



HAL
open science

Frequent delayed spontaneous seroclearance of hepatitis B virus after incident HBV infection among adult high-risk groups

Daniela Santen, Anders Boyd, Sylvia Bruisten, Gerard Jb Sonder, Maria Prins, Robin Houdt

► To cite this version:

Daniela Santen, Anders Boyd, Sylvia Bruisten, Gerard Jb Sonder, Maria Prins, et al.. Frequent delayed spontaneous seroclearance of hepatitis B virus after incident HBV infection among adult high-risk groups. *Journal of Viral Hepatitis*, 2019, 27 (1), pp.81-87. 10.1111/jvh.13205 . hal-03942132

HAL Id: hal-03942132

<https://hal.sorbonne-universite.fr/hal-03942132v1>

Submitted on 16 Jan 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Frequent delayed spontaneous seroclearance of hepatitis B virus after incident HBV infection among**
2 **adult high-risk groups**

3

4 Daniela K. van Santen*¹, Anders Boyd*^{1,2}, Sylvia Bruisten^{1,3}, Gerard JB Sonder^{1,3}, Maria Prins^{1,3}, Robin
5 van Houdt⁴

6

7 *These authors contributed equally.

8

9 **Affiliations:**

10 1. Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, the
11 Netherlands;

12 2. INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Saint Antoine Hospital, AP-HP,
13 Sorbonne Université, Paris, France;

14 3. Amsterdam UMC, University of Amsterdam, Department of Infectious Diseases, Amsterdam
15 Infection and Immunity Institute (AI&II), Amsterdam, the Netherlands;

16 4. Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Medical Microbiology and Infection
17 Control, Amsterdam, the Netherlands.

18

19 Word count manuscript: 1,987

20 Word count abstract: 200

21

22

23

1 **ABSTRACT**

2

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

7

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

12

13 147 incident HBV-infections occurred during follow-up. On initial HBsAg-testing after infection (6-12
14 months), 42 (29%) were HBsAg-positive and 105 (71%) were HBsAg-negative (“standard HBsAg-
15 seroclearance”). Of the 42 initially HBsAg-positive individuals, 22 subsequently tested HBsAg-negative
16 (of whom 7 (31.8%) were HBV-DNA positive at last visit, suggesting occult HBV). Overall, 15 became
17 HBsAg-negative and HBV-DNA-negative (“delayed HBsAg-seroclearance”), while 27 remained HBsAg
18 and/or HBV-DNA-positive (“chronic HBV”). The 5-year cumulative probability of delayed HBsAg-
19 seroclearance was 41.6% for initially HBsAg-positive individuals. Delayed HBsAg-seroclearance and
20 remaining chronically infected were associated with younger age and HIV/Hepatitis C virus (HCV)-
21 coinfection.

22

23 In conclusion, delayed HBsAg-seroclearance is common in these key adult populations at-risk for HBV,
24 while proportion developing HBV chronicity (18%) is still higher compared to the general population
25 (~5%). Given the proportion of individuals with occult HBV-infection and that HCV direct-acting
26 antivirals can lead to HBV re-activation, HBV-DNA testing in HCV co-infected MSM/PWUD are
27 warranted prior to treatment initiation.

28

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

31

32

1 **BACKGROUND**

2
3 It is estimated that 257 million individuals worldwide are chronically infected with hepatitis B virus
4 (HBV).¹ Chronic infection can lead to liver cirrhosis, liver decompensation, hepatocellular carcinoma
5 and death², while the risk of these diseases increases with uncontrolled HBV replication.³ The
6 presence of hepatitis B surface antigen (HBsAg) indicates that a person is actively infected with HBV
7 and its loss is associated with a substantially lower risk of severe morbidity and mortality.²

8
9 Most people infected in adulthood have self-limiting infection, in which HBsAg becomes detectable 2-
10 10 weeks after infection and disappears within 6 months. However, a review concluded that roughly
11 5% of infected adults are unable to clear HBsAg within six months⁴ and are defined as having chronic
12 infection. This is in contrast to newborns until the first year of age and children 6-10 years old, in
13 whom 90% and 30-50%, respectively, develop chronic infection.

