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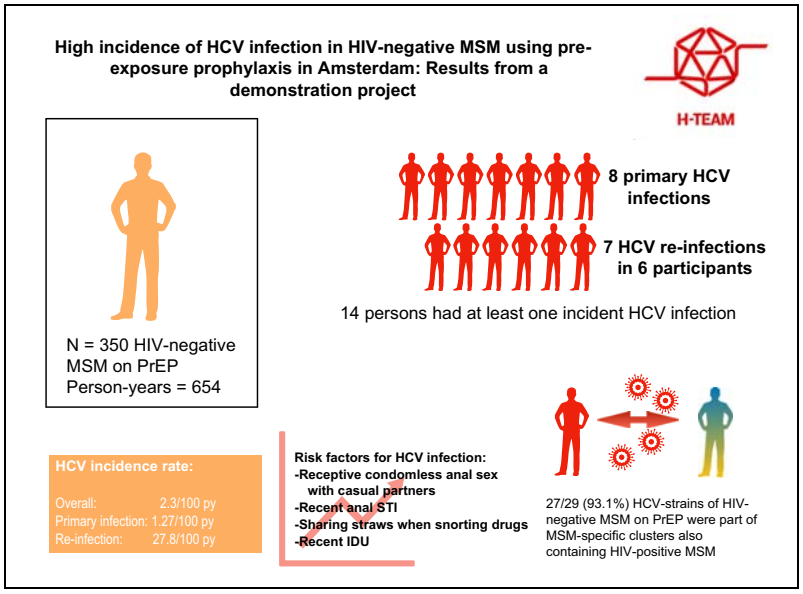
High incidence of HCV in HIV-negative men who have sex with men using pre-exposure prophylaxis

Graphical abstract



Highlights

- HIV-negative men who have sex with men while on pre-exposure prophylaxis are at risk of incident HCV infection.
- High incidence rates of both HCV primary and re-infection were observed.
- Identified HCV risk-factors were similar to those in HIV-positive men who have sex with men.
- Specific clusters of HCV strains were identified in men who have sex with men, with and without HIV.
- HCV-testing for HIV-negative men who have sex with men while on pre-exposure prophylaxis is recommended.

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Lay summary

We report that hepatitis C virus infections are frequently acquired among HIV-negative men who have sex with men (MSM) using pre-exposure prophylaxis to prevent HIV infection. New infections occurred more frequently in those reporting receptive anal sex without using condoms, having an anal sexually transmitted infection, injecting drugs, and sharing straws when snorting drugs. The viruses found in HIV-negative men using pre-exposure prophylaxis are genetically similar to those in HIV-positive men, but not in other hepatitis C risk groups, suggesting that (sexual) transmission is occurring between HIV-positive MSM and HIV-negative MSM using pre-exposure prophylaxis.

https://doi.org/10.1016/j.jhep.2019.11.022
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High incidence of HCV in HIV-negative men who have sex with men using pre-exposure prophylaxis

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Background & Aims: HCV has emerged as a sexually transmitted infection (STI) among HIV-positive men who have sex with men (MSM). We evaluated HCV incidence and its risk factors among HIV-negative MSM using HIV pre-exposure prophylaxis (PrEP).

Methods: Participants of the Amsterdam PrEP project were tested for HCV antibodies or HCV-RNA every 6 months. Participants used daily or event-driven PrEP and could switch regimens during follow-up. We calculated incidence rates (IRs) for overall HCV infection and separately for primary and re-infection. A univariable Bayesian exponential survival model was used to identify risk factors associated with incident HCV infection. The HCV NS5B gene fragment (709 bp) was sequenced and compared to HCV isolates from HIV-positive MSM and other risk groups (n = 419) using phylogenetic analysis.

Results: Among 350 participants contributing 653.6 person-years (PYs), we detected 15 HCV infections in 14 participants (IR = 2.30/100PY). There were 8 primary infections (IR = 1.27/100PY) and 7 re-infections (IR = 27.8/100PY). IR was 2.71/100PY in daily and 1.15/100PY in event-driven PrEP users. Factors associated with incident HCV infection were higher number of receptive condomless anal sex acts with casual partners (posterior hazard ratio [HR] 1.57 per ln increase; 95% credibility interval [CrI] 1.09–2.20), anal STI (posterior HR 2.93; 95% CrI 1.24–7.13), injecting drug use (posterior HR 4.69; 95% CrI 1.61–12.09) and sharing straws when snorting drugs (posterior HR 2.62; 95% CrI 1.09–6.02). We identified robust MSM-specific HCV clusters of subtypes 1a, 4d, 2b and 3a, which included MSM with and without HIV.

Conclusions: HIV-negative MSM using PrEP are at risk of incident HCV infection, while identified risk factors are similar to those in HIV-positive MSM. Regular HCV testing is needed, especially for those with a previous HCV infection and those reporting risk factors.

Lay summary: We report that hepatitis C virus infections are frequently acquired among HIV-negative men who have sex with men (MSM) using pre-exposure prophylaxis to prevent HIV infection. New infections occurred more frequently in those reporting receptive anal sex without using condoms, having an anal sexually transmitted infection, injecting drugs, and sharing straws when snorting drugs. The viruses found in HIV-negative men using pre-exposure prophylaxis are genetically similar to those in HIV-positive men, but not in other hepatitis C risk groups, suggesting that (sexual) transmission is occurring between HIV-positive MSM and HIV-negative MSM using pre-exposure prophylaxis.

