Open Forum Infectious Diseases

MAJOR ARTICLE

Direct-Acting Antiviral Treatment for Hepatitis C Genotypes Uncommon in High-Income Countries: A Dutch Nationwide Cohort Study

Cas J. Isfordink,[1,2] Thijs J. W. van de Laar,[3,4] Sjoerd P. H. Rebers,[5] Els Wessels,[6] Richard Molenkamp,[7] Marjolein Knoester,[8] Bert C. Baak,[9] Cees van Nieuwkoop,[10] Bart van Hoek,[11] Sylvia M. Brakenhoff,[12] Hans Blokzijl,[13] Joop E. Arends,[14] Marc van der Valk,[1] Janke Schinkel[5], and the HepNed Study Group

[1]Department of Infectious Diseases, Amsterdam Infection & Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands, [2]Department of Gastroenterology and Hepatology, UMC Utrecht, Utrecht, the Netherlands, [3]Department of Donor Medicine Research, Laboratory of Blood-borne infections, Sanquin Research, Sanquin Diagnostic Services, Amsterdam, the Netherlands, [4]Laboratory of Medical Microbiology, Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands, [5]Department of Medical Microbiology, Section of Clinical Virology, Amsterdam Infection & Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands, [6]Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands, [7]Department of Viroscience, Erasmus MC, University Medical Center, Rotterdam, the Netherlands, [8]Department of Medical Microbiology and Infection Control, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands, [9]Department of Gastroenterology and Hepatology, Onze Lieve Vrouwe Gasthuis locatie Oost, Amsterdam, the Netherlands, [10]Department of Internal Medicine, Haga Teaching Hospital, The Hague, the Netherlands, [11]Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, the Netherlands, [12]Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands, [13]Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands, and [14]Department of Internal Medicine and Infectious Diseases, UMC Utrecht, Utrecht, the Netherlands

Background. The majority of hepatitis C virus (HCV) infections are found in low- and middle-income countries, which harbor many region-specific HCV subtypes. Nevertheless, direct-acting antiviral (DAA) trials have almost exclusively been conducted in high-income countries, where mainly epidemically spread HCV subtypes are present. Recently, several studies have demonstrated suboptimal DAA efficacy for certain nonepidemic subtypes, which could hamper global HCV elimination. Therefore, we aimed to evaluate DAA efficacy in patients treated for a nonepidemic HCV genotype infection in the Netherlands.

Methods. We performed a nationwide retrospective study including patients treated with interferon-free DAAs for an HCV genotype other than 1a/1b/2a/2b/3a/4a/4d. The genotype was determined by NS5B region phylogenetic analysis. The primary end point was SVR-12. If stored samples were available, NS5A and NS5B sequences were obtained for resistance-associated substitutions (RAS) evaluation.

Results. We included 160 patients, mainly infected with nonepidemic genotype 2 (41%) and 4 (31%) subtypes. Most patients were from Africa (45%) or South America (24%); 51 (32%) were cirrhotic. SVR-12 was achieved in 92% (140/152) of patients with available SVR-12 data. Only 73% (8/11) genotype 3–infected patients achieved SVR-12, the majority being genotype 3b patients with 63% (5/8) SVR. Regardless of SVR, all genotype 3b patients had 30K and 31M RAS.

Conclusions. The DAA efficacy we observed in most nonepidemic genotypes in the Netherlands seems reassuring. However, the low SVR-12 rate in subtype 3b infections is alarming, especially as it is common in several HCV-endemic countries. Alongside earlier results, our results indicate that a remaining challenge for global HCV elimination is confirming and monitoring DAA efficacy in nonepidemic genotypes.

Keywords. Africa; Asia; elimination; global health; unusual subtypes.

Hepatitis C virus (HCV) infection is a global health problem, with an estimated worldwide prevalence of 71 million [1]. The virus is classified into 8 major genotypes, which are further subdivided into >67 subtypes [2]. The highest genetic diversity is observed in Sub-Saharan Africa and Asia, due to low transmission rates and centuries-long persistence in the human population [3]. In high-income countries, the majority of HCV infections are caused by a limited number of HCV subtypes that in recent centuries have rapidly spread via effective modes of transmission such as contaminated blood products, intravenous drug use, and unhygienic invasive medical procedures. In the Netherlands, these so-called epidemic subtypes, exemplified by subtypes 1a/1b/2a/2b/3a/4a/4d, account for ~90% of HCV infections, although precise data are lacking [4].

As most direct-acting antiviral (DAA) trials have been executed in high-income countries, only rarely were patients with nonepidemic HCV genotypes included [5]. This lack of nonepidemic genotypes is also seen in online HCV sequence databases, in which genomic data from low- and middle-income

Received 3 December 2020; editorial decision 29 December 2020; accepted 4 January 2021.

Correspondence: Cas Isfordink, MD, Department of Infectious Diseases, Amsterdam Infection & Immunity Institute, Amsterdam UMC, Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands (c.j.isfordink@amsterdamumc.nl).

Open Forum Infectious Diseases®2021

DOI: 10.1093/ofid/ofab006

countries (LMICs) are virtually absent [6]. Countries where these nonepidemic genotypes are endemic are among the countries with the highest HCV prevalence in the world [1]. Therefore, confirming the effectiveness of currently available DAAs in these genotypes is of utmost importance for worldwide HCV elimination.

Two of the first DAA trials ever executed in LMICs give reason to dispute the assumption that DAAs are as effective against nonepidemic HCV genotypes as they are against epidemic genotypes [7, 8]. A study from Rwanda, including mainly endemic genotype 4 infections, showed a relatively low sustained virological response (SVR) rate of 87% with sofosbuvir (SOF)/ledipasvir (LDV). This was mainly driven by a remarkably low SVR rate of 56% in 48 genotype 4r patients [7]. In a study from Asia, patients were treated with SOF/velpatasvir (VEL), resulting in a 95% SVR rate for the epidemic genotype 3a vs only 76% for the nonepidemic genotype 3b, despite similar baseline characteristics [8].

Additionally, real-life data suggest a decreased DAA efficacy in certain nonepidemic HCV genotypes, as shown for genotype 6 in an Asian cohort of 85 patients treated with SOF/LDV with an SVR rate of 74% and nonepidemic genotype 1 strains in African patients treated in London with a low SVR rate of 75% [9, 10]. Furthermore, in an analysis from France of 537 patients who failed DAA treatment, almost 10% harbored a rare nonepidemic genotype 1 strain and 5% genotype 4r, despite a low prevalence of these subtypes in the French population [11]. An explanation for the possible decreased efficacy of DAAs could be that wild-type nonepidemic strains frequently contain amino acids associated with intrinsic resistance to DAAs, in particular in the NS5A region [12–15].

