Wouthuyzen-Bakker: treating physician, critical revision of the manuscript. Robert J de Knegt: treating physician, critical revision of the manuscript. Pieter Honkoop: treating physician, critical revision of the manuscript. Omar El-Sherif: supervision of sample analysis, critical revision of the manuscript. Angela Colbers: analysis of data, critical revision of the manuscript. David J Back: critical revision of the manuscript. David M Burger: interpretation of results, critical revision of the manuscript, supervision of the case series.

Acknowledgements

The authors thank the patients for their participation in this case series. The authors also thank the laboratory personnel of the Dept of Molecular and Clinical Pharmacology of the University of Liverpool and the Dept of Pharmacy of the Radboudumc for sample analysis.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2018.10.010.

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8 weeks of sofosbuvir/ledipasvir is effective in DAA-naive non-cirrhotic HCV genotype 4 infected patients (HEPNED-001 study)

To the Editor:

In contrast to genotype 1, genotype 4 hepatitis C (HCV) infections are more often found in Central Africa and the Middle East with the highest prevalence in Egypt.[1] As the initial budget impact of HCV treatment with direct-acting antivirals (DAAs) can be substantial for countries with a high HCV prevalence,[2] shortening treatment duration could help in reaching the World Health Organization's HCV elimination goals[3] by lowering costs and expanding access.[4] The most recent EASL guideline suggests 8 weeks of therapy with sofosbuvir/ledipasvir (SOF/LDV) as an option for treatment-naive non-cirrhotic patients with chronic HCV of the genotypes 1a and 1b.[5]

Although the first clinical trials with DAA's were primarily focused on HCV genotype 1 infections, the advent of pan-genotypic DAA's give us the opportunity to study new treatment options and even treatment shortening for genotype 4 infections.[4] Indeed, LDV showed a high potency in a study that assessed the phenotypic susceptibility of various genotype 4 subtypes[6] and in the study that led to the registration of 12 weeks of SOF/LDV for genotype 4, in which 41 of the 44 (93%) patients achieved a sustained virological response (SVR).[7] Given the very comparable cure rates after 12 weeks of SOF/LDV for genotype 1 and 4, a treatment duration of 8 weeks may be appropriate for genotype 4 as well.[8] Recently,

this approach was studied in Egyptian patients and a cure rate of 95% (41/43) was observed in the 43 patients.[9] However, these patients were HIV-negative and because genotype 4a is the most prevalent HCV subtype in Egypt, these results cannot be translated to other genotype 4 subtypes.[1]

We evaluated the effectiveness of 8 weeks SOF/LDV for genotype 4 HCV-infected DAA-naive HIV-positive and -negative patients without cirrhosis in a single arm prospective open label study in 10 centers in the Netherlands and Belgium and found a high effectiveness these patients.

The primary outcome was SVR in the on-treatment (OT) study population, defined as an HCV RNA below the limit of detection 12 weeks after the end of therapy in all patients that had completed the 8-week treatment course of therapy and had an HCV RNA measurement ≥12 weeks after the end of therapy. Eligible participants were HIV-negative or HIV-positive adults chronically infected with HCV genotype 4 with a screening HCV RNA load <10 million IU/ml. Patients with a history of DAA treatment failure for the current episode of HCV, a liver biopsy with a METAVIR score above F3 or a liver stiffness measurement (FibroScan®) ≥12.5 kPa were excluded. Because HCV reinfections are frequently observed in HIV-infected men who have sex with men (MSM), it was predefined in the protocol that HCV reinfections diagnosed by a genotype switch or by phylogenetic analysis[10] will not be counted as treatment failure.

