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DOCTORAL SCHOOL OF MEDICINE

DOCTORAL FIELD: MEDICINE

EVALUATION OF THE EFFICIENCY AND TOLERABILITY OF DAA THERAPY IN C VIRUS CHRONIC HEPATITIS

SUMMARY OF THESIS

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CONTENTS

Keywords : Hepatitis C virus, direct-acting antiviral agents, sustained virologic response, quality of life.

Note: The figures and tables inserted in the summary of the doctoral thesis keep the original numbering of the work in extenso.

GENERAL PART
PRESENT STATE OF KNOWLEDGE

The hepatitis C virus (HCV) is a major problem of public health, with an estimated number of 180 million persons infected worldwide. Up to 25% of the chronically infected patients will eventually develop cirrhosis and multiple complications, including hepatocellular carcinoma [1], a chronic hepatic disease secondary to HCV infection, thus having a main indication for liver transplant in the United States [2].

There is a wide spectrum of clinical manifestations, from patients without any symptomatology or with increased transaminase levels, with mild or moderate modifications of the histological aspect of the hepatic biopsy, with a good prognosis [3], up to patients with severe hepatitis C, with elevated transaminases, with high levels of HCV-RNA, who develop hepatic cirrhosis and whose prognosis is very poor [4,310]. At the center of this disease spectrum there are patients who present few symptoms, mild increase or moderate modifications of the serom transaminases and uncertain prognosis. Hepatitis C with HCV may cause liver cancer, liver failure and hepatocellular carcinoma.

Researchers have estimated that 20% of the patients with hepatitis C with HCV develop liver cancer within 10-20 years [5,311]. After 20-40 years, a small percentage of the patients develop hepatocellular carcinoma. Liver failure caused by hepatitis C with HCV is the most common cause of liver transplant in the USA. Hepatitis C causes half of the hepatic tumors in the world. Men, alcoholics, cirrhosis patients and those infected with HCV for more than 20 years are the most predisposed to develop liver cancer [6,311].

The geographic distribution presents a great variability, in strict relation with the risk factors regarding the transmission specific to each geographic area, from very low prevalences in the United Kingdom and the Scandinavian countries (0.01-0.1%) to very high prevalences in Egypt and some African or Asian countries (>10%) [10].

From the data provided on November 4, 2016, by TESSy, it results that: [11,12,359]

In 2015, 28 member states of the EU/EEA reported 34,651 cases of hepatitis C, with the raw incidence rate of 8.6 cases in 100,000 inhabitants (a mild decrease of 4.0% as compared to the previous year)[11,12,359].

From these cases, 1.0% were reported as acute, 12.7% cases were chronic, 69.5% were “unknown”, and 16.8% were not classified [11,12,359].

The incidence rates vary from 0.1 cases in 100,000 inhabitants in Greece up to 79.1 cases in 100,000 inhabitants in Latvia, while 39.2% of all reported cases were in found the United Kingdom [11,12,359].

Austria and Germany recorded reductions of the HCV infection rate, between 2014 and 2015 (from 23.2 cases in 100,000 inhabitants to 18.6 cases in 100,000 inhabitants in Austria; from 7.2 cases in 100,000 inhabitants to 5.9 cases in 100,000 inhabitants in Germany)[12,359].

France, Liechtenstein, and Spain did not report any data. Hepatitis C is more frequently reported in men than in women, the male-female ratio having been 1.9 to 1 [11,12,359].

Figure 1. Prevalence of HCV infection worldwide [11]

The interpretation of hepatitis C related data remains problematic, with differences in the surveillance systems and difficulties in defining the cases reported as acute or chronic. In the case of hepatitis C, which is an asymptomatic disease until its tardive stages, the surveillance based on notification data is full of challenges, with information reflecting more the testing practices than the real onset of the disease [12,359].

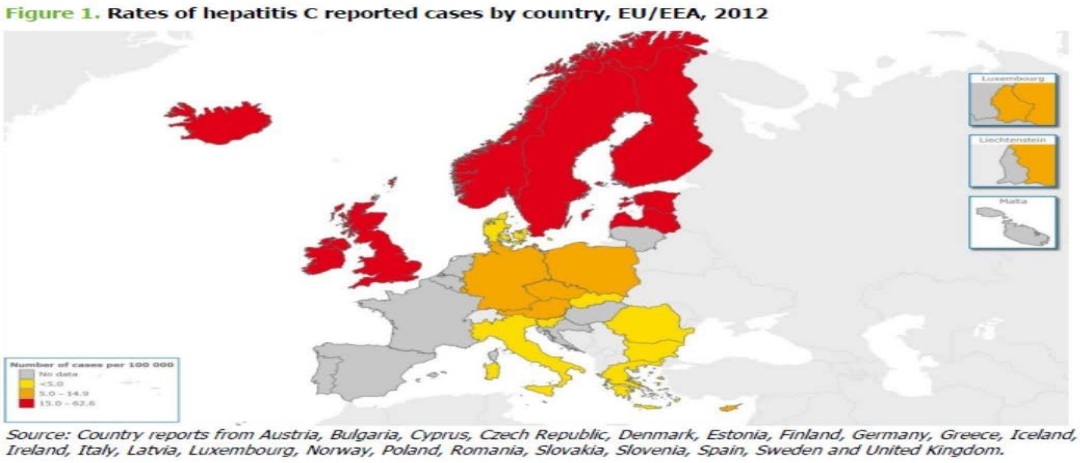


Figure 2. Prevalence of HCV hepatitis in Europe [13]

In the WHO European region, it is estimated that there are 9 million patients chronically infected with HCV, the prevalence being 2-3 times higher than in the countries outside the EU[12,359].

Approximately one adult in 50 is infected with the hepatitis C virus or has a chronic infection with HCV [12,360].

In Romania, according to the WHO, in 2016, the maximum incidence rates were recorded at the age group 45-54 years for both female (1,3%) and male (0.6%) Specific incidence per gender was 0.4% for female sex and 0.3% for the male [12,360]. The peak incidence rate has been recorded with the age group 45-54 years for the acute stage while the chronic HVC, the number of cases is too low to have a relevant comparison. The highest possible transmission category, mentioned at the highest frequency, was the nosocomial (23.6%) of master 12,360 tenths[12,360].

HCV may be transmitted in three ways:

- percutaneous: the most frequent way of transmission, of ~ 60-70%, by blood and derivatives [15], contaminated and unsterilized medical instruments, accidentally [16,17,313];
- non-percutaneous: sexually, up to 27% in risk groups [18] and perinatal, more seldom, up to 5% [19,20,314];
- sporadically: community acquired hepatitis, in 5-10% of the cases [15].

The main risk groups for contacting HCV are: polytransfused patients, hemophiliacs (~70-90%), i.v. drug abusers, hemodialyzed patients, organ recipients (~80-100% of the HCV positive organs), prostitutes, healthcare personnel, disfavored socioeconomic categories of population.

In the developed countries, there has been reported a decrease of incidence of the HCV infection, while in the poor countries, it is still at a high level due to the use of unchecked blood products and of unsterilized medical materials. An analysis made during the past decades with regard to the way of transmission demonstrated the decrease of the risk of transmission by blood products and the increase of the risk related to i.v. drug abuse [31,32,317,318].

HCV infection determines a wide spectrum of clinical manifestations:

- Usually the acute episode of HCV infection evolves subclinically, the jaundice being present in only 10% of the cases. The aspect of fulminant acute hepatitis is rare, but the disease takes a severe acute form when the infection is grafted onto the background of an unknown chronic liver disease, in case of a HBV-HCV coinfection, or in liver transplant recipients[74]. The evolution is marked by the high percentage of liver disease chronicization (70-80%) and of the development of

cirrhosis (20%) after an average interval of three decades. Hepatocellular carcinoma (3-4%) and cirrhosis explain the great majority of deaths in the patients with post transfusion hepatitis with this etiology.[74,75]

- It appears frequently after transfusions with blood or blood products. The incubation period varies from 2 to 26 weeks, with an average of 7-8 weeks. Most cases (~80%) are asymptomatic or less noisy and, when they are present, the symptoms are non-specific: asthenia, anorexia, abdominal pains, flu-like phenomena, with myalgia and arthralgia, weight loss, fever and jaundice being very rarely described, in less than 30% of the patients. It may have an extensive and severe evolution, but it seldom produces fulminant hepatocellular failure [76,330].

- Serologically, 7-8 weeks after the infection contact, aminotransferase levels increase 10-15 times over the normal limit, this being the only diagnostic criterion in some cases. Transaminases may fluctuate or may evolve into a plateau structure, in such situations the chronicization risk doubles, respectively triples [77,331].

- The negative prognostic factors related to the rapidity of progression of the disease are: genotype I b, the high level of viremia, and the degree of genetic diversity of the virus (quasispecies), HCV transmission by transfusion, immune deficiency[74], coinfection with HBV or HIV, alcohol abuse. In Romania, the prevalence of genotype 1 is of over 99%. The occurrence of cirrhosis is favored by: age 40+, daily consumption of at least 50 g of alcohol and the male sex, the infection associated with transfusion, coinfection with HBV or HIV. If with the compensated cirrhosis the survival is 5 years in 90% of the cases, the proportion is reduced to 50% at 5 years from decompensation [75].

- HCV-RNA may be detected in the patients' serum at 2 weeks after exposure. It is the single marker of the infection until the occurrence of the clinical manifestations and the increase of transaminase rates. Viremia increases rapidly at an initial stage, then slower, reaching a peak (10^5-10^7 UI/ml) right before the clinical and biological onset. Its decrease and negativization are signs of a favorable prognosis [78,79,80,331].

- Seroconversion takes place 3-12 weeks from the occurrence of viral RNA in the serum. The delay of occurrence of anti-HCV antibodies leads to diagnostic errors in the immunological window period if viral RNA is not detected as well. Anti-HCV antibodies remain for a long time in the patients serum, the high viremia being the only marker of the active infection[80,81,331].

- In case of self-limited infection (15-20%), transaminases come back to normal levels, viremia becomes undetectable, but antibodies remain in the patients serum for a couple of months.

The persistent negativization of the HCV-RNA in the serum may be associated with its absence in the liver, which means the complete elimination of the virus [80,81,331].