Clinical Trial number: Dutch trial registration number NTR5411.
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Introduction

The majority of HCV infections worldwide are transmitted via injection drug use (IDU).[1] Since the early 2000s, however, HCV has emerged as a sexually transmitted infection (STI) among HIV-positive men who have sex with men (MSM).[2,3] Sexual transmission of HCV among HIV-positive MSM has been associated with receptive condomless anal sex (CAS), recent (ulcerative) sexually transmitted infection (STI), sharing of sex toys, sexual techniques damaging the anorectal mucosa (*i.e.* fisting), and use of recreational drugs before or during sex.[4] It has been hypothesized that HIV-negative MSM have remained largely unaffected by HCV[5–8] due to i) lower biological susceptibility to HCV compared to those with HIV infection as a result of more efficient immunological responses in the gastrointestinal mucosa when small HCV inoculums are present in semen,[9,10] ii) engaging in sex with partners of the same HIV status ("serosorting") where HCV prevalence among HIV-negative MSM is low, and iii) lower

Keywords: HIV; Pre-exposure prophylaxis; Hepatitis C virus; Molecular epidemiology; Risk factors; Men who have sex with men; Homosexuality; Male.
Received 10 June 2019; received in revised form 25 October 2019; accepted 29 November 2019; available online 18 December 2019
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https://doi.org/10.1016/j.jhep.2019.11.022

sexual transmissibility of HCV compared to HIV, hence explaining why HIV infection often precedes HCV infection.[10–12] Increased uptake of pre-exposure prophylaxis (PrEP), which successfully prevents HIV infection but not HCV infection, could lead to an increasing overlap in sexual networks between HIV-negative and HIV-positive MSM, eventually resulting in an expanding HCV epidemic among MSM irrespective of HIV status. Indeed, sero-discordant CAS has increased among HIV-negative MSM since 2005.[13]

We previously reported an HCV prevalence of almost 5% among HIV-negative MSM who started PrEP in Amsterdam.[12] We aimed to determine whether this elevated risk carried over during the first 2 years of PrEP use among MSM in Amsterdam. We assessed HCV incidence over time, both overall and according to primary/re-infection or by PrEP regimen (daily/event-driven), while examining its associated risk factors. We also evaluated whether strains of incident HCV infections in HIV-negative MSM clustered with strains from HIV-positive MSM and other HCV risk groups in the Netherlands.

Materials and methods

Study population and design

The Amsterdam PrEP (AMPrEP) study is a demonstration project conducted at the Public Health Service of Amsterdam with the aim of assessing uptake and acceptability of daily and event-driven PrEP. Study design and procedures have been described previously.[14] All participants provided written informed consent. The study was approved by the institutional ethics board. Inclusion took place from 3 August 2015 to 31 May 2016 and follow-up is ongoing.

Eligible participants were offered a choice of daily or event-driven PrEP and were given the opportunity to switch PrEP regimens at each study visit.[15] We monitored participants at 3-month visits. Participant characteristics were collected every 3 months (sexual behaviour) or 6 months (behavioural risk factors for HCV infection) through computer-assisted self-reported questionnaires.[14,16] Blood samples from each 3-month visit were collected and stored. STI-testing was performed at 3-month study visits and additionally in case of partner notification or clinical indications in between visits.[17]

We restricted our analyses to participants with at least 2 HCV-test results.

HCV-testing procedures

HCV testing initially occurred every 12 months and from December 2016 every 6 months (based on analyses demonstrating high baseline HCV prevalence).[12] Additional HCV testing was performed in the event of partner notification or clinical indication. HCV-RNA positive participants were referred for further evaluation and treatment, and were counseled on prevention of HCV re-infection.

At inclusion, participants were tested for HCV-RNA (COBAS® Taqscreen MPX v2.0, Roche Diagnostics) and HCV antibodies (ARCHITECT anti-HCV, Abbott Laboratories) and if antibody-positive, immunoblot confirmation (INNO-LIA HIV I/II and HCV Score, Fujirebio).[12] During follow-up, participants without detectable HCV antibodies at the previous visit were tested for HCV antibodies (Liaison XL HCV-Ab, DiaSorin). Participants with a previous HCV antibody-positive test result and HCV-naïve participants with newly detected HCV antibodies were tested for HCV-RNA using a validated in-house HCV PCR targeting the 5' non-coding region.[18,19] Earlier samples (HCV was tested every 6 months but samples were stored every 3 months) from participants diagnosed with a new HCV (re-)infection were retrospectively tested for HCV-RNA and/or HCV antibodies to identify the first positive test (Fig. S1).

Genotyping and phylogenetic analysis

HCV-RNA isolation, reverse transcription, amplification and sequencing were performed as described previously.[12] In short, we used isolated HCV-RNA obtained from 400 μl of plasma as input for 2 separate PCR assays targeting the NS5B region, resulting in the amplification of 2 overlapping NS5B fragments. Fragment 1 (nucleotides 8001-8340) and fragment 2 (nucleotides 8228-8709) together form an NS5B fragment with a total length of 709 nucleotides (8001-8709). Phylogenetic trees were built for each HCV subtype separately containing HCV sequences from (i) HIV-negative AMPrEP participants with prevalent and/or incident HCV infection (n = 29), (ii) HIV-positive MSM acquiring HCV after 2010 obtained from 3 HIV treatment centers in Amsterdam (n = 137), (iii) HCV-RNA positive Dutch blood donors in 1997-2018 (n = 84), and (iv) all additional Dutch HCV NS5B sequences in Genbank [mainly from people who inject drugs (PWID) and HIV-positive MSM diagnosed with HCV before 2010] (n = 198). Maximum-likelihood phylogenies were constructed using a general time-reversed substitution model with γ-distribution, assuming a certain fraction of evolutionary invariable sites (GTR+G+I) in Mega v6.0. Bootstrapping (n = 500) was used to analyze the stability of tree topology (bootstrap values >70 represent robust clusters). HCV NS5B sequences from AMPrEP participants were submitted to GenBank (baseline infections: KY386877-KY386891; incident infections: MK616411-MK616424).

