

CLINICAL STUDIES

Phylogenetic analysis of acute hepatitis C virus genotype 4 infections among human immunodeficiency virus-positive men who have sex with men in Germany

Martin Vogel^{1*}, Thijs van de Laar^{2*}, Bernd Kupfer³, Hans-Jürgen Stellbrink⁴, Tim Kümmerle⁵, Stefan Mauss⁶, Gaby Knecht⁷, Annemarie Berger⁸, Sylvia Bruisten² and Jürgen K. Rockstroh¹

1 Department of Internal Medicine I, University of Bonn, Bonn, Germany

2 Public Health Laboratory, Public Health Service, Amsterdam, the Netherlands

3 Institute of Virology, University of Bonn, Bonn, Germany

4 Infektionsmedizinisches Centrum Hamburg (ICH), Hamburg, Germany

5 Department of Internal Medicine I, University of Cologne, Cologne, Germany

6 Center for HIV and Hepatogastroenterology, Düsseldorf, Germany

7 Infektiologikum Frankfurt/Main, Frankfurt, Germany

8 Institute for Medical Virology, University of Frankfurt, Frankfurt, Germany

Keywords

acute HCV – HIV – men who have sex with men (MSM) – sexual transmission – sexual transmitted disease

Correspondence

Dr Martin Vogel, Immunologische Ambulanz, Universitätsklinikum Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany
Tel: +49 228 287 16558
Fax: +49 228 287 15034
e-mail: martin.vogel@ukb.uni-bonn.de

Received 24 March 2010

Accepted 2 June 2010

DOI:10.1111/j.1478-3231.2010.02305.x

Abstract

Background: An ongoing HCV epidemic currently affects a growing proportion of HIV-positive men who have sex with men (MSM) in Europe. Recently in the North-Rhine region of Germany, we have observed an increase in acute HCV infections of genotype 4 (HCV-4). **Aims:** To characterize the current spread of HCV-4 among German MSM using a molecular epidemiological approach. **Methods:** Patient characteristics and sera were collected for HIV-positive MSM diagnosed with acute HCV-4 infections in the North-Rhine region ($n = 14$), Hamburg ($n = 14$), Frankfurt ($n = 4$) and Berlin ($n = 4$). Part of the HCV NS5B region (436 bp) was amplified, sequenced and compared with HCV-4 sequences from HIV-positive Dutch, English and French MSM ($n = 50$) as well as unrelated HCV risk groups ($n = 61$). **Results:** NS5B sequences were obtained from 35/36 (97%) of German cases, all of which were HCV subtype 4d (HCV-4d). The phylogenetic analysis of HCV sequences revealed two MSM-specific HCV-4d clusters of 71 and 12 sequences. All except one of the German MSM belonged to a large MSM-specific HCV cluster containing MSM from all four different European countries. None of the HCV-4 strains circulating among injecting drug users or in HCV-4 endemic areas were part of the MSM-specific clusters. **Conclusions:** HCV rapidly spreads among European HIV-positive MSM through a joint international transmission network, separate from that of injecting drug users. In order to contain this epidemic, non-parenteral routes of transmission, such as unsafe sex, must be taken into consideration and prevention measures should be refocused accordingly.

Since the year 2000, an increasing number of acute HCV infections has been observed in HIV-positive men who have sex with men (MSM). Outbreaks of HCV were first recognized in Europe (1–3), soon after well-defined cohorts of MSM at risk confirmed the recent increase in HCV incidence among HIV-positive MSM in western Europe (4–7). Outbreak reports from the US (8) and Australia (9) suggest that HCV has also emerged in HIV-positive communities in developed countries outside Europe, either simultaneously or through an intercon-

nected transmission network (10). Earlier studies have shown that the vast majority of recent HCV infections among HIV-positive MSM relates to percutaneous (sexual) instead of parenteral risk factors (10). Concurrent sexually transmitted infections (STI) and sexual practices with a high risk of mucosal damage may favour blood–blood contact through lesions in the rectal endothelial lineage, thereby facilitating HCV transmission (11–13).

Within our study on the treatment of acute HCV infection in HIV-positive individuals (14), we observed a high rate of HCV genotype 4 (HCV-4) infections within the North-Rhine region of Germany. Single-source

*Contributed equally.

outbreaks of HCV-4 have been described among HIV-positive MSM in France (3) and the Netherlands (7). This study uses a molecular epidemiological approach to further characterize the recent spread of HCV-4 among HIV-positive MSM in Germany.