The recently updated European Association for the Study of the Liver (EASL) HCV treatment guideline acknowledges the lack of DAA treatment data for patients infected with subtypes inherently resistant to NS5A inhibitors and mentions an urgent need for further data [16]. A suboptimal DAA efficacy in certain HCV subtypes will hamper global elimination of HCV. So far, no real-world data have been published including a nationwide cohort consisting solely of patients with nonepidemic HCV genotypes. Therefore, the aim of this study was to investigate the real-world efficacy of DAA treatment in patients with HCV genotypes other than 1a, 1b, 2a, 2b, 3a, 4a, and 4d in the Netherlands, in relation to baseline NS5A resistance–associated substitutions (RAS).

METHODS

Study Design and Population

This nationwide cohort study included patients infected with a nonepidemic HCV genotype treated with an interferon-free DAA regimen. Nonepidemic HCV genotypes were defined as genotypes and subtypes other than 1a/1b/2a/2b/3a/4a/4d. All laboratories performing HCV genotyping in the Netherlands were approached. All but 1 participated in the study: the Amsterdam University Medical Centers; Sanquin Diagnostics, Amsterdam; UMC Groningen, Groningen; LUMC, Leiden; Erasmus Medical Center, Rotterdam; and Maastricht UMC, Maastricht.

HCV Genotyping

HCV genotype was determined by sequencing and phylogenetic analysis of the NS5B region using a method and primers previously described by Murphy et al. [17]. Patients who were diagnosed with a nonepidemic subtype using a commercial assay (eg, LIPA) or based on sequencing of the highly conservative 5'UTR region were only included if the presence of a nonepidemic subtype was confirmed by NS5B sequencing of a previously stored sample. Genotype sequences were submitted to GenBank (MW205243–MW205375).

Software packages CodonCode Aligner (version 8.0.2; CodonCode Corp., Centerville, Massachusetts, USA) and ClustalX (version 2.1) [18] were used to edit and subsequently align obtained sequences against a reference set retrieved from the Los Alamos HCV sequence database [19]. Based on these alignments, genotype and subtype were determined by constructing a maximum-likelihood phylogenetic tree created in MEGA (version 6) [20]. If no subtype could be assigned using phylogenetic analysis, we used the HCV Blast tool [19] to find related sequences. A >90% match with a well-typed database sequence was considered sufficient to assign a subtype. If not, the subtype was labeled as unassigned and the closest related BLAST sequence was reported.

Data Collection

Eligible patients were selected using a database search in the laboratory information system by the local medical (molecular) microbiologist. Subsequently, the treating physician was approached to provide clinical data. Finally, both virological and clinical data were supplied anonymized to the research coordinator. Demographic variables (age, gender, country of origin), clinical variables (comorbidities, pretreatment grade of liver fibrosis as assessed by Fibroscan, HCV treatment history, and treatment outcome), and virological variables (genotype, baseline, and post-treatment RAS data if available) were collected. Patients were labeled cirrhotic if reported as such by their treating physician or if a liver stiffness measurement >12.5 kPa was reported.

Patient Consent Statement

All data were supplied anonymized to the research coordinator by the respective treating physician. According to European privacy legislations and the Dutch Code of Conduct for the Use of Data in Health Research, the need for informed consent was therefore waived. The study was approved by the Medical Ethics Committee of the Amsterdam Medical Center, the Netherlands.

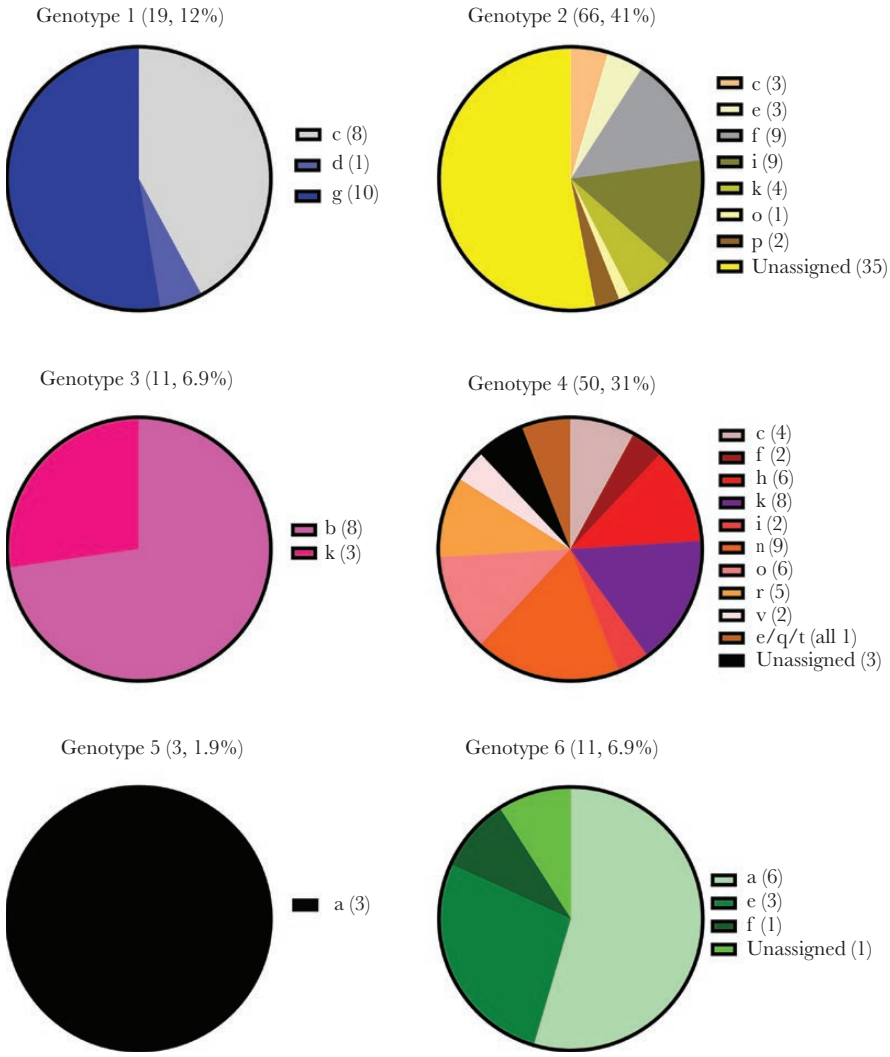


Figure 1. Overview of the included genotypes and subtypes.

