The Lisbon Cohort of Men who have sex with men: study protocol and HIV incidence

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Esta dissertação tem como base dois manuscritos, nos quais colaborei ativamente na definição das hipóteses, informatização, análise e interpretação dos dados. Fui responsável pela redação da versão inicial dos dois manuscritos:

I. The Lisbon Cohort of Men who have sex with men – Study protocol.

II. Incident risk factors as predictors of HIV seroconversion in the Lisbon cohort of MSM: 2011 – 2014.

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ACS – Amsterdam cohort studies

AIDS – Acquired immunodeficiency syndrome

ART – Antiretroviral therapy

CI – Confidence intervals

COHERE – Collaboration of Observational HIV Epidemiological Research Europe

ECDC – European centre for disease control and prevention

EIA – Enzyme immunoassay

EU/EEA – European Union/European economic area

HIV – Human immunodeficiency virus

HTC – HIV testing and counselling

IR – Incidence rate

IRR – Incidence rate ratio

MACS – Multicenter AIDS cohort studies

MSM – Men who have sex with men

NGO – Non-governmental organization

OR – Odds ratio

POC – Point-of-care

PrEP – Pre-exposure prophylaxis

PY – Person-years

STI – Sexually transmitted infection

UAI – Unprotected anal intercourse

UK – United Kingdom

US – United States

VCT – Voluntary counselling and testing

WHO – World health organization

RESUMO

Introdução: Os homens que têm sexo com homens (HSH) têm sido, desde o início da epidemia do vírus da imunodeficiência humana (VIH), uma população especialmente afetada pela infeção. Estimativas recentes mostram que nos países ocidentais a epidemia está a ressurgir nesta população. Em Portugal, após vários anos de uma epidemia maioritariamente associada ao consumo inseguro de drogas pela via injetada, a transmissão associada ao sexo entre homens tem vindo a ganhar relevância. Torna-se assim essencial o estabelecimento ferramentas de vigilância epidemiológica que permitam abordagens quer descritivas quer analíticas da distribuição e dos determinantes da infeção VIH nos HSH. Neste contexto foi implementado um estudo de coorte prospetivo – a *Lisbon Cohort of MSM* – localizado num centro comunitário para rastreio rápido do VIH e aconselhamento dirigido a HSH. O principal objetivo da coorte é monitorizar de forma dinâmica a carga da doença e os seus preditores. Também se espera que este estudo de coorte possa produzir informação importante para a melhoria dos serviços de teste e para a garantia de uma ligação atempada aos cuidados de saúde.

Objetivos: Descrever a *Lisbon Cohort of MSM* através dos seguintes objetivos: 1) descrever o desenho e as opções metodológicas do estudo, assim como as características dos participantes à entrada no estudo; 2) estimar a incidência da infeção VIH e testar as mudanças nas exposições a curto prazo como preditores da seroconversão.

Métodos: Foi estabelecido um estudo observacional prospetivo – a *Lisbon Cohort of MSM* – que funciona num centro comunitário para rastreio rápido do VIH e aconselhamento em Lisboa (CheckpointLX). Os homens que têm um teste não reativo, com idade igual ou superior a 18 anos, que reportam já ter tido sexo com outros homens são convidados a participar no estudo e são agendadas visitas de seguimento de acordo com a sua disponibilidade, idealmente com intervalos de seis meses. Cada avaliação no âmbito da coorte compreende a aplicação de um questionário estruturado e de teste rápido para o VIH e para a sífilis realizados por pares. As características dos participantes à entrada no estudo foram descritas através de frequências absolutas e proporções no caso de variáveis categóricas e de médias e desvios-padrão ou medianas e os percentis 25 e 75 no caso de variáveis contínuas. As taxas de incidência (IR) e os respetivos intervalos de confiança a 95% (IC 95%) foram calculados usando como denominador o total de pessoas-ano em risco. Os preditores da seroconversão foram identificados usando a regressão linear generalizada com

distribuição de Poisson e as associações apresentadas como razões de taxas de incidência (IRR) e respetivos IC 95%. Os dados analisados referem-se ao período de abril de 2011, data de início do estudo, a fevereiro de 2014.

Resultados: Objetivo 1: Durante o período selecionado apresentaram-se no CheckpointLX 3106 HSH elegíveis para entrada na coorte dos quais 923 (29.7%) recusaram participar. Os restantes 2183 (70.3%) HSH foram incluídos no estudo e 804 tinham pelo menos uma visita de seguimento, num total de 893 pessoas-ano de observação. Cerca de 82.3% dos participantes já tinha feito um teste ao VIH anteriormente, sendo os motivos mais referidos para o teste atual: conhecer o estado de saúde/teste de rotina (81.3%); perceção de exposição ao VIH há mais de 3 meses (50.5%) e nos últimos 3 meses (40.7%). Cerca de 12% dos HSH reportaram relações sexuais com homens VIH-positivo nos 12 meses anteriores à entrada no estudo. Aproximadamente 8% dos participantes que reportaram terem tido pelo menos um parceiro habitual referem que este era VIH-positivo, e destes 43.7% reportaram o uso inconsistente do preservativo. Dos que desconheciam o estatuto serológico do parceiro habitual 71.0% reportaram o uso inconsistente do preservativo. Dos HSH que tiveram pelo menos um parceiro ocasional, cerca 21% reportaram não ter usado o preservativo na última penetração anal e 46.4% reportaram o uso inconsistente do preservativo nos 12 meses anteriores à entrada na coorte. A razão mais referida para o não uso do preservativo na penetração anal foi a existência de um parceiro habitual (66.2%). O consumo de álcool ou drogas antes ou durante as relações sexuais nos 12 meses anteriores à entrada na coorte foi referido por 86.4% dos participantes. Objetivo 2: Durante o período de seguimento verificam-se 25 seroconversões, resultando numa incidência de 2.80 por 100 pessoas-ano (IC 95%: 1.89-4.14). Os HSH que seroconverteram tinham um maior número médio de testes por ano. A seroconversão estava significativamente associada à revelação do estatuto serológico positivo do parceiro habitual no período entre a entrada no estudo e a visita mais recente, ao abandono do uso consistente do preservativo nas relações sexuais anais com parceiro habitual durante o seguimento e ao diagnóstico de sífilis durante o seguimento. Da mesma forma, ter tido relações sexuais com homens VIH-positivo, ter tido um parceiro habitual VIH-positivo pelo menos uma vez durante o seguimento e reportar persistentemente ter tido relações anais desprotegidas com parceiro ocasional foram preditores da seroconversão.

Conclusões: A nível nacional, o seguimento desta coorte de HSH VIH-negativos constituirá uma ferramenta valiosa para a monitorização dinâmica da infeção VIH num cenário do sul da europa onde a informação prospetiva existente tem sido muito

limitada. No contexto internacional irá permitir ainda uma abordagem analítica mais aprofundada das tendências temporais a nível populacional e da identificação das mudanças nos fatores de risco a nível individual que modelam a epidemia nos HSH. Dadas as opções no desenho do estudo não se espera que amostra seja representativa de toda a população de HSH no nosso país. No entanto, uma vez que não se esperam mudanças substanciais na magnitude do viés de seleção ao longo do período do estudo, acreditamos que nossas opções metodológicas não excluem a capacidade de estimar tendências temporais da incidência ou de identificar os determinantes da seroconversão. Esta primeira estimativa da incidência do VIH nos HSH portugueses foi mais do que a observada noutros países europeus, indicando uma elevada velocidade de transmissão do vírus. O presente estudo produz evidência de que as mudanças individuais recentes nos contextos e nos comportamentos durante o seguimento, as relações serodiscordantes e as relações sexuais desprotegidas com parceiro ocasional são determinantes particularmente importantes da epidemia do VIH nesta coorte de HSH. Em última instância os resultados do estudo servirão para informar da potencial efetividade das políticas dirigidas à redução da carga do VIH na população de HSH.

ABSTRACT

Introduction: Men who have sex with men (MSM) have been, since the beginning of the HIV epidemic, a key population at higher risk. Recent trends show a resurgent epidemic in this population in Western countries. In Portugal after several years of a drug injection driven HIV epidemic, sex between men has regained relevance as a transmission mode. Surveillance tools that allow for a better description of the distribution and determinants of HIV infection in MSM are essential. In this scenario, an open prospective cohort study was set up - The Lisbon Cohort of MSM, at a community-based voluntary HIV counselling and testing service targeting the MSM population. The main goal of this cohort study is to dynamically monitor the burden of disease and its predictors. The cohort is also expected to provide important information to improve the provision of HIV testing and to ensure timely linkage to care.

Objectives: We aimed to describe the ongoing Lisbon Cohort of MSM through the following objectives: 1) to describe the design and methodological options of the study, as well as the descriptive characteristics of participants at cohort entry; 2) to estimate incidence of HIV infection and to assess how individual short-term changes in exposures predict seroconversion.

Methods: The Lisbon Cohort of MSM prospective study was set-up and conducted at a community-based voluntary HIV counselling and testing centre in Lisbon (CheckpointLX). Men testing negative, aged 18 or older who report having had sex with other men are invited to participate and reassessed at follow-up visits scheduled according to their convenience, but ideally with 6-month intervals. At each evaluation a structured questionnaire is administered and HIV and syphilis rapid testing are performed by peer counsellors. Characteristics of participants enrolled were described using absolute frequencies and proportions in the case of categorical variables. Means and standard deviation or median and percentiles 25 and 75 were used to describe continuous variables. Incidence rates (IR) with 95% confidence intervals were estimated using as the denominator the sum of person-years (PY). Predictors of HIV seroconversion were identified using Poisson generalized linear regression. Associations are presented as incidence rate ratios and 95% confidence intervals (95% CI). Data analysed refer to the period from April 2011, when the cohort study was established, to February 2014.

Results: Objective 1: During the study period 3106 MSM were eligible to enter the cohort of whom 923 (29.7%) refused to participate. The remaining 2183 (70.3%) individuals were enrolled and 804 had at least one follow-up evaluation, for a total of

893 person-years of observation. HIV testing prior to cohort entry was reported by 82.3% of participants, the most common reasons for the index HIV test were: to check health status/routine (81.3%), perception of exposure to HIV more than 3 months before (50.5%) and in the previous 3 months (40.7%). Twelve percent of MSM reported sexual intercourse with HIV-positive men in the previous 12 months. Approximately eight percent of those who reported a steady relationship had a HIV-positive partner, of whom 43.7% reported inconsistent condom use. That proportion was 71.0% among those unaware of their steady partner's HIV status. Twenty one percent of men who had at least one occasional partner reported no condom use in the last sexual encounter and 46.4% reported inconsistent use in the previous 12 months. The most referred reason for engaging in unprotected anal intercourse was with a steady partner (66.2%). Alcohol or drugs use before or during sexual intercourse in the previous 12 months was reported by 86.4% of participants. Objective 2: During follow-up 25 seroconversions were observed, yielding an overall incidence of 2.80/100 person-years (95% CI: 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 had a HIV positive steady partner at least once during follow-up and persistent UAI with occasional partners were predictors of seroconversion.

Conclusions: At a regional level the follow-up of this cohort of HIV-negative MSM will be a valuable tool for the dynamic monitoring of HIV infection in a Southern European setting where limited prospective information existed. Within the international context, it will additionally allow for a deeper analytical approach on population time trends and individual changes in risk factors shaping the epidemic among MSM. Given our sampling frame, full generalizability regarding the whole MSM population is not expected. However, since selection bias is not expected to change substantially throughout the study period, we believe that our methodological option will not preclude the estimation of time trends in incidence estimates or of the determinants of seroconversion. The first estimate of HIV incidence in Portuguese MSM yielded a higher incidence than in other countries revealing worrying HIV transmission. The present study also provides evidence that short-term contextual and behavioural changes during follow-up and serodiscordant relationships as well as persistent UAI with occasional partners are likely driving the HIV epidemic in this cohort of MSM.

Ultimately, study results are intended to inform on the real-world effectiveness of policies directed to reducing the burden of HIV among MSM.

1. INTRODUCTION

1.1. HIV EPIDEMIOLOGY

In 2012 it was estimated that 35.3 (32.2–38.8) million people were living with the human immunodeficiency virus (HIV) worldwide, an increase from previous years due to the increasing life expectancy of people living with the infection (1). At the same time the number of new HIV infections decreased from 3.4 (3.1–3.7) million in 2001 to 2.3 (1.9–2.7) in 2012 and the acquired immunodeficiency syndrome (AIDS) deaths decreased from a peak at 2.3 (2.1–2.6) million in 2005 to 1.6 (1.4–1.9) million in 2012 (figure 1) (1).

Figure 1: Estimated number of people living with HIV in 2012 and trends in the incidence of new infections from 2001 to 2012 by local region - data from UNAIDS 2013 report. Source: Maartens G. et al, 2014.

In Europe HIV infection still remains of major public health importance with an estimate of 1.5 million people living with the infection (2). In 2012, 131 202 new HIV infections were reported in 52 of the 53 countries of the WHO European Region, 29 381 of which were in the European Union and European Economic Area (EU/EEA) (2). Surveillance data estimated, for 2012, an overall incidence of 7.8 diagnoses per 100 000 population in the WHO European Region and 5.8 per 100 000 in the EU/EEA (2). The main modes of transmission varied by geographical area, illustrating the diversity of the epidemiology of HIV in Europe; heterosexual transmission was the main mode of transmission in the WHO European Region, while sexual transmission between men was the main mode in the EU/EEA and transmission due to unsafe injecting-drug use remained substantial in the Eastern region (2).

1.1.1. HIV epidemiology in MSM worldwide

Since the first cases of what is now known as AIDS were described in 1981 in 26 previously healthy homosexual men in New York and in California in the United States

(US) (3), men who have sex with men (MSM) have been a key population at higher risk for HIV infection.

In 2012, the prevalence of HIV among MSM appeared to have a slight increase globally (1). In Europe, the proportion of HIV diagnoses reported among MSM varied between from 1.2% in Eastern Europe to 41.7% in Western Europe (2). Trends by transmission mode showed that the number of HIV diagnoses among MSM have had a 2.5% annual increase from 2006 to 2011 in the EU/EEA region (2). This is of particular concern since the largest increase in new infections has been seen among younger MSM (4).

The increasing trends in new HIV cases in MSM have been described as a resurgent epidemic (5). Surveillance data from eight countries, with concentrated HIV epidemics and methodologically similar HIV case surveillance systems, Australia, Canada, France, Germany, Netherlands, Spain, United Kingdom (UK) and US, demonstrated a notably consistent pattern of changes in rates of HIV notifications among MSM in the past decade (6). After a decrease in reported cases from 1996 to 2000, a consistent increase was seen from 2000 to 2005 (6). In the US, between 2006 and 2009, the overall number of new HIV infections remained stable with the exception of MSM, in whom the estimated number of new infections increased significantly, mostly due to increases in HIV infection rates in young and black MSM (7). Similarly results from the Amsterdam Cohort Studies (ACS) showed an increase in HIV incidence in MSM from 1.0 per 100 person-years in 1992 to 2.0 per 100 person-years in 2009 (8). In low and middle income countries, MSM are also at much higher risk for HIV infection than non-MSM, with an estimated odds ratio of 10 in high prevalence countries and 60 in low prevalence countries (9).

