Outcome and adverse events in patients with chronic hepatitis C treated with direct-acting antivirals: a clinical randomized study

Christina Sølund[a,c,f], Ellen S. Andersen[d], Belinda Mössner[h], Alex L. Laursen[j], Birgit T. Røge[l], Mette S. Kjær[e,g], Jan Gerstoft[d,g], Peer B. Christensen[h,i], Martin S. Pedersen[c,f,b,k], Kristian Schønning[g,b], Ulrik Fahnøe[c,f], Jens Bukh[a,c,f] and Nina Weis[a,g]

Objective New potent direct-acting antiviral (DAA) regimens against hepatitis C virus have been approved in recent years. However, information about the rate of adverse events (AEs) across different DAA regimens is limited. We aimed to evaluate differences in AEs and treatment efficacy in patients with chronic hepatitis C (CHC), genotype (GT) 1 or 3, randomized to two different treatment arms, correspondingly.

Patients and methods We randomly assigned 96 patients in a 1 : 1 ratio, to treatment for 12 weeks with either paritaprevir/ombitasvir/ritonavir/dasabuvir/ribavirin (RBV) or ledipasvir/sofosbuvir (SOF)/RBV if infected with GT1 (72 patients) or to daclatasvir/SOF/RBV for 12 weeks or SOF/RBV for 24 weeks, if infected with GT3 (24 patients). Data on AEs were collected throughout the entire study period.

Results A total of 70 (97%) patients with CHC with GT1 and 20 (83%) patients with GT3 achieved cure. The GT3 treatment arm was prematurely terminated, owing to change in national treatment guidelines. Thus, only AEs for GT1 patients are described. AEs occurred in 70 (97%) GT1 patients, and most common AEs were anemia ($n = 56/78\%$), fatigue ($n = 53/74\%$), and headache ($n = 33/46\%$). No difference was observed in relation to treatment group ($P = 1.0$), anemia ($P = 1.0$), or liver cirrhosis ($P = 0.53$). In seven (11%) patients, AEs assessed by the investigator to be possibly related to the DAA regimen were still present 12 weeks after treatment.

Conclusions We found no difference in AEs possibly related to the DAA regimen in patients with CHC, but surprisingly, AEs possibly related to the DAA regimen persisted in a significant number of patients after treatment. This finding can be of importance for clinicians in relation to patient information concerning AEs possibly related to DAA treatment. Eur J Gastroenterol Hepatol 00:000–000

Introduction

More than 70 million people globally are chronically infected with hepatitis C virus (HCV), which causes more than 500 000 deaths yearly [1,2]. In Denmark, with a population of 5.7 million people [3], the number of HCV-infected individuals was estimated to be 16 000–18 000 (prevalence of about 0.38%) in 2007, of whom ~50% had been diagnosed [4]. According to national Danish registries, only a third of patients diagnosed with chronic hepatitis C (CHC) attended specialized clinical care, and the most prevalent HCV genotypes (GT) are 1a, 1b and 3a [4,5]. Chronically infected individuals are at risk of developing liver cirrhosis, with potential serious complications, including decompensation and hepatocellular carcinoma [6]. Owing to increasing age among HCV-infected individuals, morbidity and mortality is expected to increase the next decades [1].

Previously, the standard of care for CHC was pegylated-interferon (PEG-INF) and ribavirin (RBV) therapy with treatment durations of 24–48 weeks, with low cure rates, high rates of severe adverse events (SAE) and low tolerability [7]. Recently, HCV therapy has been revolutionized by direct-acting antivirals (DAAs) that directly target proteins involved in viral replication. The first DAAs, to be launched, were HCV NS3/4 A protease inhibitors, telaprevir and boceprevir, given in combination with PEG-INF/RBV. However, cure rates were substantially lower in a real-world setting, than in clinical

European Journal of Gastroenterology & Hepatology 2018, 00:000–000

Keywords: adverse events, direct-acting antivirals, genotype 1, hepatitis C virus, ledipasvir and sofosbuvir, ombitasvir and dasabuvir, paritaprevir, randomized, ribavirin

Departments of [a]Infectious Diseases, [b]Clinical Microbiology, [c]Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases and Clinical Research Centre, Copenhagen University Hospital, Hvidovre, Departments of [d]Infectious Diseases, [e]Hepatology, Copenhagen University Hospital, Rigshospitalet, Departments of [f]Immunology and Microbiology, [g]Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, [h]Department of Infectious Diseases, Odense University Hospital, [i]Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense, [j]Department of Infectious Diseases, Aarhus University Hospital, Skejby, [k]Department of Science and Environment, Roskilde University, Roskilde and [l]Department of Medicine, Lillebaelt Hospital, Kolding, Denmark

Correspondence to Nina Weis, PhD, MD, Department of Infectious Diseases, Copenhagen University Hospital, Kettegaard Alle 30, DK 2650 Hvidovre, Denmark

Tel: +45 386 23514; fax: +45 386 23504; e-mail: nina.weis@regionh.dk

Received 12 March 2018 **Accepted** 19 April 2018

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.eurojgh.com.

trials, and tolerability and adverse events (AEs) remained major barriers towards treatment initiation [8,9]. New potent second-generation interferon-free DAA regimens have since been approved. Clinical trials with these potent DAAs have shown improved SVR rates of greater than or equal to 90% with good tolerability, including difficult-to-treat patient groups (liver cirrhosis and liver transplantation and previous treatment failure) [10,11]. However, this rapid development has led to few systematic comparisons of different DAA regimens, usually evaluated in cohorts of patients randomized with respect to dosage, addition of RBV or treatment duration. This design provides limited information about the rate of AEs across different DAA regimens. Minimizing AEs is crucial in relation to adherence to treatment and prevention of prematurely treatment termination owing to poor tolerability.

This real-life study aimed to evaluate differences in AEs and treatment efficacy in patients with CHC, GT1, randomized to ledipasvir (LVD)/sofosbuvir (SOF) and RBV for 12 weeks versus paritaprevir (PAR)/ombitasvir (OMB)/ritonavir (rit)/dasabuvir (DAS) and RBV for 12 weeks. Patients with CHC, GT3, were randomized to daclatasvir (DAC)/SOF and RBV for 12 weeks versus SOF and RBV for 24 weeks.

Patients and methods

Patient population

Patients were screened from 1 July 2015 to 1 April 2017 at six screening sites, which covered four of five regions in Denmark (Supplemental Appendix, Supplemental digital content 1, *http://links.lww.com/EJGH/A311*). Eligible patients were 18–70 years and registered with CHC, GT1 or 3, in the Danish Database for hepatitis B and C (DANHEP) [12]. The patients had to fulfill inclusion criteria defined as follows: liver biopsy (Metavir score ≥ F2), liver stiffness measurement (LSM) greater than or equal to 10 kPa [13], clinical cirrhosis or extrahepatic manifestations of importance to treat (neuropathy caused by cryoglublinemia, porphyria cutanea tarda, glomerulonephritis, arthritis, severe debilitating fatigue, women of childbearing age with a fertility wish, specific types of B-lymphoma and vasculitis) [14]. Cirrhosis was diagnosed according to the national treatment guidelines, defined as the presence of 1 of the following: a liver biopsy with a Metavir score of F4 and/or median elasticity at TE of greater than or equal to 17 kPa [15,16]. The cutoff of 17.0 kPa was based on a prospective study reporting similar rates of liver-related complications among patients with CHC with a TE greater than or equal to 17 kPa and patients with biopsy-confirmed cirrhosis, and results from a large French multicentre study [15,16]. Both treatment-naive patients and patients previously treated with PEG-INF and RBV or discontinuation of treatment with PEG-INF, RBV and first-generation protease inhibitors, owing to AEs, could be included. Patients co-infected with HIV had to be fully suppressed on antiretroviral treatment. Exclusion criteria were decompensated liver cirrhosis (Child–Pugh B or C) or a diagnosis of hepatocellular carcinoma. Detailed eligibility criteria are provided in the Supplemental Appendix, (Supplemental digital content 1, *http://links.lww.com/EJGH/A311*).

