Prevalence of resistance-associated substitutions to NS3, NS5A and NS5B inhibitors at DAA-failure in hepatitis C virus in Italy from 2015 to 2019

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SUMMARY

Despite the high efficacy of direct-acting antivirals (DAAs), the selection of resistance-associated substitutions (RASs) after virological failure of hepatitis C virus (HCV) DAAs can impair the cure of chronic HCV. The aim of the study was to characterize RASs after virological failure of DAAs in Italy over the years. Within the Italian network VIRONET-C, the change in prevalence of NS3/4A-NS5A-NS5B RASs was retrospectively evaluated in patients who failed a DAA regimen over the years 2015-2019. NS3, NS5A and NS5B Sanger sequencing was performed using homemade protocols and the geno2pheno system was used to define HCV-genotype/subtype and predict drug resistance. The changes in the prevalence of RASs over time were evaluated using the chi-square test for trend. Predictors of RASs at failure were analysed by logistic regression. Among 468 HCV-infected patients, HCV genotype 1 was the most prevalent (1b in 154, 33% and 1a in 109, 23%). DAA regimens were: ledipasvir (LDV)/sofosbuvir (SOF) in 131 patients (28%), daclatasvir (DCV)/SOF in 109 (23%), ombitasvir/paritaprevir/ritonavir+dasabuvir (3D) in 89 (19%), elbasvir (EBR)/grazoprevir (GRZ) in 52 (10.5%), velpatasvir (VEL)/SOF in 53 (11%), glecaprevir (GLE)/pibrentasvir (PIB) in 27 (6%) and ombitasvir/paritaprevir/ritonavir (2D) in 7 (1.5%); ribavirin was administered in 133 (28%). The NS5A fasta sequence was available for all patients, NS5B and NS3/4A both for 93%. The prevalence of NS5A and NS3/4A RASs significantly declined from 2015 to 2019; NS5B RAS remained stable. Independent predictors of any RASs included older age and genotype 1a (vs G2 and vs G4). Notably, at least partial susceptibility to all the agents included in the GLE/PIB and VEL/SOF/Voxilaprevir (VOX) combinations was predicted in >95% of cases. As RASs remain common at the failure of DAAs, their identification could play a crucial role in optimizing re-treatment strategies. In Italy RAS prevalence has been decreasing over the years and susceptibility to the latest developed drug combinations is maintained in most cases.

Keywords: HCV, DAA, Resistance Associated Substitutions (RAS), HCV sequencing.

INTRODUCTION

Worldwide, current regimens combining Direct-Acting Antivirals (DAAs) targeting different key proteins in the Hepatitis C Virus (HCV) replication cycle have considerably increased the cure rate of HCV infection [1].
Despite the high rate of Sustained Virologic Response (SVR) 12 weeks after the end of treatment, approximately 2-3% of patients experienced treatment failure and did not clear HCV infection [2, 3]. Particularly, HCV DAAs failure affects those with advanced liver disease, individuals exposed to previous treatment(s), especially carriers of natural Resistance Associated Substitutions (RASs), detected at baseline, and acquired RASs, selected in HCV chronically infected patients who fail to respond to DAAs treatment [4-6]. Notably, NS5A, NS5B and NS3 RASs may affect the efficacy of HCV treatment, especially as NS5A inhibitor is included in all currently available DAAs regimens [1]. On the contrary, RASs to NS5B inhibitors are less frequently detected, even after failure to a DAA regimen containing a nucleotide inhibitor [4]. Selection of HCV DAAs RASs in individuals experienced failure to drugs might lead to transmission of RASs in communities and might impair even the efficacy of first-line DAAs in some patients [7].
Although the clinical impact of RASs on therapeutic efficacy of last generation DAAs has remained elusive, some substitutions are still associated with reduced drug sensitivity and current international guidelines suggest considering resistance patterns to select effective salvage treatments options [8-11].
The study aimed to analyse changes in RAS prevalence in Italy at DAA failure over the years. Secondary endpoints were to identify factors associated to RASs selection and to explore the predicted susceptibility to the EASL recommended DAA re-treatment strategies.

