Original Article

INCIDENCE OF HCV AND SEXUALLY TRANSMITTED DISEASES AMONG HIV POSITIVE MSM IN ANTWERP, BELGIUM, 2001-2011

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ABSTRACT

Recurrent Sexually Transmitted Infections (STIs) are an indication of unsafe sexual practices and may be associated with HCV-infection among HIV-positive men who have sex with men.

In a retrospective study we analysed the laboratory data of 99 HIV-positive MSM who acquired HCV during the observation period (cases) and 176 HIV-positive MSM who remained HCV negative during the observation period (controls), all followed at the HIV/STI-clinic in Antwerp, Belgium. All laboratory confirmed STI-episodes were recorded since the date of first consultation at our clinic, until the date of HCV-diagnosis of the cases.

The HCV incidence varied between 0.24 (2001) and 1.36 (2011) new cases per hundred person-years, with a peak of 2.93 new cases per hundred person-years in 2009.

The number of STI-episodes per person-year follow-up was significantly higher for the cases as compared to the controls for syphilis, non-LGV and LGV Chlamydia infections ($p < 0.005$). When considering the incidence of STIs that occurred 1 year prior to HCV conversion, all laboratory confirmed STIs remained more frequent among cases, but only the difference in syphilis incidence was statistically significant ($p < 0.01$).

Recurrent STIs among HIV positive MSM should be considered as a behavioural and biological risk factor for acquiring HCV and should lead to intensified screening for HCV and counselling of the patient.

Key words: HIV - HCV - STI - co-infecton - MSM

INTRODUCTION

Hepatitis-C-infection is a growing concern among MSM, since case control studies in the UK and retrospective analyses of a large HIV positive cohort in Amsterdam have indicated that HCV can be sexually transmitted (1, 2).

Over the past decade certain sexual practices and techniques have been identified as new routes of transmission, especially among HIV positive MSM: sex-associated rectal bleeding, receptive fisting and snorting cocaine/amphetamines, combined with group sex, were independently associated with HCV infection (3, 4). There is no evidence of heterosexual transmission among discordant, monogamous couples (5) provided none of the partners is HIV positive or suffers from another STI (6). HIV positivity is considered as the most important risk factor for transmission. A large cross-sectional study in Amsterdam reported that HIV-infected MSM were almost 43 times (95% CI 8.49-215.1) more likely to acquire HCV infection than HIV-uninfected MSM (7).

This has led to an epidemic of new infections in this specific risk group (8). The greatest increase was seen from 2002 onwards (9). In a cohort of HIV-infected MSM, at the Institute of Tropical Medicine in Antwerp, we saw an increase from 0.2 cases per 100 person years in 2001 to 2.9 in 2009 (10).

We calculated the most recent incidence rates for 2010 and 2011 and updated our figures in this paper. The second objective was to conduct a retrospective analysis of the incidence of 5 objectively verifiable STI's among HCV/HIV positive cases and HCV negative/HIV positive controls in a cohort of 1105 HIV-infected MSM, followed at the HIV/STI clinic of the Institute of Tropical Medicine in Antwerp, Belgium.

METHODS

Patients

At the end of 2011, 1105 MSM infected with HIV were regularly followed up, at the STI/HIV clinic of the Institute of Tropical Medicine, Antwerp. Regular follow-up includes a medical check-up by a physician and a laboratory check-up for viral load and CD4 cell count at least twice per year. In between these appointments, patients can present at the specialised clinic when they show signs and symptoms of other STIs, anytime when these occur.

Since 2006, with the rising suspicion of HCV transmission through homosexual contacts, all HIV positive MSM were routinely tested for HCV, at least yearly, more when the treating physician deemed it necessary. Before 2006, hepatitis C serology was only performed on request of the physician, when there was a clinical or laboratory or epidemiological indication.

For this study, we reviewed the files of all HIV-infected patients attending our centre who were diagnosed with a newly acquired HCV infection (= cases) between January 2000 and December 2011. Results of HCV incidence in our cohort from 2000 to 2009 have been previously reported (10). For the present study we kept our original case definition: a new case was considered as 'certain' if HCV-antibody seroconversion could be documented, meaning that a screening serological test AND a confirmation test were positive while this screening test had been found negative in the previous 24 months. A newly acquired HCV infection was considered as 'probable' if both screening and confirmation tests were found positive AND transaminase levels were elevated with exclusion of other causes of hepatitis, in a person with a negative anti-HCV result documented longer ago than the previous 24 months.

