Lack of decline in hepatitis C virus incidence among HIV-positive men who have sex with men during 1990–2014

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Abstract

Background: Hepatitis C virus (HCV) incidence among HIV-positive men who have sex with men (MSM) has increased since 2000, although there are regional differences. We aimed to 1) estimate trends in HCV incidence among HIV-positive MSM, 2) assess the association between incidence and geographical region, age and HIV-related measurements and, 3) assess temporal changes in time from HIV seroconversion to HCV infection.

Methods: Data was used from MSM with well-estimated dates of HIV seroconversion from the CASCADE Collaboration (1990-2014). Smoothly varying trends in HCV incidence over time were allowed, using restricted cubic splines. The association of calendar year, age, CD4 count (lagged), HIV RNA (lagged), geographical region and HIV infection stage (recent vs. chronic) with HCV incidence were assessed using Poisson regression.

Results: Of 5,941 MSM, 337 acquired HCV during follow-up. HCV incidence significantly increased from 0.7/1000 person-years in 1990 to 18/1000 person-years in 2014. Recent calendar years, younger age, recent HIV infection and higher HIV RNA levels were significantly associated with HCV incidence, while CD4 count was not. Trends differed by geographical region; while incidence appeared to have stabilized in Western Europe and remained stable in Southern Europe, it continued to increase in Northern Europe in recent years. Time from HIV to HCV infection significantly decreased over time ($p < 0.001$).

Conclusions: HCV has continued to spread among HIV-positive MSM in recent years, but trends differ by geographical region. Interventions to decrease the risk of HCV acquisition and increase early diagnosis are warranted.

Lay summary: Hepatitis C virus infection continues to spread among HIV-positive men who have sex with men, especially among younger individuals. However, trends seem to differ by European region in recent years. Furthermore, men who have sex with men with a higher HIV RNA load were more likely to get infected with the hepatitis C virus. During recent HIV infection, MSM appear to be at higher risk of acquiring hepatitis C.

Introduction

Since 2000, hepatitis C virus (HCV) incidence has increased among HIV-positive men who have sex with men (MSM) [1,2]. Using data from the CASCADE Collaboration (Concerted Action on SeroConversion to AIDS and Death in Europe) in EuroCoord, we previously showed that HCV incidence increased in MSM with well-estimated HIV seroconversion dates after 1990, but the main expansion of the HCV epidemic was observed from 2002 until 2007, the censoring date of the analysis [1]. A recent meta-analysis showed that HCV incidence has continued to increase, with an estimated pooled incidence of 13/1000 person-years (py) in 2010 to an extrapolated incidence estimate of 19/1000 py in 2015 [2]. However, other studies have shown varying trends in HCV incidence among MSM over the past years [3,4]. In Amsterdam, the Netherlands, HCV incidence seems to be stabilizing [3], whereas in Switzerland an increasing incidence among MSM has been observed [4].

A number of factors such as fisting, the presence of sexually transmitted infections (STIs), use of recreational drugs, and condomless anal intercourse have been shown to be significantly associated with acute HCV infection [4-10]. In addition, one study from the US reported that older age was independently associated with an acquired HCV infection [10], whereas another study from the Netherlands reported that younger MSM had a higher risk [3]. As acute HCV infections are predominantly found among HIV-positive MSM, it has been suggested that HIV facilitates sexual transmission of HCV [11]. However, contrasting results on the association between $CD4^+$ T-cell count (CD4 count) and HCV incidence have been reported [4,9,10,12]. Additionally, few studies have investigated the association with HIV RNA and, those that have, either dichotomized HIV RNA and/or could only assess the association in univariable analyses [4,9,12]. The role that HIV-related factors play in the spread of HCV among HIV-positive MSM is currently still being debated.

Using data among MSM with well-estimated dates of HIV seroconversion from the CASCADE Collaboration we aimed to 1) update trends in HCV incidence; overall and by geographical region, 2) assess the associations between HCV incidence and HIV-related measurements, geographical region, age and calendar year, and 3) assess whether the time interval between HIV seroconversion and HCV infection has changed over calendar time.

Methods

We used data from 16 out of 28 cohorts from the CASCADE Collaboration across Europe, Australia and Canada. Of the excluded cohorts, five were non-MSM cohorts and six cohorts had tested less than 50% of MSM for HCV and could not provide stored samples for HCV testing (missing HCV status data from 57.2% to 96.2%) (Fig. 1). The Kenyan cohort (IAVI; n=92) was also excluded as we believe that the HCV epidemic among MSM in Kenya differs from that in high-income countries (no incident HCV infections were observed). All cohorts include data from HIV-positive individuals with dates of HIV seroconversion that could be reliably estimated based on the midpoint between the last HIV-negative and first HIV-positive test (at most 36 months apart) or, evidence of acute HIV infection. Details of CASCADE have been previously described [13]. We only included men from the 16 cohorts who were recorded as having acquired HIV through sex between men and whose potential HIV transmission route excluded injecting drug use. For all cohorts, we used all available data, except for MSM from the French PRIMO cohort who were censored at the 31st of December 2005 as routine HCV testing was only recorded until that year. All collaborating cohorts received approval from their regulatory or national ethic boards (see Appendix) and informed consent was obtained for all participants.

HCV negative status throughout follow-up was based on at least one HCV-negative test result and never testing HCV positive. HCV infection was based on any positive HCV test (RNA, antibodies and/or antigen). Among MSM who acquired HCV during follow-up, the date of HCV infection was estimated as the midpoint between the last HCV-negative and first HCV-positive test. To optimize testing frequency, we performed additional HCV testing in cohorts that had stored specimens (8 cohorts). Stored samples from HCV-negative MSM were tested using a sample closest to the date of their last clinic visit if more than 2 years had elapsed since their last HCV-negative test date. For HCV-positive MSM without a previous HCV-negative test date, the sample closest to HIV seroconversion but up to one year of it was tested to assess whether they had become HCV infected during follow-up; if HCV negative, midpoint samples were tested until the HCV seroconversion interval was a maximum of 2 years. For MSM with a recorded HCV infection during follow-up but with an HCV test interval >2 years, samples with dates which fell in the interval between their last HCV-negative and first HCV-positive test date were tested. All cohorts provided a date of start of

routine HCV testing (defined by testing of all MSM for HCV according to prevailing guidelines or practices) and details on HCV testing strategies (e.g. retrospective testing).

HCV incidence

We estimated overall HCV incidence trends between 1990 and 2014 and stratified by European geographical region between 1997 until 2013 as not all regions have available data for the total study period. Geographical region was defined based on the United Nations classification criteria [14], namely Western (the Netherlands, Switzerland, France, Austria and Germany), Northern (United Kingdom and Norway), and Southern Europe (Italy, Spain and Greece), North America (Canada) and Australia and New Zealand (Australia) (Table 1). We only illustrate HCV incidence by geographic region for the three European regions as Canada and Australia had relatively small numbers of MSM and few HCV infections were observed. MSM were considered at risk from the latest of: HIV seroconversion, routine HCV testing date per cohort or enrolment in the cohort (Table 1). We used two methods to calculate follow-up time as previously described [1]. In both methods, MSM with one or more HCV-positive tests but without a previous HCV-negative test were excluded (Fig. 1). In method 1, follow-up time began from the moment MSM were considered at risk and will likely underestimate HCV incidence as some of the excluded MSM, who only had HCV-positive tests under active follow-up, could have become infected between the moment they were considered at risk and their first HCV test. Recognizing this possible underestimation, we applied another method (method 2) where follow-up began from the first HCV-negative test after becoming at risk (i.e. left truncation). This approach, however, leads to a shorter follow-up time for MSM who remained HCV-negative throughout follow-up as they are less likely to have been tested retrospectively compared to MSM who became HCV-positive. Consequently, this method is likely to overestimate HCV incidence. In both methods follow-up was calculated until the last HCV-negative date or, in case of HCV infection, the midpoint date. Only the first observed HCV infection during follow-up within an individual was included in the analyses. We used Poisson regression models where HCV incidence was allowed to vary smoothly over calendar time using restricted cubic splines for the overall and the stratified analyses (i.e. by geographical region). We performed a

sensitivity analysis using an interval-censored approach as previously described [1] (Please see the Supplementary material (Text 1) for further details).

HCV risk factor analyses

We used three Poisson regression models that included calendar year using the method 1 approach to calculate follow-up. We assessed variation of HCV incidence by geographical region (model 1) and the associations with age (model 2), and HIV-related measurements: CD4 count, HIV RNA and HIV infection stage (model 3). All continuous variables were included as restricted cubic splines (calendar year, current age, $\log_{10}$ HIV RNA and cube root CD4 count). The knots were chosen based on the 2.5, 25, 50, 75 and 97.5 percentiles.

Model 1

We compared the fit of three submodels by means of the Akaike information criterion (AIC): model 1.1, calendar year only; model 1.2, calendar year and region as main effects; model 1.3, calendar year, region, and their interaction.

Model 2

We then added age to the best fitting model 1. In this model, we tested the interaction between age and both region and calendar year. Significant interactions were included in this model.