Outcomes

Primary HCV infection, re-infection and possible re-infection, were defined according to the recommendations from the European Association for the Study of the Liver (EASL) (Table S1).[20] The date of infection was estimated by calculating the midpoint between last negative and first positive HCV-RNA test date. The estimated date of infection for HCV-RNA positive participants without detectable HCV antibodies was determined as 28 days prior, which is equivalent to half of the mean HCV-seroconversion time.[21]

A cured infection was defined as a sustained virological response (SVR) ≥12 weeks after the end of treatment. Spontaneously resolved infections were categorized as (i) proven: no HCV-treatment and having 2 HCV-RNA negative test results within a ≥24-week time-interval[20] or (ii) possible: no HCV-treatment and having one HCV-RNA negative test result, or having 2 HCV-RNA negative test results <24 weeks apart.

Risk factors

We evaluated the following risk factors for incident HCV infection: age; ethnicity (white *vs.* non-white); PrEP regimen (daily *vs.* event-driven); *in the past 3 months*: number of receptive CAS acts with casual partner(s), having had anal sex with an HIV-positive partner; *in the past 6 months*: sharing of sex toys, fisting without gloves, engaging in chemsex; *in the past year*: sharing straws when snorting drugs, IDU; and *at study visit*: diagnosed anal chlamydia and/or gonorrhea infections (termed herein as "recent anal STI"), diagnosed syphilis. We defined chemsex as self-

reported use of methamphetamine, γ-hydroxybutyric acid(GHB)/γ-butyrolactone(GBL) and/or mephedrone during sex.[22]

All covariates except ethnicity were time-updated. Missing data due to questions not being asked every 3 months were imputed from the subsequent visit with available data.

Statistical analysis

Follow-up began at enrolment for HCV-naïve participants or after successful treatment or spontaneous clearance for those with a prevalent HCV infection at enrolment, and ended at HIV-seroconversion, definite PrEP discontinuation, 2-year visit, or

Table 1. Characteristics at baseline and at the time of first incident HCV infection or at last visit before censoring, AMPrEP study, Amsterdam, the Netherlands, 2015-2018.

	Baseline	Follow-up	
	Total (N = 350)	First incident HCV infection n = 14[1]	No incident HCV infection[2] n = 336
	N (%)	n (%)	n (%)
Age (years), median [IQR]	40 [33-48]	37.5 [28-41]	42 [35-50.5]
≤34	115 (32.9)	5 (35.7)	82 (24.4)
35-44	105 (30.0)	7 (50.0)	106 (31.6)
≥45	130 (37.1)	2 (14.3)	148 (44.1)
Gender			
Male	348 (99.4)	14 (100.0)	334 (99.4)
Transgender female	2 (0.6)	0 (0)	2 (0.6)
Self-declared ethnicity			
White	298 (85.1)	12 (85.7)	286 (85.1)
Non-white	52 (14.9)	2 (14.3)	50 (14.9)
Residence			
Amsterdam	216 (61.7)	6 (42.7)	210 (62.5)
Other	134 (38.3)	8 (57.1)	126 (37.5)
Education level			
Less than college degree	82 (23.4)	0(0)	82 (24.4)
At least college degree	268 (76.6)	14 (100.0)	254 (75.6)
Sexual preference			
Exclusively homosexual	276 (79.1)	14 (100.0)	262 (78.2)
Not exclusively homosexual	73 (20.9)	0 (0)	73 (21.8)
PrEP regimen			
Event-driven	93 (26.6)	2 (14.3)	95 (28.3)
Daily	257 (73.4)	12 (85.7)	241 (71.7)
Receptive condomless anal sex acts with casual partners, median [IQR][@]	2 [0-6]	10 [6-17]	3 [0-11]
0-1	141 (40.3)	1 (7.7)	134 (40.6)
2-5	113 (32.3)	2 (15.4)	79 (23.9)
6-9	38 (10.9)	3 (23.1)	25 (7.6)
10-24	44 (12.6)	4 (30.8)	61 (18.5)
≥25	14 (4.0)	3 (23.1)	31 (9.4)
Having had anal sex with ≥1 HIV-positive partner[@]			
No	126 (36.0)	3 (23.1)	161 (48.9)
Yes	224 (64.0)	10 (76.9)	168 (51.1)
Recent anal STI			
No	294 (87.0)	9 (64.3)	280 (86.7)
Yes	44 (13.0)	5 (35.7)	43 (13.3)
Recent syphilis infection			
No	337 (98.8)	13 (92.9)	322 (99.1)
Yes	4 (1.2)	1 (7.1)	3 (0.9)
Sharing sex toys[$]			
No	n.a.	7 (70.0)	239 (75.4)
Yes	n.a.	3 (30.0)	78 (24.6)
Fisting without gloves[$]			
No	n.a.	7 (70.0)	238 (75.1)
Yes	n.a.	3 (30.0)	79 (24.9)
Sharing straws when snorting drugs[$]			
No	n.a.	4 (40.0)	226 (71.3)
Yes	n.a.	6 (60.0)	91 (28.7)
Injecting drug use			
No	336 (97.1)[@]	6 (60.0)[^]	301 (95.0)[^]
Yes	10 (2.9)	4 (40.0)	16 (5.1)
Reporting chemsex			
No	196 (56.8)[@]	4 (36.4)[$]	191 (58.8)[$]
Yes	149 (43.2)	7 (63.6)	134 (41.2)

PrEP, pre-exposure prophylaxis; n.a., not assessed; STI, sexually transmitted infection. Chemsex is defined as the use of GBH/GBL, crystal methamphetamine or mephedrone around the time of having sex in the past 3 (@) or 6 months ($).
[1]14 individuals were diagnosed with 15 HCV infections; [2]At last visit before censoring; [@]in the last 3 months; [$]in the last 6 months; [^]in the last 12 months.

last study visit before 30 September 2018, whichever occurred first.

