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Optimal cure rate by personalized HCV regimens in real-life: a proof-of-concept study

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Background: Pretreatment evaluation of HCV-infected patients is a complex interplay between multiple clinical and viral parameters, leading to a tailored approach that may bring real-life sustained virological response (SVR) rates close to 98%–99%.

Objectives: As proof-of-concept, we evaluated the efficacy of all-oral direct-acting antiviral (DAA) regimens in patients whose personalization included pre-therapy evaluation of natural resistance-associated substitutions (RASs), in addition to international guideline recommendations.

Methods: One hundred and thirty-one patients who started a first-line all-oral DAA regimen between April 2015 and December 2016 were tested for baseline NS3 and NS5A RASs by Sanger sequencing. SVR_{12} was defined as HCV-RNA undetectability 12 weeks after treatment discontinuation.

Results: Compatibly with a real-life context, 74.0% (97 of 131) of patients presented $\geq$2 pretreatment risk factors for failure to achieve SVR_{12} (infection by GT-1a/GT-3a; cirrhosis; previous treatment experience; HCV-RNA $\geq$800000 IU/mL) and 33.6% had $\geq$3 risk factors. Natural RASs were frequently detected (32.1% prevalence), with substantial prevalence of NS5A RASs (15.3%), mostly represented by Y93H in GT-1b (3 of 36, 8.3%) and GT-3a (3 of 25, 12.0%) and F28C in GT-2c (2 of 11, 18.2%). Overall, personalized treatment led to 100% SVR_{12}, even in those patients for whom treatment strategy was either strengthened (by ribavirin inclusion and/or duration increase) or simplified (by ribavirin exclusion and/or duration reduction), thanks to baseline RAS evaluation.

Conclusions: Even with newer DAA regimens, an integrated interpretation of clinical and virological pretreatment parameters, including natural RASs, may play a relevant role in bringing SVR rates close to the highest achievable. Treatment tailoring can be foreseen in 'hard-to-treat' patients, but also in 'easy' patients with favourable indicators, whereby a simplification/shortening of recommended regimens can be indicated.

Introduction

DAA-based treatment represents a major breakthrough in the treatment of HCV infection, bringing sustained virological response (SVR) rates beyond the threshold of 90%. Despite that, ~100000 virological failures have been predicted by 2020 in the USA,[1] the most of which will be NS5A failures with limited retreatment options due to development of resistance-associated substitutions (RASs). Therefore, management of modern direct-acting antivirals (DAAs) can still be improved.

Clinical and viral parameters were used to tailor anti-HCV treatment since the IFN era, and then reconsidered as last-generation DAAs became available. Some parameters, such as IL28B genotype and on-treatment viral response, lost importance, while others, such as HCV genotype, baseline HCV-RNA, liver disease severity and previous treatment experience, kept significance and are still used to modulate treatment schedules.[2,3] Lastly, new viral characteristics emerged as clinically significant. Among these, baseline genotypic resistance testing (GRT) for natural RASs before the first DAA course can help physicians to guide treatment decisions,[2,3] as some NS3 and (more importantly) NS5A RASs were shown to reduce significantly the SVR rates to NS3 PIs and NS5A inhibitors.[4–8]

Natural RAS relevance was shown to depend greatly upon the DAA used, HCV genotype and subtype, previous treatment experience and baseline HCV-RNA, making pretreatment evaluation of HCV-infected patients a complex interplay between multiple clinical and viral parameters. This holds true in complex patients

(comorbidities, advanced liver disease, first-generation DAA failures etc.), but also in easy-to-treat patients, in light of the tendency to decrease the duration of treatment (who should undergo short therapy, and who should not). Under these conditions, a tailored approach in some (not infrequent, though) real-life situations may help in bringing the cure rate closer to 98%–99%, which represents a reasonable and achievable target for the new, excellent DAAs.

As a proof-of-concept for a personalized approach to HCV treatment in real-life, we analysed the efficacy of several first-line all-oral regimens in patients who underwent pre-therapy assessment of multiple clinical and virological parameters, including natural RASs.