Study design

Patients with CHC were randomly assigned in a 1 : 1 ratio to treatment for 12 weeks with either PAR/OMB/rit/DAS and RBV or LVD/SOF and RBV, if infected with GT1 or to DAC/SOF and RBV for 12 weeks or SOF and RBV for 24 weeks, if infected with GT3. The study was conducted as nonblinded with randomization lists produced electronically in blocks of 4. The DAA regimens included in the treatment arms were determined according to national treatment guidelines for CHC [17]. Randomization was stratified according to liver cirrhosis status. PAR/OMB/rit/DAS and LVD/SOF were administered according to manufacturer's instructions, and RBV was given according to body weight. Any reduction or discontinuation of RBV was recorded during treatment and had to follow protocol guidelines (see Supplemental Appendix, Supplemental digital content 1, *http://links.lww.com/EJGH/A311*). The study was approved by the Danish Medicines Agency (2015-001956-31), the Regional Ethical Committee (H-15007265), and the Danish Data Protection Agency (2012-58-0004). The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines and was monitored by the Good Clinical Practice units in Copenhagen, Aarhus, and Odense. The study is registered in the European Clinical Trials Database (2015-001956-31). Written informed consent was provided by all patients, and patient data were stored in individual case report forms.

Safety and efficacy assessments

All AEs and clinical parameters were assessed and recorded by either the treating physician or a project nurse. Blood samples, AEs and clinical parameters were recorded and collected at baseline and for all patients at weeks 1, 2, 3, 4, 8, and 12 of the study period, and for patients in the 24-week treatment arm at weeks 16, 20, and 24. Clinical parameters and blood samples were recorded and collected at premature discontinuation from the treatment period for patients in either treatment arm. In the post-treatment period, AEs occurring both before and after DAA treatment completion, clinical parameters and blood samples were recorded and collected at weeks 4, 12, and 24, or at premature discontinuation from the post-treatment period. A telephone interview was conducted for patients treated for 12 weeks to obtain information on AEs 8 weeks after treatment.

The intensity of fatigue was recorded according to the common terminology criteria for AEs [18]. For all AEs, start date and end date, intensity (mild, moderate, or severe), severity (is recovering, has recovered, recovered with sequelae, still affected), relation to study drug and action taken with study drug were recorded. The severity of AEs and their relationship to treatment were assessed by the investigator. Data on SAEs were collected throughout the entire study period. The primary efficacy end point was cure, defined as SVR12 (HCV-RNA level <15 IU/ml 12 weeks after the last dose of study drug). The rate of SVR4 (SVR4), weeks after end of treatment (EOT), was also determined. HCV-RNA levels were quantified with Cobas AmpliPrep/cobas TaqMan HCV test, v2.0 (Roche; Penzberg, Germany) or Hologic Aptima HCV Dx Quant Assay (Aptima; Hologic Inc., San Diego, California, USA)

with a lower limit of quantification of 15 and 10 IU/ml, respectively [19–21], or an in-house real-time PCR method as described [22]. Definitions of treatment failure and laboratory abnormalities are shown in the Supplemental Appendix (Supplemental digital content 1, *http://links.lww.com/EJGH/A311*).

Statistical analyses

A sample size of 50 patients per group with 80% power using two-sample test for proportions with a two-sided significance level of 0.05 and the treatment arm with PAR/OMB/rit/DAS + RBV and SOF/RBV set to 30% [23–25] would detect a difference of 22% giving a proportion of 8% in the other treatment arms. Efficacy and safety analyses were performed using data from the intent-to-treat population, defined as all patients who received greater than or equal to 1 dose of study drug. Missing values were excluded from statistical analyses. Categorical variables were reported as absolute numbers and relative frequencies. Continuous data were summarized as mean ± SD or median and interquartile ranges. Patient characteristics and laboratory values for treatment groups were compared using Fisher's exact test. As anemia can be the cause of other AEs like fatigue, dyspnea, dizziness, weakness, and headache, we compared the rate of AEs in patients with and without anemia by Fisher's exact test. We hypothesized that AEs could be more common in patients with cirrhosis, owing to the affected liver function, and assessed if any difference was detectable in the incidence of AEs between cirrhotic and noncirrhotic patients by Fisher's exact test. The Wilcoxon signed-rank and rank-sum tests were applied to estimate changes in laboratory results and LSM from baseline to the EOT for all patients and the comparison of treatment groups, correspondingly. SAS 9.4 software (SAS Institute Inc., Cary, North Carolina, USA) was used for all statistical analyses, and *P* values below 0.05 (two-sided) were considered statistically significant.

Next-generation sequencing

Analysis for resistance associated substitutions (RASs) in a single GT3a patient with viral relapse was done by deep sequence analysis of recovered viruses. In short, RNA was extracted from 100 µl of serum samples and full-length ORF RT-PCR [26] was performed using HCV GT3a specific primers. The library preparation was performed and run in-house on Illumina Miseq. Data analysis was performed in-house to detect major and minor variant RASs. De-novo assembly was performed by iterative virus assembler [27], and Blast was used to verify genotyping of the isolate. Subsequently, reads were aligned to reference subtype 3a proteins NS3, NS5A and NS5B with BWA using the MEM algorithm. LoFreq [28] detected the low-frequency SNPs and translational effects by VCF annotator (Broad institute; Cambridge, Massachusetts, USA). In-house scripts were applied to detect RASs by comparing with the Geno2Pheno database [29]. Only RASs with a cut-off level greater than 15% were reported. To distinguish re-emergence of initial virus from HCV reinfection, phylogenetic analyses of the ORF sequence from baseline and post-treatment samples were performed [26].

Results

Patients

Of 130 patients assessed for eligibility, 96 were enrolled in the study. For patients with GT, 18 patients were excluded. Two patients did not wish to receive treatment containing RBV and one patient was incarcerated, whereas 15 patients did not meet inclusions criteria [severe mental illness ($n=2$), decompensated liver cirrhosis ($n=5$), hepatocellular carcinoma ($n=1$), previous stroke ($n=2$), severe pulmonary disease ($n=1$), and did not speak and/or read Danish ($n=4$)]. For patients with GT3, eight patients declined participation owing to the chance of receiving 24 weeks of treatment whereas eight patients did not meet inclusion criteria [severe mental illness ($n=2$), decompensated liver cirrhosis ($n=3$), severe pulmonary disease ($n=1$), and did not speak and/or read Danish ($n=2$)]. The treatment arm, enrolling patients with GT3, was prematurely terminated after the inclusion of 24 patients, because national treatment guidelines recommended SOF/RBV for 24 weeks to be withdrawn as an option for CHC GT3 treatment after the European approval of velpatasvir/SOF (Epclusa; Gilead Sciences Inc., Foster City, California, USA) in September 2016 [30]. We therefore report efficacy results, but no data on AEs for CHC GT3 patients. Baseline characteristics, as well as co-morbidities, were similar in the 72 CHC GT1 patients randomized to PAR/OMB/rit/DAS + RBV or LVD/SOF + RBV (Tables 1 and 2). Prescribed medication for CHC GT1 patients is shown in Supplementary Table S1 (Supplemental digital content 1, *http://links.lww.com/EJGH/A312*).

