Introduction

Hepatitis C Virus (HCV) infection is one of the leading causes of chronic liver disease worldwide [1]. The outcome of HCV infection, on a long-term basis, is variable, from minimal changes to fibrosis and cirrhosis, with or without hepatocellular carcinoma. The number of chronically infected people worldwide is estimated to be about 180 million [2], but most are not aware of the infection. Chronic Hepatitis C is the only chronic viral infection that can be cured with antiviral treatment. Importantly, successful antiviral treatment can prevent the short- and long-term complications of HCV infection in many patients [3]. Only approximately 55% of patients infected with HCV genotype 1 who tolerate the treatment with Pegylated interferon-α (PegIFN-α) and ribavirin (RBV) obtain a sustained virological response (SVR), defined as the absence of HCV RNA in serum 3 to 6 months after cessation of treatment; an SVR is almost always associated with the sustainable eradication of the virus [4,5]. 60% to 90% of patients infected with genotype 2 and 3 virus, achieve SVR after treatment with PegIFN-α and RBV. Substantial progress in understanding the mechanisms of virus entry into hepatocytes, replication and host immune response has led to the development of new therapeutic agents targeting the stages of the viral life cycle. Unfortunately, not all patients have access to Interferon Free antiviral treatment, and triple therapy hasn’t even been introduced in our country.

Material and methods

267 patients were included in the study-a group of 216 patients diagnosed with Chronic Hepatitis C viral infection in the Renasterea Medical Center and a group of 51 patients admitted in the II-nd Medical Department from the Emergency County Hospital Craiova between 2013 and 2016.

All subjects were informed about the purpose of the study and signed an informed consent before enrolling in the study. Since human subjects were involved in this study, the patients' rights established by the World Health Organization found in the Patients' Rights Act, 46/2003. We have complied with the Helsinki Declaration adopted at the 18th World Medical Assembly, Helsinki, 1964 and reviewed at the 29th World Medical Assembly, Tokyo, 1975.
The inclusion criteria were determined by the Ministry of Health criteria for inclusion in the program of antiviral treatment. All patients had compensated liver disease, tested negative for HIV/HVB (Human immunodeficiency virus/Hepatitis B Virus) co-infection, were negative for tuberculosis, had no history of any malignant tumors and did not have decompensated or even compensated liver cirrhosis. The patients were monitored for 18 months, with frequent medical visits performed at 4, 12, 24, 48 weeks from the beginning of antiviral therapy and 24 weeks after completion. Several tests were performed during these visits including a complete blood count and viral load. The evaluation of hepatic fibrosis was performed by FibroTest® test, a non-invasive alternative to hepatic biopsy that was clinically validated in patients with chronic hepatitis.

We evaluated every type of treatment response and the connection to achieving sustained virological response. Rapid virological response (RVR) was defined as undetectable hepatitis C virus RNA (ribonucleic acid) at week 4 of treatment. Early virological response (EVR) was defined as $\geq 2\log_{10}$ reduction from baseline HCV RNA, and virus is undetectable at week 12. Delayed virological response (DVR) was defined as $>2\log_{10}$ reduction, but undetectable after 24 weeks of therapy. Relapse was defined as undetectable viremia during treatment and/or at the end of treatment, but subsequent viremia following treatment cessation. Breakthrough: undetectable HCV RNA during therapy followed by reappearance of HCV RNA, in spite of continued treatment.

All the information obtained was stored in Microsoft Excel files (Microsoft Corp., Redmond, WA, USA) and was statistically analyzed in order to investigate the relationship between several host related factors and SVR by using the Microsoft Excel XLSTAT suite of programs (Addinsoft SARL, Paris, France).

Secondary data processing—calculating fundamental statistical parameters, mean and standard deviation of their report—the coefficient of variation, graphical representation and calculation of the regression coefficients was performed with Excel.