- The decrease of the detection level of HCV-RNA dropped from 500 cp/ml to 100 cp/ml, therefore some of the patients considered spontaneously healed proved to have actually presented a low viral replication [82,314]
- An important role in the elimination of the virus is played by LyT. The viral clearance is associated with an important Th1 and T $CD8^+$ response and a weak Th2 response [82,314].
- The spontaneous elimination of the virus is associated with the following favorable prognostic factors: female sex, Caucasian (white) race, presence of jaundice, low viremia, alleles: HLA DRB_1*01, HLA DRB_1*04, HLA DRB_1*1101, HLA DQA_1*03, HLA DQB_1*0301.[82,314]
- In case of evolution towards chronicization (75-80%), transaminase levels may come back to athe upper limit of the normal range, but the viremia remains detectable at high levels. There are situations in which a viremia is transiently undetectable in the serum, but subsequently becomes apparent, such event marking the evolution towards chronicization [83].
- Chronic viral hepatitis C is defined by the persistency of HCV-RNA for more than 6 months after acute hepatitis C. If to define the chronicization of the infection one considers as a parameter the absence of ALT normalization, the chronicization rate in the transfused patients is appreciated to 62-77%, and with those that acquired the infection from the community to 62 % [7, 84].
- But if the chronicization is defined by the persistence of viremia, the chronicization rate is appreciated to 80%, in some studies even more and, out of the chronic patients, 60-80% present a permanent increase of the serum enzymes [7, 84].
- In chronic infections, the HCV-RNA rate is generally stable, and the spontaneous viral clearance is especially uncommon.
- Rare are the cases where during the course of the chronic infection are detected low serum levels or transient negativizations of HCV-RNA (generally in the terminal phases of the disease) [7, 85].
- The existence of asymptomatic chronic bearers is debatable, considering the biological peculiarities of chronic hepatitis C, where 25-50% of the patients have normal transaminase levels in the presence of HCV-RNA, but the liver biopsy almost always evidences morphological modifications that cover a very wide area of modifications, from the minimal ones to hepatic cirrhosis [7, 86].

- The chronicization rate of the acute infection is of 80%, and 75-80% of these patients present high rate transaminases.
- The spontaneous resolution of the virus C infection is uncommon, but the HCV-RNA level may fluctuate or it becomes undetectable during the advances stages of the liver disease [7, 85].
- Chronicization varies from 40-50% to 90-100% according to the age (infections acquired at a younger age chronisize more seldom), sex (the female sex has a protective effects), source of the infection and the magnitude of the inoculum (chronicization is more frequent post-transfusionally) and the presence of some cofactors (coinfection with virus B, alcohol abuse, the immune status of the host) [7, 86].

Until the year 2015, the patients with HCV could benefit in Romania from the specific treatment with pegylated interferon α-2a or pegylated interferon α-2b associated with Ribavirin. This treatment is intended for patients with HCV infection, naïve (without prior specific therapy) or pretreated, diagnosed both with chronic hepatitis and with compensated hepatic cirrhosis.

Criteria for inclusion in treatment : [187]

- Perceived quantitative viremia (ARN-VHC);
- Normal or increased gas-flow rates;
- Histological confirmation (Liver biopsy driver score, FibroMax test A ≥1,F≥ 1 si/sau Fibroscan >1;
- The 65 year old threshold, initially required to perform the treatment, has been eliminated, provided that, above this age, a correct analysis of the therapeutic risk based on the patient's biological status [187].

For the past years, the treatment of the HCV infection has undergone a major revolution. Numerous orally administered DAA's were approved for the treatment of HCV infection.

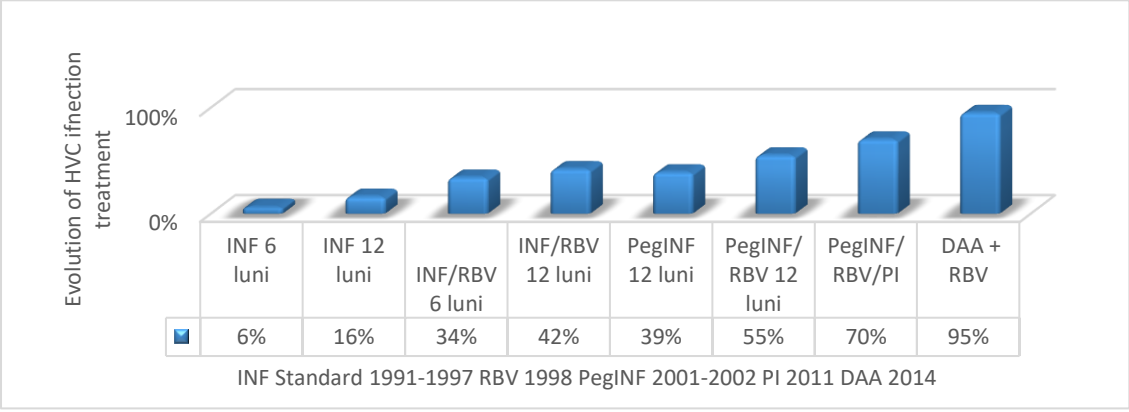


Figure 16 : The evolution of HCV infection treatment 1991-2014 [190]

Interferon free terapy in cronic viral hepatitis C:

In the past years, the treatment of HCV has undergone a major change. Numerous orally administered DAA's were approved for the treatment of HCV [190].

The treatment of the infection with hepatitis C (HCV) was revolutionized by the introduction of direct action antivirals (DAA's), which are agents that can interfere with various stages of the replication cycle of a virus [191,192].

Thus was replaced the standard treatment, based on a combination of pegylated interferon α (peg-INFα) and Ribavirin (RBV), which act in various non-specific ways in order to stimulate the antiviral immune response. DAA's lead to viral eradication in more than 90% of the patients [192].

Beside the fact that they are significantly more effective than the interferon based therapy in healing the infection, they have important additional benefits, including a tolerability profile that makes them adequate even for the patients excluded from previous treatment [193], a simplified management due to a shorter duration of the treatment, and the oral way of administration. Unfortunately, the high costs of these therapies limit the access to these drugs, which imposes a rigorous selection of patients and blocks the wide scale use of the drugs [194].

The viremia and the precocious drop of ARN-HCV levels are the strongest elements in estimating the result of the treatment and measuring the viremia during the treatment is crucial for establishing the duration of the treatment and for the evaluation of the response-guided therapy [195].

Classes of Direct Action Antivirals (DAA's)

DAA's are classified as follows: □195□

- NS3/4A protease inhibitors (PI), which function by blocking a viral enzyme (protease) which transmits the hepatitis C virus the message to survive and to reproduce within the host cells;

- NS5B nucleoside and nucleotide polymerase inhibitors, which directly target the hepatitis C virus in order to prevent its replication. It attaches to the genetic information (ARN) thereby impeding the multiplication of the virus;

- NS5A inhibitors, which block the NS5A viral protein, preventing the virus to reproduce, acting in various stages of the infection;

- NS2B non-nucleoside polymerase inhibitors, which act in order to stop HCV reproduction by its introduction into the virus, so that other parts of the HCV virus may not attach thereto.

NSE3/4A serine protease is required for self-scission during HCV replication, the NS5A region plays an important role in in the viral replication and assemblage, and the NSE5B region codifies ARN polymerase, which is necessary for HCV replication. NSE3/4A serine protease inhibitors consist of first-line linear inhibitors and macrocyclic inhibitors considered to be second-line [195].

DAA Class	Drug	Genotype
NS3/4A protease inhibitors (PI)	Glecaprevir	1-6
	Paritaprevir	1-4
	Voxilaprevir	1-6
	Grazoprevir	1-3
	Sunvepravir	1-4
NS5B nucleoside and nucleotide polymerase inhibitors	Sofosbuvir	1-4
NS5A inhibitors	Ombitasvir	1-4
	Daclatasvir	3
	Ledipasvir	1
	Velpatasvir	1-6
NS2B non-nucleoside polymerase inhibitors	Dasabuvir	1

Table 12: DAA Classes and genotype on which they act [195]

The therapy of HVC patients is difficult and depends on factors related to the virus character and to the human host. Among the viral factors there are the HCV GT genotype, the basic viral load and the virologic response to treatment, and those related to the host are age, sex, race, vicious habits, obesity, the degree of liver fibrosis and IL28B gene polymorphism [196, 350].

SPECIAL PART

PERSONAL CONTRIBUTIONS

The purpose of the study: consisted in comparing the effectiveness and tolerability of therapies for chronic viral hepatitis C, starting with the triple combination considered in 2012 to be superior to the classical therapy with interferon and ribavirin and continuing with the new DAA molecules, which promised obtaining a sustained viral response (SVR) of 100%, with the possibility of eradicating HCV infection.

The main objectives of my study consist of evaluating the effectiveness of the various therapeutic schemes used in treating patients with HCV and their long-term impact on the improvement of quality of life.

Material and method:

Selection of patients:

The study was developed within the Clinic of Infectious Diseases of Constanta, in Constanta County Hospital and in the Gastroenterology outpatient clinic.

Structure of the research in three sub-studies:

- Sub-study 1: patients under treatment with PegINF αs-2a (INF) and Ribavirin (RBV) to which was added a first generation NS3/4A protease inhibitor (DAA) - Telaprevir.
- Sub-study 2: patients under treatment with "interferon free" schemes (protease inhibitor, polymerase inhibitor, NS5A inhibitor ± Ribavirin).
- Sub-study 3: dedicated to the evaluation of the quality of life of HCV patients who benefited from the "interferon free" therapy, as compared to the patients who did not benefit from treatment for not having met the eligibility criteria.

We used for comparison the lot of 154 patients infected with HCV who did not benefit from specific antiviral therapy for various reasons, but who were evaluated from a clinical, virological, and biological point of view in order to evidence the influence of the factors pertaining to the virus or to the host in the natural evolution of the hepatic disease, as well as to evaluate the quality of life of the patient diagnosed with HVC but untreated, as compared to the quality of life of the HVC patient who benefited from "interferon free" antiviral therapy.

Work Algorithm : According to the recommendations of EASL 2018, all patients with chronic viral hepatitis C should be treated with antiviral thepapy, the access of patients to the new interferon free regimens should be increased, after a prior biological, virologycal evaluation, in order to exclude contraindication, and obtaining the patient′s consent regarding the treatment.

The patients included in the study were subdivided into groups according to the therapeutic regimen applied :

- Sub-study 1 : Peg-INF + Ribavirin + Telaprevir (1st generation protease inhibitor NS3/4A) - 24 patients.
- Sub-study 2 : Interferon free ± Ribavirin regimens – 52 patients.
- Sub-study 3 : control patients with HCV evaluated (during the period 2016 – 2018),but who did not meet the eligibility criteria – 154 patients (92 patients accepted to participate in the study regarding the quality of life of patients with chronic hepatic conditions and signed the informed consent form).

The duration of the treatment was variable,according to the treatment regimen applied,the response to treatment the patient′s tolerance.

The respons to treatment was evaluated by determining the HCV -RNA at the end of the treatment and 12 weeks after the end of the treatment (SVR).

The patients with a favorable response, respectively the patients in group A, with undetectable viremia, continued the treatment up to 48 weeks ; these patients were monitored and evaluated only for the duration of administering DAA treatment.

All the patients were monitored from a clinical, biological, histological, and virological point of view.

As a novelty, the patients included in the study in 2018, but also the patients evaluated in 2017-2018, who did not meet the eligibility criteria, were evaluated as to their quality of life, by applying the questionnaire SF-LDQOL (short form), issued for the assessment of the quality of life of patients with chronic hepatic conditions (LDQOL contains 16 questions which evaluate: the disease-related symptoms, the consequences of the hepatic disease, the concentration/memory, the problems caused by the disease, the life expectancy, the stigmata of the hepatic disease, and the sexual problems) and the questionnaire SF-36 (a generic questionnaire consisting of 11 questions, structured on various dimensions of the quality of life, including the functional status and the synthesis mental component).

There were studied some demographic data (originating environment, race, sex), personal data (age, weight), comorbidities and the importance thereof in the evolution of chronic hepatitis.