Efficacy

A total of 67 (93%) of 72 GT1 patients, available for follow-up, had SVR4, whereas 70 (97%) patients achieved SVR12. Two patients terminated treatment prematurely owing to AEs, of whom one achieved SVR12, whereas another died owing to liver failure during the study period. One patient was lost to follow-up (Fig. 1).

Cure was achieved in 20 (83%) of the GT3 patients whereas 22 (92%) patients had SVR4. Three patients, randomized to SOF/RBV for 24 weeks, terminated treatment prematurely at week 16 owing to AEs. Two of these patients still achieved cure whereas one patient was lost to follow-up. One patient emigrated during the treatment period and was lost to follow-up. One patient randomized to 12 weeks treatment terminated treatment prematurely at week 10 owing to personal issues. The patient had undetectable HCV-RNA levels at week 4 and 8 but recurrent viremia was detected at week 12. Finally, one patient had viral relapse following completed treatment (Fig. 2).

We estimated the change in LSM in 48 (67%) GT1 patients with transient elastography performed at baseline, as well as at 12–24 weeks after EOT. The baseline and post-treatment LSM were 11.8 kPa [interquartile range (IQR): 8.8–14.5] and 6.8 kPa (IQR: 4.95–9.5), with a statistically significant change of – 3.2 kPa (IQR: – 6.5 to – 1.3), *P* less than 0.001. No statistically significant difference in LSM change was seen between treatment groups ($P=0.188$).

Table 1. Baseline characteristics of the 72 chronic hepatitis C virus, genotype 1-infected patients included in the study

	PAR/OMB/rit/DAS + RBV (*N* = 38) [*n* (%)]	LVD/SOF + RBV (*N* = 34) [*n* (%)]	*P* value*
Age at inclusion (years)			
< 45	12 (32)	6 (18)	0.28
≥ 45	26 (68)	28 (82)	
Subtype			
1a	27 (71)	25 (74)	1.00
1b	10 (26)	9 (26)	
Unknown	1 (3)	0	
Ethnicity¥			
White	37 (97)	33 (97)	1.00
Non-White	1 (3)	1 (3)	
Sex			
Female	9 (24)	10 (29)	0.60
Male	29 (76)	24 (71)	
Route of infection			
IDU	25 (66)	20 (59)	0.34
Non-IDU	10 (26)	7 (20.5)	
Unknown	3 (8)	7 (20.5)	
Liver fibrosis			
Cirrhosis F4, ≥ 17 kPa, clinical diagnosed	13 (34)	13 (38)	0.07
Severe fibrosis F3/12–16.9 kPa	4 (11)	10 (30)	
Mild–moderate fibrosis F1–F2/ ≤ 11.9 kPa	21 (55)	11 (32)	
HIV status			
Negative	31 (82)	29 (85)	0.76
Positive	7 (18)	5 (15)	
Hepatitis B status			
Negative	38 (100)	34 (100)	
Previous treatment			
No	31 (82)	27 (79)	1.00
Yes	7 (18)	7 (21)	
Previous response to treatment			
Nonresponse¶	1 (3)	1 (3)	1.00
Relapse§	3 (8)	3 (9)	
Viral breakthrough¤	0	1 (3)	
Termination owing to adverse event	3 (8)	2 (6)	
Fatigue at baseline			
Grade 1	5 (13)	11 (32)	0.14
Grade 2	2 (5)	0	
BMI#	25.6 ± 4.27	25.7 ± 3.16	
HCV-RNA level (10^6 IU/ml)	2.35 ± 2.75	2.77 ± 3.39	
ALT level (U/l)			
Median	86.5	74.0	
Interquartile range	49.0–137.0	49.0–124.0	
Platelet count ($\times 10^9$/l)			
Median	206.0	187.5	
Interquartile range	161.0–237.0	126.0–227.0	
Serum albumin (g/l)			
Median	39.0	39.0	
Interquartile range	36.0–42.0	37.0–41.0	

Plus-minus values are means ± SD.
HCV, hepatitis C virus; PAR, paritaprevir; OMB, ombitasvir; rit, ritonavir; DAS, dasabuvir; LVD, ledipasvir; RBV, ribavirin; SOF, sofosbuvir; IDU, injecting drug use; ALT, alanine transaminase.
*Comparisons between treatment groups were done by Fisher's exact test.
¥Ethnicity was self-reported.
#The BMI is the weight in kilograms divided by the square of the height in meters.
¶Nonresponse to previous pegylated-interferon/ribavirin was defined as: patients received at least 12 weeks of pegylated-interferon/ribavirin for the treatment of HCV infection and did not have a reduction in the HCV-RNA level of at least 2 log10 IU/ml at week 12, or they received at least 4 weeks of pegylated-interferon/ribavirin for the treatment of HCV infection and had a reduction in the HCV-RNA level of less than 1 log10 IU/ml at week 4 [31].
§Relapse to previous pegylated-interferon/ribavirin was defined as follows: patients received at least 24 weeks of pegylated-interferon/ribavirin for the treatment of HCV infection and had an undetectable level of HCV-RNA at the end of treatment or thereafter but a detectable level within 24 weeks after treatment [31].
¤Viral breakthrough to previous pegylated-interferon/ribavirin was defined as follows: HCV-RNA levels initially decreases during treatment with pegylated-interferon/ribavirin (undetectable levels can be seen), followed by a clinical relevant increase while on treatment [31].

Table 2. Current disease of the 72 patients with chronic hepatitis C genotype 1 included in the study

	PAR/OMB/rit/DAS + RBV (*N* = 38) [*n* (%)]	LVD/SOF + RBV (*N* = 34) [*n* (%)]	*P* value[a]
Current disease			
Yes	27 (71)	28 (82)	0.28
No	11 (29)	6 (18)	
Hypertension			
Yes	3 (11)	9 (32)	0.10
No	24 (89)	19 (68)	
Diabetes type I and II			
Yes	0	3 (11)	0.24
No	27 (100)	25 (89)	
Psychiatric disease			
Yes	6 (22)	4 (14)	0.50
No	21 (78)	24 (86)	
Arthritis			
Yes	11 (41)	7 (25)	0.26
No	16 (59)	21 (75)	
Hemophilia			
Yes	2 (7)	0	0.24
No	25 (93)	28 (100)	
Gastrointestinal disease			
Yes	7 (26)	8 (29)	1.00
No	20 (74)	20 (71)	
Urological disease			
Yes	1 (4)	1 (4)	1.00
No	26 (96)	27 (96)	
Lung disease			
Yes	6 (22)	6 (21)	1.00
No	21 (78)	22 (79)	
Vasculitis			
Yes	0	2 (7)	0.49
No	27 (100)	26 (93)	
Skin disease			
Yes	5 (19)	3 (11)	0.47
No	22 (81)	25 (89)	
Thyroid disease			
Yes	0	1 (7)	1.00
No	27 (100)	26 (93)	
Edema peripheral			
Yes	1 (4)	2 (7)	1.00
No	26 (96)	27 (93)	

[a]Comparisons between treatment groups were made using Fisher's exact test.
DAS, dasabuvir; LVD, ledipasvir; OMB, ombitasvir; rit, ritonavir; PAR, paritaprevir; SOF, sofosbuvir.

