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ABSTRACT

High rates (~25%) of developing chronic hepatitis B virus (HBV)-infection (hepatitis B surface antigen (HBsAg)-positive for >6 months following infection) have been observed in people who use drugs (PWUD) and men who have sex with men (MSM). We aimed to estimate the frequency of delayed HBsAg-seroclearance, along with its determinants, and time to delayed HBsAg-seroclearance.

Data were used from MSM and PWUD enrolled in the Amsterdam Cohort Studies (1985-2002) who had anti-hepatitis B core antibody seroconversion. Potential determinants for standard HBsAg-seroclearance, delayed HBsAg-seroclearance and chronic HBV were examined using multinominal logistic regression. Time to HBsAg-seroclearance was estimated using Kaplan-Meier curves.

147 incident HBV-infections occurred during follow-up. On initial HBsAg-testing after infection (6-12 months), 42 (29%) were HBsAg-positive and 105 (71%) were HBsAg-negative ("standard HBsAg-seroclearance"). Of the 42 initially HBsAg-positive individuals, 22 subsequently tested HBsAg-negative (of whom 7 (31.8%) were HBV-DNA positive at last visit, suggesting occult HBV). Overall, 15 became HBsAg-negative and HBV-DNA-negative ("delayed HBsAg-seroclearance"), while 27 remained HBsAg and/or HBV-DNA-positive ("chronic HBV"). The 5-year cumulative probability of delayed HBsAg-seroclearance was 41.6% for initially HBsAg-positive individuals. Delayed HBsAg-seroclearance and remaining chronically infected were associated with younger age and HIV/Hepatitis C virus (HCV)-coinfection.

In conclusion, delayed HBsAg-seroclearance is common in these key adult populations at-risk for HBV, while proportion developing HBV chronicity (18%) is still higher compared to the general population (~5%). Given the proportion of individuals with occult HBV-infection and that HCV direct-acting antivirals can lead to HBV re-activation, HBV-DNA testing in HCV co-infected MSM/PWUD are warranted prior to treatment initiation.

Key words: Hepatitis B virus; Hepatitis B surface Antigen; delayed seroclearance; key populations; chronicity.

BACKGROUND

It is estimated that 257 million individuals worldwide are chronically infected with hepatitis B virus (HBV).[1] Chronic infection can lead to liver cirrhosis, liver decompensation, hepatocellular carcinoma and death[2], while the risk of these diseases increases with uncontrolled HBV replication.[3] The presence of hepatitis B surface antigen (HBsAg) indicates that a person is actively infected with HBV and its loss is associated with a substantially lower risk of severe morbidity and mortality.[2]

Most people infected in adulthood have self-limiting infection, in which HBsAg becomes detectable 2-10 weeks after infection and disappears within 6 months. However, a review concluded that roughly 5% of infected adults are unable to clear HBsAg within six months[4] and are defined as having chronic infection. This is in contrast to newborns until the first year of age and children 6-10 years old, in whom 90% and 30-50%, respectively, develop chronic infection.

Meanwhile, some chronically-infected adults become HBsAg-negative >6 months after infection. The proportion of individuals exhibiting "delayed" spontaneous seroclearance of those diagnosed with chronic infection is variable and can be up to 20% within 5 years after infection.[5] Missing delayed seroclearance could lead to overestimated prevalence of chronic hepatitis B infection.

Intriguingly, specific adult populations have exhibited high rates of chronicity.[4] In a previous study of men who have sex with men (MSM) and people who use drugs (PWUD), we observed that about 25% did not clear HBsAg within 6 months after infection and were therefore defined as having chronic infection.[6] Yet whether these infections are truly chronic or simply delayed seroclearers remains unknown. Using unique data from a prospective cohort of adult high at-risk individuals with incident HBV infection, we aimed to estimate the frequency of delayed HBsAg-seroclearance, along with its determinants, and time to delayed HBsAg-seroclearance.

