Prevalence and resistance profiles of “Unusual” HCV subtypes in Italy

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BACKGROUND

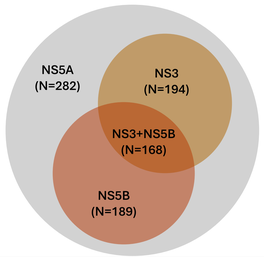
Recent data show that “unusual” hepatitis C virus (HCV) subtypes have lower responses to direct-acting antivirals (DAAs) compared to most prevalent subtypes. We aimed to investigate the prevalence and resistance profiles of “unusual” HCV subtypes in Italy.

METHODS

- Design: Multicenter real-world retrospective study within HCV Italian resistance network between 2015-2020.
- Clinical and virological data of “unusual” HCV genotype (GT)/subtypes (**defined as GT1 non-1a/b, GT2 non-2a/b, GT3 non-3a, GT4 non-4a/d**) were analyzed.
- Sanger sequencing for **NS3, NS5A and NS5B** was performed in 12 Italian laboratories, subtype assignment was confirmed by phylogenetic analyses on NS3±NS5A±NS5B sequences (Fig 1).
- The prevalence of resistance associated substitutions (**RASs**) and resistance associated positions (**PRASs**) were evaluated according to *Sorbo et al 2018, EASL guidelines 2020 and AASLD guidelines 2021.*
- Categorical variables were compared using x^2 or Fischer's exact were appropriate.

RESULTS

Figure 1: Availability of NS3, NS5A, NS5B sequences and unusual subtype distribution



Available HCV NS3, NS5A, and NS5B sequences in the study population (N=282 individuals).

Panel A: Venn diagram;

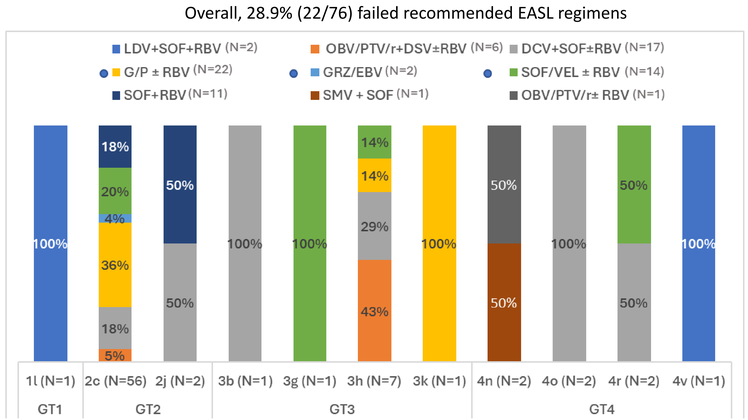
Panel B: Specificity for each gene and unusual subtype.

	Number of individuals N (%)	Overall (N=282)	DAA Naïve (N=206)	DAA Failure (N=76)
NS5A	GT1 (1g, 1i, 1l)	6 (2.1)	5 (1g=3, 1i=1, 1l=1)	1 (1l=1)
	GT2 (2c, 2j)	249 (88.3)	191 (2c=191)	58 (2c=56, 2j=2)
	GT3 (3b, 3g, 3h, 3k)	11 (3.9)	1 (3h=1)	10 (3b=1, 3g=1, 3h=7, 3k=1)
	GT4 (4c, 4i, 4l, 4m, 4n, 4o, 4r, 4v)	16 (5.7)	9 (4c=1, 4i=1, 4l=1, 4m=1, 4n=1, 4o=3, 4r=1)	7 (4n=2, 4o=2, 4r=2, 4v=1)
	Number of individuals N (%)	**Overall (N=194)**	**DAA Naïve (N=131)**	**DAA Failure (N=63)**
NS3	GT1 (1g, 1i, 1l)	5 (2.6)	4 (1g=2, 1i=1, 1l=1)	1 (1l=1)
	GT2 (2c)	167 (86.1)	117 (2c=117)	50 (2c=50)
	GT3 (3b, 3g, 3h, 3k)	6 (3.1)	1 (3h=1)	5 (3b=1, 3g=1, 3h=2, 3k=1)
	GT4 (4c, 4i, 4l, 4m, 4n, 4o, 4r, 4v)	16 (8.2)	9 (4c=1, 4i=1, 4l=1, 4m=1, 4n=1, 4o=3, 4r=1)	7 (4n=2, 4o=2, 4r=2, 4v=1)
	Number of individuals N (%)	**Overall (N=189)**	**DAA Naïve (N=128)**	**DAA Failure (N=61)**
NS5B	GT1 (1g, 1l)	2 (1.1)	1 (1g=1)	1 (1l=1)
	GT2 (2c, 2j)	165 (87.3)	119 (2c=117)	46 (2c=44, 2j=2)
	GT3 (3b, 3g, 3h, 3k)	8 (4.2)	0	8 (3b=1, 3g=1, 3h=5, 3k=1)
	GT4 (4c, 4l, 4m, 4n, 4o, 4r, 4v)	14 (7.4)	9 (4c=1, 4l=1, 4m=1, 4n=1, 4o=3, 4r=1)	6 (4n=2, 4o=2, 4r=1, 4v=1)

