Original article

The Need for Systematic Monitoring and Improved Surveillance of Hepatitis C Patients in Croatia

Leona Radmanić [1*], Nataša Cetinić Balent [1], Petra Šimičić [1], Adriana Vince [1,2], Snježana Židovec Lepej [1], Oktavija Đaković Rode [1,3]

1 University Hospital for Infectious Diseases „Dr. Fran Mihaljević", Zagreb, Croatia
2 School of Medicine, University of Zagreb, Croatia
3 School of Dental Medicine, University of Zagreb, Croatia

*Corresponding author: Leona Radmanić, leona.radmanic@gmail.com

Abstract

Aim: The aim of this study was emphasizing the need for a more systematic monitoring of patients diagnosed with HCV in Croatia.

Methods: From 2014 to 2018, at the University Hospital for Infectious Diseases, sera from 23,524 patients were tested for HCV. Confirmatory testing was performed by Western Blot. Adult patients with an anti-HCV positive screening test were analysed. HCV RNA was quantified by real-time PCR, while HCV genotypes and subtypes were determined by PCR and the reverse hybridization method.

Results: A total of 428 anti-HCV ELISA-positive adults were analysed (68.7% males, 31.3% females, median age 43 years, range 19-88 years). Hepatitis C was confirmed by WB in 390, while 28 patients had borderline WB results. Anti-HCV was not confirmed by WB in 10 patients. HCV RNA was tested in 331 patients and viremia was detected in 218 patients. There was no data on HCV RNA in 97 patients (22.66%). HCV genotypes/subtypes were determined in 185 of 218 anti-HCV WB positive patients. Genotype 1 was detected in 97/185 (52.43%), genotype 2 was detected in 3/185 (1.62%), while subtype 3a was detected in 76/185 (41.08%) and genotype 4 in 9/185 patients (4.86%).

Conclusion: In a five-year period, the HCV seroprevalence rate in subjects tested at the University Hospital for Infectious Diseases was 1.81%. According to the data analysed, almost one quarter of patients with detected anti-HCV antibodies were not treated further, which indicates the need for a systematic monitoring of patients diagnosed with HCV. It is necessary to determine viremia after a positive anti-HCV screening result in order to initiate treatment and prevent HCV-related complications.

(Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O. The Need for Systematic Monitoring and Improved Surveillance of Hepatitis C Patients in Croatia. SEEMEDJ 2020; 4(2); 28-34)

Received: Mar 12, 2020; revised version accepted: Mar 12, 2020; published: Nov 12, 2020

KEYWORDS: hepatitis C virus, monitoring, diagnosis

Introduction

Both a general practitioner and a secondary-care specialist are involved in the diagnosis of chronic viral hepatitis and in the clinical management of infected patients [1]. The initial diagnosis and management of chronic hepatitis relies on primary-care physicians to identify and screen patients who were in contact with hepatitis C, since most people with chronic hepatitis are asymptomatic until the development of cirrhosis or hepatocellular carcinoma [2]. The WHO recommends education of primary care physicians about the risks factors of acquiring HCV infection and encourages increased activity in finding new patients in screening programs.

Approximately 71 million people worldwide have chronic hepatitis C, of which 15 million in Europe, and about 400,000 people in the world die from the effects of hepatitis C per year. Croatia is a country with a low incidence rate of hepatitis C. It is estimated that 40,000 people are anti-HCV positive in Croatia. In May 2016, the World Health Assembly endorsed the Global Health Sector Strategy on viral hepatitis, which proposed to eliminate viral hepatitis as a public health threat by 2030. Elimination of viral hepatitis as a public health threat requires 90% reduction in incidence and 65% reduction in mortality in comparison with the 2015 baseline, along with the improvement of viral hepatitis diagnostic coverage up to 90% and treatment of 80% of eligible patients [4]. To reach these targets, the WHO Regional Office for Europe is encouraging Member States to plan and strengthen national responses to viral hepatitis through awareness-raising, surveillance, prevention, strengthening of laboratory capacity and provision of guidance on testing and treatment [5]. Implementation of the Global Health Sector Strategy would prevent 7.1 million deaths between 2015 and 2030 [4].