Methods

Study population

Notification of acute HCV cases in the context of the study on the treatment of acute HCV infection in HIV-positive patients is voluntary. To overcome possible under-reporting, we approached six participating centres within 4 major urban areas in Germany to participate in this study. We included all HIV-positive MSM who were diagnosed with acute HCV-4 infection at HIV-outpatient clinics in Bonn, Cologne, Düsseldorf, Frankfurt, Hamburg and Berlin in the period 2002–2009. Four patients from Berlin were described in a previous paper (10). HIV-positive patients visit these outpatient clinics every 3–6 months to check HIV-surrogate markers (CD4-cell count and HIV-RNA) as well as standard laboratory parameters (e.g. blood-count, liver transaminases and renal function). Antibody to HCV screening is performed at the first visit and at yearly intervals thereafter. In the presence of clinical symptoms or signs of hepatitis, elevated liver transaminases or recent exposure to HCV, patients are immediately checked for anti-HCV antibodies and HCV-RNA to rule out acute HCV infection. Acute HCV infection was defined as a documented anti-HCV seroconversion within 12 months and/or an increase in liver transaminases > 3.5 times the upper limit of normal with newly positive HCV-RNA and normal transaminases the year before.

RNA isolation, amplification, sequencing and phylogenetic analysis of hepatitis C virus-4

Hepatitis C virus-RNA isolation was performed on 100 µl of plasma using the Tripure method (Roche Diagnostics, Almere, the Netherlands). A 436-nucleotide fragment of the HCV NS5B region was amplified and sequenced using previously described methods (10). Viral genotype was confirmed by a phylogenetic analysis of the NS5B sequences obtained, along with established reference sequences from GenBank. An HCV phylogenetic tree was constructed by the maximum likelihood method in PHYLML version 3.0 (15), using the Hasegawa–Kishino–Yano substitution model with γ -distribution of among-site rate heterogeneity (HKY- Γ). Bootstrapping ($n=1000$) was used to analyse the stability of the tree topology. The HCV-4 NS5B sequences obtained were compared with HCV-4 strains from 50 MSM with sexually acquired acute HCV-4 infection in Amsterdam ($n=24$), Rotterdam ($n=3$), London ($n=11$), Brighton ($n=4$) and Paris ($n=8$) during the period 2000–2007 (10), as well as all other HCV-4d NS5B sequences available from GenBank ($n=61$).

Results

Study population

In the six participating HIV-outpatient clinics, 36 HIV-positive MSM were diagnosed with acute HCV-4 infection: 14 MSM in the North-Rhine region, 14 in Hamburg, four in Frankfurt and four in Berlin. One patient from the North-Rhine region did not fit our criteria for acute HCV infection, because he had no previous test results on either anti-HCV or liver transaminases. Acute HCV infection was assumed based on recent unsafe sexual contact with an HCV-positive partner.

Patient characteristics are described in Table 1. The median age at HCV diagnosis was 42 years [interquartile range (IQR): 38–47 years]. At least 89% of participants acquired their HCV infection through sexual contact, and none of the patients reported a history of injecting drug use (IDU). Their median CD4 count was 490 cells/µl (IQR: 398–643 cells/µl), and 61% of participants were on highly active antiretroviral therapy (HAART). Acute HCV infection was symptomatic in 50% of patients, although most patients reported non-specific symptoms such as fatigue (11/36) and only two patients presented with jaundice, none suffered from acute liver failure. At HCV diagnosis, 92% of patients had antibodies against HCV and the median maximum ALT level was 422 IU/L (IQR: 184–653).

Phylogenetic analysis of HCV-4

The HCV NS5B fragment was amplified and sequenced for 35/36 (97%) of the HIV-positive MSM enrolled. All sequences obtained were of HCV subtype 4d (HCV-4d). An NS5B phylogenetic tree was constructed containing 146 HCV-4d sequences: the 35 German case sequences plus 61 reference strains from GenBank and 50 sequences

Table 1. Demographics of the 36 patients with acute hepatitis C virus genotype 4 infection

| Parameters | Values |
|--------------------------|---------------|
| Male gender | 36 (100) |
| Age (years) | 42 (38–47) |
| Transmission risk | |
| Sexual | 32 (89) |
| Unknown | 4 (11) |
| Injecting drug use | 0 (0) |
| HIV status | |
| CD4 cellcount [µl] | 490 (398–643) |
| HIV-RNA [\log_{10}]* | 4.3 (3.7–5.0) |
| HAART | 22 (61) |
| HCV-infection | |
| Symptoms or signs | 18 (50) |
| Anti-HCV positive | 33 (92) |
| Maximum ALT [IU/L] | 422 (184–653) |
| HCV-RNA [\log_{10}] | 5.8 (5.3–6.2) |

*Reported only for patients off HAART.

Values given as number of patients (%) or median (interquartile range).

obtained from HIV-positive MSM diagnosed with acute HCV in other western European cities (Fig. S1).

Phylogenetic analysis revealed two strongly supported monophyletic clusters (bootstrap > 70) of MSM-specific strains. Except for one isolate from Hamburg (HA42), all HCV sequences obtained from HIV-positive German MSM were part of one large monophyletic cluster (cluster I). In total, cluster I contains 71 sequences obtained from HIV-positive MSM in Germany ($n=34$), the Netherlands ($n=26$), France ($n=8$) and the UK ($n=3$). Although isolates of one country tend to group together, only four robust subclusters with a maximum of five sequences were observed within cluster I: two were exclusively German (KO31/KO33/BO36/FR58 and HA47/HA48/HA54), one was exclusively Dutch (AM9/AM17/AM19/AM31/AM40) and one was mixed (PA6/AM14/LO101/LO102). The second MSM-specific cluster, cluster II, shows considerably less genetic diversity and contains only sequences from English MSM ($n=12$).