Results

The distribution of patients included in the study by gender was as follows: 31.46% were males representing 84 patients and 68.54% women of 183 patients. The male/female ratio was 2:1 without an additional risk factor for this population. Taking into account that the population in Dolj County consists of 48.8% men and 51.2% women, we determined that there were significantly more women that presented with HCV infection than men. Depending on the distribution of the group regarding the background, the results of the study were as follows: 82 patients had a rural residence environment, while 185 patients came from urban areas.

In our cohort, 149 (55.81%) of patients achieved SVR at 24 weeks after the end of treatment and 118 (44.19%) patients were non-responders.

![Fig.1. Sustained virological response rates at end of treatment](image)

Depending on the gender of the patients, 53 (19.85%) of those who had a favorable treatment response, were male and 96 (35.96%) were women. There was no statistical difference between the gender of patients in response to treatment. Neither women, nor men, responded better to PegIFN-α and RBV therapy ($p_{\chi^2}=0.1$).

**Table 1. The correlation between sustained virological response and gender**

| Sex    | SVR | Nonresponders | Total |
|--------|-----|---------------|-------|
| MALE   | 53  | 31            | 84    |
| FEMALE | 96  | 87            | 183   |
| Total  | 149 | 118           | 267   |

We have studied the types of treatment responses in our group. The results are as follows: 113 (42.32%) patients had EVR (early virologic response), 29 (10.86%) had a breakthrough, 17 (6.37%) were relapers, 149 (55.81%) had SVR (sustained virologic response), 167 (62.55%) had RVR (rapid virologic response) and 58 (21.72%) patients had DVR (delayed virologic response).
We further investigated the relationship between achieving any of the previous responses to achieving sustained virological response. From the total patients who achieved SVR, 75 (28.09%) patients achieved EVR. The results were statistically significant with a $p \chi^2<0.01$. In regards to the rapid response, 133 (49.81%) patients with RVR, obtained sustained virologic response, while 16 (5.99%) patients did not respond to treatment, demonstrating that the results were statistically significant with a calculated $p \chi^2<0.01$. All data is detailed in Fig.3.

Regarding the viral load, our study results were as follows: 116 (43.45%) patients had low viral load (LVL) at baseline and 151 patients had high viral load (HVL). Depending on the viral load and the type of response, the results were as follows: 73 (27.34%) patients with LVL responded to treatment, while 43 (16.10%) patients did not receive a favorable response. From a total of 151 patients (56.55%) with HVL, 76 (28.46%) patients with viral loads over 400,000 IU/ml achieved sustained virologic response and 75 (28.09%) did not respond; the results showed that the likelihood of achieving a SVR correlates strongly with the baseline viral load ($p \chi^2 0.03<0.05$).

By studying the link between the viral load and the occurrence of a rapid or early virological response, we observed that 33 (12.36%) patients who achieved RVR had a viral load <400000UI/mL, and 83 (31.09%) patients did not obtain RVR. Of the total patients with HVL, 134 (50.19%) patients achieved RVR and 17 (6.37%) patients did not obtain rapid virologic response. The results were unexpected, with a higher rate of rapid virological response for patients with high baseline viral load. The results of the study for high viral load and EVR have shown the following: 75 (28.09%) patients who achieved EVR had HVL and 38 (14.23%) had LVL; 76 (28.46%) of patients without EVR had HVL and 78 (29.21%) had LVL. The results were statistically significant ($p \chi^2=0.01$) and it can be seen that a high viral load is a negative predictive factor for obtaining an early virological response.
The determination of the fibrosis degree provided the following results in our study: 104 (38.95%) patients had grade F1 fibrosis, 92 (34.46%) patients had grade F2 fibrosis and 71 (26.59%) of patients had F3 fibrosis, calculated by FibroTest® or FibroScan®.

Of the patients who received sustained virological response, 81 (30.34%) patients had F1 fibrosis grade versus 23 (8.61) patients who did not respond, 48 (17.98%) patients had had F2 fibrosis grade versus 44 (16.48%) unresponsive patients and 20 (7.49%) patients had grade F3 versus 51 (19.10%) who did not respond. Patients with lower grades of liver fibrosis responded better to treatment.