Hepatitis C virus (HCV) diagnostics starts with determination of anti-HCV antibodies [3,6]. Each positive anti-HCV screening result requires further HCV RNA detection in order to confirm actual contact with HCV in the past. If HCV RNA is negative, positive anti-HCV results should be confirmed by Western immunoblotting (WB) [7,8]. In 2019, WHO recommended offering treatment with direct acting antivirals (DAA) to all individuals diagnosed with HCV infection who are 12 years of age or older, irrespective of disease stage (with the exception of pregnant women) [9]. Priority for treatment is defined by the stage of fibrosis, the risk of progression to advanced disease, the presence of extrahepatic manifestations and comorbidities, patients with HBV or HIV coinfection and patients with indication for organ transplantation [10].

The aim of this study was emphasizing the need for a systematic monitoring of patients diagnosed with HCV. This must include the determination of viremia after a positive anti-HCV screening result in order to administer a corresponding therapy since, according to the data analysed, almost one quarter of patients with detected anti-HCV antibodies are not treated further.

Patients and Methods

From 2014 to 2018, at the University Hospital for Infectious Diseases in Zagreb, sera from 23,524 patients were tested for anti-HCV antibodies by using either enzyme immunoassay (ELISA) for simultaneous detection of anti-HCV antibodies and capsid antigen (HCV Ag-Ab) or only anti-HCV antibody determination (BioRad, France). As required, each of the 428 positive ELISA sera was confirmed by Western Blot (INNO-LIA HCV Score Fujirebio; recomLine HCV IgG Mikrogen). HCV RNA was quantified by real-time PCR (Abbott RealTime HCV) and HCV genotypes and subtypes were determined by PCR and the reverse hybridization (INNO-LiPA HCV Genotyping) method.

Results

This study included 428 anti-HCV ELISA-positive adults who were newly diagnosed in the period from 2014 to 2018 and over 18 years of age. Median age of patients was 43 years, ranging from 19 to 88 years. Regarding gender, 68.7% of

the patients were male and 31.3% were female. According to the available diagnoses from the referral, 293 (293/428; 68.45%) patients had chronic hepatitis or elevated hepatic lesion, 52 (52/428; 12.14%) patients were injecting drug users and 83 (83/428; 19.39%) patients had other diagnoses, for example, neurological and muscular diseases and factors affecting the health system.

Anti-HCV was confirmed by Western Blot in 390 patients (91.12%), while 28 patients (6.54%) had borderline Western Blot results. When it comes to borderline Western Blot results, negative result was confirmed with molecular testing in 16 patients, HCV infection was excluded in two patients with paired sera and infection status was unknown in 10 patients. Positive anti-HCV ELISA was not confirmed by Western Blot in 10 patients (2.34%). Molecular testing for HCV RNA was performed in 331 patients – 65.86% (218/331) of the patients had measurable viremia (Table 1).

Table 1. Results of Western Blot and HCV RNA in anti-HCV (ELISA) positive patients tested at UHID

anti-HCV		HCV RNA	Patients N (%)
Enzyme-linked immunoassay	Western Blot		
Positive	Positive	Positive	218 (65.86)
		Negative	87 (26.28)
Positive	Borderline	Negative	16 (4.83)
Positive	Negative	Negative	10 (3.02)
TOTAL			331 (100.00)

Median viremia was 456.024.5 IU/ml (range 78-43.041.938 IU RNA HCV/mL). HCV RNA was not detected in 87 patients with positive HCV WB, 16 with borderline and 10 with negative HCV WB. There was no data on HCV RNA testing at the UHID for 85 patients with positive and 12 with borderline HCV WB (97/428; 22.66%) (Table 2).

Table 2. Results of Western Blot and HCV RNA in anti-HCV (ELISA) positive patients

anti-HCV		HCV RNA	Patients N (%)
Enzyme-linked immunoassay	Western Blot		
Positive	Positive	Positive	218 (50.93)
		Negative	87 (20.33)
		Unknown	85 (19.86)
Positive	Borderline	Negative	16 (3.74)
		Unknown	12 (2.80)
Positive	Negative	Negative	10 (2.34)
TOTAL			428 (100.00)

According to the available diagnoses from the referral, 48 of 97 patients with positive anti-HCV ELISA and unknown viremia were patients who had chronic hepatitis or elevated hepatic lesion (49.48%). Seventeen of 97 patients were injecting drug users (17.52%) and 32 of 97 patients had other diagnoses, for example, neurological and muscular diseases and factors affecting the health system (32.99%).