Treatment failure

Late viral relapse occurred in a 62-year-old white male with GT3a, treated with SOF/RBV for 24 weeks. The patient was treatment naive and had a LSM greater than 17 kPa, indicating liver cirrhosis; no co-infection with HIV and hepatitis B virus was confirmed. Baseline laboratory parameters revealed an elevated alanine transaminase (ALT) at 186 U/l, platelet count of 128×10^9/l and a HCV-RNA load of 773×10^3 IU/ml. HCV-RNA declined to undetectable levels at week 8 during treatment and remained undetectable in all blood samples through week 4 and 12 EOT, but was detectable again (104 IU/ml) at week 24 EOT. The viral load rapidly increased to 115×10^3 IU/ml, 4 weeks after viral relapse was confirmed. No risk behavior in relation to reinfection was observed. Phylogenetic analysis (Supplemental Fig. S1, Supplemental digital content 3, *http://links.lww.com/EJGH/A313*) revealed minimal genetic changes between the baseline and post-treatment sample, which strongly suggest that recurrent viremia had occurred with the original viral strain. No RASs against SOF was detected in the NS5B region at baseline, but the 159F substitution, conferring reduced susceptibility to SOF [32], was detected at the time of treatment

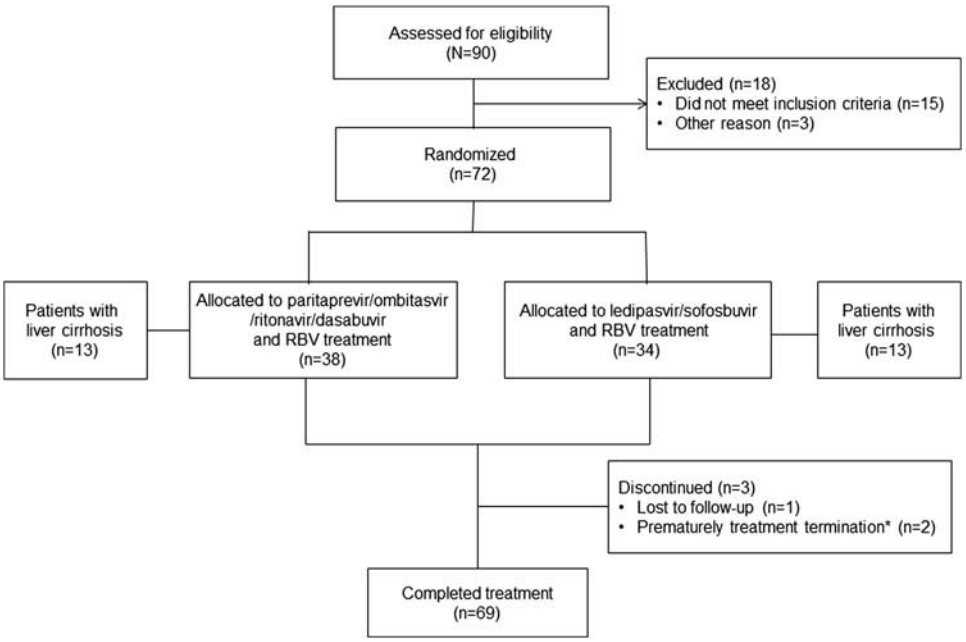

Fig. 1. Flow chart of the 72 patients with chronic hepatitis C genotype 1 included in the study. *Treatment was withdrawn after 17 days in one patient owing to liver failure, and the patient died at week 4. One patient, who terminated treatment at week 9, achieved sustained virologic response. RBV, ribavirin.

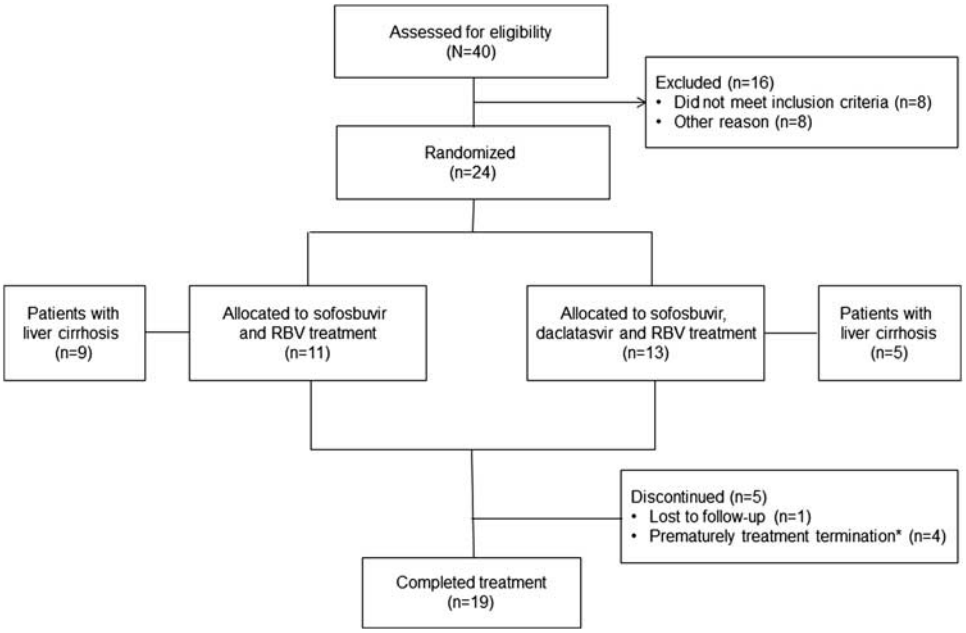

Fig. 2. Flow chart of the 24 patients with chronic hepatitis C genotype 3 included in the study. *One patient terminated treatment at week 10 and experienced recurrent viremia at week 12. Three patients discontinued treatment at week 16 owing to adverse events, of whom two patients achieved sustained virologic response, whereas one patient was lost to follow-up. RBV, ribavirin.

failure in 100% of the quasispecies. In the NS5A region, the 30E substitution was detected, knowing to confer resistance against the NS5A inhibitors DAC, LVD and OMB in GT1a and 4a patients at baseline and after treatment in 100% of the quasispecies.

Adverse events/safety

AEs occurred in 97% ($n = 70/72$) of all patients in both treatment groups, with no statistically significant difference between treatment groups (Table 3). The most common AEs in both treatment groups were anemia ($n = 56/72$, 78%), fatigue ($n = 53/72$, 74%), headache ($n = 33/72$, 46%), pruritus/eczema ($n = 33/72$, 46%), and heartburn/abdominal discomfort ($n = 27/72$, 38%). Most AEs occurred at severity grade 1, whereas for fatigue, 36 (68%) of 53 patients had grade 1, 14 (26%) had grade 2, two (4%) had grade 3, and one (2%) had grade 4 on the common terminology criteria for AEs scale.