We calculated overall, primary infection (among anti-HCV negative participants), and re-infection IRs (among participants who had been cured or spontaneously cleared HCV infection).

For all calculations, follow-up included gaps during which PrEP use was temporarily discontinued and if incident HCV infection occurred, follow-up was allowed to continue after end of successful treatment or spontaneous clearance.

In risk-factor analysis, we expected that the few observed outcomes would lead to uncertain and exaggerated parameter estimates from standard regression techniques, which could result in incorrectly detecting a risk-factor.[23] To minimize this bias, we used a "penalized" regression approach whereby uncertain estimates from the data are pulled towards more realistic ones assumed from prior knowledge.[24]

Briefly, we fit univariable exponential survival models for each covariate separately using a Bayesian approach. For each risk-factor, we first specify a prior distribution of hazard ratios (HRs) based on the most "plausible range" from both previous research and clinical observations.[25] These priors were selected on the basis of risk factors identified from studies conducted in the Netherlands and Canada (Table S2).[4,12,16,26] Since the intercept of this model estimates the IR without a given risk-factor, we based the prior distribution of this parameter on the overall HCV-IR from a study among MSM PrEP users in France, estimated at 1 per 100PY [95% credible intervals (CrI) 0.3-4.0].[27] Using these priors together with the data, we estimated a posterior distribution of HRs with Markov Chain Monte Carlo methods from the "bayes" prefix commands in Stata. The median of this distribution defined the parameter estimate (termed "posterior HR") and their 2.5% and 97.5 % quantiles defined the 95% CrI (quantifying the uncertainty in the parameter estimate).

All statistical analysis was performed using Stata 15 (Statacorp, College Station, TX, USA).

Sensitivity analysis

First, we assessed IR of re-infection while not considering possible re-infections. Second, since posterior HRs could be highly influenced by the prior distribution,[28] we repeated risk-factor analysis using (1) non-informative priors (*i.e.* those that bear minimal influence when estimating the posterior distribution) for each covariate, and (2) a different prior for daily PrEP regimen (*vs.* event-driven) and anal sex with ≥1 HIV-positive partner. Third, we restricted analyses to participants who did not report IDU in the past 12 months during follow-up visits. Fourth, we repeated the analyses using an exponential survival model without a Bayesian approach.

Results

Study population

Of the 376 enrolled participants, 26 did not have at least 2 HCV-test results and were excluded. Of the 350 analyzed participants, 348 were MSM and 2 transgender women. At enrolment, median age was 40 years (IQR 33-48), 298 (85.1%) self-identified as white, 261 (74.6%) lived in Amsterdam and 10 (2.9%) reported IDU within 3 months before enrolment (Table 1). Initially, 257 (73.4%) participants chose daily and 93 (26.6%) event-driven PrEP. There were 17/350 (4.9%) participants with HCV antibodies at baseline, 15 of whom were HCV-RNA positive.

HCV incidence

Median cumulative follow-up was 2.0 years (IQR 1.9-2.0), totaling 653.6 PY at risk, during which time we identified 12 incident HCV infections and 3 possible incident HCV infections among 14 MSM (Table 2) (overall IR 2.30/100PY, Table S3). Six of

Table 2. Characteristics of HIV-negative men who have sex with men using PrEP who acquired an HCV infection during follow-up, AMPrEP study.

Participant number	HCV incident infection (if more than one)	Initial PrEP regimen	Days between initiation of PrEP to estimated date of incident HCV infection	Genotype of prevalent HCV infection	Genotype at incident HCV infection	Days between first sample with detectable HCV-RNA[&] and HCV diagnosis	HCV outcome
Primary HCV infections							
1[*]	-	Daily	9	n.a.	2b	0	Cured
2	-	Daily	133	n.a.	1a	0	Cured
3	-	Daily	454	n.a.	1a	0	Cured
4	-	Daily	130	n.a.	1a	189	Cured
5	-	Daily	603	n.a.	1a	91	Cured
6	-	Daily	425	n.a.	1a	91	Cured
7[@]	-	Daily	662	n.a.	1a	182	Cured
8[^]	-	Daily	40	n.a.	3a	166	Cured
HCV re-infections							
9	-	Daily	253	2b	1a	62	Cured
10	-	Daily	389	1a	1a	91	Cured
11	-	Event-driven	556	-	1a	0	Cured
12	-	Daily	606	1a	1a	0	Cured
Possible HCV re-infections							
13	(1)[$]	Daily	127	-	-	0	Cleared
	(2)[#]	Daily	472	-	1a	0	Cured
14[#]	-	Daily	460	4d	4d	0	Cured

PrEP, pre-exposure prophylaxis.
[*]RNA was tested as part of follow-up during post-exposure prophylaxis use.
[@]HCV-RNA but no HCV antibodies were detected at 182 days before diagnosis, revealed by additional testing of an earlier stored sample.
[^]Intermittently detectable HCV-RNA over a 13-month period since PrEP-initiation, revealed by additional HCV-RNA testing of earlier stored samples.
[$]Only one negative HCV-RNA test.
[#]Two negative HCV-RNA tests less than 24 weeks apart.
[&]Based on retrospective testing of earlier stored samples.

