Expanding epidemic of recently acquired HCV in HIV-coinfected patients over a period of 10 years

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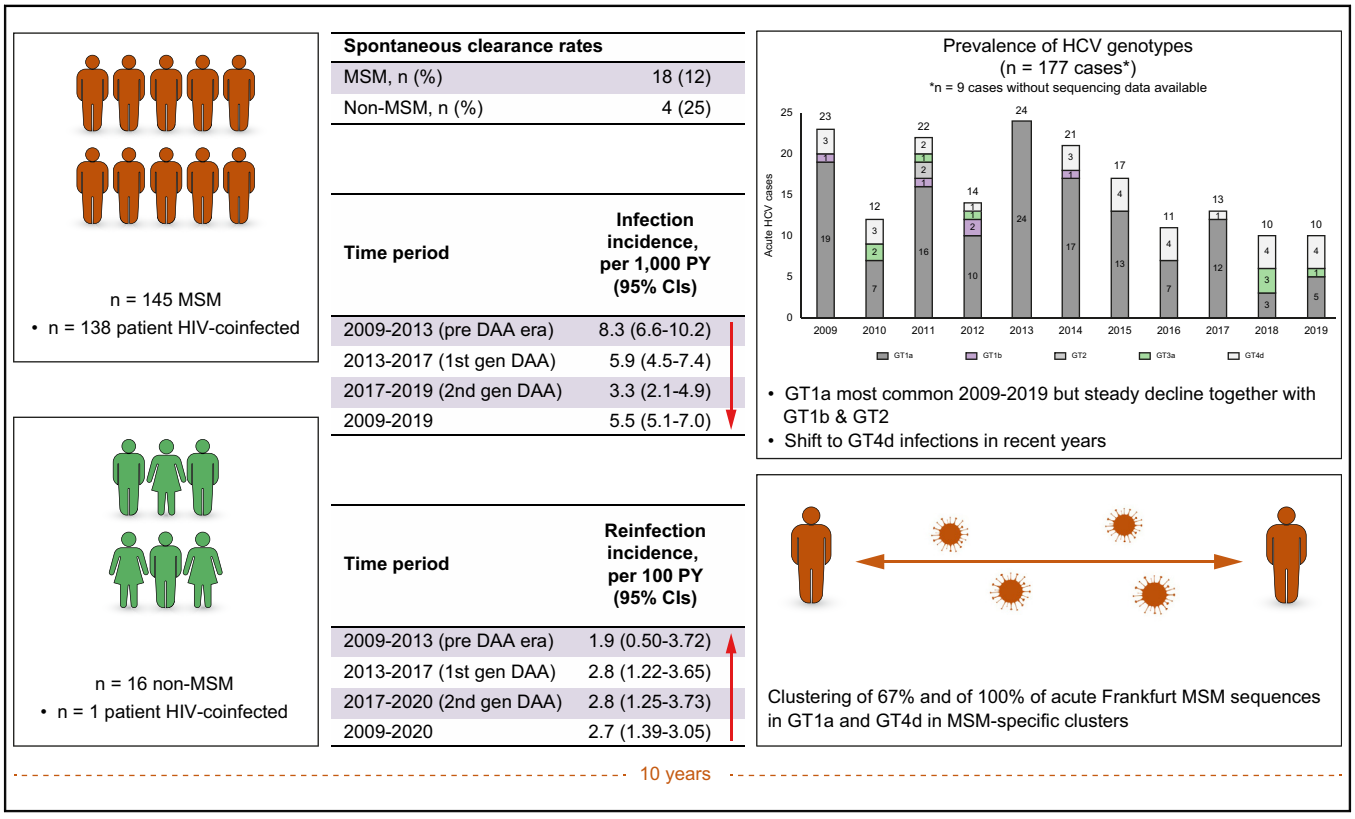
Graphical abstract



Highlights

- Recently acquired HCV infections primarily occur in HIV-coinfected MSM and PWID.
- Over 10 years, an overall decline of recently acquired HCV infections and a certain HCV genotype pattern were observed.
- Country-mixing MSM-specific clustering with a shift towards HCV GT4d infections in recent years could be identified.
- Ongoing risk of repeated HCV transmission in MSM is caused by increase of reinfection incidence and low spontaneous clearance rates.
- Our findings underscore the importance of constructive preventive strategies and early treatment initiation in MSM to achieve HCV elimination.

Impact and Implications

We evaluated the occurrence and transmission of recently acquired HCV infections (RAHCs) over a period of 10 years. Our data demonstrate that the presence of RAHC was mainly found in HIV-coinfected MSM, with internationally connected transmission networks being observed in the majority of patients. Spontaneous clearance rates were low, and reinfection rates increased mainly driven by a small subset of MSM patients with high-risk behaviour.

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Research article

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Expanding epidemic of recently acquired HCV in HIV-coinfected patients over a period of 10 years

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Background & Aims: Ongoing transmission of HCV infections is associated with risk factors such as drug injection, needlestick injuries, and men who have sex with men (MSM). Ways of transmission, the course of acute infection, changes of virologic features, and incidence over time are not well known.
Methods: Over a period of 10 years, n = 161 patients with recently acquired HCV infection (RAHC) (median follow-up 6.8 years) were prospectively enrolled. NS5B sequencing was performed to re-evaluate the HCV genotype (GT) and for phylogenetic analyses.
Results: Patients with RAHC were mainly male (92.5%), MSM (90.1%), and HIV-coinfected (86.3%). Transmission risk factors for MSM and non-MSM were sexual risk behaviour (100 and 6.3%, respectively), injection drug use (9.7 and 37.5%, respectively), and nasal drug use (15.2 and 0%, respectively). Spontaneous and interferon- or direct-acting antiviral-based clearance rates were 13.6, 84.3 and 93.4%, respectively. Mean RAHC declined from 19.8 in the first to 13.2 in the past five study years. Although the majority of infections was caused by HCV GT1a, the frequency of HCV GT4d and slightly HCV GT3a increased over time. No relevant clustering of HCV isolates was observed in non-MSM. However, 45% of HCV GT1a and 100% of HCV GT4d MSM cases clustered with MSM isolates from other countries. Travel-associated infections were supported by personal data in an MSM subgroup. No international clustering was detected in MSM with HCV GT1b or HCV GT3a.
Conclusions: RAHCs were mainly diagnosed in HIV-coinfected MSM patients and were associated with sexual risk behaviour. Spontaneous clearance rates were low, and phylogenetic clusters were observed in the majority of patients.
Impact and Implications: We evaluated the occurrence and transmission of recently acquired HCV infections (RAHCs) over a period of 10 years. Our data demonstrate that the presence of RAHC was mainly found in HIV-coinfected MSM, with internationally connected transmission networks being observed in the majority of patients. Spontaneous clearance rates were low, and reinfection rates increased mainly driven by a small subset of MSM patients with high-risk behaviour.

