New Epidemiologic Data Regarding Hepatitis C Virus Infection in Romania

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ABSTRACT

Background & Aims: Literature data suggest that HCV genotype-1b is present in 93-99% of the Romanian patients infected with hepatitis C virus (HCV). We present the genotyping tests recently performed on patients with HCV and advanced fibrosis eligible for the Direct-Acting Antiviral (DAA) therapy, as well as the prevalence of these cases across Romania.

Methods: The genotyping method was performed on 7,421 HCV patients with advanced fibrosis. The detection method was automatic real time PCR platform M2000 (Abbott). Every subject was introduced into a database including age, sex, county and address.

Results: Genotype 1b was almost exclusively present: 7,392/7,421 (99.6%). Genotype 1b patients were 19.6% from Bucharest, 49% were males, with a median age of 60 years. Genotype non-1b was encountered in 29/7,421 subjects (0.4%), 62% were males, 69% from Bucharest and the median age was 52 years. Most of the subjects (75%) were in the 6th and 7th age decade. The prevalence of these cases varied significantly across Romanian counties: the highest was in Bucharest (61.3/10⁵), Bihor (47/10⁵), Iasi (46/10⁵) and Constanța (43/10⁵), and the lowest in Ilfov (2.8/10⁵), Harghita (3.7/10⁵), Covasna (5.4/10⁵) and Maramureș (8.8/10⁵) (p<0.001).

Conclusions: Genotype 1b is encountered in 99.6% of patients with chronic hepatitis C and advanced fibrosis from Romania. The presence of genotypes non-1b is more common in Bucharest, in males and at a younger age. There are significant differences regarding the distribution of these cases across Romania: the highest rates are in Bucharest, Bihor, Iasi and Constanța.

Key words: liver cirrhosis – hepatitis C virus (HCV) – epidemiology – direct-acting antiviral agents - DAA.

Abbreviations: BMI: body mass index; DAA: direct-acting antiviral agent; GT: genotype; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IDU: intravenous drug users; MELD: model for end stage liver disease; NASH: non-alcoholic steatohepatitis; SVR: sustained virologic response.

INTRODUCTION

Hepatitis C virus (HCV) is one of the largest epidemic problems worldwide, affecting approximately 180 million people [1-3]. There are seven major genotypes of the virus and every genotype (GT) has different subtypes, being pathogenic only to humans [4-6]. According to different studies from the literature, 3 to 4 million people are newly infected every year worldwide, and 350,000 patients die every year due to HCV-related diseases [7-15]. The HCV prevalence varies around the world: there is a higher prevalence in Africa, Eastern Mediterranean, South-East Asia and the West Pacific, and a lower one in North America, Northern and Western Europe and Australia [16]. In Europe the prevalence of the infection increases with age, with a peak in 55–64-year-old patients [17]. Not only the prevalence varies around the world, but also the GTs have a certain distribution: in Europe the most common is subtype 1b, on the American continent it is subtype 1a, in North and Central Africa and in the Middle East GT 4 is prevalent and in Asia GT 3 [18-21]. The acute HCV infection is rarely diagnosed because there are no symptoms or the symptoms are mild. Unfortunately, 75–80% of the infections become chronic resulting in liver damage with varying degrees of inflammation and fibrosis, develop complications such as liver cirrhosis, hepatocarcinoma, and lead to liver transplant [16, 22-30]. The epidemiology of HCV in Europe is evolving due to an increasing number of intravenous drug users (IDU)
and immigration from endemic areas [31-34]. According to the current estimates based on HCV serology, the prevalence of 4.5% - 4.9% of HCV infection in the general population in Romania represents one of the highest figures in Europe [35-36]. Currently GT 1, and probably its subtype 1b is almost exclusively present in Romania [35-41]. The aim of this study is to present new data regarding HCV infection in Romania, as our country is one of the most important sources of migrant population towards Western Europe, in particular to countries such as Spain and Italy, therefore the prevalence of GT 1b might have changed. There is a need for a national strategy for the active detection and control of the silent epidemic of HCV-infected population in Romania and for treating chronic infection, which represents one of the highest prevalence rates in Europe.

