New Microbiologica, 41, 1, 26-29, 2018, ISSN 1121-7138

FULL PAPER

Epidemiology and risk factors for hepatitis C virus genotypes in a high prevalence region in Italy

Antonio Riccardo Buonomo[1], Riccardo Scotto[1], Biagio Pinchera[1], Nicola Coppola[2], Caterina Monari[2], Margherita Macera[2], Guglielmo Borgia[1], Ivan Gentile[1]

[1]*Department of Clinical Medicine and Surgery, Section of Infectious Diseases, University of Naples "Federico II", Italy;*
[2]*Department of Mental Health and Public Medicine, Infectious Disease Unit, University of Campania Luigi Vanvitelli*

SUMMARY

Hepatitis C virus (HCV) is globally widespread. Southern Italy is a high prevalence region where the distribution of the HCV genotypes (GTs) is changing. Intravenous drug abuse is the only risk factor associated with a specific HCV GT (GT3). The aim of this study was to evaluate the distribution and the risk factors for specific HCV GTs. A total of 682 patients with measurable serum HCV-RNA were enrolled between January and March 2017. We recorded clinical information and the presence of risk factors for HCV. GT1b was the prevalent genotype in our patients (59.8%). HCV GT1a and GT3 infections were more frequent among patients aged ≤60 years (14.9% vs 2.2%, p<0.01 and 13.6% vs 0.8%, p<0.01, respectively). At multivariate analysis, intravenous drug abuse and age ≤60 years were associated with GT1a infection (OR: 4.79; 95% CI: 2.43-9.47, p <0.001 and OR: 5.07; 95CI: 2.25-11.40, p<0.001, respectively), while age ≤60 years was the only risk factor for GT3 (OR: 15.81; 95CI: 4.76-52.54, p <0.001). In the Campania region, we observed an increase in GT1a and GT3 rates compared with those observed in previous years. Age ≤60 was an independent risk factor for GT1a and GT3 infection. Intravenous drug use was independently associated with GT1a infection.

Received July 5, 2017 *Accepted* September 25, 2017

INTRODUCTION

About 80 million people worldwide are estimated to be chronically infected with hepatitis C virus (HCV) (Gower *et al.,* 2014), which is one of the leading causes of cirrhosis and hepatocellular carcinoma (HCC) worldwide (Poynard *et al.,* 2000; Seeff *et al.,* 2001). Risk factors for HCV infection include injection-drug use, haemodialysis, tattoos in an unregulated setting, exposure to HCV-infected blood, recipient of blood products and homosexual intercourse (Stroffolini *et al.,* 2004; Gentile *et al.,* 2013; Sagnelli *et al.,* 2014; Messina *et al.,* 2015). The recently available direct acting antivirals (DAA) will probably change this scenario (Gentile *et al.,* 2014c; Gentile *et al.,* 2015).

HCV is characterized by a remarkable genetic variability: seven genotypes (GTs) with a genomic identity greater than 65%, and at least 67 subtypes have been recognized so far (Simmonds *et al.,* 1994). The GT distribution varies according to geographic area. Indeed, GT1 prevails in Europe (subtype 1b) and in the United States (subtype 1a), GTs 2 and 3 are ubiquitous, GT4 is predominant in North Africa, particularly in Egypt, while GT5 is almost peculiar to South Africa (Messina *et al.,* 2015). In western Europe, GT1 is the most prevalent GT (55.1%), followed by GT3 (29%), GT2 (8.9%) and GT4 (5.8%) (Petruzziello *et al.,* 2016). In Italy, where HCV infection is endemic, it is estimated that about one million people are infected with HCV (Mancusi *et al.,* 2016). The prevalence rates of the various HCV GTs in Italy differ slightly from those recorded in other western European countries. In fact, GT1 infection rates are higher and GT2 infection rates are lower in Italy than in other western European countries (GT1a: 4.2%, GT1b: 57.5%, GT2: 3.0%, GT3: 26%, GT4: 3.6%) (Gower *et al.,* 2014). In Southern Italy, the prevalence of GTs 1a and 3 is higher in patients aged ≤60 years, while GT2 infection seems to be more frequent among patients aged >60 years (Petruzziello *et al.,* 2013). Furthermore, the rate of GT2 infection is higher in the Campania region, which is a high-prevalence area for HCV infection in Southern Italy, than in Northern Italy (GT1a: 5.5%, GT1b: 50.7%, GT2: 29.4%, GT3: 6.2%, GT4: 0.8%) (Petruzziello *et al.,* 2014).