This case definition excluded patients newly diagnosed simultaneously with HIV and HCV infections and patients without any previous anti-HCV testing before HCV diagnosis. We also did not include the probable cases, in whom no HCV testing had occurred within the previous 24 months prior to HCV diagnosis.

Controls were patients that visited the clinic on the same day that the case was diagnosed. For each case 2 controls were chosen, i.c. HIV positive, HCV negative MSM, no further matching was done.

All laboratory confirmed STI-episodes (syphilis, gonorrhoea, lymphogranuloma venereum, non-LGV chlamydial infections, herpes simplex) were recorded since the date of first consultation at our clinic, until the date of HCV-diagnosis of the cases, or selection as a control.

Testing

The practice of syphilis testing underwent the same evolution: only on indication before 2006, routinely with every HIV check-up after 2006. All other STIs were diagnosed on clinical indication. Only laboratory confirmed STIs were included in this analysis: STI episodes that were only based on symptoms, but that were not confirmed through lab tests were excluded. Routine HIV follow-up is done only on appointment, but the clinic also offers a drop-in centre, in which patients are seen by a doctor for any problem that is related to HIV and/or STIs. Acute STI problems are typically seen in this drop-in centre. All STI episodes were treated according to CDC treatment guidelines.

Laboratory procedures

STI episodes were defined on the basis of laboratory results: the screening HCV test used throughout the study period was Vitros ECI Immunodiagnostic System (Ortho Clinical Diagnostics, Rochester, USA) and the confirmation test was INNO-Line Immuno Assay HCV Score (Innogenetics, Belgium). Qualitative determination of HCV-RNA was performed in each patient found with positive confirmation test (COBAS Amplicor HCV test, version 2.0, Roche Diagnostics, England; limit of detection 50 IU/mL), and if this test was positive, HCV-RNA was quantified by PCR (COBAS Amplicor HCV Monitor Test, version 2.0, Roche Diagnostics, England; limit of detection 600 IU/mL). Since November 2006, HCV-RNA was determined by the use of Abott RealTime HCV assay (Abott Park, USA). Viral genotyping was performed at baseline by line probe assay (first INNO-LiPA, Innogenetics, Belgium, and since 2006 Versant HCV genotype 2.0 assay LiPA, Siemens, Germany).

Syphilis was diagnosed by using both the Rapid Plasma Reagin (Macro-Vue RPR card test (Becton Dickinson BD Microbiology systems, USA) and the Treponema pallidum particle agglutination test (Serodia-TPPA, Fujirebio, Tokyo, Japan). A primo-infection was considered in case of a seroconversion of both the nontreponemal and treponemal antibodies and a re-infection was defined by a fourfold or greater increase of RPR-titer in the presence of pre-existing treponemal antibodies. N. gonorrhoea was cultured on GC-Lect TM agar plates (Becton-Dickinson, USA).

To detect *C. trachomatis* DNA in clinical samples Nucleic Acid Amplification Tests (NAATs) were used. During the period of 2000-2011 different assays in a testing algorithm were used. All clinical samples were first tested with a commercial assay which was the BD ProbeTec ET assay (Becton Dickinson, Sparks, USA) till 2007. From 2007 and from 2010, the Gen-Probe Aptima assay (Gen-Probe, San Diego, USA) and Abbott Realtime CT/NG (Abbott Molecular, Des Plaines, USA) has been used, respectively. Positive results were confirmed with a second NAAT which was the Amplcor CT/NG assay (Roche molecular Systems, Branchburg,USA) till 2010. Since then an in-house Real Time-Polymerase Chain Reaction (RT-PCR) assay based on the publication by Chen et al was used (11). Besides the confirmation of *C. trachomatis* the RT-PCR distinguishes the *C. trachomatis* L serovar from the non L serovars. Before 2010 the L serovar types were identified by RFLP. A target of 1050 bp of the *omp1 gene* was amplified by nested PCR. The amplicons were digested by the restriction enzymes; Alu I, BstU I, CFO I, EcoR I/Dde I, and Hinf I, r and the fragments were separated on a polyacrylamide gel.

In-House PCR assays were performed to detect Herpes Simplex viruses (HSV). For screening the specimens were tested by a PCR as described by Lakeman et al (12). The specimens containing HSV DNA were then retested with a PCR which differentiated HSV-1 from HSV-2 DNA (13) (Fang et al.) Since 2010 the specimens were tested using a commercial assay, the Argene Herpes Simplex Real-Time (Argene, Verniolle, France).

