidence rates in both groups were similar. Unprotected anal intercourse (UAI) was defined as not always having used a condom in receptive or insertive anal sex. Incidence rates with 95% confidence intervals (95% CI) were estimated with time at risk defined as the period between

recruitment and the most recent follow-up visit. In MSM who seroconverted, half of the period between the last HIV-negative test and the first HIV-positive test was subtracted.

TABLE 1A

Characteristics at entry of participants followed in the cohort of men who have sex with men, Lisbon, Portugal, 2011–2014 (n=804)

Participants followed-up	804
Background characteristics	
Age (years), mean (SD)	30.3 (8.9)
Missing	0
Sexual identity, n (%)	
Homosexual	692 (86.1)
Bisexual/heterosexual/other	109 (13.6)
Prefer not to answer	3 (0.4)
Missing	0
Country of origin, n (%)	
Portugal	575 (75.0)
Other country	190 (24.7)
Prefer not to answer	2 (0.3)
Missing	37
Educational level (schooling years), n (%)	
Less than higher education (≤12 years of school)	317 (39.5)
Higher education (>12 years of school)	483 (60.1)
Other/Prefer not to answer	3 (0.3)
Missing	1
HIV testing	
Previous HIV testing, n (%)	
No	115 (15.2)
Yes	636 (84.1)
Did not know	5 (0.7)
Missing	48
Number of previous tests ^a , median (P25-P75)	4 (2–7)
Missing	16
Reasons for index test, n (%)	
To check health status/routine	602 (77.9)
Perception of HIV exposure more than 3 months before	426 (54.0)
Perception of HIV exposure in the previous 3 months	357 (44.8)
Accident with condom use (rupture/left inside)	65 (8.4)
Partner diagnosed HIV+ /Disclosed HIV+ status	59 (7.6)
Possible window period by the time of the last test	55 (7.2)
To stop using condom with my partner	38 (5.0)
My partner asked me to test for HIV	34 (4.4)
Symptoms / Medical indication	20 (2.6)

^a Among participants who had had a previous HIV test (n=636).

^b Among participants who had a steady partner in the previous 12 months (n=501).

^c Among participants who had an occasional partner in the previous 12 months (n=713).

^d Among participants who had an HIV-positive steady partner (n=46).

TABLE 1B

Characteristics at entry of participants followed in the cohort of men who have sex with men, Lisbon, Portugal, 2011–2014 (n=804)

Participants followed-up	804
Sexual life and partners	
Age at first anal intercourse, median (P25-P75)	18.0 (16.0–21.0)
Missing	37
Role in anal intercourse, n (%)	
Only insertive	192 (24.1)
Only receptive	72 (9.0)
Versatile	525 (66.0)
Prefer not to answer	7 (0.9)
Missing	8
Sex with at least one of the following in the previous 12 months, n (%)	
Bisexual men	
No	420 (53.1)
Yes	271 (34.3)
Did not know	98 (12.4)
Prefer not to answer	2 (0.2)
Missing	13
Men with different sex partners	
No	148 (18.7)
Yes	588 (74.2)
Did not know	54 (6.8)
Prefer not to answer	2 (0.3)
Missing	12
Sex workers (even if not paid)	
No	707 (89.4)
Yes	51 (6.4)
Did not know	31 (3.9)
Prefer not to answer	2 (0.3)
Missing	13
HIV-positive men	
No	401 (50.7)
Yes	107 (13.5)
Did not know	281 (35.5)
Prefer not to answer	2 (0.3)
Missing	13
People who inject drugs	
No	719 (90.9)
Yes	4 (0.5)
Did not know	65 (8.2)
Prefer not to answer	3 (0.4)
Missing	13
Women	
No	690 (87.2)
Yes	99 (12.5)
Did not know	0
Prefer not to answer	2 (0.3)
Missing	13
Trios/group sex	
No	563 (71.2)
Yes	224 (28.3)

Participants followed-up	804
Sexual life and partners	
Did not know	1 (0.1)
Prefer not to answer	3 (0.4)
Missing	13
Steady partner in the previous 12 months, n (%)	
No	301 (37.4)
One steady partner	449 (55.8)
More than one steady partner	52 (6.5)
Prefer not to answer	2 (0.2)
Missing	0
HIV status of steady partner^b, n (%)	
HIV negative	310 (62.5)
HIV positive	46 (9.3)
Did not know	139 (28.0)
Prefer not to answer	1 (0.2)
Missing	5
Occasional partners in the previous 12 months, n (%)	
No	89 (11.1)
Yes	713 (88.7)
Prefer not to answer	2 (0.2)
Missing	0
Number of occasional partners in the previous 12 months ^c , median (P25-P75)	5 (2–10)
Missing	19
Having sex for money or drugs in the previous 12 months^c, n (%)	
No	693 (97.3)
Yes	19 (2.7)
Missing	1
Venues used to meet occasional partners^c, n (%)	
Internet	522 (73.9)
Other venues (discos/gay bars, gym, outdoor cruising venues)	458 (57.6)
Only sexual venues (saunas, dark room, sex clubs)	166 (20.9)

^a Among participants who had had a previous HIV test (n=636).

^b Among participants who had a steady partner in the previous 12 months (n=501).

^c Among participants who had an occasional partner in the previous 12 months (n=713).

^d Among participants who had an HIV-positive steady partner (n=46).