The evolution of hepatic fibrosis was evaluated in all patients by FibroMax, before initiating the antiviral therapy.

The viral genotype was determined for a limited number of patients, but sufficient to draw pertinent conclusions on its influence on the evolution of chronic hepatitis.

Criteria for including patients in sub-study 1:

- Patients with HCV infection, with ages between 18 and 70 years.
- Patients with HCV infection, with F3-F4 fibrosis, evaluated by hepatic biopsy or non-invasive expectancy evaluations, such as FibroScan, elastography, or FibroTest.
- The patient must have had a hepatic biopsy before the screening (or between the screening and the baseline visit), except for the case where s/he would have had a contraindication for such procedure or any proof of portal hypertension. For the patients who had a hepatic biopsy done more than 2 years before the screening or without any biopsy (in case of a contraindication or portal hypertension), it was required a non-invasive evaluation by FibroScan, elastography, or FibroTest, which should not be older than 6 months before the screening.
- The chronic infection with the hepatitis virus C (HCV) must be confirmed by one of the following: the presence of anti-HCV antibodies and/or the HCV-RNA for at least 6 months before the screening visit and/or the presence of fibrosis evidenced by the biopsy.
- Genotype 1 of the HCV infection with plasmatic HCV-RNA > 10.000 UI/ml (both confirmed upon screening).
- Patients with partial and zero response to the previous treatment with PegINF, PegINFα-2a, or PegINFα-2b in association with Ribavirin (RBV).
- The patient must have had at least one treatment regimen, documented with PegINFα-2a or PegINFα-2b in association with Ribavirin (RBV) (at least 12 weeks for a zero response and 20 weeks for a partial response).
- Patients (male and female) will use double barrier contraception.

Criteria for the patients' exclusion from sub-study 1:

- Decompensated hepatic cirrhosis, proved by modifications of the lab tests of other active pathological conditions which contraindicate the treatment.
- No hepatic carcinoma or other malignant conditions.
- Infection with the human immune deficiency virus (HIV) or with non-genotype 1 hepatitis C, or hepatic conditions not related with the hepatitis C virus infection.

- ❖ Other previous treatment for the viral hepatitis C, other than PegIFN and RBV.
- ❖ Pregnancy or breastfeeding.

The patients were initially evaluated both physically and paraclinically, by lab tests (complete blood count, biochemistry, urinalysis, genotyping), hepatic biopsy or FibroScan/FibroMax, abdominopelvic ultrasound, electrocardiogram.

Subsequent evaluations were made at week 1, 2, 4, 6, 8, and 12 by lab tests (complete blood count, ALT, AST, BT, BD, lipid panel, uric acid, electrolytes), urinalysis, EKG, complete physical examinations, and women took pregnancy tests.

The therapeutic response was evaluated by analyzing:

1. the virological response:

- ❖ quantitative HCV-RNA at week 4 of treatment (facultative RVR);
- ❖ quantitative HCV-RNA at week 12 of treatment (RVP);
- ❖ quantitative HCV-RNA at 24 weeks from the end of treatment (RVS);
- ❖ quantitative HCV-RNA at week 48 of treatment (EOT);

We evaluated the therapeutic response at 12 weeks of antiviral treatment by associating a protease inhibitor (Telaprevir) to the standard therapy PegINF + Ribavirin, the patients continuing their treatment up to 48 weeks only with Peg-INF and Ribavirin.

The patients were monitored and evaluated only for the period of administration of the triple therapy.

2. the biochemical response: - ALT/AST at 4 weeks, 12 weeks, 24 weeks;

3. the histological response: - pathology tests and METAVIR score upon initiation of the therapy and optionally after 2 years.

For the patients diagnosed with chronic viral hepatitis C, but untreated, for various reasons, used in the control group, the clinical, biological, serological, and imaging evaluation was carried out at a 3-month interval. We monitored the evolution, having as main aim to evidence the appearance of the decompensated hepatic cirrhosis and of the hepatic carcinoma in the untreated patients.

<u>Criteria for including patients in sub-study 2:</u>

- ❖ Naïve patients with F3 (without any previous antiviral treatments);
- ❖ Interferon treatment-experienced patients with F3;
- ❖ Naïve patients with F4 (compensated cirrhosis, Child Pugh <= 6);
- ❖ Interferon treatment-experienced patients with F4 (compensated cirrhosis, Child-Pugh <= 6);
- ❖ Patients with coinfection HCV-HIV;

- Patients with coinfection HCV-HBV;
- Patients with hepatic carcinoma (HCC) ;
- Patients with extrahepatic malignant conditions curatively treated in early stages, with low relapse rate, after an imagining evaluation and with the consent of the oncology specialist;
- Naïve and treatment-experienced patients with F2 and the following associated/concomitant conditions: mixed cryoglobulinemia, renal disease associated with HCV infection (membranoproliferative glomerulonephritis), non-Hodgkin B cell lymphoma where during the standard curative treatment there can be recorded an exacerbation of the C virus replication, hemophilia, major thalassemia, hepatic carcinoma.

Criteria for excluding patients from sub-study 2 :

- decompensated hepatic cirrhosis (defined by a Child-Pugh score > 6 points or the presence of complications: medically documented ascites and/or hydrothorax, spontaneous bacterial peritonitis, hepatorenal syndrome, digestive hemorrhage, hepatic encephalopathy at present or previous);
- hepatic cancer without therapeutic indication with curative potential (transplant, resection, ablation) and hepatic carcinoma treated by resection, ablation, less than 6 months post-procedure or in the presence of imaging criteria (CT/MRI) demonstrating an incomplete response or post-procedure relapse;
- malignant extrahepatic conditions that do not benefit from any treatment with curative potential;
- drug contraindications.

All patients were evaluated at the beginning of the treatment by a physical and paraclinical examination (complete blood count, serum transaminases, serum albumin, bilirubin, prothrombin time, serum creatinine, α-fetoprotein, HCV-RNA, FibroMax), abdominal ultrasound, EDS, CT/MRI if the value of α-fetoprotein is > 50 ng/dl, to exclude hepatocarcinoma) to evaluate the coinfection with the virus HVB or HIV.

There were listed the extrahepatic (pulmonary, cardiac, renal etc.) comorbidities, as well as the pertaining medication before initiating the antiviral therapy and during the therapy, if considered necessary, with a view to establish the contraindications or the eventual drug interactions.

The treatment was monitored by:

- evaluating the response at the end of the therapy: ALT, AST, Child-Pugh score (F4), HCV-RNA at the end of the 12^{th} week of treatment;
- evaluating the sustained virological response (SVR): HCV-RNA at 12 weeks from the end of treatment;

❖ the patients with hepatic cirrhosis (F4) will be monitored every 6 months for an eventual diagnosis of decompensation, evaluation of the variceal bleeding risk, developing hepatocellular carcinoma (HCC).

The evaluation criteria of the therapy result are:

❖ sustained virological response (SVR): HCV-RNA undetectable at the end of treatment and 12 weeks after the end of therapy;

❖ tardive response: HCV-RNA detectable at the end of treatment with values < 15 UI/ml, but undetectable 12 weeks after the end of therapy;

❖ lack of response: HCV-RNA detectable with values above 15 UI/ml at the end of treatment (in this case it is no longer needed to determine viremia 12 weeks after the end of therapy);

❖ relapse: HCV-RNA undetectable at the end of treatment and detectable 12 weeks after the end of therapy, irrespective of the value of HCV-RNA.

Statistical data analysis: The data collected from the group studied served to complete a database, by using SPSS V.21.0, the same software being also used for the statistical processing of the data. The baseline values of the continuous quantitative values were reported as an average and as standard deviation, and the category variables were reported as number of patients (%).To compare the intergroup scaled variables we applied the Mann-Whitney U test.

The Pearson correlation index analyses the dependency relationship among the variability series of the sets of data. It may range between +1 and –1, being the higher in absolute value, the tighter the correlation. Thus, in the case of a correlation coefficient that is very close to +1, we are talking about a very high dependency of the phenomena, a positive dependency, while for a coefficient that is very close to –1, we are talking about a very high dependency, but a negative one. The more the value thereof gets away from the limits, closing to zero, the more the intensity of the relationship is lower.

The mathematical formula for the correlation coefficient is:

$$r= \Sigma(x_i-x)(y_i-y)/\sqrt{\Sigma(x_i-x)^2 \Sigma (y_i-y)^2}$$

The statistical significance of the tests done was interpreted according to the value of *P*:

a) if $0,01 \leq P < 0,05 \rightarrow$ the results are statistically significant;

b) if $0,001 \leq P < 0,01 \rightarrow$ the results are statistically highly significant;

c) if $P < 0,001 \rightarrow$ the results are statistically very highly significant;

d) if $P \geq 0,05 \rightarrow$ the results are statistically insignificant.

Results:

Patient enrolment for the antiviral treatment program:

As mentioned in the previous chapter, the study period was subdivided into intervals on years of study, to evidence the efficiency and tolerability of the new antiviral therapies in the DAA era and the patients' evolution under the antiviral treatment and the quality of life in patients treated with latest generation DAA's.

The study was carried out both at the Constanta Hospital for Infectious Diseases and at the Internal Medicine Clinic of the Department Hospital of Constanta and, subsequently, for the past 12 months (the year 2018) in the outpatient service of Gastroenterology as well.

Study interval	*Number of subjects*
October 2012 – December 2013	*110*
January 2014 – December 2015	*128*
January 2016 – December 2017	*136*
January 2018 – June 2018	*48*

Table 1: Patients diagnosed with HC with HCV on years of study

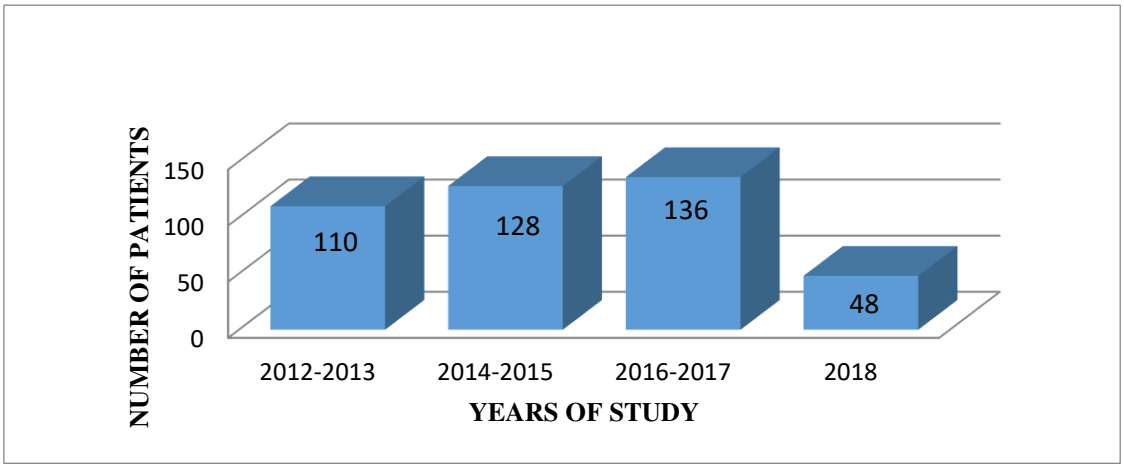


Figure 1: Patients diagnosed with HC with HCV on intervals of study

The interval of time between January 2018 – June 2018 was reserved to the selection of patients that met the inclusion criteria for la antiviral therapy, respectively their inclusion for treatment until June 2018, and their subsequent monitoring by determining HCV-RNA at the end of treatment, the end of the 12th week, respectively day 85-91, and at 3 months after the end of treatment, day 169-175 (SVR), as well as for the statistical analysis of the data collected for the whole duration of the study.