From January 2016 until June 2017, 63 patients were screened for eligibility of whom 44 were enrolled. Four patients never started therapy and 30 HIV-positive and 10 HIV-negative patients started treatment (Fig. S1). All patients completed the 8 weeks of therapy, but 1 HIV-negative patient was lost to follow-up before SVR could be evaluated (last HCV viral load <15 IU/ml). In the on-treatment population, 33 of the 39 patients were HCV RNA negative 12 weeks after therapy and 6 were HCV RNA positive. However, 4 of them had a proven reinfection (Fig. S2). These 4 patients were all MSM and had ongoing unprotected sex, underlining the urgent need for effective interventions to decrease the risk of reinfection in this subpopulation. In total, 37 of 39 patients (95%; 95% CI 83–99%) of the on-treatment population were successfully treated for the HCV that was present at baseline. Stratified to HIV-status, 28 of the 30 HIV-positive patients (93%; 95% CI 80–99%) and 9 of the 9 HIV-negative patients (100%) reached SVR12 ($p = 1.0$) (Table 1). In the 2 treatment failures, the baseline HCV viral loads were 9.8E5 and 8.7E6 IU/ml. The subtype was 4c in one patient, but in the other

Table 1. Baseline characteristics and outcome according to HIV-status.

Baseline characteristics	All (n = 40)	HIV-positive (n = 30)	HIV-negative (n = 10)	*p* value
Age (yr)[a], mean ± SD	51 (± 9.9)	51 (± 10.4)	51 (± 8.7)	0.971
Male[b], % (n)	85% (34/40)	86,7% (24/30)	80% (8/10)	1.000
Caucasian[b], % (n)	80% (32/40)	76,7% (23/30)	90% (9/10)	0.653
Transmission mode HCV[b]				0.068
MSM, % (n)	52.5% (21/40)	63.3% (19/30)	20% (2/10)	
IVDU, % (n)	12.5% (5/40)	10% (3/30)	20% (2/10)	
Other, % (n)	7.5% (3/40)	6.7% (2/30)	10% (1/10)	
Missing, % (n)	27.5% (11/40)	20% (6/30)	50% (5/10)	
Previous treatment[b], % (n)				0.011
Naive (no treatment)	80% (30/40)	83,3% (25/30)	70% (7/10)	
PegIFN ± ribavirin	20% (8/40)	16.7% (5/30)	30% (3/10)	
Baseline viral load (IU/ml)[c], median (IQR)	1.05 E6(3.36 E5–3.64 E6)	1.21 E6(3.97 E5–3.37 E6)	6.9 E5(1.75 E5–2.00 E6)	0.235
Time since diagnosis of HCV infection (yr)[c], median (IQR)	4.2 (2.1–9.8)	4.4 (2.8–10.1)	4.4 (4.0–4.9)	0.331
HCV subtype[b], % (n)				0.304
4a	15% (6/40)	10% (3/30)	30% (3/10)	
4c	2.5% (1/40)	3.3% (1/30)	0%	
4d	37.5% (15/40)	40% (12/30)	30% (3/10)	
4t	2.5% (1/40)	0%	10% (1/10)	
Unknown	42.5% (17/40)	46.6% (14/30)	30% (3/10)	
Liver stiffness measurement (FibroScan®)				
pKa[c], median (IQR)	5.6 (4.5–7.6)	5.3 (4.2–6.8)	8.8 (6.5–10.8)	0.004
F3 (>9.5 kPa)[b], % (n)	15% (6/40)	3.3% (1/30)	50% (5/10)	0.002
CD4 cell count (cells/μl), mean ± SD				
Nadir	n.a.	397.9 ± 53.9	n.a.	
At start of HCV therapy	n.a.	807.0 ± 69.0	n.a.	
On Cart, % (n)	n.a.	100% (30/30)	n.a.	
HIV viral load <40 copies/ml at start of HCV therapy, % (n)	n.a.	97% (29/30)	n.a.	
Outcomes in on-treatment population[d]				
Effectiveness OT population				
%, n	95% (37/39)	93% (28/30)	100% (9/9)	
95% exact CI[e]	83–99%	80–99%	–	
HCV RNA negative 12 weeks after therapy	33	24	9	
HCV RNA positive 12 weeks after therapy				
Reinfection (genotype switch)	1	1	–	
Reinfection (phylogenetically distinct genotype 4 virus)	3	3	–	
Relapse	2	2	–	

cART, combined antiretroviral therapy; HCV, hepatitis C virus; IVDU, intra-venous drug use; MSM, men who have sex with men; n.a., not applicable; OT, on-treatment.
[a] T-test. [b] Fisher's exact test. [c] 2-sided Mann-Whitney *U* test. [d] Reinfections are not considered treatment failure. [e] 2-sided Clopper Pearsons confidence interval.

patient the subtype was not typable. No resistance associated mutations in NS5a or NS5b were detected at the time of HCV relapse.