These increasing rates of new HIV notifications among MSM are unlikely to be explained solely by changes in surveillance practices. Neither is it expected that changes in HIV testing patterns among MSM could be accountable for such trends (6). Therefore, after excluding the most probable sources of artificial trends, observed data point to a true increase in the risk of infection in this population. Such findings call for research into accurate data and up to date quantitative estimation of the burden of new infections as driving the current epidemic.

1.1.2. HIV epidemiology in MSM in Portugal

In Portugal, by the end of 2012, there were 42 580 HIV cases notified of which 31 255 (73.4%) were in men (10). The majority of cumulative cases were sexually transmitted,

43.4% of heterosexual transmission and 13.8% of sexual transmission between men. Unsafe use of injected drugs accounted for 37.8% of all HIV cases (10). Throughout the first 30 years of the HIV epidemic a change in the epidemiological patterns of transmission has been observed. Whereas the first 20 years most cases were related with unsafe injection practices, in the last decade heterosexual transmission became reportedly the major driver of the epidemic in Portugal (11). Nevertheless, an increase in the absolute number of notified cases related with sexual transmission between men as well as a decrease in median age of notified cases have been observed (11). Between 2005 and 2011 there was a 6% annual increase in the number of newly diagnosed HIV cases attributed to sexual transmission between men, while in the same period cases due to unsafe injection behaviours and heterosexual transmission decreased by 22% and 4% respectively (10). HIV cases notified in MSM tend to be in younger individuals, mostly those resident in Lisbon and those born in Portugal (11). The trends of increasing new cases in MSM may reflect not only higher transmission rates in this group but also earlier diagnosis which may result from increased self-perception of risk and higher uptake of HIV testing (11).

1.1.3. Implications of the current HIV epidemics in MSM

Many accomplishments in terms of scientific advances during the past three decades have been achieved. Still, from this portrayal of the HIV epidemic in MSM, it is clear that this group remains a key population at higher risk of HIV infection, posing a number of challenges for the different stakeholders in this field.

Surveillance data should be used within countries and across countries to help set priorities for prevention research and for allocation of resources. In fact data, suggests that MSM communities in North America, Western Europe, and Australia might share some important features that should be considered on HIV research and prevention efforts overcoming country-specific programs (1, 6).

The resurgent epidemics among MSM highlights the need for additional research to update estimates of the incidence of HIV globally and to identify the factors that currently influence transmission, including risk management and sexual network characteristics (5, 6).

1.2. HIV TESTING AND COUNSELLING

HIV testing and treatment remains a cornerstone of HIV prevention and management (12) with major individual and public health benefits. The advent of effective antiretroviral therapy (ART) has improved prognosis and life expectancy and reduced

AIDS-related deaths of persons infected with HIV. Widespread and early access to treatment may reduce HIV incidence by reducing viral load at the population level, although this has not yet been shown for MSM (13). Nevertheless, knowledge of one's HIV status has been associated with a reduction in risk behaviour in HIV-positive individuals (14). Early diagnosis is therefore key to ensure benefit from ART and to decrease HIV transmission.

In Europe the number of late presenters, defined as patients with a CD4 count below 350cell/µL at the time of diagnosis or ART initiation within 3 months of diagnosis (15), has decreased over time but remains a significant issue in all HIV exposure groups (16). Within the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) network in 2011, 53.8% of all persons and 43.7% of MSM presenting for care were late presenters (16). A previous research that studied testing practices among recently diagnosed HIV-positive persons from 4 European countries including Portugal found an overall proportion of late diagnosis of 41% (17). Portugal had a higher rate of late diagnosis estimated at 63%, when compared with Estonia (10%), Belgium (34%) and Finland (50%) (17). Also noteworthy is the finding that most important barriers to testing were centred on denial of risk, indicating the need for raising awareness about HIV and the benefits of testing (17).

Earlier and more widespread testing strategies targeting all populations at risk both within the health care system and as part of community-based programs are key in the response to HIV epidemics (18).

1.2.1. Guidelines for HIV testing and counselling

WHO guidelines for HIV testing and counselling (HTC) are that mandatory or compulsory testing is never appropriate. Exceptions to this however are screening for HIV and other blood-borne infections of all blood destined for transfusion or for manufacture of blood products and screening of donors prior to all procedures involving transfer of bodily fluids or body parts, such as artificial insemination, corneal grafts, and organ transplant (19).

HTC services, regardless of the model of delivery, should adhere to the five Cs: Consent, Confidentiality, Counselling, Correct test results and linkage to Care (20). For concentrated epidemics, HIV testing and counselling should not be recommended to all people attending all health facilities, but rather target all adults and children who present with signs and symptoms suggestive of underlying HIV infection and to children known to have been exposed to HIV in the perinatal period (21).

To increase the uptake of HIV testing at the European region level, the European Centre for Disease Control and Prevention (ECDC) has identified a number of core principles for national HIV testing strategies and programmes (22):

- HIV testing should be voluntary, confidential, and undertaken with informed consent;
- access to treatment, care, and prevention services should be ensured;
- governments need to make HIV testing a priority, which should be part of national strategies, developed and implemented with the participation of stakeholders;
- stigma must be reduced;
- legal and financial barriers have to be removed.

Only a few countries indicate any national guidance detailing how often MSM should be screened for sexually transmitted infections (STI) and HIV, while a few others, indicate that guidelines are under development (23). European guidelines regarding frequency of HIV/STI screening for MSM are lacking, although risk-taking behaviours in MSM and the need to offer an increased screening frequency for these men seems to be recognized (23). A clearer national and pan-European guidance that provides a framework for risk profiling and screening intervals and more coherent and explicit guidelines on screening of MSM would be helpful for less experienced healthcare professionals and for new providers that are emerging and who may benefit from guidelines that require less interpretation (23).

In Portugal HIV testing is non-mandatory, voluntary, confidential, and undertaken under informed consent (24). HIV testing is recommended to all patients whose clinical presentation may result from an underlying HIV infection, and more selectively in certain subpopulations that includes sexual partners of persons with a HIV diagnosis, MSM and female partners of MSM amongst other key populations (24). In the case of MSM annual testing is recommended, however frequency can be increased if an acute HIV infection is suspected or in case of high risk exposure to HIV (24).

1.2.2. Type of HIV tests

In 1985 the first HIV tests were introduced based on enzyme immunoassay (EIA) techniques and, shortly after, Western blot was approved to confirm HIV infection (25). EIAs were originally developed to screen blood for HIV and have evolved to detect HIV earlier during the course of infection. First and second generation EIAs detect IgG antibodies against HIV-1. Third generation EIAs use “antigen sandwich” techniques

that can also detect IgM antibodies against HIV-1, which develop earlier after infection (25). Fourth generation combination EIAs identify HIV infection even earlier because these simultaneously detect HIV antibody and p24 antigen (25, 26).

Rapid diagnostic HIV tests are single-use EIAs that contain all necessary reagents and that yield results in less than 30 minutes (25). Because rapid tests have sensitivities and specificities similar to those of conventional EIAs, a negative result of a rapid test is conclusive and generally requires no further confirmatory testing. A reactive rapid test result requires further testing to confirm the diagnosis, usually with Western blot or indirect immunofluorescence assay (25, 27). The widespread adoption of rapid antibody tests has many advantages. Rapid HIV tests make it possible to perform the test in a timely manner, without the need for a return visit, thereby substantially increasing the number of persons who receive their test results (28) and who have access to effective treatment earlier (25). Rapid HIV tests are also essential when immediate results are necessary to make decisions about treatment (25). As rapid HIV antibody tests are easy-to-use they are feasible in a variety of clinical and nonclinical settings playing an important role in expanding access to testing (25).

Point-of-care (POC) tests do not have a universally accepted definition but they are often described as any test designed to be used directly at the site of patient care providing a rapid result to guide clinical decisions and follow-up during the same encounter (29, 30). POC tests have been used in genitourinary clinics, outreach settings, community clinics, sex venues for MSM, and in needle and syringe exchange facilities. POC tests can also be used in cases of women at high risk presenting late in pregnancy or delivery, or in case of a non-occupational post-exposure prophylaxis (PEP) for recipient and possible index subjects (31).

In order to be used in such settings the abovementioned POC tests have to meet specific criteria including high sensitivity and specificity (>99%) (31). These tests should also be reproducible, and be easy to learn, perform and read, as well as give accurate results and be as non-invasive as possible (31). Other important issues are storage conditions, such as refrigeration, and quality assurance of both the test kits used and procedures (31). Beyond the technical advantages of rapid tests mentioned above, other advantages have been described, such as high acceptability by staff and preference by many patients, and confirmed cost effectiveness in many situations (31). These tests also have the potential to overcome many of the barriers that patients experience in HIV testing, such as a fear of venepuncture, anxiety between the time of the test and the time of the result, the inconvenience of having to return for results,

stigma, and confidentiality issues (31, 32). These tests have also been described as critically improve access to HIV testing by allowing more individuals to know their HIV status (29).

1.2.3. HIV testing and counselling services

HTC services are the gateway for HIV treatment and care, as well as for prevention at the community level. Robust HTC services that are acceptable, accessible, and provide successful linkage to prevention and care services, are essential for a successful public health response to HIV.

HTC services are mainly available through two delivery models - facility-based, (whether in clinical settings or in voluntary counselling and testing (VCT) sites), and community-based (20).

The WHO defines VCT as HTC services in sites besides health-care facilities that have some features that make them similar to community-based services (20). VCTs are outside clinical settings and are usually situated in the community, and are thus accessible to their target populations, often offering comprehensive counselling, same-day results, staffing, and linkages to care or prevention services (20). Nevertheless, because of their potential for biased coverage, client-initiated approaches should not be relied upon as the single strategy for offering HIV tests, but should rather be part of an integrated effort to promote increasing levels of HIV status awareness in the population (20).

Community-based HTC as defined by the WHO are services that are home-based, door-to-door, index-case, mobile and outreach, workplace, and school-based (20). This definition leaves out those VCT services that are situated in the community and directed to a specific key population.

A different perspective regarding community-based HTC came from a recent European project aimed at promoting early diagnosis of HIV infection in Europe, by improving the implementation and evaluation of community-based testing practices. This group has come to a widely accepted definition of community-based HTC, namely defining it as: ‘any program or service that offers HIV counselling and testing on a voluntary basis outside formal health facilities and that has been designed to target specific groups of the population most at risk and is clearly adapted for and accessible to those communities. Moreover, these services should ensure the active participation of the community with the involvement of community representatives either in planning or implementing HIV testing interventions and strategies (33).

The WHO nonetheless has recognized the need for a rapid scaling up of innovative models of HTC beyond health-care-facility based approaches (34). The provision of HIV testing and counselling using community-based approaches can improve the equality of access and greater uptake of services, while simultaneously contributing to the reduction of stigma and discrimination (20, 21).

A systematic review assessing feasibility, acceptability, and effectiveness of HIV testing in community settings in resource-rich countries, found that the most commonly targeted group were MSM (35). MSM clients prefer non-clinical settings, non-invasive methods, receiving results faster, supportive and non-judgmental services (35, 36). Aspects regarding anonymity, confidentiality and free-of-charge tests were also cited as important in choosing to test for HIV (35). However, issues have been raised about possible breaches in confidentiality, as well as stigma and the ability of community services to provide a high professional standard of care (35). Among MSM there was concern that providing adequate post-test counselling would be difficult in community settings such as bars and clubs. Staff training was highlighted as an important component of community testing as it increased the levels of comfort about both the testing process and the provision of results in this setting (35).

In summary, HTC services are crucial to improve access to HIV-related care and prevention services, and to provide opportunities for people to reduce their risk of acquiring or transmitting HIV (34). The WHO recommends a strategic mix of service delivery models to achieve universal and equitable access to HTC, including expanded community-based options and innovated strategies reaching facilities, strong linkages to prevention, care, and treatment, and support services after testing and the use of new HIV testing strategies in high and low prevalence epidemics to assure accurate and valid test results (20).

1.2.3.1. The CheckpointLX model

CheckpointLX is a community-based centre for anonymous and free rapid HIV testing and counselling directed at MSM. Testing and counselling is provided by trained peer-counsellors (37). It is a project from GAT – *Grupo Português de Activistas sobre Tratamentos de VIH/SIDA*, a Portuguese non-governmental organization (NGO) advocating legal and political changes that can have a positive effect on the rights and quality of life of those living with HIV, or those most at risk of acquiring the infection (38).

The centre was established in April 2011 aiming to:

1. Perform free, anonymous, or confidential HIV screening with rapid tests and counselling especially directed to MSM in a peer-based approach;
2. Early detection of HIV infection in MSM and assurance of effective linkages to care to the National Health Service;
3. Providing specific information for MSM about sexual health and HIV, or other sexually transmitted infections;
4. Produce and disseminate information materials on HIV or other STI prevention;
5. Contribute to epidemiological and behavioural knowledge regarding MSM in Portugal by collecting anonymized data.

CheckpointLX is located at a Lesbian Gay Bisexual Transgender socializing quarter, it is publicized in MSM socializing sites such as bars, discos, saunas, sex shops and guesthouses, at parties and events of the MSM community, at cruising areas and online social networks. The location and the shop-front style promote walk-ins (figure 2). Promotion materials include flyers, videos, stickers, banners at online social networks and prevention kits containing condoms, lubricant and an information card about CheckpointLX.

Figure 2: CheckpointLX shop-front style.

Although CheckpointLX is intentionally devoted to MSM, the centre also receives any individual who may seek for the services offered. These services, although more limited than those available for MSM, may include HIV testing, and pre- and post-test counselling.

MSM, when coming to CheckpointLX, find a team of trained peer counsellors that provide pre- and post-test counselling and who perform HIV and syphilis rapid tests. A protocol for the referral of individuals who have a reactive test for HIV was made with a HIV/Infectious diseases clinic in a central hospital in Lisbon (*Hospital Santo António dos Capuchos*). In case of a reactive test for syphilis, an internal referral is made to a parallel STI clinic service called “Checklist”. It consists of a medical appointment for MSM, addressing sexual health and screening for other STI.

1.3. COHORT STUDIES IN HIV-NEGATIVE MSM

1.3.1. Basic concepts of cohort studies

Cohort studies are observational investigations that can be thought of as natural experiments in which outcomes are measured in real world settings. These cohort studies have been used since the 19th century as a powerful tool to study determinants of diseases (39, 40). In a cohort study, members of a defined population are characterized in terms of relevant risk factors or exposures, placed under observation, and followed for some time until the disease either develops or not. This design allows for the evaluation of both the risk and the rate of disease or disease-related outcomes (39). Cohort studies can be classified in two major categories depending on the decision to go forward in time from the present or to go back in time to assemble the study sample. The first category being concurrent or prospective cohorts, where the study is done by going ahead in time from the present, and the second category being non-concurrent or retrospective cohorts where the study goes back in time to constitute the cohort and follow them up to the present (39, 41). A cohort study is the best way to accurately estimate incidence and to characterize the natural history of a disease, and can be used to examine multiple outcomes after a single exposure (41). Despite disadvantages regarding higher cost and complexity, cohort studies remain of vital importance to public health research (39, 40).

1.3.2. Cohort studies among HIV-negative MSM

In the context of the HIV epidemic in MSM, several cohort studies have been implemented since 1983. A summary of the main characteristics of these cohort studies using a prospective design that estimated HIV incidence in MSM who were HIV-negative at the time of recruitment are presented in table 1.

Cohort studies are now implemented in a variety of contexts from high income to low and middle income countries, and are being conducted using several approaches in recruitment strategies and settings.