Methods

One hundred and thirty-one patients who started a first-line all-oral DAA regimen between April 2015 and December 2016 were included. The choice of treatment was based on DAA availability. Ribavirin use and treatment duration were at the discretion of the investigator.

All patients were tested for natural NS3 and NS5A RASs by standard population sequencing as described elsewhere,[9] while testing for resistance to sofosbuvir or dasabuvir was optional, and performed in 91 of 131 patients. RASs were defined according to latest updates available.[3,10,11]

A phylogenetic analysis by neighbour-joining method in MEGA v5.1 excluded laboratory contaminations, and assigned HCV genotype and subtype.

SVR was defined as HCV-RNA undetectability 12 weeks after treatment discontinuation (SVR_{12}).

Ethics

This study was conducted in accordance with the principles of the Declaration of Helsinki, and approval by local Ethics Committees, as well as patient written informed consents, were obtained.

Results

Study population

Our study population was representative of a quite complex real-life clinical context (Table 1). Infection by GT-1a and GT-3a were frequent (35.9% and 19.1%, respectively), concordantly with a high prevalence of patients with a history of drug addiction (28.2%), in 50% of cases still active (19 of 37). In addition, 67.2% of patients had an F4 cirrhosis with remarkable stiffness [median (IQR) = 20.2 (13.4–30.4) kPa]; seven had liver decompensation, three with hepatocellular carcinoma. Nearly half of patients (43.5%) were IFN experienced (including 27 previous non-responders) and 10 (7.6%) had previously failed a first-generation PI (boceprevir/telaprevir). Median (IQR) baseline HCV-RNA was 5.8 (5.2–6.4) log IU/mL and 46.2% of patients had values ≥800000 IU/mL.

Overall, 74.0% of patients presented ≥2 risk factors for failure to achieve SVR_{12} (infection by HCV GT-1a or GT-3a; cirrhosis; previous treatment with IFN-based regimens; baseline HCV-RNA ≥800000 IU/mL) and 33.6% had ≥3 factors.

Comorbidities included non-Hodgkin lymphoma (3.8% prevalence), β-thalassaemia (3.8%), type 2 diabetes (3.1%), cryoglobulinaemia (2.3%), psoriasis (2.3%), end-stage kidney failure (1.5%) and monoclonal gammopathy (1.5%). Prevalence of HIV coinfection was 6.9%.

Baseline resistance

Before the start of treatment, the presence of at least one RAS for currently recommended DAA regimens was found in 42 of 131 patients (32.1%) (Table S1, available as Supplementary data at *JAC* Online). No RASs were detected in the coinfected GT-1a/GT-3a patient or in the patient infected with GT-4a.

NS3 RASs for simeprevir/paritaprevir/grazoprevir were found in 25 of 131 patients (19.1%), mainly GT-1b infected (14 of 36, 38.9%), due to frequent detection of Y56F grazoprevir RAS (10 of 36, 27.8%).

NS5A RASs for daclatasvir/elbasvir/ledipasvir/ombitasvir/velpatasvir were found in 20 of 131 patients (15.3%), including 4 of 47 GT-1a (8.5%), 7 of 36 GT-1b (19.4%), 2 of 11 GT-2c (18.2%) and 7 of 25 GT-3a (28.0%). Double RASs were detected in six patients and triple RASs in one GT-1a (M28V + Q30R + L31M). Most frequent NS5A RASs were Y93H in GT-1b (3 of 36, 8.3%) and GT-3a (3 of 25, 12.0%) and F28C in GT-2c (2 of 11, 18.2%).

NS5B RASs were detected only in 7 of 91 patients (7.7%), of whom 6 were GT-1b. The sofosbuvir RAS S282T was never detected.

Phylogenetic analysis of HCV sequences disclosed the wrong genotype classification in one patient (GT-1a instead of GT-3a) and resolved undetermined genotyping results by commercial assays in three other cases (GT-1a, GT-1b and GT-3a, respectively).

Treatment personalization and efficacy

According to GRT results, HCV genotype and subtype assigned by phylogenetic analysis, liver status, previous treatment experience and other clinical predictors of response, patients started a first-line, tailored DAA regimen (Figure 1).