AEs only lead to treatment discontinuation in two (3%) of 72 patients with GT1. One patient treated with

Table 3. Adverse events and laboratory abnormalities for 72 patients with chronic hepatitis C genotype 1 included in the study

Variables	PAR/OMB/rit/DAS + RBV ($n = 38$) [n (%)]	LVD/SOF + RBV ($n = 34$) [n (%)]	Total ($N = 72$)	P value[a]
Any adverse event	37 (97)	33 (97)	70 (97)	1.00
Adverse event leading to				
Treatment discontinuation	1 (2.6)	1 (3)	2 (3)	
Serious adverse event	3 (8)	6 (18)	9 (12.5)	0.29
Death	1 (2.6)	0	1 (1.4)	
Change in ribavirin dose treatment week 1–12	4 (11)	8 (24)	12 (17)	0.21
Common adverse events				
Anemia[b]	29 (76)	27 (79)	56 (78)	0.78
Fatigue	27 (71)	26 (77)	53 (74)	0.79
Headache	19 (50)	14 (41)	33 (46)	0.49
Pruritus, dry skin or eczema	20 (53)	13 (38)	33 (46)	0.25
Heartburn/abdominal pain/abdominal distention	16 (42)	11 (32)	27 (38)	0.47
Nausea/vomiting	14 (37)	11 (32)	25 (35)	0.81
Upper respiratory infection	13 (34)	11 (32)	24 (33)	1.00
Asthenia/malaise/tremor	13 (34)	9 (26)	22 (31)	0.61
Dyspnea	11 (29)	10 (29)	21 (29)	1.00
Irritability/mood swings/depression	8 (21)	12 (35)	20 (28)	0.20
Insomnia	9 (24)	8 (24)	17 (24)	1.00
Decreased appetite	11 (29)	4 (12)	15 (21)	0.09
Diarrhea	8 (21)	6 (18)	14 (19)	0.77
Dizziness	5 (13)	3 (9)	8 (11)	0.71
Arthralgia	3 (8)	4 (12)	7 (10)	0.70
Muscle spams	3 (8)	2 (6)	5 (7)	1.00
Memory impairment/absent minded	1 (3)	3 (9)	4 (6)	0.34
Affected vision	3 (8)	1 (3)	4 (6)	0.62
Increased appetite	1 (3)	3 (4)	4 (6)	0.34
Tinnitus	3 (8)	0	3 (4)	0.24
Herpes outbreak	2 (5)	1 (3)	3 (4)	1.00
Constipation	2 (5)	1 (3)	3 (4)	1.00
Chest pain	1 (3)	1 (3)	2 (3)	1.00
Fungal infection (mouth or vaginal)	2 (5)	0	2 (3)	0.49
Laboratory abnormalities[c]				
Alanine transaminase, grade 2 or 3	0	0	0	
Alkaline phosphatase, grade 2 or 3	1 (2.6)	0	1 (1.4)	1.0
Total bilirubin, grade 2 or 3	1 (2.6)	0	1 (1.4)	1.0
Hemoglobin				
Grade 1	29 (76)	23 (68)	52 (72)	0.047
Grade 2	0	4 (12)	4 (6)	
Grade 3	0	0	0	

For alanine transaminase, a level of grade 2 was defined as a value that was 5–10 times the upper limit of normal range, and grade 3 was defined as a value that was more than 10 times the upper limit of normal. For alkaline phosphatase, a level of grade 2 was defined as a value 2–4 times the upper limit of the normal range, and grade 3 was defined as more than four times the upper limit of the normal range. A total bilirubin level of grade 2 was defined as 3–10 times the upper limit of the normal range, and grade 3 as a value that was more than 10 times the upper limit of the normal range.
For hemoglobin, a level of grade 1 was defined as 6 mmol/l to less than the lower limit of the normal range, grade 2 as 5.0–5.9 mmol/l, and grade 3 as less than 5.0 mmol/l.
DAS, dasabuvir; LVD, ledipasvir; OMB, ombitasvir; PAR, paritaprevir; rit, ritonavir; SOF, sofosbuvir.
[a]Comparisons between treatment groups were made using Fisher's exact test.
[b]Anemia was defined as hemoglobin levels below the lower limit of normal range (male: 8.3 mmol/l, female: 7.3 mmol/l).
[c]The abnormalities here reflect postbaseline laboratory values, regardless of baseline values.

LVD/SOF + RBV had severe dizziness, dyspnea and impaired memory and a grade 1 level decline in hemoglobin observed at treatment week 3 and 4. Despite reduction in RBV and normalization of hemoglobin levels at week 8, the patient terminated treatment at week 9. The other patient experienced a suspected unexpected serious adverse reaction, during treatment with PAR/OMB/rit/DAS + RBV, and discontinued treatment owing to progressive liver failure. A total of 29 (76%) of 38 patients treated with PAR/OMB/rit/DAS + RBV and 23 (68%) of 34 patients treated with LVD/SOF + RBV experienced a grade 1 decline in hemoglobin levels, whereas a grade 2 decline in hemoglobin level was seen in four (12%) of 34 patients treated with LVD/SOF + RBV ($P = 0.047$). Anemia occurred in 83% ($n = 24/29$) and 82% ($n = 22/27$) of patients treated with PAR/OMB/rit/DAS + RBV and LVD/SOF + RBV before week 4 of treatment and in the remaining 17% ($n = 5/29$) and 19% ($n = 5/27$) between treatment week 4 and 8, respectively. Dose reduction of RBV occurred in 12 (17%) patients owing to anemia ($n = 8$) or AEs ($n = 4$). We found no statistically significant difference between patients with and without anemia and common AEs (Supplementary Table S2, Supplemental digital content 4, *http://links.lww.com/EJGH/A314*). All 26 patients with cirrhosis experienced AEs during treatment, but no statistically significant differences were found in the rate of AEs between cirrhotic and noncirrhotic patients ($P = 0.53$), neither was any relation between cirrhosis and anemia ($P = 0.56$) or reduction of RBV dosage ($P = 0.75$) seen (Supplementary Table S3, Supplemental digital content 5, *http://links.lww.com/EJGH/A315*).

AEs possibly related to the DAA regimen were still present in 45% ($n = 30/72$) and 11% ($n = 7/72$) of patients available for follow-up at week 4 and 12 EOT, respectively (Supplementary Table S4, Supplemental digital content 6, *http://links.lww.com/EJGH/A316*), with no difference between treatment groups ($P = 1.0$ and 0.69). The most common AEs (multiple AEs were possible for each patient) at week 12 EOT were nausea/vomiting ($n = 4$), fatigue ($n = 3$), and heartburn/abdominal discomfort ($n = 2$).

We observed no statistically significant difference in median changes in ALT, albumin, platelets and hemoglobin from baseline to treatment week 12 between treatment groups (Supplementary Table S5, Supplemental digital content 7, *http://links.lww.com/EJGH/A317*). Statistically significant decrease in ALT and hemoglobin levels and increase in platelet count were seen in both treatment groups whereas an increase in albumin only occurred in patients treated with PAR/OMB/rit/DAS + RBV.

Severe adverse events and suspected unexpected serious adverse reaction

Severe AEs occurred in six patients treated with LVD/SOF + RBV and in three patients receiving PAR/OMB/rit/DAS + RBV during the study period (Supplementary Table S6, Supplemental digital content 8, *http://links.lww.com/EJGH/A318*). The severity of the SAEs ranked from mild to severe, and the DAA regimen was, due to SAEs, discontinued temporarily in three patients. All nine patients completed the treatment course and eight patients achieved SVR12, whereas one patient was lost to follow-up. The investigators assessed that there was a possible relation to the study drugs (most likely RBV) in four of the nine patients.