Statistical analysis

Because HCV is a relatively rare event, a case-control design was used. In a first analysis, all newly HCV infections that fulfilled the case definition of a new HCV infection between the period of January 2000 and December 2011 were extracted from the clinic database of HIV-positive MSM.

Poisson regression was used to assess the difference in STI episodes between cases and controls. In a second analysis, the time at risk for STI episodes was limited from 1 year prior to the HCV infection of the case to the day of the HCV infection itself (for both cases and controls), and was expressed as person months.

Incidence rates for the exposure of interest were expressed as number of STI episodes per 100 person-years (for the whole period) or person-months (for the one year analysis) of follow-up. Incidence ratios together with 95% confidence intervals were reported for the five STIs examined separately and for the total number of STI episodes. *p*-values below 0.05 were considered statistically significant.

Ethical considerations

All patients followed at the STI/HIV clinic are asked to sign a general consent form with regard to the use of laboratory data and data retrievable from their medical file. The ethical review board of the institute decided that this general consent form covered the data that are presented here.

RESULTS

Characteristics at inclusion

Ninety nine patients fulfilled the case definition. Twenty two of 198 selected controls were rejected because the HIV infection occurred after the date of the HCV infection of the case, leaving 176 files for further analysis (Figure 1).

There was no difference between the cases and the controls in terms of age, proportion of patients on HAART, proportion of patients that had a detectable HIV viral load, or the CD4 cell count at the time of inclusion (Table 1). Cases however were significantly more tested for HCV serology, in the year prior to the diagnosis, as compared to the controls: 1.6 times for the cases and 0.8 times for the controls ($p < 0.001$).

Incidence of HCV infection

During the study period, a total of 99 incident cases of certain new HCV infections among the study population were diagnosed.

Taking the total number of HIV positive MSM followed at the clinic at the end of each year as the denominator, this led to an incidence between 0.24 (2001) and 1.36 (2011) new cases per hundred person-years (table 2), with a peak of 2.93 new cases per hundred person-years in 2009 (Figure 2).

Incidence of STIs during the whole period of follow-up

We observed 175 periods of syphilis, 39 periods of gonorrhoea, 13 periods of LGV, 47 periods of rectal or urethral non-LGV Chlamydia trachomatis, 21 periods of HSV, during 467.5 and 850.5 person-years of follow-up of the cases and controls respectively. The total number of STI episodes prior to acquisition of HCV, per person-year follow-up, was significantly higher for the cases as compared to the controls ($p < 0.001$). When considering each STI separately, all five diseases were observed more frequently among cases. This difference was