TABLE 1C

Characteristics at entry of participants followed in the cohort of men who have sex with men, Lisbon, Portugal, 2011–2014 (n=804)

Participants followed-up		804
Unprotected anal intercourse (UAI), n (%)		
UAI with a steady partner in the previous 12 months ^b		
No		130 (27.4)
Yes		344 (72.4)
Prefer not to answer		1 (0.2)
Missing		26
UAI in the previous 12 months with an HIV-positive steady partner ^d		
No		26 (59.1)
Yes		18 (40.9)
Missing		2
UAI with occasional partners in the previous 12 months ^c		
No		375 (56.1)
Yes		292 (43.7)
Prefer not to answer		1 (0.1)
Missing		45
Recreational drugs, n (%)		
Used recreational drugs before or during sexual intercourse in the previous 12 months		
Never		552 (69.9)
Always/often/occasionally/rarely		238 (30.1)
Missing		14
Post-exposure prophylaxis (PEP), n (%)		
Does not know about PEP		411 (54.7)
Knows but never used		317 (42.2)
Knows and used		23 (3.1)
Missing		53
Sexually transmitted infections, n (%)		
In the previous 12 months:		
Gonorrhoea		20 (2.5)
Syphilis		13 (1.6)
Condyloma or genital warts		10 (1.3)
Chlamydia		7 (0.9)
Genital herpes		1 (0.1)
Trichomonas		1 (0.1)
History of hepatitis, n (%)		
Hepatitis B		18 (2.3)
Hepatitis C		3 (0.4)

^a Among participants who had had a previous HIV test (n=636).

^b Among participants who had a steady partner in the previous 12 months (n=501).

^c Among participants who had an occasional partner in the previous 12 months (n=713).

^d Among participants who had an HIV-positive steady partner (n=46).

Poisson generalised linear regression was used to identify predictors of HIV seroconversion with the default log link and offset in the variable follow-up time (t). To measure the magnitude of associations, crude and adjusted incidence rate ratios (IRR and

aIRR) and respective 95% CI were computed. Variables whose regression coefficient through the Wald test had $p < 0.10$ in the univariate analyses were further adjusted for UAI with a steady partner and UAI with occasional partners to estimate their direct effects, even though we acknowledge that UAI may be an intermediate step in the causal mechanism. For the multivariate analysis, significance level was set at $p < 0.05$. All statistical analyses were computed with Statistical Package for Social Sciences (SPSS) for Windows, version 22.0 (SPSS Inc., Chicago, Illinois, US).

Results

Characteristics of participants at cohort entry

Background and behavioural characteristics at entry for the 804 participants who came for a follow-up visit between April 2011 and February 2014 are summarised in Table 1. Briefly, mean (SD) age was 30.3 (8.9) years; 86.1% (692/804) of MSM self-identified as homosexual; 75.0% (575/767) were born in Portugal and 60.1% (483/803) had over 12 years of schooling. HIV testing before cohort entry was reported by 84.1% (636/756) of participants. Slightly less than two thirds (501/804) of participants had at least one steady partner, of whom 9.3% (46/496) were in a serodiscordant couple. UAI with a steady partner in the year before cohort entry was reported by 72.4% (344/475); in particular, 40.9% (18/44) of MSM who had an HIV-positive partner had UAI in the same period; UAI with one or more occasional partners was reported by 43.7% (292/668) in the same period. Almost one third (238/790) of men reported having used recreational drugs before or during sexual intercourse in the previous year. Over 2% (20/804) of MSM had a diagnosis of gonorrhoea during the previous 12 months, in the same period a little less than 2% (13/804) of MSM had a diagnosis of syphilis and 0.4% (3/804) were hepatitis C positive.

HIV incidence

Between April 2011 and February 2014, 804 MSM were followed for a total of 893.37 person-years (ranging from six days to 2.84 years). During follow-up, 25 seroconversions were recorded, yielding an overall incidence of 2.80 per 100 person-years (95% CI: 1.89–4.14). From these 25 newly-identified cases, 19 (76.0%) were effectively linked to care via CheckpointLX. Of the remaining six individuals who did not accept referral, three preferred to use their own means to access health services and three did not provide information on clinical follow-up. Participants who seroconverted had a mean age of 31.2 (9.4) years: not significantly different from those who did not (30.2 (8.9) years, $p = 0.598$), and a significantly shorter average follow-up time than those who did not seroconvert (0.79 years vs 1.12 years, $p = 0.018$), but approximately the same number of visits, resulting in a higher mean number of tests per year (4.8 vs 3.9, $p = 0.012$) (Table 2).

TABLE 2

Comparison of follow-up time and number of visits between participants who seroconverted and those who did not, cohort of men who have sex with men, Lisbon, Portugal, 2011–2014 (n=804)

	HIV-positive	HIV-negative	p value ^a
N	25	779	
Minimum and maximum of follow-up time	56 days – 1.91 years	6 days – 2.84 years	n.a.
Mean time of follow-up (SD) (years)	0.79 (0.50)	1.12 (0.68)	0.018
Mean number of visits (SD)	2.76 (1.05)	2.85 (1.21)	0.816
Mean number of visits per year (SD)	4.8 (3.0)	3.9 (5.6)	0.012

n.a.: not applicable; SD: standard deviation.

^a p value for independent samples, Mann-Whitney test

Predictors of HIV infection

Being born before 1970 had a strong point estimate of association, though non-significant, with seroconversion, whereas the remaining background indicators had negligible associations. Variables that were directly associated with HIV incidence even after adjustment for UAI were: reporting partner disclosure of HIV positive status between first and the most recent visit (aIRR=5.25; 95% CI 1.60–17.24; p=0.006); sexual intercourse with HIV-positive men whether only reported at first visit (aIRR=3.79; 95%CI 1.17–12.24; p=0.026), or only at the most recent visit (aIRR=5.99; 95%CI 2.28–15.71; p<0.001); having had an HIV-positive steady partner at least once during follow-up (aIRR=3.28; 95%CI 1.24–8.68; p=0.017); newly-adopted UAI with a steady partner regardless of their HIV status between cohort entry and the most recent visit (aIRR=3.85; 95%CI 1.26–11.78; p=0.018); persistent UAI with occasional partners during follow-up (aIRR=3.63; 95%CI 1.38–9.58; p=0.009) and having been newly diagnosed with syphilis between cohort entry and HIV seroconversion (aIRR=4.71; 95%CI 1.07–20.71; p=0.040).

Even though non-significant, having had sex with sex workers at least once during follow-up (aIRR=2.60; 95%CI 0.92–7.36; p=0.072) and newly adopting UAI with occasional partners between cohort entry and the most recent visit (aIRR=2.79; 95%CI 0.87–8.92; p=0.084) were associated with HIV incidence. Crude associations with more generic markers of exposure (having started to have sex with men four to eight years before cohort entry, reporting recent sexual intercourse with bisexual men or women and persistent use of recreational drugs during follow-up) lost significance after adjustments. Detailed results of HIV predictors are presented in Table 3.