Model 3

This multivariable model included: age, calendar year, region, HIV RNA and CD4 count. The CD4 count and HIV RNA value from the previous visit were used, but had to be no more than one year before. Missing HIV RNA and CD4 count data were imputed based on individual predicted values from random-effects models adjusted for age and stratified by combination antiretroviral therapy (cART) use: treatment naïve, on cART, and during cART interruption among cART-experienced (Supplementary text 2). For this model we defined a treatment interruption as a stop of cART for >1 week. When a person had no CD4/HIV RNA values throughout follow-up, we used the predicted values based on the fixed effects. We defined cART as a 3 drug ART regimen containing two different classes, or three nucleoside reverse transcriptase inhibitors, provided tenofovir or abacavir were included in the regimen. In additional analyses we assessed whether a recent HIV infection (defined as the period from estimated HIV seroconversion to less than 0.7 years hereafter) was associated with HCV incidence using model 3. We also tested the interaction between

HIV RNA and HIV infection stage (recent vs. chronic). We used the likelihood ratio test to test significance in model 2 and 3. Instead of reporting incidence ratios, we illustrate the association between age, CD4 count and HIV RNA and incidence by plotting the absolute incidence with 95% confidence intervals, choosing representative values (e.g. median values) for the other covariates.

Sensitivity analyses

We performed four sensitivity analyses. As we imputed missing CD4 and HIV RNA values, first, we performed the analyses using predicted values instead of using a combination of predicted and observed values. Second, we performed a complete case analyses in which only observed values were included. Third, an analysis was performed where the antepenultimate CD4 count and HIV RNA value were used. The reason for the third analysis is that antibody development might be delayed in HIV-positive individuals [15,16] and in our study 83.4% (n=281) of HCV infections were based on HCV antibody seroconversion and 15.7% (n=53) were based on a positive HCV RNA test and an HCV-antibody negative test result. Lastly, although additional HCV testing was performed in the Italian cohort (ICoNA), we performed the overall HCV incidence analyses without this cohort as currently there is no routine HCV testing in place.

Time from HIV to HCV

Kaplan-Meier curves were constructed applying the method 1 follow-up calculation to compare cumulative HCV incidences by calendar period of HIV seroconversion. We modelled whether HCV incidence depended on calendar year using a Cox proportional hazards model, including calendar year of HIV seroconversion as a continuous variable using restricted cubic splines.

Statistical analyses were performed using R [17] and Stata [18] software.

Results

Of 17,429 HIV-positive MSM, 7,368 MSM were excluded from six cohorts with more than 50% missing HCV status data and that could not provide stored samples for HCV testing (Fig. 1). Of the remaining 10,061 MSM, 9,014 had at least one HCV test result of whom 8,311 tested only HCV negative and 703 had at least one HCV-positive test result. MSM with HCV test results did not differ by ethnicity from MSM without test results, but were more likely to have a post-secondary education (37% vs. 32%). The median and mean number of HCV tests during follow-up among cohorts that routinely and prospectively collected HCV data (n=13) was 3.0 (Interquartile range (IQR)=2-6) and 4.1 (Standard deviation=3.6), respectively. A total of 7,864 MSM had follow-up and at least one HCV test result (Table 1). Among these MSM, 57.0% were white and median age was 34 years (IQR=28-41) at inclusion. The median year of HIV seroconversion was 2004 (IQR=1999-2008). Over the total study period, the median observed CD4 count was 509 cells/µl (IQR=367-684), median observed HIV RNA was 70 copies/ml (IQR=50-15522) and 70.3% started or were on cART.

A total of 5,941 and 4,326 MSM were eligible according to method 1 and 2, respectively (Fig. 1;Table 1). These MSM accounted for a total of 28,600 and 19,480 py and 337 and 279 HCV infections in method 1 and 2, respectively. The median follow-up time was 4.0 (IQR=1.7-7.2) and 3.9 (IQR=2.0-6.3) years in method 1 and 2, respectively. Of the 337 incident HCV infection observed during follow-up, 25 (7.4%) occurred during recent HIV infection.

HCV incidence

HCV incidence significantly increased from 1990 onwards ($p_{method1}<0.001$;$p_{method2}=0.04$); with an estimated incidence ranging from: 0.7/1000 py (95% confidence interval (CI)=0.1-5) in 1990 to 18/1000 py (95%CI=9-37) in 2014 in method 1 and from 3/1000 py (95%CI=0.4-18) in 1990 to 21/1000 py (95%CI=10-42) in 2014 in method 2 (Fig. 2). The interval-censored method showed a similar increasing trend (Supplementary Fig. 1). Excluding one cohort (ICoNA) from the overall analyses, led to similar statistically significant increasing trends by both methods, although the estimations were slightly lower (Supplementary Fig. 2). The stratified analyses by geographical region showed that in recent years HCV incidence seems to have increased in Northern Europe, but calendar year was only statistically

significant in method 2 (p=0.02) (Fig. 3). In Southern Europe, a stable trend was observed and calendar year was not significant. In Western Europe the trend was significant in both methods ($p_{method1}$=0.001;$p_{method2}$=0.005); based on method 1, HCV incidence increased sharply from 14/1000 py (95%CI=10-20) in 2006 to 23/1000 py (95%CI=17-31) in 2009, but declined thereafter to 9/1000 py (95%CI=3-27) in 2013 (Fig. 3).

HCV risk factor analyses

The first analysis showed that the model with region and calendar year as main effects only (model 1.2) had the lowest AIC of the three submodels, thus the best fit.

The second model showed that younger HIV-positive MSM had a higher risk of HCV infection (p=0.005) (Fig. 4A). The interaction term between age and region was borderline significant (p=0.05). Based on the model with the interaction term, in Western Europe, HCV incidence remained highest and stable until around age 35 and declined thereafter (Supplementary Fig. 3). In Northern and Southern Europe, HCV incidence increased until age 35, and declined thereafter.

In the third model, a higher HIV RNA was associated with higher HCV incidence (p=0.001) (Fig. 4C), especially when $\log_{10}$ HIV RNA was ≥5 copies/ml, whereas CD4 count (Fig. 4B) was not (p=0.53). When we added “HIV infection stage” to the model, the association between HIV RNA and HCV incidence was attenuated (p=0.01) (Fig. 4D). HCV incidence was higher during recent HIV infection than during chronic HIV infection (Incidence Rate $Ratio_{recent\ vs.\ chronic}$=1.8, 95%CI=1.1-2.7, p=0.02). The interaction term between HIV infection stage and HIV RNA was not significant (p=0.60), and was left out of the model. The association with CD4 count remained non-significant (p=0.53).

Sensitivity analyses

All sensitivity analyses showed comparable associations of HIV RNA, CD4 count and calendar year with HCV incidence and the conclusions were not altered. However, in the complete case analyses, HIV RNA was non-significant (p=0.25) (Supplementary Fig. 4).

In the model that included HIV infection stage, two sensitivity analyses (i.e. antepenultimate and predicted values) showed comparable associations between HIV RNA and HCV incidence, but when antepenultimate HIV RNA values were used, the

association was no longer statistically significant (p=0.09). In the complete case analyses, there was no association (p=0.40).

Time from HIV to HCV

Among 5,680 MSM who seroconverted for HIV at or after 1990, median time from HIV seroconversion to HCV infection was 5.2 years. The time from HIV seroconversion until HCV infection significantly decreased over calendar periods ($p_{log\text{-}rank}$<0.001). At 3 years after HIV seroconversion, the cumulative HCV incidence was 5.9% (95%CI=3.8-9.2%) in 2010-2014 compared to 2.0% (95%CI=0.5-7.8%) in 1990-1994 (Fig. 5). The Cox model showed that MSM who seroconverted for HIV in 2010, had a 6.1 (95%CI=2.8-13.3) times higher hazard of acquiring HCV than MSM who seroconverted in 1990 (p<0.001) (Supplementary Fig. 5).

Discussion

Using data from the CASCADE Collaboration among HIV-positive MSM with well-estimated dates of HIV seroconversion, we showed that HCV incidence significantly increased from 1990 onwards and no decline was observed in recent years. This suggests on-going transmission of HCV among HIV-positive MSM. However, trends seem to differ by geographical region. While HCV incidence appears to have stabilized in Western Europe and remained stable in Southern Europe, a recent increase in HCV incidence was observed in Northern Europe. Interestingly, higher HIV RNA levels, recent HIV infection and younger age were associated with higher HCV incidence. The time from HIV seroconversion to HCV infection has significantly shortened in recent years. Hence, routine and continued surveillance following HIV diagnosis is needed.