Introduction

Ongoing transmission of HCV infection is associated with high numbers of patients with acute hepatitis C globally.[1,2] Intravenous drug abuse (IVDA) is a major risk factor for HCV transmission.[2] However, over the last two decades, a persistent epidemic of recently acquired HCV infections (RAHCs) has also been observed in HIV-positive men who have sex with men (MSM) in several metropolitan areas worldwide.[3] Depending on various factors, RAHC spontaneously cleared in 15–30% of patients, with higher rates being reported in HCV-monoinfected patients.[4] In patients who initiated antiviral treatment with direct-acting antivirals (DAAs) during the acute HCV (aHCV) infection phase, high sustained virologic response (SVR) rates have been achieved, even after using a shortened treatment duration.[5] However, multiple recently acquired HCV reinfections after successful virus elimination have been observed in MSM.[6] Data from the interferon era indicated that reinfection incidence ranges between 7 and 15 per 100 patient-years in MSM living with HIV.[7] Comprehensive data on HCV reinfection incidence from the DAA era are still scarce, but first longitudinal reports provide evidence that despite high treatment uptake and excellent treatment outcomes, HCV reinfection rates in HIV-positive MSM continue to be comparably high[3,6,8] Therefore, questions remain about what impact DAA availability will have on the epidemic and whether elimination will be achieved in the short term as intended by the World Health Organization agenda. One of the major reasons for the ongoing epidemic in

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MSM may be their maintained risk behaviour consisting of traumatic sexual practices, and recreational intravenous and nonintravenous drug use, recently referred to as chemsex.[6–8]

In recent years, an increasing number of HCV outbreaks among MSM and people who inject drugs (PWID) has been observed in large urban centres.[9,10] Several studies showed a separate phylogenetic clustering of HCV strains in patients with IVDA, sexual networks, and HIV-coinfected patients, implicating different transmission networks.[10–12] Interestingly, MSM-specific transmission networks were not restricted to single countries but largely overlapped in an interconnected international transmission network.[9]

Overall, there is a lack of data concerning the investigation of patients with RAHC over extended time periods. In the present study, we analysed patients with recently acquired hepatitis C presented at large liver and infectiology centres in Frankfurt am Main, Germany, over a period of up to 10 years. In addition to epidemiological and clinical parameters, virologic outcome and detailed phylogenetic analyses were conducted to identify possible HCV transmission clusters.

Patients and methods

Study population

All patients aged 18 and older who were diagnosed with an acute hepatitis C infection at the participating centres (liver and HIV centres at Goethe-University Hospital and Infektiologikum, Frankfurt, Germany) were prospectively enrolled between January 2009 and December 2019 and followed up until October 2020. Acute hepatitis C was diagnosed if one of the following criteria was met: (1) documented HCV antibody seroconversion within 12 months and a positive HCV RNA, (2) positive HCV RNA and a documented prior negative HCV antibody or HCV RNA test within 12 months, and (3) positive HCV RNA and an increase in liver transaminases more than five times the upper limit of normal and normal transaminases the year before. SVR was defined by a negative HCV PCR at least 12 weeks after the end of treatment. The definition of a spontaneous hepatitis C clearance is described elsewhere.[13] HCV reinfection was defined by a detectable positive HCV RNA level after documented HCV RNA negativity, by a switch in HCV genotype (GT), or by phylogenetic analysis suggesting HCV reinfection following SVR. Serum and EDTA samples were collected at the time of diagnosis of an acute hepatitis C infection (baseline) and longitudinally at monthly intervals during the follow-up routine medical visits. Collection of samples was stopped as soon as an SVR or a spontaneous clearance was documented. Sera from patients with chronic hepatitis C infection, who were used as a control cohort, were obtained from the European Resistance Database, which was established based on a non-interventional study.[14] According to the international European AIDS Clinical Society guidelines, screening on HCV coinfection was performed annually in HIV-positive patients.[15] Further screens were conducted based on individual risk behaviour and in cases of a suspected HCV coinfection. Monitoring of transaminases in HIV-positive individuals was performed every 3–12 months. Demographic and clinical data were recorded at baseline and at each follow-up visit. Moreover, the history of sexually transmitted diseases within 6 months before the onset of RAHC as well as the history of a prior hepatitis C infection were assessed by medical chart review. Patients were followed up to the last medical visit with available biochemical parameters at their treatment centre. All participants provided written informed consent before inclusion in the study. Ethical approval of this study as well as for usage of patient blood samples for research purposes was obtained from the local ethics committee at the University Hospital Frankfurt, Germany (ethics committee reference number: 58-09). All investigations were performed in accordance with the Declaration of Helsinki.

Statistical analyses

Values of demographic and clinical characteristics are expressed as mean (range) or median (IQR). Baseline comparisons between groups were performed using a *t* test (for variables with an assumed Gaussian distribution) or a Mann–Whitney *U* test (for non-normally distributed variables).

Incidence rates of a first HCV infection and of a reinfection during follow-up of the study period were calculated using person time of observation. Details on the calculation of incidence rates and the corresponding denominators can be found in the Supplementary material. CIs for incidence rates (reinfections and new infections) were assessed using a Poisson model. A p value of ≤0.05 was considered statistically significant. Statistical analysis was performed using SPSS (version 22; IBM; Armonk, NY, USA) and R (version 4.0.4; R Foundation for Statistical Computing, Vienna, Austria).

NS5B amplification and sequencing analyses

Extraction of HCV RNA and cDNA synthesis were conducted as described previously.[14] We amplified a 465- to 494-bp fragment of NS5B using nested PCR and primers described elsewhere.[16] Further details on the PCRs and sequencing analyses can be found in the Supplementary methods.

Phylogenetic analyses

We compared the NS5B sequences from 161 patients with RAHC in the current study with a set of 369 published sequences obtained from patients with RAHC and a sexual transmission mode (MSM). The latter sequences are available from GenBank (https://www.ncbi.nlm.nih.gov/genbank/).[3,10,17–22] As a control, 61 sequences from patients with chronic hepatitis C infection and non-sexual transmission were included in the analysis. For each GT, the maximum likelihood phylogenetic tree with 100 bootstrap replicates was inferred using IQ-TREE (version 2.0.3).[23] The best-fit substitution models for each GT were determined according to the Bayesion information criterion (BIC) (GT1a: TIM2e + I + G4, GT1b: K2P + I + G4, GT3a: K2P + I + G4, and GT4d: K2P + I + G4) by the built-in method ModelFinder.[24] Further details on the construction of phylogenetic trees for each GT, alignment, derivation of phylogenetic clusters, and accession numbers from published sequences are available in the Supplementary information.

Phylogenetic analysis of reinfections

Phylogenetic analyses to detect potential reinfections were conducted for 13 patients with recently acquired HCV GT1a infection and 2 patients with HCV GT4d infection. Samples from patients with chronic hepatitis C and a documented relapse to a DAA-based treatment were used as controls. Relapse was defined as HCV RNA negativity at the end of DAA treatment followed by detectable HCV RNA within 12 weeks after the end of treatment. Control patient samples were obtained from the European Resistance Database, and sequence data at baseline and at the time of relapse were used for phylogenetic analyses (HCV GT1a,

Table 1. Baseline characteristics of MSM and non-MSM diagnosed with acute HCV infection between 2008 and 2019 (n = 161).