HCV genotypes and subtypes were determined in 185 of 218 anti-HCV WB positive patients with HCV RNA >1.000 IU/ml in the serum. Genotype 1 was detected in 97 (97/185; 52.43%) patients, genotype 2 in 3 (3/185; 1.62%) patients, subtype 3a in 76 (76/185; 41.08%) /185 and genotype 4 in 9 (9/185; 4.86%) patients. Subtypes 1a and 1b of genotype 1 were further distinguished. Subtype 1a was detected in 57 (57/97; 58.76%) patients and subtype 1b in 36 (36/97; 37.11%) patients. In four patients, the subtype of genotype 1 could not be determined (Figure 1).

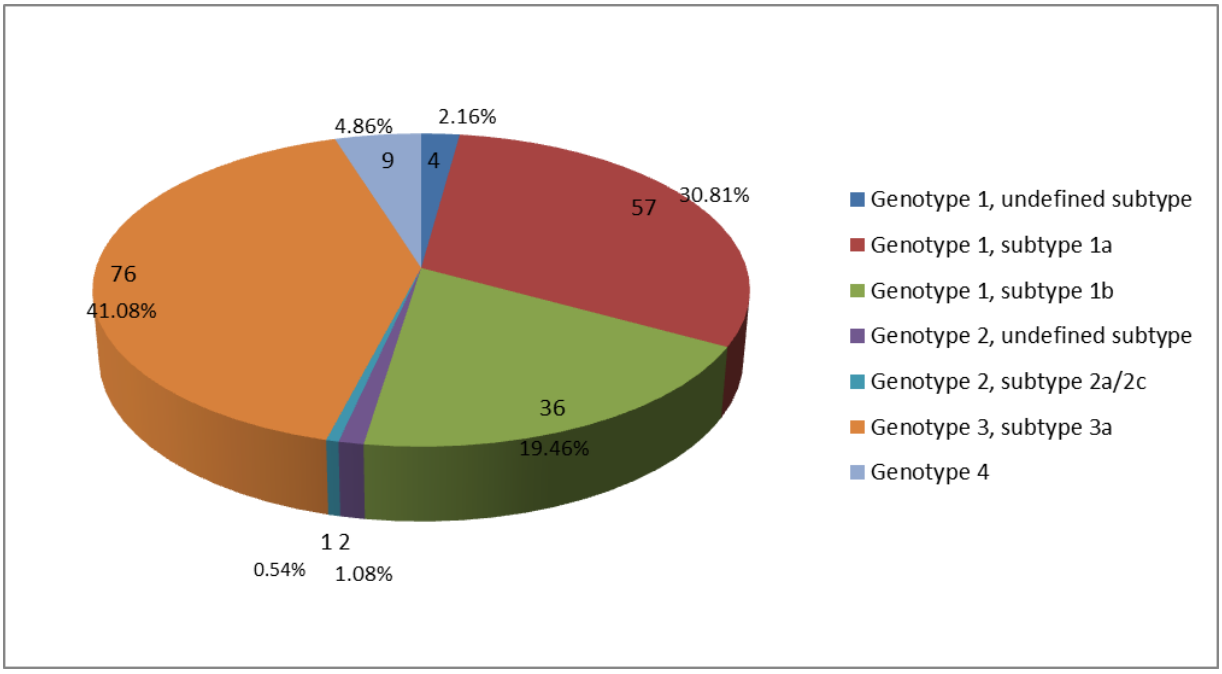

Figure 1. HCV genotype/subtype distribution in HCV RNA-positive patients (n = 185)

Discussion

HCV prevalence estimates range from 0.4% to 5.2% in the world, with countries in the north and west of Europe having lower estimates (0.9%) than countries in the east of Europe (3.3%) [11,12]. In a five-year period, HCV seroprevalence rate in subjects diagnosed at the UHID was 1.81%, which was slightly higher than the estimate (0.8-1.0%) for the Croatian population [13]. However, most of the data on prevalence was obtained through the serological testing of samples from voluntary blood donors, who were a strictly controlled group. HCV prevalence in blood donors continuously declined from 1.38% in 1992 to 0.0009% in the last decade. Therefore, it is to be expected that the actual prevalence in the general population is higher [13,14]. Nevertheless, this data suggests that HCV seroprevalence in Croatia is most similar to the seroprevalence rate in western European countries.