14
15 Meanwhile, some chronically-infected adults become HBsAg-negative >6 months after infection. The
16 proportion of individuals exhibiting “delayed” spontaneous seroclearance of those diagnosed with
17 chronic infection is variable and can be up to 20% within 5 years after infection.⁵ Missing delayed
18 seroclearance could lead to overestimated prevalence of chronic hepatitis B infection.

19
20 Intriguingly, specific adult populations have exhibited high rates of chronicity.⁴ In a previous study of
21 men who have sex with men (MSM) and people who use drugs (PWUD), we observed that about 25%
22 did not clear HBsAg within 6 months after infection and were therefore defined as having chronic
23 infection.⁶ Yet whether these infections are truly chronic or simply delayed seroclearers remains
24 unknown. Using unique data from a prospective cohort of adult high at-risk individuals with incident
25 HBV infection, we aimed to estimate the frequency of delayed HBsAg-seroclearance, along with its
26 determinants, and time to delayed HBsAg-seroclearance.

1 **METHODS**

2

3 **Study population and procedures**

4

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

13

14 **Testing procedures**

15

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

25

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

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

6 **Statistical analyses**

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

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

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

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

1 **RESULTS**

2

3 A total of 148 individuals had evidence of incident HBV infection during follow-up in the cohort (61
4 MSM, 87 PWUD). In analysis, one participant was excluded as no HBsAg result was available following
5 incident HBV infection. Among 147 included participants, median year of incident HBV infection was
6 1993 (interquartile range (IQR)=1989-1996), and median follow-up was 9.0 years (IQR:4.5-16.1) since
7 incident HBV infection until last cohort visit. Follow-up differed across the three HBV groups (Kruskal-
8 Wallis $p=0.03$): standard HBsAg-seroclearance, 10.7 years (IQR=4.8-16.9); delayed HBsAg-
9 seroclearance, 6.3 (IQR=3.0-8.8); chronic HBV, 7.8 (IQR=2.2-15.6). Median age at baseline was 31
10 years (IQR=27-36). At baseline, 61.6% (53/86) of all PWUD were HCV co-infected and 20.9% (18/86)
11 HIV/HCV co-infected. Of the PWUD, 89.5% (77/86) had ever injected drugs. Within the MSM group,
12 47.5% (28/61) were coinfecting with HIV (Table 1). During follow-up, 13 participants seroconverted for
13 HIV and/or HCV: only HCV (n=9), only HIV (n=3), both HIV/HCV (n=1).

14

15 Based on the first HBsAg test following incident HBV infection, 71.4% (105/147) cleared the virus and
16 28.6% (42/147) had initial chronic HBV infection (Figure 2). On subsequent HBsAg testing within the
17 initial chronic HBV group, 52.3% (22/42) tested HBsAg negative at their last available serum sample
18 and 20 tested HBsAg positive. Following HBV DNA testing of the HBsAg-negative individuals with initial
19 chronic infection, 31.8% (7/22) were in fact HBV-DNA positive suggesting occult HBV infection. Based
20 on combined HBsAg and HBV DNA testing, 15 individuals were re-classified as delayed HBsAg-
21 seroclearance and 27 remained in the chronically infected HBV group.

22

23 Based on the Kaplan-Meier curve including the total population (n=147), 120 individuals (105
24 standard HBsAg-seroclearance and 15 delayed HBsAg-seroclearance) were able to achieve HBsAg-
25 seroclearance in a median time of 7.3 months (95%CI=6.4-8.7) (Figure 2, Panel A). Based on the
26 Kaplan-Meier curve among the 42 participants with initial chronic HBV infection, 41.6% (95%CI=22.0-
27 56.3%) were able to clear HBsAg within 5 years from incident HBV infection (Figure 2, Panel B).

28

29 Of the 15 individuals with delayed HBsAg-seroclearance, 53.3% (8/15) were MSM (5 HIV/HCV-
30 uninfected and 3 HIV co-infected) and 46.6% (7/15) were PWUD (3 with HCV co-infection and 4 with
31 HIV/HCV co-infection). Of the 27 individuals who remained chronically infected with HBV, 25.9%
32 (7/27) were MSM (3 HIV/HCV-uninfected and 4 HIV co-infected) and 74.1% (20/27) were PWUD (2
33 HIV/HCV-uninfected, 9 HCV co-infected and 9 HIV/HCV co-infected).