PATIENTS AND METHODS

HCV sequences from patients who failed to DAA regimens between 2015 and 2019 were retrieved from the observational cohort VIRONET-C, a large Italian collaboration network enrolling HCV-infected individual from multiple clinical centres (https://www.vironetc.org/). The VIRONET-C database was queried to retrieve the data of HCV-infected patients with (I) DAAs failure and (II) at least 1 NS5A fasta sequence. Demographic (age, gender) and clinical data (HBVand HIV co-infection, HCV-RNA, HCV genotype, liver fibrosis, previous treatment history) were collected.
The database was approved by Ethics Committees at each clinical centre and written informed consent was obtained from all patients before participation. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki (7th revision) and with the International Conference on Harmonization - Good Clinical Practice guidelines (ICH-GCP).
NS3, NS5A and NS5B genomic regions were previously amplified by RT-PCR homemade protocols specific for the genotype/subtypes, covering positions involved in drug resistance, and PCR products were sequenced by Sanger sequencing with homemade protocols in different laboratories.
Geno2pheno system and Sorbo MC et al. Drug Resistance Updates 2018 were used to confirm HCV-genotype/subtype and to predict drug resistance [12, 13]. The susceptibility to currently employed Single Tablet Regimens (STRs) including DAAs was defined according to the latest literature data available. It was reported as I) full resistance: resistance to all compounds in STR, II) 1 DAA resistance, or 2 DAA resistance for sofosbuvir/velpatasvir/voxilaprir: resistance to one or two compounds in STR, III) reduced susceptibility to at least one compound, IV), full susceptibility to all compounds [12, 13]. Results are expressed as median value and interquartile range for continuous variables and as number and percentage for categorical values. Changes in the prevalence of RASs over time were evaluated by $\chi 2$ test for trend. Logistic regression models were used to assess predictors of RASs at failure. Each variable was included in univariable models and then the statistically significant variables ($p<0.05$) were fitted simultaneously in a multivariable model. All analyses were performed using SPSS for Macintosh (Statistical Package for the Social Sciences, version 25.0, Armonk, New York, NY, USA).