The increasing trend in HCV incidence over time is comparable with the trend observed in a recent meta-analysis [2]. We estimated that in 2014 HCV incidence was between 18 and 21/1000 py and in the meta-analysis the extrapolated estimate was 19/1000 py in 2015 [2]. A recent study from EuroSida, not restricted to HIV seroconverters, also reported that HCV incidence differed by European geographical region; Eastern, Northern and Southern Europe had higher odds for HCV seroconversion than Western Europe [19]. Interestingly, no HCV infections were observed among MSM from the Kenyan cohort, while another Kenyan study reported that 10% (30/300) of HIV-positive male and female patients were HCV-coinfected [20]. This might suggest that HCV has not yet been introduced in the Kenyan MSM population. The decline in HCV incidence that we observed after 2009 in Western Europe might be ascribed to earlier introduction or recognition of HCV. Consequently, as previously suggested [3], this might have led to a saturation effect among MSM at higher risk for HCV infection and/or increased HCV awareness, leading to more HCV testing and treatment, as well as safer-sex practices. Conversely, since the introduction of cART, condom use has declined among MSM [21,22], which probably led to the increase in syphilis incidence across European countries in recent years, especially among HIV-positive MSM [23]. In Northern Europe (UK and Norway) HCV incidence seems to have increased in recent years, although the overall effect of calendar year was only significant when method 2 was used. A European survey among MSM in 2010 showed that the prevalence of drug use associated with 'chemsex', i.e. drug use to enhance sexual arousal [24] – was highest in three UK

cities [25]; as injecting and non-injecting drug use have been associated with acute HCV among HIV-positive MSM [5,7-9], differences in HCV trends might be partly explained by differences in drug use across European countries. However, we cannot discern whether that study is representative for MSM across Europe. Given the overall continued rise of HCV incidence, HCV-treatment guidelines should consider recommending direct-acting antivirals during acute HCV infection – when registered [26] - to prevent on-going transmission. As suggested by modelling studies, the greatest population benefit among HIV-positive MSM can be achieved when HCV treatment is provided within 1 year of HCV diagnosis, together with behavioural interventions [27,28].

Furthermore, we found that younger MSM, peaking at around age 35, are at higher risk for HCV infection, in line with findings from the Netherlands [3] but in contrast to a study in the USA, where older MSM had a higher risk of HCV infection [10]. Regional differences in the HCV epidemic among HIV-positive MSM could explain this discrepancy, in line with our finding of a borderline significant interaction between age and region.

HIV RNA was significantly associated with HCV incidence, especially when $\log_{10}$ HIV RNA was ≥5 copies/ml. Few studies have assessed the association between HIV RNA and HCV incidence [4,9,12] and, to the best of our knowledge, this is the only study to have modelled HIV RNA as a continuous variable in multivariable analysis. In univariable analyses, two observational cohort studies [4,9] found a significant association between HIV RNA with HCV incidence, whereas a clinical HIV cohort did not [1]. Although, in the Swiss Cohort study, this association was no longer significant in multivariable analysis [4]; but ART use was included in that multivariable model which may mask the effect of HIV RNA as it may lie on the causal pathway. However, in our study, the association between HIV RNA and HCV incidence was attenuated when HIV infection stage was included in the model. The overlap in risk behaviour between HIV and HCV might result in the acquisition of both viruses simultaneously. We found that HCV infection is more likely during recent HIV infection and this is a period characterized by high HIV RNA levels, which might explain the stronger association between HCV incidence and HIV RNA when HIV infection stage is not included in the model. Additionally, until recently, these individuals might not be on

cART. Our finding underscores the importance of monitoring HCV incidence and risk factors among HIV seroconverters.

Yet HIV RNA remained statistically significant. HIV RNA might partly explain why HIV-positive MSM have a higher risk of HCV infection than HIV-negative MSM [11]. The biological mechanism behind the association with HIV RNA may be through the activation of Langerhans cells (LCs) that results in the facilitation of HCV transmission, as immature LCs capture but do not transmit HCV, while activated LCs (due to HIV replication) are able to transmit the virus [29]. Alternatively, having an STI, a risk factor for HCV infection [4,6,9,10,12] leads to an increase in HIV RNA levels [30]. In that case, HIV RNA would be merely a proxy for having an STI. Also, higher HIV RNA levels might be surrogate for poor adherence to cART. Unfortunately, we could not assess the effect of STIs and cART adherence on HCV incidence, as most cohorts do not collect these data.

We found no association between HCV incidence and CD4 count, which is in line with most studies [3,4,6,12]. However, one showed that HIV-positive MSM with lower CD4 counts had a higher risk of acquiring HCV [9] while another study only found an association with CD4 count below 500 cell/μl [10]. However, both studies did not exclusively include HIV seroconverters and did not account for time since HIV infection.

A previous study using data from the CASCADE Collaboration and the same estimating procedures, reported a similar increasing trend in HCV incidence until 2007 [1]. However, HCV incidence in 2007 using method 2 was considerably higher than our estimation (51/1000 vs. 21/1000 py); although confidence intervals were wide after 2005 and our estimates fall within this confidence intervals previously estimated [1]. The present study provides a more accurate estimate of HCV incidence after 2000 as additional HCV testing was performed to minimize bias related to selective testing and more MSM were included.

Our study has some limitations. First, as HCV infection was based on any kind of HCV test, an observed HCV infection might be a re-infection; although 99.1% (334/337) of HCV infections in our study were based on HCV-antibody seroconversion or evidence

of acute/recent primary HCV infection. Also, since we lacked data on the mode of HCV transmission, we could not assess whether all HCV infections were sexually transmitted and whether changes in risk behaviour over time (e.g. increase in injecting drug use (IDU)) are driving the HCV epidemic. However, studies have reported HCV acquisition in the absence of traditional HCV risk factors, such as IDU, in the majority of MSM [4,5,7-10,12]. Hence, the increase in sexual risk behaviour among MSM (e.g. condomless anal intercourse [21]) is likely to partly explain the observed trends. Furthermore, although recreational drug use is common among MSM [25], recent studies have reported a low percentage of IDU among HIV-positive MSM with acute HCV infection (5.8% and 12.2%) [6,9]. To the best of our knowledge, evidence of an increase in IDU is scarce as only one study assessed trends in recent years; an increase in IDU, from 45.1% in 2005 to 53.8% in 2014, was observed among HIV-positive MSM reporting methamphetamine use in Australia [31]. Further research is needed to assess changes over time in HCV-related risk factors and the proportion of HCV acquisition attributable to sexual practices and drug use among MSM. Despite the lack of behavioural data, the main focus of our study was to assess temporal trends in HCV incidence, irrespective of the mode of HCV transmission. Furthermore, it is important to bear in mind that clinicians may have monitored patients at risk for HCV infection better over time, leading to more HCV-positive test results in recent years. To account for this possible bias we performed additional HCV testing and we only included data from the date of routine testing onwards. However, the median and mean number of tests was 3 and 4, respectively, over a median follow-up time of 4 years, suggesting that current guidelines [32] might not be followed consistently. This is in line with results from EuroSida where only a median of three tests were performed per patient between 2002 and 2013 [19]. In addition, due to a lack of country specific HCV testing guidelines (e.g. Italy), HCV testing practices may not be systematic.

The strengths of our study are that we had data from HIV seroconversion onwards for a large group of MSM, and extensive follow-up that enabled us to assess temporal changes in time from HIV seroconversion to HCV infection and the association between HCV incidence and HIV infection stage. We also applied different estimating methods to calculate HCV incidence and various sensitivity analyses. All methods showed comparable results suggesting that our results are robust.

To conclude, no decline in HCV incidence was observed in recent years, although trends seem to differ by geographical region. Hence, HCV screening among HIV-positive MSM should be continued and routinely and frequently offered. Furthermore, targeted preventive measures should be implemented and/or scaled-up to decrease the risk of HCV acquisition. Other than recent calendar year, younger age, recent HIV infection and high HIV RNA levels were all associated with HCV incidence.

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Conflict of interest

Kholoud Porter has served on the Dolutegravir Advisory Board, reports grants from EU FP7 and personal fees from ViiV, outside the submitted work. Dr. Price reports other from International AIDS Vaccine Initiative (IAVI) during the conduct of the study. The other authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors' contributions

DvS performed the statistical analyses together with RG, also interpreted the data, and wrote the manuscript. JvdH provided substantial contributions to the analyses and interpretation of the data as well as the manuscript. MP and RG designed and supervised the overall study, and substantially contributed to the analyses, interpretation of the data and manuscript. KP obtained funding for the study. All authors contributed to the design, additional HCV testing, interpretation of the data, subsequent drafts and approved the final version of the manuscript.