It is well-known that the distribution of HCV genotypes differs among different groups at risk for HCV infection (Petruzziello *et al.,* 2013; Petruzziello *et al.,* 2014). For example, genotype 3 is very common among people who inject drugs (PWID) (Wiessing *et al.,* 2014). Conversely, no clear association has yet been identified between genotype 1a and PWID. Given the correlation between the distribution of HCV genotypes and risk factors for HCV infection, information on genotype prevalence and its temporal trend could shed light on epidemiological changes in these risk factors, and in turn may impact on health policies for the prevention of HCV infection.

The aim of our study was to determine the prevalence

Key words:
HCV, Epidemiology, Risk factors, People who inject drugs, Southern Italy.

Corresponding author:
Prof. Guglielmo Borgia
E-mail: borgia@unina.it

of the different HCV GTs in two university centers in the Campania region and to evaluate the association between risk factors for infection and the different genotypes.

MATERIALS AND METHODS

Consecutive anti-HCV positive and HCV-RNA-positive inpatients and outpatients referring to two University Infectious Diseases Departments (Department of Clinical Medicine and Surgery – Section of Infectious Diseases of University of Naples "Federico II" and Department of Mental Health and Public Medicine, Infectious Diseases Unit of the University of Campania "Luigi Vanvitelli") between January 2016 and March 2017 were enrolled. Patients with HIV co-infection were excluded from the study due to the different HCV genotypes distribution among this population (Loko *et al.,* 2010). At enrollment, the following demographic and clinical data of patients were recorded: presence of cirrhosis, presence of hepatocellular carcinoma (HCC), HCV genotype and co-infection with Hepatitis B virus (HBV). Patients were invited to repeat the HCV genotype test if such a test had been performed more than three years prior to enrolment. All patients completed a questionnaire concerning risk factors for HCV transmission at enrolment, a history of intravenous drug use, blood transfusions, surgical interventions, medical procedures, hemodialysis, dental surgery or accidental punctures, and piercings/tattoos or a mother-to-child transmission of the HCV infection.
HBV serum markers were sought using commercial immunoenzymatic assays (Abbott Laboratories, North Chicago, IL, USA, for HBsAg, anti-HBs and anti-HBc). The anti-HCV antibody was sought using a 3rd generation commercial immunoenzymatic assay (Ortho Diagnostic Systems, Neckargemund, Germany). Antibodies to HIV 1 and 2 were sought using a commercial ELISA (Abbott Lab., North Chicago, IL, USA). Viral RNA was extracted from 140 ul of plasma samples using a microspin column (QIAamp RNA viral kit, Qiagen GmbH, Hilden, Germany). HCV-RNA was quantified by performing a real-time polymerase chain reaction (PCR) in a Light cycler 1.5 (Roche Diagnostics, Branchburg, NJ, USA) (Macera *et al.,* 2017); the detection limit of this method in plasma samples is estimated at around 40 IU/mL. HCV genotypes were determined with the HCV genotype Lipa assay II (Bayer, France) according to the manufacturer's instructions.

Ethical aspects

The study was conducted in accordance with Good Clinical Practices and with the latest revision of the Helsinki Declaration. Data handling was in accordance with the Italian law on privacy. The patients were managed according to best routine clinical practice.