As a result of the rapid treatment uptake of DAAs in HIV-infected MSM in the Netherlands and Belgium,[11] the inclusion of additional patients was not possible because after the screening of 63 and the treatment of 40 genotype 4 patients, no eligible patients were left in any of the participating centers. Therefore, we did not reach the intended sample size of 41 patients as stated in the protocol of our study (as described supplementary information). However, although relatively small, our sample size was comparable to the number of patients included in phase III trials of SOF/LDV that led to the registration of 12 weeks SOF/LDV therapy for HCV genotype 4.[5]

Our study showed that 8 weeks of SOF/LDV could be an effective therapy for non-cirrhotic HCV genotype 4 infected patients with an HCV RNA load <10 million IU/ml and is the first to evaluate the efficacy of 8 weeks of SOF/LDV in a substantial number of HIV-coinfected patients. Our results further strengthen the observation made among Egyptian mono-infected patients.[9] Therefore, 8 weeks of SOF/LDV could be considered a treatment option in DAA-naïve genotype 4 patients without cirrhosis, thereby expanding access to therapy to a larger number of patients.

The extended version of the methods and ethics statement (S1), the flow diagram of the study (S2) and the phylogenetic analysis (S3) can be found in the online supplements.

Financial support

The authors received no financial support to produce this manuscript.

Conflict of interest

AB: has nothing to disclose. TV: reports grants from Foundation Against Cancer Belgium (No. 2014-087), during the conduct of the study; non-financial support from Roche Diagnostics, grants from Gilead Sciences, grants from Bristol-Myers-Squibb, grants from Janssen, outside the submitted work; and Advisory Board membership for Abbvie, Gilead, Janssen and BMS. MvdV: reports grants and other from Abbvie, grants and other from Gilead, grants and other from Johnson & Johnson, grants, non-financial support and other from MSD, other from ViiV, outside the submitted work. GvdB: has nothing to disclose. MvK: has nothing to disclose. DP: reports personal fees from Luminex – MTE session, outside the submitted work. AD: has nothing to disclose. BvH: reports personal fees from Bristol-Meyers Squibb, personal fees from Merck, personal fees from Abbvie, personal fees from Norgine, for advisory boards outside the submitted work. DR: reports grants from AbbVie, personal fees from Abbvie, personal fees from Abbvie, personal fees from Bristol Myers Squib, outside the submitted work. JK: has nothing to disclose. JS: reports grants from Gilead Sciences, grants from Abbvie, grants from MSD, grants from Janssen Pharmaceuticals, outside the submitted work. EF: reports grants from Janssen, grants from BMS, grants from Gilead, grants from ViiV Healthcare, other from Janssen, other from ViiV Healthcare, outside the submitted work. JA: reports other from Gilead Sciences, other from MSD, other from Janssen, other from Abbvie, other from ViiV, grants from Abbvie, grants from MSD, grants from ViiV, grants from MSD, outside the submitted work. BR: reports grants from MSD, during the conduct of the study; grants from Gilead, other from MSD, Jansen-Cilag, BMS, Gilead, Pfizer, ViiV, outside the submitted work.

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

Authors' contributions

AB: study conception and design, acquisition of data, analysis and interpretation of data, drafting of manuscript, critical revision. TV: acquisition of data, critical revision. MvdV: acquisition of data, critical revision. GvdB: acquisition of data, critical revision. MvK: acquisition of data, critical revision. DP: acquisition of data, critical revision. AD: acquisition of data, critical revision. BvH: acquisition of data, critical revision. DR: acquisition of data, critical revision. JK: acquisition of data, analysis and interpretation of data. JS: acquisition of data, analysis and interpretation of data, critical revision. EF: acquisition of data, critical revision. JA: study conception and design, acquisition of data, interpretation of data, critical revision. BR: study conception and design, analysis and interpretation of data, drafting of manuscript, critical revision.