Table 1: Number of MSM per cohort with and without HCV test results in the CASCADE Collaboration

Cohorts	At least one HCV test result	MSM with follow-up[a] & at least one HCV test result			At-risk set[b]				Start routine testing date
			HCV+[d]	HCV-[e]	Method 1		Method 2		
N[c]	n (%)	Total, n (%)	n (%)	n (%)	n	HCVsc - Pys	n	HCVsc - Pys	
Southern Europe									
AMA; n=177	172 (97.2%)	167 (94.4%)	2 (1.2%)	165 (98.8%)	128	0 - 526.3	87	0 - 347.2	1-1-1991
COR; n=365	353 (97.7%)	310 (84.9%)	5 (1.6%)	302 (97.4%)	184	3 - 246.2	68	3 - 87.7	1-1-2005[f]
ICO; n=1018	914 (89.8%)	848 (83.3%)	49 (5.8%)	770 (90.8%)	497	29 - 1926.3	411	29 - 1705.7	AT
MAD; n=342	308 (90.1%)	293 (85.7%)	16 (5.5%)	274 (93.5%)	213	3 - 1047.9	30	3 - 56.8	1-1-1993
VAL; n=165	89 (53.9%)	85 (51.5%)	13 (15.3%)	71 (83.5%)	65	1 - 66.9	2	1 - 2.9	1-1-1998
Total; n=2067	**1,836 (88.8%)**	**1,703 (82.4%)**	**85 (5.0%)**	**1,582 (92.9%)**	**1,087**	**36 - 3813.6**	**598**	**36 - 2200.4**	
Western Europe									
AQU; n=788	730 (92.6%)	707 (89.7%)	29 (4.1%)	657 (92.9%)	486	21 - 3053.1	360	19 - 2389.3	1-1-1991
AUS; n=212	206 (97.2%)	201 (94.8%)	3 (1.5%)	193 (96.0%)	181	5 - 682.3	150	4 - 575.7	1-1-2006
GER; n=1912	1,848 (96.7%)	1,543 (80.7%)	63 (4.1%)	1,393 (90.3%)	1,025	87 - 4557.0	764	51 - 2665.5	RT
LYO; n=62	60 (96.8%)	59 (95.2%)	1 (1.7%)	57 (96.6%)	11	1 - 40.2	0	0 - 0	1-1-1999[f]
NEM; n=239	239 (100%)	239 (100%)	2 (0.8%)	215 (90.0%)	224	22 - 1841.6	144	21 - 1098.1	RT
PRI; n=966	894 (92.5%)	401 (41.5%)	15 (3.7%)	381 (95.0%)	211	6 - 791.8	190	5 - 748.5	1-1-1996[f]
SWI; n=343	338 (98.5%)	320 (93.3%)	4 (1.3%)	294 (91.9%)	274	22 - 1532.6	236	17 - 1210.2	1-1-2000
Total; n=4522	**4,315 (95.4%)**	**3,470 (76.7%)**	**117 (3.1%)**	**3,190 (91.9%)**	**2,412**	**164 - 12498.5**	**1,844**	**117 - 8687.4**	
Northern Europe									
NOR; n=383	378 (98.7%)	349 (91.1%)	10 (2.9%)	328 (94.0%)	305	11 - 2165.9	258	11 - 1489.6	1-1-1995
UKR; n=2714	2,209 (81.4%)	2,073 (76.4%)	50 (2.4%)	1,903 (91.8%)	1,937	120 - 9395.2	1,582	110 - 6871.6	1-1-2004
Total; n=3097	**2,587 (83.5%)**	**2,422 (78.2%)**	**60 (2.5%)**	**2,231 (92.1%)**	**2,242**	**131 - 11561.1**	**1,840**	**121 - 8361.2**	
North America									
SAL; n=138	**138 (100%)**	**131 (94.9%)**	**4 (3.1%)**	**122 (93.1%)**	**67**	**5 - 327.2**	**43**	**4 - 230.0**	1-1-2000
Australia									
PHA; n=145	**138 (95.2%)**	**138 (95.2%)**	**5 (3.6%)**	**132 (95.7%)**	**133**	**1 - 399.4**	**1**	**1- 0.8**	1-1-2002
Total; n=9,969	**9,014 (90.4%)**	**7,864 (78.9%)**	**271 (3.4%)**	**7,257 (92.3%)**	**5,941**	**337- 28599.9**	**4,326**	**279 - 19479.8**	

Abbreviations: N=number; n=number; HCVsc=HCV seroconverters; PYs=person years of observation; HCV+=HCV-positive; HCV-=HCV-negative; RT=retrospective testing; AT=additional testing only (no routine testing); AMA= AMACS cohort, Greece; AQU: Aquitaine cohort, France; AUS: Austrian HIV cohort study, Austria; COR=CoRis cohort, Spain; GER=German cohort, Germany; IAV=IAVI, Kenya; ICO=ICONA cohort, Italy; LYO= Lyon cohort, France; MAD=Madrid cohort, Spain; NEM=Amsterdam Cohort Study among MSM, the Netherlands; NOR=Oslo and Ulleval hospital cohorts, Norway; PHA=PHAEDRA cohort, Australia; PRI=PRIMO cohort, France; SAL=Southern Alberta Clinic, Canada; SWI=Swiss HIV cohort, Switzerland; UKR=UK Register of HIV seroconverters, UK; VAL=Valencia cohort, Spain; NA=not applicable.
a MSM with a clinic visit, and thus follow-up, after becoming at risk, being the latest of: enrolment in the cohort, HIV seroconversion or routine testing per cohort. HCV test results irrespective of the moment of becoming at risk.
b MSM included in the analyses from 1990 until 2014.
c Number of MSM per cohort irrespective of the moment of becoming at risk, HCV test, year and length of follow-up.
d HCV-positive MSM without a previous HCV negative test result (i.e. excluding HCV seroconverters).
e MSM who remained HCV negative throughout follow-up (from becoming at risk until last clinic visit).
d,e Out of all MSM with follow-up & at least one HCV test result (third column).
f Start of routine testing date before individuals were enrolled in the cohort.

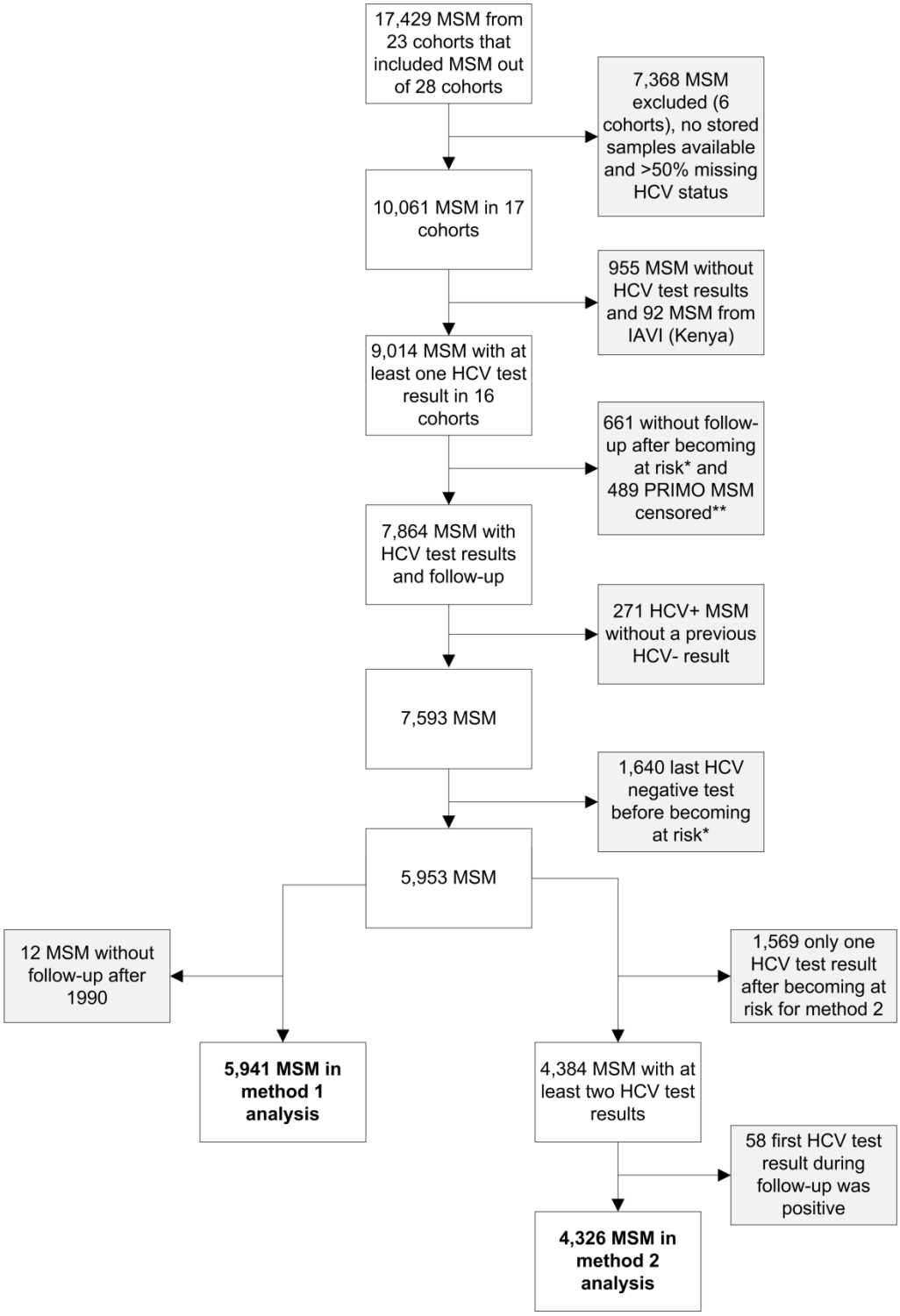

Fig. 1: Flow diagram of the study population selection for method 1 and 2 of the HCV incidence analyses

* Becoming at risk being the latest of: enrolment in the cohort, routine HCV testing date per cohort or HIV seroconversion.

** MSM from the French Primo cohort were censored at the 31st of December 2005 as HCV testing was only systematically recorded until that year.