We stratified the analysis of the main determinants of HIV incidence by HIV status of steady partner (Figure). Overall, we observed that MSM who had an HIV-positive steady partner during follow-up had higher incidence

rates than MSM who did not have an HIV-positive partner. The greatest increases in HIV incidence were found for MSM reporting newly-adopted UAI with a steady partner (IRR=17.29; 95% CI: 5.00–59.70) and MSM reporting persistent UAI with occasional partners during follow-up (IRR=14.19; 95% CI: 2.75–73.12).

Discussion

The Lisbon Cohort of MSM provides the first quantification of HIV incidence in Portuguese MSM. The overall estimate of 2.80 per 100 person-years is higher than those obtained in other European settings [4,6,8], and shows worrying ongoing transmission of HIV among MSM, consistent with routine surveillance data [23].

In this cohort, having an HIV positive steady partner increased the risk of seroconversion, particularly after newly-adopted UAI with that partner and regardless of UAI with occasional partners. The role of serodiscordant steady relationships in newly acquiring HIV infection is well-recognised [24]. Previous studies suggest that men within a steady relationship are more likely to engage in UAI and have lower rates of HIV testing as a result of lower risk perception and increased confidence of remaining HIV-negative [25]. As for the timing of transmission, among MSM who seroconverted and had an HIV positive steady partner, approximately half reported their disclosure of HIV (whether previously diagnosed or not) during follow-up. This suggests that a substantial fraction of transmission to the index partner might occur during the acute infection stage of the steady partner, when the risk of transmission is highest [26]. Nevertheless, we cannot exclude the contribution of older infections. Indeed, 37.1% of HIV-positive MSM in Portugal presented to care with CD4 count < 350/mm³ and, and 39.0% either had detectable or unknown viral load [27].

Persistent UAI with occasional partners was associated with HIV seroconversion, as extensively described [28]. Our study adds that being newly diagnosed with

TABLE 3A

Predictors of HIV incidence, cohort of men who have sex with men, Lisbon, Portugal, 2011–2014 (n=804)

	HIV cases	PY	HIV incidence	IRR (95% CI)	p value	aIRR ^a (95%CI)	p value
Mean number of tests per year during follow-up							
Less than 2	1	166.74	0.6	1		1	
2	5	367.90	1.4	2.27 (0.26–19.39)	0.455	3.40 (0.40–29.33)	0.266
3	6	215.20	2.8	4.65 (0.56–38.62)	0.155	4.70 (0.51–42.92)	0.170
4	3	83.49	3.6	5.99 (0.62–57.60)	0.121	10.59 (1.09–103.27)	0.042
More than 4	10	60.05	16.7	27.77 (3.56–216.92)	0.002	45.30 (5.62–365.00)	<0.001
Background characteristics							
Birth cohort							
Before 1970	5	109.26	4.6	2.81 (0.76–10.47)	0.123	n.a.	n.a.
1970–1979	4	245.75	1.6	1		n.a.	
1980–1989	12	373.74	3.2	1.97 (0.64–6.12)	0.239	n.a.	n.a.
1990 or after	4	164.63	2.4	1.49 (0.37–5.97)	0.571	n.a.	n.a.
Country of birth							
Portugal	18	648.27	2.8	1		n.a.	
Other	7	211.54	3.3	1.19 (0.50–2.85)	0.694	n.a.	n.a.
Education (schooling years)							
Less than higher education (≤12 years)	11	357.42	3.1	1.17 (0.53–2.58)	0.692	n.a.	n.a.
Higher education (>12 years)	14	533.74	2.6	1		n.a.	
Sexual identity							
Homosexual	22	789.81	2.8	1		n.a.	
Bisexual/heterosexual/other	3	100.31	3.0	1.07 (0.32–3.59)	0.908	n.a.	n.a.
HIV testing							
Number of HIV previous tests at cohort entry							
0	0	120.98	0.0	n.a.		n.a.	n.a.
1 to 5	14	476.42	2.9	1		n.a.	
More than 5	10	234.47	4.3	1.45 (0.65–3.27)	0.368	n.a.	n.a.
Reasons for HIV test during follow-up							
Concerned with exposure to HIV throughout follow-up							
Never	2	163.21	1.2	1		n.a.	
At least once	22	716.67	3.1	2.51 (0.59–10.65)	0.214	n.a.	n.a.
Partner was diagnosed with HIV/disclosed HIV status throughout follow-up							
Persistent No	18	758.15	2.4	1		1	
Changed: Yes to No	2	33.42	6.0	2.52 (0.58–10.86)	0.215	1.91 (0.24–15.01)	0.537
Changed: No to Yes	5	38.48	13.0	5.47 (2.03–14.74)	0.001	5.25 (1.60–17.24)	0.006
Persistent Yes	0	12.22	0.0	n.a.	n.a.	n.a.	n.a.
Sexual life and partners							
Age at first anal intercourse							
More than 15	21	693.42	3.0	1		n.a.	
15 or less	3	136.57	2.2	0.73 (0.22–2.43)	0.603	n.a.	n.a.
Time since the beginning of sexual life with other men							
4 years or less	5	238.32	2.1	1		1	
4 to 8 years	10	185.97	5.4	2.56 (0.88–7.50)	0.086	2.57 (0.77–8.54)	0.123
more than 8 years	9	405.71	2.2	1.06 (0.35–3.16)	0.920	1.09 (0.32–3.70)	0.887
Role in anal sex							
Insertive only	8	213.54	3.7	1		n.a.	
Receptive/both	17	658.75	2.6	0.69 (0.30–1.60)	0.385	n.a.	n.a.

aIRR: adjusted incidence rate ratio; CI: confidence interval; IRR: incidence rate ratio; n.a.: not applicable; PEP: post-exposure prophylaxis; PY: person-years; STI: sexually transmitted infection; UAI: unprotected anal intercourse.