A suspected unexpected serious adverse reaction occurred in a 66-year-old treatment-naive woman with compensated liver cirrhosis (Child–Pugh A, MELD score 8, no ascites on abdominal ultrasound, and 2–3 grade 1 esophageal varices), chronic obstructive pulmonary disease and previous alcohol use, treated with PAR/OMB/rit/DAS + RBV. The patient developed clinical jaundice 7 days after treatment initiation, and blood samples revealed a minor decline in hemoglobin from 7.5 to 7.2 mmol/l, normal ALT (24 U/l), rise in total bilirubin from 15 to 157 μmol/l and rise in international normalized ratio from 1.2 to 1.3. Ultrasound of the abdomen showed small amounts of ascites, no focal pathology and dilation of the common bile duct. RBV was discontinued on day 8 and the patient was, due to nausea, malaise and continuous rise in bilirubin, admitted to hospital on day 11. The patient experienced a grade 2 and 3 elevation in total bilirubin and alkaline phosphatase levels, which did not occur in other patients in this study, and further diagnostics, including combined MR cholangiopancreatography and MR scan of the liver, were performed to rule out intrahepatic and extrahepatic bile obstruction. Treatment with PAR/OMB/rit/DAS was discontinued on day 17 (HCV-RNA titer 19 IU/ml) owing to further rise in total bilirubin to greater than 400 μmol/l and signs of hepatic encephalopathy and acute liver failure. The patient was transferred to a tertiary hepatology unit, but died on day 24 owing to liver failure. Autopsy of the patient revealed acute cholestatic hepatitis.

Discussion

This real-life study is to the best of our knowledge the first to examine the rate of AEs in patients with HCV GT1 infection, randomized to one of two different DAA regimens in a real-world setting. We found no statistically significant difference in AEs, neither between treatment groups nor in relation to anemia or cirrhosis. The overall rate of AEs was comparable to other real-world studies, but the frequency of the most common AEs, such as anemia (78%), fatigue (74%), headache (74%), pruritus/eczema (46%), and heartburn/abdominal discomfort (38%), was higher in our study [33,34]. The negative effect of RBV on physical parameters is well-known, and the frequency of AEs in studies, where DAA treatment was administered without RBV, has also been lower compared with studies where treatment was given in combination with RBV [34,35]. However, we could not detect any difference in frequency of AEs between patients with and without anemia, and neither was anemia more frequent in patients with cirrhosis, as seen in a previous real-world study [36].

Surprisingly, we found that 45 and 11% of the patients still had AEs possibly related to the DAA regimen at 4 and 12 week EOT, respectively. We consider this an important finding that can be used when informing the patient about AEs that might be caused by the DAA regimen, despite the number of patients included in our study being relatively small.

The overall cure rate was 97% for patients with HCV GT1, similar to the SVR12 found in other studies presenting real-world data [33,36]. The SVR12 for GT3 patients was 83% which is also similar to what has been found in clinical trials and real-world studies with SOF/RBV treatment and treatment with SOF/DAC ± RBV in patients with cirrhosis [37–40], but lower than the SVR12 rate seen after SOF/DAC ± RBV treatment in noncirrhotic patients [38]. However, we cannot exclude that the SVR12 rate might be higher, as two (8%) patients were lost to follow-up. In this study, two (3%) patients with GT1 discontinued treatment owing to AEs, which is slightly higher than what has been seen in clinical trials [41], but similar to other real-world data [33,36]. Three (13%) GT3 patients discontinued treatment owing to AEs, which was substantially higher than what has been seen in a clinical trial [37] also including patients with previous treatment failure and liver cirrhosis. This probably reflects that the real world of patients has a greater array of medical comorbidities and prior treatment exposures that can influence the discontinuation rate.

Severe AEs occurred in six (18%) patients treated with LVD/SOF + RBV compared with three (8%) patients treated with PAR/OMB/rit/DAS + RBV. This difference was not statistically significant in relation to treatment group, anemia or cirrhosis. Including all SAEs, we found that our incidence was higher than what had been seen in clinical trials and real-world studies [24,36,42], but if nonrelated SAEs were excluded, our incidence was comparable to other real-world studies [33,34].

We found that cure was accompanied by a significant decrease in LSM in 48 patients with HCV GT1 infection, consistent with the findings of previous studies [43–45].

We found an expected median decrease in ALT and a median increase in albumin level and platelet count, suggesting an improvement in liver function after DAA treatment. The median change from baseline to treatment week 12 was significant, but no statistically significant difference was found between treatment groups. A grade 2 level decline in hemoglobin only occurred in four (12%) patients treated with LVD/SOF + RBV and was successfully managed with modification of RBV dose. The difference in hemoglobin decline was statistically significant

between treatment groups, but as grade 2 level decline in hemoglobin has previously been seen for both treatment regimens in clinical trials [24,42,46], our finding might be owing to coincidence. Although all patients received RBV, known to cause hemolysis, a grade 2 or grade 3 level increase in total bilirubin only occurred in one patient treated with PAR/OMB/rit/DAS + RBV who died at treatment week 4 owing to acute liver failure. Based on 26 worldwide cases of hepatic decompensation and liver failure in patients with advanced cirrhosis treated with PAR/OMB/rit/DAS and PAR/OMB/rit/DAS plus RBV, the FDA changed the recommendations and discouraged the use of these regimens in patients with liver cirrhosis Child–Pugh B or C [47]. Our patient had Child–Pugh A at treatment initiation, but the finding of severe rise in total bilirubin level, coagulation disorder and mild elevation in ALT is consistent with an idiosyncratic pattern of drug-related acute liver failure [48], which was supported by the autopsy report stating that the acute hepatitis presumably was caused by the DAA treatment. Other similar cases have been reported in patients with Child–Pugh A [49,50], and our finding highlights the need for close monitoring of patients with liver cirrhosis who experience elevation in total bilirubin during DAA treatment, as early discontinuation is crucial in cases where the patient develops signs of acute liver failure.

A low rate (1%; $n = 1/96$) of virologic failure was observed in this study and only occurred in one patient with GT3 who experienced late viral relapse 24 weeks after treatment. Phylogenetic analysis revealed that no reinfection had occurred and that the original HCV had persisted in the liver or another compartment and reemerged despite that blood samples revealed undetectable levels of HCV-RNA since treatment week 8. Recently, only five patients, all treated with SOF/RBV for either 12 or 24 weeks, were shown to experience late viral relapse among 3004 patients treated with LVD/SOF or SOF/RBV, with or without PEG-INF, in 11 clinical phase III trials [51].

Our finding supports the prevalence of late recurrent viremia, after achievement of SVR, in patients treated with SOF-based treatment regimens to be low, but phylogenetic analysis of baseline and post-treatment sequences is needed to distinguish reinfection from viral relapse. Clinical trials have shown that most patients with CHC with treatment failure harbor RASs and that baseline RASs in the NS5A region might decrease the chance of SVR in patients with GT1a or 3, liver cirrhosis and previous treatment failure [52, 53]. Therefore, analysis for RASs post-treatment is valuable to optimize re-treatment options. In this case, the patient harbored a RAS in the NS5A region known to cause resistance against DAC, LVD and OMB in patients with GT1a and 4a and a substitution in the NS5B region causing reduced susceptibility SOF after treatment [32,52,54]. These findings should be considered when choosing a DAA regimen for re-treatment, hereby optimizing the chance of cure.