Following the application of the exclusion criteria, out of the total number of 422 patients diagnosed with HCV in the interval October – June 2018, 76 patients underwent antiviral therapy with the mention that, during the interval 2016-2018 were evaluated only patients from the Internal Medicine Clinic of the Constanta Departmental Hospital and from the outpatient service of Gastroenterology.

Study interval	*Number of subjects treated*
October 2012 – December 2013	*24*
January 2014 – December 2015	*22*
January 2016 – June 2018	*30*
2012 - 2018	*76*

Table 2: Enrolment for treatment of patients with HC with HCV on years of study

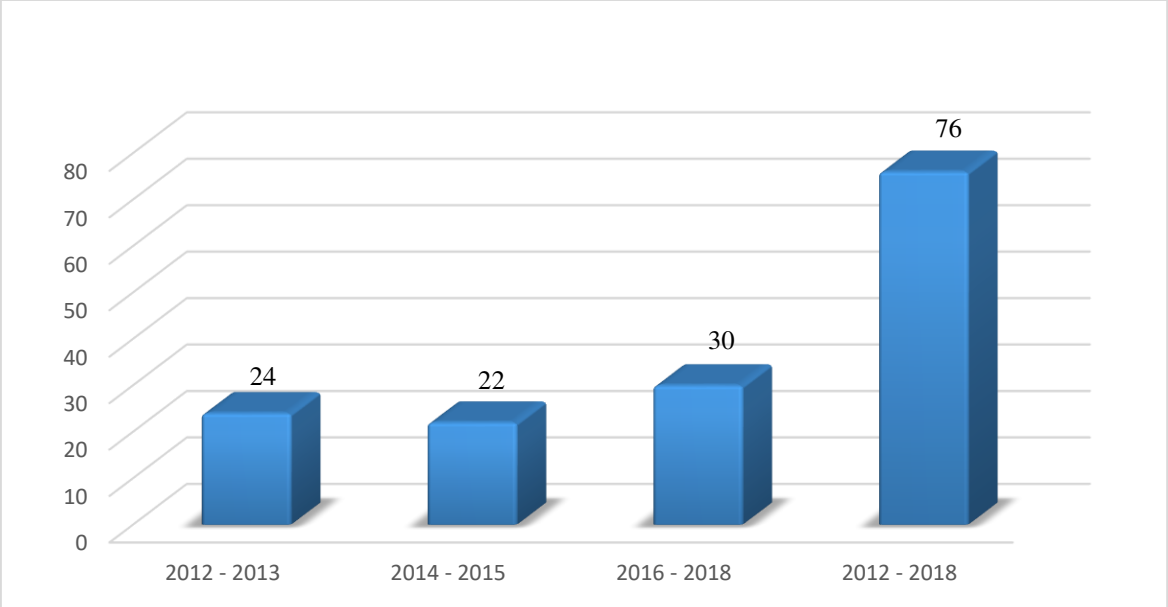


Figure 2: Curve of enrolment for treatment of treated patients with HC with HCV

The rest of patients were excluded as a result of applying the exclusion criteria, or they changed their mind about undergoing the therapy after analyzing, together with their attending physician, of the possible adverse effects of the therapy, as well as due to their comorbidities and to the concomitant medication administered to them which contraindicated the antiviral therapy.

The data analysis evidenced a greater number of patients treated during the last period of the study, as compared to the first period thereof, namely 2012-2013, with the mention that the patients were selected based on eligibility criteria and study year, considering the therapeutic regimen chosen at the moment of including the patients in the study, which was valid also for the patients

excluded from the treatment, considering the various exclusion criteria according to the therapeutic regimen used at the relevant moment in time.

Study period	Subjects diagnosed with HCV		Cases eligible for treatment		Cases excluded from treatment	
	No.	%	No.	%	No.	%
October 2012 – December 2013	110	100%	24	21.81%	86	78.18%
January 2014 – December 2015	128	100%	22	17.18%	106	82.81%
January 2016 – June 2018	184	100%	30	16.30%	154	83.69%

Table 3: Patients with HCV diagnosed and treated, on years of study

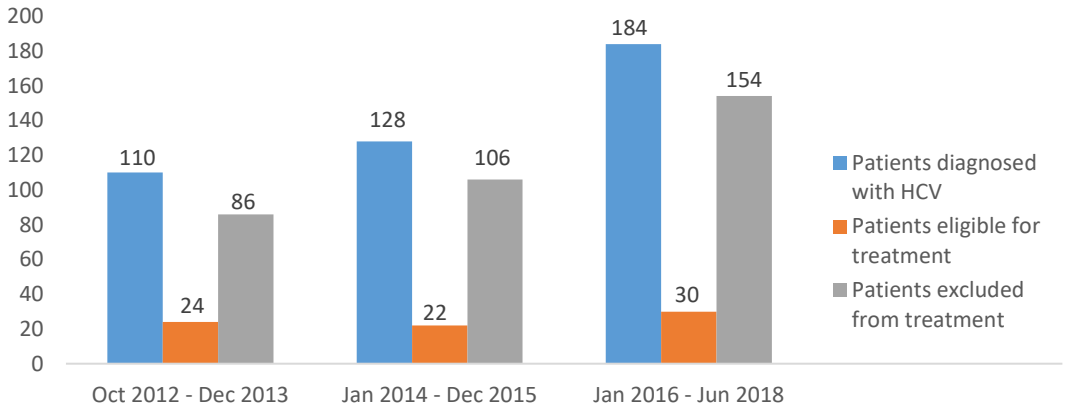


Figure 3: Correlations between the years of study with regard to the patients diagnosed and treated

The initial purpose of the treatment was to demonstrate the efficiency and tolerability of the DAA therapy in patients with chronic viral hepatitis C, considering the launching in 2011 of two preparations with direct antiviral action from the first generation of the class of protease inhibitors, namely Boceprevir and, respectively, Telaprevir, which in combination with PegIFN and Ribavirin determined a SVR of 73% and, respectively, 67% in patients with genotype 1 HCV.

Triple therapy had a short life from its launching until the moment when it was no longer recommended both by the European Association for the Study of Liver (EASL), and by the American Association for the Study of the Liver Diseases (AASLD) in 2014, due to the appearance of the new DAA's, which are extremely effective and safe.

The thesis aims to highlight the current, effective and tolerability of triple therapies compared to "interferon free" therapy, as well as the quality of life of patients treated with "interferon free" schemes compared to untreated patients for various reasons, Eligibility criteria, associated co-morbidities, i.e. refusal of the patient.

Considering the severe adverse effects of the antiviral medication, we strictly observed all the contraindications that interfered with the administration of the therapy. There were excluded 346 (81.99%) patients in the order of frequency, due to their lack of meeting the eligibility criteria at the moment of the initial evaluation, respectively those mainly due to the comorbidities, the patient's refusal, the patient's age (criterion valid for patients in sub-study 1).

Patients excluded due to contraindications	Causes for exclusion from the treatment		
	Comorbidities	Patient's refusal	Age
Number	312	26	8
Percentage	90,17	7,51	2,31

Table 4: Causes for exclusion from the antiviral therapy

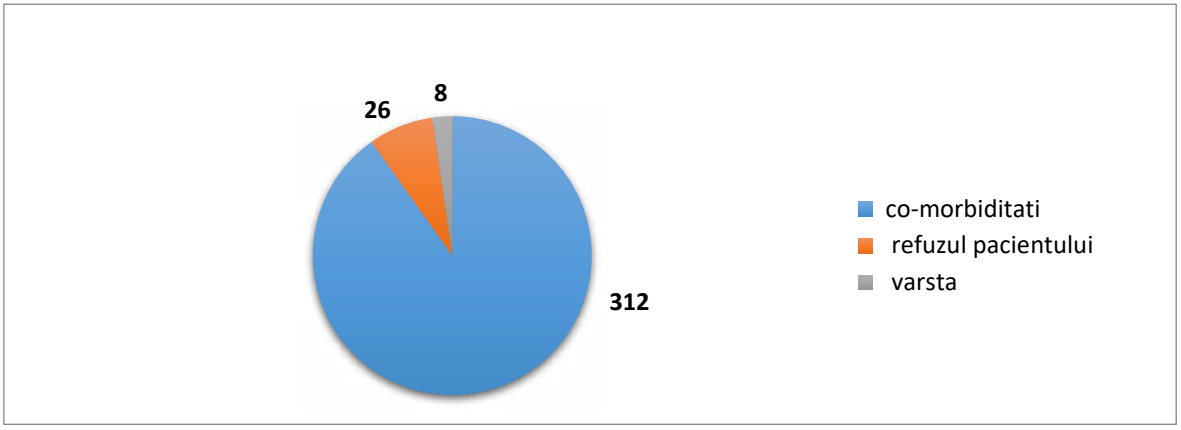


Figure 4: Causes for exclusion from the study (Comorbidities: 312; Patient's refusal: 26; Age: 8)

The comorbidities that interfered in a negative way with the inclusion for treatment of HCV patients were, in the order of their frequency, the following: hepatic cirrhosis of the Child class B/C, serious cardiovascular and respiratory conditions, diabetes mellitus insufficiently controlled by treatment and diet, psychiatric disorders, coinfections with HIV.

Patients	Comorbidities				
	CH CHILD B/C	Severe cardiorespiratory conditions	Uncontrolled diabetes mellitus	Psychoses / Depression	Coinfection with HIV
Number	128	74	58	56	4
Percentage %	40,1	23,71	18,58	17,94	1,28

Table 5: Important comorbidities that interfered with the inclusion of patients in the study

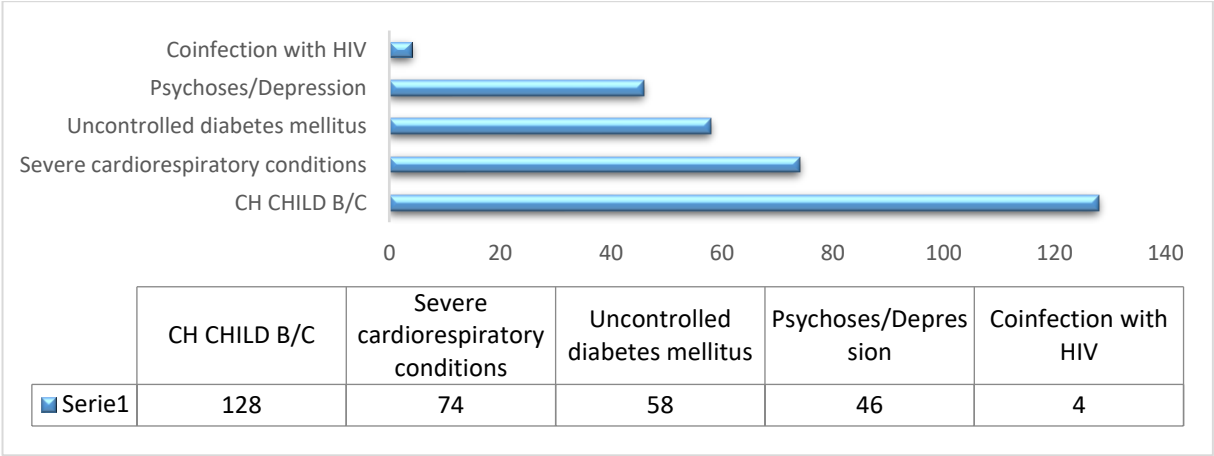


Figure 5: Comorbidities that interfered with the inclusion of patients in the treatment

With regard to the age of the patients, this was an important selection criterion at the moment of evaluating the opportunity of initiating the antiviral therapy, for the group A of patients, according to the treatment inclusion criteria, which recommend that the age range between 18 and 70 years, thus there were excluded from the treatment 8 patients aged over 70, the interval of ages of the excluded patients ranging between 75 and 78 years.