METHODS

Study population and procedures

We used data from PWUD and MSM participating in the Amsterdam Cohort Studies (ACS) on human immunodeficiency virus (HIV) who acquired HBV during follow-up. In summary, the ACS is a prospective cohort study initiated in 1984 and continued until 2016 for PWUD and is still on-going for MSM.[7] The ACS recruited MSM and PWUD at sexually-transmitted infection clinics, local methadone outposts, and by word-of-mouth. Participation was voluntary and written informed consent was obtained at intake. Follow-up visits took place at the Public Health Service of Amsterdam every 3-6 months. At each study visit, standardised questionaries about health, sexual and drug-use behaviour were collected and blood samples were drawn for testing and storage at -80°C.

Testing procedures

At each visit, blood samples were tested for HIV-antibody testing (Ag/Ab Combo test, Axsym; Abbott Laboratories and bioMerieux, France). Stored blood samples were retrospectively tested for hepatitis B core antibodies (anti-HBc) (AxSYM Core, Abbott, Germany and Hepanostika; Organon Technika, The Netherlands). Of all MSM or PWUD testing anti-HBc negative at cohort entry between 1984 and 2002, the sample taken at their last visit before 2003 was tested for anti-HBc (Figure 1). If this sample tested anti-HBc positive, samples between these two visits were tested to estimate the moment of incident HBV infection, which was calculated as the midpoint date between the last anti-HBc negative and first positive anti-HBc test date. Since 2003, all susceptible individuals participating in the ACS were vaccinated against HBV, which made it unnecessary to systematically test for anti-HBc after that year.

Among individuals with incident HBV infection, the first sample available ≥6 months after incident HBV infection was tested for HBsAg. As we aimed to study delayed HBsAg-seroclearance, we defined initial chronic HBV infection as a positive HBsAg test result within 6 to 12 months of incident HBV infection. Participants with an initial chronic HBV infection were subsequently tested for HBsAg at their last available sample (>12 months after incident HBV infection) (Figure 1). If this test was negative, all available intermediate samples between positive and last negative HBsAg result were tested for HBsAg. We then determined the moment of delayed spontaneous HBsAg-seroclearance (herein referred as ‘delayed HBsAg-seroclearance’), defined as the midpoint date between the last positive HBsAg and subsequent negative HBsAg test date. Additionally, to distinguish occult HBV

infection from HBsAg-seroclearance, the last HBsAg negative sample from participants with an initial chronic infection were also tested for HBV DNA. Similar to the HBV testing procedure, all participants were retrospectively tested for hepatitis C virus (HCV) antibodies (AxSYM HCV version 3.0, Abbott, Germany) up to 2005. HCV testing was included afterwards as part of study visits.

Statistical analyses

Individuals with incident HBV infection were classified into one of three groups: 1) "standard" HBsAg-seroclearance, not identified with initial chronic HBV infection; 2) delayed HBsAg-seroclearance, identified with initial chronic HBV infection but became HBsAg- and HBV-DNA-negative during subsequent follow-up, and 3) chronic infection, identified with initial chronic HBV infection and subsequently testing either HBsAg-positive or HBsAg-negative with an HBV-DNA positive test result at their last available serum sample (Supplementary Figure 1). In this study, participants were only included if they could be classified into one of the three HBV groups, meaning that they had at least one sample tested for HBsAg following the first anti-HBc positive test result.

Kaplan-Meier curves were used to estimate cumulative probability of HBsAg-seroclearance over time. For this analysis, the time origin was defined as the estimated date of incident HBV infection (Supplementary Figure 1). Follow-up continued until the midpoint date between estimated date of incident HBV infection and first HBsAg-negative result (group 1), estimated date of delayed HBsAg-seroclearance (group 2), or date of last available serum sample tested for HBsAg (group 3).

Potential baseline clinical, behavioural and socio-demographic determinants for delayed HBsAg-seroclearance and chronic HBV were examined using univariable multinomial logistic regression with standard HBsAg-seroclearance as the reference category. Baseline data were obtained from the first visit an individual tested anti-HBc positive. Due to low numbers of HIV-positive individuals across HBV groups, only descriptive analyses were performed on HIV-related variables.

Analysis was conducted using Stata (v13.1, College Station, TX).