Cohort studies have the potential to deliver important and valuable a most valuable public health knowledge (42). In fact, most established knowledge related with HIV transmission and pathogenesis has originated from several cohorts targeting key populations at higher risk of HIV infection (5). These findings have significantly improved our understanding of HIV epidemiology and HIV pathogenesis, by characterizing risk factors associated with disease acquisition and progression, and the effects of therapy (5, 43). Many of these results have guided public health policies (43).

Table 1: Summary of main characteristics of cohort studies using a prospective design with HIV incidence estimates for MSM.

COHORT NAME DESIGN SPECIFICITIES	CITY, COUNTRY	N TOTAL PERSON-YEARS OF FOLLOW-UP	PERIOD OF DATA COLLECTION	MAIN OBJECTIVES	RECRUITMENT STRATEGIES	SETTING	FOLLOW-UP FREQUENCY MEASUREMENTS	HIV INCIDENCE
EUROPE								
Amsterdam Cohort Studies (ACS) – Protocol 2 (8, 44, 45) Open cohort	Amsterdam, The Netherlands	1642 MSM (with at least 2 visits to December 2009) 11 223 PY	October 1984-present	1. Investigate prevalence, incidence and risk factors of HIV-1 infection and AIDS 2. Investigate natural history and pathogenesis of HIV-1 infection 3. Investigate the effects of interventions	1. 'Convenience sampling' (brochures/advertisements in the STI clinic and other MSM meeting places) 2. 'Chain referral sampling'	The Public Health Service	3- to 6-monthly follow-up 1. Epidemiological and social questionnaires 2. Physical examination 3. Virological and immunological tests and cell storage (selected groups)	2.0 (1.0-3.7) per 100 PY in 2009
Rotterdam MSM-Cohort study (46, 47) 3-year cohort	Rotterdam, The Netherlands	286 MSM aged 18 to 75 190 MSM followed for 3 years	Recruitment between February 1999 and February 2000 – end of follow-up in 2003	1. Monitor the cumulative incidence of STDs and HIV infection in MSM 2. Investigate longitudinal behavioural changes 3. Monitor the increase in both HIV infection and STDs together with behavioural determinants 4. Study whether perceived susceptibility to and severity of infection with HIV and STDs, as well as knowledge of HIV infection and STDs, were associated with incident HIV infection and STDs	1. At STD clinics, gay bars and saunas 2. Advertisements in newspapers and gay periodicals	STD clinic of the Department of Dermatology and Venereology, Erasmus University Medical Centre	6-monthly follow-up 1. Medical history 2. Demographic and sexual information 3. Self-administered questionnaire on potential psychological predictors 4. Laboratory testing for HIV, syphilis, hepatitis B, Chlamydia and genital herpes	3.2% (cumulative incidence) over 3 years
(48)	Amsterdam, The Netherlands	770 homosexual men 675 injecting drug abusers	1984/1985 to 1995	1. Assess the HIV epidemic in this high-risk groups	Not available	Not available	Not available	1.0 per 100 PY in 1995 (homosexual

								men)
(49)	Amsterdam, The Netherlands	429 young MSM	1995-October 1996	1. Surveillance of HIV prevalence and incidence 2. Surveillance of sexual risk behaviour among young homosexual men	Not available	Municipal Health Service	1. Sexual behaviour questionnaire 2. Laboratory determination of HIV, hepatitis B and syphilis antibodies	1.0% per year
NORTH AMERICA								
The Multicenter AIDS Cohort Study (MACS) (43, 50) Ongoing and multicentre cohort	Los Angeles, Chicago, Baltimore and Pittsburgh, in the United States	As of May 2011: 6972 MSM (3501 HIV-positive of who 617 incident) 86.883 PY of follow-up	Initiated in 1983 Recruitment: April 1984 to March 1985 (4954 MSM) April 1987 to September 1991 (668 MSM) October 2001 to August 2003 (1350 MSM)	1. Elucidate the natural history of the infection causing AIDS 2. Identify risk factors for occurrence and clinical expression of the infection 3. Establish a repository of biologic specimens for future study	1. Media publicity 2. Personal connections of both the gay activists and early participants in the study 3. previous clinical or research contacts	UCLA Northwestern University in Chicago University of Pittsburgh Johns Hopkins University in Baltimore	6-monthly follow-up 1. Questionnaire about medical history, behaviour, quality of life, depression, activities of daily living and medications. 2. Neuropsychological screening. 3. Laboratory testing for HIV antibody (only HIV-negative MSM), T-cell subsets and viral load. 4. Storage of cells, serum, plasma and Epstein-Barr virus-transformed B-cell lines and peripheral blood mononuclear cell pellets.	Cumulative incidence of 11.3% as up 1989 (51)
The San Francisco Cohort Study (52, 53) 6-year cohort	San Francisco, US	6875 homosexual and bisexual men who had sought evaluation for STD (397 had follow-up assessment of whom 360 were seronegative)	Recruitment between 1978 and 1980, end of follow-up 1984	1. Study the prevalence, incidence and prevention of hepatitis B virus infection 2. Determine the incidence and prevalence of the AIDS, related conditions, and infection with the human T-lymphotropic virus, type III/lymphadenopathy-associated virus (HTLV-III/LAV).	Convenience sampling	San Francisco City Clinic	6- year follow-up 1. Blood specimens collected and tested for HTLV-III/LAV after	Cumulative incidence of AIDS of 4.8% in men over 35 years and 2.2% in younger men. Cumulative incidence of HTLV-III/LAV of 66.4%.
The San Francisco Men's Health Study (54, 55)	San Francisco, US	1034 single men aged between 25 to 54 years 411 were HIV-negative and homo/bisexual	Recruitment between June 1984 and January 1985, end of follow-up December 1987	1. Elucidate epidemiology and natural history of AIDS	Multistage stratified cluster sampling	Study clinic University of California Survey Research Centre	Semi-annually follow-up 1. Intensive interview on demographics and behaviour 2. Physical examination 3. Laboratory testing for HIV	5.9 (2.8-9.0) per 100 PY in the first six months of 1985 to 0.7 per 100 PY (0.002-3.8) in

								the last six months of 1987
(56) 12-month cohort study	Seattle, Washington, US	578 MSM, aged 18 or older and who have engaged in anal sex in the previous year 384.62 PY of follow-up	1995 to 1996	1. Ascertain the prevalence of STD and incidence of HIV-1 and STD 2. Determine risk factors acquiring STD, including HIV-1	Variety of outreach methods	Not available	6-monthly follow-up 1. HIV/STD risk reduction counselling 2. Standardized interviews conducted by trained interviewers on demographics and behavioural 3. Laboratory testing at baseline for HIV, other STI and hepatitis B 4. STD physical examination	1.3 per 100 PY
EXPLORE Study (57-59) Longitudinal randomized behavioural intervention trial	Boston, Chicago, Denver, New York, San Francisco and Seattle, US	4295 MSM aged 16 or older and who reported having engaged in anal sex with 1 or more men in the previous year	Recruitment between January 1999 and February 2001 End of follow-up 2003	1. Test a behavioural intervention in preventing acquisition of HIV infection among MSM in the United States. 2. Estimate prevalence of risk behaviours	1. Advertising 2. Street and gay socializing outreach 3. Referrals from other cohort studies, current study participants, community agencies and clinics 4. Internet sites targeting MSM, community forums, mailings 5. Recruitment video	Not available	6-monthly follow-up 1. Standardized questionnaires on demographics, reasons for participating in the study, history of sexually transmitted diseases, use of post-exposure prophylaxis, and histories of counselling and psychotherapy. 2. Audio computer-assisted self-interviewing (ACASI) on attitudes toward safer sex, social activities within the gay community, depression, alcohol and drug use, and sexual behaviours. 3. HIV pre-test counselling 4. Laboratory testing for HIV antibody for herpes simplex virus-2 antibody. Screening for urethral and rectal gonorrhoea 2 weeks after screening post-test counselling to receive HIV test result	2.1 (1.9-2.4) per 100 PY
The BROTHERS Study – HTPN 061 (60) Multi-site cohort	Atlanta, Boston Los Angeles, New York City, San Francisco and Washington, DC, US	1553 enrolled black MSM aged 18 or older reporting UAI with a men in the past 6 months and 1164 followed-up 926 PY of follow-up	Recruitment: July 2009 to October 2010 End of follow-up: December 2011	1. Evaluate feasibility and acceptability of a multi-component HIV prevention intervention for Black MSM 2. Prepare for a community-level randomized trial to test the efficacy of the intervention in reducing HIV incidence among black MSM	1. Directly from the community or as sexual network partners referred by index participants. 2. Community outreach, engagement of key informants, local community-based groups 3. Print advertising 4. Online strategies	Not available	1. Interview administered questionnaire on demographics and health-care related information 2. ACASI questionnaire on behaviours, perceived racism and sexual discrimination, internalised homophobia, depression, social support and internalised HIV stigma 3. HIV rapid testing and pre-test risk reduction counselling 4. Laboratory testing for HIV reactive tests confirmation and for gonorrhoea, chlamydia and syphilis	3.0 (2.0-4.4) per 100 PY
The Vanguard Project (61-63)	Vancouver, Canada	1024 self-identified gay and bisexual men aged 15 to 30 years For HIV incidence estimates 674 men were considered	May, 1995 to March 2002 (last update)	1. Determine whether social determinants were independent predictors of high-	1. Physician's offices, clinics 2. Outreach	Clinics, Community and Health Centres	6-monthly follow-up 1. Self-administered questionnaire on demographics, sexual behaviours and substance use 2. HIV testing	1.9 (1.3–2.5) per 100 PY as of December 2001

		1894.34 PY of follow-up		risk sexual behaviour 2. Identify sociocultural and transmission factors associated with seroconversion				
Omega Cohort study (64, 65) Open cohort	Montreal, Canada	1890 MSM aged 16 or older who have had sex with another men in the preceding year recruited, 1846 enrolled and 1587 5121 PY of follow-up	October 1996 to July 2003	1. Estimate HIV incidence and identify factors associated with seroconversion among MSM (particularly men of less than 30 years of age) recruited mainly outside clinical settings of Montreal; 2. Characterize changes in sexual behaviours over time; 3. Identify psychosociosexual factors associated with each stage of sexual behaviour in a quantitative perspective; 4. Facilitate the transfer of knowledge to community groups involved in HIV prevention	Bilingual publicity campaign carried through the general and gay press before recruitment	1. Community organization Centre des gais et lesbiennes de Montréal 2. Three private medical clinics serving a large MSM clientele 3. Community health clinic	6-monthly follow-up 1. Self-administered and interview-administered questionnaires on demographics, sexual behaviours and psychosocial data 2. Pre-test counselling 3. Laboratory testing of HIV, hepatitis B (HBV) and syphilis 4. Three weeks after the first appointment, participants return to receive their test results and post-test counselling.	0.62 per 100 PY (0.41-0.84)
The Vancouver Lymphadenopathy - AIDS Study (66)	Vancouver, Canada	726 homosexual men of whom 345 were seronegative	November 1982 through October 1985	1. Report on the epidemiologic features of HIV seroconversion in this population	At 6 primary care practices	Primary care practices	6-monthly follow-up 1. Administered questionnaire 2. Physical examination 3. Immunologic and HIV antibody testing	19% (crude cumulative incidence)
AUSTRALIA								
Health in Men Study (67-70) Open community-based cohort	Sydney, Australia	1427 gay men who reported having sex with other men within the 5 years before enrolment in the study living in Sidney or regularly participating in the gay	July 2001 to December 2004 18-month extension of the study starting from	1. Establish a baseline of risk practice among potential vaccine trial participants	1. Gay venues, events and organisations 2. Snowballing' through friends of participants 3.Referrals from earlier	Not available	1. Annual face-to-face interviews and semi-annual telephone interviews between annual visits on demographics, sexuality and identity, sexual behaviour, gay community involvement, contact with HIV epidemics,	0.87 (0.65–1.14) per 100 PY

		community in Sidney	January 2006	2. Monitor risk practice and HIV incidence in the context of vaccine initiatives 3. Inform community-based organisations and other agencies engaged in health promotion about men's current understandings of vaccine initiatives	studies and medical practitioners 4. Via magazine advertisements and websites		health, drug use, discrimination, HIV vaccines attitudes and understandings 2. Annual testing for HIV	
LATIN AMERICA								
Project *Horizonte* (71) Multicentre open cohort	Minas Gerais - Belo Horizonte, Brazil	470 sexually active homosexual and bisexual men aged 18 and 59 years 10866 PY of follow up	October 1994 to May 1999	1. Evaluation of seroincidence of HIV 2. Ascertain the role of counselling on behaviour modification 3. Assess willingness to participate in future HIV vaccine trials	1. Pamphlets, notices in community newspapers, radio, and television 2. At anonymous testing centres 3. By word of mouth	Infectious and Parasitic Diseases Service of the *Universidade Federal de Minas Gerais*	1. Standardized form 2. Face-to-face core interview on risk behaviour for HIV infection and knowledge about HIV/AIDS and HIV vaccines 3. Standardized clinical interview 4. Physical examination 5. Laboratory testing for HIV and cell storage	1.99 (1.18-3.14) per 100 PY for 48 months of follow-up
Project Rio (72-74) Multicentre open cohort	Rio de Janeiro, Brazil	647 homosexual or bisexual men ranging in age from 18 to 50 years 631 PY of follow-up	January 1994 to December 1998		1. At Municipal health centres 2. Public information 3. Community activities 4. Volunteers (snowball)	*Servidores do Estado Hospital* and a sexually transmitted diseases clinic, Fluminense Federal University	1. Initial visit form 2. Psychological profile 3. Social behavioural questionnaire 4. Physical examination 5. Laboratory testing for HIV, syphilis and hepatitis	3.33 (1.93-4.67) per 100 PY
Project *Bela Vista* (75) Multicentre open cohort	São Paulo, Brazil	MSM aged 18 or older 50 years who have not used drugs in the previous 6 months to recruitment	August 1994		1. Pamphlets, notices in community newspapers, radio, and television 2. At anonymous testing centres 3. By word of mouth	*Instituto Adolfo Lutz* and AIDS and STD state programme	1. Socio-behavioural interview 2. Physical examination 3. Laboratory testing for HIV, syphilis and hepatitis	Not available
Project *Praça Onze* (76)	Rio de Janeiro, Brazil	753 men with homosexual behaviour aged between 18 and 50 years Mean follow-up of 1.5 years	July 1995 to May 1997	1. Identify a high-risk population for prevention trials 2. Determine suitability of this population for vaccine and non-vaccine intervention studies	At HIV testing sites and places frequented by gay men	*Hospital Escola São Francisco de Assis,* a teaching hospital of the Federal University of Rio Janeiro	6-monthly follow-up 1. Extensive face-to-face interview on risk behaviours, signs and symptoms of acute HIV infection and STD, and attitudes about participating in a HIV vaccine trial 2. Physical examination 3. Laboratory testing for HIV, other STI and Hepatitis	3.1 (2.1-4.1) per 100 PY