Characteristics	MSM (n = 145)	Non-MSM (n = 16)
Median age (years)	43 (39–47)	32 (25–51)
Male sex, n (%)	145 (100)	4 (25)
Caucasian ethnicity	136 (93.8)	14 (87.5)
BMI (kg/m^2), median (range)	24.0 (21–26)	20.8 (20–23)
HIV coinfection, n (%)	138 (95.2)	1 (6.3)
CDC stage C, n (%)	15 (10.9)	0 (0)
HIV RNA viral load (log_{10}) (IU/ml), median (range)	0 (0–33.5)	0 (0)
CD4$^+$ cell count (/μl), median (range)	588 (433–766)	612
Antiretroviral treatment, n (%)	133 (96.4)	1 (100)
Cured HCV episodes before inclusion, n (%)	16 (11)	1 (6)
STI identified at HCV diagnosis, n (%)	108 (74.5)	0 (0)
History of STI lifetime, n (%)	88 (60.7)	1 (6.3)
Transmission risk factors		
Injecting drug use, n (%)	14 (9.7)	6 (37.5)
Sexual risk behaviour, n (%)	145 (100)	1 (6.3)
Needlestick injury, n (%)	0 (0)	3 (18.8)
Transfusion/surgery, n (%)	0 (0)	3 (18.8)
Others/unknown, n (%)	0 (0)	3 (18.8)
Substance abuse		
Injecting drug use, n (%)*	14 (17.0)	6 (46.2)
Nasal drug use, n (%)*	34 (41.5)	6 (46.2)
Alcohol abuse, n (%)**	74 (81.3)	5 (38.4)
Traumatic sexual practices		
Sex party, n (%)	42 (29.0)	0 (0)
Fisting, n (%)	19 (13.1)	0 (0)
HCV genotype, n (%)***		
1a	116 (80)	6 (38)
1b	1 (1)	4 (25)
2b	0 (0)	2 (13)
2c	0 (0)	1 (6)
3a	3 (2)	3 (19)
4d	25 (17)	0 (0)
Biochemical parameters of HCV infection		
Baseline HCV RNA (log_{10} IU/ml), median (range)	6.1 (1.5–9.6)	5.3 (1.1–7.4)
Baseline ALT (IU/ml), median (range)	319 (28–4,332)	172 (6–1,943)
Maximum ALT (IU/ml), median (range)	484 (27–4,754)	146 (11–1,943)
Maximum bilirubin (IU/ml), median (range)	1.0 (0.3–23)	0.9 (0.2–13.1)
Clinical course of HCV infection		
Chronic HCV, treatment pending, n (%)	1 (1)	1 (6)
Spontaneous HCV clearance, n (%)	18 (12)	4 (25)
LTFU before treatment, n (%)	6 (4)	0 (0)
Therapy initiation, n (%)	120 (83)	11 (69)
PEG/RBV, n (%)	51 (43)	7 (64)
PEG/RBV/SOF, n (%)	7 (6)	0 (0)
PEG/RBV + BOC, n (%)	1 (1)	0 (0)
PEG/RBV + TVR, n (%)	2 (2)	2 (13)
SOF/RBV, n (%)	1 (1)	0 (0)
SOF/LDV ± RBV, n (%)	30 (25)	2 (13)
SOF/DCV/RBV, n (%)	1 (1)	0 (0)
SOF/VEL ± RBV, n (%)	8 (7)	0 (0)
PTV/r/OBV ± RBV, n (%)	4 (3)	0 (0)
PTV/r/OBV/DSV ± RBV, n (%)	1 (1)	0 (0)
GLE/PIB, n (%)	14 (12)	0 (0)
SVR achieved, n (%)	103 (86)	7 (64)
Relapse/non-response§, n (%)	5 (4)	0 (0)
Treatment discontinuation, n (%)	5 (4)	0 (0)
LTFU during therapy, n (%)	7 (6)	4 (36)

ALT, alanine aminotransferase; GLE/PIB, glecaprevir/pibrentasvir; LTFU, lost to follow-up; MSM, men who have sex with men; PEG/RBV + BOC, pegylated interferon/ribavirin + boceprevir; PEG/RBV + TVR, pegylated interferon/ribavirin + telaprevir; PEG/RBV, pegylated interferon/ribavirin; PEG/RBV/SOF, pegylated interferon/ribavirin/sofosbuvir; PTV/r/OBV ± RBV, paritaprevir/ritonavir/ombitasvir ± ribavirin; PTV/r/OBV/DSV ± RBV, paritaprevir/ritonavir/ombitasvir/dasabuvir ± ribavirin; SOF/DCV/RBV, sofosbuvir/daclatasvir/ribavirin; SOF/LDV ± RBV, sofosbuvir/ledipasvir ± ribavirin; SOF/RBV, sofosbuvir/ribavirin; SOF/VEL ± RBV, sofosbuvir/velpatasvir ± ribavirin; STI, sexually transmitted infection; SVR, sustained virologic response.

* Data on intravenous and nasal drug use available in n = 82 MSM and n = 13 non-MSM.

** Data on alcohol abuse available in n = 91 MSM and n = 13 non-MSM.

*** Determined hybridisation assay (VERSANT HCV Genotype 1.0 and 2.0, Assay Siemens, Germany).

§ Virologic failure to interferon-based therapy in four cases and to DAA-based treatment in one case.