Owing to the high costs of DAA treatment, national treatment guidelines for CHC restricted treatment initiation to patients with moderate fibrosis to cirrhosis during the study period. This was a severe limitation to our study that meant that fewer patients than expected according to the power calculation were included. This could have influenced our statistical analyses, and we cannot exclude the possibility that our failure to demonstrate any difference in AEs between treatment groups could be owing to lack of statistical power. The included patients all received DAA treatment in combination with RBV, and it is likely that the rate of AEs might have been different without administration of RBV. A total of 26 (36%) patients with CHC with liver cirrhosis were included in the study, which was less than expected. National treatment guidelines had before the study period restricted DAA treatment to patients with severe fibrosis or cirrhosis, which meant that most patients with cirrhosis, who were most in need of cure, had already been treated.

In conclusion, we found no difference in frequency of AEs possibly related to the DAA regimen in relation to randomized treatment group or liver cirrhosis in patients with chronic HCV GT1 infection. The well-known adverse effect of anemia, in patients treated with RBV, and associated symptoms, such as fatigue, headache and dyspnea, were among the most frequent AEs in both treatment groups. However, we did not find any difference when comparing the rate of AEs between patients with and without anemia in the study. The low discontinuation rate reflects that AEs possibly induced by DAA treatment are rarely treatment limiting, even when DAA treatment is given in combination with RBV. We found that 45 and 11% of the patients still had AEs possibly related to the DAA regimen at 4 and 12 weeks after EOT, respectively. We believe that this finding can be of importance for clinicians in relation to patient information concerning AEs that might be caused by the DAA regimen.

Acknowledgements

The authors thank the patients and their families for participation in this study. Thanks to project nurses Lene K. Jensen, Ann Bach, Yordanos Yehdego, Bente Ramskover, Bitten Konradsen, Philippa Collins, Lene P. Jensen, Bente Baadegaard, and Anne A. Bayer for recording patient data and their dedication to the project. Thanks to statisticians Håkon Sandholt and Steen Ladelund and MD, PhD, Sofie Hallager and data manager Lars Smith-Hansen for statistical advice and help with data set-up.

This work was supported by a PhD stipend from Faculty of Health and Medical Sciences, University of Copenhagen (C.S.) and by Hvidovre Hospital Research Foundation (C.S.), the Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre (C.S.), the Capital Region of Denmark's Research Foundation (J.B.), the Innovation Fund Denmark project 060-2009-3 (J.B.), the Novo Nordisk Foundation (J.B.), and the Danish Research Council (J.B.).

Conflicts of interest

Nina Weis has received honorarium, paid to her institution, for being clinical investigator, member of advisory boards and lecturer for Abbvie, Bristol–Myers Squibb, and Merck, and member of advisory board and lecturer for Gilead; Alex L. Laursen has been member of advisory boards for Abbvie, Gilead, and Merck; Peer B. Christensen has received research grants from Abbvie, Gilead, and Merck; Jan Gerstoft has received honorarium, paid to his institution, for being clinical investigator, member of

advisory boards and lecturer, and developing educational material and received grants from Abbvie, BMS, Bohringer, Gilead, Janssen, Merck, Sanofi-Pasteur, and Viiv; Kristian Schønning has received speakers honorary and travel grants from Hologic and Roche and has served at advisory board for Abbott; and for the remaining authors there are no conflicts of interest.