Delayed HBsAg-seroclearance among high-risk groups

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

6

7 Among the 46 HIV-positive participants, with or without HCV co-infection, 5 were undergoing
8 antiretroviral therapy (ART) at first anti-HBc positive test (4 with anti-HBV-containing ART). All 5
9 belonged to the HBV chronic group. At the end of follow-up, 10 participants ever received ART (9 with
10 anti-HBV-containing ART): 3 belonging to the standard HBsAg-seroclearance group and 7 to the
11 chronic HBV group. Median cumulative time on ART at the end of follow-up was 2.4 years (IQR=1.1-
12 10.1). Median CD4 count at baseline was 430 cells/ μ l (IQR=280-590) and did not significantly differ
13 between HBV groups (Kruskal Wallis test, $p=0.89$).

14

1 **DISCUSSION**

2
3 A substantial proportion (cumulative probability 42% at 5 years) of MSM and PWUD initially
4 categorized as having chronic HBV infection after incident HBV infection were able to spontaneously
5 clear HBV. However, roughly 18% of the total study population remained with chronic HBV infection
6 at the end of an almost 9-year median follow-up.⁵ This study provides a rare insight into the
7 phenomenon of delayed spontaneous HBsAg-seroclearance, particularly among key populations at
8 risk of HBV infection in high-income countries.

9
10 Of the determinants studied, younger age was associated with developing chronic infection and
11 delayed HBsAg-seroclearance. The inverse relationship between age and higher risk of chronicity has
12 been demonstrated in several studies⁴ and is thought to be explained by the increasingly complex
13 innate and adaptive immune responses as individuals become older.⁸ Our data would suggest that the
14 risk of chronicity remains elevated particularly for younger adults at high-risk of infection.

15
16 We found HIV/HCV co-infection to be associated with delayed HBsAg-seroclearance and chronic HBV
17 infection, while coinfection with HIV and HCV only were not. Both HIV/HBV- and HCV/HBV-
18 coinfection, compared to HBV mono-infection, have been associated with accelerated liver disease
19 progression and higher HBV DNA levels⁹, which are strongly associated with delayed HBsAg-
20 seroclearance and progression to chronicity after acute HBV infection.¹⁰ The interaction between
21 these viruses is likely complicated with alternating dominance in circulating virus over time.¹¹ On the
22 other hand, only PWUD were HIV/HCV co-infected in our study. Sharing needles, the main route of
23 HCV transmission in this group, with the same HBV source partner may have led to continuous
24 reintroduction of the virus and impeded successful antiviral immune responses. However, the same
25 could be true for MSM repeatedly engaging in condomless sexual intercourse with HBV-infected
26 partners. From our analysis, we cannot disentangle the immunological or behavioral causes behind
27 the association of HIV/HCV co-infection on HBsAg-seroclearance.

28
29 Importantly, 32% of individuals initially classified as chronically infected with a subsequent negative
30 HBsAg result had in fact occult infection (HBV DNA positive in the absence of HBsAg positivity). Others
31 have indeed observed occult HBV profiles (HBV DNA+/HBsAg-) that were congruent with chronic or
32 recovered occult HBV infection¹², similar to individuals in our study. Nevertheless, the findings of
33 occult HBV after HBsAg-seroclearance are difficult to explain. A range of host adaptive anti-HBV
34 immune responses has been implicated in both occult infection and acute versus chronic infection¹³,

Delayed HBsAg-seroclearance among high-risk groups

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

5
6 Re-activation of HBV has been associated with direct-acting antivirals (DAA) when treating HCV
7 infection.¹⁵ MSM and PWUD are at high risk for both HBV and HCV infection, and seem to be less
8 likely to clear HBV than other adult populations.⁶ Therefore, HBsAg testing alone prior to DAA
9 initiation might be insufficient in assessing the risk of HBV reactivation for these high-risk populations,
10 and instead a serological battery including at least anti-HBc antibodies and HBV DNA should be
11 performed. Nevertheless, a small body of research has shown that HBV reactivation is not likely to
12 occur during DAA-treatment for HCV in individuals with occult HBV infection.¹⁶ Yet, this observation
13 needs to be confirmed in larger studies.