^a Adjusted for UAI with a steady partner and UAI with occasional partners during follow-up.

TABLE 3B

Predictors of HIV incidence, cohort of men who have sex with men, Lisbon, Portugal, 2011–2014 (n=804)

	HIV cases	PY	HIV incidence	IRR (95% CI)	p value	aIRR ^a (95%CI)	p value
Sexual life and partners							
Sexual intercourse throughout follow-up with any of the following:							
HIV-positive men							
Persistent No	11	672.50	1.6	1		1	
Changed: Yes to No	5	78.05	6.4	3.92 (1.36–11.27)	0.011	3.79 (1.17–12.24)	0.026
Changed: No to Yes	8	74.57	10.7	6.56 (2.64–16.31)	<0.001	5.99 (2.28–15.71)	<0.001
Persistent Yes	0	33.72	0.0	n.a.		n.a.	
Bisexual men							
Persistent No	10	478.84	2.1	1		1	
Changed: Yes to No	2	152.34	1.3	0.63 (0.14–2.87)	0.549	0.71 (0.15–3.32)	0.660
Changed: No to Yes	3	79.66	3.8	1.80 (0.50–6.55)	0.370	2.23 (0.59–8.42)	0.236
Persistent Yes	8	147.29	5.4	2.60 (1.03–6.59)	0.044	2.12 (0.79–5.66)	0.136
Men with different sexual partners							
Persistent No	3	113.13	2.7	1		n.a.	
Changed: Yes to No	5	194.75	2.6	0.97 (0.23–4.05)	0.965	n.a.	n.a.
Changed: No to Yes	2	85.45	2.3	0.88 (0.15–5.28)	0.891	n.a.	n.a.
Persistent Yes	13	462.90	2.8	1.06 (0.30–3.72)	0.929	n.a.	n.a.
Sex workers (even if not paid)							
Never	18	779.22	2.3	1		1	
At least once	5	78.92	6.3	2.74 (1.02–7.39)	0.046	2.60 (0.92–7.36)	0.072
Women							
Persistent No	18	743.54	2.4	1		1	
Changed: Yes to No	4	64.21	6.2	2.57 (0.87–7.60)	0.087	2.22 (0.74–6.71)	0.156
Changed: No to Yes	0	11.83	0.0	n.a.	n.a.	n.a.	
Persistent Yes	1	38.55	2.6	1.07 (0.14–8.03)	0.946	0.69 (0.09–5.34)	0.723
Trios/group sex							
Persistent No	13	508.19	2.6	1		n.a.	
Changed: Yes to No	0	129.60	0.0	n.a.		n.a.	
Changed: No to Yes	3	84.82	3.5	1.38 (0.39–4.85)	0.613	n.a.	n.a.
Persistent Yes	7	134.39	5.2	2.04 (0.81–5.10)	0.129	n.a.	n.a.
Steady partner during follow-up							
Persistent No	5	180.52	2.8	1		n.a.	
Changed: Yes to No	2	192.56	1.0	0.38 (0.07–1.93)	0.241	n.a.	n.a.
Changed: No to Yes	4	145.44	2.8	0.99 (0.27–3.70)	0.992	n.a.	n.a.
Persistent Yes	13	360.75	3.6	1.30 (0.46–3.65)	0.617	n.a.	n.a.
HIV-positive steady partner during follow-up							
Never	16	777.93	2.1	1		1	
At least once	8	90.14	8.9	4.32 (1.85–10.08)	0.001	3.28 (1.24–8.68)	0.017
Occasional partners during follow-up							
Persistent No	2	40.46	4.9	1		n.a.	
Changed: Yes to No	2	146.52	1.4	0.28 (0.04–1.96)	0.198	n.a.	n.a.
Changed: No to Yes	1	46.70	2.1	0.43 (0.04–4.78)	0.495	n.a.	n.a.
Persistent Yes	18	644.85	2.8	0.56 (0.13–2.43)	0.443	n.a.	n.a.
Number of occasional sexual partners in the previous 12 months at cohort entry							
≤ 1	3	125.50	2.4	1		n.a.	
2 to 9	12	408.48	2.9	1.30 (0.35–4.36)	0.749	n.a.	n.a.
> 10	6	242.20	2.5	1.03 (0.26–4.14)	0.960	n.a.	n.a.
Having sex for money or drugs during follow-up							
Never	22	854.61	2.6	1		n.a.	
At least once	1	21.84	4.6	1.78 (0.24–13.19)	0.573	n.a.	n.a.

aIRR: adjusted incidence rate ratio; CI: confidence interval; IRR: incidence rate ratio; n.a.: not applicable; PEP: post-exposure prophylaxis; PY: person-years; STI: sexually transmitted infection; UAI: unprotected anal intercourse.

^a Adjusted for UAI with a steady partner and UAI with occasional partners during follow-up.

TABLE 3C

Predictors of HIV incidence, cohort of men who have sex with men, Lisbon, Portugal, 2011–2014 (n=804)