References

1 Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastrotenterol Hepatol* 2017; 2:161–176.
2 Bukh J. The history of hepatitis C virus (HCV): basic research reveals unique features in phylogeny, evolution and the viral life cycle with new perspectives for epidemic control. *J Hepatol* 2016; 65:S2–S21.
3 Danmarks statistik. Befolkning. 2017. Available at: *www.dst.dk*. [Accessed 1 July 2017].
4 Christensen PB, Hay G, Jepsen P, Omland LH, Just SA, Krarup HB, *et al*. Hepatitis C prevalence in Denmark – an estimate based on multiple national registers. *BMC Infect Dis* 2012; 12:178.
5 Corbet S, Bukh J, Heinsen A, Fomsgaard A. Hepatitis C virus subtyping by a core-envelope 1-based reverse transcriptase PCR assay with sequencing and its use in determining subtype distribution among Danish patients. *J Clin Microbiol* 2003; 41:1091–1100.
6 Hallager S, Brehm CP, Ladelund S, Rye CM, Lund LA, Moller A, *et al*. Mortality rates in patients with chronic hepatitis C and cirrhosis compared with the general population: a Danish cohort study. *J Infect Dis* 2017; 215:192–201.
7 Lange CM, Sarrazin C, Zeuzem S. Review article: specifically targeted anti-viral therapy for hepatitis C – a new era in therapy. *Aliment Pharmacol Ther* 2010; 32:14–28.
8 Yau AH, Lee T, Ramji A, Ko HH. Rate, delay and predictors of hepatitis C treatment in British Columbia. *Can J Gastroenterol Hepatol* 2015; 29:315–320.
9 Solund C, Krarup H, Ramirez S, Thielsen P, Roge BT, Lunding S, *et al*. Nationwide experience of treatment with protease inhibitors in chronic hepatitis C patients in Denmark: identification of viral resistance mutations. *PLoS One* 2014; 9:e113034.
10 Naggie S, Muir AJ. Oral combination therapies for hepatitis C virus infection: successes, challenges, and unmet needs. *Annu Rev Med* 2017; 68:345–358.
11 Falade-Nwulia O, Suarez-Cuervo C, Nelson DR, Fried MW, Segal JB, Sulkowski MS. Oral direct-acting agent therapy for hepatitis C virus infection: a systematic review. *Ann Intern Med* 2017; 166:637–648.
12 Hansen N, Obel N, Christensen PB, Krarup H, Laursen AL, Clausen MR, *et al*. Predictors of antiviral treatment initiation in hepatitis C virus-infected patients: a Danish cohort study. *J Viral Hepat* 2009; 16:659–665.
13 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24:289–293.
14 Christiansen PB, Clausen MR, Krarup H, Laursen AL, Schlichting P, Weis N. Treatment of hepatitis B virus and hepatitis C virus infection. *Dan Med J* 2017; 59:C4465.
15 Christiansen KM, Mossner BK, Hansen JF, Jarnbjer EF, Pedersen C, Christensen PB. Liver stiffness measurement among patients with chronic hepatitis B and C: results from a 5-year prospective study. *PLoS One* 2014; 9:e111912.
16 Degos F, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, *et al*. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). *J Hepatol* 2010; 53:1013–1021.
17 Danish Society for Infectious Diseases, Gastroenterology and Hepatology. Treatment of hepatitis B and C infection. 2015. Available at: *www.infmed.dk*. [Accessed 6 November 2017].
18 National Institutes of Health (NCI). Common terminology criteria for adverse events (CTCAE). 2010 (online 16 October 2017).
19 Pas S, Molenkamp R, Schinkel J, Rebers S, Copra C, Seven-Deniz S, *et al*. Performance evaluation of the new Roche cobas AmpliPrep/cobas TaqMan HCV test, version 2.0, for detection and quantification of hepatitis C virus RNA. *J Clin Microbiol* 2013; 51:238–242.
20 Schonning K, Pedersen MS, Johansen K, Landt B, Nielsen LG, Weis N, *et al*. Analytical and clinical performance of the Hologic Aptima HCV Quant Dx Assay for the quantification of HCV RNA in plasma samples. *J Virol Methods* 2017; 248:159–165.
21 Zitzer H, Heilek G, Truchon K, Susser S, Vermehren J, Sizmann D, *et al*. Second-generation Cobas AmpliPrep/Cobas TaqMan HCV quantitative test for viral load monitoring: a novel dual-probe assay design. *J Clin Microbiol* 2013; 51:571–577.
22 Krarup HB, Drewes AM, Madsen PH. A quantitative HCV-PCR test for routine diagnostics. *Scand J Clin Lab Invest* 1998; 58:415–422.
23 Ferenci P, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, *et al*. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med* 2014; 370:1983–1992.
24 Poordad F, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, *et al*. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med* 2014; 370:1973–1982.
25 Gilead Sciences Inc. Sovaldi (sofosbuvir) prescribing information; 2013. Available at: *www.gilead.com*. [Accessed 21 November 2017].
26 Billerbeck E, Wolfisberg R, Fahnoe U, Xiao JW, Quirk C, Luna JM, *et al*. Mouse models of acute and chronic hepacivirus infection. *Science* 2017; 357:204–208.
27 Hunt M, Gall A, Ong SH, Brener J, Ferns B, Goulder P, *et al*. IVA: accurate de novo assembly of RNA virus genomes. *Bioinformatics* 2015; 31:2374–2376.
28 Wilm A, Aw PP, Bertrand D, Yeo GH, Ong SH, Wong CH, *et al*. LoFreq: a sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. *Nucleic Acids Res* 2012; 40:11189–11201.
29 Kalaghatgi P, Sikorski AM, Knops E, Rupp D, Sierra S, Heger E, *et al*. Geno2pheno[HCV] – a web-based interpretation system to support hepatitis C treatment decisions in the era of direct-acting antiviral agents. *PLoS One* 2016; 11:e0155869.
30 European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. *J Hepatol* 2017; 66:153–194.
31 Halfon P, Locarnini S, Hepatitis C. virus resistance to protease inhibitors. *J Hepatol* 2011; 55:192–206.
32 Svarovskaia ES, Gane E, Dvory-Sobol H, Martin R, Doehle B, Hedskog C, *et al*. L159F and V321A sofosbuvir-associated hepatitis C virus NS5B substitutions. *J Infect Dis* 2016; 213:1240–1247.
33 Terrault NA, Zeuzem S, Di Bisceglie AM, Lim JK, Pockros PJ, Frazier LM, *et al*. Effectiveness of ledipasvir-sofosbuvir combination in patients with hepatitis C virus infection and factors associated with sustained virologic response. *Gastroenterology* 2016; 151: 1131–1140.
34 Flisiak R, Janczewska E, Wawrzynowicz-Syczewska M, Jaroszewicz J, Zarebska-Michaluk D, Nazzal K, *et al*. Real-world effectiveness and safety of ombitasvir/paritaprevir/ritonavir +/− dasabuvir +/− ribavirin in hepatitis C: AMBER study. *Aliment Pharmacol Ther* 2016; 44:946–956.
35 Younossi ZM, Stepanova M, Marcellin P, Afdhal N, Kowdley KV, Zeuzem S, *et al*. Treatment with ledipasvir and sofosbuvir improves patient-reported outcomes: results from the ION-1, -2, and -3 clinical trials. *Hepatology* 2015; 61:1798–1808.
36 Pogorzelska J, Flisiak R. Real-world experience with ombitasvir/paritaprevir boosted with ritonavir and possibly combined with dasabuvir and ribavirin in HCV infection. *Clin Exp Hepatol* 2016; 2:34–37.
37 Zeuzem S, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, *et al*. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; 370:1993–2001.
38 Nelson DR, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, *et al*. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 2015; 61:1127–1135.
39 Wehmeyer MH, Ingiliz P, Christensen S, Hueppe D, Lutz T, Simon KG, *et al*. Real-world effectiveness of sofosbuvir-based treatment regimens for chronic hepatitis C genotype 3 infection: results from the multicenter German hepatitis C cohort (GECCO-03). *J Med Virol* 2017; 90: 304–312.
40 Hezode C, Lebray P, de Ledinghen V, Zoulim F, Di MV, Boyer N, *et al*. Daclatasvir plus sofosbuvir, with or without ribavirin, for hepatitis C virus genotype 3 in a French early access programme. *Liver Int* 2017; 37:1314–1324.
41 Deeks ED. Ombitasvir/paritaprevir/ritonavir plus dasabuvir: a review in chronic HCV genotype 1 infection. *Drugs* 2015; 75:1027–1038.
42 Lawitz E, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, *et al*. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; 383:515–523.

43 Hezode C, Castera L, Roudot-Thoraval F, Bouvier-Alias M, Rosa I, Roulot D, *et al*. Liver stiffness diminishes with antiviral response in chronic hepatitis C. *Aliment Pharmacol Ther* 2011; 34:656–663.

44 Facciorusso A, Del Prete V, Turco A, Buccino RV, Nacchiero MC, Muscatiello N. Long-term liver stiffness assessment in HCV patients undergoing antiviral therapy: results from a 5-year cohort study. *J Gastroenterol Hepatol* 2017; 33:942–949.

45 Chekuri S, Nickerson J, Bichoupan K, Sefcik R, Doobay K, Chang S, *et al*. Liver stiffness decreases rapidly in response to successful hepatitis C treatment and then plateaus. *PLoS One* 2016; 11:e0159413.

46 Feld JJ, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, *et al*. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med* 2014; 370:1594–1603.

47 US Food and Drug Administration. FDA drug safety communication: FDA warns of serious liver injury risk with hepatitis C treatments Viekira Pak and Technivie. 22 October 2015. Available at: *www.fda.gov*. [Accessed 6 November 2017].

48 Masetti M, Magalotti D, Martino E, Andreone P, Scuteri A, Zoli M. A case of acute liver failure during ritonavir-boosted paritaprevir, ombitasvir and dasabuvir therapy in a patient with HCV genotype 1b cirrhosis. *J Gastrointestin Liver Dis* 2016; 25:559–561.

49 Buzas C, Tantau M, Ciobanu L. Fatal acute liver failure during ritonavir-boosted paritaprevir, ombitasvir and dasabuvir plus ribavirin therapy. *J Gastrointestin Liver Dis* 2017; 26:93–94.

50 Fofiu C, Dobru D, Boeriu A. Potential pitfalls of Viekira Pak therapy in patients with HCV genotype 1b cirrhosis. *J Gastrointestin Liver Dis* 2017; 26:94–95.

51 Sarrazin C, Isakov V, Svarovskaia ES, Hedskog C, Martin R, Chodavarapu K, *et al*. Late relapse versus hepatitis C virus reinfection in patients with sustained virologic response after sofosbuvir-based therapies. *Clin Infect Dis* 2017; 64:44–52.

52 Pawlotsky JM. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. *Gastroenterology* 2016; 151:70–86.

53 Perez AB, Chueca N, Garcia F. Resistance testing for the treatment of chronic hepatitis C with direct acting antivirals: when and for how long? *Germs* 2017; 7:40–44.

54 Sarrazin C. The importance of resistance to direct antiviral drugs in HCV infection in clinical practice. *J Hepatol* 2016; 64:486–504.