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RESULTS

Sequences from 468 patients failing a DAA based regimen between 2015 and 2019 were collected. The NS5A region was successfully amplified in all the patients, NS5B in 436/468 (93%) and NS3/4A in 437/468 (93%). Table 1 shows the characteristics of the patients enrolled. Notably, most patients were affected by liver cirrhosis (277/429, 64.6%) and one third had a previous hepatitis B virus infection (46/150). Interferon-based strategies were previously used in 23.5% of cases (109/468). HCV genotype 1b was the most prevalent (154/468, 32.9%), followed by genotype 3 (131/468, 28%).
Treatment was completed as scheduled in 434/468 patients (92.7%), namely 246/276 (89.1%) in F4 Metavir stage carriers and 110/111 (99%) in the others. All patients were exposed to NS5A inhibitors, 189/468 (40.4%) were exposed to NS3/4A protease inhibitors and 382/468 (81.6%) to NS5B polymerase inhibitors. DAAs regimens were: ledipasvir (LDV)/sofosbuvir (SOF) in 131 patients (28%), daclatasvir (DCV)/SOF in 109 (23%), ombitasvir/paritaprevir/ritonavir +dasabuvir (3D) in 89 (19%), elbasvir (EBR)/grazoprevir (GRZ) in 52 (10.5%), velpatasvir (VEL)/SOF in 53 (11%), glecaprevir (GLE)/pibrentasvir (PIB) in 27 (6%) and ombitasvir/paritaprevir/ritonavir (2D) in 7 (1.5%); ribavirin was administered in 133 (28%).
We found at least one RAS in 86.5% (405/468) of cases and the cumulative number of RASs detected was 1,025 with a median value of 2 per patient (IQR 1-3).
RASs were detected in 83.3% (390/468) of NS5A sequences, with Y93H as the most prevalent change (53.2%). Substitutions in NS3/4A were also quite frequent (152/437, 34.8%), as the most frequent mutation S122T (12.2%), with the exception of N174S (15.2%) relevant only for telaprevir susceptibility. Among NS5B sequences, 25.7% (112/436) of cases had RASs, with C316N at the highest rate (47.6%). Among 437 cases with NS3/4A and NS5A sequences available, 141 (32.3%) showed at failure complex NS5A and/or NS3 RASs patterns.
Table 2 shows the distribution of RASs for specific target region according to genotype and viral subtype. NS5A mutations were highly prevalent in all HCV genotypes and comparably in the most common genotypes 1a (82.0%, 89/109) and 1b (96.1%, 148/154). NS5B RASs were most prevalent in genotype 1b (56.8%, 84/148), while genotype 1a had more NS3/4A RASs (68.0%, 71/105).
Complete sequence information on the three target regions was available for the majority of cases (88%, 412/468); RASs in all the three target regions were detected in 14.6% (60/412), while 13.1% (54/412) of isolates showed no RASs.
Patients without RASs were similar to those with RASs for gender, median of calendar year, baseline viral load and previous treatment history. Notably, 54 patients without RASs were younger (median age 54 years, 48-57 *vs* 56 years, 53-63, among 358 with RASs, $p<0.001$). In patients without RAS liver cirrhosis was reported less frequently (24/54, 44% *vs* 224/332, 67%, $p=0.02$), while HIV coinfection was most common (14/37, 26% *vs* 23/299, 8%, $p<0.001$). According to HCV genotype, among patients without RASs genotype 3 prevailed (23/54, 43%), followed by 2 and 4 (both 10/54, 19%), 1a (7/54, 13%) and 1b (3/54,

Table 1 - Baseline clinical and virological population characteristics (n=468).

Male gender, % (n/N)	73.5 (344/468)
Age, median in years (IQR)	56.0 (51.7-62.3)
Metavir fibrosis stage	
F0-F1, % (n/N)	11.6 (45/387)
F2, % (n/N)	8.5 (33/387)
F3, % (n/N)	8.5 (33/387)
F4, % (n/N)	71.3 (276/387)
Liver cirrhosis, % (n/N)	64.6 (277/429)
HBsAg positivity, % (n/N)	6.0 (8/133)
HBcAb positivity, % (n/N)	30.7 (46/150)
HIV coinfection, % (n/N)	10.9 (41/373)
Previous ribavirin use, % (n/N)	23.5 (110/468)
Previous interferon use, % (n/N)	23.5 (109/468)
Previous DAA use, % (n/N)	5.8 (27/468)
HCV genotype	
1b, % (n/N)	32.9 (154/468)
3a/g/h/k, % (n/N)	28.0 (131/468)
1a, % (n/N)	23.3 (109/468)
4a/d/n/o/r/v, % (n/N)	9.6 (45/468)
2a/c, % (n/N)	6.2 (29/468)
HCVRNA log10 baseline, median (IQR)	6.14 (5.7-6.50)
Calendar genotype year, median (IQR)	2016 (2015-2017)

Abbreviations: IQR, interquartile range; HBsAg, HBV surface antigen; HBcAb, Anti HBV core antigen antibodies; HIV, human immunodeficiency virus; HCV hepatits C virus; DAA, direct acting antivirals; HCVRNA, hepatitis C viremia.

Table 2 - Resistance associated substitution detected at virological failure according to viral genotype.