Statistical analysis

Among-group comparisons were made using the chi-squared test or the Fisher's exact test when appropriate. The cut-off for statistical significance was set at 5% in two-tailed test. Univariate and multivariate analysis for the evaluation of risk factors were made using the logistic regression function. The variables that showed a significant association at the univariate analysis were included in the multivariate model analysis. The cut-off for statistical significance of the logistic regression function was set at 5%.

RESULTS

A total of 682 patients with chronic HCV infection were enrolled in the study; half of them were female (344/682, 50.4%); median age was 62 years (IQR: 52-70), with about half of the patients being >60 years old (374/682, 54.8%). About one-third of patients had liver cirrhosis (233/682, 34.2%), 18/682 (2.6%) had HCC, only 7/682 (0.7%) had HBV co-infection. Regarding risk factors for HCV transmission, 125/682 (18.4%) patients reported a history of surgical interventions other than dental surgery, 284/682 (41.6%) reported a history of dental surgery, 56/682 (8.2%) had at least one previous blood transfusion and 58/682 (8.5%) were PWID. Only 5/682 (0.7%) and 6/682 (0.9%) patients had a history of accidental puncture or at least one tattoo/piercing, respectively. Finally, 16/282 (2.3%) patients were born from an HCV-positive mother. No patient was on haemodialysis.
The distribution of HCV GTs among the enrolled patients is shown in *Table 1*. GT1 was the predominant genotype (n=485/682, 71%) and GT1b was the most prevalent subtype (408/682, 59.8%). HCV GT1a and GT3 infection were significantly more frequent among patients aged ≤60 years compared to those aged >60 years (14.9% vs 2.2%, p<0.01 and 13.6% vs 0.8%, p<0.01, respectively), while the rate of GT1b infection was significantly higher in patients aged >60 years compared with those aged ≤60 years (68.4% vs 48.4%, p<0.01) *(Figure 1)*. Notably, PWID were more frequent among patients aged ≤60 years than among patients aged >60 years (18.4% vs 0.5%, p<0.001). No other significant distribution patterns of HCV GTs were found in relation to the age of patients, although the prevalence of GT2 tended to be higher in patients aged >60 years versus those aged ≤60 years (23.5% vs 17.7%, p=0.065).
Among PWID, GT1a (20/58, 34.5%) and GT3 (11/58, 19%) were the most frequent GTs. In fact, PWID were found

Table 1 - *Distribution of HCV genotypes according to age and risk factors.*

HCV Genotype	*All Patients (n=682)*	*Age ≤60 years (n=308)*	*Age >60 years (n=374)*	*Previous surgical interventions (n=125)*	*Previous blood transfusions (n=56)*	*Intravenous drug use (n=58)*
1* (n, %)	24 (3.5)	9 (2.9)	16 (4.3)	13 (10.4)	1 (1.8)	5 (8.6)
1a (n, %)	53 (7.8)	46 (14.9)	8 (2.2)	4 (3.2)	5 (8.9)	20 (34.5)
1b (n, %)	408 (59.8)	149 (48.4)	256 (68.4)	62 (49.6)	27 (48.2)	15 (25.9)
2 (n, %)	143 (21)	54 (17.5)	88 (23.5)	38 (30.4)	14 (25)	6 (10.3)
3 (n, %)	44 (6.4)	42 (13.6)	3 (0.8)	6 (4.8)	8 (14.3)	11 (19)
4 (n, %)	10 (1.5)	8 (2.6)	3 (0.8)	2 (1.6)	1 (1.8)	1 (1.7)

*Genotype 1c, or not known whether Genotype 1a or 1b.