The grey boxes depict MSM whose data were excluded from the analyses.

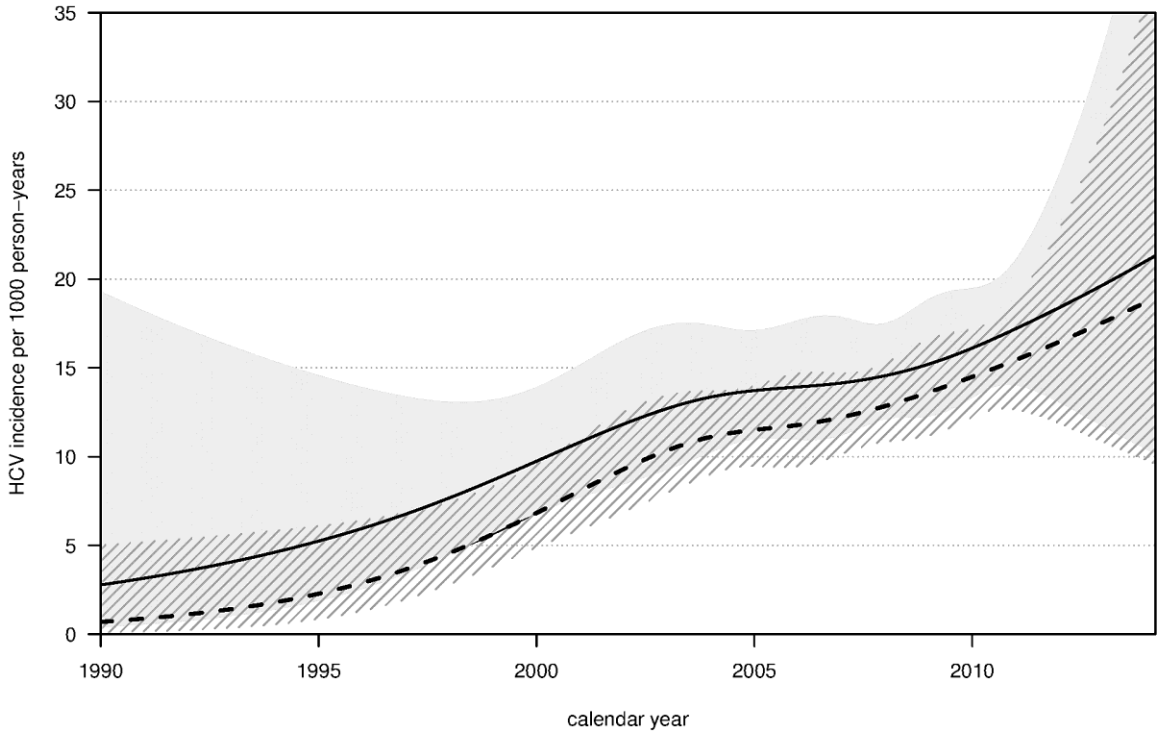

Fig. 2: HCV incidence among HIV-positive MSM using two methods to estimate follow-up in the CASCADE Collaboration; 1990-2014

Method 1: dashed line, 95%CI: dashed area. Method 2: solid line, 95%CI: grey solid area.

Poisson regression was used to test the overall effect of calendar year on HCV incidence between 1990 and 2014.

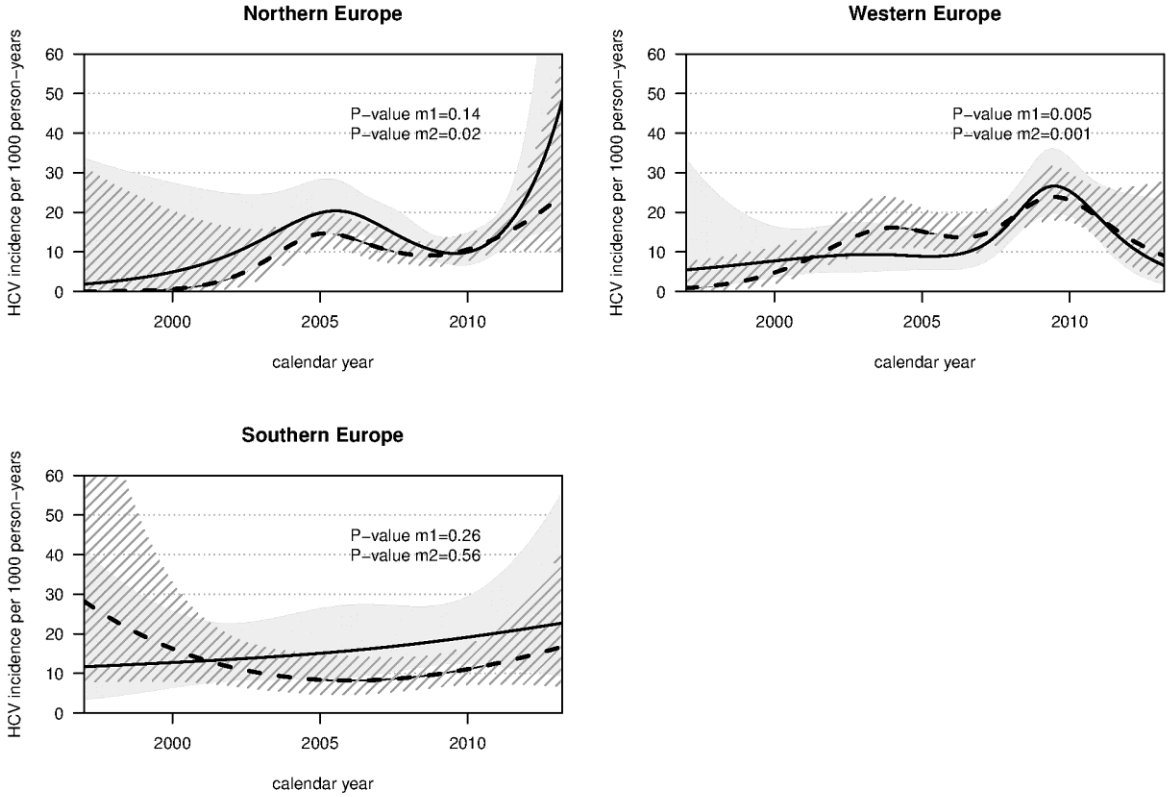

Fig. 3: HCV incidence among HIV-positive MSM by European UN geographical region in the CASCADE Collaboration; 1997-2013

Abbreviations: m1= method 1; m2= method 2

Method 1: dashed line, 95%CI: dashed area. Method 2: solid line, 95%CI: grey solid area.

P-values: overall effect of calendar year on HCV incidence between 1997 and 2013 obtained from Poisson regression models.

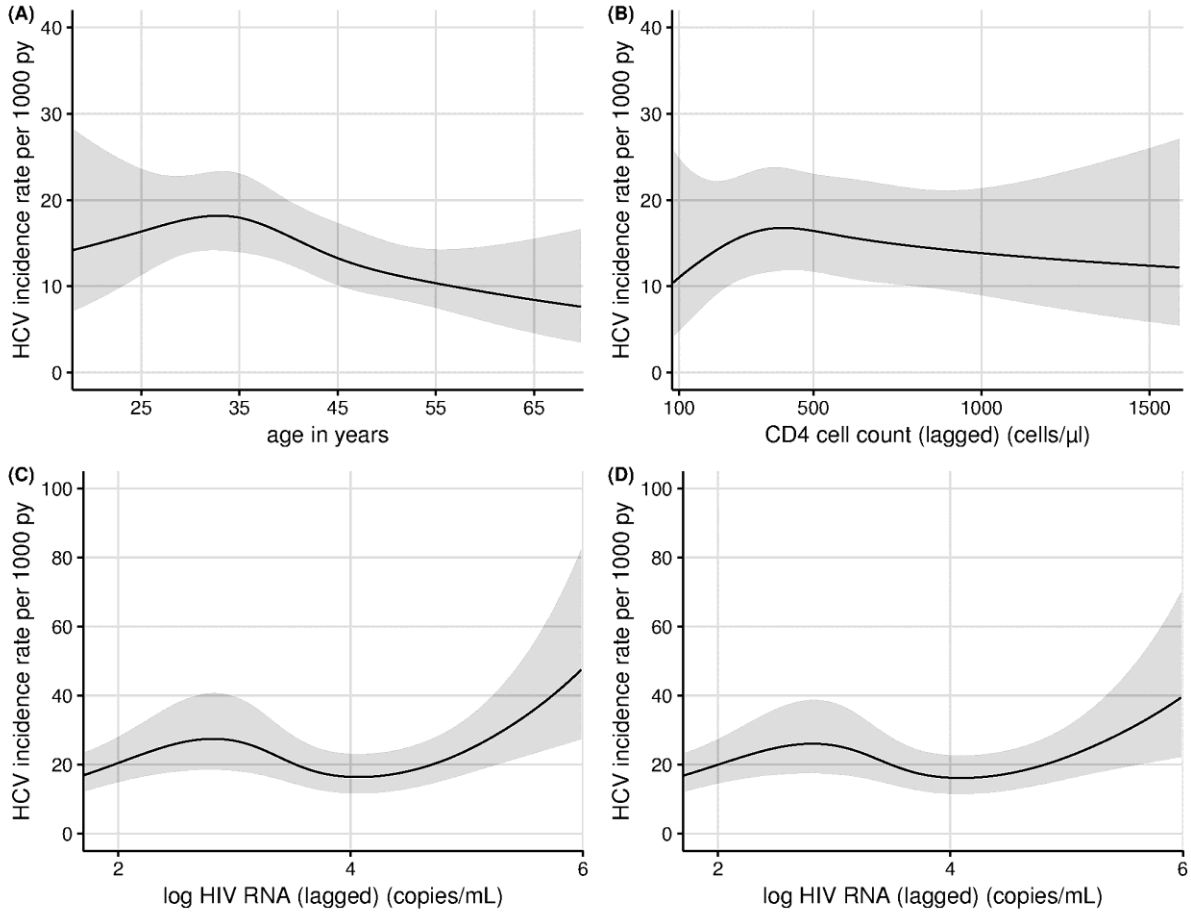

Fig. 4. HCV incidence by age, CD4 cell count and HIV RNA among HIV-positive MSM from the CASCADE Collaboration, in year 2007 in Western Europe[a]