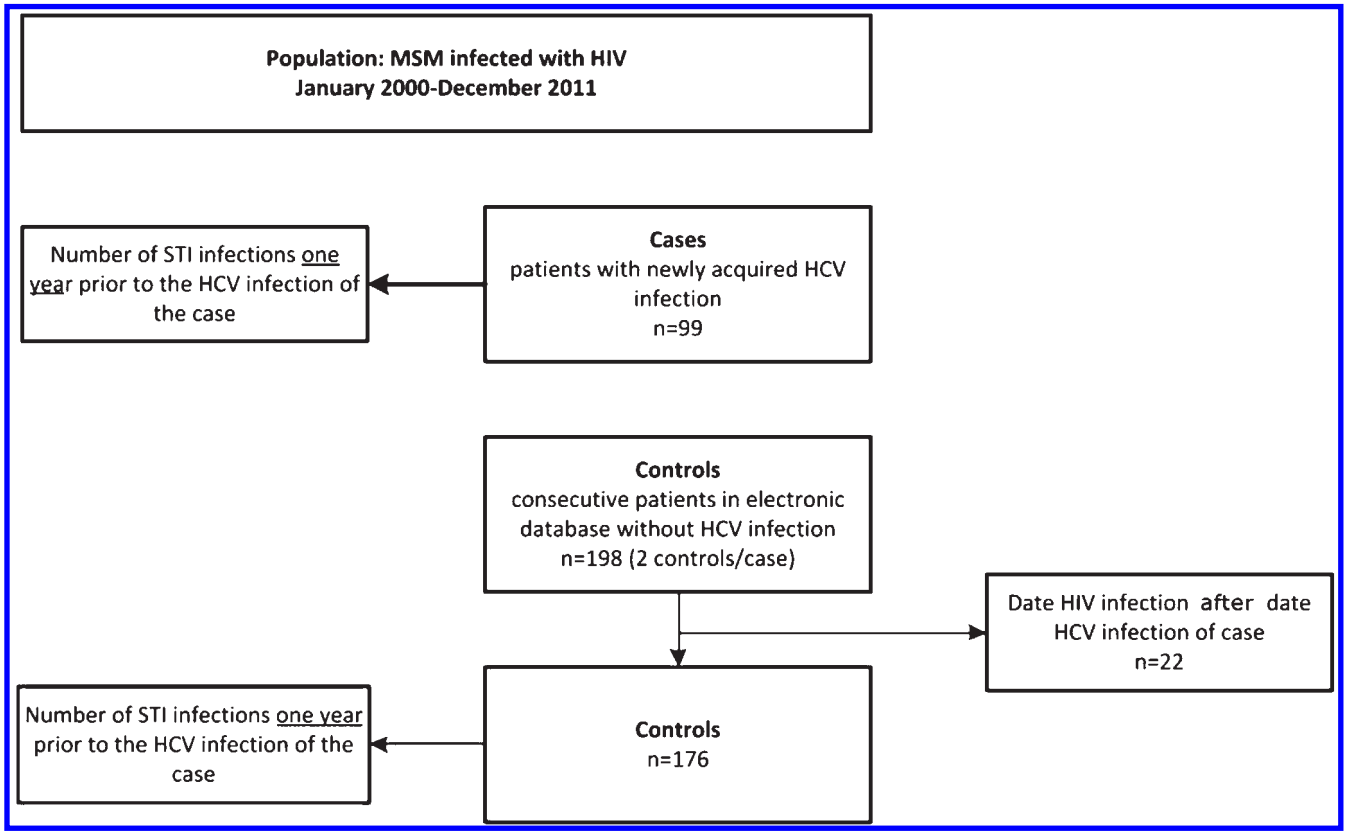

Figure 1: *Patient accounting and design.*

Table 1: Characteristics at inclusion

	Total n = 275	Cases n = 99	Controls n = 176	*p*-value
Age (years): mean (SD)	40.8 (8.6)	41.0 (8.1)	40.7 (8.9)	0.839
HCV tests performed(/patient), mean (range)	1.0 (0-5)	1.6 (0 -5)	0.8 (0-5)	< 0.001
On HAART, n(%)	140 (50.9)	51 (51.5)	89 (50.6)	0.901
CD4 cell count (cells/μL), median (IQR)	499 (343-707)	477 (362-726)	504 (336-686)	0.668
Detectable HIV viral load (VL > 50 copies/mL), n (%)	127 (55.7)	50 (54.4)	77 (56.6)	0.786

Table 2: Newly diagnosed HCV cases among HIV positive MSM: 2000-2011

Year	Total number of HIV + patients in follow-up	Number of HIV + MSM in follow-up	Number of HCV + among HIV + MSM	% HCV among HIV + MSM
2000	810	373	0	0.00
2001	915	418	1	0.24
2002	990	450	2	0.44
2003	1147	508	1	0.20
2004	1235	539	3	0.56
2005	1333	607	3	0.49
2006*	1411	686	9	1.31
2007	1539	756	10	1.32
2008	1698	858	13	1.52
2009	1812	922	27	2.93
2010	1996	1000	16	1.60
2011	2147	1105	15	1.36

*Introduction of routine HCV testing, before on clinical suspicion only

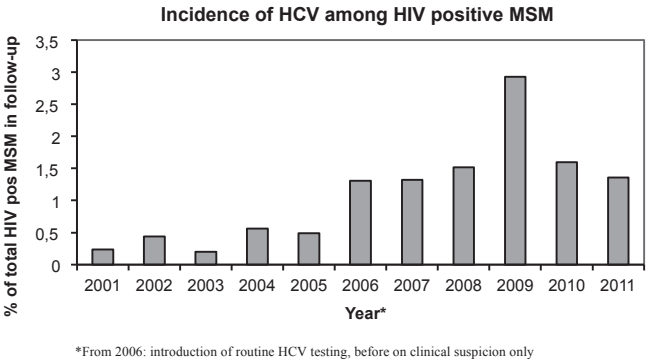

Figure 2: *Incidence of HCV among HIV positive MSM: 2000-2011*
**From 2006: introduction of routine HCV testing, before on clinical suspicion only .*

statistically significant for syphilis, non-LGV and LGV Chlamydia infections ($p < 0.05$) (Table 3).