The Buenos Aires Cohort (77, 78)	Buenos Aires, Argentina	327 MSM who did not report injecting drug use in the previous year	February 2003 to December 2004	1. Assess the prevalence, incidence, risk factors, and molecular genotyping of HIV-1	At NEXO's through a convenience sample	NEXO Asociación Civil a large, gay-supporting NGO	6- and 12-monthly follow-up 1. Sociodemographic, sexual risk behaviour data 2. Laboratory HIV testing 3. Pre and post-testing counselling. Returning 2 week later to receive serological results	3.9 (2.0-6.7) per 100 PY
The ALASKA Cohort (79) Prospective cohort and a nested case-control study	Lima, Peru	1056 high-risk MSM aged 18 or older 971 PY follow-up	October 1998 to May 2000	1. Prospectively evaluate both HIV and ulcerative STI incidence estimates 2. Evaluate its behavioural and biological correlates	1. Snowball techniques 2. Outreach by peer educators	At five "gay-friendly" primary health care public clinics	6-monthly follow-up 1. Counsellor driven standardized clinical, demographics and behavioural interview 2. Risk reduction counselling 3. Laboratory testing for HIV, syphilis and other STI.	3.5 (2.3-4.7) per 100 PY
AFRICA								
(80) Pilot cohort	Dakar, Senegal	119 MSM who reported having anal sex with a man in the previous 12 months, aged 18 or older, and members of one of the known MSM organizations in Dakar and lived there for at least six months Followed for 15 months	June 2011 to October 2012	1. Assess the feasibility of implementing and retaining participants in a community-driven HIV prevention study in Senegal. 2. Describe the study participants in terms of HIV and STI prevalence and incidence, risk behaviours and indicators of social capital at baseline	Conducted by members of a community-based organization	Not available	1. Structured survey on demographics and behavioural 2. Medical examination 3. Laboratory testing for HIV, syphilis and hepatitis B virus at two time points	16.0 (4.6- 27.4) per 100 PY
ASIA								
(81)	Bangkok, Thailand	1744 Men aged 18 or older, Thai national, Bangkok residents who had penetrative male-to-male sex in the previous six months	April 2006 and December 2011	1. Assess HIV-prevalence, incidence and risk factors	1. At HIV testing services and, entertainment venues (bars, discos, saunas) 2. Internet 3. By word of mouth	A dedicated study clinic in a central Bangkok hospital	4-monthly follow-up 1. ACASI on demographic and behavioural data 2. Pre- and post-test HIV and risk behaviour counselling 3. Laboratory testing for hepatitis, anti-herpes simplex virus type-1 and 2, syphilis, *N. gonorrhoea* and *C. trachomatis* and drug use	5.9 (5.2-6.8) per 100 PY
(82) 12 month cohort	Shenyang, China	218 MSM aged 18 or older who reported at least one male sexual partner 111 PY of follow-up	August and September 2006	1. Analyse incident rates of HIV and syphilis 2. Clarify the dynamics of the 2 epidemics 3. Provide rationale	1. At a non-governmental organization (NGO) 2.Referral from MSM peers	Hospital of China Medical University	12 month follow-up 1. Interview-administered questionnaires on demographics, sexual behaviour, drug use and history of STD 2. Laboratory testing for HIV and Syphilis	5.4 (2.0-11.3) per 100 PY

				for building interventions				
(83) 12 month cohort	Beijing, China	507 sexually active HIV-negative MSM aged 18 or older	November 2006 to February 2007	1. Assess correlates of the incident infections for HIV, syphilis and hepatitis B virus	1. Website advertisement 2. Peer recruiters 3. Peer referrals	District HIV testing and counselling clinic	6- and 12-month follow-up 1. HIV-STD risk assessment interview administered by trained health professionals on sociodemographic and behavioural information and history of HIV-related prevention, counselling and testing 2. Client-centred pre-testing counselling and risk reduction on HIV/syphilis, HBV and other STD 3. Testing for HIV, syphilis and HBV 4. Post-test counselling when returning for test results	2.6 (1.1-4.1) per 100 PY
(84) 6 month cohort	Nanjing, China	397 MSM HIV-seronegative aged 18 or older who have engaged in anal or oral sex with a men in the previous 12 months 136.81 PY of follow-up	May to July 2007	1. Estimate HIV incidence 2. Investigate the mediating effects of same sex-related risk factors in explaining the relationships between particular risk factors and seroconversion	Respondent driven sampling	At a HIV clinic at the Jiangsu Provincial Central for Disease Control and Prevention	6-monthly follow-up 1. In-person interviews on sociodemographic, sexual behaviour information and history of STD 2. Volunteer counselling and rapid testing for HIV and syphilis	5.12 (1.33-8.91) per 100 PY
(85) 12 month cohort	Beijing, China	797 MSM aged 18 or older who have had anal or oral sex with men in the previous 6 months 592.98 PY of follow-up	August to December 2009	1. Assess the HIV, herpes simplex virus-2 and syphilis incidence rate among MSM 2. Identify sociodemographic and behavioural risk factors of HIV seroconversion among MSM	1. Website advertisements by a NGO AIDS volunteer group 2. Distribution of flyers with study-related information at MSM- frequented venues by trained peer recruiters 3. Referral from peers study participants		6- and 12 month follow-up 1. Socio-demographic and sexual behaviour questionnaire-based interviews 2. HIV voluntary and counselling testing 3. Clinical STD examination 4. Laboratory testing for HIV, syphilis and herpes simplex virus-2 available after one week	8.09 (6.92-9.26) per 100 PY
(86) 12 month cohort	Beijing, Shanghai, Kunming, Guiyang, Chongqing, Chengdu, Urumqi and Nanning, China	1102 young MSM aged 18 to 25 years who had anal or oral sex with men in the previous 6 months 1168.40 PY follow-up	August 2009 to December 2010	1. Assess the HIV incidence rate 2. Identify risk factors for HIV infection	Snowball sampling	Local Centres for Disease Control and Prevention	6-monthly follow-up with 878 YMSM and 12-month follow-up with 902 YMSM 1. Structured questionnaire-based performed by health workers on sociodemographic and behavioural information and knowledge related to HIV 2. Laboratory testing for HIV and syphilis infections	Overall - 6.7 per 100 PY Guiyang - 18.9 per 100 PY Beijing - 10.6 per 100 PY Shanghai - 5.6 per 100 PY Kunming - 5.3 per 100 PY Chongqing - 4.9 per 100 PY

								Nanning - 4.8 per 100 PY Urumqi - 4.3 per 100 PY Chengdu - 3.9 per 100 PY
(87) 18 month cohort	Kunming capital city of Yunnan Province, China	378 aged 18 or older who report receptive and/or insertive anal sex in the past 12 months 312.10 PY of follow-up	June 2009 and March 2011	1. Evaluate the current HIV incidence 2. Evaluate the correlation between HIV infection and factors including nationalities, syphilis, illicit drug use behaviours etc., and HIV subtypes and primary drug resistance of seroconverted MSM	Approached from NGO staff at various venues	Local MSM NGO	3-monthly follow-up 1. Interviewer-administrated questionnaires on demographics, sexual behaviour, illicit drug use and other HIV related factors 2. Laboratory testing for syphilis and HIV	3.5 (1.8-6.2) per 100 PY

2. AIMS

The aim of this thesis is to describe the ongoing Lisbon MSM cohort by providing the first estimates of HIV incidence in MSM in Portugal through the following objectives:

1. To describe the setup and design of the Lisbon cohort of MSM and to provide the descriptive characteristics at baseline for the participants recruited between April 2011 and February 2014.

2. To estimate incidence of HIV infection in a cohort of MSM and to assess how changes in risk factors over time predict HIV seroconversion.

3. CHAPTER I

The Lisbon Cohort of Men who have Sex with Men– Study protocol

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Abstract

Background: In recent years, sex between men is gaining special relevance as transmission mode of HIV infection in Portugal. Surveillance tools that allow for a better description of the distribution and determinants of HIV infection in men who have sex with men (MSM) are essential. We set up an open prospective cohort study, The Lisbon Cohort of MSM, at a community-based voluntary HIV counselling and testing service targeting the MSM population. The main goal of this cohort study is to dynamically monitor the burden of disease and its predictors. The cohort is also expected to provide important information to improve the provision of HIV testing and to ensure timely linkage to care. In this paper, we aimed to describe the design and methodological options of the study, as well as the descriptive characteristics at cohort entry of participants enrolled between April 2011 and February 2014.

Methods/Design: The Lisbon Cohort of MSM, established in April 2011, is an observational prospective study conducted at a community-based voluntary HIV counselling and testing centre in Lisbon, Portugal (CheckpointLX). Men testing negative, aged 18 or older who report having had sex with other men are invited to participate at follow-up visits scheduled according to their convenience. At each evaluation a structured questionnaire is applied by trained peer counsellors and HIV and syphilis rapid tests are performed. From April 2011 to February 2014, 3106 MSM were eligible to enter the cohort of whom 923 (29.7%) refused to participate. The remaining 2183 (70.3%) individuals were enrolled and 804 had at least one follow-up evaluation, for a total of 893 person-years of observation. HIV testing prior to cohort entry was reported by 82.3% of participants, the most common reasons for the index HIV test were: to check health status/routine (81.3%), perception of exposure to HIV more than 3 months before (50.5%) and in the previous 3 months (40.7%). Twelve percent of MSM reported sexual intercourse with HIV positive men in the previous 12 months. Approximately eight percent of those who reported a steady relationship had a HIV positive partner, of whom 43.7% reported inconsistent condom use. That proportion was 71.0% among those unaware of their steady partner's HIV status. Twenty one percent of men who had at least one occasional partner reported no condom use in the last sexual encounter and 46.4% reported inconsistent use in the previous 12 months. The most referred reason for engaging in unprotected anal intercourse was a steady partner (66.2%). Alcohol or drugs use before or during sexual intercourse in the previous 12 months was reported by 86.4% of participants.

Discussion: Regionally, the follow-up of this cohort of HIV-negative MSM will be a valuable tool for the dynamic monitoring of HIV infection in a Southern European setting where limited prospective information existed. Within the international context, it will additionally allow for a deeper analytical approach on population time trends and individual changes in risk factors shaping the epidemic among MSM. Given our sampling frame, full generalizability regarding the whole MSM population is not expected. However, since selection bias is not expected to change substantially throughout the study period, we believe that our methodological option will not preclude the estimation of time trends in incidence estimates or of the determinants of seroconversion. Ultimately, study results are intended to inform on the real-world effectiveness of policies directed to reducing the burden of HIV among MSM.

Key-words: HIV; Men who have sex with men; Cohort studies; Incidence.

Background

Since the beginning of the HIV/AIDS epidemic in the early 80s, gay, bisexual and other men who have sex with men (MSM) have been a core population affected by the disease, but also key contributors to the response to it [1, 2]. During the past three decades, significant scientific advances and societal efforts in the fields of prevention, treatment, care and support have renewed the hope in achieving an AIDS-free generation. However, in many high income countries where a decline in overall HIV incidence has been observed, a concurrent increase in the number of new cases among MSM has been documented [3], namely in the European Union /European Economic Area, where the largest increase in new infections in the last decade was observed among young MSM [4].

In Portugal, HIV/AIDS followed a concentrated epidemic pattern, where the prevalence of HIV in the general population is estimated around 0.5%, but it is over 5% in some key populations at such as MSM, people who inject drugs, prisoners, and commercial sex workers [5]. Among MSM, the prevalence of self-reported infection was estimated at 10.9% in the European MSM Internet Survey (EMIS) [6]. This was very similar to the 10.3% estimate found in a previous interview survey (S Dias, personal communication).

Portuguese official surveillance data show a 6% annual increase in the number of newly-diagnosed HIV cases among MSM from 2005 to 2011, while cases due to unsafe injection behaviour and heterosexual intercourse decreased by 22% and by 4% respectively, in the same period [7]. In 2012, sex between men accounted for 34.1% of all HIV cases reported in men, and 24.1% of all cases. Hence, there is an urgency to establish dynamic instruments to monitor HIV incidence and determinants in this population if, in fact, we want to succeed in the response to HIV among MSM [1].

HIV surveillance must be tailored to the state of the epidemic in each setting and this includes the promotion of decentralized surveillance tools that are capable of capturing HIV trends and behavioural changes in a more timely and analytical fashion than national surveillance systems, which are necessarily heavier structures with limited applicability for behavioural research [8].

Community-based studies of MSM present great challenges, namely when it comes to defining a sampling frame [9] due to the clear difficulty in establishing the boundaries of the target population itself because of cultural, anthropological, and sociological reasons. Traditional sampling strategies designed to ensure representativeness and external validity, such as simple random or cluster sampling, are often not efficient to

recruit and follow MSM [8-11]. Alternative sampling techniques such as convenience sampling in community-based facilities devoted to MSM can be substantially more feasible and improve crucial attributes for the success of integrated epidemiological surveillance such as simplicity, acceptability of participants, and stability [8-12].

The Lisbon Cohort of MSM was assembled as a facility-based open prospective cohort in a community-based voluntary HIV counselling and testing service directed to MSM. The main objectives of the study are: to quantify the burden of the disease by estimating the incidence of HIV infection in MSM and monitoring trends in primary and secondary prevention; and to identify strategies to improve the provision of HIV testing and timely linkage to care of those who have a reactive test. In this paper we describe the design and methodological options of the study and present the descriptive characteristics at cohort entry of the participants recruited between April 2011 and February 2014.

Methods/Design

The Lisbon Cohort of MSM is an observational prospective study established in April 2011, designed as an open cohort. Eligible participants are men who have sex with men aged 18 or older, who voluntarily seek CheckpointLX for HIV testing and counselling and have a negative HIV test result at recruitment.

Setting

The cohort is a joint project of GAT Portugal (GAT) and the Institute of Public Health of University of Porto (ISPUP). GAT is a non-governmental organization advocating legal and political changes that can have a positive effect on the rights and quality of life of those living with HIV, or most at risk of acquiring the infection. One of GAT's projects is materialized in CheckpointLX, where the Lisbon Cohort of MSM is recruited. CheckpointLX is a community-based centre for anonymous and free rapid HIV testing and counselling, directed at MSM, and provided by trained peer MSM counsellors. ISPUP is an advanced training and research institution in the Public Health domain. In respect to the cohort study, CheckpointLX is responsible for recruitment and data collection, while ISPUP provides scientific support, data management and analysis. Both institutions were involved in the design and implementation of the cohort protocol and both have established an official partnership to guarantee a shared commitment to the follow-up of cohort participants and to the periodic dissemination and evaluation of research outputs.

Ethics

The data collected are confidential, and participants give their written informed consent prior to inclusion. The study protocol was approved by the Ethics Committee of São João Hospital and Medical School, University of Porto (ID 104/12).

Funding

From April 2011 to March 2014, all direct funding for the cohort was provided through CheckpointLX, with 80% initially funded by the National HIV/AIDS Coordination (Portuguese Ministry of Health), and the remaining 20% from sponsors of GAT which include: AIDS Healthcare Foundation; Gilead Science, Lda; Bristol-Myers Squibb; Janssen; Abbott Portugal; Merck Sharp and Dohme; ViiV Healthcare; ANF – *Associação Nacional de Farmácias*, *Delta Cafés* and private donations. Since April 2014, additional specific funding has been obtained as part of the European Commission DG SANCO - Health and Consumers funded Euro HIV EDAT project

(grant no. 20131101). From inception, ISPUP has provided contribution through the allocation of research staff time and information technology support (programming, software, and hardware) to the project.

Recruitment and follow-up of participants

Recruitment is generally made on the first visit to CheckpointLX where peer counsellors invite all eligible clients to enter the cohort. Eligibility criteria for entering the cohort is being a man aged 18 or older, reporting having had sex with other men, and having a HIV negative test result. CheckpointLX is publicized in MSM socializing sites such as bars, discos, saunas, sex shops and guesthouses, parties and events of the MSM community, cruising areas, and online social networks. The centre itself, once located at an Lesbian Gay Bisexual Transgender socializing quarter, promotes walk-ins. Promotion material includes flyers, videos, stickers, banners at online social networks and prevention kits containing condoms, lubricant, and an information card about CheckpointLX.