According to the latest analysis on the distribution of HCV genotypes and subtypes in Croatia, Croatian patients were mostly infected with HCV genotype 1 (56.63%), followed by

genotype 3 (37.23%), genotype 4 (4.21%) and genotype 2 (1.83%). This is very similar to our data, according to which genotype 1 was detected in 52.43% patients, genotype 2 in 1.62% patients, subtype 3a in 41.08% and genotype 4 in 4.86% patients [15]. However, genotype 1 subtyping showed 58.76% of subtype 1a infections and 37.11% subtype 1b infections in our study, while national studies suggest a higher prevalence of subtype 1b in Croatia [15,16].

Our cohort study tested 23,524 patients for anti-HCV antibodies to show how many adults with a positive anti-HCV screening result were accurately diagnosed, including determination of viremia, in the period from 2014 to 2018. Epidemiological and clinical data from the referrals and the UHID database suggest that 218 of 331 patients who were molecularly tested at the UHID had measurable viremia, while there was no data for 97 anti-HCV-positive patients. This suggests the possibility they were tested at another institution or that they were simply not treated further. This indicates the need for a more systematic monitoring and determination of viremia in potential patients in order to initiate treatment. According to the available diagnoses from the referral, 17.50% of the patients were people who injected drugs and, probably due to their lifestyle, were lost to secondary specialist care. Furthermore, 49.50% of the patients were people who had chronic hepatitis or elevated hepatic lesion and 33% of the patients were people with other diagnoses, for example, neurological and muscular diseases and factors affecting the health system. Since injecting drug use is one of the most efficient routes for HCV transmission, there is a very high prevalence of HCV in people who inject drugs in most European countries, while in Croatia it ranges from 29% to 65%, depending on the geographical region [13].

According to Becchini et al., general practitioners' role and referral back to primary care vary within and between countries – most general practitioners are rarely involved in monitoring clinical outcomes other than some side effects among patients undergoing antiviral treatment [1,8]. A lack of uniform practice suggests that in some patients with serologically confirmed presence of the infection, additional nucleic acid testing may not always be performed. Therefore, the role of the general practitioner and specialists involved in clinical management of chronically infected patients should be clarified in order to ensure that the patients are followed-up and accurately diagnosed [1]. The Croatian Reference Centre for Diagnostics and Treatment of Viral Hepatitis performs initial check-ups of patients with acute and chronic hepatitis B and hepatitis C infection, serological and molecular testing to detect parameters of virus infection, pretreatment evaluation of the patients (liver biopsy, Fibroscan) along with treatment and monitoring of patients after the end of treatment. National Reference Centre also provides strategies and guidelines for optimization of diagnostics and standardization of treatment of viral hepatitises [17]. However, in Croatia, there is no National Hepatitis Treatment Registry used by all physicians prescribing DAAs and patients are diagnosed and treated at various institutions across the country. Such decentralization enables easier access to care for patients, whereas an early diagnosis and successful treatment not only prevent HCV-related complications, but also stop infectiousness. On the other hand, it is possible that the monitoring of patients and overview of the treatment process is better in specialized, centralized settings than in unspecialized, decentralized settings [18].

Conclusion

It is necessary to improve the monitoring and surveillance of hepatitis C patients in Croatia since almost one quarter of patients with detected anti-HCV antibodies are not treated further. Determination of viremia after positive anti-HCV screening would ensure adequate treatment of the patients and prevent HCV-related complications.

Acknowledgement. None.

Disclosure

Funding. This publication was supported by the grant "Strengthening the capacity of CerVirVac for research in virus immunology and vaccinology", KK.01.1.1.01.0006, awarded to the Scientific Centre of Excellence for Virus Immunology and Vaccines and co-financed by the European Regional Development Fund.

Competing interests. None to declare.

References

1. Bechini A, Levi M, Falla A, Ahmad A, Veldhuijzen I, Tiscione E, Bonanni P. The role of the general practitioner in the screening and clinical management of chronic viral hepatitis in six EU countries. J Prev Med Hyg 2016; 57(2):E51–E60.