Evaluation of the general data of the studied groups:

The analysis of the general data of the studied patients is as follows:

- sub-study 1, consisting of patients who underwent treatment with Peg-INF + Ribavirin + Telaprevir (1st generation protease inhibitor, NS3/4A) = 24 patients ;
- substudy 2, consisting of patients who underwent treatment with interferon free regimens (polymerase inhibitor, NS5A inhibitor ± Ribavirin) = 52 patients ;

- sub-study 3, with 2 lots patients :

L0 - HCV patients, physically and paraclinically evaluated during the period 2016-2018, but who did not meet the eligibility criteria in order to be included for the antiviral therapy, 154 patients, among which 94 patients participated in the evaluation of the quality of life of HCV patients;

L1 - Patients with HVC who were treated with interferon free schemes accepting participation in the study on quality of life, before and after treatment.

To evaluate the quality of life of HCV patients, we used the SF-LDQOL Questionnaire issued for the assessment of the hepatic patient's quality of life. [257,259]

This questionnaire is useful for the assessment of the quality of life of patients with chronic hepatic diseases and for the evaluation of the way in which such patient becomes aware of the improvement of his/her quality of life after undergoing the treatment. For a result as eloquent as possible, it is useful to do this questionnaire before and after undergoing the antiviral treatment.

The results and the analysis of the data obtained from the SF-LDQOL Questionnaire confirm the need to periodically evaluate the quality of life of patients with chronic hepatic diseases, periodic, before and after undergoing the antiviral treatment, but it is also useful to be done at distance from the end of treatment, during the checkup at 12 weeks from the end of treatment (SVR), with a view to monitor in the most effective way possible the patient's quality of life and the psychological impact of the hepatic disease on the quality of life.

Sub-study I:

It consists of 24 patients with chronic viral hepatitis C, genotype 1, who underwent antiviral therapy with PegINF-α and Ribavirin, to which NS3/4A protease inhibitor (Telaprevir) was added.

The treatment with direct acting antiviral medication (DAA's) increased the rate of sustained virological response (SVR) in patients with chronic viral hepatitis C, genotype 1, and determined the shortening of the treatment duration in many patients, but these agents have low-strength barrier.

The initial monotherapy studies evidenced a prompt suppression of the virus and the fast appearance of the resistance, requiring he administration of the two together with α-interferon and Ribavirin to prevent strength, at 12 weeks and at 24 weeks after the end of treatment with DAA [258,259].

The study carried out on this group of patients analyzes the triple therapy in point of tolerability and efficiency only for the duration of DAA administration (Telaprevir for 12 weeks).

The duration of the association of the DAA therapy (Telaprevir 2250 mg/day, 750 mg every 8 hours, after meals) to the standard treatment was of 3 months, with remarkable virological results, which we want to demonstrate by our study.

The patients included in the study were evaluated physically and biologically both before and after the therapy.

There were carried out:

- diagnostic tests to detect HCV antibodies by the ELISA (Enzyme Linked Immuno Sorbent Assay) isoenzyme method [261]

- molecular tests to detect the viral genotype and to evaluate the viral load by the PCR method (Cobas 6800, Roche Molecular Diagnostics) to confirm the diagnosis [261]

The viral load was determined at the beginning of the therapy and at the end thereof.

There were done blood tests (complete blood count), biochemical tests (GOT/ALT, GPT/AST), total gamma globulin, FA, urea, creatinine [261], INR, serum albumin;

The evaluation of the hepatic fibrosis grade was made by hepatic biopsy or by noninvasive tests (FibroScan, FibroTest).

Results of Sub-study I:

Assessing the evolution of patients sub-study 1 from the point of view of response to treatment, efficiency and tolerability.

The main purpose of the administration of antiviral therapy with DAA (1st generation NS3/4A protease inhibitor) was to obtain a sustained viral negativization. In patients infected with HCV, genotype 1, the chances of a sustained virological response (SVR) with the previous standard treatment (Peg-IFN-α + Ribavirin) are of 40-50% only.

The triple therapy with DAA in association with Peg-IFN-α + Ribavirin was considered as the new standard care for the treatment of chronic hepatitis C in patients infected with genotype 1 [275,276,277].

The treatment with direct acting antiviral medication (DAA's) increased the rate of for the sustained virological response (SVR) in the infection with genotype 1 and the shortening of the duration of treatment in many patients, but these agents have a low barrier to resistance.

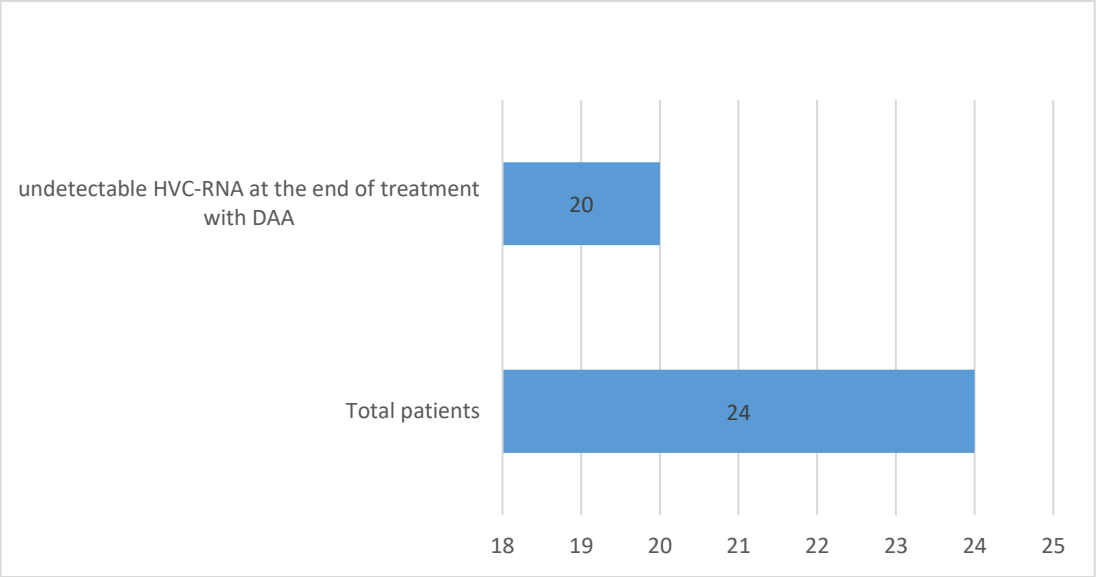
The initial monotherapy studies with DAA presented a prompt suppression of the viral activity and a fast appearance of the resistance, which required that these should be administered together with an α-interferon and Ribavirin to prevent resistance, at 12 weeks and at 24 weeks after the end of treatment with DAA [267,268].

The response to treatment is evaluated by the negativization of the viremia, i.e. the quantitative HCV-RNA undetectable by the PCR technique.

We analyzed the effect of the triple therapy, including DAA (Telaprevir administered for 12 weeks) in point tolerability and efficiency.

We determined the early virological response, namely negativization of the viremia or the decrease thereof with 2 log after 12 weeks of treatment (RVP).

Figura 17. The distribution of patients according to RNA – undetectable HCV at the end of treatment.

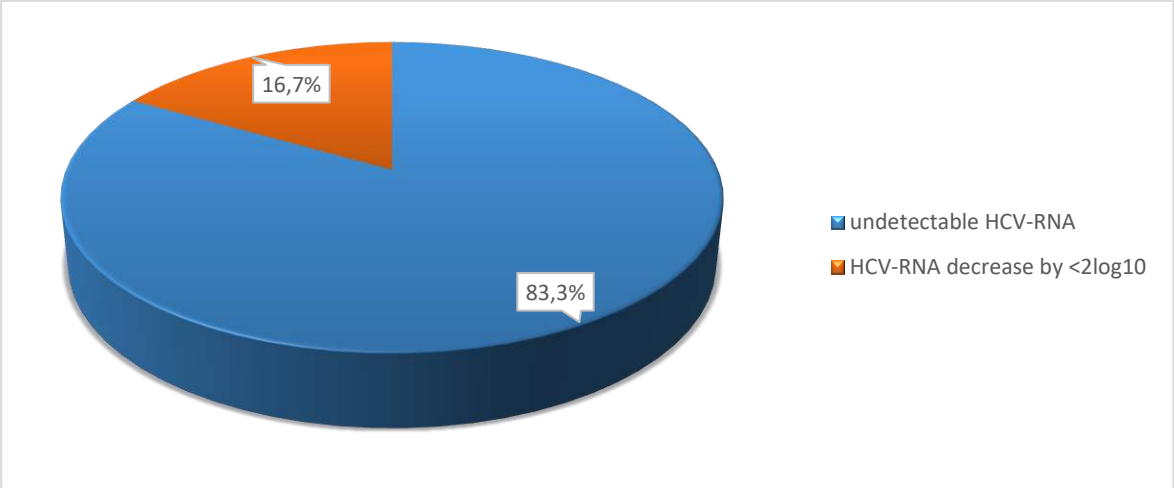


Out of the total number of 24 patients who underwent the treatment, 20 patients obtained undetectable HCV-RNA after 12 weeks of treatment, which represents a percentage of 83.3%, as compared to the 4 patients where HCV-RNA had a value of < 2 log, which represents a percentage of 16.6%.

Table 12 : *Distribution of patients according to their response to therapy*

Treatment regimen	ARN-VHC at 12 weeks of therapy	
	undetectable	decrease with<2log10
PegINF + Ribavirin + Telaprevir	20 pacients 83,3%	4 pacients 16,7%

Figure 18: Distribution of patients according to their response to therapy



Before the DAA era, the sustained virological response (SRV), with the standard therapy PegINF and Ribavirin, was of 40-50% only.

The triple therapy with DAA in combination with PegINFα and Ribavirin were considered standard therapy for the patients with chronic viral hepatitis C genotype 1, due to obtaining of a SVR of 83.3% after 12 weeks of treatment.

Tolerability was variable. I will present the most frequent adverse reactions of the triple therapy found in the group of patients studied.