14
15 Given the period during which this study was conducted, these findings would be most generalizable
16 to settings where HBV vaccination coverage is low, HBV transmission is ongoing and/or where anti-
17 HBV containing ART uptake for HIV-positive individuals is suboptimal or not provided. How these data
18 could relate to current populations in the Netherlands is debatable. Tenofovir (TDF)-containing ART in
19 HIV-positive individuals is likely to reduce the risk of HBV acquisition¹⁷, and most HIV-positive
20 individuals in the Netherlands are treated with a TDF-containing regimen.¹⁸ Targeted HBV vaccination
21 campaigns have helped decrease HBV incidence in the HIV-negative MSM population since 2002, yet
22 HBV vaccination uptake from 2012 was around 40%.¹⁹ Meanwhile, there were 114 notified acute HBV
23 infections in 2017 across the Netherlands, almost half of which were sexually transmitted among
24 MSM.²⁰ No acute HBV infections have been observed in Dutch people who inject drugs over the last 5
25 years²⁰, in line with trends of HIV and HCV incidence.^{21,22} Since HIV-negative MSM in the Netherlands
26 are still acquiring HBV infection, delayed HBsAg-seroclearance could still be considered problematic for
27 this key population.

28
29 Our study has certain limitations. First, we were unable to quantify transaminases, HBV-DNA viral
30 loads, and HBsAg levels at the time of incident HBV infection or anti-HBs antibodies at the end of
31 follow-up. In previous research, these markers at first HBsAg-positive serology were associated with
32 reduced risk of delayed HBsAg-seroclearance and developing chronic infection.^{5,10} Second, anti-HBV
33 containing ART was infrequently given to HBV infected individuals during the study period and the
34 treatments provided (mostly lamivudine) were unlikely to provide a major prophylactic effect on
35 either HBV acquisition¹⁷ or HBsAg-seroclearance during the acute phase.²³ As such, we were unable to

Delayed HBsAg-seroclearance among high-risk groups

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

6

7 In conclusion, more than one-third of adult MSM and PWUD were able to spontaneously clear HBsAg
8 within five years after established chronic HBV infection, yet a higher proportion of HBV chronicity
9 (~18%) was observed in this population compared to the general population (~5%). The latter finding
10 highlights the importance of prompt HBV vaccination in these key populations. Importantly, 32% of
11 those initially categorized as having chronic HBV infection were later found to have cleared HBsAg but
12 remained HBV DNA positive. As DAA treatment can lead to HBV re-activation, testing for HBV DNA
13 along with anti-HBc antibodies in MSM and PWUD co-infected with HCV is warranted prior to DAA-
14 treatment.

15

1 **FOOTNOTE PAGE**

2

3 **Conflict on of interest:**

4 None of the other authors had any conflict to declare.

5 **Funding:**

6 This work was supported by the Research and Development fund from the Public Health Service of
7 Amsterdam, the Netherlands.

8

9 **Previously presented at the:**

- 10 - 20th International Workshop on HIV and Hepatitis Observational Databases (IWHOD), Lisbon,
11 Portugal 2017
- 12 - 11th Netherlands Conference on HIV Pathogenesis, Epidemiology, Prevention and Treatment
13 (NCHIV), Amsterdam, the Netherlands 2017
- 14 - 6th International Symposium on Hepatitis Care in Substance Users (INHSU), New York, US
15 2017
- 16 - European Association for the Study of the Liver (EASL), Amsterdam, the Netherlands 2017