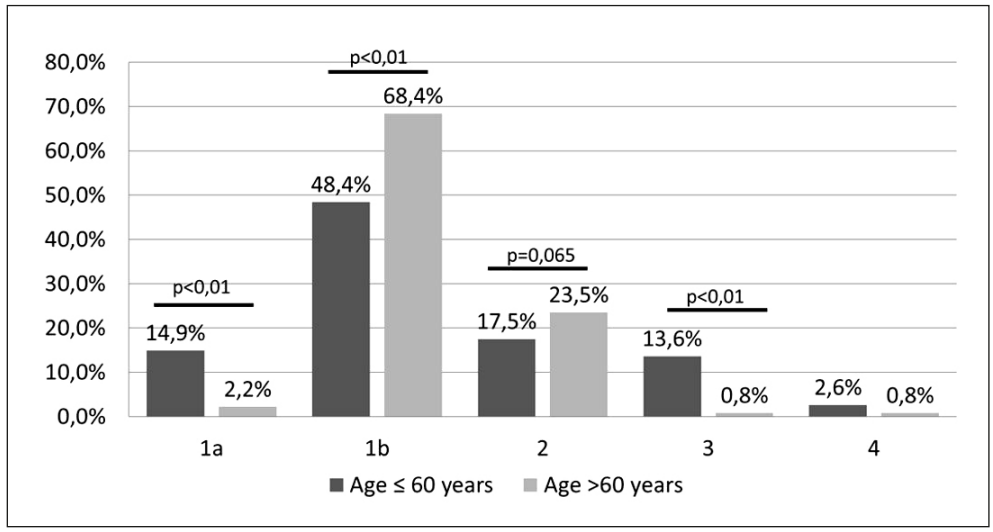

Figure 1 - *Prevalence of HCV genetypes according to age.*

to be at risk for both GT1a (OR: 9.41; 95CI: 4.94-17.94, p<0.001) and GT3 infection (OR: 4.18; 95CI: 1.99-8.80, p<0.001). Other associations between specific GT infection and risk factors for HCV are shown in *Table 2*. As shown in *Table 3*, when PWID, tattoo/piercing and age ≤60 were included in the multivariate analysis of risk factors for GT1a infection, only PWID (OR: 4.79; 95CI: 2.43-9.47, p<0.001) and age ≤60 (OR: 5.07; 95CI: 2.25-11.40, p<0.001) remained significantly associated with GT1a infection. Furthermore, when PWID, previous blood transfusion and age ≤60 were included in the multivariate analysis of risk factors for GT3 infection, only the latter remained significantly associated with GT3 infection (OR: 15.81; 95CI: 4.76-52.54, p<0.001).

DISCUSSION

The availability of IFN-free antiviral therapies for HCV (namely, DAA) that have few side-effects and result in a high percentage of sustained virological responses (SVR), and the aging of previously untreated or non-responder HCV-infected patients will probably change the epidemiology of HCV genotypes (Gentile *et al.,* 2014a; Gentile *et al.,* 2014b). We found that the prevalence of HCV GT1a infec-

Table 2 - *Univariate analysis of risk factors for specific HCV genotypes infection.*

	Genotype 1a			*Genotype 1b*			*Genotype 2*			*Genotype 3*			*Genotype 4*		
	OR	*95CI*	*p*	*OR*	*95CI*	*p*	*OR*	*95CI*	*p*	*OR*	*95CI*	*p*	*OR*	*95CI*	*p*
Surgical Interventions	0.34	0.12-0.97	<0.05	0.60	0.40-0.88	0.01	*1.90*	*1.23-2.94*	*<0.05*	0.69	0.28-1.66	0.406	1.11	0.23-5.31	0.892
Dental Surgery	1.30	0.73-2.33	0.374	*1.58*	*1.16-2.17*	*<0.05*	0.67	0.46-0.99	<0.05	1.30	0.71-2.40	0.398	0.93	0.26-0.34	0.915
Blood Transfusions	1.18	0.45-3.09	0.739	1.68	0.97-2.90	0.065	1.29	0.69-2.44	0.426	*2.73*	*1.20-6.19*	*<0.05*	1.24	0.15-10.0	0.837
PWID	*9.41*	*4.94-17.94*	*<0.001*	0.24	0.11-0.38	<0.001	0.41	0.17-0.98	<0.05	*4.18*	*1.99-8.80*	*<0.001*	1.20	0.15-9.62	0.866
Tattoo/Piercing	*6.12*	*1.09-34.20*	*<0.05*	1.34	0.24-7.38	0.735	#	#	#	#	#	#	#	#	#
Age ≤60	*7.83*	*3.63-16.89*	*<0.001*	0.44	0.32-0.60	<0.001	0.70	0.48-1.02	0.065	*19.0*	*5.82-62.0*	*<0.001*	2.87	0.74-11.2	0.129