			Frequency, n(%)					
REGION	*POSITION*	*MUTATION*	*OVERALL*	*GT1a n=109*	*GT1b n=154*	*GT2 n=29*	*GT3 n=131*	*GT4 n=45*
NS3/4A	36	V36L	6/253 (2.4)	3/105 (2.9)	3/148 (2.0)			
		V36M	4/253 (1.6)	4/105 (3.8)	0			
	43	F43L	1/105 (0.9)	1/105 (0.9)				
	54	T54S	18/253 (7.1)	6/105 (5.7)	12/148 (8.2)			
	55	R55A/I	3/105 (2.9)	3/105 (2.9)				
		R55I	3/105 (2.9)	3/105 (2.9)				
	56	Y56F	8/253 (3.2)	0	8/148 (5.4)			
		Y56H	20/411 (4.9)	8/105 (7.6)	12/148 (8.2)	0	0	
	80	Q80K	37/369 (10.0)	31/105 (29.5)	2/148 (1.4)	4/116 (3.4)		
		Q80L	7/253 (2.8)	4/105 (3.8)	3/148 (2.0)			
		Q80R	1/411 (0.2)	0	0		1/116 (0.8)	0
	122	S122G	11/253 (4.4)	8/105 (7.6)	3/148 (2.0)			
		S122N	3/105 (2.9)	3/105 (2.9)				
		S122T	18/148 (12.2)		18/148 (12.2)			
	155	R155K	6/295 (2.0)	6/105 (5.7)	0			0
		R155S	1/147 (0.7)	1/105 (0.9)				0
		R155T	1/253 (0.4)	1/105 (0.9)	0			
	156	A156G	4/411 (1.0)	0	0		4/116 (3.4)	0
		A156S	1/295 (0.3)	0	0			1/42 (2.4)
		A156T	3/437 (0.7)	1/105 (0.9)	1/148 (0.7)	0	0	1/42 (2.4)
	168	D168A	9/321 (2.8)	5/105 (4.8)	4/148 (2.7)	0		0
		D168E	5/321 (1.6)	1/105 (0.9)	3/148 (2.0)	0		1/42 (2.4)
		D168G	1/253 (0.4)	0	1/148 (0.7)			
		Q168K	1/116 (0.8)				1/116 (0.8)	
		Q168R	1/116 (0.8)				1/116 (0.8)	
		D168V	31/321 (9.7)	5/105 (4.8)	20/148 (13.6)	2/26 (7.7)		4/42 (9.5)
		D168Y	1/253 (0.4)	1/105 (0.9)	0			
	170	V170A	1/148 (0.7)		1/148 (0.7)			
		I170V	5/105 (4.8)	5/105 (4.8)				
	174	N174S	16/105 (15.2)	16 (15.24)				
NS5A	24	K24R	1/109 (0.9)	1/105 (0.9)				
	28	M28A	1/109 (0.9)	1/105 (0.9)				
		L/F28C	15/29 (51.7)			15/29 (51.7)		
		M28I	1/131 (0.8)				1/131 (0.7)	
		M28K	1/131 (0.8)				1/131 (0.7)	
		L28M	26/154 (16.9)		26/148 (16.9)			
		L/I28M	6/45 (13.3)					6/45 (13.3)
		L28S	2/138 (1.4)	0		2/29 (6.9)		
		L/I28S	4/45 (8.9)					4/45 (8.9)
		M28T	7/109 (6.4)	7/109 (6.4)				
		L/I28T	1/199 (0.5)		1/154 (0.6)		0	