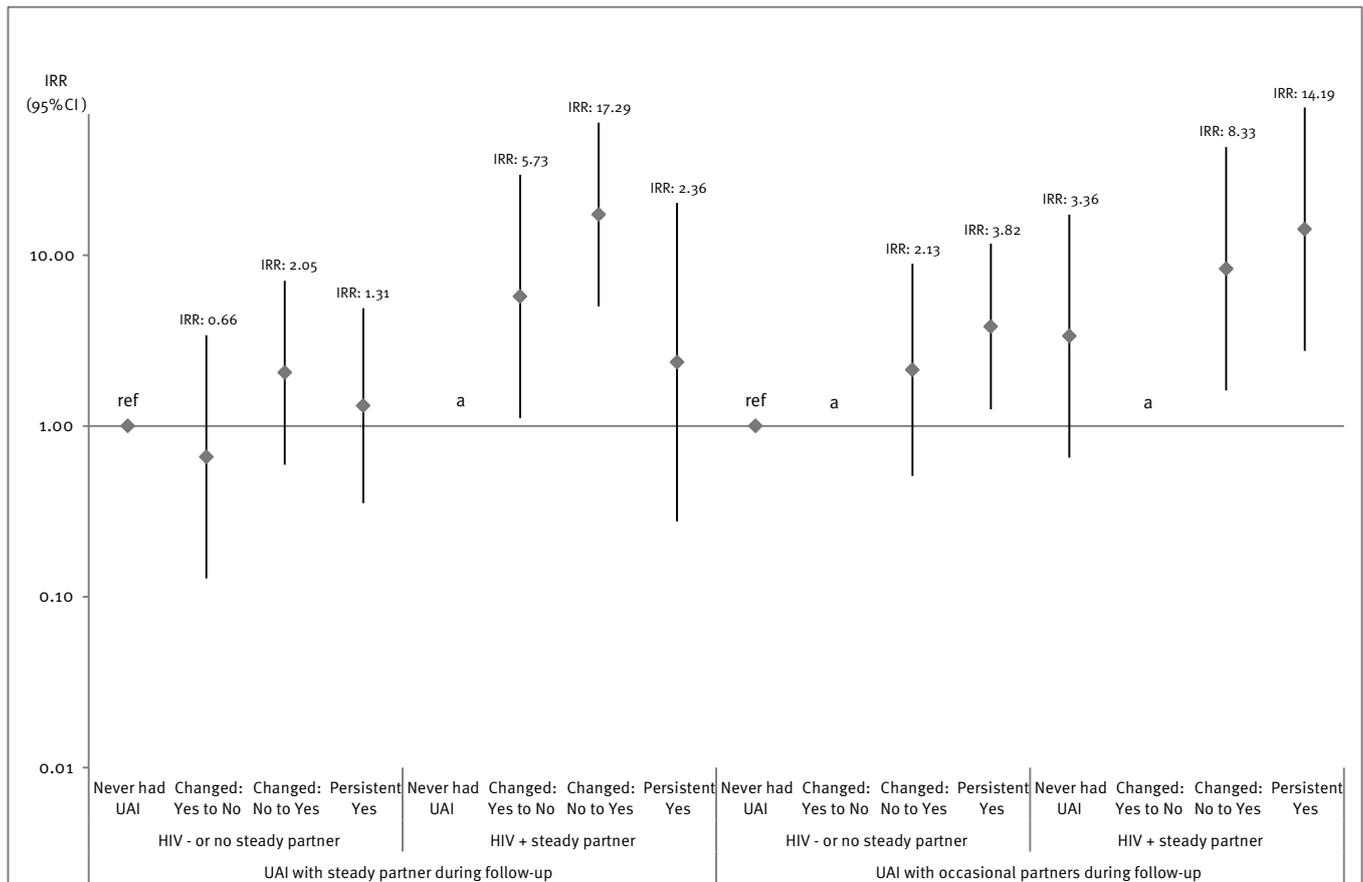
	HIV cases	PY	HIV incidence	IRR (95% CI)	p value	aIRR ^a (95%CI)	p value
UAI during follow-up							
UAI with a steady partner							
Persistent No	5	305.85	1.6	1		1	
Changed: Yes to No	4	191.38	2.1	1.28 (0.34–4.76)	0.714	1.10 (0.29–4.11)	0.892
Changed: No to Yes	10	150.54	6.6	4.06 (1.39–11.89)	0.010	3.85 (1.26–11.78)	0.018
Persistent Yes	5	194.26	2.6	1.57 (0.46–5.44)	0.473	1.83 (0.53–6.38)	0.340
UAI with occasional partners							
Persistent No	7	388.18	1.8	1		1	
Changed: Yes to No	0	148.83	0.0	n.a.		n.a.	
Changed: No to Yes	5	115.29	4.3	2.41 (0.76–7.58)	0.134	2.79 (0.87–8.92)	0.084
Persistent Yes	10	162.34	6.2	3.42 (1.30–8.97)	0.013	3.63 (1.38–9.58)	0.009
Venues used to meet occasional partners at cohort entry							
Only sexual venues (saunas, dark rooms, sex clubs)							
No	19	681.40	2.8	1		n.a.	
Yes	6	195.90	3.1	1.1 (0.44–2.76)	0.841	n.a.	n.a.
Other venues (discos/gay bars, gym and outdoor cruising venues)							
No	10	368.51	2.7	1		n.a.	
Yes	15	513.79	2.9	1.08 (0.48–2.40)	0.858	n.a.	n.a.
Internet							
No	6	313.65	1.9	1		n.a.	
Yes	19	567.72	3.3	1.75 (0.70–4.38)	0.232	n.a.	n.a.
STIs and hepatitis							
Recent history of syphilis during follow-up							
Persistent No	22	858.10	2.6	1		1	
Changed: Yes to No	1	12.16	8.2	3.21 (0.43–23.79)	0.254	3.89 (0.47–31.91)	0.206
Changed: No to Yes	2	21.95	9.1	3.55 (0.84–15.12)	0.086	4.71 (1.07–20.71)	0.040
Persistent Yes	0	0.00	-	-		-	
Recent history of gonorrhoea during follow-up							
Persistent No	24	835.79	2.9	1		n.a.	
Changed: Yes to No	0	25.77	0.0	n.a.		n.a.	n.a.
Changed: No to Yes	1	30.08	3.3	1.16 (0.16–8.56)	0.886	n.a.	n.a.
Persistent Yes	0	0.00	n.a.	n.a.		n.a.	n.a.
Lifetime history of hepatitis C reported at cohort entry							
No/does not know	25	874.81	2.9	1		n.a.	
Yes	0	2.76	0	n.a.		n.a.	
Lifetime history of Hepatitis B reported at cohort entry							
No/does not know	24	862.89	2.8	1		n.a.	
Yes	1	20.64	4.8	1.74 (0.24–12.88)	0.587	n.a.	n.a.
Drug use before or during intercourse							
Use of recreational drugs before or during intercourse during follow-up							
Persistent No	9	507.25	1.8	1		1	
Changed: Yes to No	2	91.08	2.2	1.24 (0.27–5.73)	0.785	0.92 (0.19–4.38)	0.915
Changed: No to Yes	5	117.90	4.2	2.39 (0.80–7.13)	0.118	1.63 (0.42–6.28)	0.477
Persistent Yes	8	155.99	5.1	2.89 (1.12–7.49)	0.029	1.90 (0.70–5.17)	0.209
PEP at cohort entry							
Does not know about	14	437.41	3.2	1		n.a.	
Knows about but never used	10	392.47	2.5	0.80 (0.35–1.79)	0.582	n.a.	n.a.
Knows and used	1	21.15	4.7	1.48 (0.19–11.23)	0.706	n.a.	n.a.

aIRR: adjusted incidence rate ratio; CI: confidence interval; IRR: incidence rate ratio; n.a.: not applicable; PEP: post-exposure prophylaxis; PY: person-years; STI: sexually transmitted infection; UAI: unprotected anal intercourse.