I will briefly present the definitions of the adverse effects from the point of view of the United States Food and Drugs Administration. An adverse effect was defined as any untoward medical experience associated with the use of a drug in humans, irrespective if it was considered to be drug-related or not.

The severe adverse effects were defined as death, any life-threatening event, admission to hospital, prolonged hospitalization, persistent or significant incapacity or a substantial discontinuance of the capacity to develop normal life functions, a congenital anomaly/defect or any other event considered medically serious [278].

For pacients sub – study 1 , the most common adverse reactions are presented in the following table below :

Table 13. Distribution of patients depending on the presence of adverse reactions

No	Adverse reaction	frequency	percentage %
1.	Anemia	17 pacients	70,8%
2.	Leucopenia	10 pacients	41,6%
3.	Thrombocytopenia	14 pacients	58,3%
4.	Hyperbilirubinemia	3 pacients	12,5%
5.	Hyperuricemia	8 pacients	33,3%
6.	Hypocalcemia	4 pacients	16,6%
7.	Weight loss	4 pacients	16,6%
8.	Anal pruritus	4 pacients	16,6%

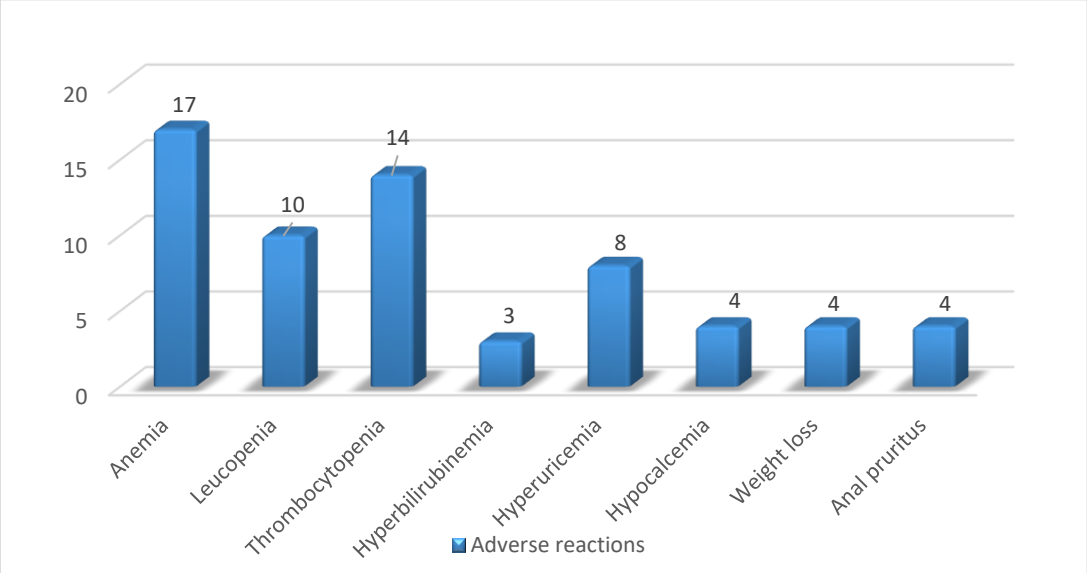


Figure 19. Distribution of patients according to the presence of adverse reactions

From the point of view of general adverse reactions, the most common was anemia 17 pacients (70,8%), followed by thrombocytopenia 14 pacients (58,3%), leucopenia 10 pacients (41,6%), hyperuricemia 8 pacients (33,3%), hypocalcemia 4 pacients (16,6 %), weight loss 4 pacients (16,6%) anal pruritus 4 pacients (16,6%),hyperbilirubinemia 3 pacients (12,5%).

Conclusions and discussions for sub – study I :

- Triple therapy with DAA in combination with PegINF-α and ribavirin was considered standard therapy at the time of study, for patients with chronic viral hepatitis C and genotype 1 due to obtaining an SVR of 83.3% after 12 weeks of treatment, Compared to the standard therapy at which RVS was only 40 – 50%.
- Tolerability was variable, in the first time due to the presence of adverse reactions, anemia being evidenced at 50% of patients (70.8%). The moderate form of anaemia (47%, 8 patients) was predominant, followed by the mild form (20.8%, 5 patients), the severe form being present in 4 patients (24%).
- Patients who experienced severe anaemia and had a combination of Ribavirin decreased the dose of Ribavirin at 600 mg/day and no interruption of Telaprevir administration was necessary. A patient with Hb = 7.5 g/dl required dose reduction of ribavirin but also administration of Isogroup izoRh.
- Leukopenia was present in our study in approximately 40% of patients with the predominance of mild form without implications for therapy.
- Mild thrombocytopenia was present in 50% of patients without requiring adjustment of the dose of antiviral treatment.
- Hyperuricemia in severe form was present in 5 patients with a predominant in male sex requiring the combination of Allopurinol treatment at a dose of 300 mg/day during the entire treatment period.
- Hypocalcemia in severe form was present in 2 patients without requiring discontinuation of medication, calcium preparations with favorable evolution were administered.
- The weight loss was present in 4 patients with a predominance of mild form and a higher weight in female type 3:1. No discontinuation of medication or modification of the dose of antiviral therapy was required.
- Abnormal events were reported in all clinical studies in 29% of subjects treated with DAAs. Most of these ano-rectal events included hemorrhoids, ano-rectal discomfort, rectal pruritus and rectal burning that were mild to moderate in severity [265,266,267]. In our study, the rectal prutum was present in 4 patients (16.6%), which was completely resolved at the end of treatment.

❖ Triple therapy had a short lifetime since launch until it was no longer recommended by the European Association for the Study of Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) in 2014, in Princip Al due to the emergence of new highly efficient and safe DAA molecules.

Sub – Study II :

Three new DAA HCV were licensed in the EU in 2014, for use as part of combination therapies for HCV infection. Sofosbuvir is a pan-genotopic inhibitor of NS5B RNA, polymerase dependent on HCV RNA, which has a pivotal role in viral replication [282] and is indicated in combination with other medicinal products for the treatment of hepatitis C chronic [282], was approved in January 2014. Simeprevir, a second DAA, the first generation NS3/4A active protease inhibitor against genotypes 1 and 4 was approved in May 2014. Daclatasvir, a pangenotypical NS5A inhibitor, was approved in August 2014. Each of these three DAA, can be used as a component of a triple therapy, in combination with PegINF-α and ribavirin, producing SVR rate of 60 – 100% depending on the DAA used, HCV genotype, severe liver disease [283].

Sub – Study II includes 54 patients with chronic viral hepatitis C who underwent interferon free treatment regimens for the period 2015-2018, containing:

- Viekirax (Ombitasvir 12.5 mg / Paritaprevir 75 mg / Ritonavir 50 mg) in a single dose administered in the morning, after meals.
- Exviera (Dasabuvir 250 mg), administered every 12 hours, after meals.

The duration of the treatment was 12 weeks (84 days), with the observance of the inclusion and exclusion criteria as well as of the drug contraindications of the product.

The patients included in the study were evaluated physically and biologically both before and after the treatment.

There were carried out:

- diagnostic tests to detect HCV antibodies by the ELISA (Enzyme Linked Immuno Sorbent Assay) isoenzyme method [261];

- molecular tests to detect the viral genotype and to evaluate the viral load by the PCR method (Cobas 6800, Roche Molecular Diagnostics) to confirm the diagnosis [261];

The viral load was determined at the beginning of the therapy and at the end thereof, as well as at 12 weeks after the end of treatment.

There were done blood tests (complete blood count), biochemical tests (GOT/ALT, GPT/AST), total gamma globulin, FA, urea, creatinine, INR, serum albumin.

The ultrasound examination of the liver was made with a view to evidence the chronic hepatic disease, the size of the hepatic lobes, in order to describe the appearance of the liver with focus on echogenicity, the presence of the regeneration nodules, the caliber of the vena porta (sign of portal hypertension), evident signs of hepatic cirrhosis.

The evaluation of the hepatic fibrosis grade was made by hepatic biopsy or by noninvasive tests (FibroScan, FibroTest).

Results for the Sub-study II

Evaluation of patients from the point of view of response to anti-viral therapy interferon free,evaluation of the efficiency and tolerability terapy.

The evaluation criteria of the therapy result are:

a) Sustained viral response (SVR): HCV-RNA undetectable at the end of treatment and at 12 weeks after the end of therapy;

b) Tardive response: HCV-RNA detectable at the end of treatment with values < 15 UI/ml, but undetectable 12 weeks after the end of therapy;

c) Lack of response: HCV-RNA detectable with values above 15 UI/ml at the end of treatment; in this case it is no longer needed to determine viremia 12 weeks after the end of therapy.

d) Relapse: HCV-RNA undetectable at the end of treatment and detectable 12 weeks after the end of therapy, irrespective of the value of HCV-RNA.

Evaluation of patients from the point of view of TGO/AST and TGP/ALT at final treatment

From the point of view of serum transaminases when initiating interferon free therapy and at the end treatment reveal significant differences, at the end of treatment all patients presented values within the normal limits of TGO/AST respectively TGP/ ALT.

Figure 51. Distribution of patients according to the TGO/AST values at final treatment

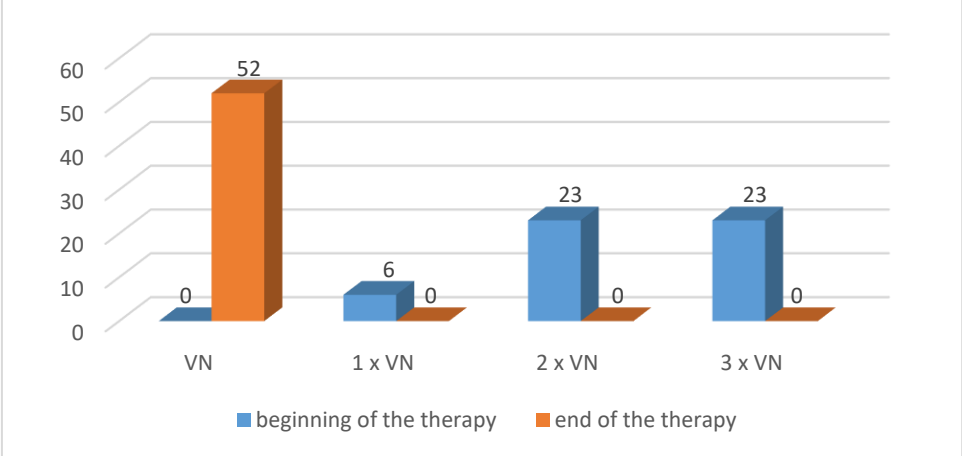
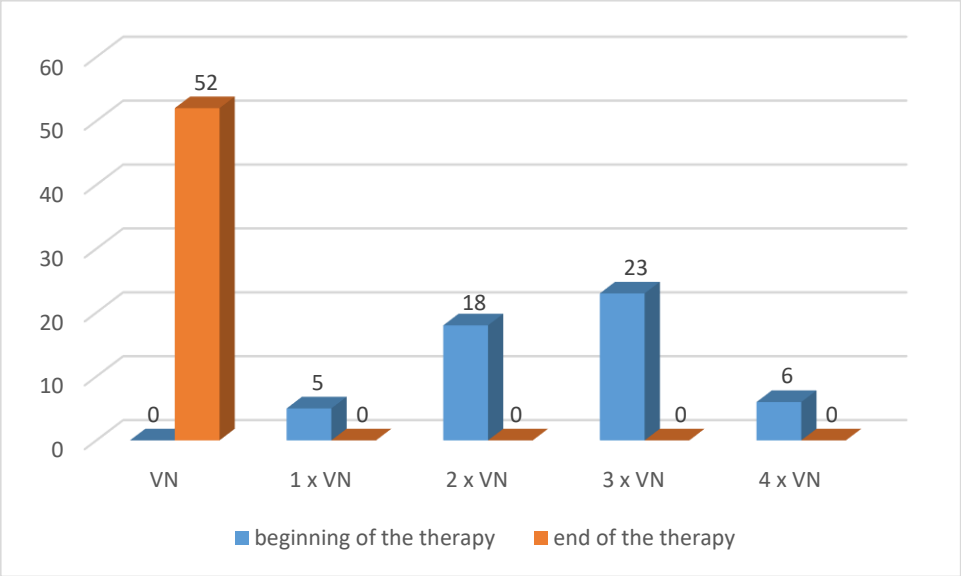


Figure 52. Distribution of patients according to the TGP/ALT values at final treatment



Distribution of patients according to the HCV-RNA at the end end of the 12th week of the terapy:

At the end of the 12th week of treatment, i.e. days 85-91, HCV-RNA was determined and the result was undetectable for all the patients in the study group.