Acknowledgements

Hannah Muylle, Michelle Mutschelknauss, Frank Pijnappel, Imke Hooijenga, Danielle Vos, Inge de Kroon, Danielle van Elst, Robin Ackens, Karin Grintjes, Lida Beneken Kolmer, Marien Kuipers and Renée Dierx for local study coordination. Sjoerd Rebers for phylogenetic analysis.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2018.10.032.

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Over-gap PCR amplification to identify presence of replication-competent HBV DNA from integrated HBV DNA: An updated occult HBV infection definition

To the Editor:

With great interest, we read the manuscript "Quantitation of HBV cccDNA in anti-HBc-positive liver donors by droplet digital PCR: a new tool to detect occult infection" by Caviglia *et al.* published in *Journal of Hepatology*.[1] Using a highly sensitive in-house droplet digital PCR assay (ddPCR) method, the authors indicated that intrahepatic HBV covalently closed circular (cccDNA) was detectable in about half (52%, 27/52) of the defined cases of occult HBV infection (OBI). We wonder whether the pretreatment with plasmid-safe ATP dependent DNase (PSAD) plus double-over-gap cccDNA 'specific' primers spanning the HBV relaxed circular DNA (rcDNA) gap region used in this paper could totally eliminate the interference of rcDNA, though this method had been widely used in the detection of cccDNA.[2] Here we evaluated the capacity of the above approach to discriminate between the cccDNA, the rcDNA and the integrated double strand linear HBV DNA (dslDNA). In addition, several sets of mono-over-gap rcDNA primers (Table S1) were also tested, which theoretically can amplify both rcDNA and cccDNA.[3]

First, to exclude the likely cccDNA contaminant leaked from cells, the supernatant of HepAD38[2] and serum specimens from patients with HBV infection[4] were treated with DNase I prior to viral DNA extraction and PCR amplification. The elimination efficiency was confirmed by the failed amplification of plasmid DNA containing 1.2xHBV genome (Fig. 1A). In contrast, the HBV rcDNA in Dane particles could still be detected by using the supposed cccDNA 'specific' primers, which provided a similar result compared to rcDNA primers. Moreover, the gradual increase of HBV DNA level was observed in parallel with the increased amount of rcDNA, when either the supposed cccDNA primers or the rcDNA primers were used (Fig. 1B). As previously reported,[5] the rcDNA could not be eliminated completely pretreatment by PSAD and this was further confirmed by T5 Exonuclease and Exonuclease III, respectively (Fig. 1C, D). Hence, PSAD digestion plus double-over-gap PCR may not guarantee the discrimination of cccDNA from rcDNA.

The term 'occult hepatitis B virus infection' has been introduced to describe a status characterized as an absence of serum HBV surface antigen and presence of replication-competent HBV DNA in the liver.[6–8] Since cccDNA is the resource for viral replication and the reason for HBV infection persistence, the presence of cccDNA for OBI is indispensable. The integrated HBV DNA fragments, on the other hand, have an incomplete viral genome which lost the capacity to serve as the template for HBV replication. Therefore, it is reasonable to postulate that the detection of cccDNA, but not the presence of integrated HBV DNA fragment, is essential for true OBI. Moreover, it may not be necessary to distinguish cccDNA from rcDNA for the definition of OBI because the rcDNA originates solely from the transcriptionally active cccDNA.[9]

Integration of HBV DNA fragments is a common event during HBV infection. Our previous study revealed that the breakpoints of the integrated HBV DNA fragments were mainly found within the DR1 and DR2 regions (Fig. 1E).[4] This is in accordance with the suggestion that HBV dslDNA is the preferred form for viral DNA integration into the host genome.[10] To test if the integrated