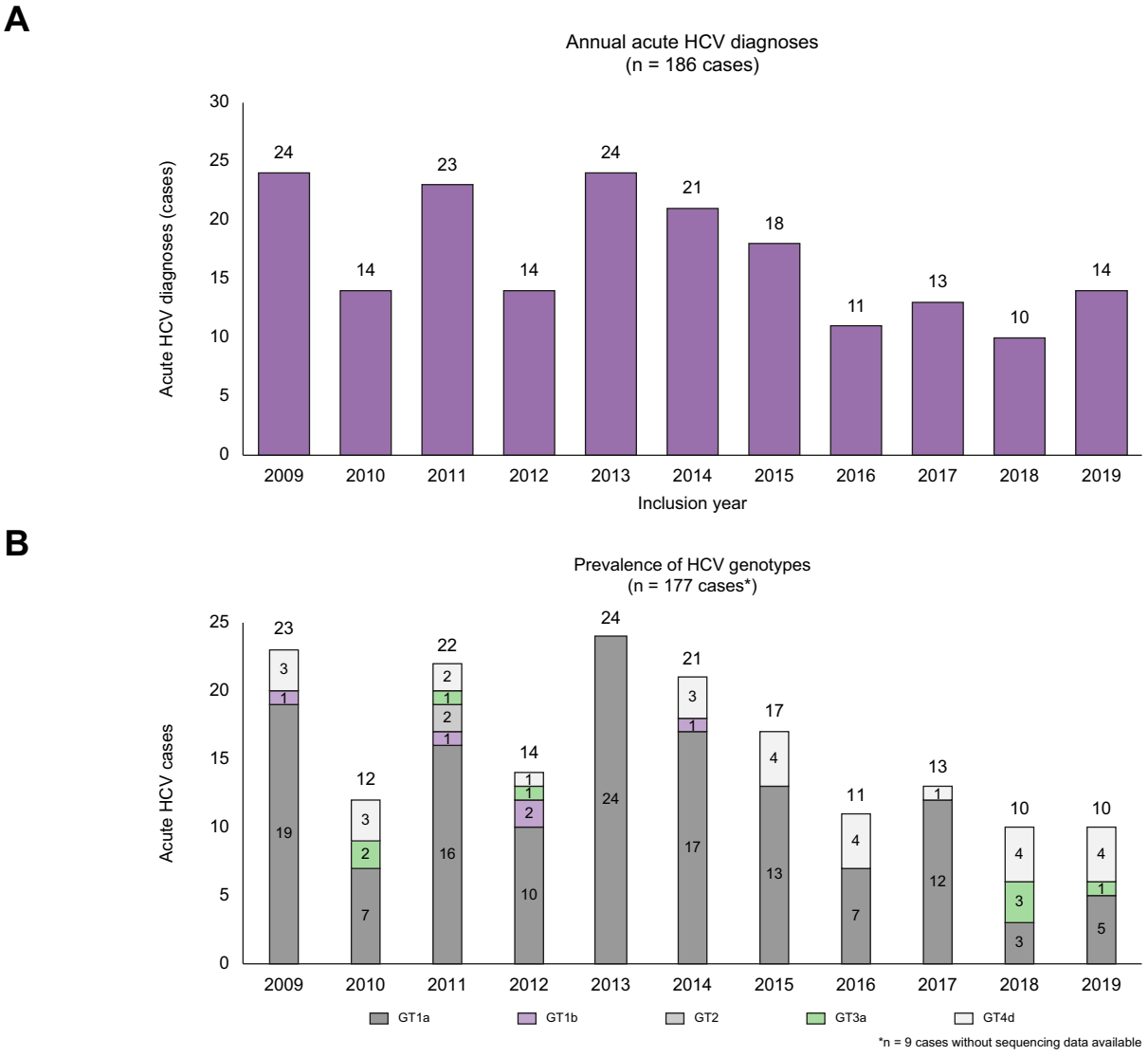

Fig. 1. Characteristics of HCV-monoinfected and HCV/HIV-coinfected patients with recently acquired hepatitis C infection. (A) Annual recently acquired hepatitis C cases and (B) prevalence of different HCV genotypes and subtypes over the study time period. GT, genotype.

n = 4; HCV GT4d, n = 1).[14] The baseline and post-treatment consensus sequences for subtypes 1a and 4d were aligned separately using BioEdit (version 7.2.5, Ibis Biosciences, Carlsbad, CA, USA). For each subtype, IQ-TREE (version 2.0.3) was used to infer maximum likelihood phylogenetic trees with 100 bootstrap replicates.[23] The best-fit substitution models were determined according to the BIC (GT1a: K2P + G4, GT4d: K2P) by using ModelFinder.[24]

Results

Study population

Demographic and clinical characteristics of the study population are shown in Table 1. Between January 2009 and December 2019, a total of 161 patients diagnosed with RAHCs were included in the study, of whom 145 (90.1%) were MSM. The majority of patients were Caucasian (93.2%), and 1.9% were Asian and 2.5% were Hispanic. At the time of study inclusion, the median age was 43 years (IQR 38–48 years), and 149 (92.5%) participants were male. Symptomatic disease was observed in a total of 59 patients (36.7%); 20 patients had jaundice, and nine had a bilirubin level >10 mg/dl. Furthermore, 24 patients reported fatigue, and 13 patients had abdominal pain at the time of HCV diagnosis. Median alanine aminotransferase (ALT) level at baseline was 463 IU/ml. Median maximum of ALT and bilirubin values was 4,754 IU/ml and 23 mg/dl, respectively. Median HCV RNA viral load at baseline was 6.1 $\log_{10}$ IU/ml (range 1.2–9.6 $\log_{10}$ IU/ml). No patient experienced fulminant disease with progression to acute liver failure. The overall prevalence of an active or previous HBV coinfection was 1.9 and 31.7%, respectively. Among 34 patients with available information, none of them suffered from an active HEV coinfection during the follow-up period and two patients were tested positive for a resolved HEV infection. Moreover, 139 (86.3%) individuals were living with HIV, of whom 134 received highly active antiretroviral therapy. The median CD4 cell count at the time of study inclusion was 590/μl (IQR 432–766/μl), and

Table 2. Characteristics of HCV reinfections.

	First reinfection	Second reinfection	Third reinfection
Patients included, n	22	2	1
HCV GTs, n (%)			
1a	13 (59)	1 (50)	1 (100)
3a	3 (14)	0 (0)	
4d	6 (27)	1 (50)	
HCV GT switch, n (%)	14 (64)	1 (50)	
No treatment initiation*, n (%)	9 (41)	0 (0)	
Spontaneous clearance, n (%)	1 (4)	0 (0)	
Treatment initiation, n (%)	12 (55)	2 (100)	1 (100)
SVR proportion (%)†, n (%)	10/12* (83)‡	2/2 (100)	1/1 (100)

GT, genotype; LDV/SOF, ledipasvir/sofosbuvir; P/R, paritaprevir/ritonavir; SVR, sustained virologic response.
* During study observation period.
† In patients with final treatment outcome available (12-week follow-up).
‡ Two patient relapsed following P/R and LDV/SOF treatment and achieved SVR after re-therapy.

63.3% of HIV/HCV-coinfected patients had an HIV viral load less than 50 copies/ml. For 17 individuals, at least one prior infection, either effectively treated or spontaneously cleared, was documented before being included in the study with the actual HCV episode. With regard to the last available laboratory measurements, the total follow-up time was 1,035 patient-years, and the median follow-up time for each patient was 82 months (range 1–142 months).

Twenty-two episodes (13.6%) resulted in a spontaneous clearance of RAHC. Six patients were lost to follow-up before treatment initiation. HCV treatment was initiated in 131 patients (81.4%) with a median time from recently acquired HCV diagnosis to treatment initiation of 5 months (range 0–79 months). The overall intention-to-treat SVR rate following interferon-based and DAA-based therapy was 84.3% (59/70) and 93.4% (57/61), respectively.

Annual recently acquired HCV diagnoses and prevalence of HCV GTs in MSM and non-MSM

Overall, the highest number of HCV infections was found in the years 2009 to 2014, which can be assigned to the interferon era (Fig. 1A). With regard to the distribution of the HCV GTs, we observed different frequencies during the observation period. In the years 2009 to 2017, HCV GT1a was always the most common (Fig. 1B). However, GT1a decreased in 2018 and 2019. The proportion of GT4d cases increased over the years. Although no GT4d cases were diagnosed in 2013, the proportion rose to 40% in 2018 and 2019. There was also a trend towards more GT3a cases in 2018 and 2019, whereas for GT1b and GT2 only individual cases were detected between 2009 and 2014.

HCV reinfections

In total, 25 HCV reinfections occurred in 22 MSM, resulting overall in 186 cases of HCV infection (Table 2). Of note, no HCV reinfection was observed in non-MSM patients with RAHC.

Reinfection incidence was 2.7 per 100 person-years (PY; 95% CI 1.39–3.05), and the median duration to reinfection was 17 months (IQR 0–130 months). Patients with reinfections were exclusively HIV-positive MSM and were significantly younger than patients without reinfection ($p = 0.03$).

Of the 22 patients with an HCV reinfection, 14 (64%) had a different HCV GT at the time of reinfection compared with the first infection (Table 2). In the remaining eight patients, no HCV GT switch could be detected (all HCV GT1a). However, HCV reinfection was suspected in these cases owing to clinical data, as sexual risk behaviour was documented at the time of suspected reinfection. In additional seven cases in HCV GT1a and GT4d with detectable HCV RNA after previous achievement of HCV RNA negativity 12 weeks after the end of treatment (SVR12), late virologic relapse was supposed, as the same HCV isolate could but be detected as before treatment. However, reinfection with a different HCV isolate could not be ruled out.

To distinguish HCV reinfections from a virologic relapse in patients with GT1a and GT4d, we conducted phylogenetic analyses and used samples of 13 patients with RAHC and patient samples from the European Resistance Database with a proven relapse to DAA treatment as a control for the genetic drift over time.