4(A). Incidence by age in years (model 2)[b]

4(B). Incidence by CD4 count for an individual with a HIV RNA = 1000, aged 35 (model 3)

4(C). Incidence by HIV RNA for an individual with a CD4 cell count = 500, aged 35 (model 3)

4(D). Incidence by HIV RNA for an individual with a HIV RNA = 1000, aged 35, in the chronic HIV infection stage (model 3)

[a] The relative hazards obtained from the regression models were translated into the predicted incidence and this is illustrated for certain values of the covariates (e.g. only for Western Europe).

[b] Obtained from model 2 without the interaction term between age and region.

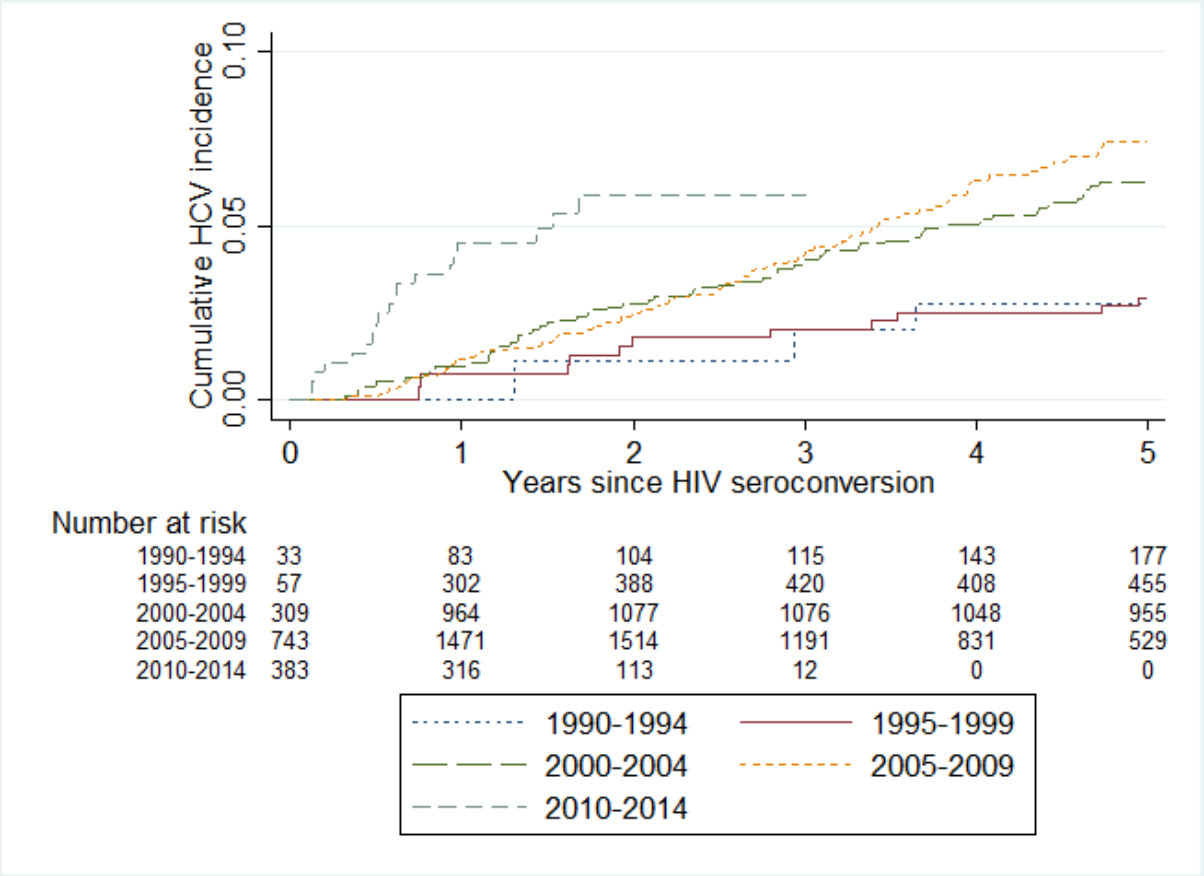

Fig. 5: Time from HIV seroconversion until HCV infection over time: Kaplan-Meier curves by calendar period of HIV seroconversion in the CASCADE Collaboration (1990-2014)[a]

[a] Curves were truncated when less than 10 individuals were at risk for HCV infection. The log-rank test was used to assess changes in the time from HIV seroconversion to HCV infection among calendar periods.

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Supplementary text

1. Interval-censored data method

The likelihood is maximized based on the interval-censored HCV status information. As the current available version of the software cannot correct for left-truncated data, follow-up was divided in 5 calendar periods of 5 years each, from 1990 to 2014. This method has the advantage that we can also include data from MSM with only HCV-positive test results who have a left censored infection time. MSM were included in a calendar period if they were considered at risk and had been (at least partly) followed during that period, irrespective of whether or not they had HCV results in that particular calendar period. Follow-up in the interval censored method was calculated from the moment MSM became at risk (as previously described in the main text of the manuscript) until the last clinic visit. Some MSM only had HCV test results before becoming at risk. For these MSM, if the last result was positive, they were categorized a HCV positive at the moment of becoming at risk. MSM with only HCV-negative test results, but with the last negative results at most two years before becoming at risk, were assumed to remain HCV negative until that moment. When the last HCV negative test took place more than 2 years before becoming at risk, the individual was excluded (n=77). The likelihood maximizes the cumulative incidence. This estimate was transformed into the hazard using kernel smoothing.

A total of 7,787 MSM were included of whom 336 acquired HCV during follow-up (i.e., had HCV negative and positive test results between being considered at risk and last clinic visit), 271 had HCV-positive results without HCV-negative test results and 7,180 MSM had only HCV-negative test results. Among these 7,180 HCV-negative MSM, 809 had their last HCV negative test within two years before becoming at risk. The

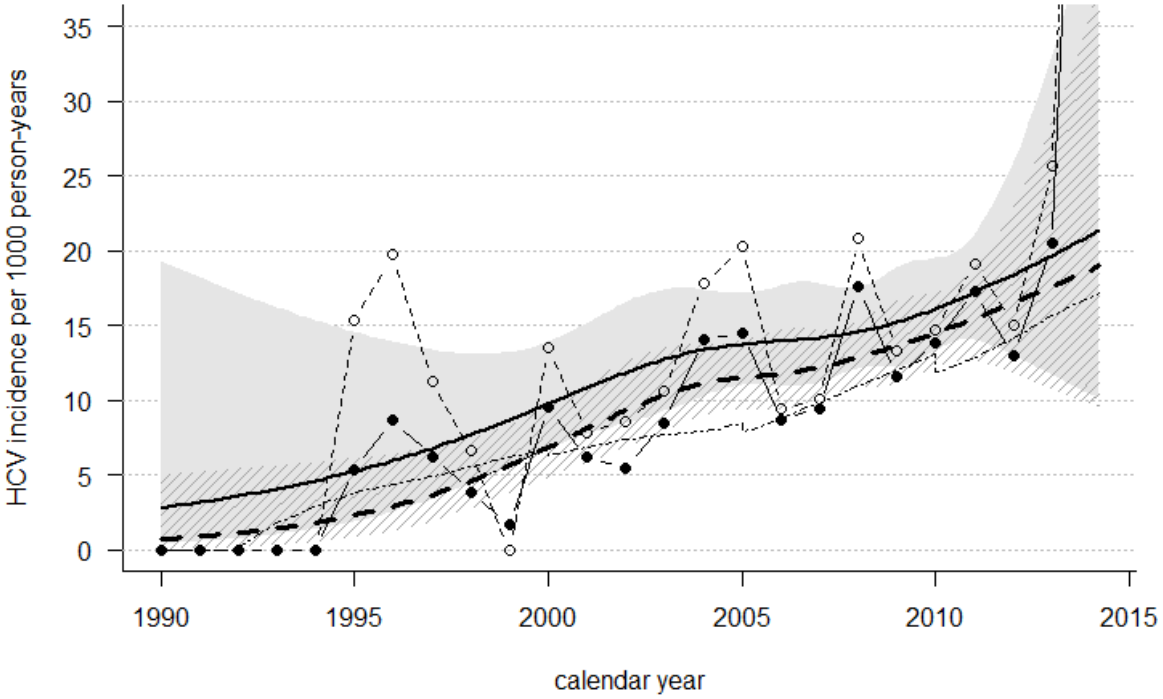
median time between the last HCV-negative test and becoming at risk among the 809 MSM was 0.08 years (IQR=0.03-0.23). The number of MSM included in each of the five calendar periods was 231 in 1990–1994, 1,005 in 1995–1999, 3,517 in 2000-2004, 5,691 in 2005-2009 and 5,760 in 2010-2014.