17

18 **Acknowledgments:**

19

20 The authors wish to thank the participants of the ACS for their contribution, research nurses of the
21 ACS for data collection and cohort management, and Margreet Bakker, Marja de Ridder and Petra
22 Blom for laboratory support. The Amsterdam Cohort Studies on HIV infection and AIDS, which is a
23 collaboration between the Public Health Service of Amsterdam (Gemeentelijke Gezondheidsdienst
24 Amsterdam; GGD Amsterdam), Department of Infectious Diseases, Research and Prevention,
25 Amsterdam, The Netherlands, Amsterdam University Medical Centers (UMC), University of
26 Amsterdam (Department of Medical Microbiology, Experimental Immunology, Department of Internal
27 Medicine, Division of Infectious Diseases, Emma's Childrens hospital (Emma Kinderziekenhuis), HIV
28 treatment centre), Dutch Monitoring Foundation (Stichting HIV Monitoring; SHM), Jan van Goyen
29 Medical Centre, Department of Internal Medicine , HIV Focus Centre (DC Klinieken), and Sanquin
30 Blood Supply Foundation financially supports the maintenance of the biobank. The ACS is financially
31 supported by the Center for Infectious Disease Control of the Netherlands National Institute for Public
32 Health and the Environment (RIVM), Bilthoven, The Netherlands.

33

1 REFERENCES

- 2
- 3 1. World Health Organization GHP. Global hepatitis report. World Health Organization.
4 <http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1>.
5 Published 2017. Accessed 10 July 2018.
- 6 2. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the
7 management of hepatitis B virus infection. *J Hepatol* 2017;67:370–398.
- 8 3. Chen CJ, Yang HI, Iloeje UH, Group R-HS. Hepatitis B virus DNA levels and outcomes in chronic
9 hepatitis B. *Hepatology*. 2009;49(5 Suppl):S72-84.
- 10 4. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. *Clin Infect*
11 *Dis*. 1995;20(4):992-1000.
- 12 5. Ito K, Yotsuyanagi H, Yatsunami H, et al. Risk factors for long-term persistence of serum
13 hepatitis B surface antigen following acute hepatitis B virus infection in Japanese adults.
14 *Hepatology*. 2014;59(1):89-97.
- 15 6. van Houdt R, Bruisten SM, Speksnijder AG, Prins M. Unexpectedly high proportion of drug
16 users and men having sex with men who develop chronic hepatitis B infection. *J Hepatol*.
17 2012;57(3):529-533.
- 18 7. van den Hoek JA, Coutinho RA, van Haastrecht HJ, van Zadelhoff AW, Goudsmit J. Prevalence
19 and risk factors of HIV infections among drug users and drug-using prostitutes in Amsterdam.
20 *AIDS*. 1988;2(1):55-60.
- 21 8. Revill P, Yuan Z. New insights into how HBV manipulates the innate immune response to
22 establish acute and persistent infection. *Antivir Ther*. 2013;18(1):1-15.
- 23 9. Singh KP, Crane M, Audsley J, Avihingsanon A, Sasadeusz J, Lewin SR. HIV-hepatitis B virus
24 coinfection: epidemiology, pathogenesis, and treatment. *AIDS*. 2017;31(15):2035-2052.
- 25 10. Yotsuyanagi H, Ito K, Yamada N, et al. High levels of hepatitis B virus after the onset of disease
26 lead to chronic infection in patients with acute hepatitis B. *Clin Infect Dis*. 2013;57(7):935-
27 942.
- 28 11. Raimondo G, Brunetto MR, Pontisso P, et al. Longitudinal evaluation reveals a complex
29 spectrum of virological profiles in hepatitis B virus/hepatitis C virus-coinfected patients.
30 *Hepatology*. 2006;43(1):100-107.
- 31 12. Brojer E, Grabarczyk P, Liszewski G, et al. Characterization of HBV DNA+/HBsAg- blood donors
32 in Poland identified by triplex NAT. *Hepatology*. 2006;44(6):1666-1674.
- 33 13. Said ZN. An overview of occult hepatitis B virus infection. *World J Gastroenterol*.
34 2011;17(15):1927-1938.