^a Adjusted for UAI with a steady partner and UAI with occasional partners during follow-up.

FIGURE

Stratified analysis of the main determinants of HIV incidence by HIV status of steady partner, cohort of men who have sex with men, Lisbon, Portugal, 2011–2014 (n=804)



IRR: incidence rate ratio; 95% CI: 95% confidence interval; UAI: unprotected anal intercourse; ref: reference category.

^a No seroconversions were observed in the category

syphilis during follow-up was a strong predictor of HIV incidence, independently of self-reported UAI. An additional red flag was the observation that MSM who seroconverted had shorter intervals between follow-up visits and higher mean number of tests per year, which highlights the use of testing as a risk management strategy.

Our findings suggest that, in addition to the pattern of service use itself, incident circumstances (newly-adopted UAI with a steady partner, newly-disclosed HIV-positive partner, and newly-diagnosed syphilis) may be useful markers of the short-term risk of infection. Yet, it is important to note that we cannot assume that any incident circumstance or change in the information provided between visits represents a sustained behavioural change but rather indicates varying behavioural options that may influence seroconversion risk.

Other behavioural factors, such as time since the beginning of sexual life, intercourse with bisexual men or sex

workers and persistently using recreational drugs, may be regarded as less specific predictors of incident HIV, even though such effects were probably largely mediated by UAI. The number of sexual partners in the year before cohort entry was not associated with increased HIV incidence. These findings highlight that, rather than extensively characterising the type or number of partners, targeted inquiries about UAI in this context seem to be more accurate for predicting HIV risk.

So far, none of the background variables predicted HIV risk in this cohort of Portuguese MSM. However, higher HIV incidence was found in MSM born before 1970. Older MSM were previously described at higher risk of acquiring HIV from a steady partner [8] and may underestimate vulnerability since they have remained uninfected up to the present [29]. In contrast with previous studies and national and European surveillance data [5,30], younger MSM were not clearly identified as being at higher risk for HIV, but that could be related

to different patterns of use of the CheckpointLX by younger generations.

Methodological options and limitations of this study should be addressed. First, this design option is unlikely to result in a representative sample of the source MSM population, which limits the generalisability of our findings. When compared with data from the 2007 National Health and Sexuality Survey (HSS) [31], MSM in our sample are younger, more self-identified as homosexual (86.1% vs 35.9% of men reporting some kind of sexual contact with men in the HSS) and report more frequently history of HIV testing (84.1% vs 61.0% in HSS). Nevertheless, by setting up a cohort study in a community-based voluntary counselling and testing service we expect to reach MSM on average at higher risk of infection than the general MSM community. Thereby it seems reasonable to admit that we are focusing our attention on a priority subset of the population in terms of HIV risk (even if potentially more aware than those not reached by the service). Additionally, since CheckpointLX promotion strategies remained similar during follow-up, we do not expect that the extent of selection bias will change substantially over time, which is particularly important for estimating secular trends of infection and behaviours in the source population [32-34]. Finally, the fact that the recruitment site is a service which aims to anticipate diagnosis and to provide evidence-based and adapted information may itself modify the risk of acquiring HIV and the consequent incidence estimates. However, we expect that newly-recruited clients reflect the overall incidence of infection in the community.

Another important issue is participation bias: the fact that around 30% of eligible MSM chose not to enter the cohort implies that informative data may be missing on a harder-to-reach subset of the target population. However, the frequency of prior testing was similar between groups, suggesting that both may have similar perceived risk of acquiring HIV [35]. Moreover, the observed attrition means that information about possible seroconversions is missing in half of participants, which is a clear limitation. Follow-ups depend on the frequency of service uptake, which can itself be influenced by perceived risk of infection. Efforts have been made to minimise dropout rates, including active reminders of follow-up visits by peer counsellors. However, we still found differences in mean age between MSM who appeared for follow-up and those who did not, although the absolute difference was small. No differences were found in the frequency of behaviours associated with higher probability of seroconversion. This leads us to hypothesise that our incidence rate might not be substantially affected by losses to follow-up.

Self-reported information is always subject to limitations in validity and reliability. However, we are confident that a relevant strength comes from the involvement of community peer counsellors, since this

strategy increases participation and improves validity and completeness of information as well as disclosure of risk, as supported by previous research [34,36].

Despite the high incidence observed, the absolute number of infections is still low, resulting in suboptimal statistical power for some comparisons. In the future, with larger sample size and longer follow-up periods, we expect increased precision of estimates. Nevertheless, these first estimates are important for two main reasons: i) they draw a first picture of HIV incidence and its drivers in Portuguese MSM about whom little was known; ii) they add evidence on the role of changes in individual circumstances in newly acquiring HIV to the existing body of prospective evidence from a variety of settings.

In conclusion, we found high HIV incidence in this cohort of Portuguese MSM likely to be driven by short-term contextual and behavioural changes, namely newly-adopted UAI with a steady partner, newly-disclosed HIV-positive partner and newly-diagnosed syphilis. History of serodiscordant steady relationships and persistently reporting UAI with occasional partners also played a major role in predicting HIV seroconversion.

Acknowledgements

The Lisbon Cohort of MSM teams thanks to all participants, to CheckpointLX team of peer counsellors: Jesus Rojas, Miguel Rocha, Fernando Ferreira, Nuno Pinto and Julio Esteves, and to Dina Cosme from ISPUP.