RAS Analysis

For RAS analysis, a fragment of the NS5A and NS5B region was sequenced if stored plasma or serum was available. The sequenced fragment length was dependent on the specific primer set used but was minimally stretched from amino acids 23–129 for NS5A and 150–321 for NS5B sequences. It is debatable whether resistance-associated amino acid sequences that are wild-type for a specific subtype can be labeled RAS, as they are not necessarily substitutions. However, both in literature and clinical practice, these are often labeled as such. Therefore, we chose to define RAS as an amino acid substitution relative to the H77 genotype 1a reference sequence at a position associated with resistance, regardless of whether this amino acid was wild-type for the specific subtype. Positions associated with resistance were extracted from the Geno2pheno HCV database and the EASL guideline [21, 22]. RAS sequences were submitted to GenBank (MW205376–MW205507).

Outcome

The primary outcome was the SVR-12 rate for the first interferon-free DAA treatment in all patients for whom the SVR-12 result was available. SVR-12 was defined as an undetectable level of HCV RNA 12 weeks after completion of DAA treatment. For subanalyses, we calculated the SVR-12 rate per genotype, cirrhotic vs noncirrhotic, per region of origin (according to the standard area codes of the United Nations statistics division), for DAA regimens with and without an NS5A inhibitor, and for pangenotypic second-generation DAAs (SOF/VEL and glecaprevir [GLE]/pibrentasvir [PIB]) vs older NS5A inhibitor–containing DAA regimens.

Statistical Analysis

Data were analyzed using IBM SPSS statistics (version 25.0; IBM Corp., Armonk, NY, USA). Descriptive data are reported as either percentages, mean (SD), or median (interquartile range).

Table 1. Patient and Treatment Characteristics

Patient Characteristics	n = 160	Treatment Characteristics	n = 160
Female gender	62 (39)	PEG-IFN treatment experience[a]	32 (20)
Age, median (IQR)	56 (49–64)	DAA regimen	
Hiv co-infection	5 (3)	Sofosbuvir/ledipasvir	37 (23)
Cirrhosis	51 (32)	Sofosbuvir/velpatasvir	31 (19)
Child-Pugh A/B/C	43 / 7 / 1	Sofosbuvir/daclatasvir	30 (19)
Region of origin		Glecaprevir/pibrentasvir	18 (11)
Northern Africa	40 (25)	Sofosbuvir/simeprevir	18 (11)
South America	39 (24)	Sofosbuvir + ribavirin	14 (9)
Middle Africa	16 (10)	Elbasvir/grazoprevir	6 (4)
Western Europe	16 (10)	Ombitasvir/paritaprevir/ritonavir	4 (3)
Eastern Africa	11 (7)	Ombitasvir/paritaprevir/ritonavir/dasabuvir	1 (1)
Southeastern Asia	11 (7)	Sofosbuvir/glecaprevir/pibrentasvir	1 (1)
Eastern Asia	6 (4)	NS5A inhibitor-containing regimen	128 (80)
Southern Asia	6 (4)	Ribavirin added to DAA regimen	28 (18)
Western Africa	5 (3)		
Western Asia	4 (3)		
Southern Europe	3 (2)		
Unknown	3 (2)		

Data are number (%) unless otherwise noted.

Abbreviations: DAA, direct-acting antiviral; IQR, interquartile range; NS5A, nonstructural protein 5a; PEG-IFN, pegylated interferon.

[a]Three patients were treated unsuccessfully with PEG-IFN + DAA.

RESULTS

Nonepidemic Genotypes

We included 160 patients treated with an interferon-free DAA regime for a nonepidemic HCV genotype. Three patients were treated in a trial setting in 2012 or 2013, whereas the remaining 157 patients were treated between 2015 and 2019. Phylogenetic analysis revealed 28 different HCV subtypes in 121 patients, predominantly of subtypes 2 and 4 (Figure 1). In the remaining 39 patients, neither phylogenetic analysis nor BLAST search was able to assign a recognized HCV subtype. Twenty-five out of these 39 belonged to 1 of 5 previously described but officially unassigned genotype 2 clades originating from Suriname [23]. For the remaining 14 unassigned subtypes, BLAST results showing the closest related NS5B sequence with an assigned subtype are shown in Supplementary File 2.

Baseline

Fifty-one (32%) of the included patients had liver cirrhosis, the vast majority Child-Pugh A (84%) (Table 1). Most patients originated in Northern Africa (25%), South America (24%), or Sub-Saharan Africa (20%). At country level, most common origins were Suriname (23%), Egypt (18%), the Netherlands (10%), Democratic Republic Congo (7%), and Morocco (6%). Fifty individuals (31%) were treated with a pangenotypic second-generation DAA regime, 78 (49%) patients received a nonpangenotypic regime containing an NS5A inhibitor, and 32 (20%) patients were treated without an NS5A inhibitor. The latter were either genotype 2 infections treated with SOF + ribavirin (n = 14, 9%) or patients treated with SOF/simeprevir (SIM; n = 18, 11%).

Treatment Results

SVR-12 data were available for 152 (95%) patients, of whom 140 (92%) achieved SVR-12. The 8 patients without available SVR-12 results were either awaiting SVR-12 measurement at the time of data collection (n = 5), were lost to follow-up (n = 2), or died before SVR-12 measurement (n = 1). Treatment results per genotype and subtype are shown in Table 2 (further stratification per DAA regime is available in Supplementary File 3). Nonepidemic genotype 3 infections showed the lowest SVR-12 rate, with 73% (8/11) being cured at the first treatment attempt. All 3 failures were genotype 3b infections, of whom 1 was cirrhotic. The SVR-12 rate in genotype 3b patients was 63% (5/8). Notably, for 3 of the 8 successfully treated genotype 3 infections, the intended treatment regime was optimized after baseline RAS analysis. One genotype 3b–infected patient was treated successfully with GLE/PIB/SOF as first-line treatment, another genotype 3b–infected patient was treated with GLE/PIB + ribavirin instead of the intended SOF/VEL, and a genotype 3k–infected, noncirrhotic patient had ribavirin added to 12 weeks of SOF/DAC. The SVR rate of genotype 3b patients with ribavirin added to their DAA regimen was 75% (3/4, all cirrhotic), compared with 50% without ribavirin (2/4, 1 cirrhotic), Besides genotype 3b, genotype 4n infections also showed a low SVR rate of 75% (6/8) due to 2 cirrhotic patients failing DAA treatment.