Incidence of STIs 1 year prior to HCV conversion

We observed 49 periods of syphilis, 9 periods of gonorrhoea, 4 periods of LGV, 12 periods of rectal or urethral non-LGV Chlamydia trachomatis, 4 periods of HSV during 961.8 and 1871.3 person-months of follow-up of the cases and controls respectively. The total number of STI episodes one year prior to acquisition of HCV, per person-month of follow-up, remained significantly higher for the cases as compared to the controls ($p < 0.001$). When considering each STI separately, all five diseases were observed more frequently among cases. This difference remained statistically significant for syphilis only ($p < 0.01$) (Table 4).

DISCUSSION

Sexual transmission of HCV is now widely accepted as a mode of getting infected with this virus, predominantly among HIV co-infected patients, and episodes of STI's have been consistently observed prior to HCV seroconversion. In this retrospective analysis we looked at a cohort of well over 1000 HIV positive MSM that are at least twice a year routinely seen at the HIV/STI clinic of the Institute of Tropical Medicine in Antwerp, as part of their HIV care. Patients are encouraged to have a general practitioner for first line health services. As such, it is not proven that all STIs that occurred among the study population during the study period were notified in the clinic. On the other hand, HIV positive patients are often reluctant to discuss issues related to their sexual health with their GP (14). This is a limitation of the study that may have led to an underestimate of the true incidence of STIs, except hepatitis C and syphilis, of which the changed serology shows a retrospective diagnosis. We have no reasons to assume that this underreporting of Chlamydia infections, gonorrhoeae and/or herpes infections would differ among the cases and the controls.

HCV Incidence

The incidence of HCV among HIV positive MSM ranged between 0.20% (2003) and 2.93% (2009). A sharp increase in incidence was observed in 2006. The diagnosis may have been missed more frequently in the first years of the study period since HCV testing was performed on clinical suspicion only at that time. Later on, awareness of ongoing outbreaks in neighbouring cities prompted our HIV physicians to perform a standard baseline HCV screening and to re-test regularly MSM at risk,

Table 3: Risk factors for HCV infection in HIV+MSM: STI incidence during total period of follow-up, prior to HCV seroconversion

	Total number of patients n=275	Cases n=99	Controls n=176	Incidence ratio (95% CI)	*p*-value
Total number of syphilis episodes	175	96	79		
Number of syphilis episodes/100 person-years	13.3	20.5	9.2	2.2 (1.6-3.0)	< 0.001
Total number of gonorrhoea episodes	39	18	21		
Number of gonorrhoea episodes/100 person-years	3.0	3.8	2.4	1.6 (0.8-2.9)	0.167
Total number of non-LGV Chlamydia trachomatis episodes	47	24	23		
Number of non-LGV Chlamydia trachomatis episodes/100 patient-years	3.6	5.1	2.7	1.9 (1.1-3.4)	0.028
Total number of LGV Chlamydia trachomatis episodes	13	10	3		
Number of LGV Chlamydia trachomatis episodes/100 patient-years	1.0	2.1	0.3	6.1 (1.7-22.0)	0.006
Total number of HSV infections	21	9	12		
Number of HSV episodes/100 patient-years	1.6	1.9	1.4	1.4 (0.5-3.5)	0.481
Total number of STI infections	295	157	138		
Person-years of follow-up	1318	467.5	850.5		
Number of STI episodes/100 person-years	22.4	33.6	16.2	2.1 (1.6-2.6)	< 0.001

Table 4: Risk factors for HCV infection in HIV+MSM: STI incidence 1 year prior to HCV seroconversion

	Total number of patients n=275	Cases n=99	Controls n=176	Incidence ratio (95% CI)	*p*-value
Total number of syphilis episodes	49	33	16		
Number of syphilis episodes/100 person-months	1.7	3.4	0.9	4.0 (2.1-7.8)	< 0.001
Total number of gonorrhoea episodes	9	5	4		
Number of gonorrhoea o episodes/100 person- months	0.3	0.5	0.2	2.4 (0.5-12.3)	0.185
Total number of non-LGV Chlamydia trachomatis episodes	4	4	0		
Number of non-LGV Chlamydia trachomatis episodes/ 100 patient-months	1.4	4.2	0	/	/
Total number of LGV Chlamydia trachomatis episodes	12	7	5		
Number of LGV Chlamydia trachomatis episodes/100 patient- months	4.2	7.3	2.7	2.7 (0.7-10.9)	0.087
Total number of HSV infections	4	2	2		
Number of HSV episodes/100 patient-months	0.1	0.2	0.1	1.9 (0.1-26.8)	0.506
Total number of STI infections	78	51	27		
Person-months of follow-up	2833.1	961.8	1871.3		
Number of STI episodes/100 person-months	2.8	5.3	1.4	3.7 (2.3-6.1)	< 0.001