Between April 2011 and February 2014, there were 3301 potential eligible individuals, 195 (5.9%) of whom had a HIV reactive test at entry and therefore were not included in the cohort. The remaining 3106 were eligible to the cohort. Among those 923 (29.7%) refused to participate, and 2183 (70.3%) were enrolled in the cohort. As of February 2014, 804 of the 2183 participants had been re-evaluated at least once, yielding approximately 2300 interviews (figure 1).

Follow-up is intended to take place with intervals of six months, although the exact time between visits is adjusted according to the convenience of the participant. Men who leave their contact details are invited to come back for follow-up visits through text messages or email from CheckpointLX staff. All the remaining participants are observed whenever they decide to appear again for testing. End points for follow-up are the acquisition of HIV infection or death. In the almost three years since the recruitment began, we followed 804 participants for a total of 893 person-years. Median time between visits was 208 days (approximately 7 months) and 25th to 75th percentiles were 148 to 308 days (approximately 5 to 10 months).

Study procedures

Questionnaire

At each visit a questionnaire is administered by a trained CheckpointLX peer counsellor. The questionnaire applied at cohort entry is divided into the following

sections: sociodemographic characteristics, HIV testing history, sexual life and partners, condom use, use of alcohol and drugs, post-exposure prophylaxis (PEP) and other sexually-transmitted infections (STI). Follow-up questionnaires update time-varying information on all sections. Detailed questionnaire content is presented in table 1. From those eligible MSM who refuse to participate but agree to provide some baseline data, information is collected concerning age, gender, country of origin, educational level, HIV testing history, date and result of previous HIV test, sexual identity, screening for HIV and syphilis at the index visit to CheckpointLX, and reasons for refusal to participate. Questionnaires are identified through a sequential number and each participant is identified with a six-digit and four-letter unique code corresponding to their date of birth (YYMMDD) and the first two letters of their first and last names, which allows for data linkage during follow-up while protecting personal identity [9]. Periodically, questionnaires are sent to ISPUP where they are processed into a computer-based data management system, and where data are stored and analysed.

Rapid HIV testing

Rapid testing for HIV-1 and HIV-2 is performed at each visit by the same peer counsellor that applies the questionnaire. From April 2011 to April 2012 two rapid tests were used, namely the *Retrocheck HIV®* (QUALPRO DIAGNOSTICS, Goa, India) (manufacturer reported Sensitivity= 100.00% and Specificity= 99.75%) *and Hexagon HIV* (Human GmbH, Wiesbaden, Germany) (Sensitivity= 100.00% and Specificity= 99.50%). Since then only the Alere Determine™ HIV-1/2 (Alere Medical Co., Ltd. Chiba, Japan) (Sensitivity= 100.00% and Specificity= 100.00%) has been used according to the instructions provided by the manufacturer. In case of a reactive test, an outpatient appointment is scheduled for every participant that accepts it at the HIV/Infectious diseases clinic at Santo António dos Capuchos Hospital in Lisbon, where a confirmatory test is performed. The peer counsellor offers to accompany the participant to that appointment. Pre and post-test counselling is offered at every visit.

Syphilis rapid testing

Rapid testing for detection of *Treponema pallidum* antibodies is proposed to every individual who reports no prior history of syphilis infection or who is unaware of previous infection; in this instance the *Alere Determine™ Syphilis TP* (Alere Medical Co., Ltd. Chiba, Japan) (Sensitivity= 92.31% and Specificity= 100.00%) is used according to the instructions provided by the manufacturer. In case of a reactive test a medical appointment is proposed and scheduled at CheckpointLX as part of the

Checklist STI clinic where a confirmatory test is performed and treatment is prescribed, if needed.

Statistical procedures

Characteristics of participants at cohort entry are 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) are used, as appropriate, to describe continuous variables. Comparisons between groups were performed using the chi-square test or Fisher's exact test when variables were categorical. For continuous variables Student's t-test or ANOVA and Mann-Whitney or Kruskall-Wallis tests were used, as appropriate. In data analysis, all possible answer categories are described but the missing answers are excluded from the denominator of proportions for each item.

Characteristics of enrolled population between April 2011 and February 2014

From April 2011 to February 2014, 2183 participants were enrolled in the study. In the same period there were 923 refusals to participate in the cohort. The most common reasons for refusing to participate were having no interest in the study (25.7%), not having the time (23.5%), and not living in Portugal (18.0%).

As summarized on table 2, there were significant differences between participants and refusals: participants self-identified more frequently as homosexual (83.9% vs. 78.3% in refusals, $p<0.001$); mean (SD) age of participants was 30.8 (9.5) years, significantly younger than individuals who refused (mean=31.8, SD=10.6 years, $p=0.010$); participants were more frequently born in Portugal (75.7% vs. 59.0% in refusals, $p<0.001$); and 58.1% of participants had a university degree compared to 51.4% among refusals. The proportion of individuals who had a previous HIV test was similar between groups (81.9% in participants vs. 83.8% in refusals).

Characteristics of cohort participants

Median (P25-P75) number of HIV tests prior to cohort entry was 3 (2-6) and the most common reasons for the index HIV test were: to check health status/routine (81.3%), perception of exposure to HIV more than 3 months before (50.5%) and in the previous 3 months (40.7%) (Table 3A).

Median (P25-P75) age at first anal intercourse (AI) (receptive or insertive) was 18 (16-22) years, and 1409 (65.2%) men reported having a versatile role on AI, while 553 (25.6%) reported having only an insertive role and 177 (8.2%) only a receptive role.

Twelve percent reported sexual intercourse with HIV positive men in the previous 12 months (Table 3B).

In the previous 12 months, 1373 (63.0%) participants had at least one steady partner, of whom 108 (7.9%) had a HIV-positive partner, 338 (24.8%) were unaware of their steady partner's HIV status and the remaining 913 (67.0%) had HIV-negative partner. More than half of men who had at least one steady partner reported no condom use in the last sexual encounter (LSE) and approximately 72.0% reported inconsistent use. Among those in a serodiscordant relationship, 43.7% reported inconsistent use of condom and that proportion was 71.0% among those unaware of their steady partner's HIV status (Table 3B1).

Sexual intercourse with at least one occasional partner in the previous 12 months was reported by 1860 (85.2%) participants and the median (P25-P75) number of partners was 4 (2-10). Twenty one percent of men who had at least one occasional partner reported no condom use in the LSE and 46.4% reported inconsistent use. The most referred venues where participants usually met their occasional partners were the internet (72.2%), discos and gay bars (48.4%) and cruising sites (23.2%) (Table 3B2).

Condoms were always used for oral sex by 2.3% of participants. Always using condoms for AI in lifetime was reported by 652 (32.9%) participants. Among the 1318 (66.5%) participants who reported not having always used condom for AI the most common reasons for engaging in unprotected anal intercourse (UAI) were a steady partner (66.2%), a steady partner after testing negative for HIV (47.9%), "reliable" persons (39.8%), and being too aroused (37.1%) (Table C).

Lifetime use of alcohol or drugs before or during intercourse was reported by 1520 (69.7%) participants, among whom 86.4% reported consumption in the previous 12 months. The most frequently reported psychoactive substances were alcohol (82.7%), poppers (25.6%), and cannabis (23.4%) (Table 3D).

A little over one third of participants had heard about PEP, and 54 participants (2.7%) knew about and had used PEP (Table 3E).

History of symptoms or diagnosis of any STI in the previous 12 months was reported by 216 (9.9%) participants and 1368 (62.8%) never had any symptom or STI. A diagnosis of gonorrhoea was reported by 226 (28.9%) participants, of which 54 (20.9%) were diagnosed in the previous 12 months. Syphilis had been diagnosed in the previous 12 months in 38 (4.7%) participants and before that in 116 (14.3%)

participants. Having been diagnosed with Hepatitis C was reported by 10 (0.5%) participants (Table 3F), none of whom admitted to inject drugs.

Discussion

The Lisbon Cohort of MSM is the first Portuguese prospective study of MSM in the context of HIV incidence, testing, and linkage to care. As an open prospective study, it will provide information on the trends of HIV infection and other STIs among MSM in Portugal and it will contribute to identify and monitor determinants of infection, including risk-taking behaviours.

Until recently, serological and behavioural evidence on HIV among MSM in Portugal had been scarce, apart from the necessarily succinct indicators obtained through routine national HIV surveillance. Two recent cross-sectional studies [13, 14] targeting MSM in Portugal provided the first population-based estimates of self-reported prevalence: 10.9% [6] and 10.3% [14]. In addition to these alarming estimates, both studies have raised important concerns regarding the future of the epidemic in Portugal supporting the need for a closer monitoring of behavioural and serological indicators within a dynamic framework.

A few cohorts follow seronegative MSM internationally, for instance the Amsterdam Cohort Studies (ACS) on HIV infection and AIDS that started shortly after the first cases of AIDS had been diagnosed in the Netherlands [15], the Multicenter AIDS Cohort Study (MACS) initiated in 1983 in four cities in the United States [16], the Omega Cohort Study in Montreal, Canada carried out from October 1996 to July 2003 [17], the Health in Men (HIM) in Sidney, Australia established in July 2001 [18] and, more recently, in 2008 the ITACA Cohort in Barcelona established in a community-based voluntary HIV testing and counselling centre - the BCN Checkpoint [19]. These cohorts have significantly contributed to our understanding of the HIV/AIDS epidemic and will likely enable comparisons of findings with those of our newly-developed infrastructure. This cohort has the potential to serve as a modern decentralised surveillance structure that will provide dynamic information about the burden of the epidemics and its determinants in this group. Within our geographical setting, it has the potential to enable locally-adapted responses in terms of service provision, namely on the development of effective strategies to anticipate diagnosis, and to improve linkage to care for individuals who have recently been diagnosed with HIV. The cohort will also allow comparisons of behavioural indicators drawn from entry and follow-up questionnaires within the international context, since it collects the set of indicators for behavioural surveillance among MSM defined by the European Centre for Disease Control and Prevention (ECDC) [20]. Finally, a set of specific analytical research

objectives will be pursued, with strong emphasis on how contextual and behavioural trajectories throughout follow-up may be used to predict the risk of seroconversion.

The Lisbon Cohort of MSM has a relevant strength in the peer-based approach provided by CheckpointLX, in an attempt to promote an adequate response to MSM needs, and to be a non-judgmental and an inclusive service, reportedly the preference of gay and other MSM for testing services [21]. From a research point of view, this approach can also help reduce social desirability bias with regard to information collected, and can be more cost effective than interventions based on clinical staff [22]. Another strength of the cohort is the assurance of anonymity which is expected to influence completeness of reporting and disclosure of risk [9]. In addition, in line with the ethical guidelines for surveillance in populations most at risk for HIV, the Lisbon Cohort of MSM offers to all participants: timely results, information about HIV and AIDS, counselling on HIV prevention and on other health or social needs, linkage to treatment and care to the extent possible with local resources and protocols with health services for referrals [9]. Furthermore, CheckpointLX peer counsellors accompany newly-identified HIV-positive participants to their first appointment at a HIV/Infectious disease clinic to boost linkage to care, following the experience of other community-based centres dedicated to MSM in European countries that have shown have high efficiency in HIV detection and linkage to care [23, 24].

The Lisbon Cohort of MSM, as a facility-based structure is unlikely to result in a representative sample of the source MSM population, which limits the generalizability of our findings to the whole community. This is a frequent concern in studies with non-probabilistic samples, but should not be an argument for not attempting to generate the best scientific evidence within real-world constraints. Additionally, by following only MSM who seek HIV testing, we are arguably selecting a subgroup that might be on average at a higher risk of infection than the general MSM community, thereby focusing our attention among a priority subset of the population (even if potentially more aware than those not reached by the service). The following comparisons are useful to assess the extent of selection bias (Table 4). In the 2007 National Health and Sexuality Survey (HSS) [25], which included a representative sample of the Portuguese population, 4.7% of adult male individuals reported some kind of sexual contact with other men in their lifetime, 3.0% of sexually active men had sex with men in the previous 12 months, and 0.9% reported homosexual identity. Despite the heteronormative frame still persistent in the Portuguese society [25] the proportion of men reporting sex with other men is quite similar to that estimated by the EMIS study, where approximately 3.0% of the adult male population living in Portugal were

estimated to be MSM [26]. Men in our sample are clearly younger than in the HSS, where about 31% were less than 25 years old, while in the HSS, men who have had some kind of sexual contact with men in that age strata represent only 9.8%. Men in the Lisbon cohort reported more frequently on having a previous HIV test (82.3% vs. 61.0% in HSS). When compared with EMIS results [13] from a sub-analysis including only participants aged 18 or more living in the Lisbon region, men in our sample have lower mean age (30.8 vs. 33.4) and lower educational level (58.3% with an university degree vs. 61.9%), but report more frequently homosexual identity (84.3% vs. 73.6%), migrant status (24.0% vs. 22.6%), previous HIV test (82.3% vs. 77.0%) and lifetime use of PEP (2.7% vs. 2.1%). We may assume that we are capturing men who are more self-identified as homosexual, which was expected once CheckpointLX is directed to this group, and perhaps more aware of HIV risk once frequency of uptake of HIV testing is higher than in previous studies. It is important to stress that since CheckpointLX promotion strategies remained similar during follow-up, we do not expect a change in the extent of selection bias over time which is particularly important for the estimation of secular trends of infection and behaviours in the source population [8-10].

Participation bias is also a key methodological issue in epidemiological studies. In fact, participants in our study are more self-identified as homosexual, more frequently born in Portugal, and more educated than those who refused to participate. This implies that important data may be missing on a harder to reach subset of the target population. However, it is interesting to note that the proportion of a previous HIV test is similar between groups suggesting that both groups may have similar perceived high risk of acquiring HIV [27, 28].

Attrition is a main concern in prospective investigations, due to the fact that this is not an interval cohort with fixed follow-up times the ability to estimate it in a short time frame is limited. However, efforts have been made to minimize dropout rates. CheckpointLX peer counsellors ask all participants to provide their email or mobile phone contact at the first interview and to update contacts in the follow-up assessments. These contacts are used with the consent of participants to send reminders at the month of an intended follow-up.

One other ongoing challenge is the possible behavioural modification by cohort participants due to their participation in an investigation, known as Hawthorne effect. This aspect also relates to the dual role of CheckpointLX as a healthcare/counselling provider and a research structure. Checkpoint's first priority is that appropriate and high-quality pre- and post-test information or counselling is offered [29], and hopefully

that will produce a change towards better health empowerment, likely to influence the risk of the outcomes being studied [30].

Additional note

We encourage interest from scientists, researchers or students from graduation or post-graduation to get involved in data collection and/or analyses, and to raise new scientific questions. We also look forward to external collaborations with other cohort studies. Requests for data analysis, presentation or publication, must be submitted to the Lisbon Cohort of MSM scientific coordination, and will require acknowledgement that Lisbon Cohort of MSM has the copyright to the data. All Information is available at http://www.checkpointLX.com.