RESULTS

A total of 148 individuals had evidence of incident HBV infection during follow-up in the cohort (61 MSM, 87 PWUD). In analysis, one participant was excluded as no HBsAg result was available following incident HBV infection. Among 147 included participants, median year of incident HBV infection was 1993 (interquartile range (IQR)=1989-1996), and median follow-up was 9.0 years (IQR:4.5-16.1) since incident HBV infection until last cohort visit. Follow-up differed across the three HBV groups (Kruskal-Wallis p=0.03): standard HBsAg-seroclearance, 10.7 years (IQR=48-16.9); delayed HBsAg-seroclearance, 6.3 (IQR=3.0-8.8); chronic HBV, 7.8 (IQR=2.2-15.6). Median age at baseline was 31 years (IQR=27-36). At baseline, 61.6% (53/86) of all PWUD were HCV co-infected and 20.9% (18/86) HIV/HCV co-infected. Of the PWUD, 89.5% (77/86) had ever injected drugs. Within the MSM group, 47.5% (28/61) were coinfected with HIV (Table 1). During follow-up, 13 participants seroconverted for HIV and/or HCV: only HCV (n=9), only HIV (n=3), both HIV/HCV (n=1).

Based on the first HBsAg test following incident HBV infection, 71.4% (105/147) cleared the virus and 28.6% (42/147) had initial chronic HBV infection (Figure 2). On subsequent HBsAg testing within the initial chronic HBV group, 52.3% (22/42) tested HBsAg negative at their last available serum sample and 20 tested HBsAg positive. Following HBV DNA testing of the HBsAg-negative individuals with initial chronic infection, 31.8% (7/22) were in fact HBV-DNA positive suggesting occult HBV infection. Based on combined HBsAg and HBV DNA testing, 15 individuals were re-classified as delayed HBsAg-seroclearance and 27 remained in the chronically infected HBV group.

Based on the Kaplan-Meier curve including the total population (n=147), 120 individuals (105 standard HBsAg-seroclearance and 15 delayed HBsAg-seroclearance) were able to achieve HBsAg-seroclearance in a median time of 7.3 months (95%CI=6.4-8.7) (Figure 2, Panel A). Based on the Kaplan-Meier curve among the 42 participants with initial chronic HBV infection, 41.6% (95%CI=22.0-56.3%) were able to clear HBsAg within 5 years from incident HBV infection (Figure 2, Panel B).

Of the 15 individuals with delayed HBsAg-seroclearance, 53.3% (8/15) were MSM (5 HIV/HCV-uninfected and 3 HIV co-infected) and 46.6% (7/15) were PWUD (3 with HCV co-infection and 4 with HIV/HCV co-infection). Of the 27 individuals who remained chronically infected with HBV, 25.9% (7/27) were MSM (3 HIV/HCV-uninfected and 4 HIV co-infected) and 74.1% (20/27) were PWUD (2 HIV/HCV-uninfected, 9 HCV co-infected and 9 HIV/HCV co-infected).

In univariable analysis, HIV/HCV-coinfection and younger age at HBV infection were significantly associated with both delayed seroclearance and remaining chronically infected (Table 2). Ethnicity, risk group and gender were not significantly associated with either delayed HBsAg-seroclearance or chronic HBV. Multivariable analysis was precluded by the small sample size and high collinearity between variables (e.g. PWUD and HIV/HCV co-infection).

Among the 46 HIV-positive participants, with or without HCV co-infection, 5 were undergoing antiretroviral therapy (ART) at first anti-HBc positive test (4 with anti-HBV-containing ART). All 5 belonged to the HBV chronic group. At the end of follow-up, 10 participants ever received ART (9 with anti-HBV-containing ART): 3 belonging to the standard HBsAg-seroclearance group and 7 to the chronic HBV group. Median cumulative time on ART at the end of follow-up was 2.4 years (IQR=1.1-10.1). Median CD4 count at baseline was 430 cells/µl (IQR=280-590) and did not significantly differ between HBV groups (Kruskal Wallis test, p=0.89).