Achieving a comparison with the patients of substudy I (patients treated with PegINF + ribavirin regimens in which a first-generation, NS3/4A protease inhibitor was added, Telaprevir) there were significant differences in the RVS that were 83.3% in the case of triple therapies compared to interferon free therapy (Viekirax + Exviera) to which the RVS was 100%, while noting very good tolerability with minimal adverse reactions, which will be described later.

Table 30. The HCV-RNA value at the end of the 12th week of interferon free therapy as compared to the beginning of the therapy

Number of patients	HCV-RNA value at the beginning of the therapy	HCV-RNA value at the end of the therapy
18	HCV-RNA < 600.000 UI/ML	undetectable
34	HCV-RNA > 600.000 UI/ML	undetectable
Total 52		

Distribution of patients according to the tolerability interferon free terapy :

In all clinical studies, the most frequent adverse reactions of the interferon free therapy were described as minimal, represented by headaches, vertigo, diarrhea, insomnia, fatigue, skin pruritus, reported by 1-2% of the patients [274].

In our study, adverse reactions were present in 22 patients (42.3%), categorized with minimal severity grade, as they did not require any treatment or the discontinuance of administering the medication, with the mention that they subsided completely after the end of the antiviral therapy.

Table 31. Distribution of the patients according to the presence of adverse reactions

No	Adverse reactions	Frequency	Percentage
1.	Headaches	10	19,2%
2.	Skin pruritus	5	9,6%
3.	Vertigo	2	3,84%
4.	Insomnia	1	1,92%
5.	Fatigue	1	1,92%
6.	Diarrhea	1	1,92%
7.	Hyperglycemia	1	1,92%
8.	Hypertriglyceridemia	1	1,92%

The most frequent adverse reactions were:

- headaches, present in 10 patients (19.2%), age group 50-59 years, more predominantly with the female gender;

- skin pruritus was present in 5 patients (9.6%), age group 40-49 years, more predominantly with the female gender;

- 2 patients (3.84%) presented vertigo, age group 50-59 years, more predominantly with the female gender;

- 1 female patient (1.92%) presented insomnia, age group 60-69 years;

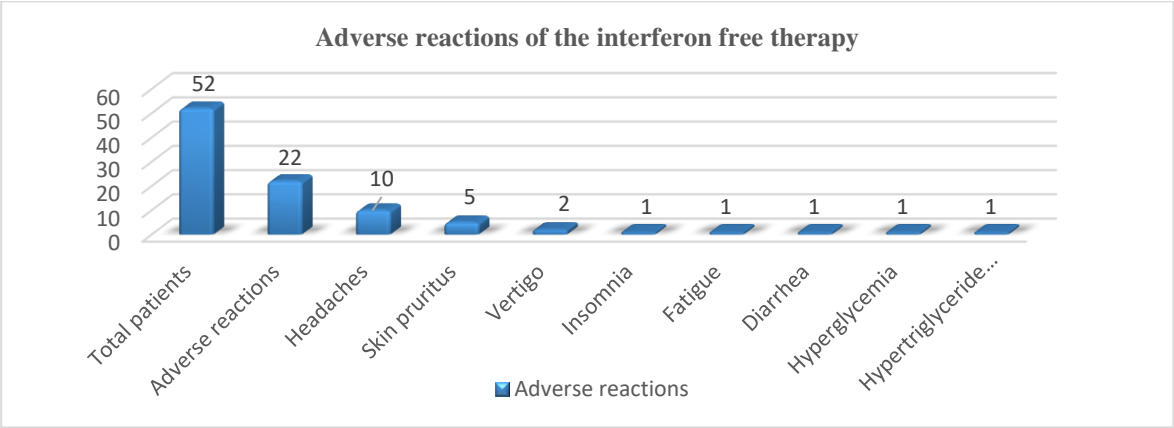
- 1 male patient (1.92%) presented fatigue, age group 50-59 years;

- 1 male patient (1.92%), presented diarrhea (3-4 semiconsistent stools in 24 hours), age group 50-59 years;

- 1 female patient (1.92%) presented hyperglycemia (a patient with type II diabetes mellitus under treatment with oral antidiabetics, with blood glucose values 2 x VN), age group 50-59 years;

- 1 female patient (1.92%) presented hypertriglyceridemia (adverse reaction found in a patient with mixed dyslipidemia and obesity grade 2), age group 50-59 years.

Figure 56. Distribution of the patients according to the presence of adverse reactions



Assessment of the patients from the viewpoint of the HCV-ARN at 12 weeks from the end of the therapy.

It was performed assessment of the patients from the viewpoint of the HCV-ARN at 12 weeks from the ent of the therapy,day 169 – day 175 from the beginning therapy.

The result was HCV-ARN undetectable for all patients.

Table 33. The value of HCV-RNA at the beginning of the antiviral therapy, at the end of the therapy, and at 12 weeks from the end of the therapy

Number of patients	HCV-RNA value at the beginning of the therapy	HCV-RNA value at the end of the therapy (12 weeks therapy)	HCV-RNA at 12 weeks from the end of the therapy
18	< 600.000 UI/ML	undetectable	undetectable
34	> 600.000 UI/ML	undetectable	undetectable

All patients sub-study II presented values of HCV – RNA undetectable at the end of the 12 th week end of therapy end 12 week from the completion of therapy,proving the efficiency of interferon free therapy.

Conclusions end discussions for sub-study II.

❖ The rapid evolution of the approved DAAs molecules and included in the treatment of patients with HVC, proves the usefulness of our study as well as the interest from continuation with the purpose of demonstrating the utility of these DAAs, the need to expand patients access to antiviral therapy due to the results obtained, remarkable by the reduced percentage of adverse events being minimised and due to RVS 100%.

❖ The distribution of patients differentiated on age groups, originating environment, and gender shows a predominance of the female gender for the urban originating environment and age group 30-39 years, followed by the male gender; for the age group 40-49 years and the urban originating environment predominates the female gender, while for the rural originating environment and the same age group, the male gender predominates, with some statistically significant differences ($p < 0.005$); for the age group 50-59 years and the urban originating environment predominates the female gender, as compared to the rural originating environment and the same age group , where the male gender predominates, with some statistically significant differences ($p < 0.002$); for the age group 60-61 years and the urban originating environment predominates the female gender, as compared to the rural originating environment and the same age group, where the male gender predominates, but without any statistically significant differences.

❖ With regard to the fibrosis stage at the moment of initiating the therapy, fibrosis stage F3 is predominant in more than half of the total number of study patients (63.5%), followed by fibrosis stage F2 (25%), the fibrosis stage F4 being present in 6 patients (11.5%), with some statistically significant differences ($p < 0.002$).

❖ The chronic hepatic disease was evaluated in patients with fibrosis stage F4 based on the Child-Pugh score. All the patients evaluated were categorized in the Child-Pugh Class A with a value of 5-6 points.

❖ The distribution of patients according to the previous therapy with PegINF and Ribavirin (experienced patients) or patients who were not previously treated (naïve patients) revealed a predominance of naïve patients (61.5%) as compared to the experienced ones (38.5%), which goes to show that there is an increase in the number of new cases of patients infected with hepatitis virus C, who did not receive any specific therapy, explained by the increase in the recent years of accessibility to diagnosis and therapy specified according to the therapeutic guidelines.

❖ The most frequent adverse reactions were represented by: headaches, present in 10 patients (19.2%), predominant in age group 50-59 years, with a higher frequency in the female patients, followed by skin pruritus, present in 5 patients (9.6%), with a higher frequency in the female patients and age group 40-49 years, vertigo in 2 patients (3.84%), more predominantly with the female gender and age group 50-59 years, respectively 1 female patient (1.94%) presented insomnia, in the age group 60-69 years, 1 male patient (1.94%) presented fatigue, in age group 50-59 years, 1 male patient (1.94%) presented diarrhea (3-4 semiconsistent stools in 24 hours), in age group 50-59 years, a female patient presented hyperglycemia, in the age group 50-59 years (a patient with type II diabetes mellitus under treatment with oral antidiabetics, with blood glucose values 2 x VN, whose blood glucose at the end of therapy was 1 x VN), hypertriglyceridemia in a female patient in the age group 50-59 years (with history of mixed dyslipidemia and obesity grade 2).

❖ It is worth noting that we should underline the fact that the serum transaminases TGO/TGP had high values at the beginning of the therapy and, after 12 weeks of treatment, presented normal values in all patients as a result of the effectiveness of therapy.

❖ The initiation of therapy was revealed the low values of serum thrombocytes, but not less than 100,000/mm3, nevertheless at the end of the therapy, the values of the thrombocytes were within normal limits in all patients included in the sub-study II.

❖ The triple therapy with DAA's added to the standard treatment proved its utility at the moment of the study, but the appearance of new DAA molecules proved the necessity to continue this study and to demonstrate the efficiency and the tolerability of the interferon free regimens, and to increase the access of patients to a new antiviral interferon free therapy.

Sub-Study III:

It includes the results of the evaluation of the quality of life of patients with chronic hepatic diseases, on a group of 122 patients divided into two lots:

- Lot 0 includes 92 patients diagnosed with chronic viral hepatitis C who did not meet the inclusion criteria for the interferon free therapy, but were physically and biologically evaluated (control group);
- Lot 1 includes 30 patients with chronic viral hepatitis C who finished the interferon free therapy (patients of the study group B).

The 92 patients making up the control lot come from the total number of 154 patients diagnosed with chronic hepatic diseases during the period 2016-2018.

The patients were physically and biologically evaluated (complete blood count, TGO (ALT), TGP (AST), total gamma globulins, FAS, INR, urea, creatinine, serum albumin).