METHODS

For analysing the epidemiology of viral C compensated liver cirrhosis in Romania, we used the database of the National Health Agency with patients evaluated to be included in the Direct-Acting Antiviral (DAA) therapy in December 2015-October 2016. For HCV genotyping, the National Health Agency contacted the central laboratories that performed genotyping on patients considered to be eligible for this therapy: Bioclinica and Synevo. In total, 7,421 with advanced fibrosis underwent genotyping. The real-time polymerase chain reaction (PCR) platform M2000 automatic (Abbott) was used: an amplification assay using as targets of a sequence of 5'-UTR region of the viral genome for the detection of genotypes 1-6 and the NS5B region for the separate identification of subtypes 1a and 1b; 4 sets of primers were used because 3 separate types of reactions are required.

The inclusion criteria were: compensated liver cirrhosis (Child-Pugh score ≤6), detectable HCV-RNA viral load, absence of significant drug-drug interactions, and lack of significant alcohol consumption in the previous 3 months.

Patients co-infected with hepatitis B virus (HBV) received anti-HCV therapy if they had an HBV-DNA viral load below 2000 IU/ml. Those with hepatocellular carcinoma (HCC) were included if they had no relapse of cancer 6 months after their last session of therapy (surgery or radiofrequency ablation or transarterial chemoembolization).

Advanced fibrosis (Metavir stage F4) was confirmed by Fibromax testing in most of the patients; in a minority of cases liver biopsy or Fibroscan was further utilized if clinical judgement suggested that Fibromax testing had initially misclassified the patient.

Only the serious adverse events leading to the discontinuation of therapy were reported.

The study was approved by the National Ethics Committee of Medicines and Medical Devices. All patients signed a written informed consent before entering in the study.

The recorded patient data available for analysis were: residence (city/village, county, province), age, sex, prior antiviral therapy (and the patients status if he was pre-treated, i.e. non-responder or relapser), weight, height, body mass index, parameters calculated by Fibromax (fibrosis stage, steatosis score, necroinflammatory activity), presence of significant co-morbidities, use of concomitant medications. Laboratory data were recorded 3 months before starting the antiviral therapy. Parameters recorded included platelet count, albumin, INR, total bilirubin, creatinine, AST, ALT, glucose level, HBs antigen, alfa-fetoprotein, HCV-RNA viral load.

In order to estimate the population of Romania and in each county, we used the data available on the portal http://www.recensamantromania.ro.

**Statistical analysis**

Results were summarized as median and range for non-normally distributed scale or ordinal variables or as numbers and percentages for categorical variables. We looked for differences concerning the independent variables by an outcome in bivariate analysis (Mann-Whitney U test or Fisher's exact test, depending on variables). A logistic regression model was computed, and all independent variables associated with P<0.10 with the dependent variable in bivariate analysis were introduced in a stepwise manner. The model with the independent variables retained by both the forward and the backward stepwise method was kept. A two-sided P value of < 0.05 was noted as statistically significant. Data analyses were performed with statistical software (Stata 11 from StataCorp LP, College Station, TX, USA, and SPSS version 20.0 from IBM Corporation, Armonk, NY, USA).

**RESULTS**

Genotype 1b was almost exclusively present among the patients tested: 7,392/7,421 (99.6%); 1,449 out of the 7,392 patients with genotype 1b (19.6%) were from Bucharest and 3,622/7,421 (49%) were males, with a median age of 60 years. A total of 4,418 of the subjects (75%) were in the 6th and 7th age decade (Fig. 1).

**Fig. 1.** Number of cases of HCV compensated liver cirrhosis treated with DAA based of age decades.

Genotype 1a was encountered in 7/7,421 of subjects: 6 (86%) were males, 5 (71%) were from Bucharest; the median age was 38 years. Unknown subtype of GT 1 was encountered in 7 cases: 4 (57%) were males with a median age of 50 years; 3 (42%) of them were from Bucharest. Four patients had a GT combination (1b and 3, 1b and 4): 3 (75%) of them were females; 3 (75%) were from Bucharest, the median age was 67 years. Another 4 subjects had GT 3, all were from Bucharest.
and all were males with a median age of 51 years. Genotype 4 was encountered in 4 patients: 2 (50%) of them were males; 3 (75%) were from Bucharest, the median age was 49 years. Genotype 2 was the least common, encountered in only 3 cases: 2 (67%) were females; 2 (67%) were from Bucharest, the median age was 70 years.

The demographic and clinical characteristics of the 5,891 patients with compensated liver cirrhosis who were treated with DAA therapy are illustrated in Table I. The proportion of male patients was 49.2%, the median age was 60 years. Most patients (67%) were interferon pre-treated (most of them non-responders), and the rate of co-morbidities was 37%. A few (1.6%) were co-infected with HBV and 0.7% had a history of treated HCC. Most of the patients were overweight or obese, with a median BMI of 27.2 kg/m². With regard to the severity of liver disease, 90% of the subjects included in this cohort had a Child Pugh A score of 5.