List of abbreviations:

AI –Anal Intercourse

AIDS – Acquired Immunodeficiency Syndrome

ACS – Amsterdam Cohort Studies

ECDC – European Centre for Disease Control and Prevention

EMIS - European MSM Internet Survey

GAT – GAT Portugal

HIM – Health in Men Study

HIV – Human Immunodeficiency Virus

HSS - Health and Sexuality Survey

ISPUP – Institute of Public Health of the University of Porto

LSE – Last sexual encounter

MACS – Multicenter AIDS Cohort Study

MSM – Men who have Sex with Men

P25-P75 – percentiles 25 and 75

PEP – Post-exposure prophylaxis

SD – Standard deviation

STI – Sexually transmitted infections

UAI – Unprotected anal intercourse

Competing interests

No competing interests.

Author's contributions

PM drafted the manuscript and performed the descriptive 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. AM participated in analysis and interpretation of data, and 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.

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Table 1: Content of the questionnaire.

	Entry	Follow-up
1. Sociodemographic characteristics		
Date of birth	✓	–
Gender	✓	–
Country of birth	✓	✓
Educational level	✓	✓
Employment status	✓	✓
2. HIV testing		
Ever tested for HIV	✓	–
Access to HIV testing result	✓	–
Reasons for not testing or not having HIV test result	✓	–
Number of previous HIV tests	✓	–
Place, date and result of previous HIV test	✓	✓
Reasons for index test	✓	✓
3. Sexual life and partners		
Sexual identity	✓	–
Age at first anal intercourse	✓	–
Role in anal intercourse	✓	–
Characteristics of sexual partners in the previous 12 months/since the previous visit	✓	✓
a. Steady partner		
Steady partner in the previous 12 months/since the previous visit	✓	✓
Duration of the relationship with steady partner	✓	–
Gender of steady partner	✓	✓
Sexual practices with steady partner	✓	✓
Sexual intercourse with other partners	✓	–
HIV status of the steady partner	✓	✓
b. Occasional partner		
Occasional partner in the previous 12 months/since the previous visit	✓	✓
Number of occasional partners in the previous 12 months/since the previous visit	✓	✓
Sexual practices with an occasional partner	✓	✓
Venues used to meet occasional partners	✓	–
c. Sex work		
Having sex for money or drugs in the previous 12 months/since previous visit	✓	✓
4. Condom use		
Condom use with a steady partner in the previous 12 months/since the previous visit	✓	✓
Condom use with a steady partner in the last anal intercourse	✓	✓
Condom use with an occasional partner in the previous 12 months/since the previous visit	✓	✓
Condom use with an occasional partner in the last anal intercourse	✓	✓
Condom use for oral sex	✓	–
Reasons for not using condom	✓	✓
Lubricant use for anal intercourse	✓	–
5. Alcohol and drugs		
Lifetime use of alcohol or drugs before or during intercourse	✓	–

Frequency of use of alcohol or drugs before or during intercourse in the previous 12 months/since the previous visit	✓	✓
Perception of reduction in condom use due to use of alcohol or drugs	✓	–
6. Post exposure prophylaxis		–
Knowledge of PEP	✓	–
Lifetime use of PEP	✓	–
Use of PEP in the previous 12 months/since the previous visit	✓	✓
7. Sexually transmitted infections and Hepatitis		
Lifetime history of STI (symptoms or diagnosis)	✓	–
Symptoms of STI in the previous 12 months/since the previous visit	✓	✓
Lifetime history of STI diagnosis	✓	–
Diagnosis of STI in the previous 12 months/since the previous visit	✓	✓
Immunization status for Hepatitis A and Hepatitis B	✓	–
Lifetime history of Hepatitis virus A, B or C diagnosis	✓	–

* bisexual men; men with different partners; sex workers; HIV-positive men; injecting drug users; women; trios/group sex

Abbreviations: HIV – Human Immunodeficiency virus; PEP – Post-exposure Prophylaxis; STI - Sexually transmitted infections

Table 2: Sociodemographic characteristics of participants in the cohort and refusals.

	PARTICIPANTS	REFUSALS	p-value
	2183 (70.3)	**923 (29.7)**	
Sexual identity, n (%)			<0.001
Homosexual	1831 (83.9)	709 (78.3)	
Bisexual	306 (14.0)	151 (16.7)	
Heterosexual	28 (1.3)	37 (4.1)	
Other/Did not know/Rather not say	17 (0.8)	8 (0.9)	
Missing	1	18	
Age, mean (SD)	30.8 (9.5)	31.8 (10.6)	0.010
Country/Region of origin			<0.001
Portugal	1573 (75.7)	539 (59.0)	
Brazil	231 (11.1)	160 (17.5)	
Other European country	139 (6.7)	141 (15.4)	
African country	89 (4.3)	27 (3.0)	
Other American country	31 (1.5)	30 (3.3)	
Asia / Middle east / Oceania	9 (0.4)	16 (1.8)	
Rather not answer	5 (0.2)	1 (0.1)	
Missing	107	9	
Educational level			<0.001
Basic education or less	78 (3.6)	101 (11.3)	
Secondary education	564 (25.9)	288 (32.3)	
Professional training	260 (11.9)	36 (4.0)	
Bachelor	896 (41.0)	341 (38.2)	
Master or Doctoral	373 (17.1)	118 (13.2)	
Other/Rather not answer	10 (0.5)	9 (1.0)	
Missing	2	30	
Previous HIV testing			0.167
Yes	1650 (81.9)	766 (83.8)	
No	354 (17.6)	145 (15.9)	
Did not know	11 (0.5)	2 (0.2)	
Rather not answer	0 (0.0)	1 (0.1)	
Missing	168	9	

Abbreviations: HIV: human immunodeficiency virus, SD: standard deviation

Table 3A – 3F: Descriptive characteristics of participants at cohort entry

(3A) HIV TESTING	N (%)	Missing
Previous HIV testing (n= 2183)		168
Yes	1650 (81.9)	
No	354 (17.6)	
Did not know	11 (0.5)	
Rather not answer	0 (0.0)	
Number of previous tests, median (P25-P75)	3 (2-6)	31
Local of last HIV test (n=1650)		2
Public network of VCT centres (CAD)	506 (30.7)	
Family doctor (National health service)	311 (18.9)	
Public Hospital (National health service)	182 (11.0)	
Abroad	152 (9.2)	
Private Laboratory	150 (9.1)	
Private Hospital or Clinic	144 (8.7)	
CheckpointLX	79 (4.8)	
Blood donation	45 (2.7)	
Mobile unit	28 (1.7)	
Other	49 (3.0)	
Did not know	2 (0.1)	
Reasons for index test (n=2183) (multiple options possible)		
To check health status / Routine	1736 (81.3)	49
Perception of HIV exposure more than 3 months before	1084 (50.5)	38
Perception of HIV exposure in the previous 3 months	884 (40.7)	9
Accident with condom use (rupture / left inside)	183 (8.6)	56
My partner asked me to test for HIV	158 (7.4)	57
To stop using condom with my partner	149 (7.0)	64
Partner diagnosed HIV+/Disclosed HIV+ status	138 (6.5)	56
Possible window period by the time of the last test	136 (6.4)	61
Symptoms / Medical indication	58 (2.7)	61
Other reason	159 (7.3)	0

(3B) SEXUAL LIFE AND PARTNERS		N (%)	Missing
Age at first anal intercourse, median (P25-P75)		18 (16-22)	216
Role on anal intercourse			22
	Only insertive	553 (25.6)	
	Only receptive	177 (8.2)	
	Versatile	1409 (65.2)	
	Did not know	2 (0.1)	
	Rather not answer	20 (1.0)	
Intercourse with at least one of the following in the previous 12 months:			
Bisexual men			31
	Yes	732 (34.0)	
	No	1145 (53.2)	
	Did not know	262 (12.2)	
	Rather not say	13 (0.6)	
Men with different sex partners			32
	Yes	1475 (68.6)	
	No	491 (22.8)	
	Did not know	172 (8.0)	
	Rather not say	13 (0.6)	
Sex workers (even if not payed)			32
	Yes	133 (6.2)	
	No	1920 (89.3)	
	Did not know	85 (4.0)	
	Rather not say	13 (0.6)	
HIV positive men			
	Yes	259 (12.0)	32
	No	1181 (54.9)	
	Did not know	698 (32.5)	
	Rather not say	13 (0.6)	
Injecting Drug Users			32
	Yes	16 (0.7)	
	No	1958 (91.0)	
	Did not know	164 (7.6)	
	Rather not say	13 (0.6)	
Women			32
	Yes	287 (13.3)	
	No	1851 (86.1)	
	Did not know	0 (0.0)	
	Rather not say	13 (0.6)	
Trios / Group sex			33
	Yes	585 (27.2)	
	No	1549 (72.0)	
	Did not know	1 (0.0)	
	Rather not say	15 (0.7)	

(3B1) STEADY PARTNER	N (%)	Missing
Steady partner in the previous 12 months (n=2183)		2
Yes, one	1254 (57.5)	
Yes, more than one	119 (5.5)	
No	798 (36.6)	
Did not know	0 (0.0)	
Rather not answer	10 (0.5)	
HIV status of steady partner (n=1373)		11
HIV negative	913 (67.0)	
HIV positive	108 (7.9)	
Did not know	338 (24.8)	
Rather not say	3 (0.2)	
Condom use with steady partner		
In the last sexual encounter (n=1373)		70
Yes	572 (43.9)	
No	718 (55.1)	
Did not know	5 (0.4)	
Rather not say	8 (0.6)	
Frequency in the previous 12 months (n=1373)		69
Always	364 (27.9)	
Often/Occasionally/Rarely/Never	931 (71.4)	
Rather not say	9 (0.7)	
Frequency in the previous 12 months with HIV positive steady partner (n=108)		5
Always	57 (55.3)	
Often/Occasionally/Rarely/Never	45 (43.7)	
Rather not say	1 (1.0)	
Frequency in the previous 12 months with unknown HIV status steady partner (n=338)		10
Always	95 (29.0)	
Often/Occasionally/Rarely/Never	233 (71.0)	
Rather not say	0 (0.0)	

(3B2) OCCASIONAL PARTNERS	N (%)	Missing
Occasional partners in the previous 12 months (n=2183)		0
Yes	1860 (85.2)	
No	312 (14.3)	
Rather not say	11 (0.5)	
Number of occasional partners in the previous 12 months: Median (P25-P75) (n=1860)	4 (2-10)	45
Having sex for money or drugs in the previous 12 months (n=1860)		1
Yes	62 (3.3)	
No	1796 (96.6)	
Did not know	1 (0.1)	
Condom use with occasional partner		
In the last sexual encounter (n=1860)		124
Yes	1360 (78.3)	
No	367 (21.1)	
Did not know	8 (0.5)	
Rather not say	1 (0.1)	
Frequency in the previous 12 months (n=1860)		123
Always	925 (53.3)	
Often/Occasionally/Rarely/Never	806 (46.4)	
Did not know	2 (0.1)	
Rather not say	4 (0.2)	
Venues used to meet occasional partners (n=1860) (multiple options possible)		
Internet	1338 (72.2)	8
Discos and gay bars	897 (48.4)	7
Cruising sites	430 (23.2)	10
Saunas	356 (19.3)	11
Gymnasium	232 (12.6)	14
"Dark rooms" (including sex-shops)	129 (7.0)	11
Sex clubs	92 (5.0)	10
Other	445 (23.9)	0

(3C) CONDOMS	N (%)	Missing
Lifetime condom use on oral sex (n=2183)		7
Always	49 (2.3)	
Often /Occasionally/Rarely/Never	2106 (96.8)	
Rather not say	21 (1.0)	
Lifetime condom use on anal intercourse (n= 2183)		202
Always	652 (32.9)	
Often /Occasionally/Rarely/Never	1318 (66.5)	
Rather not say	11 (0.6)	
Reasons for not using condom on anal intercourse (n=1318) (multiple options possible)		
with steady partner	870 (66.2)	3
with steady partner after testing for HIV and both were negative	629 (47.9)	5
with a “reliable” person	523 (39.8)	3
being too aroused	487 (37.1)	6
condom reduces pleasure	360 (27.4)	5
with a partner that declares he is HIV negative	303 (23.1)	7
not having condoms in that moment	261 (19.9)	5
if he has used alcohol or drugs	226 (17.2)	5
condom interrupts sexual intercourse	201 (15.3)	5
does not like using condoms	205 (15.6)	5
condom makes him loose erection	188 (14.3)	4
with a partner that does not want to use	124 (9.4)	5
being in a sex venue without condoms available	59 (4.5)	6
condoms are expensive	40 (3.0)	6
with a partner that declares undetectable viral load	37 (2.8)	5
allergy to latex	24 (1.8)	6
other reasons	77 (5.8)	0

(3D) ALCOHOL AND DRUGS	N (%)	Missing
Lifetime use of alcohol or drugs before or during intercourse (n=2183)		1
Yes	1520 (69.7)	
No	662 (30.3)	
Use of alcohol or drugs before or during intercourse in the previous 12 months (n=1520)		62
Yes	1260 (86.4)	
No	175 (12.0)	
Did not know	4 (0.3)	
Rather not answer	19 (1.3)	
Ever used alcohol or drugs before or during intercourse in the previous 12 months (n=1520) (multiple options possible)		
Alcohol	1254 (82.7)	4
Poppers	389 (25.6)	2
Cannabis	329 (23.4)	113
Cocaine	236 (15.5)	1
Ecstasy	123 (8.1)	3
Viagra/Cialis/similar	89 (5.9)	2
Mephedrone	76 (5.0)	3
Amphetamines	72 (4.7)	3
GHB	37 (2.4)	2
Ketamine	32 (2.1)	2
LSD	31 (2.0)	3
Heroine	7 (0.5)	3
Methadone	8 (0.5)	2
Others	49 (3.2)	0

(3E) POST-EXPOSURE PROPHYLAXIS (n=2183)	N (%)	Missing
Did not know about PEP	1228 (61.2)	175
Knows but never used	726 (36.2)	
Knows and used	54 (2.7)	

(3F) SEXUAL TRANSMITTED INFECTIONS AND HEPATITIS	N (%)	Missing
Lifetime history of STI (symptoms or diagnosis) (n=2183)		6
Yes, in the previous 12 months	216 (9.9)	
Yes, more than 12 months before	593 (27.2)	
No	1368 (62.8)	
STI diagnosed (n=809)		
History of gonorrhoea		
Yes, in the previous 12 months	57 (7.0)	
Yes, more than 12 months before	169 (20.9)	
No	552 (68.2)	
Did not know	31 (3.8)	
History of syphilis		
Yes, in the previous 12 months	38 (4.7)	
Yes, more than 12 months before	116 (14.3)	
No	640 (79.1)	
Did not know	15 (1.9)	
History of condyloma or genital warts		
Yes, in the previous 12 months	68 (8.4)	
Yes, more than 12 months before	22 (2.7)	
No	710 (87.8)	
Did not know	9 (1.1)	
History of chlamydia		
Yes, in the previous 12 months	64 (7.9)	
Yes, more than 12 months before	14 (1.7)	
No	700 (86.5)	
Did not know	31 (3.8)	
History of genital herpes		
Yes, in the previous 12 months	4 (0.5)	
Yes, more than 12 months before	20 (2.5)	
No	771 (95.3)	
Did not know	14 (1.7)	
History of trichomonas		
Yes, in the previous 12 months	3 (0.4)	
Yes, more than 12 months before	1 (0.1)	
No	791 (97.8)	
Did not know	14 (1.7)	
History of lymphogranuloma venereum		
Yes, in the previous 12 months	2 (0.2)	
Yes, more than 12 months before	0 (0.0)	
No	797 (98.5)	
Did not know	10 (1.2)	
Lifetime history of hepatitis diagnosis (n=2183)		12
History of hepatitis A		
Yes	127 (5.8)	
No	1897 (87.4)	
Did not know	137 (6.3)	
Rather not answer	10 (0.5)	
History of hepatitis B		13
Yes	52 (2.4)	

	No	2002 (92.3)	
	Did not know	106 (4.9)	
	Rather not answer	10 (0.5)	
History of hepatitis C			15
	Yes	10 (0.5)	
	No	2032 (93.7)	
	Did not know	116 (5.4)	
	Rather not answer	10 (0.5)	
Vaccination (n=2183)			8
Hepatitis A			
	Yes	827 (38.0)	
	No	742 (34.1)	
	Did not know	596 (27.4)	
	Rather not answer	10 (0.5)	
Hepatitis B			6
	Yes	1603 (73.6)	
	No	312 (14.3)	
	Did not know	252 (11.6)	
	Rather not answer	10 (0.5)	

Abbreviations: HIV: Human Immunodeficiency Virus; VCT: Voluntary counselling and testing GHB: Gamma-hydroxybutyric acid; LSD: Lysergic acid diethylamide; PEP: Post-exposure prophylaxis; STI: Sexually transmitted infection.