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			Frequency, n(%)					
REGION	*POSITION*	*MUTATION*	*OVERALL*	*GT1a n=109*	*GT1b n=154*	*GT2 n=29*	*GT3 n=131*	*GT4 n=45*
NS5A	28	L/I28V	5/45 (11.1)					5/45 (11.1)
		M28V	13/263 (4.9)	10/109 (9.2)	3/154 (1.9)			
	30	Q30D	1/109 (0.9)	1/109 (0.9)				
		Q30E	2/263 (0.7)	2/109 (1.8)	0			
		Q30G	1/394 (0.2)	0	0		1/131 (0.7)	
		R30H	2/154 (1.3)		2/154 (1.3)			
		L/R30H	2/45 (4.4)					2/45 (4.4)
		Q30H	15/439 (3.4)	11/109 (10.1)	2/154 (1.3)	0	2/45 (4.4)	
		Q30K	19/240 (7.9)	5/109 (4.6)			14/131 (10.7)	
		Q30L	3/109 (2.7)	3/109 (2.7)				
		Q30N	1/109 (0.9)	1/109 (0.9)				
		R30Q	18/199 (9.0)		18/154 (11.7)		0	
		L30R	3/45 (6.7)					3/45 (6.7)
		Q30R	35/109 (32.1)	35/109 (32.1)				
		L/R30S	1/45 (2.2)					1/45 (2.2)
		Q30V	1/176 (0.6)				1/131 (0.7)	0
	31	L31F	5/394 (1.7)	0	3/154 (1.9)	2/131 (1.5)		
		L31I	12/423 (2.8)	0	12/154 (7.8)	0	0	
		L/M31I	1/45 (2.2)					1/45 (2.2)
		M31L	1/45 (2.2)					1/45 (2.2)
		L31M	63/423 (14.9)	16/109 (14.7)	43/154 (27.9)	2/29 (6.9)	2/131 (1.5)	
		L/M31V	6/45 (13.3)					6/45 (13.3)
		L31V	19/423 (4.5)	5/109 (4.6)	14/154 (9.1)	0	0	
	58	H58D	6/109 (1.9)	6/109 (5.5)				
	62	A62L	16/131 (12.2)				16/131 (12.2)	
	92	A92K	1/263 (0.4)	0	1/154 (0.6)			
		C92S	2/29 (6.9)			2/29 (6.9)		
		A92T	3/263 (1.1)	0	3/154 (1.9)			
	93	Y93C	9/308 (2.9)	2/109 (1.8)	2/154 (1.3)		5/45 (11.1)	
		Y93H	251/ (53.6)	16/109 (14.7)	136/154 (88.3)	0	91/131 (69.5)	8/45 (17.8)
		Y93N	9/468 (1.9)	8/109 (7.34)	1/154 (0.6)	0	0	0
		Y93R	1/439 (0.2)	0	1/154 (0.6)	0	0	
		Y93S	3/308 (0.9)	1/109 (0.9)	0			2/45 (4.4)
NS5B	159	L159F	63/395 (15.9)	1/102 (1.0)	61/148 (41.5)	0	1/123 (0.8)	
	282	S282T	11/436 (2.5)	0	4/148 (2.7)	0	3/123 (2.4)	4/41 (9.5)
	316	C316N	70/148 (47.6)		70/148 (47.6)			
		C316Y	4/250 (1.6)	2/102 (1.9)	2/148 (1.4)			
	414	M414I	4/250 (1.6)	0	4/148 (2.7)			
		M414T	1/250 (0.4)	0	1/148 (0.6)			
		M414V	1/250 (0.4)	0	1/148 (0.7)			
	445	C445F	1/148 (0.7)		1/148 (0.7)			
	448	Y448H	1/250 (0.4)	0	1/148 (0.7)			

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Frequency, n(%)								
REGION	*POSITION*	*MUTATION*	*OVERALL*	*GT1a n=109*	*GT1b n=154*	*GT2 n=29*	*GT3 n=131*	*GT4 n=45*
NS5B	451	C451S	9/148 (6.1)		9/148 (6.1)			
	553	A553T	1/102 (1.0)	1/102 (1.0)				
		A553V	1/250 (0.4)	1/102 (1.0)	0			
	556	S556G	38/250 (15.3)	14/102 (13.7)	24/148 (16.3)			
		S556N	2/102 (1.9)	2/102 (1.9)				
	558	G558R	1/102 (1.0)	1/102 (1.0)				

6%), while among those with RASs genotype 1b was more frequent (140/358, 39%), followed by 1a (92/358, 25.6%), 3 (87/358, 24.2%), 4 (28/358, 7.8%) and 2 (12/358, 3.3%) (p<0.001).