The necroinflammatory activity, estimated by Fibromax in the cohort, showed that most of the patients had severe inflammatory activity (67%), 24% had moderate inflammation and in only 9% the necroinflammatory activity was mild or absent (Table I).

Fibromax testing also allowed assessment of coexisting non-alcoholic steatohepatitis (NASH) changes, with 3,063 (almost 60%) of patients showing moderate-severe coexisting NASH. Regarding the severity of steatosis, the distribution of severity was similar to that of NASH, with 3,535 (almost 60% of patients) showing moderate to severe steatosis.

**Table I.** Demographic and clinical features of patients with compensated liver cirrhosis treated with direct-acting antiviral therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proportion (%) or Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender male</td>
<td>2898/5891 (49.2%)</td>
</tr>
<tr>
<td>Pre-treated patients (non-responders/relapers)</td>
<td>67.3% (57.3/39.1%)</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td>2197/5891 (37.3%)</td>
</tr>
<tr>
<td>HBs-Ag positive patients</td>
<td>94/5891 (1.6%)</td>
</tr>
<tr>
<td>Patients with HCC</td>
<td>40/5891 (0.7%)</td>
</tr>
<tr>
<td>Child Pugh A 6 points patients</td>
<td>615/5891 (10.44%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60 (25-82)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 (16.84-44.9)</td>
</tr>
<tr>
<td>Platelets (/mm³)</td>
<td>133,000 (12,000 – 650,000)</td>
</tr>
<tr>
<td>AST (IU/ml)</td>
<td>77 (20-431)</td>
</tr>
<tr>
<td>ALT (IU/ml)</td>
<td>88 (19-604)</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>105 (60-454)</td>
</tr>
<tr>
<td>e Cl Cr (ml/min)</td>
<td>98 (15 - 347)</td>
</tr>
<tr>
<td>INR</td>
<td>1.11 (0.34-2)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.9 (0.1 - 2.61)</td>
</tr>
<tr>
<td>MELD score</td>
<td>8.09 (6-22)</td>
</tr>
<tr>
<td>Severe necroinflammatory activity (grade 3 or 4 by Fibromax)</td>
<td>3976/5891 (67.5%)</td>
</tr>
<tr>
<td>Severe NASH</td>
<td>2951/5891 (50.1%)</td>
</tr>
<tr>
<td>Stage 4 fibrosis by Fibromax</td>
<td>5272/5891 (89.5%)</td>
</tr>
</tbody>
</table>

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; e Cl Cr, estimated clearance of creatinine; MELD: model for end stage liver disease; NASH: non-alcoholic steatohepatitis.

Regarding county distribution, the highest prevalence of cases with HCV compensated liver cirrhosis treated with DAA was encountered in Bucharest (61.3/10⁵), Bihor (47/10⁵), Iasi (46/10⁵) and Constanța (43/10⁵), and the lowest one was in Ilfov (2.8/10⁵), Harghita (3.7/10⁵), Covasna (5.4/10⁵) and Maramures (8.8/10⁵) (p<0.001).

Analyzed by geographical regions, we found that Muntenia and Dobrogea (37/10⁵) had the highest prevalence, followed by Banat, Crisana (34/10⁵) and Moldavia (31/10⁵) and the lowest prevalence was found in Maramures (9/10⁵) and Transylvania (19/10⁵) (p<0.001) (Fig. 2).

**DISCUSSION**

Our analysis offers important epidemiological data regarding the HCV infection in Romania, focusing on genotyping HCV for including patients in DAA therapy. The reimbursed DAA therapy was available only for GT 1a, 1b and 4, and the duration of therapy was different according to GT [43].

Previous data suggested that HCV GT 1 was present in 93-99% of the Romanian patients with chronic hepatitis C, but the studies included a relatively low number of cases (241,614) [5, 35, 36]. The present governmental program that included patients with HCV and advanced fibrosis (mainly F4 and less than 10% F3) gave us the opportunity to have new data regarding GT in 7,421 individuals, and these data confirm a prevalence of 99.6% for GT 1b. Given these results, identical to the previous estimation, the expert Commission of National Health Agency recommended that for the next therapeutic program, which will include about 10,000 patients with F3 fibrosis and some categories of F2 patients, genotyping is not mandatory, as it is not cost-effective.