Baseline and relapse sequences of these controls were each located on a single branch in the phylogenetic tree (Fig. 2A and B). In all seven cases with suspected virologic relapse, minimal genetic changes between baseline and post-treatment NS5B consensus sequences could be detected, as indicated by clustering and separation from other sequences by a supported branch (aHCV8, aHCV10, aHCV11, and aHCV12, and aHCV13, aHCV14, and aHCV15). Thus, infection with the same HCV isolate in these patients is plausible (relapse or intermediate HCV RNA negative course). In further six cases, phylogenetic analyses of NS5B sequences showed that the baseline sequence and post-treatment sequence were located on different branches and did not cluster with each other (aHCV1, aHCV3, aHCV5, aHCV6, aHCV7, and aHCV9). These results strongly suggest that patients were reinfected with a new HCV strain. In two further patients, we assumed HCV reinfection because the patients were sexual partners and an infection event was documented, although all sequences were located on the same branch (aHCV2 and aHCV4).

Incidence analyses

To study the incidence of HCV infections as a result of different treatment levels over a period of 10 years, we compared the cumulative incidence for primary HCV infections and HCV reinfections among MSM patients in the second-generation DAA era (primary HCV infections: 2017–2019; HCV reinfections: 2017–2020) with the first-generation DAA era (2013–2017) and

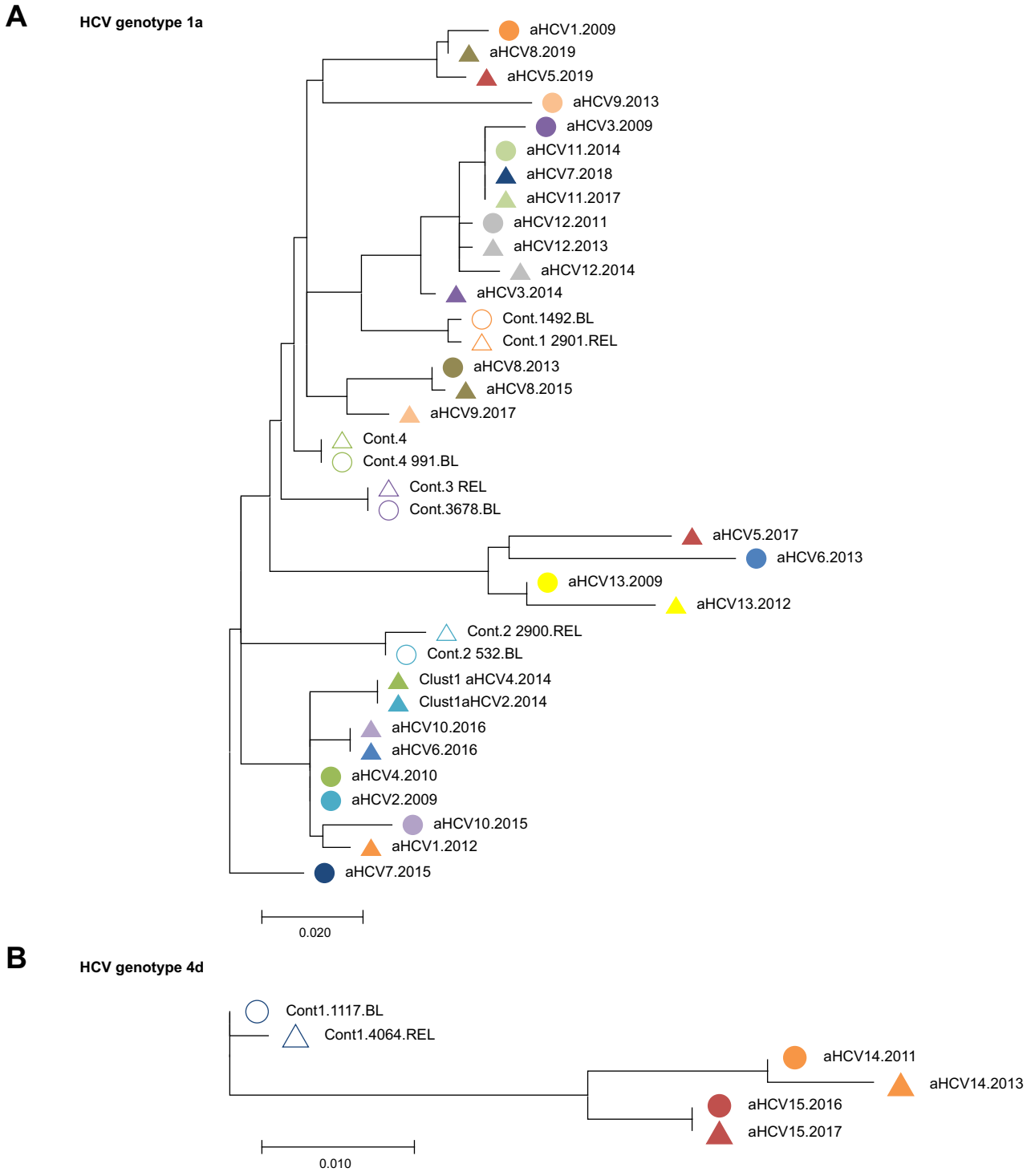

Fig. 2. Differentiation of viral relapse *vs.* HCV reinfection by phylogenetic trees in acute hepatitis C infections with (A) HCV genotype 1a and (B) HCV genotype 4d. Baseline and posttreatment sequences are marked by a circle and triangle each in the same colour, respectively. Control samples are shown in white and marked as BL and REL. aHCV, acute HCV; BL, baseline; Cont., control; REL, relapse.

interferon era (2009–2013) (Tables 3 and 4). The incidence rate for a first HCV infection substantially declined in the period between 2017 and 2019 (3.3 per 1,000 PY) compared with that in the DAA era between 2013 and 2017 (5.9 per 1,000 PY) and that in the interferon era (2009–2012; 8.3 per 1,000 PY) (Table 3). Conversely, the incidence of HCV reinfections remained a frequent finding among MSM during the whole study period and increased slightly from 1.9 per 100 PY to 2.8 per 100 PY over the 10-year study period (Table 4).

Phylogenetic analyses to compare recently acquired hepatitis C in MSM *vs.* non-MSM

To investigate whether larger HCV infection networks exist in patients with RAHC, we conducted phylogenetic analyses

Table 3. Incidence of first HCV infections over time.

Time period	PY follow-up*	No. of first HCV infections	Infection incidence, per 1,000 PY (95% CI)
2009–2013 (pre-DAA era)	9,932	82	8.3 (6.6–10.2)
2013–2017	10,756	63	5.9 (4.5–7.4)
2017–2019 (second-generation DAA)	6,635	22	3.3 (2.1–4.9)
2009–2019	25,402	139	5.5 (5.1–7.0)

DAA, direct-acting antiviral; MSM, men who have sex with men; PY, person-years.
* PY using the annual total number of HIV-positive MSM of one tertiary centre (Infektiologikum Frankfurt) as denominator.

Table 4. Reinfection incidence over time.

Time period	PY follow-up*	No. of reinfections	Reinfection incidence, per 100 PY (95% CI)
2009–2013	208	4	1.9 (0.50–3.72)
2013–2017	461	13	2.8 (1.22–3.65)
2017–2020	461	13	2.8 (1.25–3.73)
2009–2020	929	25	2.7 (1.39–3.05)

MSM, men who have sex with men; PY, person-years.
* PY using MSM of the study cohort as denominator.

for each HCV GT. HCV NS5B sequences of Frankfurt MSM patients and non-MSM patients with RAHC were compared with published NS5B reference sequences obtained from MSM. Moreover, we generated sequences from patients with chronic hepatitis C and other non-sexual transmission routes (*e.g.* blood transfusion, needlestick injury, or IVDA) that served as controls (see Patients and methods).