and systematically after a STI had been diagnosed. The figures reported in the second half of the decade are more likely to reflect the true incidence in this cohort, with maybe a marginal catch-up effect. The catastrophic incidence that was observed in 2009 prompted us to alert the medical fraternity and the prevention organisations, and through the latter, the risk groups at large, as described previously (10). In how far this led to the –so far- reduced incidence in 2010 and 2011 can not be deducted from the figures.

Of note also is that up till the writing of the paper no new HCV infections were diagnosed in HIV negative MSM who attend the clinic for STI-screening.

Incidence of other STIs

A strong association has been described between STIs and acquisition of HCV[15]. In this cohort, we have previously reported the occurrence of STIs, six months prior to HCV seroconversion: 50% of the 69 cases detected at the end of 2009 had a proven syphilis infection in that period (10).

For this study we first looked at the whole period of follow up, and observed a significantly higher incidence amongst HCV cases as compared to controls. Two conclusions press forward, one behavioural, one biological: the higher incidence among cases is an indicator of higher risky sexual behaviour as compared to controls. Secondly, STIs may lead to a higher probability of transmission due to mucosal damage, ulcers and/or inflammation. The latter is illustrated by the also significantly higher incidence of STI's among cases, if limited to one year prior to the HCV seroconversion. It was only syphilis however, that was significantly more frequent. In a recent study in Taiwan in a similar cohort, recent syphilis was also identified as an independent risk factor associated with HCV seroconversion (15).

All patients in this cohort are HIV positive, and are necessarily aware of their infectiousness. They are seen at 3 to 4

monthly intervals by at least a physician, but if needed, they can be referred to a member of a multidisciplinary team consisting of social nurses, a psychologist and a sexologist. If condom use is a problem, the patient is offered the possibility of an intensive programme of DVD assisted counselling sessions (computerised intervention for safer sex).

Still 295 STI episodes were diagnosed during 1318 person-years of follow-up. This is alarming. The availability of Highly Active Antiretroviral Treatment (HAART) has been associated with increased sexual risk behaviour (16). The proportion of patients on HAART was similar in cases and in controls in our study and hence may not have contributed to increased high sexual risk behaviour.

In a recent review, Tohme et al (17) identified HIV infection as the real risk for sexual transmission of HCV, especially unprotected anal intercourse. The role of the gastrointestinal tract is well established in the pathogenesis and transmission of HIV-infection (18). Studies reported partial restoration of epithelial barrier function, central memory T cells, Th17 cells, and polyfunctional T-cell responses in patients on long-term suppressive HAART. Early treatment is associated with less severe tissue damage and more significant repair and T-cell reconstitution.

Serosorting (practicing unprotected anal intercourse with partners of the same HIV serostatus), a widely applied practice among HIV positive people, increased the risk of STIs in a recent study in Germany by a factor three (19). Serosorting was found to be considered as 'protective' behaviour by 39.1% of MSM in San Francisco (20). In the same cohort, 51.1% of HIV-positive MSM were involved in serosorting, as compared to 40.5% of HIV negative MSM (21). This so-called seroadaptive behaviour can explain the high rate of STIs among this cohort of HIV patients, eventually leading to HCV infection among those patients with the highest rates of STIs.

In this article we have focussed on the incidence of HCV in a cohort of HIV positive MSM in an HIV/STI clinic. The trend we observe over the last two years is a stabilisation of the epidemic. We demonstrated that incidence of STIs among the HIV/HCV co-infected patients, prior to HCV acquisition was higher than among HIV mono-infected controls, significantly so for non-LGV and LGV Chlamydia infections and for syphilis.

Behavioural factors specifically leading to acquisition of syphilis and LGV as well as biological features linked to any of these conditions appear to substantially contribute to HCV transmission in the HIV-infected MSM population.

This study was presented as a poster at the International Conference of the International Society for Sexually Transmitted Diseases Research at Québec, 2011.

No sponsorship was sought to carry out this research.

CONFLICT OF INTEREST: None

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