KEY FINDINGS

- GT2c was the most frequent unusual subtype in Italians and GT3 infected individuals were more prone to failure (91%), p<0.0001
- Number of RASs/PRASs varied by genotype, subtype and treatment exposure
- NS5A-RAS/PRAS were most prevalent (88.2% at failure and 95.1% in naïve population), NS3-RASs/PRASs were present in all GT3 failures, NS5B-RASs/PRASs had variable patterns
- NS5-RASs at positions 93H/F/S were detected only at failure in GT3h/k and GT4o/v
- Sofosbuvir mutation S282C was present only in one GT4o DAA failing individual

Figure 2. Treatments used by the 76 patients infected with unusual HCV subtypes who failed DAA therapy



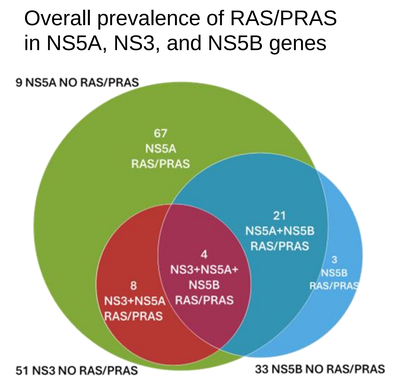
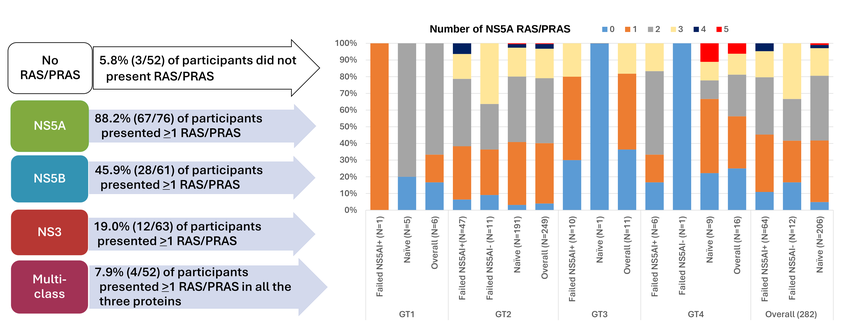
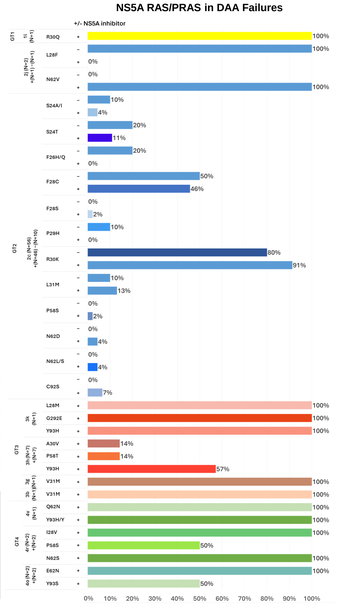
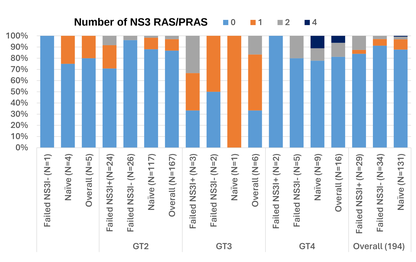
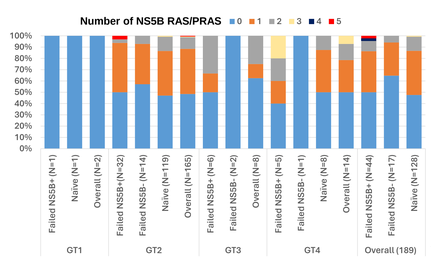


Figure 3. Detailed prevalence and frequencies of RAS/PRAS in NS5A, NS3, and NS5B among the 76 patients infected with unusual HCV subtypes who failed DAA therapy









#All infected individuals with GT3b and GT3g who failed NS5A inhibitor containing regimens had the V31M RAS (100%), and also the 30K as *wild-type* amino acid for their specific subtype (100%).