SVR-12 was 93% (112/120) for first treatment with an NS5A inhibitory–containing regime and 88% (28/32) without an NS5A inhibitor–containing regime. For patients treated with a pangenotypic second-generation DAA regimen, the SVR-12 rate was 98% (44/45), compared with 91% (68/75) for

Table 2. Treatment Results Stratified for Included Subtypes (n = 160)

Genotype (No., %)	Subtype (No.)	SVR-12 Result, % (No./No.)[a]
1 (19, 12)		**100 (18/18)**
	c (8)	100 (7/7)
	d (1)	100 (1/1)
	g (10)	100 (10/10)
2 (66, 41)		**93 (57/61)**
	c (3)	100 (3/3)
	e (3)	100 (3/3)
	f (9)	83 (5/6)
	i (9)	89 (8/9)
	k (4)	100 (4/4)
	o (1)	100 (1/1)
	p (2)	100 (2/2)
	Clade I (12)[b]	92 (11/12)
	Clade II (5)	100 (5/5)
	Clade III (5)	100 (5/5)
	Clade IV (1)	100 (1/1)
	Clade V (2)	100 (2/2)
	Unassigned (10)	88 (7/8)
3 (11, 7)		**73 (8/11)**
	b (8)	63 (5/8)
	k (3)	100 (3/3)
4 (50, 31)		**92 (44/48)**
	c (4)	100 (4/4)
	e (1)	100 (1/1)
	f (2)	100 (2/2)
	h (6)	100 (6/6)
	k (8)	88 (7/8)
	l (2)	100 (2/2)
	n (9)	75 (6/8)
	o (6)	100 (6/6)
	q (1)	100 (1/1)
	r (5)	100 (5/5)
	t (1)	100 (1/1)
	v (2)	0 (0/1)[c]
	Unassigned (3)	100 (3/3)
5 (3, 2)		**100 (3/3)**
	a (3)	100 (3/3)
6 (11, 7)		**91 (10/11)**
	a (6)	100 (6/6)
	e (3)	100 (3/3)
	f (1)	0 (0/1)
	Unassigned (1)	100 (1/1)
Total (160)		**92 (140/152)**

Data in bold represent SVR rates per genotype.

Abbreviation: SVR, sustained virological response.

[a]Number of patients with SVR-12 result can be lower than number of included patients, as not all SVR-12 results were known at the moment of data collection.

[b]These unassigned genotype 2 infections belong to previously described clades from Suriname [23].

[c]This patient had a detectable viral load of 38 IU/mL at SVR-12, and an undetectable viral load at SVR-24

patients treated with another NS5A inhibitor–containing regimen. SVR-12 was 89% (42/47) in cirrhotic and 93% (98/105) in noncirrhotic patients. SVR-12 in cirrhotic patients treated with SOF/VEL or GLE/PIB was 100% (16/16). SVR-12 after treatment with SOF + ribavirin for nonepidemic genotype 2 infections was 79% (11/14) of cases, vs 98% (47/48) after NS5A inhibitor–containing DAAs for genotype 2. Patient characteristics of the 12 patients who failed treatment are shown in Table 3.

Figure 2 shows the SVR-12 percentage per region of origin. The lowest SVR-12 rate was seen in patients originating in Southern Asia, with a 50% SVR-12 rate (3/6) due to 2 genotype 3b failures from Pakistan and 1 genotype 6f patient from India failing DAA treatment. In patients originating from Sub-Saharan Africa, the SVR-12 rate was 90% (27/30); however, 93% (28/30) were cured with the first DAA regimen, as 1 patient with a detectable viral load at SVR-12 achieved SVR-24. All 5 patients with subtype 4r achieved SVR-12. Patients infected with 1 of the unassigned genotype 2 clades from Suriname had an SVR-12 rate of 96% (24/25).

Resistance-Associated Substitutions

Baseline NS5A and NS5B RAS sequences were obtained for 69 and 28 patients, respectively (Tables 4 and 5). Prevalent NS5A RAS in the sequenced nonepidemic genotypes were 24S for genotype 2, 30K and 31M for genotype 3, and 30R and 58P for genotype 4. Only 1 sample contained RAS at position 93, which was a successfully treated subtype 4n infection with Y93C. Regarding the NS5B region, none of the samples contained RAS at the main resistance-harboring NS5B positions 150, 159, 282, and 321.

In all 4 nonepidemic genotype 2 infections that failed DAA therapy, the 24S NS5A RAS were present, although from the genotype 2f sample only a post-treatment sequence was available. The 24S NS5A RAS were also present in all but 1 of the 17 successfully treated patients with a nonepidemic genotype 2 subtype and available baseline NS5A sequences. The 3 genotype 3b infections that did not reach SVR-12 had post-treatment 30K and 31M RAS, which are known to be dominant amino acids at these positions for genotype 3b and were also present in the 5 successfully treated genotype 3b infections. In 1 of the genotype 3b infections that failed treatment, NS5B 159F RAS developed during treatment. In all 7 genotype 4n infections, the 30R RAS was present at baseline. In 1 of the 2 non-SVR 4n patients, the 28M RAS was also demonstrated at baseline, which was found in 2 of the 5 successfully treated genotype 4n infections with available RAS data. 58T was present in 7 genotype 4 NS5A sequences, all subtype 4n, of whom only 5 were successfully treated.

DISCUSSION

In this study, we report DAA treatment outcomes of 152 patients infected with a nonepidemic HCV genotype in the Netherlands. Overall, the SVR-12 rate was 92%, which is reassuring, as the majority of patients were treated with older DAA regimens with lower efficacy. However, only 73% (8/11) of patients with a genotype 3 infection achieved SVR-12, due to a 63% (5/8) SVR rate

Table 3. Characteristics of the 12 Patients Failing DAA Therapy

Genotype	Country of Origin	Cirrhosis	Failed DAA Regimen(s)	Successful Retreatment	Baseline RAS	Post-treatment RAS
2	Guinea	CP-A	SOF+rbv 16w	SOF/DAC+rbv 12w	NS5A: 24S NS5B: none	NS5A: 24S NS5B: none
2 clade I	Suriname	No	SOF+rbv 12w, SOF+rbv 24w, SOF/DAC+rbv 12w	SOF/GLE/PIB 16w	NS5A: 24S, 31M, 92S NS5B: none	Before & after SOF/DAC failure:NS5A: 24S, 31M, 92S NS5B: none
2i	Morocco	No[a]	SOF+rbv 12w	SOF/LDV+rbv 24w	NS5A: 24S, 31M NS5B: None	NA
2f	Guyana	No	SOF/DAC 12w	No retreatment	NA	NS5A: 24S, 31M NS5B: None
3b	Myanmar	No	SOF/VEL 12w	SOF/GLE/PIB 16w	NA	NS5A: 30K, 31M NS5B: None
3b	Pakistan	No	SOF/DAC 12w	SOF/GLE/PIB+rbv 16w	NA	NS5A: 30K, 31M NS5B: None
3b	Pakistan	CP-A	SOF/DAC+rbv 24w	SOF/VEL/VOX 12w	NS5A: NA NS5B: none	NS5A: 30K, 31MNS5B: 159F
4k	Rwanda	No	SOF/LDV 12w	SOF/VEL/VOX 12w	NA	NA
4n	Egypt	CP-A	SOF/LDV+rbv 12w	SOF/SIM+rbv 24w	NS5A: 28M, 30R NS5B: NA	NS5A: 28M, 30R NS5B: NA
4n	Egypt	CP-B	SOF/SIM+rbv 24w	SOF/DAC+rbv 12w	NS5A: 30R NS5B: NA	NA
4v[b]	Burundi	No	EBR/GZR 12w	No retreatment	NA	NA
6f	India	CP-A	EBR/GZR 12w[c]	SOF/VEL/VOX+rbv 12w	NA	NS5A: 28M, 31M NS5B: NA