2. Random-effect models

Missing data:

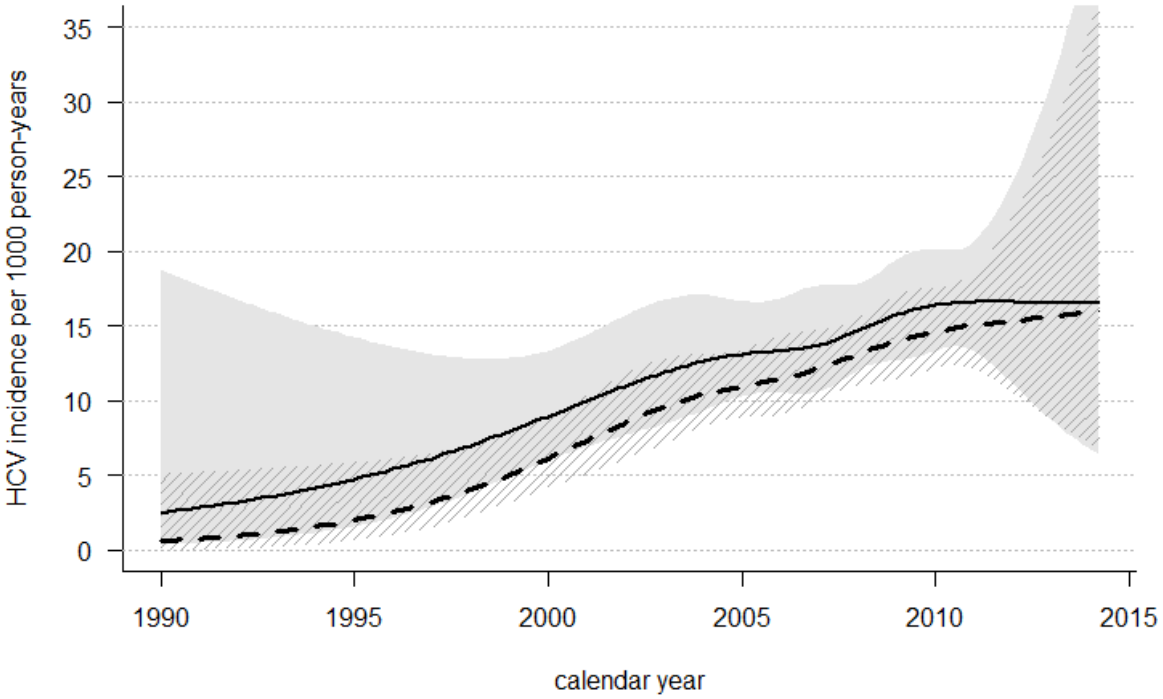
In model 3, if individuals had a clinic visit, but a missing value for CD4 and/or HIV RNA, we imputed missing values using a separate random-effects model for each marker, stratified by cART usage (as described in the main text). In each model, we allowed for a random intercept and a random slope per individual. When an individual did not have a clinic visits in a calendar year and hence CD4 and HIV RNA were not measured, we created an additional record in between when consecutive visits were less than 2 years apart; this additional record was also imputed.

Supplementary figures



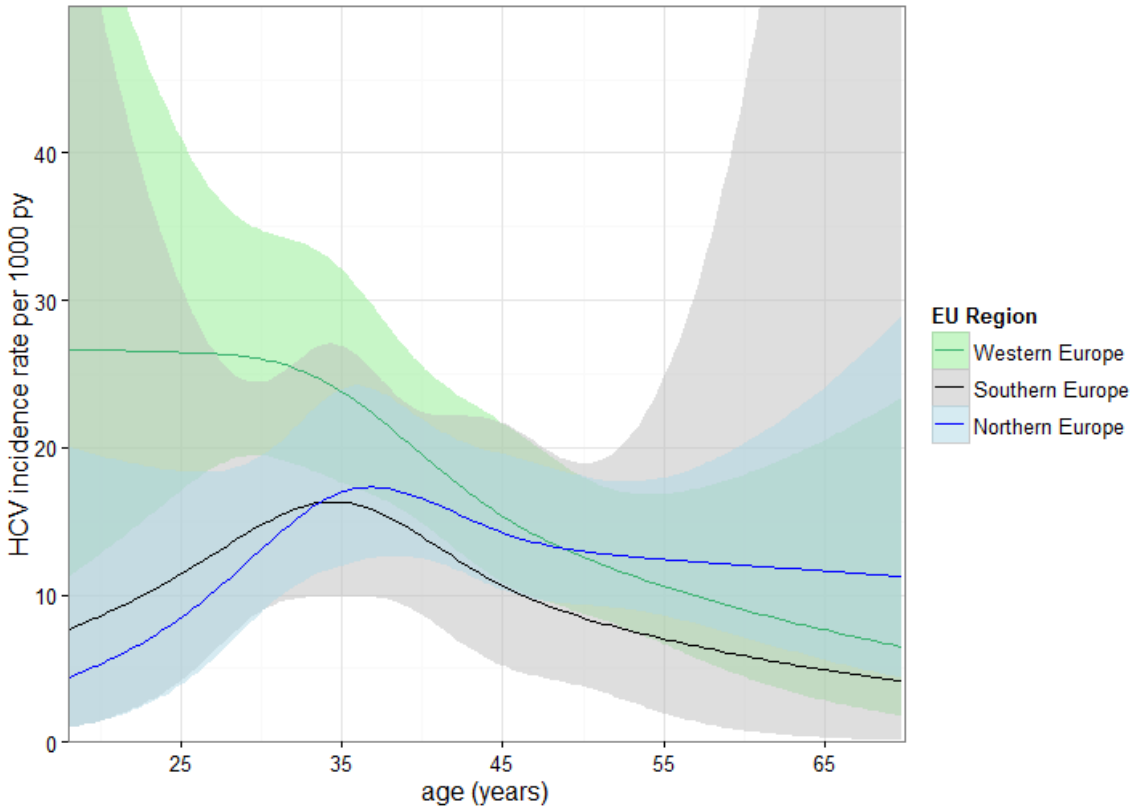
Supplementary fig. 1: HCV incidence among HIV-positive MSM using three methods to estimate follow-up in the CASCADE Collaboration; 1990-2014

Method 1: dashed line; 95%CI: dashed area. Method 2: solid line; 95%CI: grey area. Interval censored data method (IC): point-dash line. Black points are the observed incidence per year in method 1 and white points are the observed incidence per year in method 2.



Supplementary Fig. 2: Sensitivity analyses: HCV incidence among HIV-positive MSM excluding the Italian cohort (ICoNA) in the CASCADE Collaboration; 1990-2014

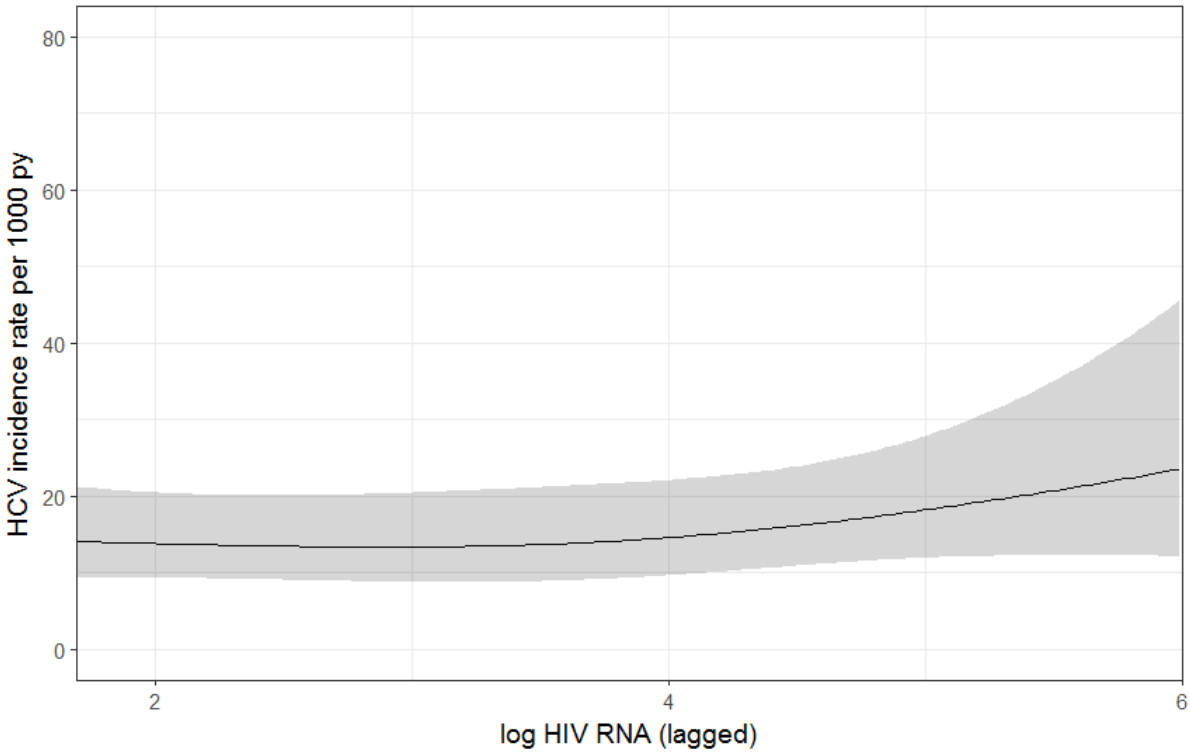
Method 1: dashed line, 95%CI: dashed area. Method 2: solid line, 95%CI: grey solid area.