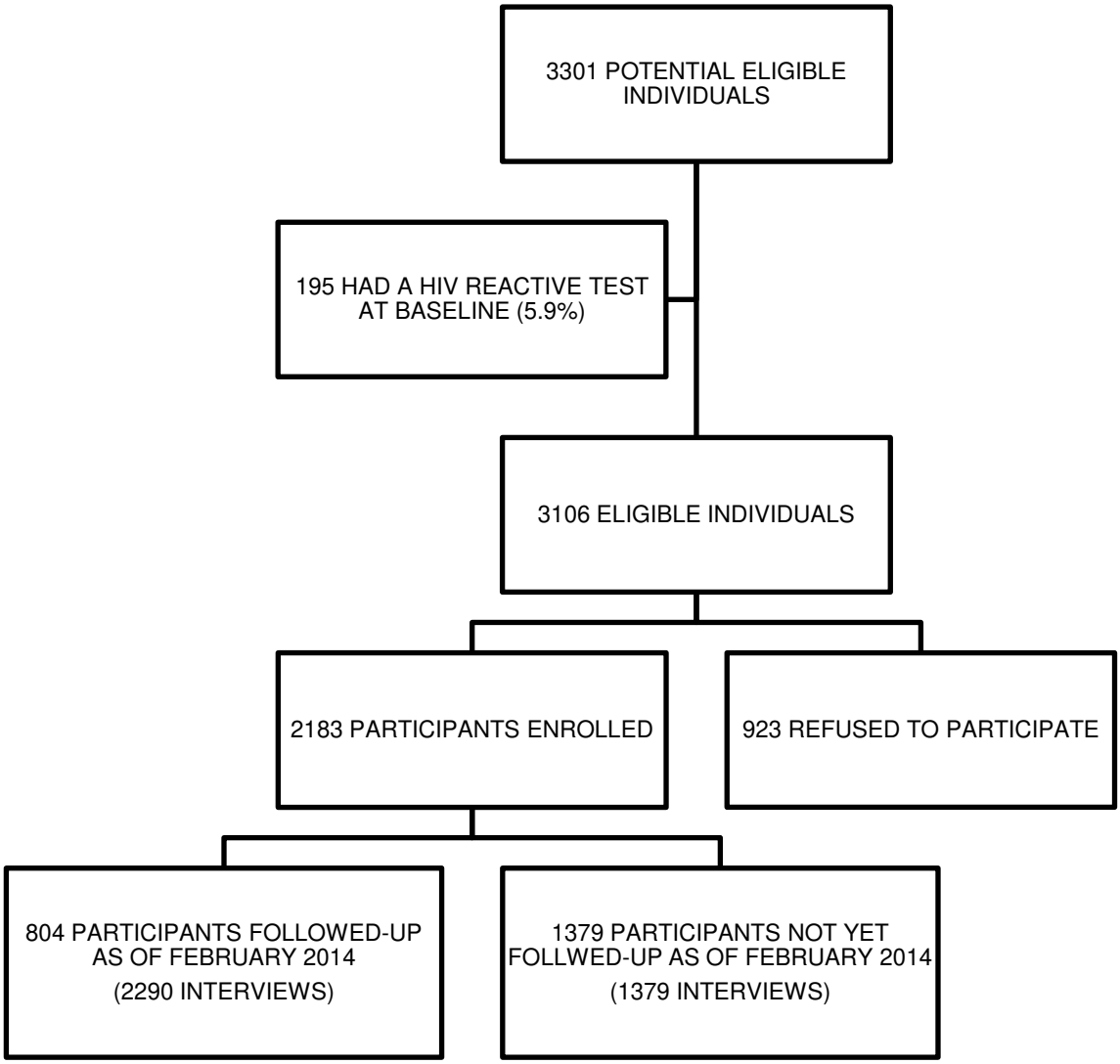
Table 4: Comparison of Lisbon Cohort of MSM with previous studies in Portugal.

	Lisbon Cohort of MSM	HSS*	EMIS study**
Age			
Mean	30.8	not available	33.4
Up to 24 (%)	30.9	9.8	28.0
University degree (%)	58.3	not available	61.9
Self-reported homosexual identity (%)	84.3	35.9	73.6
Migrant status	24.0	not available	22.6
HIV previous test (%)	82.3	61.0	77.0
Lifetime use of PEP (%)	2.7	not available	2.1

*between only those men who have had some kind of sexual contact with men
**sub-analysis of participants aged 18 or more living in the Lisbon region

Abbreviations: MSM: Men who have sex with men; HSS: Health and Sexuality Survey; EMIS: European men who have sex with men survey; HIV: Human immunodeficiency Virus; PEP: Post-exposure prophylaxis.

Figure 1: Flowchart of enrolments between April 2011 and February 2014



Abbreviations: HIV: Human Immunodeficiency virus.

4. CHAPTER II

Incident risk factors as predictors of HIV seroconversion in the Lisbon cohort of MSM: 2011 – 2014

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Abstract

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 generalized linear regression. The overall seroincidence was 2.80/100 person-years (95% CI: 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 UAI with a steady partner and being newly-diagnosed with syphilis during follow-up. Likewise, sexual intercourse with HIV-positive men, having had a 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 of Portuguese MSM is likely to be driven by short-term contextual and behavioural changes during follow-up.

Key-words: HIV; Men who have sex with men; Cohort studies; Incidence; Risk factor.

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 2012 was attributable to sex between men (40.4%), followed by heterosexual transmission (33.8%), and finally by unsafe injection practices (6.1%) (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 24.1% of all reported cases in 2012 (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% (12).

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

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, being aged 18 years or more, reporting having sex with other men and having a negative HIV test result at recruitment. Suspicion of being in the window period was an exclusion criterion (n=5 among MSM who filled all inclusion criteria). 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. At each visit a structured questionnaire was administered and a rapid HIV test was performed by a trained CheckpointLX peer counsellor. Data reported in this study refer to the period from April 2011 to February 2014, during which 3301 potential eligible individuals presented for testing, 195 (5.9%) of whom had a HIV reactive test at entry and therefore were not included in the cohort. The remaining 3106 were eligible to the cohort. Among those, 2183 (70.3%) were enrolled in the cohort of whom 804 had at least one follow-up evaluation (893.37 person-years of observation) and 923 (29.7%) refused to participate. Refusals were less self-identified as homosexual, less frequently born in Portugal, and less educated than those who accepted to participate but had a similar proportion of HIV testing prior to cohort entry. 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).

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 then, 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* – 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 by trained peer counsellors at entry and at each follow-up visit collecting data on background and behavioural characteristics, according to ECDC and UNGASS guidelines for HIV surveillance (21, 22). 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, gender, 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;
- Sexual life and partners: 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, gender and HIV status, if known) and occasional partners in the previous year, having been paid for sex and venues used to meet occasional partners;
- Condom use: frequency of condom use for anal intercourse with steady and occasional partners.
- Use of alcohol or recreational drugs (cannabis, LSD, poppers, heroin and drugs typically used at (sex) parties such as ecstasy, amphetamines, mephedrone, GHB, ketamine and cocaine) before or during intercourse;
- Knowledge and use of post-exposure prophylaxis (PPE);
- 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 trajectories of 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: 1) the majority (53.8%) of participants had only 2 visits, and 2) 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 of exposure trajectories during follow-up. For this purpose we created new variables for time-varying information that

compiled responses from the first visit and the most recent visit, categorized 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 2 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 with characteristics of sexual partners the “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 (IR) 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.

Poisson generalized 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 analysis were computed with SPSS for Windows, version 22.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Characteristics of participants at cohort entry

Background and behavioural characteristics at entry are summarized in table 1. Briefly, mean (SD) age was 30.3 (8.9) years; 86.1% of MSM self-identified as homosexual; 75.0% were born in Portugal and 60.2% had higher education (over 12 years of schooling). HIV testing prior to cohort entry was reported by 84.1% of participants. Slightly more than two thirds of participants had at least one steady partner, of whom 46 (9.3%) were in a serodiscordant couple. UAI with a steady partner in the year prior to cohort entry was reported by 72.4%; in particular, 40.9% of MSM who had a HIV-positive partner had UAI in the same period; UAI with one or more occasional partners was reported by 43.7% in the same period. Almost one third of men reported having used recreational drugs before or during sexual intercourse in the previous year. Approximately 2% of MSM had a diagnosis of syphilis in the previous year and 0.4% 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 means of their own to access health services and three did not provide information on clinical follow-up. Participants who seroconverted had significantly shorter average follow-up time than those who did not seroconvert, 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).

Predictors of HIV infection

Being born before 1970 had a strong though non-significant association with increased HIV incidence, whereas the remaining background indicators (country of birth, education, sexual identity) had negligible associations with seroconversion. 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 a

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 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). 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 borderline significance with HIV incidence.

Significant crude associations with more generic markers of exposure (having started to have sex with men four to eight years prior to cohort entry, reporting recent sexual intercourse with bisexual men or women and persistent use of recreational drugs at both assessments) lost significance after adjustment for UAI with steady and occasional partners. 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 (graph 1). Overall, we observed that MSM who had a HIV-positive steady partner during follow-up had higher incidence rates than MSM who did not have a HIV-positive partner for all levels of each additional determinant. When compared to the lowest risk category of each variable, 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 MSM in Portugal. 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 in Portugal consistently with routine surveillance data. The present study also provides evidence that intraindividual changes in the sexual life context - particularly partner disclosure of HIV status allied with newly-adopted or persistent UAI - are likely driving the HIV epidemic in this cohort of MSM.

Increased risk of HIV among MSM stems mainly from unprotected anal intercourse, and indeed self-reported UAI was an important predictor of HIV seroconversion in this cohort, as extensively described in the literature (23). More specifically, persistent UAI with occasional partners predicted seroconvertion. Additionally our study specifies that changes in UAI adoption with a steady partner during follow-up predicted increased HIV incidence, i.e., MSM at higher risk were those who probably abandoned primary prevention with a steady partner between cohort entry and the most recent visit. Likewise, those that newly adopted UAI with occasional partners were at higher risk, even though this association was only marginally significant and of lower magnitude than for UAI with a steady partner.

The role of serodiscordant steady relationships in newly acquiring HIV infection is well-recognized (24). Safe intercourse between serodiscordant partners requires consistent primary prevention, which seems to be to some extent bypassed in this cohort of MSM. There is evidence that men within a steady relationship have been overlooked by prevention efforts which may have resulted in lower perceptions of risk and increased confidence of remaining HIV-negative (25). Previous studies have concluded that MSM with steady partners engage in UAI as way to show their love, intimacy, and trust toward one another as well as to strengthen their relationship commitment and satisfaction (25). It is unknown whether MSM might benefit from prevention strategies addressing the couple's joint serostatus (concordant positive, concordant negative, or serodiscordant).

As for the timing of transmission, among MSM who seroconverted and had a HIV positive steady partner during follow-up, approximately half reported their disclosure of HIV (whether previously diagnosed or not) between baseline and the most recent visit. This suggests that a substantial fraction of transmission to the index partner in this cohort might occur during the acute infection stage of the steady partner, when the risk of transmission is highest (26, 27). Nevertheless, we cannot exclude the contribution of

older infections from partners with suboptimal testing or treatment coverage. Indeed, previous studies in Portuguese MSM found that 37.1% of HIV-positive MSM presented to care with CD4 count<350/mm3 and, and 39.0% either had detectable viral load or were unaware of viral load at the time of the questionnaire (28).

Regardless of the relative contribution of older or newer infections to incident transmission, our results clearly indicate some degree of bypassing of primary prevention and a shift towards risk management. This is also reflected in our finding of associations between being newly diagnosed with syphilis during follow-up and HIV seroconversion. Those incident circumstances (newly-adopted UAI with a steady partner, newly-disclosed HIV-positive partner, and newly-diagnosed syphilis) may then function as quantitative markers of the short-term risk of infection.

Conversely, other behavioural factors whose associations with seroconversion were independent of measurement timing may be regarded as less specific predictors of incident HIV. Such examples were the time since the beginning of sexual life, sexual intercourse with bisexual men or sex workers and persistently using recreational drugs. These were also associated with higher HIV incidence, even though such associations were probably largely mediated by UAI. The number of sexual partners in the year prior to cohort entry was not associated with increased HIV incidence. These findings highlight that, rather than extensively characterizing the type or number of partners, targeted inquiring about UAI in this context seems to be more accurate to predict HIV risk.

In line with this, accurate self-perception of HIV risk and use of testing as a risk management strategy are plausible in this cohort, as also shown by the observation that MSM who seroconverted had shorter intervals between follow-up visits and higher mean number HIV tests per time unit.

So far, none of the background variables predicted HIV risk in this cohort of Portuguese MSM. However it should be highlighted that 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 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 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. The cohort is a facility-based sample, where men are invited to participate when presenting for testing. This raises two main issues, one that relates to self-selection and participation and the other to service provision itself.

First, this design option is unlikely to result in a representative sample of the source MSM population, which limits the generalizability of our findings to the whole community. When compared with data from the 2007 National Health and Sexuality Survey (HSS) (31), MSM in our sample are clearly younger, much more self-identified as homosexual (86.3% vs. 35.9% of those men who reported some kind of sexual contact with men) and report a history of HIV testing more frequently (84.7% vs. 61.0% in HSS). Nevertheless, by setting up a cohort study in a community-based voluntary counselling and testing site 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 the estimation of secular trends of infection and behaviours in the source population (32-34). Another important issue is participation bias: the fact that approximately 30% of potentially eligible MSM refused to participate 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).

In addition, this is not an interval cohort with fixed follow-up times, rather depending on the frequency of service uptake which limits the ability to estimate losses to follow up in a short time frame such as the one considered so far in our study. Nevertheless, efforts have been made to minimize dropout rates, including active reminders of follow-up visits by peer counsellors. 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 stakeholders as technicians and key informants, 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, 37).

Finally, 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 estimates are important for two main reasons: 1) they draw a first picture of HIV incidence and its drivers in Portuguese MSM about whom little was known; 2) 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.