According to DAAs regimens, patients without RASs were treated as follows: DCV+SOF in 19 (35%), VEL/SOF in 13 (24%), LDV/SOF in 9 (17%), GLE/PIB 7 (13%), 3D in 6 (11%), while among those with RASs the treatments were: LDV/SOF in 108 (30%), DCV+SOF in 76 (21.2%), 3D in 76 (21.2%), EBR/GRZ in 44 (12%), VEL/SOF in 34 (9.5%), GLE/PIB 15 (4.2%), 2D 6 (1.7%) (p<0.001).

During the study period the prevalence of any RASs significantly decreased: 13/13, 100% in 2015, 126/145, 87% in 2016, 122/130, 94% in 2017, 122/154, 79% in 2018, 22/26, 85%, in 2019 (p=0.004). The RAS prevalence decreased especially in NS3/4A (9/12, 75% in 2015, 53/131, 86% in 2016, 44/123, 91% in 2017, 45/147, 73% in 2018 and 1/24, 4% in 2019; p<0.001) and the NS5A region (13/13, 100% in 2015, 125/145, 86% in 2016, 118/130, 91% in 2017, 113/154, 73% in 2018, 21/26, 81% in 2019; p=0.001), while it tended to slowly decrease for NS5B (4/12, 33% in 2015, 37/137, 27% in 2016, 41/123, 33% in 2017, 27/142, 19% in 2018, 3/22, 14% in 2019; p=0.05) (Figure 1).

In a multivariate model, adjusting for calendar year, gender, liver cirrhosis, and previous HCV treatment, we detected a higher rate of RASs in older patients and a lower risk with genotype 2 and 4 compared to genotype 1a (Table 3).

Complete resistance to the DAA regimens recommended by current guidelines ranged from 0%

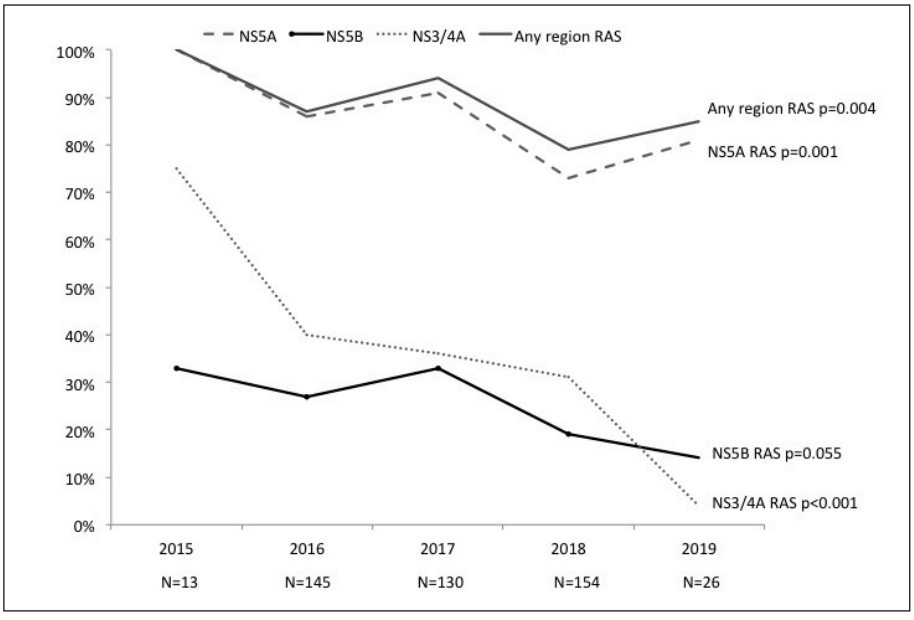

Figure 1 - Resistance Associated Substitutions trends over the study period.