Table 2. Liver fibrosis does not influence the achievement of RVR, EVR, DVR or SVR

| Fibroscan | EVR | F1  | F2  | F3  | Total |
|-----------|-----|-----|-----|-----|-------|
| Yes       |     | 38  | 40  | 35  | 113   |
|           |     | (14.23%) | (14.98%) | (13.11%) | (28.46%) |
| No        |     | 66  | 52  | 36  | 154   |
|           |     | (24.72%) | (19.48%) | (13.48%) | (71.54%) |

| Fibroscan | RVR | F1  | F2  | F3  | Total |
|-----------|-----|-----|-----|-----|-------|
| Yes       |     | 73  | 54  | 40  | 167   |
| No        |     | 31  | 38  | 31  | 100   |

| Fibroscan | DVR | F1  | F2  | F3  | Total |
|-----------|-----|-----|-----|-----|-------|
| Yes       |     | 29  | 20  | 9   | 58    |
| No        |     | 75  | 72  | 62  | 209   |

| Fibroscan | RVS | F1  | F2  | F3  | Total |
|-----------|-----|-----|-----|-----|-------|
| Yes       |     | 81  | 48  | 20  | 149   |
| No        |     | 23  | 44  | 51  | 118   |

We studied whether the degree of fibrosis influenced different virological responses as follows: in terms of EVR: 38 F1 grade patients responded to treatment, compared to 40-F2 patients and 35 were grade F3 patients. In terms of RVR, the results of the study were as follows: 73 (27.34%) patients who achieved RVR were F1 fibrosis, 54 (20.22%) had grade F2 and 40 (14.98 %) grade F3 of fibrosis. Regarding the DVR, the results were as follows: 29 (10.86%) of patients who achieved DVR had a determined F1 fibrosis score, 20 (7.49%) patients were F2 and 9 (3.37%) patients had grade F3 of fibrosis. In our study, it was not possible to demonstrate a statistical link between achieving early, rapid or delayed virological response and the degree of hepatic fibrosis, but we investigated whether the decline rate of the viral load is influenced by the baseline fibrosis stage. We found that the highest decline was recorded for the patients that tested as F1 score for liver fibrosis.

Fig.6. The rapid decline of viral load in patients with F1 grade of liver fibrosis

Discussions

The results of the clinical and epidemiological study presented in our paper are largely consistent with the research literature on the same subject.

The number of cases with chronic hepatitis C virus infection is increasing globally, rising from 122 million cases in 1990 to 184 million cases in 2005. Data on the prevalence of viral hepatitis C in Romania are insufficient, but it is estimated that about 5.6% of the Romanian population tested positive for anti-HCV antibodies [1], which represents over 1 million of the country's population, according to the latest data available from the Ministry of Health and specialized journal data [2].

The number of cases of chronic hepatitis C has increased in recent years worldwide, with an upward trend observed in the country between 2006 and 2012 [6], [7], with a slight downward slope with small year-to-year variations on the analysis carried out between 2006-2014 [7,8] and a reduction of the number of HCV infected patients on the last analysis carried out by the National Institute of Public Health Romania in 2015 [9,10].

In our study, 267 patients diagnosed with chronic viral hepatitis C, aged 29-74 years, with
an average age of 55 years with a standard deviation of 10.76, were included.

By analyzing the ages of the patients included, we noticed that the highest percentage was represented by patients aged 50-59 years (80 patients, 29.96%), with small variations of patients falling in decades 4 and 6 of life. The largest number of patients in our study diagnosed with chronic HCV infection belonged to the 40-49, 50-59 and 60-69 age groups, with a total of 82.77% of the patients. The data obtained are similar to the results listed in the literature; In a study on epidemiological observational studies, it has been demonstrated that globally the most affected age is between 50 and 64 years [1,10,11].