15 infections were diagnosed among 35 participants who reported IDU at least once during follow-up (IR 10.49/100PY). Nine infections were diagnosed among 314 participants who never reported IDU during follow-up (IR 1.51/100PY, Table S3). HCV was not diagnosed among transgender women. Primary infection occurred in 8 of 333 at-risk participants over 628.4 PY of follow-up (IR 1.27/100PY) and re-infection occurred in 7 of 21 at-risk participants over 25.2 PY of follow-up (IR 27.8/100PY). When excluding the 3 possible re-infections, the overall and re-infection IRs were 1.84/100PY and 16.6/100PY, respectively. No participant with incident HCV infection acquired HIV.

The overall IRs for daily and event-driven PrEP regimens were 2.71/100PY and 1.15/100PY, respectively (Table S3). IRs of primary infection were 1.51/100PY in daily PrEP and 0.61/100PY in event-driven PrEP, and IRs of re-infection 41.4/100PY and 9.3/100 PY, respectively. One person with incident HCV infection (Table 2, participant 11) was likely infected during PrEP discontinuation, while PrEP was re-continued at HCV diagnosis.

Of the 14 MSM with incident HCV infection(s), 13 had chosen daily PrEP and one event-driven PrEP at baseline. At the estimated date of first incident HCV infection, 12 were using daily and 2 event-driven PrEP. For 7/15 incident HCV infections, HCV-RNA was detectable in stored samples 62 to 189 days prior to HCV diagnosis.

Of the 4 proven re-infections, all had been treated with direct-acting antivirals for their primary HCV infection and had achieved SVR12. The 3 possible re-infections occurred in 2 persons. In these cases, we could not exclude relapse of a previous infection because the 2 viremic episodes were either separated by only one HCV-RNA negative visit (Table 2, participant 13, first infection) or 2 subsequent HCV-RNA negative visits were <24 weeks apart (participant 13 [second infection] and participant 14; Table S4) without a documented switch in genotype.

Risk factors for incident HCV infection

We found that HCV incidence was associated with increased number of receptive CAS acts with casual partners in the past 3 months (posterior HR 1.57 per ln increase, 95% CrI 1.09-2.20) and recent anal STI diagnosis (posterior HR 2.93, 95% CrI 1.24-7.13). Higher IRs were also found among those who shared straws when snorting drugs (posterior HR 2.62, 95% CrI 1.09-6.02) and those who injected drugs in the past 12 months (posterior HR 4.69, 95% CrI 1.61-12.09) (Table 3 and Fig. 1). Multivariable analysis was precluded by the small numbers of events.

Table 3. Prior and posterior estimates of risk factors for incident HCV infection in HIV-negative MSM using PrEP, AMPrEP study.

Risk-factor[1]	n (cases)[2] n = 15	PY	Prior HR (95% CrI)	Posterior HR (95% CrI)
Age (per 10 year increase)[3]			0.90 (0.23-3.60)	0.76 (0.50-1.12)
Ethnicity				
White	13	557.9	1	1
Non-white	2	95.6	1.00 (0.25-4.00)	0.98 (0.33-2.58)
PrEP regimen				
Event-driven	2	174.1	1	1
Daily	13	479.5	1.00 (0.25-4.00)	1.56 (0.85-2.83)
Receptive condomless anal sex acts (per ln increase)[6,7]			1.25 (0.31-5.00)	1.57 (1.09-2.20)
Having had anal sex with ≥1 HIV-positive partner[6]				
No	4	273.9	1	1
Yes	10	367.1	1.50 (0.38-6.00)	1.91 (0.81-4.33)
Recent anal STI				
No	10	573.9	1	1
Yes	5	79.7	2.00 (0.50-8.00)	2.93 (1.24-7.13)
Recent syphilis infection				
No	14	638.8	1	
Yes	1	14.8	2.00 (0.50-8.00)	2.26 (0.64-7.14)
Sharing of sex toys[5]				
No	8	406.9	1	1
Yes	3	121.0	2.00 (0.50-8.00)	1.64 (0.64-4.07)
Fisting without gloves[5]				
No	8	393.6	1	1
Yes	3	134.3	2.00 (0.50-8.00)	1.51 (0.59-3.90)
Sharing straws when snorting drugs[4]				
No	5	372.5	1	1
Yes	6	155.4	2.00 (0.50-8.00)	2.62 (1.09-6.02)
Injecting drug use[4]				
No	7	498.4	1	1
Yes	4	29.5	2.00 (0.50-8.00)	4.69 (1.61-12.09)
Reporting chemsex[5]				
No	5	297.2	1	1
Yes	7	200.2	1.50 (0.38-6.00)	2.02 (0.83-4.89)

CrI, credible interva; HR, Hazard ratiol; ln, natural log; PrEP, pre-exposure prophylaxis; PY; person-years; STI, sexually transmitted infection.
HRs and corresponding 95% CrI were obtained from univariable Bayesian exponential survival models.
[1]Risk-factor as reported or measured at the earliest visit after estimated date of HCV infection, with the exception of ethnicity (data collected at enrolment).
[2]Cases denote infections.
[3]Cases per age category: <35: n = 6, 35-44: n = 7, ≥45: n = 2; age was centered at 18 years.
[4]In the last 12 months; [5]in the last 6 months; [6]in the last 3 months.
[7]With casual partners, cases per category: 0-1: n = 1, 2-5: n = 3, 6-9: n = 3, 10-24: n = 4, ≥25: n = 3, missing: n = 1.