In this personalized-treatment cohort, the PP SVR_{12} rate was 100% (130 of 130) (Figure 1). One GT-4d patient died due to stage IV mantellar lymphoma after 8 weeks of treatment; viraemia at that time was stably undetectable.

Personalized regimens were in accordance with current recommendations from the European Association for the Study of the Liver (EASL; update of September 2016) in 84 of 131 patients (64.1%), of whom 28 had baseline RASs and 56 did not. Thirty-three other patients (25.2%) who started therapy before September 2016 were treated with DAA regimens no longer considered optimal by EASL recommendation 2016–17 (i.e. sofosbuvir + simeprevir for GT-1, sofosbuvir + ribavirin for GT-2), but that were recommended by EASL indications of 2015.

Baseline RASs contributed to a modification of treatment schedule in 14 patients (10.7%) for whom a DAA combination was specifically chosen among those available ($n = 3$), and/or strengthened (by ribavirin inclusion and/or duration increase, $n = 4$) or simplified (by ribavirin exclusion and/or duration reduction, $n = 8$).

In two GT-1a patients and one GT-1b non-cirrhotic patient, DAA regimens were chosen based on the resistance profile among those available. Indeed, one GT-1a patient received 24 weeks of daclatasvir + sofosbuvir + ribavirin due to the presence of Q80K in NS3 and Y93C in NS5A, while the other GT-1a patient was treated with sofosbuvir + simeprevir + ribavirin for 12 weeks due to the presence of M28V NS5A RAS. The GT-1b patient received 12 weeks of sofosbuvir/ledipasvir due to the presence of dasabuvir RAS C316H.

Table 1. Study population ($N = 131$)

HCV genotype, *n* (%)	1a	47 (35.9)
	1a/3a coinfection	1 (0.8)
	1b	36 (27.5)
	2c	11 (8.4)
	3a	25 (19.1)
	4a	1 (0.8)
	4d	10 (7.6)
Treatment naive, *n* (%)		74 (56.5)
Previous virological failure, *n* (%)	breakthrough	3 (2.3)
	non-responder	27 (20.6)
	relapse	17 (13.0)
Previous treatment discontinuation, *n* (%)	adverse events	5 (3.8)
	drop-out	1 (0.8)
	unknown	4 (3.1)
Previous PI experience, *n* (%)		10 (7.6)
Liver stiffness (kPa), median (IQR)		13.6 (10.8–25.1)
Metavir, *n* (%)	F1	1 (0.8)
	F2	3 (2.3)
	F3	39 (29.8)
	F4	88 (67.2)
Child–Turcotte–Pugh class, *n* (%)[a]	CTP-A	70 (53.4)
	CTP-B	7 (5.3)
HIV coinfection, *n* (%)		9 (6.9)
Hepatocellular carcinoma, *n* (%)		3 (2.3)
HCV-related extrahepatic manifestations, *n* (%)	cryoglobulinaemia	3 (2.3)
	monoclonal gammopathy	2 (1.5)
	non-Hodgkin lymphoma	5 (3.8)
	psoriasis	3 (2.3)
	type 2 diabetes	4 (3.1)
	end-stage kidney failure	2 (1.5)
Haemophilia A, *n* (%)		1 (0.8)
β-Thalassaemia, *n* (%)		5 (3.8)
HCV-RNA (log IU/mL), median (IQR)		5.8 (5.2–6.4)
ALT (IU/mL), median (IQR)		78 (46–131)
AST (IU/mL), median (IQR)		56 (33–97)
Platelets (n/mm^3), median (IQR)		161000 (118000–241000)
Creatinine (mg/dL), median (IQR)		0.8 (0.7–1.0)
Bilirubin (mg/dL), median (IQR)		0.8 (0.53–1.23)
Albumin (mg/dL), median (IQR)		4 (3.9–4.0)

[a]Information available for 77 patients.