None of the 61 GenBank reference strains were part of the two MSM-specific clusters. Of those 61 strains, 53 (87%) were obtained from European injecting drug users, five (8%) from individuals with an unknown transmission risk, four (7%) from endemic areas in Africa/Middle East and one (2%) from a Dutch HIV-negative MSM. The singleton HCV sequence obtained from an HIV-positive MSM in Hamburg (HA42) was most similar to strains found among European injecting drug users.

Discussion

Phylogenetic analysis of incident HCV-4 infections among HIV-positive MSM confirms the existence of a single recent ancestor connecting MSM populations from distinct urban areas within Germany. German HCV-4 sequences form a monophyletic cluster with HCV-4 sequences obtained from HIV-positive MSM throughout Western Europe, which suggests that HCV has rapidly spread among MSM populations in neighbouring countries via a joint international HCV transmission network (10). Previous evolutionary analyses have placed the phylogenetic history of HCV in HIV-positive MSM on a timescale, suggesting an occasional introduction and transmission of HCV between 1975 and 1996, followed by a rapid expansion within the HIV-positive MSM population after 1996 (10). Interestingly, this sudden emergence of HCV coincides with an increase in sexual risk behaviour and increased STI rates among MSM due to a decrease in the perceived threat of HIV/AIDS in the HAART era (16). Except for one German patient, HCV-4 strains obtained from European MSM were different from HCV-4 strains circulating among European injecting drug users, strengthening the hypothesis that transmission of HCV occurs within the HIV-positive MSM community via a network other than injecting drug use.

Thirty-two of 36 German HIV-positive MSM participating in this study reported unprotected anal sex (or sexual practices) as their sole risk factor for acquiring HCV; in the remaining four patients, the source of infection remained unknown. Importantly, all patients denied IDU, which is still the most common route of HCV transmission in Europe, the US and Australia (17–19). Although sexual transmission of HCV has been described previously in HCV serodiscordant heterosexual couples, prospective cohort studies suggest that sexual transmission of HCV is a relatively rare event in monogamous heterosexual partnerships and varies from 0 to 0.6% per year (20, 21). A slightly higher risk has been reported for heterosexuals with multiple partners or those at risk for STI (0.4–1.8% per year) (20), as well as in non-IDU HIV-positive women with a male IDU sexual partner (22). Since the sudden emergence of HCV among HIV-positive MSM, several groups have studied behavioural predictors of HCV transmission in MSM, by comparing parenteral and sexual risk behaviour of HCV-infected and HCV-uninfected MSM (6, 11, 13, 23). Strong evidence exists indicating that the current outbreak relates to perimucosal risk factors associated with (traumatic) sexual practices, concurrent ulcerative STI and non-injecting recreational drug use, all of which are associated with a higher risk of mucosal damage in the rectum. Disruption of the rectal endothelial lineage may favour blood–blood contact, thereby providing an efficacious route for the transmission of HCV (12).

Our study focuses primarily on the genetic relationship between HCV isolates obtained from HIV-positive MSM with acute HCV infection. Information on risk behaviour was limited, and disclosed during face-to-face interviews between the patient and his treating physician. Underreporting of parenteral risk factors, because of a lack of standardized questionnaires or the unwillingness of the patient to disclose IDU (e.g. incidental), cannot be excluded. Nevertheless, phylogenetic analysis suggests a non-IDU-related common source of infection, which fits the hypothesis that HCV emerges as an STI among HIV-positive MSM. Such an epidemic can have serious clinical complications: HIV/HCV co-infection has been associated with a less favourable treatment outcome and an accelerated liver disease (24, 25), in particular because HCV is acquired after HIV and at an older age (> 40 years) (8). Targeted prevention, including raising awareness among MSM as well as clinicians, regular HCV screening and treatment of acute and chronic HCV are required to mitigate the further spread of HCV in the HIV-positive MSM community.

Acknowledgements

The authors kindly thank Martin Stürmer, Frankfurt University, for performing part of the sequencing and Christian Noah of IPM Biotech GmbH Hamburg for kindly providing us specimens for the analysis.

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Supporting information

Additional supporting information may be found in the online version of this article:

Fig. S1. NS5B Phylogenetic tree of HCV genotype 4d. Monophyletic MSM-specific clusters are shaded, and the country of origin is coded: (●) Germany, (□) the Netherlands, (✦) United Kingdom and (+) France; taxa reveal the city of residence: (AM) Amsterdam, (BE) Berlin, (BO) Bonn, (BRI) Brighton, (DU) Dusseldorf, (FR) Frankfurt, (HA) Hamburg, (KO) Cologne, (LO) London, (PA) Paris and (RO) Rotterdam.

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