Table 1. Characteristics of the study population infected with unusual HCV subtypes

Number of individuals	Overall (N=282)	DAA naive (N=206)	DAA failure (N=76)
Male, N (%)	164 (58.2)	107 (51.9)	57 (75.0)
Median age, Years (IQR)[a]	69.7.5 (53.6-76.5)	70.0 (53.4-78.1)	69.0 (53.8-74.9)
Italian origin, N (%)[b]	229 (91.6)	184 (93.9)	45 (83.3)
Cirrhotic, N (%)[c]	57 (28.9)	45 (28.3)	12 (31.6)
HCV genotype/subtype, N (%)			
GT1 (1g, 1i, 1l)	6 (2.1)	1g=3, 1i=1, 1l=1	1l=1
GT2 (2c[d], 2j)	249 (88.3)	2c=191	2c=56, 2j=2
GT3 (3b, 3g, 3h, 3k)	11 (3.9)	3h=1	3b=1, 3g=1, 3h=7, 3k=1
GT4 (4c, 4i, 4l, 4m, 4n, 4o, 4r, 4v)	16 (5.7)	4c=1, 4i=1, 4l=1, 4m=1, 4n=1, 4o=3, 4r=1	4n=2, 4o=2, 4r=2, 4v=1

[a]The median and interquartile percentile were performed on 248 individuals with data available (189 DAA naive and 59 DAA failures). [b]The prevalence was performed on 250 individuals with data available (196 DAA naive and 54 DAA failures; non-Italian origin as follows: 18 (7.2%) African and 3 (1.2%) Asian. [c]The prevalence was performed on 197 individuals with data available (159 DAA naive and 38 DAA failures). [d] The GT2c is the most prevalent subtype in Italy.

Abbreviation, DAA; direct-acting antiviral, GT; genotype

Table 2. Retreatment and outcome of 13 DAA failures infected with unusual HCV subtypes

ID	Subtype	Failed DAA Regimen	Duration (weeks)	Cirrhosis	Age (years)/ sex	Failure	RAS at failure (baseline of retreatment) NS5A RAS	NS3 RAS	NS5B RAS	Retreatment DAA Regimen	Duration (weeks)	Outcome
1	2c	SOF/VEL	12	No	61/F	No response	None	None	T150A	G/P	12	No response
2	2c	OBV/PTV/r+ DSV	12	No	77/M	Relapse	F28C, R30K	None	None	SOF+RBV	12	SVR
3	2c	OBV/PTV/r+ DSV	8	Yes	70/M	No response	F28C, R30K, C92S	L36V, D168V	None	SOF/VEL	24	SVR
4	2c	G/P	8	No	28/M	Relapse	R30K	F56Y	None	SOF/VEL/VOX	12	SVR
5	2c	G/P	8	No	63/M	Relapse	R30K	None	NA	SOF/VEL/VOX+RBV	12	SVR
6	2c	SOF/VEL	12	NA	75/F	No response	None	None	None	SOF/VEL/VOX	12	SVR
7	2c	G/P	8	No	73/M	Relapse	S24T, R30K	None	T150A	SOF/VEL/VOX+RBV	12	SVR
8	2c	G/P	8	No	48/M	Relapse	R30K	None	None	SOF/VEL/VOX	12	SVR
9	2c	SOF+RBV	20	Yes	NA/M	Relapse	S24T, K26H, R30K	None	None	SOF+DCV	12	SVR
10	2c	SOF+RBV	8	Yes	88/F	Relapse	R30K	None	S368Y	SOF+DCV	12	SVR
11	2c	G/P+RBV	8	No	70/M	Relapse	S24T, R30K	None	N444I	SOF/VEL/VOX+RBV	12	SVR
12	2c	SOF+DCV	12	No	78/F	Relapse	S24T, R30K	None	None	SOF/VEL+RBV	24	SVR
13	4o	SOF+DCV	12	Yes	61/M	Relapse	E62N	None	None	SOF/VEL/VOX	12	SVR

Abbreviations: DAA, direct-acting antiviral; RAS, Resistance associated substitution; SVR, sustained virological response; SOF/VEL, Sofosbuvir/Velpatasvir; G/P, Glecaprevir/Pibrentasvir; RBV, Ribavirin; SOF, Sofosbuvir; DCV, Daclatasvir; OBV/PTV/r, Ombitasvir/Paritaprevir/ritonavir; DSV, Dasabuvir; VOX, Voxilaprevir; NA, non-available; F, female; M, male.

CONCLUSIONS

In this Italian setting, GT1-GT4 unusual subtypes were frequent, predominated by GT2c in Italians, and with failure rates highest within GT3, followed by GT4. Most DAA-failures carried complex NS5A-RAS patterns, some conferring high-level of resistance. For some unusual subtypes, resistance is natural with *wild-type* amino acid being a RAS (e.g GT3b 30K). Our results advise for closer surveillance and further studies to better characterize the impact of “unusual” HCV subtypes on DAA efficacy.

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