Phylogenetic trees were constructed for each HCV GT separately. Phylogenetic analyses revealed 44 monophyletic clusters of MSM-specific strains containing a total of 377 MSM isolates (Fig. 3). Moreover, 139 singleton MSM sequences that were not closely related to any other strain in this study population were identified. The 44 MSM clusters ranged in size from 2 to 99 sequences and consisted predominantly of HCV GT1a and GT4d.

Phylogenetic analyses in HCV GT1a-infected patients

The GT1a phylogeny is illustrated in Fig. 3A and reveals a total of 35 transmission clusters, of which 34 were MSM-specific. Infection with GT1a was the most prevalent among MSM patients as well as among non-MSM patients. Interestingly, all but one of the GT1a sequences obtained from non-MSM patients appeared to be unrelated to any of the clusters. In contrast, the majority of GT1a infections in the Frankfurt MSM individuals (n = 86/126, 68%) could be assigned to 18 different MSM-specific clusters. Ten of these 18 clusters exclusively contained sequences from the Frankfurt MSM patients and German reference MSM sequences from Bonn, but no sequences from other countries (clusters 1, 3, 4, 9, 10, 13, 19, 21, 27, and 32; Fig. 3A, Fig. S1, and Tables S1.1 and S1.2). The remaining eight transmission clusters contained published MSM sequences from different European countries in addition to Frankfurt MSM sequences and are therefore considered as country-mixing clusters (clusters 5, 6, 15, 17, 18, 20, 31, and 34; Tables S1.1 and S1.2). Overall, 45% (n = 57/126) of the Frankfurt MSM GT1a sequences were assigned to these eight international clusters.

Four of these international clusters contained sequences from Germany and one further European country (clusters 5 and 31, the Netherlands; cluster 20, UK; cluster 24, Switzerland). The remaining four clusters consisted of sequences obtained from German MSM and sequences from further two (cluster 6, the Netherlands and Switzerland; cluster 15, the Netherlands and UK) or three European countries (clusters 17 and 18, the Netherlands, UK, and Switzerland). All country-mixing clusters contained only sequences obtained from European MSM. Australian MSM isolates clustered separately and were never part of an HCV GT1a cluster with European sequences (Fig. 3A).

Of the 15 sequences of patients with chronic hepatitis C and other HCV transmission routes, only one control sequence of a patient with IVDA transmission clustered with one MSM sequence obtained from the Netherlands (cHCV.2679.2015.IVDA, cluster 11). Besides that, no clustering of chronic HCV sequences with those of patients with RAHC was observed (Fig. 3A).

Phylogenetic analyses of HCV GT1b-infected cases

Neither the one Frankfurt MSM sequence nor the three non-MSM sequences clustered with any reference sequence. In contrast, a clustering of published MSM sequences obtained from the Netherlands and UK was observed (clusters 1–3). Control sequences of our study obtained from patients with chronic hepatitis C and other transmission routes such as blood transfusion or IVDA could also not be assigned to any cluster (Tables S2.1 and S2.2).

Phylogenetic analyses in HCV GT3a-infected patients

Overall, 83% (n = 5/6) of Frankfurt HCV GT3a MSM cases were assigned to a transmission cluster. Interestingly, Frankfurt MSM sequences showed a separate clustering in one single cluster, which did not contain reference sequences obtained from other European countries (cluster 2; Fig. 3C, Fig. S3, and Tables S3.1 and S3.2). In addition to the Frankfurt MSM cluster, we detected three additional HCV GT3a clusters, which exclusively consisted of published MSM sequences obtained from other European countries (Table S3.2). Sequences from non-MSM patients with RAHC (aHCV.1.2009.a, aHCV.242.2011.a, and aHCV.394.2012.a) as well as sequences obtained from patients with chronic hepatitis C were not assigned to any cluster (Fig. 3C).

Phylogenetic analyses in HCV GT4d-infected patients

Strikingly, all HCV GT4d sequences (n = 27/27, 100%) obtained from Frankfurt MSM patients clustered with published MSM isolates from other countries (Switzerland, the Netherlands, UK, and Canada).

The Frankfurt MSM sequences were part of two transmission clusters (Fig. 3D, Fig. S4, and Tables S4.1 and S4.2): sequences