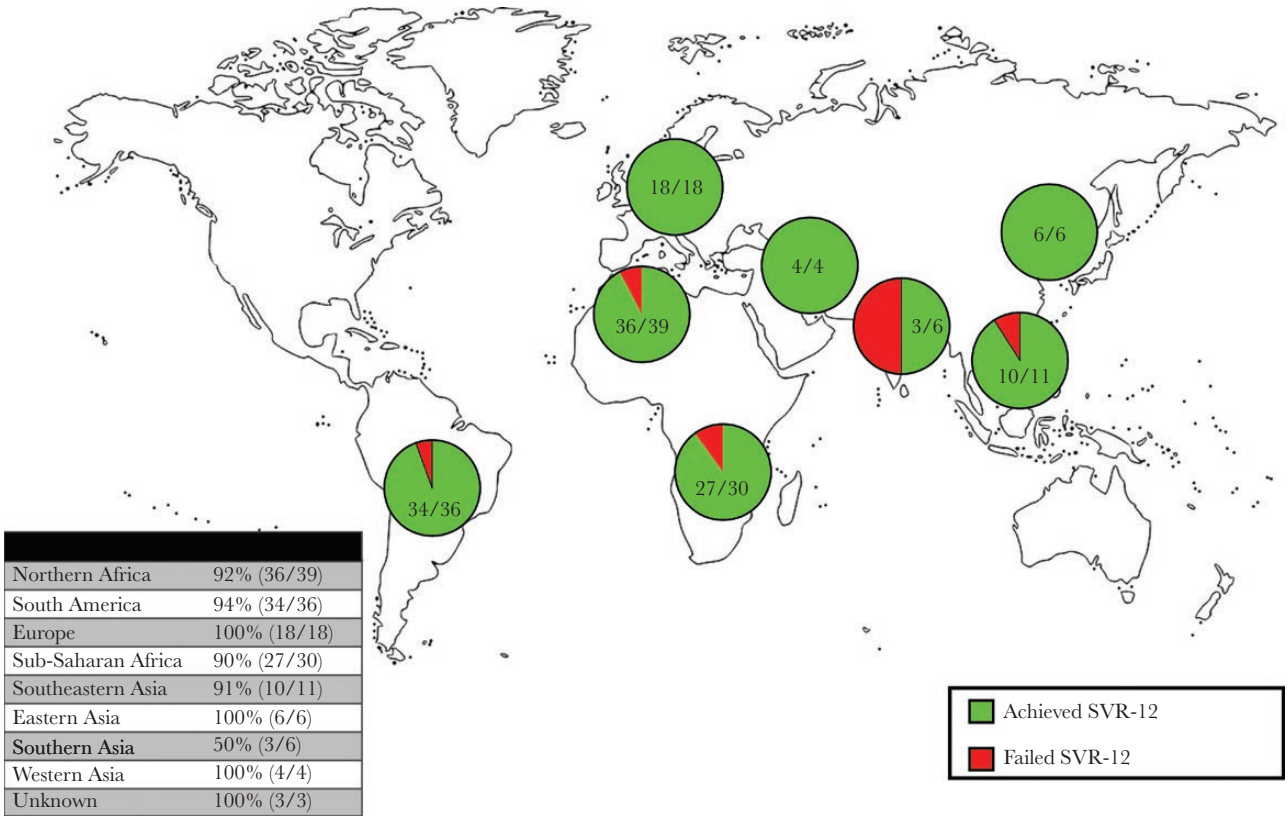
RAS are based on the European Association for the Study of the Liver guideline [22].

Abbreviations: CP, Child-Pugh class; DAA, direct-acting antiviral; DAC, daclatasvir; EBR, elbasvir; GLE, glecaprevir; GZR, grazoprevir; IFN, interferon; LDV, ledipasvir; NA, not available; NS, nonstructural protein; PEG, pegylated; PIB, pibrentasvir; RAS, resistance-associated substitutions; rbv, ribavirin; SIM, simeprevir; SOF, sofosbuvir; SVR, sustained virological response; VEL, velpatasvir; VOX, voxilaprevir.

[a]This patient had a detectable viral load of 38 IU/mL at SVR-12 and an undetectable viral load at SVR-24, and was thus not retreated.

[b]This patient had a noncirrhotic liver after orthotopic liver transplantation due to cirrhosis and HCC.

[c]This patient was treated in a phase III study.



Northern Africa	92% (36/39)
South America	94% (34/36)
Europe	100% (18/18)
Sub-Saharan Africa	90% (27/30)
Southeastern Asia	91% (10/11)
Eastern Asia	100% (6/6)
Southern Asia	50% (3/6)
Western Asia	100% (4/4)
Unknown	100% (3/3)

Figure 2. SVR-12 rate per region. Abbreviation: SVR-12, sustained virological response 12 weeks after cessation of treatment.

in genotype 3b. Of note, in 3 of the successfully treated patients with either genotype 3b or 3k, the intended first-line DAA regime was optimized after detection of RAS at baseline and subsequently tailored accordingly. These 3 patients were all treated in the same academic center, where baseline genotyping of all patients and baseline RAS analysis for nonepidemic genotype 3 infections are routinely performed.

A decreased DAA efficacy for genotype 3b will have serious implications for HCV elimination in Asia, as this subtype is endemic in several countries with a high HCV prevalence, such as China, India, Myanmar, and Pakistan [24–27]. In China, the country with the highest HCV prevalence in the world [1], genotype 3b accounts for 7% of all HCV infections [24]. A possible explanation for decreased DAA efficacy in genotype 3b could be that wild-type HCV-3b infections contain several resistance-associated amino acids in the NS5A region, most importantly 30K and 31M [8, 12]. This combination is associated with decreased efficacy against all NS5A inhibitors [12]. In fact, a recently published in vitro study demonstrated that PIB was the only NS5A inhibitor with high antiviral activity against subtype 3b [14].