DISCUSSION

A substantial proportion (cumulative probability 42% at 5 years) of MSM and PWUD initially categorized as having chronic HBV infection after incident HBV infection were able to spontaneously clear HBV. However, roughly 18% of the total study population remained with chronic HBV infection at the end of an almost 9-year median follow-up.[5] This study provides a rare insight into the phenomenon of delayed spontaneous HBsAg-seroclearance, particularly among key populations at risk of HBV infection in high-income countries.

Of the determinants studied, younger age was associated with developing chronic infection and delayed HBsAg-seroclearance. The inverse relationship between age and higher risk of chronicity has been demonstrated in several studies[4] and is thought to be explained by the increasingly complex innate and adaptive immune responses as individuals become older.[8] Our data would suggest that the risk of chronicity remains elevated particularly for younger adults at high-risk of infection.

We found HIV/HCV co-infection to be associated with delayed HBsAg-seroclearance and chronic HBV infection, while coinfection with HIV and HCV only were not. Both HIV/HBV- and HCV/HBV-coinfection, compared to HBV mono-infection, have been associated with accelerated liver disease progression and higher HBV DNA levels[9], which are strongly associated with delayed HBsAg-seroclearance and progression to chronicity after acute HBV infection.[10] The interaction between these viruses is likely complicated with alternating dominance in circulating virus over time.[11] On the other hand, only PWUD were HIV/HCV co-infected in our study. Sharing needles, the main route of HCV transmission in this group, with the same HBV source partner may have led to continuous reintroduction of the virus and impeded successful antiviral immune responses. However, the same could be true for MSM repeatedly engaging in condomless sexual intercourse with HBV-infected partners. From our analysis, we cannot disentangle the immunological or behavioral causes behind the association of HIV/HCV co-infection on HBsAg-seroclearance.

Importantly, 32% of individuals initially classified as chronically infected with a subsequent negative HBsAg result had in fact occult infection (HBV DNA positive in the absence of HBsAg positivity). Others have indeed observed occult HBV profiles (HBV DNA+/HBsAg-) that were congruent with chronic or recovered occult HBV infection[12], similar to individuals in our study. Nevertheless, the findings of occult HBV after HBsAg-seroclearance are difficult to explain. A range of host adaptive anti-HBV immune responses has been implicated in both occult infection and acute versus chronic infection[13],

and perhaps immune responses from these individuals were sufficient enough to clear HBsAg but not HBV DNA replication. Other evidence has suggested that occult infection is related to mutations on the "a" determinant of the S-gene .[14] Unfortunately, we did not have data to support the hypotheses above.

Re-activation of HBV has been associated with direct-acting antivirals (DAA) when treating HCV infection.[15] MSM and PWUD are at high risk for both HBV and HCV infection, and seem to be less likely to clear HBV than other adult populations.[6] Therefore, HBsAg testing alone prior to DAA initiation might be insufficient in assessing the risk of HBV reactivation for these high-risk populations, and instead a serological battery including at least anti-HBc antibodies and HBV DNA should be performed. Nevertheless, a small body of research has shown that HBV reactivation is not likely to occur during DAA-treatment for HCV in individuals with occult HBV infection.[16] Yet, this observation needs to be confirmed in larger studies.

Given the period during which this study was conducted, these findings would be most generalizable to settings where HBV vaccination coverage is low, HBV transmission is ongoing and/or where anti-HBV containing ART uptake for HIV-positive individuals is suboptimal or not provided. How these data could relate to current populations in the Netherlands is debatable. Tenofovir (TDF)-containing ART in HIV-positive individuals is likely to reduce the risk of HBV acquisition[17], and most HIV-positive individuals in the Netherlands are treated with a TDF-containing regimen.[18] Targeted HBV vaccination campaigns have helped decrease HBV incidence in the HIV-negative MSM population since 2002, yet HBV vaccination uptake from 2012 was around 40%.[19] Meanwhile, there were 114 notified acute HBV infections in 2017 across the Netherlands, almost half of which were sexually transmitted among MSM.[20] No acute HBV infections have been observed in Dutch people who inject drugs over the last 5 years[20], in line with trends of HIV and HCV incidence.[21,22] Since HIV-negative MSM in the Netherlands are still acquiring HBV infection, delayed HBsAg-seroclearance could still be considered problematic for this key population.