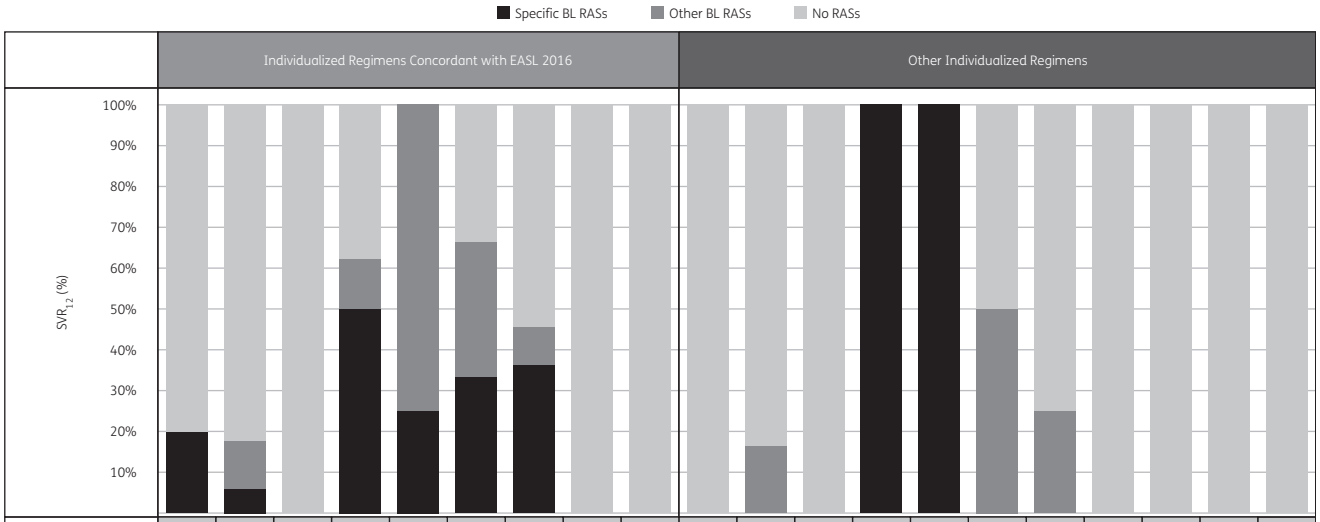
Similarly, in three other patients with natural RASs, currently recommended DAA regimens were 'strengthened' by prolonging treatment duration to 24 weeks or by adding ribavirin. In particular, one GT-3a patient received 24 weeks of daclatasvir + sofosbuvir + ribavirin due to the presence of A62L NS5A RAS, while two GT-1b patients received daclatasvir + sofosbuvir for 24 weeks and sofosbuvir/ledipasvir + ribavirin for 12 weeks due to double-class NS5A + NS5B resistance (NS5A:P58A; NS5B:C445F + V321A) in one patient and the presence of L159F NS5B RASs plus a very high viral load and complex clinical situation in the other patient.

On the other hand, regimens were simplified by ribavirin exclusion and/or duration reduction from 24 weeks to 12, in eight cirrhotic GT-1a or GT-3a patients without baseline NS5A RASs.

Lastly, in two patients with no baseline RASs, other negative predictors of response contributed to the choice of a more intense regimen (longer and/or with ribavirin) than those suggested by EASL indications 2016. These included previous PI experience, high baseline HCV-RNA and cirrhosis with high stiffness values.

Discussion

In this study, we provided a proof-of-concept for a 'tailored' DAA regimen that associates baseline evaluation of HCV resistance to DAAs to the other clinical parameters universally considered essential for treatment personalization. Treatment personalization