Supplementary Fig. 3: HCV incidence by age and European geographical region among HIV-positive MSM from the CASCADE Collaboration; 1990-2014[a]

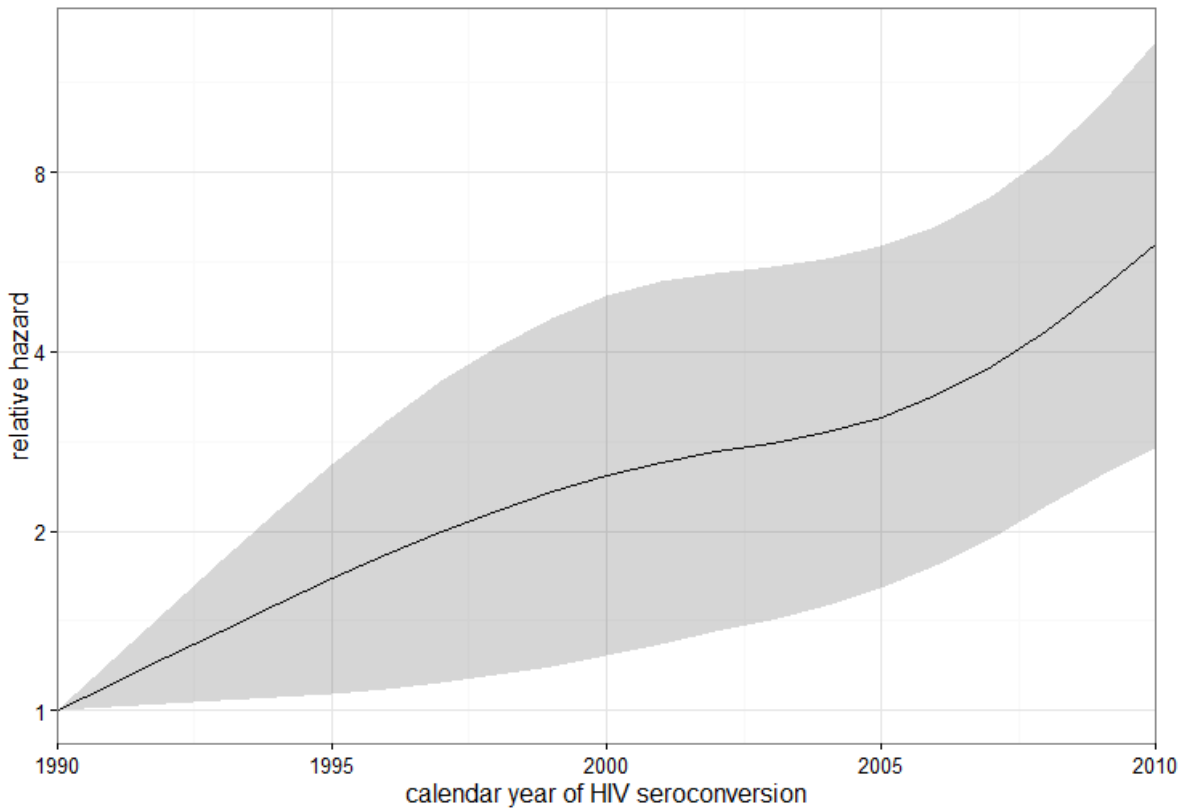
[a]Canada and Australia are included in the model but are not depicted in this graph.

EU = European



Supplementary Fig. 4 (Complete case analyses): HCV incidence by HIV RNA value among HIV-positive MSM from the CASCADE Collaboration; 1990-2014

Grey solid areas depict the 95% confidence interval.



Supplementary Fig. 5: Relative hazard of HCV infection by calendar year of HIV seroconversion among MSM from the CASCADE Collaboration; 1990-2014

Grey solid areas depict the 95% confidence interval

Appendix 1:

CASCADE Steering Committee: Julia Del Amo (Chair), Laurence Meyer (Vice Chair), Heiner C. Bucher, Geneviève Chêne, Osamah Hamouda, Deenan Pillay, Maria Prins, Magda Rosinska, Caroline Sabin, Giota Touloumi.

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CASCADE Clinical Advisory Board: Heiner C. Bucher, Andrea De Luca, Martin Fisher, Roberto Muga

CASCADE Collaborators: Australia PHAEDRA cohort (Tony Kelleher, David Cooper, Pat Grey, Robert Finlayson, Mark Bloch) Sydney AIDS Prospective Study and Sydney Primary HIV Infection cohort (Tony Kelleher, Tim Ramacciotti, Linda Gelgor, David Cooper, Don Smith); Austria Austrian HIV Cohort Study (Robert Zangerle); Canada South Alberta clinic (John Gill); Estonia Tartu Ülikool (Irja Lutsar); France ANRS CO3 Aquitaine cohort (Geneviève Chêne, Francois Dabis, Rodolphe Thiebaut), ANRS CO4 French Hospital Database (Dominique Costagliola, Marguerite Guiguet), Lyon Primary Infection cohort (Philippe Vanhems), French ANRS CO6 PRIMO cohort (Marie-Laure Chaix, Jade Ghosn), ANRS CO2 SEROCO cohort (Laurence Meyer, Faroudy Boufassa); Germany German HIV-1 seroconverter cohort (Osamah Hamouda, Karolin Meixenberger, Norbert Bannert, Barbara Bartmeyer); Greece AMACS (Anastasia Antoniadou, Georgios Chrysos, Georgios L. Daikos); Greek Haemophilia cohort (Giota Touloumi, Nikos Pantazis, Olga Katsarou); Italy Italian Seroconversion Study (Giovanni Rezza, Maria Dorrucci), ICONA cohort (Antonella d'Arminio Monforte, Andrea De Luca.) Netherlands Amsterdam Cohort Studies among homosexual men and drug users (Maria Prins, Ronald Geskus, Jannie van der Helm, Hanneke

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Ethics committees:

Ethics approval has been granted by the following committees: Austrian HIV Cohort Study: Ethik-Kommission der Medizinischen Universität Wien, Medizinische Universität Graz – Ethikkommission, Ethikkommission der Medizinischen Universität Innsbruck, Ethikkommission des Landes Oberösterreich, Ethikkommission für das Bundesland Salzburg; PHAEDRA cohort: St Vincent's Hospital, Human Research Ethics Committee; Southern Alberta Clinic Cohort: Conjoint Health Research Ethics Board of the Faculties of Medicine, Nursing and Kinesiology, University of Calgary; Aquitaine Cohort: Commission Nationale de l'Informatique et des Libertés; French PRIMO Cohort: Comite Consultatif de Protection des Personnes dans la Recherché Biomedicale; German HIV-1 Seroconverter Study: Charité, University Medicine Berlin; AMACS: Bioethics & Deontology Committee of Athens University Medical School and the National Organization of Medicines; Greek Haemophilia Cohort: Bioethics & Deontology Committee of Athens University Medical School and the National Organization of Medicines; ICoNA cohort: San Paolo Hospital Ethic Committee; Amsterdam Cohort Studies in Homosexual Men: Academic Medical Centre, University of Amsterdam; Oslo and Ulleval Hospital Cohorts: Regional komite for medisinsk forskningsetikk - Øst- Norge (REK 1); CoRIS-scv: Comité Ético de Investigación Clínica de La Rioja; Madrid Cohort: Ethics Committee of Universidad Miguel Hernandez de Elche; Swiss HIV Cohort Study: Kantonale Ethikkommission,

spezialisierte Unterkommission Innere Medizin, Ethikkommission beider Basel, Kantonale Ethikkommission Bern, Comité départemental d'éthique de médecine et médecine communautaire, Commission d'éthique de la recherche clinique, Université de Lausanne, Comitato etico cantonale, Ethikkommission des Kantons St.Gallen; UK Register of HIV Seroconverters: South Birmigham REC; Early Infection Cohorts: Kenya Medical Research Institute, Kenyatta National Hospital.