2. Ferrante JM, Winston DG, Chen PH, de la Torre AN. Family Physicians' Knowledge and Screening of Chronic Hepatitis and Liver Cancer. Fam Med 2008; 40(5):345-51.

3. https://www.hzjz.hr/aktualnosti/virusni-hepatitisi/

4. World Health Organization, Combating hepatitis B and C to reach elimination by 2030, https://apps.who.int/iris/bitstream/handle/10665/206453/WHO_HIV_2016.04_eng.pdf?sequence=1 (2016), accessed date: November 2019.

5. WHO Regional Office for Europe, Hepatitis C in the WHO European Region, http://www.euro.who.int/en/health-topics/communicable-diseases/hepatitis/data-andstatistics/fact-sheet-hepatitis-c-in-the-who-european-region1 (2019), accessed date: November 2018.

6. Cetinić Balent N, Mikulić R, Đaković Rode O. Imunoenzimski test za određivanje protutijela na pojedinačne antigene virusa hepatitisa C kao potvrdni test u dijagnostici hepatitisa C. Infektološki glasnik 2013; 33(3),109-15.

7. EASL Clinical Practice Guidelines: Management of hepatitis C virus infection. J Hepatol 2014; 60:392–420.

8. Albeldawi M, Ruiz-Rodriguez E, Carey WD. Hepatitis C virus. Prevention, screening, and interpretation of assay. Cleve Clin J Med 2010; 77:616–26.

9. World Health Organization, Guidelines for the care and treatment of persons diagnosed with chronic hepatitis C virus infection, https://apps.who.int/iris/bitstream/handle/10665/273174/9789241550345-eng.pdf (2018), accessed date: November 2019.

10. Recommendations for treatment of Hepatitis C, http://www.bfm.hr/page/hepatitis-c (2019), accessed date: November 2019.

11. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol 2014; 61(1) Suppl; S 45-57.

12. European Centre for Disease Prevention and Control (ECDC). Technical Report. Hepatitis B and C in the EU neighbourhood: prevalence, burden of disease and screening policies. Stockholm: ECDC; 2010. Available from: http://ecdc.europa.eu/en/publications/Publications/TER_100914_Hep_B_C%20_EU_neighbourhood.pdf.

13. Vilibić Čavlek T, Kučinar J, Kaić B, Vilibić M, Pandak N, Barbić LJ, Stevanović V, Vraneš J. Epidemiology of hepatitis C in Croatia in the European context. World Journal of Gastroenterology 2015; 21(32):9476-93.

14. Civljak R, Kljakovic-Gaspic M, Kaic B, Bradaric N. Viral hepatitis in Croatia. Viral Hepat J 2014; 20:49-56.

15. Vince A, Židovec Lepej S, Bingulac-Popović J, Miletić M, Kuret S, Sardelić S., Baća-Vrakela I., Kurelac I. Distribution of hepatitis C virus genotypes and subtypes in Croatia 2008-2015, Cent, Eur, J, Publ, Health 2018; 26(3):159–63.

16. Vince A., Iščić-Beš J., Židovec Lepej S., Baća-Vrakela I., Bradarić N., Kurelac I., Vince DB. Distribution of hepatitis C virus genotypes in Croatia – a 10 years retrospective study of four

geographic regions, Coll Antropol 2006; 30(Suppl 2):139–43.

17. Reference centers of Ministry of Health in Croatia http://www.bfm.hr/en_GB/page/referentni-centri (2020). Accessed date: January 2020.

Bregenzer A, Conen A, Knuchel J, Friedl A, Eingenmann F, Näf M, Ackle P, Roth M, Fux CA. Management of hepatitis C in decentralized versus centralized drug substitution programmes and minimally invasive point-of-care tests to close gaps in the HCV cascade. Swiss Med Wkly 2017; 147:w14544.

[i] **Author contribution:** Acquisition of data: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Administrative, technical or logistic support: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Analysis and interpretation of data: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Conception and design: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Critical revision of the article for important intellectual content: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Drafting of the article: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Final approval of the article: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Guarantor of the study: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Obtaining funding: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Provision of study materials or patients: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O
Statistical expertise: Radmanić L, Cetinić Balent N, Šimičić P, Vince A, Židovec Lepej S, Đaković Rode O