Conflict of interest

None declared

Authors' contributions

PM drafted the manuscript and performed the data analysis. RL participated in the study design, helped draft the manuscript, participated in analysis and interpretation of data, and reviewed the manuscript for important intellectual content. CC reviewed the manuscript for important intellectual content. RF and JB participated in the study design and data collection, and reviewed the manuscript for important intellectual content. MJC conceived the study, participated in the study design and coordination, and reviewed the manuscript for important intellectual content. LM conceived the study, participated in the study design and coordination, and reviewed the manuscript for important intellectual content. HB conceived the study, participated in the study design and coordination, and reviewed the manuscript for important intellectual content. All authors read and approved the final manuscript.

References

1. Sullivan PS, Carballo-Diéguez A, Coates T, Goodreau SM, McGowan I, Sanders EJ, et al. Successes and challenges of HIV prevention in men who have sex with men. *Lancet*. 2012;380(9839):388-99. Epub 20120724. PMID:22819659
2. Morris SR, Little SJ. MSM: resurgent epidemics. *Curr Opin HIV AIDS*. 2011;6(4):326-32. <http://dx.doi.org/10.1097/COH.0b013e3283476c29> PMID:21537172

3. Sullivan PS, Hamouda O, Delpuch V, Geduld JE, Prejean J, Semaille C, et al.; Anancy MSM Epidemiology Study Group. Reemergence of the HIV epidemic among men who have sex with men in North America, Western Europe, and Australia, 1996-2005. *Ann Epidemiol.* 2009;19(6):423-31. Epub20090523. PMID:19460672
4. Phillips AN, Cambiano V, Nakagawa F, Brown AE, Lampe F, Rodger A, et al. Increased HIV incidence in men who have sex with men despite high levels of ART-induced viral suppression: analysis of an extensively documented epidemic. *PLoS One.* 2013;8(2):e55312. Epub20130305. PMID:23457467
5. Prejean J, Song R, Hernandez A, Ziebell R, Green T, Walker F, et al.; HIV Incidence Surveillance Group. Estimated HIV incidence in the United States, 2006-2009. *PLoS One.* 2011;6(8):e17502. Epub20110810. PMID:21826193
6. Ndawinz JD, Costagliola D, Supervie V. New method for estimating HIV incidence and time from infection to diagnosis using HIV surveillance data: results for France. *AIDS.* 2011;25(15):1905-13. Epub20110804. PMID:21811147
7. Rosinska M, Janiec J, Niedzwiedzka-Stadnik M. Increase of HIV diagnoses among men who have sex with men in Poland, 2000 to 2011. *Euro Surveill.* 2013 Nov 28;18(48):20642. <http://dx.doi.org/10.2807/1560-7917.ES2013.18.48.20642>
8. Jansen IA, Geskus RB, Davidovich U, Jurriaans S, Coutinho RA, Prins M, et al. Ongoing HIV-1 transmission among men who have sex with men in Amsterdam: a 25-year prospective cohort study. *AIDS.* 2011;25(4):493-501. Epub20101231. PMID:21192230
9. European Centre for Disease Prevention and Control (ECDC)/WHO Regional Office for Europe. HIV/AIDS surveillance in Europe 2013. Stockholm: ECDC; 2014. Available from: <http://ecdc.europa.eu/en/publications/Publications/hiv-aids-surveillance-report-Europe-2013.pdf>.
10. Departamento de Doenças Infecciosas do INSA. Unidade de Referência e Vigilância Epidemiológica. Núcleo de Vigilância Laboratorial de Doenças Infecciosas; colab. Programa Nacional para a Infecção VIH/SIDA. Infecção VIH/SIDA: a situação em Portugal a 31 de dezembro de 2013 [HIV/AIDS: the situation in Portugal – December 31, 2013]. Portuguese. 2014 [Accessed December 2014]. Available from: http://repositorio.insa.pt/bitstream/10400.18/2448/3/INSA-Relatorio_Infecao_HIV-SIDA_2013.pdf.
11. The EMIS Network. The European Men-Who-Have-Sex-With-Men Internet Survey. Findings from 38 countries. 2013. Available from: http://www.emis-project.eu/sites/default/files/public/publications/emis-2010_european_msm_internet_survey_38_countries_v5.pdf
12. Dias S, Mendão L, Gama A, Barros H. How to access vulnerable and hard-to-reach populations? Methodological challenges in HIV and STIs epidemiological and behavioural research with sex workers [abstract]. *Eur J Epidemiol.* 2012; (27):S1-197.
13. Marcus U, Hickson F, Weatherburn P, Schmidt A, The EMIS Network. Prevalence of HIV among MSM in Europe: comparison of self-reported diagnoses from a large scale internet survey and existing national estimates. *BMC Public Health.* 2012;12:978. <http://dx.doi.org/10.1186/1471-2458-12-978> PMID:23151263 PMCID:PMC3526585
14. Lavoie E, Alary M, Remis RS, Otis J, Vincelette J, Turmel B, et al. Determinants of HIV seroconversion among men who have sex with men living in a low HIV incidence population in the era of highly active antiretroviral therapies. *Sex Transm Dis.* 2008;35(1):25-9. Epub20070928. PMID:17898678
15. Koblin BA, Husnik MJ, Colfax G, Huang Y, Madison M, Mayer K, et al. Risk factors for HIV infection among men who have sex with men. *AIDS.* 2006;20(5):731-9. Epub20060304. PMID:16514304
16. Buchbinder SP, Vittinghoff E, Heagerty PJ, Celum CL, Seage GR, 3rd, Judson FN, et al. Sexual risk, nitrite inhalant use, and lack of circumcision associated with HIV seroconversion in men who have sex with men in the United States. *J Acquir Immune Defic Syndr.* 2005 May 1;39(1):82-9. <http://dx.doi.org/10.1097/01.qai.0000134740.41585.f4> PMID:15851918