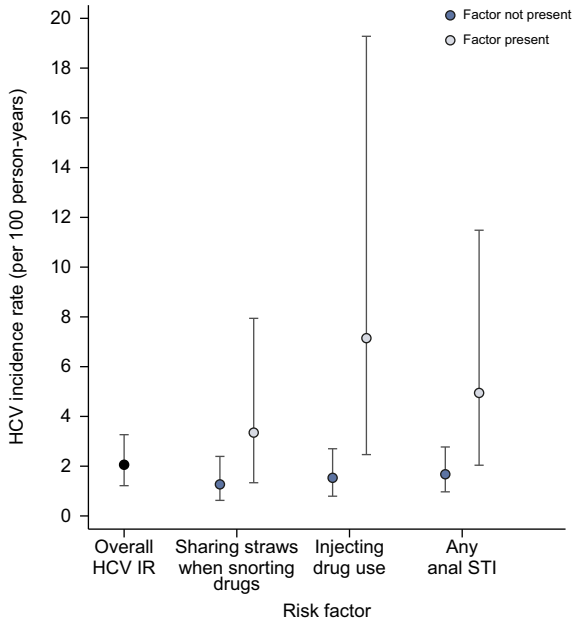

Fig. 1. Modeled HCV incidence rates, overall and by levels of risk factors, identified among HIV-negative MSM using PrEP, AMPrEP study, Amsterdam, the Netherlands, 2015-2018. The number of receptive CAS acts with casual partners, identified as a risk-factor, was not included. IRs and corresponding 95% CrI were obtained from univariable Bayesian exponential survival models.

Using non-informative priors resulted in CrI with increased uncertainty (Table S5), yet all identified factors for incident HCV infection were retained with the exception of sharing straws when snorting drugs. A stronger prior for daily PrEP regimen and for reporting sex with at least one HIV-positive partner (prior HR 2 for both) did not substantially alter posterior HRs (1.78, 95% CrI 0.95-3.32 and 2.07, 95%CrI 0.92-4.83, respectively). HRs estimated from the non-penalized regression approach resulted in much higher HRs with larger 95% confidence intervals as expected (Table S6). We did not identify risk factors when we restricted the analysis to participants who did not report IDU during follow-up, although all factors had similar directions of effect compared to the main analysis (Table S7). The proportion engaging in HCV risk factors did not increase over time since initiation of PrEP (Table S8). Number of receptive condomless anal sex acts with casual partners, proportion having had anal sex with an HIV-positive partner, having a recent anal STI and sharing sex toys were all lower in the event-driven *vs.* daily PrEP group (Table S9).

Hepatitis C virus genotyping and phylogenetic analysis

Of the 8 primary infections, 6 were subtype 1a, one 2b and one 3a. All 4 proven re-infections were subtype 1a. Of the 3 possible re-infections, one was subtype 4d, one 1a and one could not be genotyped.

Fig. 2 shows the maximum-likelihood phylogenies of HCV-1a, HCV-2b, HCV-3a and HCV-4d, including all incident and prevalent HCV infections among AMPrEP participants together with HCV strains from HIV-positive MSM, PWID and blood donors in the Netherlands. Phylogenetic trees revealed 10 robust MSM-specific clusters of HCV-1a (n = 7), HCV-2b (n = 1), HCV-3a (n = 1) and HCV-4d (n = 1), which ranged in size from 3 to 53 sequences. HCV sequences from HIV-negative MSM were highly interspersed with those of HIV-positive MSM: 27/29 (93.1%) strains of HIV-negative MSM were part of 10 MSM-specific clusters also containing HIV-positive MSM, one formed a homologous pair with a strain from an HIV-positive MSM, and one strain from an HIV-negative MSM had a singleton sequence.

Six of 17 participants with prevalent or resolved HCV infection were diagnosed with a re-infection during follow-up. Of those, 2 had resolved their initial infection prior to enrolment, leaving 4 participants for whom we could compare the primary and re-infecting HCV strain. Phylogenetic analysis confirmed that 3 participants indeed had 2 different HCV strains over time: participant 10: HCV 1a-1 and HCV 1a-2, participant 12: HCV 1a-1 and HCV 1a-8, and participant 9: HCV 2b-1 and HCV 1a-1 (Fig. 2). Participant 14 was classified as possibly re-infected and had 2 viraemic episodes with 2 highly similar HCV 4d-1 strains separated by 2 HCV-RNA negative visits more than 2 months apart.

Discussion

Among HIV-negative MSM enrolled in the Amsterdam PrEP project, we found a high incidence of HCV primary and re-infection, with a much lower IR for primary than re-infection. Factors associated with HCV infection were receptive CAS with casual partners, being diagnosed with an anal STI, IDU and sharing straws when snorting drugs. Phylogenetic analyses revealed that HCV sequences of HIV-negative MSM starting or using PrEP were highly interspersed with HCV sequences from HIV-positive MSM.

Several studies have reported small numbers of incident HCV infections among HIV-negative MSM.[8,15,29–35] HCV-IRs among HIV-negative MSM using PrEP were assessed in 3 cohorts, with observed IRs varying from 0/100PY in Canada to 3.0/100PY in France in 2017.[27,35,36] In our study, the overall HCV-IR was more comparable to the French study. The differences in reported IRs across the different studies may be explained by variations in sexual networks and risk behaviour, geographic region, calendar time, and whether re-infections have been included in IR estimations.[37]

The IRs of overall HCV infection and re-infection observed in HIV-negative MSM using PrEP are comparable to those observed in HIV-positive MSM.[2,5,38–41] In contrast, HIV-negative MSM not on PrEP have a 19-fold lower HCV incidence compared to HIV-positive MSM according to a recent meta-analysis.[42] The prevalence of HCV antibody-positive serology among HIV-negative MSM in Amsterdam participating in cross-sectional surveys at our STI clinic varied between 0.0% and 1.7% and did not change from 2007-2017.[43] These findings imply that there is no overflow of HCV infections observed in HIV-positive MSM and HIV-negative MSM starting or using PrEP to the larger HIV-negative MSM population, at least for the time being. Taken together, this evidence adds to the need for routine HCV-screening among MSM if initiating or using PrEP, as well as continued surveillance of HCV infections within the broader HIV-negative MSM population.