In a study conducted in our country, the mean age of the cohort was 33 years, which is not in line with our findings, the average age of our group being 55 years[12].

In terms of gender distribution, our group comprised 84 men and 183 women, with a ratio of 2: 1 women: men. In a study in Japan in an endemic area, it was shown that the number of men infected with hepatitis C virus was higher than that of women [13]. Similar results were also reported in a study in our country [12] on a group of 327 patients, where the female-male ratio was 1.14: 1 in favor of males and another study conducted on a larger group of patients in the United States demonstrates a higher rate of male infection compared to women with a ratio of 2.24: 1 in favor of males [14]. However, many other studies on patients with chronic hepatitis C confirm the results of our study with regard to increased seroprevalence among female patients [15]. The latest available data from our country, however, confirms that the maximum rates of incidence were recorded in the 55-64 age groups and the female gender as the majority with 0.69 cases per 100000 inhabitants versus 0.53 cases per 100,000 for men [9]. Corroborating the data obtained with the available data on the gender distribution for Dolj county, where the study was conducted, we can say that there is a significant difference between the two genders, with a calculated p value of 0.01.

From our study, 185 (69.29%) patients came from the urban area and 82 (30.71%) patients came from rural areas.

Due to the fact that we did not find data available on the distribution of patients diagnosed with chronic viral hepatitis C by gender and the background, we decided to compare the background with positive HCV patients, with the distribution of the population from Dolj county regarding the residence environment. The latest data available after the 2011 census showed that 52.1% of the Dolj county has an urban residential environment and 47.9% has a rural environment. Taking this into account, we can say that there is a significant difference between these proportions and those calculated for the patients included in our study with a p value of 0.01, which means that the number of patients in the urban environment is significantly higher than in rural areas.

Hepatitis C virus is characterized by a high genetic variability and therefore an exceptional capacity to persist in most infected individuals. Currently, seven genotypes and more than 200 subtypes are described. In our country, genotype 1b and a mixture of genotypes 1a and 1b prevail. Knowing the genotype, we are provided with predictive information on the efficacy of therapy in patients with chronic hepatitis C. For example, genotype 1b (often acquired after transfusion) is associated with a poor response to treatment and an increased risk of cirrhosis and hepatocellular carcinoma.

On the other hand, genotypes 2 and 3 (predominantly acquired through iv drug use) are associated with a favorable response to antiviral therapy [16,17,18]. A 42-46% SVR rate is seen in patients infected with HCV genotype 1, while a 76-82% SVR rate is seen in those with genotypes 2 and 3 [19,20,21].

A recent study conducted in the Clinical Emergency Hospital “Professor Dr. Octavian Fodor”, Cluj-Napoca, Romania, on 461 patients, showed a prevalence of over 99% of the HCV genotype 1 virus [22]. Extrapolating from these results, we considered for our group, as a majority, the HCV genotype 1. The results of our study were in line with previous findings; the SVR rate in our study was 55.81%, slightly exceeding the published values for viral genotype 1.

We noticed that a proportion of 28.46% of patients had an early virological response. We investigated whether obtaining EVR can be used as a predictive factor for SVR. Of the patients who achieved EVR, 88.16% of them had undetectable viremia at the end of therapy and 6 months after the end of treatment. According to other similar studies [23], our study confirms that obtaining an early virological response as well as obtaining a rapid virological response is a positive predictive factor for obtaining a sustained virological response. Discontinuation of treatment for patients who did not achieve
EVR could have led to a reduction in treatment costs of perhaps 15-20%.

Interestingly, in many other studies, it has been demonstrated that there is a greater chance that response to dual therapy with PegIFN and RBV is dependent on the viral load at the start of treatment [24]. It has been shown that patients with a high viral load have less chance of responding to treatment than those who have had a low viral load at initiation of therapy. In our group, the proportions were retained according to reported data, with patients with high viral load being those who achieved higher rates of SVR.