In a real-world cohort study from Myanmar, genotype 3b patients were treated with either SOF/DAC or SOF/VEL, showing favorable SVR-12 rates of 96% (115/120) and 91% (50/55), respectively [27]. Conversely, in a recent SOF/VEL phase 3 trial conducted in multiple Southeastern Asian countries, only 76% (32/42) of included genotype 3b patients achieved SVR-12 [8]. Likewise, another recent Asian trial reported 70% (14/20) efficacy of GLE/PIB in genotype 3b patients [28]. In both trials, resistance-associated polymorphism 31M was present in all genotype 3b NS5A sequences [8, 28]. Furthermore, 4 other Asian studies, albeit with only a small number of genotype 3b patients, showed low SVR-12 rates of 75% (9/12), 33% (2/6), 75% (3/4), and 50% (2/4) [29–32]. Notably, in multiple of these studies all patients were treated with GLE/PIB, indicating that despite PIB having the highest antiviral activity against subtype 3b, its effectiveness is not indisputable [30–32]. Perhaps some of the differences in efficacy could be related to ribavirin use, as in contrast to the other studies many of the Myanmar genotype 3b patients had ribavirin added to their therapy [27]. In a large Italian genotype 3 cohort, a beneficial effect of ribavirin was seen when added to SOF/DAC, or to SOF/VEL in case of cirrhosis, although the genotype 3 subtypes and origins of patients were not reported [33].

So far, most studies reporting decreased DAA efficacy in nonepidemic HCV genotypes have focused on subtypes endemic in Sub-Saharan Africa [7, 10, 11]. In our study, we were not able to confirm these findings. In a London cohort with African patients, a suboptimal SVR rate in mainly West African non-1a/1b genotype 1 subtypes was seen [10], whereas

Table 4. SVR-12 Rate (No./No.) per NS5A RAS and Presence of Baseline RAS Per Genotype

AA at NS5A RAS positions[a]	Genotype									
	1 (n = 4)		2 (n = 21)		3 (n = 6)		4 (n = 30)		6 (n = 7)	
SVR-12	No.	100% (4/4)	No.	86% (18/21)	No.	100% (6/6)	No.	93% (28/30)	No.	100% (7/7)
K24F			1	1/1						
K24G					1	1/1				
K24K							31	29/31	5	5/5
K24R	2	2/2								
K24Q									2	2/2
K24S	2	2/2	20	17/20	5	5/5				
M28C			1	1/1						
M28F			11	8/11					1	1/1
M28L	4	4/4	6	6/6	1	1/1	18	15/16	2	2/2
M28M					5	5/5	10	9/10	1	1/1
M28S			1	1/1						
M28V							3	3/3	3	3/3
M28F/I			1	1/1						
M28L/R			1	1/1						
Q30C							1	1/1		
Q30K			20	17/20	6	6/6				
Q30Q	1	1/1								
Q30R	2	2/2					24	22/24	3	3/3
Q30S							3	3/3	4	4/4
Q30T							3	3/3		
Q30K/R			1	1/1						
Q30G/R	1	1/1								
L31I			1	1/1						
L31L	4	4/4	4	3/4			12	11/12	7	7/7
L31M			16	14/16	6	6/6	19	18/19		
P32P	4	4/4	21	18/21	6	6/6	31	29/31	7	7/7
S38S	4	4/4	21	18/21	6	6/6	31	29/31	7	7/7
H58A							1	1/1		
H58P	4	4/4	19	17/19	5	5/5	22	22/22	4	4/4
H58S			1	1/1	1	1/1				
H58T			1	0/1			6	4/6	2	2/2
H58A/T							1	1/1	1	1/1
H58P/S							1	1/1		
E62A			1	1/1					1	1/1
E62D					3	3/3	1	1/1		
E62E					1	1/1	19	18/19	1	1/1
E62G	1	1/1								
E62K							3	3/3		
E62L					1	1/1				
E62N			16	13/16			3	3/3		
E62Q	3	3/3					3	2/3		
E62R									1	1/1
E62S			2	2/2			1	1/1		
E62V									4	4/4
E62A/V			1	1/1						
E62D/E					1	1/1				
E62N/S			1	1/1						
E62N/T							1	1/1		
A92A	4	4/4					31	29/31	7	7/7
A92C			17	15/17						
A92E					6	6/6				
A92S			4	3/4						
Y93F	4	4/4								
Y93T									7	7/7
Y93Y			21	18/21	6	6/6	30	28/30		
Y93Y/C							1	1/1		

Abbreviations: AA, amino acids; RAS, resistance-associated substitution.

[a]Reference amino acid originates from the H77 genotype 1a sequence. Analyzed subtypes (No.): 1g (4), 2 unassigned (5), 2 clade I (3), 2 clade III (2), 2 clade V (1), 2c (1), 2f (2), 2i (4), 2k (1), 2o (1), 2p (1), 3b (5), 3k (1), 4unassigned (1), 4c (4), 4h (3), 4k (5), 4n (8), 4o (5), 4r (4), 4t (1), 6unassigned (1), 6a (3), 6e (3).

Table 5. SVR-12 Rate (No./No.) per NS5B RAS and Presence of Baseline RAS per Genotype

AA at NS5B RAS Positions[a]	Genotype			
	2 (n = 22)		3 (n = 6)	
SVR-12	No.	86% (19/22)	No.	83% (5/6)
N142N	22	19/22	5	5/5
N142?[b]			1	0/1
E150A	11	9/11	6	5/6
E150I	1	1/1		
E150S	2	1/2		
E150T	6	6/6		
E150V	2	2/2		
L159L	22	19/22	6	5/6
Q206E			2	2/2
Q206H	1	1/1		
Q206K			4	3/4
Q206Q	14	12/14		
Q206R	7	6/7		
E237E	22	19/22	6	5/6
S282S	22	19/22	6	5/6
C289F			6	5/6
C289M	22	19/22		
L320L	22	19/22	6	5/6
V321V	22	19/22	6	5/6

Abbreviations: AA, amino acids; RAS, resistance-associated substitution.

[a]Reference amino acid originates from the H77 genotype 1a sequence.

[b]Position 142 was not included in this sequence. Analyzed subtypes (No.): 2unassigned (5), 2 clade I (4), 2 clade III (2), 2 clade V (1), 2f (2), 2k (2), 2o (1), 2p (1), 3b (6).

the mainly Egyptian genotype 1 infections in our cohort were all successfully treated. Likewise, all 5 patients in our cohort infected with the 4r subtype were successfully treated, despite that in 4 out of 5 baseline NS5A RAS were present (28V/M, 30R, 58P). Besides the low number of included patients, differences in used treatment regimens are likely to have contributed to differences between cohorts, given the fact that VEL and PIB have better in vitro antiviral activity against known NS5A RAS [12, 14].