Our study has certain limitations. First, we were unable to quantify transaminases, HBV-DNA viral loads, and HBsAg levels at the time of incident HBV infection or anti-HBs antibodies at the end of follow-up. In previous research, these markers at first HBsAg-positive serology were associated with reduced risk of delayed HBsAg-seroclearance and developing chronic infection.[5,10] Second, anti-HBV containing ART was infrequently given to HBV infected individuals during the study period and the treatments provided (mostly lamivudine) were unlikely to provide a major prophylactic effect on either HBV acquisition[17] or HBsAg-seroclearance during the acute phase.[23] As such, we were unable to

provide any assessment on the role of anti-HBV-containing ART in our study. Third, individuals with established chronic infection had a shorter follow-up than those with standard HBsAg-seroclearance, which may have led to unobserved events, thus underestimating the probability of delayed HBsAg-seroclearance. Finally, we had a small sample size which did not allow us to study determinants of delayed HBsAg-seroclearance in multivariable analysis.

In conclusion, more than one-third of adult MSM and PWUD were able to spontaneously clear HBsAg within five years after established chronic HBV infection, yet a higher proportion of HBV chronicity (~18%) was observed in this population compared to the general population (~5%). The latter finding highlights the importance of prompt HBV vaccination in these key populations. Importantly, 32% of those initially categorized as having chronic HBV infection were later found to have cleared HBsAg but remained HBV DNA positive. As DAA treatment can lead to HBV re-activation, testing for HBV DNA along with anti-HBc antibodies in MSM and PWUD co-infected with HCV is warranted prior to DAA-treatment.

FOOTNOTE PAGE

Conflict on of interest:

None of the other authors had any conflict to declare.

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- European Association for the Study of the Liver (EASL), Amsterdam, the Netherlands 2017