Each patient included in the study was tested for hepatic fibrosis grading. We wanted to determine if the degree of fibrosis influences the response to treatment. Patients with low grade liver fibrosis have been shown to have improved virologic response rates. There have been similar studies in which similar results have been shown [25], demonstrating the degree of fibrosis as an individual predictor of response to treatment. Interestingly, in our study, the degree of hepatic fibrosis did not correlate with any of the early virological, rapid or delayed responses. As we have demonstrated that a higher proportion of patients with grade F1 of hepatic fibrosis, those with EVR and those who achieve RVR are more likely to respond to antiviral therapy, extrapolating, links between RVR or EVR and the degree of fibrosis can be established. The results obtained cannot, however, support this theory. Further studies and wider groups of patients are needed to determine if the hypothesis formulated can be confirmed or not.

Conclusions

Although there has been remarkable progress in the treatment of HCV infection until recently, and it is not incorrect to believe that also currently, dual therapy with PegIFN and RBV remain the standard-of-care for HCV in Romania. Unfortunately, treatment with PegIFN and RBV results in SVR rates of only 42-52% for viral genotype 1. In this paper, we have demonstrated that there are several factors that can be taken into consideration regarding the possibility to achieve sustained virological response. We have shown that the highest positive predictive value for obtaining SVR, was certified for patients who achieved RVR, EVR, were females, had low baseline viral load and had an F1 grade of hepatic fibrosis. These tools can further help in selecting suitable patients for dual antiviral therapy.

Abbreviations

HCV Hepatitis C Virus
SVR Sustained Virological Response
RVR Rapid Virological Response
EVR Early Virological Response
DVR Delayed Virological Response
LVL Low Viral Load
HVL High Viral Load
HIV Human Immunodeficiency Virus
HBV Hepatitis B Virus
RNA Ribonucleic Acid
CBC-Complete Blood Count
PegIFN-Pegylated Interferon
RBV-Ribavirin

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References

1. Constantinescu I. Studii privind epidemiologia hepatitiei de tip C. Rev Română Med Lab; 2005; 1(1): 63-66
2. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect; 2011;17(2):107-115.
3. van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Roque A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Kegt R, Hansen BE, Janssen HL. Association Between Sustained Virological Response and All-Cause Mortality Among Patients With Chronic Hepatitis C and Advanced Hepatic Fibrosis. JAMA; 2012; 308(24):2584-2493
4. Swain MG, Lai MY, Shiffman ML, Cooksley WG, Zeuzem S, Dieterich DT, Abergel A, Pessoa MG, Lin A, Tietz A, Connell EV, Diago M. A Sustained Virologic Response Is Durable in Patients With Chronic Hepatitis C Treated With Peginterferon Alfa-2a and Ribavirin. Gastroenterology; 2010; 139(5):1593-1601.
5. Manns MP, Pockros PJ, Norkrans G, Smith CI, Morgan TR, Häussinger D, Shiffman ML, Hadziyannis SJ, Schmidt WN, Jacobson IM, Bárcena R, Schiff ER, Shaikh OS, Bacon B, Marcellin P, Deng W, Esteban-Mur R, Poynard T, Pédiçone LD, Brass CA, Albrecht JK, Gordon SC. Long-term clearance of hepatitis C virus following interferon α-2b or peginterferon α-2b, alone or in combination with ribavirin. J Viral Hepat; 2013; 20(8):524-529.
6. Analiza evoluției bolilor transmisibile aflate în supraveghere Raport pentru anul 2012 [Internet Institutul Național de Sănătate Publică. Available from:https://cnscbt.ro/index.php/rapoarte-anuale/546-analiza-evolutiei-bolilor-transmisibile-aflate-in-supraveghere-raport-pentru-anul-2012/file
7. Analiza evoluției bolilor transmisibile aflate în supraveghere Raport pentru anul 2013 [Internet]; Institutul Național de Sănătate Publică; Available from: https://cnscbt.ro/index.php/rapoarte-anuale/547-analiza-evolutiei-bolilor-transmisibile-aflate-in-supraveghere-raport-pentru-anul-2013/file
8. Adina M. Kamal et.al. - Predictive Factors for Treatment Response in Patients with HCV infection

9. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology; 2013; 57(4):1333-1342.