The reasons explaining why HIV-negative PrEP-using MSM acquire HCV may be twofold: their sexual networks are more likely to overlap with those of HIV-positive MSM, in whom HCV is more prevalent, and after initiating PrEP, the protection against HIV provided by PrEP makes the use of other prevention strategies, such as serosorting and condom use, less important. Several studies have indeed shown decreases in condom use

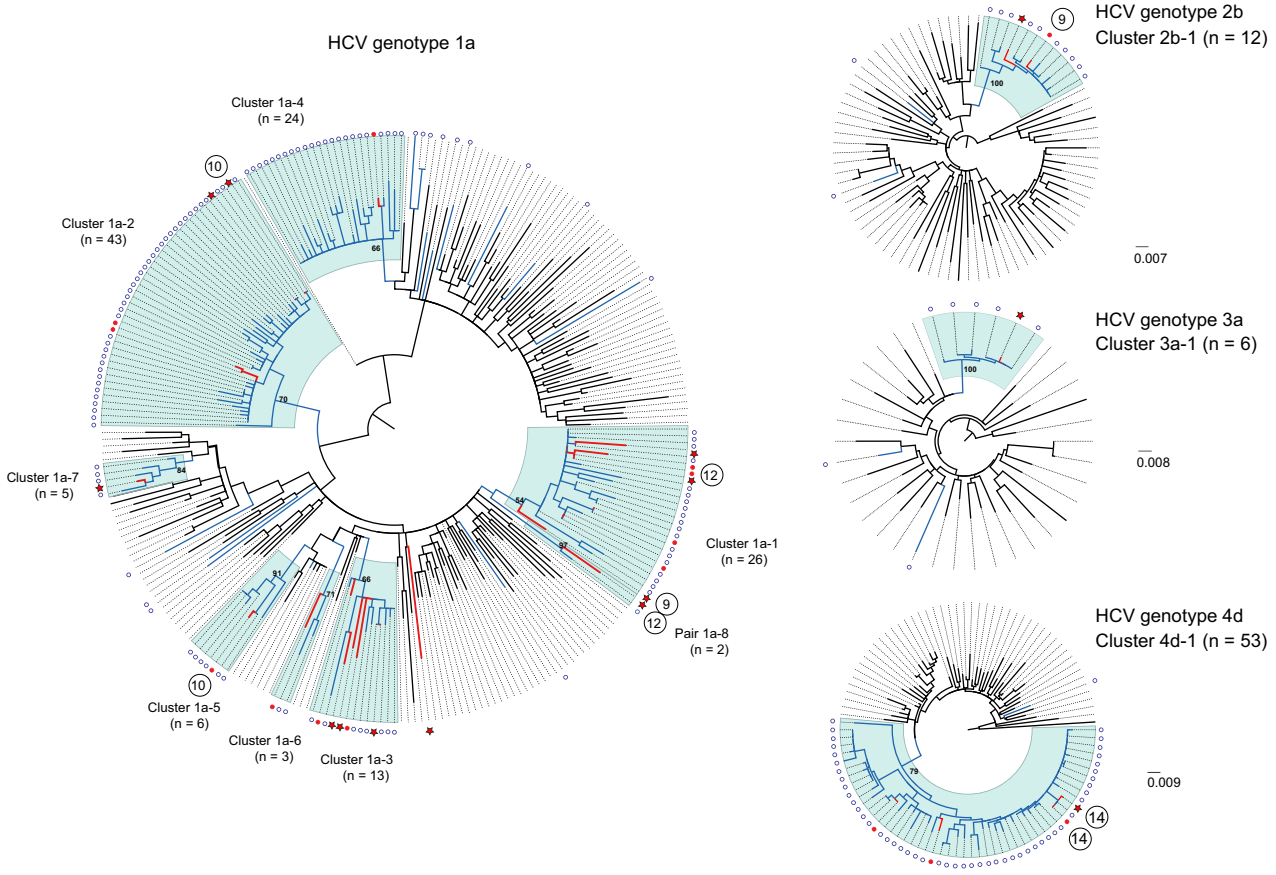

Fig. 2. HCV NS5B fragment 2 phylogenetic trees for HCV subtypes 1a, 2b, and 4d comparing HCV sequences from HIV-negative MSM who had prevalent HCV infection upon PrEP-initiation (red branches, red dots), and incident infection during PrEP use (red branched, red stars) with HCV sequences obtained from HIV-positive MSM (blue branches, blue dots) and unrelated HCV-positive individuals other than MSM, predominantly people who inject drugs, (black branches) in the Netherlands. Numbers in circles correspond to numbers in Table 2 and indicate participants with prevalent or resolved HCV infection and diagnosed with a re-infection during follow-up. Maximum-likelihood phylogenies were constructed using a general time-reversed substitution model with γ-distribution.

during the course of PrEP use,[17,44] which likely results in increased exposure to HCV and susceptibility to HCV-transmission when STI are present.[3] We demonstrated that factors associated with incident HCV infection were related to both sex (receptive CAS with casual partners and a recent anal STI) and drug use (IDU and sharing straws when snorting drugs). These factors are largely comparable to those found in HIV-positive MSM.[2,5] HCV incidence was higher among those on daily PrEP than among those on event-driven PrEP, most likely resulting from higher levels of sexual risk behaviour in this group. Although several HCV infections were among participants reporting IDU, the majority (9/15) of infections was not related to IDU, indicating that sexual behaviour is, next to IDU, an important target for intervention.