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Tables and figures

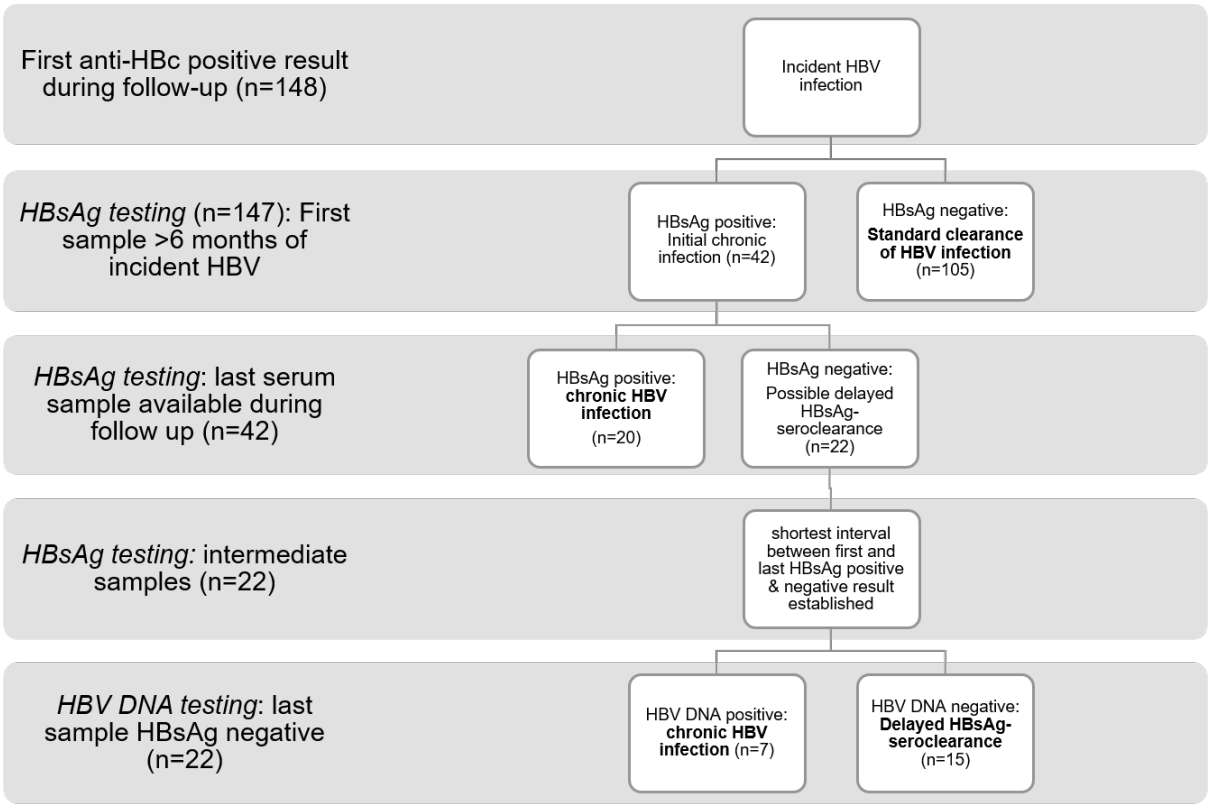
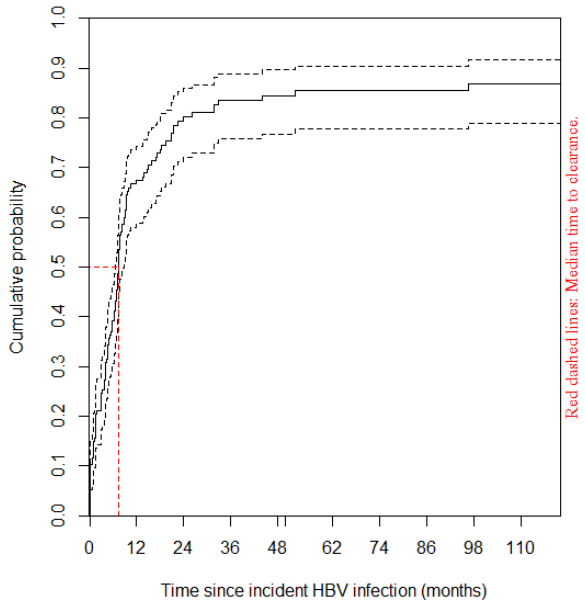


Figure 1. Flow chart of testing HBV replicative markers among adult MSM and PWUD participating in the Amsterdam Cohort Studies between 1985-2002

Sequential steps for testing hepatitis B surface antigen (HBsAg) and hepatitis B virus (HBV) DNA in the study are provided. Boldface text indicates the comparisons groups used in analysis.

A



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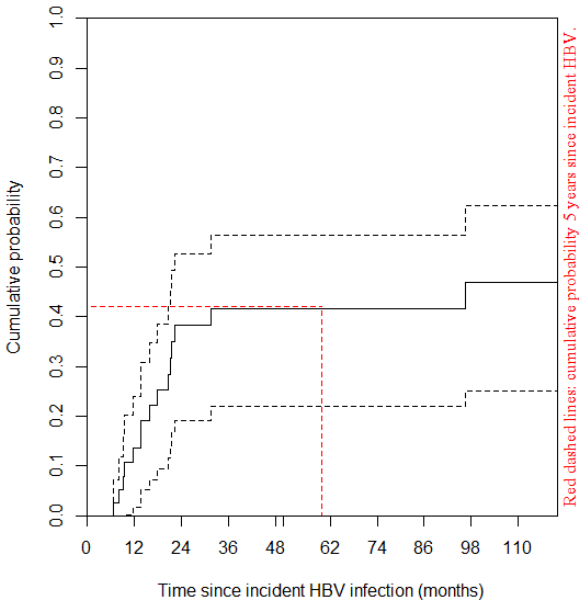

Figure 2: Time to HBsAg-seroclearance after incident HBV infection among adult MSM and PWUD participating in the Amsterdam Cohort Studies

Abbreviations: HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; MSM, men who have sex with men; PWUD, people who use drugs.

Panel A illustrates time to HBsAg-seroclearance among 147 ACS participants with evidence of incident HBV infection. Dashed lines represent 95% confidence intervals.