Table 3 - Predictors of RASs.

Variable	*Univariate analysis*			*Multivariate analysis*		
	OR	*95% CI*	*P value*	*aOR*	*95% CI*	*P value*
Age, per 10 years increase	1.66	1.27-2.18	<0.001	1.49	1.06-2.09	0.02
Female gender vs male	2.65	1.22-5.75	0.01	2.06	0.80-5.25	0.13
Liver cirrhosis	2.09	1.19-3.68	0.01	1.54	0.76-3.15	0.23
Previous HCV treatment	2.47	1.18-5.16	0.02	2.04	0.89-4.67	0.09
Treatment calendar year	0.73	0.55-0.96	0.03	1.15	0.61-1.24	0.46
Genotype 1a	1			1		
Genotype 1b vs 1a	2.97	0.87-10.12	0.08	2.41	0.59-9.91	0.22
Genotype 2 vs 1a	0.11	0.04-0.31	<0.001	0.13	0.04-0.46	<0.001
Genotype 3 vs 1a	0.29	0.13-0.67	0.01	0.33	0.14-0.79	0.93
Genotype 4 vs 1a	0.24	0.09-0.66	0.01	0.25	0.08-0.72	<0.001

Table 4 - Predicted susceptibility to DAAs-treatment strategies recommended by current guidelines.

REGIMEN			*According to HCV genotype n/N (%)*				
Susceptibility		*Overall*	*G1a*	*G1b*	*G2*	*G3*	*G4*
VEL/SOF	Full resistance	4/439 (0.9)	0/102 (0)	2/148 (1.4)	0/22 (0)	2/124 (1.6)	0/43 (0)
	1 DAA resistance	254/439 (57.9)	31/102 (30.4)	131/148 (88.5)	0/22 (0)	85/124 (68.5)	7/43 (16.3)
	Reduced susceptibility	89/439 (20.3)	55/102 (53.9)	5/148 (3.4)	5/22 (22.7)	13/124 (10.5)	11/43 (25.6)
	Full susceptibility	92/439 (21.0)	16/102 (15.7)	10/148 (6.8)	17/22 (77.3)	24/124 (19.4)	25/43 (58.1)
EBR/GRZ	Full resistance	23/295 (7.8)	13/105 (12.4)	6/148 (4.1)	n.a	n.a	4/42 (9.5)
	1 DAA resistance	192/295 (65.1)	62/105 (59.0)	127/148 (85.8)	n.a	n.a	3/42 (7.1)
	Reduced susceptibility	40/295 (13.6)	14/105 (13.3)	6/148 (4.1)	n.a	n.a	20/42 (47.6)
	Full susceptibility	40/295 (13.6)	16/105 (15.2)	9/148 (6.1)	n.a	n.a	15/42 (35.7)
GLE/PIB	Full resistance	1/437 (0.2)	0/105 (0)	0/148 (0)	0/26 (0)	1/116 (0.9)	0/42 (0)
	1 DAA resistance	21/437 (4.8)	13/105 (12.4)	1/148 (0.7)	0/26 (0)	7/116 (6.0)	0/42 (0)
	Reduced susceptibility	110/437 (25.2)	70/105 (66.7)	18/148 (12.2)	2/26 (7.7)	16/116 (13.8)	4/42 (9.5)
	Full susceptibility	305/437 (69.8)	22/105 (21.0)	129/148 (87.2)	24/26 (92.3)	92/116 (79.3)	38/42 (90.5)
VEL/SOF/VOX	Full resistance	0 (0)	0/99 (0)	0/143 (0)	0/22 (0)	0/111 (0)	0/40 (0)
	2 DAA resistance	10/415 (2.4)	1/99 (1.0)	7/143 (4.9)	0/22 (0)	1/111 (0.9)	1/40 (2.5)
	Reduced susceptibility	168/415 (40.5)	57/99 (57.6)	86/143 (60.1)	0/22 (0)	20/111 (18)	5/40 (12.5)
	Full susceptibility	237/415 (57.1)	41/99 (41.4)	50/143 (35.0)	22/22 (100)	90/111 (81.1)	34/40 (85)