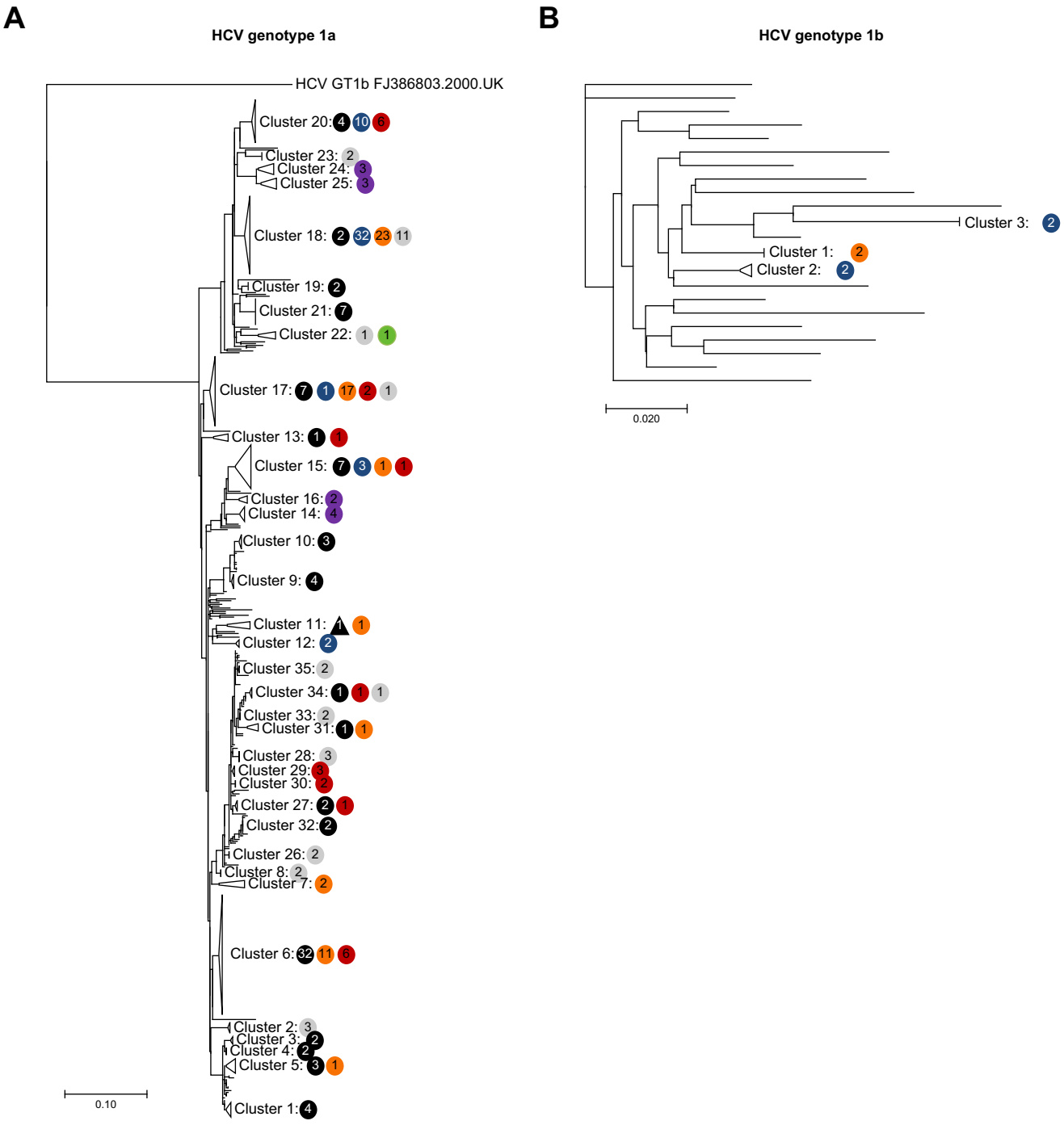

Fig. 3. Phylogenetic trees based on HCV NS5B sequencing analyses conducted at the time point of acute hepatitis C diagnosis in patients with (A) HCV genotype 1a, (B) HCV genotype 1b, (C) HCV genotype 3a, and (D) HCV genotype 4d. Clusters are illustrated by triangles, and numbers in the triangles represent the number of sequences in each cluster. Country of origin is shaded as follows: (▲) non-MSM, Frankfurt, Germany; (●) MSM, Frankfurt, Germany; (red) MSM (published sequences), Germany; (dark blue) MSM (published sequences), UK; (orange) MSM (published sequences), Netherlands; (grey) MSM (published sequences), Switzerland; (yellow) MSM (published sequences), Spain; (light blue) MSM (published sequences), USA; (pink) MSM (published sequences), Canada; (purple) MSM (published sequences), Australia; (green) MSM (published sequences), France. GT, genotype.

from two Frankfurt MSM patients represented cluster 1, and further 25 Frankfurt MSM sequences (n = 25/27, 93%) were part of a very large country-mixing cluster together with 73 European (the Netherlands, Spain, Canada, France, Germany [Bonn], and the UK) and 13 Canadian sequences (cluster 2). The third cluster consisted exclusively of English MSM reference strains (cluster 3) (Tables S4.1 and S4.2).

As previously observed for the other HCV GTs, control sequences obtained from patients with chronic hepatitis C without a sexual transmission route were not

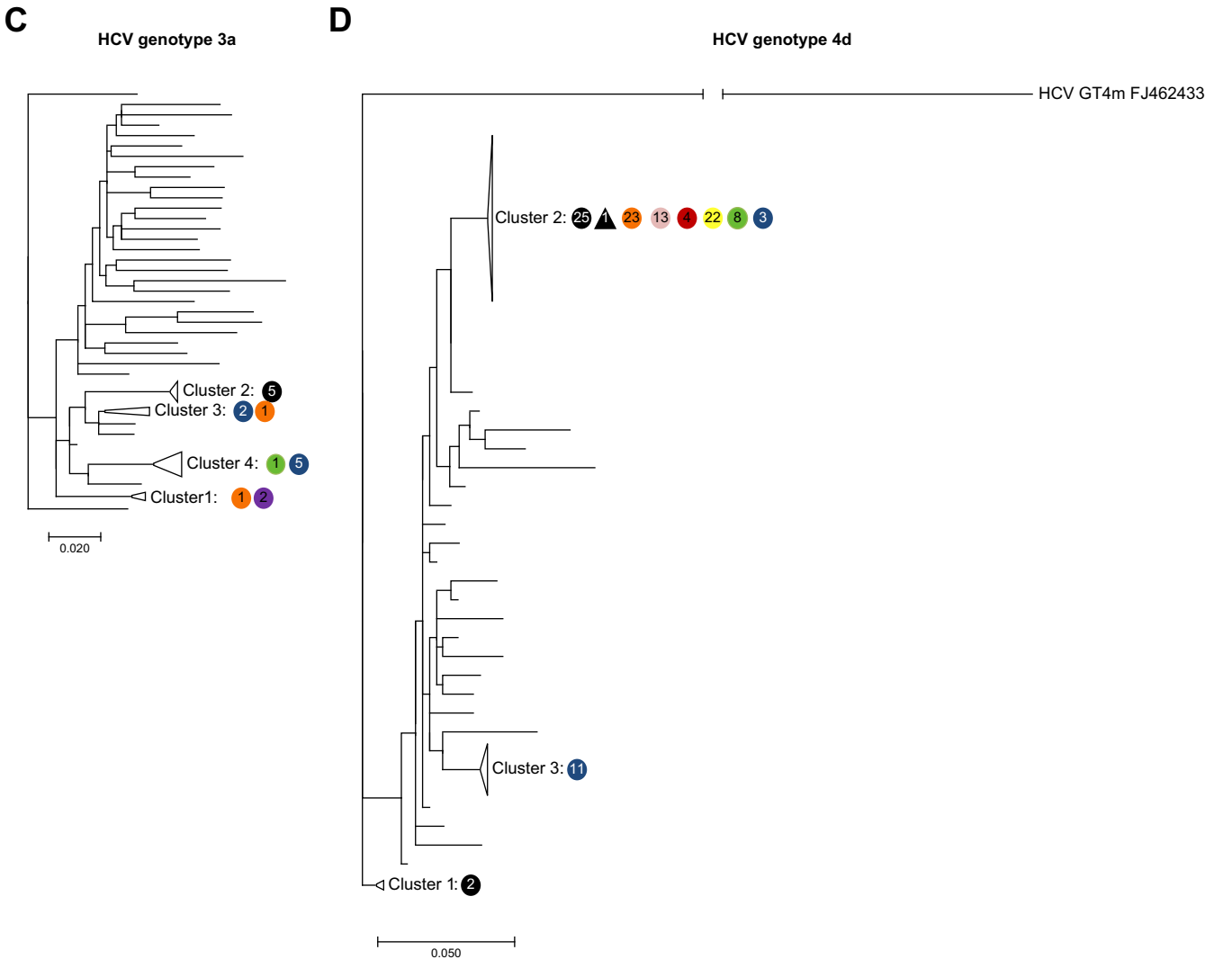

Fig. 3 (*continued*).

assigned to any cluster, except for one (cHCV.6036.2017.IVDA, cluster 2).

Clinical data supporting phylogenetic analysis results

Phylogenetic analysis results could be confirmed in a subgroup of patients with additional information on social, transmission, and travel history (Tables S5–S7). In 15 cases, partnerships or sexual contacts within our MSM cohort could be identified within the clusters. In country-mixing clusters, eight Frankfurt MSM reported to have frequent stays abroad to carry out business activities (such as flight attendants) and two MSM had a partner working abroad in countries they clustered with. Moreover, 10 MSM who lived or stayed in a foreign country or national region that was part of the respective cluster could be identified. Concerning transmission histories, in country-mixing clusters, seven cases could be identified, in which sexual contacts of our MSM cohort with MSM from clustering countries were documented. Finally, in eight HCV cases, at least two infection events within

one cluster corresponded in terms of time, transmission route, and geographic region.

Discussion

Excellent treatment results in RAHC and chronic HCV infection have prompted the World Health Organization to incorporate HCV elimination until 2030 in its agenda. However, the burden of disease, especially attributed to RAHC, is still high among key at-risk populations such as PWID and HIV-infected MSM. Thus, we aimed to investigate whether high-risk networks exist that facilitate an ongoing HCV transmission over a long time period of 10 years and what impact DAA availability may have on the HCV epidemic among MSM.