Our study has several limitations. In particular, the inclusion of some (sub)types is limited due to the low prevalence of these genotypes in the Netherlands. Also, as we report real-world data spanning multiple years, a variety of 10 different DAA regimens was used including older regimens such as SOF + ribavirin, which had a low SVR-12 rate of 79% in our cohort. Furthermore, due to limited availability of stored samples, we were not able to obtain baseline and post-treatment RAS sequences for all patients who failed DAA therapy. However, to our knowledge, our study is the first study to evaluate DAA efficacy of all nonepidemic HCV genotypes in a country or region. Moreover, for the first time DAA efficacy in unassigned genotype 2 clades prevalent in Suriname has been assessed. As these HCV subtypes reached Suriname and the Caribbean area through historic slave trade from Western Africa [23], one could argue that these are in fact distinct Sub-Saharan African subtypes. Furthermore, an important strength of our study is the reliable method of genotype determination, which allows for accurate classification of subtypes. By contrast, the widely used commercial assay INNO-LiPa frequently fails to report accurate subtypes for genotype 2, 4, and 6, with rates of 51%, 5.8%, and 9.3%, respectively [13].

Our results show that despite availability of pangenotypic DAA, genotyping remains necessary for patients originating from countries where nonepidemic genotypes are present. Furthermore, in order to advance global HCV elimination, and not only HCV elimination in high-income countries, we believe that more studies reliably assessing the unique prevalence of HCV subtypes for each region of LMICs are needed, preferably including RAS analysis. It is important that these studies be conducted at a regional level, as genotype distribution can vary greatly between regions in a country. For example, a review of 26 genotype distribution studies from several regions of Pakistan reported a wide range of 0.2%–22.3% for genotype 3b prevalence [26]. Alongside the local availability of DAAs, these data should be used to develop tailored regional HCV treatment guidelines taking baseline RAS into account. We believe that, given the prevalence of baseline RAS and low SVR-12 rates in genotype 3b, SOF/VEL/VOX or SOF/GLE/PIB as first-line treatment, as well as the standard addition of ribavirin, should be investigated. Importantly, this would require accelerated low-price access to the most recent NS5A inhibitor DAA regimes in low-income countries.

In conclusion, the DAA treatment results we observed in most nonepidemic genotypes in the Netherlands seem reassuring. However, the low SVR-12 rate in genotype 3b infections is alarming, especially as this genotype is common in several countries with high HCV prevalence. Alongside earlier published results, these results indicate that one of the remaining challenges for global HCV elimination is confirmation and monitoring of DAA treatment effectiveness in nonepidemic genotypes.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

The authors would like to thank all the physicians who contributed to data collection. These are: Dr. Pieter Honkoop, Albert Schweitzer Ziekenhuis. Dr. Sunje Abraham, Alrijne zorgroep. Dr. Karen Steenhuisen—van Erp, Bernhoven. Dr. Svend Rietdijk, BovenIJ. Dr. Ton Dofferhoff, Canisius-Wilhelmina Ziekenhuis. Dr. Pieter Friederich, Catharina Ziekenhuis. Dr. Frank ter Borg, Deventer Ziekenhuis. Dr. Xander Vos, Dr. Johannes Schmidt-Böhmer, Dr. Joost Vermeulen Dijklander Ziekenhuis. Dr. Hanna Telleman, Dr. Ulrike Schlüter,

Dr. Judith Branger, Flevoziekenhuis. Dr. Robert Roomer, Fransiscus Gasthuis & Vlietland. Dr. Paul Groeneveld, Dr. Jolanda Lammers, Isala. Dr. Henk-Marijn de Jonge, Jeroen Bosch Ziekenhuis. Dr. Eric de Goede, Laurentius Ziekenhuis. Dr. Jan den Hollander, Maasstad Ziekenhuis. Dr. Philip Friederich, Meander MC. Dr. Kamilla Lettinga, Dr. Jan Veenstra, Dr. Willem Blok, Dr. Guido van den Berk, Marijke Spelbrink, Onze Lieve Vrouwe Gasthuis. Dr. Nicole van Gerven, Rode Kruis Ziekenhuis. Dr. Tim Schreuder Treant zorggroep. Dr. Khalida Soufidi, Zuyderland MC.

HepNed study group. Dr. Sophie Willemse, Robin Erken, Dr. Marije Bomers, Dr. Dewkoemar Ramsoekh, Amsterdam UMC. Dr. Rob de Knegt, Prof. Dr. Rob de Man, Dr. Bart Rijnders, Erasmus MC. Dr. Hanneke van Soest, Haaglanden MC. Dr. Ger Koek, Dr. Dirk Posthouwer, Ozgür Koc, Maastricht UMC. Prof. Dr. David Burger, Marleen van Dijk, Prof. Dr. Joost Drenth, Daan von den Hoff, Radboud UMC. Dr. Johannes Brouwer, Reinier de Graaf Gasthuis. Dr. Greet Boland, Dr. Karel van Erpecum, UMC Utrecht.

Financial support. No financial support or funding was received in relation to this paper.

Potential conflicts of interest. C.I. has received research funding from Gilead. B.B. has received fees for advisory boards for AbbVie, Gilead, and Norgine and received research grants from Gilead and MSD. B.v.H. participated in advisory boards of WillPharma and received research grants from Zambon Pharma, Astellas, and Chiesi Pharma. S.B. has received research funding from Gilead. H.B. has participated in advisory boards for Gilead. J.A. has participated in advisory boards for Gilead, MSD, Janssen, and AbbVie. M.v.d.V. has participated in advisory boards (fees paid to institution) for AbbVie, Gilead, Johnson & Johnson, MSD, and ViiV and has received independent research grants from AbbVie, Johnson & Johnson, Gilead, and MSD. J.S. has participated in advisory boards for Gilead and received research grants from Gilead and AbbVie. All other authors declare no conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author contributions. Concept and design: C.I., M.v.d.V., J.S. Sample collection, genotyping, and/or RAS analysis: T.v.d.L., S.R., E.W., R.M., M.K., J.S. Data collection: C.I., T.v.d.L., E.W., R.M., M.K., B.B., C.v.N., B.v.H., S.B., H.B., J.A. Drafting of the first version of the manuscript: C.I., M.v.d.V., J.S. All authors contributed to the interpretation of results and preparation and review of the manuscript. All authors read and approved the final manuscript.