Abbreviations: VEL/SOF, velpatasvir/sofosbuvir; EBR/GRZ, elbasvir/grazoprevir; GLE/PIB, glecaprevir/pibrentasvir; VEL/SOF/VOX, velpatasvir/sofosbuvir/voxilaprevir.

with SOF/ VEL/VOX to 7.8% with EBR/GRZ. Notably, GLE/PIB and SOF/VEL/VOX remained at least partially active in >95% of isolates (Table 4).

DISCUSSION

Despite the excellent efficacy of current DAAs, some patients still fail to eliminate HCV [1-3]. Detection of RASs at failure is frequent, but its impact on virological efficacy of subsequent treatment current strategies is controversial [4, 6, 9]. Characterization of the resistance profile can be helpful in guiding re-treatment choice [10, 11].

We analysed the impact of baseline virological and clinical characteristics on RASs development in a large Italian real-life setting of HCV infected patients failing treatment over the years 2015 to 2019 and explored the potential efficacy of rescue treatment strategies.

The strengths of our study are the national representativeness of a real-life cohort and the large time period analysed. The main limitations of the study are the lack of data regarding pre-treatment RASs and patients' adherence.

In line with other studies [14, 15], this work confirms high frequency of RASs at DAA failure, heterogeneously distributed on the basis of drug exposure and genotype.

Similar to other studies, the main polymorphism observed in NS5A was Y93H, which confers high levels of resistance to all NS5A inhibitors, especially associated with another mutation [16].

In genotype 1a carriers, the NS3 Q80K polymorphism was highly prevalent and it was known for conferring lower response rates to simeprevir. Regarding newer NS3 inhibitors, voxilaprevir could be slightly impacted by the Q80K polymorphism during 8 weeks of treatment with sofosbuvir/velpatasvir, but no impact was reported with 12-weeks sofosbuvir/velpatasvir re-treatment strategies [17-19].

Other NS3 RASs including S122T, with the exception of N174S relevant only for telaprevir susceptibility, was detected at high frequencies in genotype 1b sequences.

The real impact of RASs depends on some factors, such as liver fibrosis, drug regimens and treatment adherence.

Interestingly, we documented decreasing prevalence of RASs at DAA failure over calendar years, probably reflecting the increasing potency of newer drug combinations. In line with previous reports, RASs were associated with DAA exposure, with the exception of NS5B RAS [20-22].

The clinical impact of DAA resistance must be analysed in relation to the availability of retreatment options. In this context, it is reassuring that 95% of cases were predicted to remain treatable with the latest developed drug combinations recommended for re-treatment. Accordingly, a recent real-life study in a large cohort of Italian patients demonstrated excellent efficacy of SOF/VEL/VOX in patients with previous DAA failure harboring drug-resistant virus population [21].

Resistance testing for all 3 HCV regions (NS3, NS5A and NS5B) after DAAs regimens virological failure was suggested by the results of an Italian study to individualize re-treatment strategies [20].

In conclusion, such as individual complex failure cases may remain highly challenging, recommended and in some cases personalized re-treatment regimens are both relevant step towards HCV eradication.

Conflict of interest

BR received speakers' honoraria and support for travel to meetings from Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme (MSD), ViiV Healthcare, and fees for attending advisory boards from ViiV Healthcare, Janssen-Cilag, Merck Sharp & Dohme (MSD), Gilead Sciences, Bristol-Myers Squibb (BMS).

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