The demographical data of GT non-1b patients suggest that these genotypes are more frequent in the Bucharest region, in male patients and at younger age; due to the very few cases, the statistical significance was not reached. These data are concordant with previous data from Romania [36] showing that non-1b GTs were evidenced mainly in younger patients with a history of IDU. We also appreciate that this is the category of patients in whom it is mandatory to perform genotyping before starting antiviral therapy.

No other East European country has reported that GT 1b is almost exclusively present in the HCV infected population. The most prevalent HCV genotype in the Czech Republic is HCV GT 1b (80-85%) followed by GT 3 (mostly 3a, 10-15%) [44, 45]. The data from Hungary suggest that GT 1b is present in more than 90% of infected persons [44, 45]. In Serbia and Montenegro, the GT 1b (58%) and 3a (23%) are predominant in patients with chronic HCV infection. In Russia, the distribution of HCV GTs is the following: 1b -77%; 2a - 3.8% and 3a - 17% [21]. In Poland, recent data were reported from 9,800 patients: almost 82% of patients were infected with GT 1b, with a prevalence showing an increasing tendency, accompanied by a decrease of GTs 3 (11.3%), 4 (3.5%) and 1a (3.2%) [37].

Our cohort of patients is not fully representative for Romania, because only patients with advanced fibrosis were selected. These are usually older subjects, infected a long time before the availability of DAA therapy.
ago mainly through inadequately screened blood products and unsterilized needles or devices or syringes.

Our study offers important data regarding the distribution of the 5,891 patients with HCV and advanced fibrosis that received DAA therapy with Paritaprevir/Ombitasvir/ritonavir and Dasabuvir with Ribavirin between December 2015 and October 2016. This program led to a 96.6% sustained virologic response (SVR) by intention-to-treat analysis, data comparable with other real-life cohorts [46-48]. Serious adverse events related to therapy were reported in 2.9%, most of them liver decompensation (1.9%), related to hepatic dysfunction, and low platelet count [46].

The analysis of these patients gave us a unique opportunity to study the prevalence of the advanced cases in Romania, which is related to the prevalence of HCV infection in Romania, the addressability of the patients to therapy (which includes also the awareness of therapeutic possibilities by patients and physicians, and the will to be treated and to treat, respectively), the number of prescriptors in different counties, and the level of access to the County Health Agency through the territory. The most striking feature is the very high prevalence in Bucharest, and this may be not only the consequence of higher addressability, but also reflects the concentration of these cases in our densely populated capital city. In the following counties the prevalence of these patients is also increased: Bihor, Iasi and Constanta (Supplementary Fig. 1). The „green areas”, with the lowest numbers are the counties Harghita, Covasna and Maramures, reflecting both a low addressability as well as a low prevalence of HCV infection.

This is the first complete report of the county prevalence of HCV infected patients. In part, our results confirm the data published by Gheorghe L. et al. in 2010, according to which the prevalence of HCV infection in Romania varies significantly, ranging from 0.56% in Covasna county (Transylvania) to 7.19% observed in Tulcea county (Dobrogea) [31]. The discrepancy regarding the low prevalence of cases with HCV infection and advanced fibrosis in Tulcea county in our study is for certain explained by the low addressability of the people in this geographical region. Regarding the distribution of the HCV cases according to geographical regions, our results are similar with those obtained by Gheorghe L. et al.: Muntenia, Dobrogea, Moldavia, Banat and Crisana demonstrated almost an equal prevalence of cases (31-37/105), presenting an increased risk for HCV infection, while Transylvania and Crisana had the lowest prevalence of HCV infected cases.

These results will have a major impact on clinical practice: for prescribing DAA therapy, for future screening policies and for the application of prevention measures.

CONCLUSIONS

Genotype 1b is encountered in 99.6% of the patients with chronic hepatitis C and advanced fibrosis in Romania. The presence of genotypes non-1b is more common in Bucharest, in males and at a younger age. There are significant differences regarding the distribution of these cases across Romania.

Conflicts of interest: No conflicts to disclose.

Authors’ contributions: M.M. designed and carried out the study. C.M.P. and D.P. drafted the manuscript. C.B. performed the statistical analysis. A.O. designed the study and contributed by critical revision of the manuscript. The other authors contributed by critical revision of the manuscript. All authors read and approved the final manuscript.

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Supplementary material: To access the supplementary material visit the online version of the J Gastrointestin Liver Dis at http://www.jgld.ro/wp/archive

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