Delayed HBsAg-seroclearance among high-risk groups

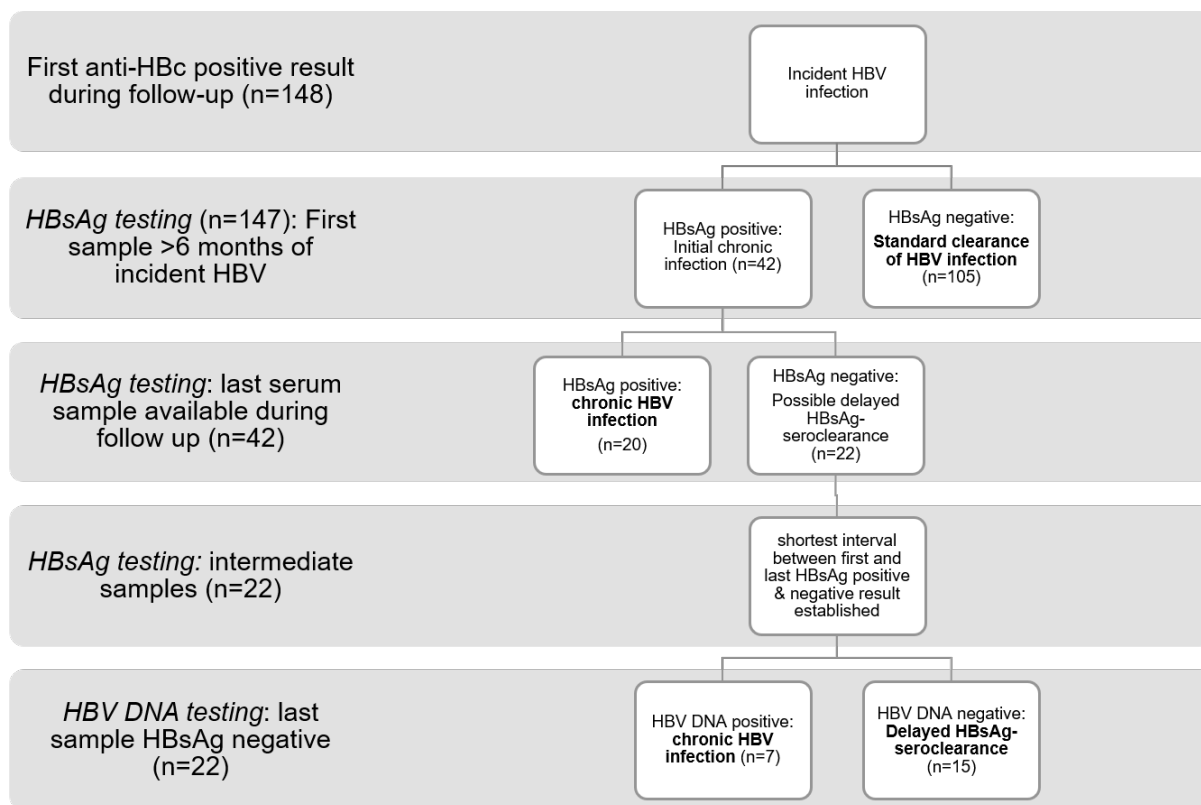
- 1 14. Zhu HL, Li X, Li J, Zhang ZH. Genetic variation of occult hepatitis B virus infection. *World J Gastroenterol*. 2016;22(13):3531-3546.
- 2
- 3 15. Chen G, Wang C, Chen J, et al. Hepatitis B reactivation in hepatitis B and C coinfecting patients
4 treated with antiviral agents: A systematic review and meta-analysis. *Hepatology*.
5 2017;66(1):13-26.
- 6 16. Ozaras R, Mete B, Tabak F. Occult Hepatitis B and Risk of Reactivation After Hepatitis C
7 Treatment With Direct-Acting Antivirals. *Clin Gastroenterol Hepatol*. 2017;15(4):605.
- 8 17. Heuft MM, Houba SM, van den Berk GE, et al. Protective effect of hepatitis B virus-active
9 antiretroviral therapy against primary hepatitis B virus infection. *AIDS*. 2014;28(7):999-1005.
- 10 18. van Sighem AI, Boender TS, Wit FWNM, Smit C, Matser A, Reiss P. *Monitoring Report 2018*.
11 *Human Immunodeficiency Virus (HIV) Infection in the Netherlands*. Amsterdam: Stichting HIV
12 Monitoring;2018.
- 13 19. van Rijckevorsel G, Whelan J, Kretzschmar M, et al. Targeted vaccination programme
14 successful in reducing acute hepatitis B in men having sex with men in Amsterdam, the
15 Netherlands. *J Hepatol*. 2013;59(6):1177-1183.
- 16 20. Slurink IAL, van Aar F, Op de Coul ELM, et al. *Sexually transmitted infections in the*
17 *Netherlands in 2018*. Bilthoven: National Institute for Public Health and the Environment
18 (RIVM);2018.
- 19 21. Grady BP, Vanhommerig JW, Schinkel J, et al. Low incidence of reinfection with the hepatitis C
20 virus following treatment in active drug users in Amsterdam. *Eur J Gastroenterol Hepatol*.
21 2012;24(11):1302-1307.
- 22 22. de Vos AS, van der Helm JJ, Matser A, Prins M, Kretzschmar ME. Decline in incidence of HIV
23 and hepatitis C virus infection among injecting drug users in Amsterdam; evidence for harm
24 reduction? *Addiction*. 2013;108(6):1070-1081.
- 25 23. Mantzoukis K, Rodriguez-Peralvarez M, Buzzetti E, et al. Pharmacological interventions for
26 acute hepatitis B infection: an attempted network meta-analysis. *Cochrane Database Syst*
27 *Rev*. 2017;3:CD011645.