Panel B illustrates time to delayed HBsAg-seroclearance among 42 ACS participants with initial chronic HBV infection following incident HBV infection (15 with delayed seroclearance and 27 remaining chronic). Dashed lines represent 95% confidence intervals. Time origin starts 6 months following incident HBV infection.

Table 1: Socio-demographic and clinical characteristics and HBV outcome of 147 MSM and PWUD with incident HBV infection by HBV risk group in the Amsterdam Cohort Studies (1984-2002)

	MSM, n=61	PWUD, n=86
Follow-up in years[#], m (IQR)	7.2 (3.5-12.1)	12.7 (5.1-18.1)
Socio-demographic characteristics[$]		
Age at HBV infection median (IQR)	31 (26-36)	31 (27-35)
Male, n (%)	61 (100%)	53 (61.6%)
Western Ethnicity, n (%)	58 (95.1%)	75 (87.2%)
Clinical characteristics		
HIV/HCV co-infection status[$]		
HIV/HCV uninfected	31 (52.5%)	15 (17.4%)
HIV co-infected	28 (47.5%)	0 (0%)
HCV co-infected	0 (0%)	53 (61.6%)
HIV/HCV co-infected	0 (0%)	18 (20.9%)
HBV group at the end of follow-up		
Standard clearance	46 (75.5%)	59 (68.8%)
Delayed clearance	8 (13.1%)	7 (8.1%)
Chronic HBV	7 (11.5%)	20 (23.3%)

Abbreviations: m, median, IQR, interquartile range, HBVsc, Hepatitis B virus seroconversion; MSM, men who have sex with men, PWUD, people who use drugs, HCV: hepatitis C virus. Missing values: HIV/HCV co-infection status, n=2.

[#] From incident HBV infection until the last cohort visit.

[$] At the first anti-HBc positive cohort visit (i.e. baseline).

Table 2: Baseline factors associated with delayed HBsAg-seroclearance and chronic HBV infection among MSM and PWUD with incident HBV infection in univariable analyses

	Delayed seroclearance group, n=15				HBV chronic group, n=27				
	n[$]	%[^]	OR	p-value	n[$]	%[^]	OR	p-value	overal p-value
Sex									0.59
Female	5	15.2	1	0.29	6	18.2	1	0.43	
Male	10	8.8	0.53 [0.16,1.71]		21	18.4	0.93 [0.33,2.58]		
Ethnicity									0.89
Western	14	10.5	1	0.72	24	10.1	1	0.81	
Non-Western	1	7.1	0.68 [0.08,5.71]		3	21.4	1.19 [0.30,4.65]		
Risk group									0.14
MSM	8	13.1	1	0.49	7	11.5	1	0.08	
PWUD	7	8.1	0.68 [0.23,2.02]		20	23.3	2.23 [0.87,5.72]		
HIV/HCV co-infection									0.01
HIV/HCV uninfected	5	10.9	1	0.06	5	10.9	1	<0.01	
HIV co-infected	3	10.7	1.03 [0.22,4.75]		4	14.3	1.37 [0.33,5.68]		
HCV co-infected	3	5.7	0.53 [0.12,2.36]		9	17	1.58 [0.49,5.15]		
HIV/HCV co-infected	4	22.2	5.76 [1.15,28.92]		9	50	12.96 [3.07,54.63]		
Age at HBV infection[#]									<0.01
19-28	7	14.3	1	0.14	19	38.9	1	<0.01	
28-34	3	6.1	0.23 [0.06-1.00]		4	8.2	0.15 [0.05-0.44]		
35-55	5	10.2	0.51 [0.15-1.79]		4	8.2	0.11 [0.03-0.42]		

Abbreviations: OR, Odds Ratio, 95%CI, 95% confidence interval, HCV, Hepatitis C virus; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; MSM, men who have sex with men; PWUD, people who use drugs.

[$] Number of events in the delayed seroclearance of chronic HBV groups per variable.

^ The denominator of the percentage is the total number of individuals per category (i.e. females only) and the numerator is the number of events.

[#] Based on tertiles of age at the first anti-HBc positive results.

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