17. Lloyd S, Operario D. HIV risk among men who have sex with men who have experienced childhood sexual abuse: systematic review and meta-analysis. *AIDS education and prevention: official publication of the International Society for AIDS Education.* *AIDS Educ Prev.* 2012;24(3):228-41. <http://dx.doi.org/10.1521/aeap.2012.24.3.228> PMID:22676462
18. Ostrow DG, Plankey MW, Cox C, Li X, Shoptaw S, Jacobson LP, et al. Specific sex drug combinations contribute to the majority of recent HIV seroconversions among MSM in the MACS. *J Acquir Immune Defic Syndr.* 2009;51(3):349-55. <http://dx.doi.org/10.1097/QAI.0b013e3181a24b20> PMID:19387357 PMCID:PMC3074969
19. Poynten IM, Jin F, Prestage GP, Kaldor JM, Kippax S, Grulich AE. Defining high HIV incidence subgroups of Australian homosexual men: implications for conducting HIV prevention trials in low HIV prevalence settings. *HIV Med.* 2010;11(10):635-41. Epub20100512. PMID:20456511
20. World Health Organization (WHO). Service delivery approaches to HIV testing and counselling (HTC): a strategic HTC policy framework. Geneva: WHO; 2012. Available from: http://apps.who.int/iris/bitstream/10665/75206/1/9789241593877_eng.pdf?ua=1
21. Elford J, Jeannin A, Spencer B, Gervasoni JP, van de Laar MJ, Dubois-Arber F. HIV and STI behavioural surveillance among men who have sex with men in Europe. *Euro Surveill.* 2009;14(47). pii: 19414. PMID:19941807
22. Joint United Nations Programme on HIV/AIDS (UNAIDS). Monitoring the Declaration of Commitment on HIV/AIDS: guidelines on construction of core indicators: 2010 reporting. March 2009. Geneva: UNAIDS; 2009. Available from: http://www.unaids.org/en/media/unaids/contentassets/dataimport/pub/manual/2009/jc1676_core_indicators_manual_09_en.pdf.
23. Martins HC. Infecção VIH e SIDA em homens que têm sexo com homens em Portugal (1983-2012): caracterização dos casos notificados [HIV/AIDS in Men who have Sex with Men in Portugal (1983-2012): characterization of notified cases]. *Boletim Epidemiológico Observações.* 2013;2(5):19-21. Portuguese. Available from: http://www.insa.pt/sites/INSA/Portugues/PublicacoesRepositorio/Documents/observacoesN52013_artigo7.pdf
24. Xiridou M, Geskus R, De Wit J, Coutinho R, Kretzschmar M. The contribution of steady and casual partnerships to the incidence of HIV infection among homosexual men in Amsterdam. *AIDS.* 2003;17(7):1029-38. Epub20030418. PMID:12700453
25. Stephenson R, White D, Darbes L, Hoff C, Sullivan P. HIV Testing Behaviors and Perceptions of Risk of HIV Infection Among MSM with Main Partners. *AIDS Behav.* 2015;19(3):553-60. <http://dx.doi.org/10.1007/s10461-014-0862-4> PMID:25081599
26. Hollingsworth TD, Anderson RM, Fraser C. HIV-1 transmission, by stage of infection. *J Infect Dis.* 2008;198(5):687-93. Epub20080730. PMID:18662132
27. Carvalho C, Fuertes R, Lucas R, Martins A, Campos MJ, Mendão L, et al. HIV testing among Portuguese men who have sex with men--results from the European MSM Internet Survey (EMIS). *HIV Med.* 2013;14(Suppl 3):15-8. Epub20130927. PMID:24033897
28. Baggaley RF, White RG, Boily MC. HIV transmission risk through anal intercourse: systematic review, meta-analysis and implications for HIV prevention. *Int J Epidemiol.* 2010;39(4):1048-63. Epub20100422. PMID:20406794
29. Dufour A, Alary M, Otis J, Remis RS, Masse B, Turmel B, et al. Risk behaviours and HIV infection among men having sexual relations with men: baseline characteristics of participants in the Omega Cohort Study, Montreal, Quebec, Canada. *Can J Public Health.* 2000;91(5):345-9. PMID:11089286
30. Janiec J, Haar K, Spiteri G, Likatavicius G, Van de Laar M, Amato-Gauci AJ. Surveillance of human immunodeficiency virus suggests that younger men who have sex with men are at higher risk of infection, European Union, 2003 to 2012. *Euro Surveill.* 2013;18(48):20644. <http://dx.doi.org/10.2807/1560-7917.ES2013.18.48.20644>
31. Ferreira PM, Cabral MV. Sexualidades em Portugal: Comportamentos e Riscos [Sexualities in Portugal: Behaviours and Risks] Lisboa: Editorial Bizâncio: 2010. Portuguese.
32. Paquette D, De Wit J. Sampling methods used in developed countries for behavioural surveillance among men who have sex with men. *AIDS Behav.* 2010;14(6):1252-64. Epub20100709. PMID:20614177
33. World Health Organization/Joint United Nations Programme on HIV/AIDS (WHO/UNAIDS). Second generation surveillance for HIV: The next decade 2000. Geneva: WHO/UNAIDS; 2000. Available from: http://www.who.int/reproductivehealth/publications/rtis/CDS_CSR_EDC_2000_5/en/.
34. World Health Organization/Joint United Nations Programme on HIV/AIDS (WHO/UNAIDS). Guidelines on surveillance among populations most at risk for HIV. Geneva: WHO/UNAIDS; 2011. Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2011/20110518_Surveillance_among_most_at_risk.pdf.
35. Deblonde J, Hamers FF, Callens S, Lucas R, Barros H, Rüütel K, et al. HIV testing practices as reported by HIV-infected patients in four European countries. *AIDS Care.* 2014;26(4):487-96. Epub20131005. PMID:24090396
36. Lorenc T, Marrero-Guillamón I, Llewellyn A, Aggleton P, Cooper C, Lehmann A, et al. HIV testing among men who have sex with men (MSM): systematic review of qualitative evidence. *Health Educ Res.* 2011;26(5):834-46. Epub20110830. PMID:21873612