Number of patients	5	17	1	8	4	3	11	2	4	4	6	1	1	1	12	4	3	2	1	3
HCV genotype	1a		1a/3a	1b		2c	3a	4d		1a			1b			2c	3a		4a/d	
DAA regimen	3D +RBV	SOF/ LDV ±RBV	DAC +SOF	3D ±RBV	SOF/ LDV	DAC+ SOF	DAC+ SOF ±RBV	SIM+ SOF	SOF /LDV	3D +RBV	SIM+ SOF	SOF/ LDV +RBV	3D +RBV	DAC+ SOF	SIM+ SOF	SOF +RBV	DAC+ SOF	SOF +RBV	SIM+ SOF	SOF/ LDV ±RBV
Patients treated with 12 week regimens, *n* (%)	2 (40.0)	9 (52.9)	1 (100)	8 (100)	3 (75.0)	3 (100)	2 (18.2)	2 (100)	3 (75.0)	4 (100)	5 (83.3)	0 (0.0)	1 (100)	0 (0.0)	11 (91.7)	3 (75.0)	0 (0.0)	0 (0.0)	1 (100)	1 (33.3)
Patients with regimen-specific BL RASs, *n* (%)	1 (20.0)	1 (5.9)	0 (0.0)	4 (50.0)	1 (25.0)	1 (33.3)	4 (36.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Patients with cirrhosis, *n* (%)	2 (40.0)	11 (64.7)	0 (0.0)	6 (75.0)	2 (50.0)	2 (66.7)	9 (81.8)	2 (100)	2 (50.0)	4 (100)	6 (100)	1 (100)	1 (100)	0 (0.0)	11 (91.7)	2 (50.0)	3 (100)	1 (50.0)	1 (100)	3 (100)

Figure 1. SVR rates according to treatment regimen. Histograms represent individual PP SVR rates for each DAA regimen, categorized according to current EASL recommendations (2016) and HCV genotype/subtype. Within each regimen category, the percentages of patients receiving a 12 week regimen are reported, along with percentages of patients with baseline RASs for the selected regimens and of patients with cirrhosis. 3D, paritaprevir/ritonavir, ombitasvir and dasabuvir; BL, baseline; DAC, daclatasvir; LDV, ledipasvir; RBV, ribavirin; SIM, simeprevir; SOF, sofosbuvir.

following this approach led to 100% PP SVR, even in patients whose 'tailored' treatment did not precisely meet current international recommendations.[3] Indeed, baseline RAS presence or absence contributed to the selection of a different DAA combination or to 'simplified' or 'strengthened' treatment protocols by ribavirin use and/or modulation of treatment.

Our population was representative of a quite complex real-life clinical context, with a high prevalence (74.0%) of patients showing $\geq$2 risk factors for treatment failure and with important extrahepatic comorbidities that could significantly complicate anti-HCV treatment.[3] A significant number of the patients we included had a history of drug addiction (28.2%, of whom 50% were still active drug-users), which accounted for the high prevalence of GT-3a and GT-1a infection in our population.

Thanks to baseline GRT, we were able to disclose natural RAS presence in 32.1% of patients analysed and more frequently in those infected by GT-1b, GT-2c and GT-3a. In addition, we also correctly attributed infecting HCV genotype in four patients, highlighting a case of wrong genotype assignation by commercial assays that would have led to an inappropriate treatment regimen (GT-3a versus GT-1a).

From our perspective, 'personalization' of first-line DAA regimens can take advantage of the evaluation of viral resistance profile, provided that a reliable methodology and proper results' interpretation flowchart are developed and easily accessible (as correctly proposed in a recent review).[12] In this case, resistance information may contribute to a reliable *a priori* evaluation of the likelihood of response at the individual patient level. In specific situations, this can help identify those patients, who for social reasons or comorbidities, may benefit from the use of short and/or ribavirin-free therapies. In these patients, a baseline profile of no viral resistance would support treatment 'simplification'. Similarly, it can be fruitfully used to define which patients can take advantage of increasing drug pressure by a proper DAA regimen, and/or of adding ribavirin or prolonging treatment, as previously suggested by clinical trials.[4,8,13,14] Further evidences is required to support the observations obtained in this proof-of-concept study, and we have to be aware that our GRT-guide approach to simplification of DAA regimens (shortening of therapy and no use of ribavirin with current drugs) was applied only for first-line IFN-free regimens. Currently, no data are available for DAA-experienced subjects in real-life, in whom retreatment 'simplification' would potentially be risky.

In conclusion, in DAA-naive patients, baseline resistance evaluation integrated with clinical and virological parameters may play a relevant role in bringing the rate of success close to the highest achievable rate.

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Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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