10. Guadagnino V, Stroffolini T, Rapietza M, Costantino A, Kondili LA, Menntti-Ippolito F, Caroleo B, Costa C, Griffo G, Loiacono L, Pisani V, Foca A, Piazza M. Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: A community-based survey in southern Italy. Hepatology; 1997; 26(4):1006-1011.

11. Drăgănescu M, Iancu A. Prevalența hepatitelor acute virale în ultimii ani la Spitalul clinic de Boli Infecțioase “Sf. Cuv. Paraschieva” din Galați. Revista Română de Boli Infectioase “Sf. Cuv. Paraschieva” din Galați; 2015; 17(2-3):99-102.

12. Kuboki M, Shinzawa H, Shao L, Ishibashi M, Yoshii E, Suzuki K, Saito K, Saito T, Togashi H, Takahashi T, Yasumura S, Fukao A. A cohort study of hepatitis C virus (HCV) infection in an HCV epidemic area of Japan: age and sex-related seroprevalence of anti-HCV antibody, frequency of viremia, biochemical abnormality and histological changes. Liver; 1999; 19(2):88-96.

13. Evans JL, Hahn JA, Page-Shafer K, Lum PJ, Stein ES, Davidson PJ, Moss AR. Gender Differences in Sexual and Injection Risk Behavior Among Active Young Injection Drug Users in San Francisco (the UFO Study). J Urban Health; 2003; 80(1):137-146.

14. Campello C, Poli A, Dal Molin G, Besozzi-Valentini F. Seroprevalence, Viremia and Genotype Distribution of Hepatitis C Virus: A Community-Based Population Study in Northern Italy. Infection; 2002; 30(1):7-12.

15. Nguyen MH, Keeffe EB. Prevalence and treatment of hepatitis C virus genotypes 4,5, and 6. Clin Gastroenterol Hepatol; 2005; 3(10 Suppl 2):97-101.

16. Pawlotsky J-M. Hepatitis C virus genetic variability: pathogenic and clinical implications. Clin Liver Dis; 2003; 7(1):45-66.

17. Seeff LB, Hoofnagle JH. The National Institutes of Health Consensus Development Conference Management of Hepatitis C 2002. Clin Liver Dis; 2003; 7(1):261-287.

18. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet; 2001; 358(9286):958-965.

19. Friedman MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçalves FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon Alfa-2a plus Ribavirin for Chronic Hepatitis C Virus Infection. N Engl J Med; 2002; 347(13):975-982.

20. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM; PEGASYS International Study Group. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med; 2004; 140(5):346-355.

21. Grigorescu M. HCV Genotype 1 is Almost Exclusively Present in Romanian Patients with Chronic Hepatitis C. J Gastrointestin Liver Dis; 2009; 18(1):45–50.

22. Davis G, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology. 2003; 38(3):645-652.

23. Asselah T1, Estrabaud E, Bieche I, Lapalus M, De Muynck S, Vidaud M, Saadoun D, Soumelis V, Marcellin P. Hepatitis C: Viral and host factors associated with non-response to pegylated interferon plus ribavirin. Liver Int; 2010; 30(9):1259-1269.

24. Poynard T, Munteanu M, Colombo M, Bruix J, Schiff E, Terg R, Flamm S, Moreno-Otero R, Carrillo F, Schmidt W, Berg T, McGrattan T, Heathcote EJ, Gonçalves F, Diago M, Craxi A, Silva M, Boparai N, Griffel L, Burroughs M, Brass C, Albrecht J. FibroTest is an independent predictor of virologic response in chronic hepatitis C patients retreated with pegylated interferon alfa-2b and ribavirin in the EPIC3 program. J Hepatol; 2011; 54(2):227-235.

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