In our 10-year observational study, we found a steady and substantial decline in RAHC incidence rates among HIV-positive MSM from 8.3 per 1,000 PY in the interferon to 3.3 per 1,000 PY in the DAA era, which is in line with another study.[25] Our study cohort was predominantly characterised by HIV-coinfected MSM, who reported high-risk sexual behaviour and recreational drug use. The minority of patients were HIV-negative non-MSM individuals with RAHC, with one-third of them reporting IVDA followed by other transmission routes such as transfusion or needlestick injury.

The decline in incidence rates in conjunction with the high SVR rates observed in this cohort suggests that scale-up of DAAs together with behavioural interventions may have the potential to reduce HCV transmission.[26,27] However, our findings also demonstrate an ongoing risk of repeated HCV transmission among MSM individuals: in line with previous findings in the German Hepatitis C Cohort (GECCO) study, 15.2% of MSM were diagnosed with an HCV reinfection during the observation period.[6] Moreover, the resulting reinfection incidence rate slightly increased in the DAA era compared with that in the interferon era, suggesting that a small group of patients with high-risk behaviour mainly contributes to an ongoing HCV epidemic. Accordingly, higher HCV reinfection rates have been reported in cohorts of individuals with recently acquired HCV, which mainly consist of PWID and HIV-positive MSM, compared with individuals treated for chronic HCV infection.[28–30] Aggravated are these observations by low spontaneous clearance rates detected in HIV-positive individuals of our study cohort (12%). These findings might have been biased by the relatively early treatment uptake in our study population. However, our results of lower spontaneous resolution rates in HIV-infected patients are consistent with several previous findings and might be caused by the compromised immune response in HIV coinfections, which limits the ability to clear an aHCV infection in HIV-coinfected patients.

Regarding phylogenetic analysis, our study results suggest the existence of a large MSM-specific network connecting national outbreaks of RAHC in different European, Australian, and Canadian countries with each other. The phylogenetic contrast between largely clustering sequences of MSM populations and unrelated sequences obtained from patients with other transmission routes reflects an expanding epidemic of HCV among MSM, transmitted permucosally. Moreover, all identified clusters in our study proved to be MSM-specific, which strengthens the hypothesis that transmission of HCV within MSM and IVDA communities occurs in distinct clusters. The results of our phylogenetic analysis revealed that most of the HCV strains have rapidly spread among MSM populations in different countries via a joint international HCV transmission network. Interestingly, travel-associated infections could be supported in several of these cases, highlighting the existence of a well-connected and rapidly spreading network. Growing MSM tourism, cheaper international travel, and increasing digitisation seem to extend HCV transmission networks and increase the connectivity of high-risk MSM communities. We observed clustering of Frankfurt MSM sequences for GT1a, GT3a, and GT4d. However, overlap of Frankfurt MSM cases with networks from different countries was exclusively detected in GT1a and GT4d: 45% and 100% of Frankfurt MSM sequences were part of international clusters, respectively. Interestingly, these clusters contained published sequences from more than 10 years ago as well as more recent sequences from patients in our study. This suggests that large international transmission networks still exist in GT1a and GT4d. Our analysis over an extended period of 10 years reveals that especially GT4d appears to play a major role in the changes of HCV GT distributions over time, as GT4d considerably increased during the last 5 study years. Some studies have shown a phylogenetic clustering of GT4 sequences not only within one country but also between countries such as France, the Netherlands, UK, and Germany for MSM patients with GT4d.[10,31–33] Remarkably, we were also able to confirm this overlap with sequences from these countries for current sequences obtained from 2019. In addition, we demonstrate phylogenetic clusters between the Frankfurt MSM sequences with Spanish and even Canadian MSM sequences, which has not been shown to this extent before. This emphasises the rapid spread of GT4d among MSM as well as the immense influence of international joint networks on HCV transmission.

In contrast, we did not observe any country mixing or clustering of GT1b and GT3a, which confirms observations from another study.[34]

One major limitation of our study is the lack of a standardised protocol consisting of regular testing intervals to trace reinfections after cure of a first HCV infection. Reinfection cases were detected by the treating physicians, so we are not able to rule out differences in testing frequencies and missing cases if patients did not stay in care after DAA treatment. In addition, we did not collect behavioural data systematically by standardised questionnaires. Thus, potential transmission chains within clusters could only be drawn carefully, and we were not able to identify specific risk behaviours as possible predictors for HCV infections. Moreover, we focused on HIV-positive MSM although the proportion of other high at-risk patients such as PWID with RAHC was small, limiting the results to the MSM population.

In conclusion, our data demonstrate the presence of RAHC mainly in association with HIV-coinfected MSM and injection drug use with an overall decline of cases and a certain HCV GT pattern over the observation period of 10 years. In addition, the

existence of large and internationally connected transmission networks among MSM, which unite the majority of RAHCs in the form of clusters and are rapidly spreading throughout different countries, could be demonstrated. Spontaneous clearance rates were observed to be low, and reinfection rates increased, mainly driven by a small subset of MSM patients with high-risk behaviour. Thus, these data highlight the importance of implementing regular screening for HCV in the clinical routine care of HIV-positive MSM. Beyond that, further constructive preventive strategies such as scaling-up of DAAs in high-risk groups, early treatment initiation to avert secondary infections, raising awareness among MSM, PWID, and clinicians, and behaviour interventions are inevitable to achieve HCV elimination.

Abbreviations

aHCV, acute HCV; ALT, alanine aminotransferase; BOC, boceprevir; DAA, direct-acting antiviral; DCV, daclatasvir; DSV, dasabuvir; GLE, glecaprevir; GT, genotype; IVDA, intravenous drug abuse; LDV, ledipasvir; LTFU, lost to follow-up; MSM, men who have sex with men; OBV, ombitasvir; PEG, pegylated interferon; PIB, pibrentasvir; PTV/r, paritaprevir/ritonavir; PWID, people who inject drugs; PY, person-years; RAHC, recently acquired HCV infection; RBV, ribavirin; SOF, sofosbuvir; STI, sexually transmitted infection; SVR, sustained virologic response; TVR, telaprevir; VEL, velpatasvir.

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Conflicts of interest

CG has received speaking and/or consulting fees from AbbVie and Gilead. FF has received travel support from Abbvie and Novartis, speaker fees from Abbvie and MSD, and consulting fees from Fresenius and Ibsen. GD has received speaking and/or consulting fees from AbbVie and Gilead. SZ has received speaking and/or consulting fees from Abbvie, BMS, Gilead, Janssen, and Merck/MSD. JD has received research support from Gilead. CZ has received speaking and/or consulting fees from Abbvie, BMS, Gilead, and Merck/MSD, and research support from Abbvie and Gilead.

LH, TL, CS, GK, PG, MB, KHP, AM, NW, NF, EH, and NB have no conflicts to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Designed the study concept: CS, JD. Performed data collection: JD, CS, CG, TL, PG, CS, GK, MB, KHP, FF, GD, AM, NW, SZ. Performed analysis and interpretation of data: CG, JD, CS, LF, NB, NF, EH. Performed drafting of the manuscript: CG, JD, CS, LF, NB. Provided the critical revision of the manuscript: CS, SZ, NW, NB.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2023.100701.

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