The second main issue is the fact that the recruitment site is itself a service whose intervention aims not only to anticipate the diagnosis of HIV infection but also to provide evidence-based and adapted information which hopefully may modify the risk of acquiring HIV. Given the open cohort design we expect that newly-recruited clients are especially important to reflect the overall incidence of infection in the community. Additionally, regarding the study of the determinants of infection, there seems to be no evidence to support that the magnitude or shape of associations will change substantially even if the background risk does.

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

Competing interests

No competing interests.

Author's contributions

PM drafted the manuscript and performed the descriptive 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.

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Table 1: Characteristics at entry of participants followed in the cohort.

PARTICIPANTS FOLLOWED-UP	**804**
BACKGROUND CHARACTERISTICS	
Age (years), mean (SD)	30.3 (8.9)
Sexual identity, n (%)	
Homosexual	692 (86.1)
Bisexual/Heterosexual/Other	109 (13.6)
Rather not answer	3 (0.4)
Country of origin, n (%)	
Portugal	575 (75.0)
Other country	190 (24.7)
Rather not answer	2 (0.3)
Educational level, n (%)	
Less than higher education (≤ 12 years of school)	317 (39.5)
Higher education (> 12 years of school)	483 (60.2)
Other/Rather not answer	3 (0.3)
HIV TESTING	
Previous HIV testing, n (%)	
No	115 (15.2)
Yes	636 (84.1)
Did not know	5 (0.7)
Number of previous tests, median (P25-P75)	4 (2-7)
Reasons for index test, n (%) (multiple options possible)	
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)
SEXUAL LIFE AND PARTNERS	
Age at first anal intercourse, median (P25-P75)	18.0 (16.0-21.0)
Role on anal intercourse, n (%)	
Only insertive	192 (24.1)
Only receptive	72 (9.0)
Versatile	525 (66.0)
Rather not answer	7 (0.9)
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)
Rather not answer	2 (0.2)
Men with different sex partners	
No	148 (18.7)
Yes	588 (74.2)

Did not know	54 (6.8)
Rather not answer	2 (0.3)
Sex workers (even if not payed)	
No	707 (89.4)
Yes	51 (6.4)
Did not know	31 (3.9)
Rather not answer	2 (0.3)
HIV positive men	
No	401 (50.7)
Yes	107 (13.5)
Did not know	281 (35.5)
Rather not answer	2 (0.3)
People who inject drugs	4 (0.5)
No	785 (99.2)
Yes	4 (0.5)
Did not know	
Rather not answer	2 (0.3)
Women	
No	690 (87.2)
Yes	99 (12.5)
Did not know	0
Rather not answer	2 (0.3)
Trios / Group sex	
No	563 (71.2)
Yes	224 (28.3)
Did not know	1 (0.1)
Rather not answer	3 (0.4)
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)
Rather not answer	2 (0.2)
HIV status of steady partner (n=501), n (%)	
HIV negative	310 (62.5)
HIV positive	46 (9.3)
Did not know	139 (28.0)
Rather not answer	1 (0.2)
Occasional partners in the previous 12 months, n (%)	713 (88.7)
No	89 (11.1)
Yes	713 (88.7)
Rather not answer	2 (0.2)
Number of occasional partners in the previous 12 months, median (P25-P75)	5 (2-10)
Having sex for money or drugs in the previous 12 months (n=713), n (%)	19 (2.7)
No	693 (97.3)
Yes	19 (2.7)
Venues used to meet occasional partners (n=713), n (%) (multiple options possible)	
Internet	522 (73.9)
Discos and Gay bars	359 (50.8)
Cruising sites	173 (24.5)

Saunas	142 (20.2)
Gymnasium	94 (13.4)
"Dark rooms" (including sex-shops)	49 (7.0)
Sex clubs	32 (4.5)
UNPROTECTED ANAL INTERCOURSE (UAI), n (%)	
UAI with a steady partner in the previous 12 months (n=501)	
No	130 (27.4)
Yes	344 (72.4)
Rather not say	1 (0.2)
UAI in the previous 12 months with a HIV-positive steady partner (n=46)	
No	26 (59.1)
Yes	18 (40.9)
UAI with occasional partners in the previous 12 months (n=713)	
No	375 (56.1)
Yes	292 (43.7)
Rather not say	1 (0.1)
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)
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)
SEXUALLY TRANSMITTED INFECTIONS, n (%)	
In the previous 12 months:	
Gonorrhea	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)

Abbreviations: HIV: Human Immunodeficiency Virus; UAI: Unprotected anal intercourse; PEP: Post-exposure prophylaxis.

Table 2: Comparison of follow-up time and number of visits between MSM who seroconverted and those who did not seroconverted.

	HIV-positive	HIV-negative	p-value*
N	25	779	
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

*p-value for independent samples Mann-Whitney test

Abbreviations: HIV: human immunodeficiency virus; SD: standard deviation.

Table 3: Predictors of HIV Incidence.

	HIV cases	PY	HIV Incidence	IRR (95% CI)	p-value	aIRR* (95%IC)	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		
1970-1979	4	245.75	1.6	1			
1980-1989	12	373.74	3.2	1.97 (0.64-6.12)	0.239		
1990 or after	4	164.63	2.4	1.49 (0.37-5.97)	0.571		
Country of birth							
Portugal	18	648.27	2.8	1			
Other	7	211.54	3.3	1.19 (0.50-2.85)	0.694		
Education (schooling years)							
Less than higher education (<= 12 years)	11	357.42	3.1	1.17 (0.53-2.58)	0.692		
Higher education (>12 years)	14	533.74	2.6	1			
Sexual identity							
Homosexual	22	789.81	2.8	1			
Bisexual/Heterosexual/Other	3	100.31	3.0	1.07 (0.32-3.59)	0.908		
HIV testing							
Number of HIV previous tests at cohort entry							
0	0	120.98	0.0	-			
1 a 5	14	476.42	2.9	1			
More than 5	10	234.47	4.3	1.45 (0.65-3.27)	0.368		

Reasons for HIV test during follow-up							
Concerned with exposure to HIV throughout follow-up							
Never	2	163.21	1.2				
At least once	22	716.67	3.1	2.51 (0.59-10.65)	0.214		
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	-			
Sexual life & partners							
Age at first anal intercourse							
More than 15	21	693.42	3.0	1			
15 or less	3	136.57	2.2	0.73 (0.22-2.43)	0.603		
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 on anal sex							
Insertive only	8	213.54	3.7	1			
Receptive/Both	17	658.75	2.6	0.69 (0.30-1.60)	0.385		
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	-			
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			
Changed: Yes to No	5	194.75	2.6	0.97 (0.23-4.05)	0.965		
Changed: No to Yes	2	85.45	2.3	0.88 (0.15-5.28)	0.891		
Persistent Yes	13	462.90	2.8	1.06 (0.30-3.72)	0.929		
Sex workers (even if not payed)							
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	-			
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			
Changed: Yes to No	0	129.60	0.0	-			
Changed: No to Yes	3	84.82	3.5	1.38 (0.39-4.85)	0.613		
Persistent Yes	7	134.39	5.2	2.04 (0.81-5.10)	0.129		
Steady partner during follow-up							
Persistent No	5	180.52	2.8	1			
Changed: Yes to No	2	192.56	1.0	0.38 (0.07-1.93)	0.241		
Changed: No to Yes	4	145.44	2.8	0.99 (0.27-3.70)	0.992		
Persistent Yes	13	360.75	3.6	1.30 (0.46-3.65)	0.617		
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			
Changed: Yes to No	2	146.52	1.4	0.28 (0.04-1.96)	0.198		
Changed: No to Yes	1	46.70	2.1	0.43 (0.04-4.78)	0.495		
Persistent Yes	18	644.85	2.8	0.56 (0.13-2.43)	0.443		
Number of occasional sexual partners in the previous 12 months at cohort entry							
<=1	3	125.50	2.4	1			
2 to 9	12	408.48	2.9	1.30 (0.35-4.36)	0.749		
>=10	6	242.20	2.5	1.03 (0.26-4.14)	0.960		
Having sex for money or drugs during follow-up							
Never	22	854.61	2.6	1			
At least once	1	21.84	4.6	1.78 (0.24-13.19)	0.573		
Unprotected anal intercourse (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	-			
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							
Discos & gay bars							
No	12	472.08	2.5	1			
Yes	13	410.22	3.2	1.25 (0.57-2.73)	0.582		
Saunas							
No	21	705.35	3.0	1			

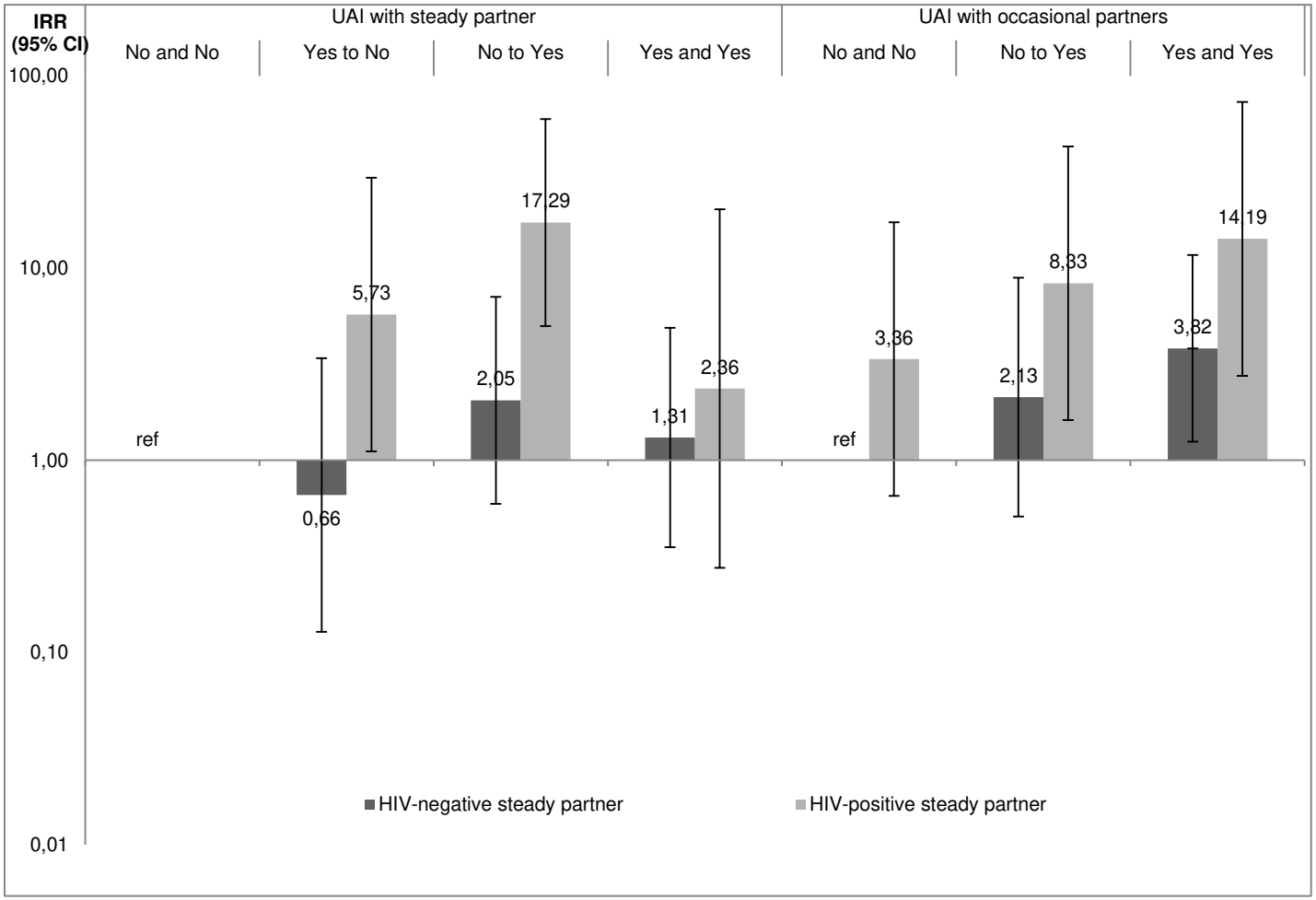
Yes	4	173.20	2.3	0.78 (0.27-2.26)	0.641		
"Dark rooms" (including sex-shops)							
No	24	815.21	2.9	1			
Yes	1	60.58	1.7	0.56 (0.08-4.14)	0.571		
Sex clubs							
No	24	840.34	2.9	1			
Yes	1	39.61	2.5	0.88 (0.12-6.53)	0.904		
Internet							
No	6	313.65	1.9	1			
Yes	19	567.72	3.3	1.75 (0.70-4.38)	0.232		
Cruising sites							
No	19	675.79	2.8	1			
Yes	6	204.17	2.9	1.05 (0.42-2.62)	0.925		
Gymnasium							
No	21	771.94	2.7	1			
Yes	4	103.01	3.9	1.43 (0.49-4.16)	0.514		
Sexually transmitted infections & 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			
Changed: Yes to No	0	25.77	0.0	-			
Changed: No to Yes	1	30.08	3.3	1.16 (0.16-8.56)	0.886		
Persistent Yes	0	0.00	-	-			
Lifetime history of Hepatitis C reported at cohort entry							
No/Does not know	25	874.81	2.9	1			

Yes	0	2.76	0	-			
Lifetime history of Hepatitis B reported at cohort entry							
No/Does not know	24	862.89	2.8	1			
Yes	1	20.64	4.8	1.74 (0.24-12.88)	0.587		
Drugs 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
Post-exposure prophylaxis at cohort entry							
Does not know	14	437.41	3.2	1			
Knows but never used	10	392.47	2.5	0.80 (0.35-1.79)	0.582		
Knows and used	1	21.15	4.7	1.48 (0.19-11.23)	0.706		

Abbreviations: HIV: human immunodeficiency virus; PY: person-years; IRR: incidence rate ratio; aIRR: adjusted incidence rate ratio; CI: confidence interval; UAI: unprotected anal intercourse.

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

Graph 1: Stratified analysis of the main determinants of HIV incidence by HIV status of steady partner



Abbreviations: IRR: incidence rate ratio; 95% CI: 95% confidence interval; UAI: unprotected anal intercourse; HIV: human immunodeficiency virus; ref: reference category

6. CONCLUSIONS

The setup of the Lisbon Cohort of MSM is a stepping-stone for the maintenance of a modern decentralised surveillance structure specifically addressing seronegative MSM in the context of HIV occurrence, risk factors, testing, and linkage to care.

Regionally, the follow-up of this cohort of HIV-negative MSM will be a valuable tool for the dynamic monitoring of HIV infection in a Southern European setting where limited prospective information existed. Within the international context, it will additionally allow for a deeper analytical approach on population time trends and individual changes in risk factors shaping the epidemic among MSM. Given our sampling frame, full generalizability regarding the whole MSM population is not expected. However, since selection bias is not expected to change substantially throughout the study period, we believe that our methodological option will not preclude the estimation of time trends in incidence estimates or of the determinants of seroconversion.

We found a first estimate of HIV incidence of 3 per 100 MSM-years a high HIV incidence. HIV epidemic in this cohort of Portuguese MSM likely to be driven by short-term contextual and behavioural changes during follow-up, namely, newly-adopted UAI with a steady partner, newly-disclosed HIV-positive partner and newly-diagnosed syphilis. Serodiscordant steady relationships played a major role in predicting HIV seroconversion as well as persistently reporting UAI with occasional partners.

Ultimately, study results are intended to inform on the real-world effectiveness of policies directed to reducing the burden of HIV among MSM.

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