28

29

1 Tables and figures

2



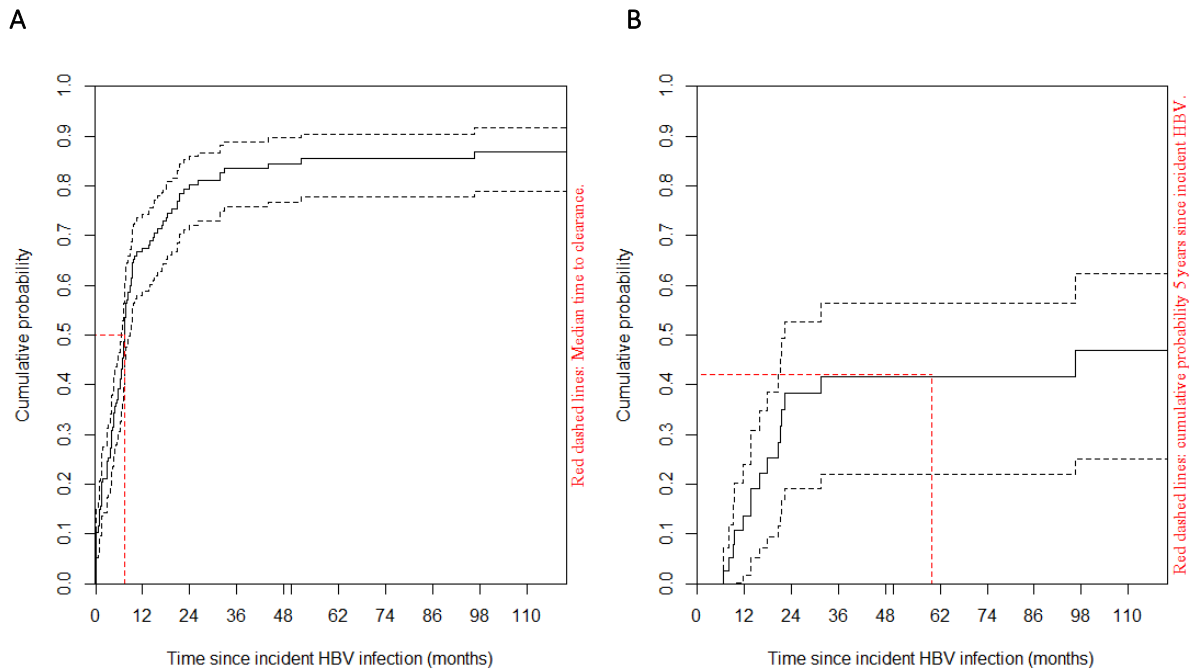
3

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

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

Delayed HBsAg-seroclearance among high-risk groups

1



2

3

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

6

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

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

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

15

16

17

18

19

1 **Table 1: Socio-demographic and clinical characteristics and HBV outcome of 147 MSM and PWUD**
 2 **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%)

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

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

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

8

9

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

	Delayed seroclearance group, n=15				HBV chronic group, n=27				overall p-value
	n [§]	% [^]	OR	p-value	n [§]	% [^]	OR	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]		

3

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

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

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

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

11

12

13

14

15

16

17

18

19

Delayed HBsAg-seroclearance among high-risk groups

- 1
- 2