OR: Odds Ratio. 95CI: 95% Confident Intervals. *p:* p-value. PWID: people who inject drug.
Not measurable: no patients with HCV GT2, 3 or 4 reported a history of tattoo/piercing.

Table 3 - *Multivariate analysis of risk factors for HCV genotype 1 and genotype 3 infection.*

	Genotype 1a			*Genotype 3*		
	OR	*95CI*	*p*	*OR*	*95CI*	*p*
Surgical Interventions	#	#	#	#	#	#
Dental Surgery	#	#	#	#	#	#
Blood Transfusions	#	#	#	2.05	0.87-4.85	0.102
PWID	*4.79*	*2.43-9.47*	*<0.001*	1.88	0.87-4.04	0.108
Tattoo/Piercing	2.49	0.39-15.79	0.332	#	#	#
Age ≤ 60	*5.07*	*2.25-11.40*	*<0.001*	*15.81*	*4.76-52.54*	*<0.001*

OR: Odds Ratio. 95CI: 95% Confident Intervals. *p:* p-value. PWID: people who inject drug.
Variables not associated with specific HCV GT infection at univariate analysis, they were not included in the multivariate model.

tion in the Campania region was higher than that reported four years earlier (7.8% vs 5.5%) (Petruzziello *et al.,* 2014). This may be related to the low rates of SVR in previous years with the poorly tolerated IFN-based treatments in patients with chronic HCV GT1a infection (Gentile *et al.,* 2005; Tosone *et al.,* 2007), and their consequent failure to attend follow-up appointments. GT1a was, in fact, one of the most difficult-to-treat HCV genotypes and patients with GT1a infection often had comorbidities that contraindicated treatment with IFN (namely, psychiatric disorders or intravenous drug abuse). Moreover, the abuse of intravenous drugs among younger people is probably contributing to the increase in GT1a infection. In fact, in our analysis, PWID and age ≤60 years were independent risk factors for GT1a infection, which highlights a strong correlation between PWID/younger age and infection with this genotype. A history of tattooing/piercing was also a risk factor for GT1a infection in our study, which confirms the association between GT1a infection and behaviours usually related to young people. On the other hand, our study found that the prevalence of HCV GT3 infection was very similar to that recorded in the immediate pre-DAA era, i.e., in 2013 (6.4% vs 6.2%) (Petruzziello *et al.,* 2014), but it was higher than that recorded in the years 2009-2011 (6.4% vs 4.2%) (Petruzziello *et al.,* 2013). We also found a reduction of the prevalence of HCV GT2 compared to the rate recorded in 2013 in the Campania region (21% vs 29.4%) (Petruzziello *et al.,* 2013), as well as high prevalence rates of HCV GT1b and GT2 in older patients (age >60 years). Interestingly, patients with HCV GT2 infection had high rates of SVR with IFN-based treatment strategies in the pre-DAA era (von Wagner *et al.,* 2005; Marotta *et al.,* 2016). This probably explains the low prevalence of GT2 in our cohort.

A history of dental surgery or surgical interventions were the only risk factors for HCV GT1b and GT2, respectively. These results may be explained by the less rigorous hygiene protocols and controls applied to surgical and dental interventions during past years, which allowed the spread of HCV GT1b and GT2 infection.

In conclusion, the distribution of HCV GTs is changing in the Campania region of Southern Italy. In fact, GT1a and GT3 infections are increasing at the expense of HCV GT2. Age ≤60 is an independent risk factor for GT1a as well as for GT3 infection. PWID was independently associated with GT1a infection.

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