Similar to our study, 2 studies from France have reported that HCV isolates from HIV-negative MSM, of whom the majority was using PrEP, were infected with HCV strains also found in HIV-positive MSM.[31,35] Our phylogenetic analysis, using longer HCV NS5B fragments and larger subsets of HCV isolates obtained from various risk groups of both MSM and non-MSM, confirms the existence of robust MSM-specific HCV clusters. This emphasizes the overlap between sexual networks of MSM with and without HIV.

The major strengths of our study are that we report not only HCV-IRs but also factors associated with incident HCV infection and phylogenetic clusters from a PrEP demonstration study. In addition, we report HCV-IRs of MSM using daily and event-driven PrEP, which have yet to be reported by other trials or prospective cohorts.

This study does have some limitations. Risk factors for HCV re-infection in MSM have not been studied extensively and all relevant risk factors might not have been included. We tested for HCV-RNA every 6 months and re-infections lasting shorter than this interval could have gone undiagnosed, leading to underestimated IRs. This limitation is shared, however, with other studies reporting irregular or unclear HCV-testing frequency.[8,15,27,32–34] One participant had 2 HCV infections with viral strains that, genetically, were highly comparable. It remains uncertain whether he was re-infected via a related source (*i.e.* the same sexual partner) or had chronic HCV infection with low intermittent viraemia.

PrEP prevents HIV, but not HCV infection. Currently, sexually active MSM intending to use PrEP are recommended to be screened for HCV infection at PrEP-initiation, while the advised testing frequency during PrEP use varies with 3-month intervals,[45,46] regularly,[47] annually[48] or even 'consider annual testing'.[49] Based on the observed incidence rate, we suggest that all MSM be tested for HCV antibodies at PrEP-initiation, followed by a minimum of 6-month testing intervals during PrEP. More

frequent testing (*e.g.* at 3-month intervals) for HCV-RNA should be considered for those with previous HCV infection and those reporting HCV risk factors.

In conclusion, we demonstrate ongoing sexual transmission of HCV and high IR of primary and re-infection in a cohort of HIV-negative MSM using PrEP. Both rates and risk factors are comparable to those among HIV-positive MSM. Phylogenetic analysis suggests shared sexual networks of MSM with and without HIV. Our findings highlight the importance of an integrated sexual health approach, which includes professional education concerning HCV, frequent provider- or client-initiated testing and treatment of those with detectable HCV-RNA. Additional preventive interventions are certainly needed, such as campaigns to inform MSM about the risks of HCV and effective behavioural interventions, particularly for those at risk of (re-) infection.

Abbreviations

CAS, condomless anal sex; CrI, credibility interval; HR, hazard ratio; IDU, injection drug use; IR, incidence rates; MSM, men who have sex with men; PrEP, pre-exposure prophylaxis; PWID, people who inject drugs; PY, person-years; STI, sexually transmitted infection.

Financial support

The AMPrEP study received funding as part of the H-team initiative from ZonMw (grant number: 522002003), the National Institute for Public Health and the Environment, GGD research funds, Gilead Sciences, and the H-TEAM. The study drug and an unrestricted research grant for AMPrEP was provided by Gilead Sciences. The H-TEAM initiative is supported by the Aidsfonds Netherlands (grant number: 2013169), Stichting Amsterdam-Dinner Foundation, Gilead Sciences Europe Ltd (grant number: PA-HIV-PREP-16-0024), Gilead Sciences (protocol numbers: CO-NL-276-4222, CO-US-276-1712), Janssen Pharmaceuticals (reference number: PHNL/JAN/0714/0005b/1912fde), M.A.C. AIDS Fund, and ViiV Healthcare (PO numbers: 3000268822, 3000747780).

Conflicts of interest

The study drug was donated by Gilead Sciences. EH obtained advisory board fees from Gilead Sciences and speaker fees from Janssen-Cilag; both of which were paid to her institute.

HdV obtained a research grant from Medigene and advisory board and speaker fees from Gilead Sciences, Medigene, Abbvie, Janssen-Cilag and Willpharma, all of which were paid to his institution. MP obtained unrestricted research grants and speaker fees from Gilead Sciences, Roche, Abbvie and MSD; all of which were paid to her institute. The other authors reported no conflicts of interests.

Part of the findings were previously reported at the International Symposium on Viral Hepatitis and Liver Diseases (ISVHLD) held from June 14th to 17th, 2018, in Toronto, Canada (https://onlinelibrary.wiley.com/doi/full/10.1111/jvh.03_12935), and at the international AIDS conference, held from July 23th to 27th in Amsterdam, the Netherlands (https://onlinelibrary.wiley.com/doi/full/10.1002/jia2.25148). Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: EH, TvdL, MP. Acquisition, analysis or interpretation of the data: LC, EH, AB, MSvdL, TvdL, RA, MP, HdV. Drafting of the manuscript: EH, LC, TvdL. Critical revision of the manuscript: all authors.

Acknowledgements

The authors wish to thank all AMPrEP participants. In addition, they thank the following persons for their support to this study: Joop Arends, Marjo Broeren, Udi Davidovich, Yvonne van Duijnhoven, Mark van den Elshout, Ertan Ersan, Princella Felipa, Arjan Hogewoning, Marije Hofstra, Kees de Jong, Michelle Kroone, Myra van Leeuwen, Dominique Loomans, Ilya Peters, Martijn van Rooijen, Janke Schinkel, Adriaan Tempert, Marc van der Valk, and Annemarie Wensing. Furthermore, they thank the members of the AMPrEP advisory board and community engagement group, and all of those who contributed to the H-TEAM.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.11.022.

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