References

1. Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol **2017**; 2:161–76.
2. Smith DB, Bukh J, Kuiken C, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology **2014**; 59:318–27.
3. Simmonds P. The origin of hepatitis C virus. In: R. Bartenschlager, ed. Hepatitis C Virus: From Molecular Virology to Antiviral Therapy. Vol 369. Heidelberg, Germany: Springer; **2013**:1–15.
4. de Vries MJ, te Rijdt B, van Nieuwkerk CM. Genotype distribution amongst hepatitis C patients in the Netherlands. Neth J Med **2006**; 64:109–13.
5. Davis C, Mgomella GS, da Silva Filipe A, et al. Highly diverse hepatitis C strains detected in Sub-Saharan Africa have unknown susceptibility to direct-acting antiviral treatments. Hepatology **2019**; 69:1426–41.
6. Niebel M, Singer JB, Nickbakhsh S, et al. Hepatitis C and the absence of genomic data in low-income countries: a barrier on the road to elimination? Lancet Gastroenterol Hepatol **2017**; 2:700–1.
7. Gupta N, Mbituyumuremyi A, Kabahizi J, et al. Treatment of chronic hepatitis C virus infection in Rwanda with ledipasvir-sofosbuvir (SHARED): a single-arm trial. Lancet Gastroenterol Hepatol **2019**; 4:119–26.
8. Wei L, Lim SG, Xie Q, et al. Sofosbuvir-velpatasvir for treatment of chronic hepatitis C virus infection in Asia: a single-arm, open-label, phase 3 trial. Lancet Gastroenterol Hepatol **2019**; 4:127–34.
9. Lim SG, Phyo WW, Shah SR, et al. Findings from a large Asian chronic hepatitis C real-life study. J Viral Hepat **2018**; 25:1533–42.
10. Childs K, Davis C, Cannon M, et al. Suboptimal SVR rates in African patients with atypical genotype 1 subtypes: implications for global elimination of hepatitis C. J Hepatol **2019**; 71:1099–105.
11. Fourati S, Rodriguez C, Hézode C, et al. Frequent antiviral treatment failures in patients infected with hepatitis C virus genotype 4, subtype 4r. Hepatology **2019**; 69:513–23.
12. Smith D, Magri A, Bonsall D, et al; STOP-HCV Consortium. Resistance analysis of genotype 3 hepatitis C virus indicates subtypes inherently resistant to nonstructural protein 5A inhibitors. Hepatology **2019**; 69:1861–72.
13. Welzel TM, Bhardwaj N, Hedskog C, et al. Global epidemiology of HCV subtypes and resistance-associated substitutions evaluated by sequencing-based subtype analyses. J Hepatol **2017**; 67:224–36.
14. Nguyen D, Smith D, Vaughan-Jackson A, et al. Efficacy of NS5A inhibitors against unusual and potentially difficult-to-treat HCV subtypes commonly found in sub Saharan Africa and South East Asia. J Hepatol **2020**; 73:794–9.
15. McPhee F, Ueland J, Vellucci V, et al. Impact of preexisting hepatitis C virus genotype 6 NS3, NS5A, and NS5B polymorphisms on the in vitro potency of direct-acting antiviral agents. Antimicrob Agents Chemother **2019**; 63:e02205-18.
16. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C – final update of the series. J Hepatol **2020**; 73:1170–218.
17. Murphy DG, Willems B, Deschênes M, et al. Use of sequence analysis of the NS5B region for routine genotyping of hepatitis C virus with reference to C/E1 and 5' untranslated region sequences. J Clin Microbiol **2007**; 45:1102–12.
18. Larkin MA, Blackshields G, Brown NP, et al. Clustal W and Clustal X version 2.0. Bioinformatics **2007**; 23:2947–8.
19. Kuiken C, Yusim K, Boykin L, Richardson R. The Los Alamos hepatitis C sequence database. Bioinformatics **2005**; 21:379–84.
20. Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol **2013**; 30:2725–9.
21. Kalaghatgi P, Sikorski AM, Knops 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.
22. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2018. J Hepatol **2018**; 69:461–511.
23. Markov PV, van de Laar TJ, Thomas XV, et al. Colonial history and contemporary transmission shape the genetic diversity of hepatitis C virus genotype 2 in Amsterdam. J Virol **2012**; 86:7677–87.
24. Chen Y, Yu C, Yin X, et al. Hepatitis C virus genotypes and subtypes circulating in Mainland China. Emerg Microbes Infect **2017**; 6:e95.
25. Solomon SS, Boon D, Saravanan S, et al. Diversity of hepatitis C virus infection among HIV-infected people who inject drugs in India. Virusdisease **2019**; 30:490–7.
26. Umer M, Iqbal M. Hepatitis C virus prevalence and genotype distribution in Pakistan: comprehensive review of recent data. World J Gastroenterol **2016**; 22:1684–700.
27. Hlaing NKT, Nangia G, Tun KT, et al. High sustained virologic response in genotypes 3 and 6 with generic NS5A inhibitor and sofosbuvir regimens in chronic HCV in Myanmar. J Viral Hepat **2019**; 26:1186–99.
28. Wei L, Wang G, Alami NN, et al. Glecaprevir-pibrentasvir to treat chronic hepatitis C virus infection in Asia: two multicentre, phase 3 studies—a randomised, double-blind study (VOYAGE-1) and an open-label, single-arm study (VOYAGE-2). Lancet Gastroenterol Hepatol **2020**; 5:839–49.
29. Hu C, Yuan G, Liu J, et al. Sofosbuvir-based therapies for patients with hepatitis C virus infection: real-world experience in China. Can J Gastroenterol Hepatol **2018**; 2018:3908767.
30. Nozaki A, Atsukawa M, Kondo C, et al; KTK49 Liver Study Group. The effectiveness and safety of glecaprevir/pibrentasvir in chronic hepatitis C patients with refractory factors in the real world: a comprehensive analysis of a prospective multicenter study. Hepatol Int **2020**; 14:225–38.
31. Tamori A, Inoue K, Kagawa T, et al. Intention-to-treat assessment of glecaprevir + pibrentasvir combination therapy for patients with chronic hepatitis C in the real world. Hepatol Res **2019**; 49:1365–73.
32. Kumada H, Watanabe T, Suzuki F, et al. Efficacy and safety of glecaprevir/pibrentasvir in HCV-infected Japanese patients with prior DAA experience, severe renal impairment, or genotype 3 infection. J Gastroenterol **2018**; 53:566–75.
33. Soria A, Fava M, Bernasconi DP, et al. Comparison of three therapeutic regimens for genotype-3 hepatitis C virus infection in a large real-life multicenter cohort. Liver Int **2020**; 40:769–77.