Diagnostic tests were done to detect HVC antibodies by the isoenzyme method ELISA (Enzyme Linked Immuno Sorbent Assay), the viral load was assessed by PCR (Cobas 6800, Roche Molecular Diagnostics) to confirm the diagnosis. The hepatic fibrosis stage was evaluated by noninvasive tests (FibroMax).

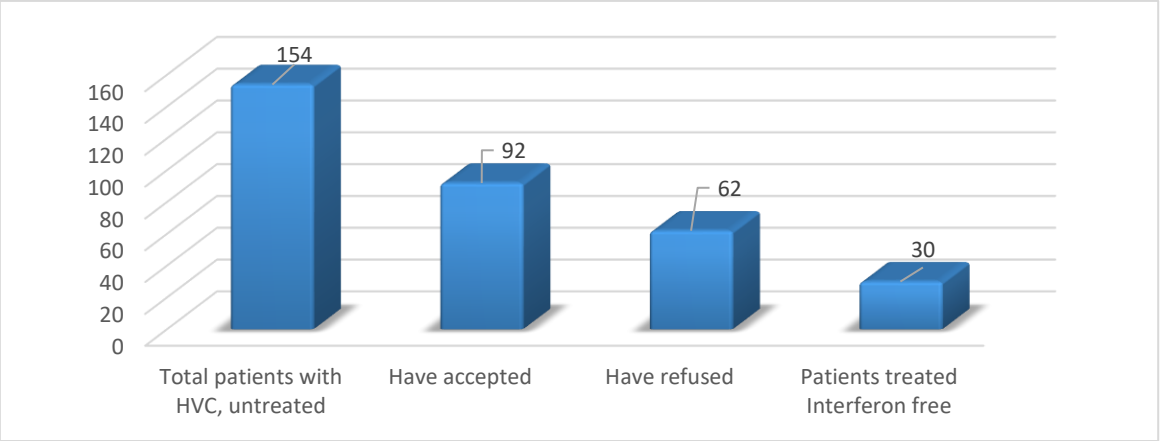


Figure 57. Distribution of the patients in Group C for the evaluation of the quality of life of the patient with hepatic disease

Results for the Sub-study III. Results for the evaluation of the quality of life

This study represents a complex evaluation, from an observational, clinical and sociological perspective, of the quality of life of patients with chronic hepatic diseases, being structured on several phases, such as establishing the groups of patients, the data collection and interpretation.

As we have specified, the sub-study includes a number of 122 patients, divided into two distinct lots:

- Lot 0 (L0) – the control lot, consisting of 92 patients who did not meet the inclusion criteria for the interferon free therapy, but were evaluated according to the CNAS (National Health Insurance House) protocol.
- Lot 1 (L1) consists of 30 patients who underwent the interferon free antiviral therapy during the period April – June 2018, having been evaluated in point of their quality of life before and after the therapy.

Our aim was to study the impact of the antiviral therapy on the patients' quality of life before and after the interferon free antiviral therapy, as well as to study the quality of life of the patients with chronic viral hepatitis C who did not receive interferon free antiviral therapy because they did not meet the eligibility criteria, but accepted to participate in the study.

The patients' quality of life was evaluated after obtaining the informed consent for the participation in the study from all the patients.

One of the instruments used to measure the quality of life was the approved generic questionnaire SF-36 (Annex 1), a test that was validated for the Romanian population within a project developed by the Romanian Association for Public Health and Medical Management (ARSPMS) and the Center for Urban and Regional Sociology (CSUR) Bucharest [275, 289, 290].

The questionnaire SF- 36 consists of 36 questions that make up the 8 scales: PF – Physical Functioning); PR – Physical Role Functioning; BP – Bodily Pain; GH – General Health perceptions; VT – Vitality; SF –Social Role Functioning; ER – Emotional Role Functioning; MH – Mental Health; PCS – Physical Component Summary; MCS – Mental Component Summary [259,303].

The results of these scores were quantified and appreciated by: [259,303]

- the simple way that uses a scale from 0 to 100 for each question and answer and where the maximum value is the most favorable status; each question is coded in several phases/steps and receives a score which, cumulated, gives the maximum value of 100;

- the software scoring system, which reached the same final point, offering a graph of the cumulated scores.

Interpretation of results:

- a good quality of life (71-100 points);

- an average quality of life (36 – 70 points);

- a seriously deteriorated quality of life (0 – 35 points).

The SF-LDQOL questionnaire is an instrument especially designed to evaluate the quality of life of the patients with chronic hepatic diseases (main author: Fasiha Kanwal, 2008), consisting of two main parts, adapted to the objectives pursued by us [303].

The first part consists of 5 items, that evaluate four therapeutic fields: [259,303]

1. – symptoms of the hepatic disease (6 items, e.g. edema of the lower limbs, gum bleeding etc.);

2. – consequences of the hepatic disease (3 items, e.g. work around the house, drug administration etc.);

3. – concentration and memory (4 items, e.g. capacity of concentration, memory problems etc.);

4. – problems caused by the disease (2 items, e.g. frustration, fatigue etc.);

The second part presents 5 fields 36 items related to everyday life: “sleep”, “isolation”, “hope”, “hepatic disease stigma”, “sex life”, each of them having a certain weight by the sum of the weighted values and which, depending on the severity of such symptom, may vary from 0 (most affected health) to 100 (perfect health) [259,303].

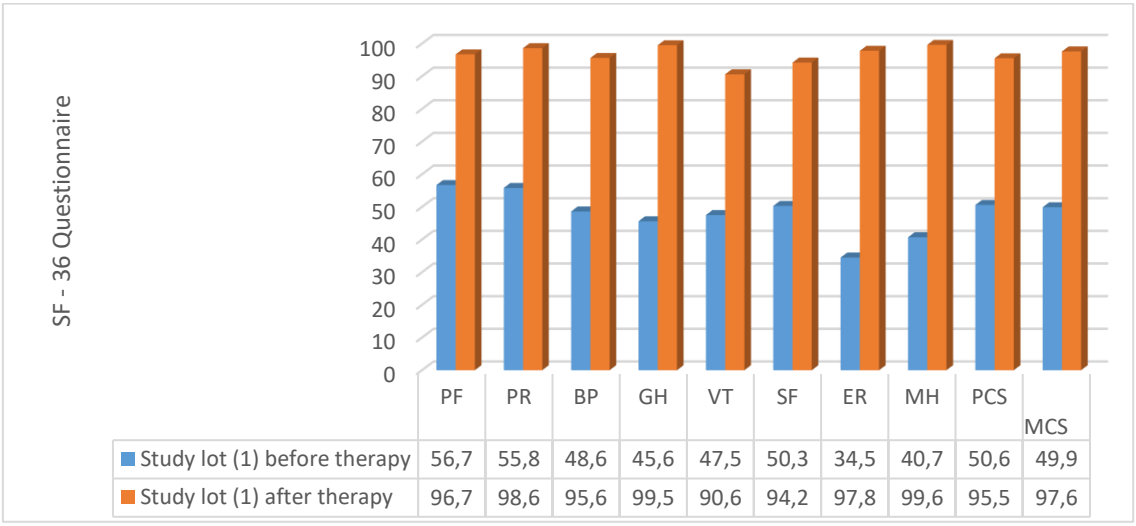
The phases of monitoring and evaluation of the quality of life were carried out at the moment of initiating the antiviral therapy and at the end of the 12th week of treatment (day 85-91), for the patients who received antiviral therapy and, for the patients who did not meet the criteria for getting the treatment and received non-viral medication, the evaluation of the quality of life by questionnaires was carried out at the moment when the patient came in for the physical and biological evaluation of his/her chronic hepatic disease, only with his/her written consent [259,303].

To evaluate the quality of olife, we used the SF-LDQOL questionnaire (short version) translated into Romanian with the author’s consent (Darii E), which includes instruments oriented towards the impact of the chronic hepatic disease on the quality of life; the answers were centralized on the following scales: physical health and mental health, self-assessment of symptoms, consequences, and problems caused by the chronic hepatic disease, reporting the troubles of concentration and memory, sleep, stigma of the hepatic disease, isolation – hope, sexual function [259,303].

At a first stage, we evaluated based on the SF-LDQOL questionnaire patients who met the evaluation criteria for the interferon free therapy (L1), the questionnaire being filled out on the day of the approval and registration for treatment.

Concomitantly there were also evaluated some patients that were not eligible for interferon free therapy (L0 / control group).

At the end of the therapy (the end of the 12th week of treatment), the patients included in the study lot (L1) were reevaluated based on the SF-LDQOL questionnaire in order to evidence the impact of the therapy on the quality of life of these patients, as compared to that of patients who did not receive antiviral therapy.



	PF	PR	BP	GH	VT	SF	ER	MH	PCS	MCS
Study lot (1) before therapy	56,7	55,8	48,6	45,6	47,5	50,3	34,5	40,7	50,6	49,9
Study lot (1) after therapy	96,7	98,6	95,6	99,5	90,6	94,2	97,8	99,6	95,5	97,6

Figure 64. Results of the SF-36 score calculated for the Study Lot (1) before and after the interferon free therapy

Key: PF – Physical Functioning; PR – Physical Role Functioning; BP – Bodily Pain; GH – General Health perceptions; VT – Vitality; SF – Social Role Functioning; ER – Emotional Role Functioning; MH – Mental Health; PCS – Physical Component Summary; MCS – Mental Component Summary.

The analysis of the lot structures with regard to the quality of life, also considering the patients of Lot 1 (study lot) before initiating the interferon free therapy and at the end of the therapy found statistically significant differences of the values of the quality of life ($p < 0.0075$), which indicates a severe deterioration of the patients' quality of life before initiating the therapy, affecting first of all the life expectancy.

The self-assessment of the quality of life at the initial moment, before the initiation of the therapy, shows that the patients presented a low degree of physical functioning ($p < 0.06$), asserting severe symptoms of the hepatic disease ($p < 0.003$) and a seriously affected cognitive functioning ($p < 0.004$). Before the initiation of the therapy it was evidenced a significant deterioration of the psycho-emotional state, determined by the presence of the chronic hepatic disease, manifesting difficulties in carrying our social activities, asserting physical discomfort, along with the fact that the chronic hepatic disease affected to a great extent their capacity to maintain interhuman relations, both in the social and in the family environment.

Our study shows that the chronic hepatic disease alters to a great extent the patient's quality of life.

The results of the research evidence statistically significant differences of the quality of life of patients with chronic hepatic disease, upon initiating the antiviral therapy (L1 prior to therapy) as

compared to the end of the therapy (L1 post-therapy). Thus, the questioned patients appreciate the present quality of life at the initial moment of the therapy as being severely affected (25 patients – 83.3%), moderately affected (5 patients – 16.6%), no patient affirmed a mild affectation of his quality of life. The score obtained ranged between 22 and 68 points.

Subsequently, at the end of the therapy, we identified statistically significant differences ($p < 0.001$), the majority of patients asserting a mild affectation of their quality of life after receiving the interferon free therapy, a significant boost of their self-confidence and of the psychological and physical satisfaction, predominantly within the age groups 30-39 years and 40-49 years.

The impact of the quality of life of the patients who did not receive antiviral interferon free therapy (L0 – control lot) evidences the following aspects: the quality of life is severely affected (67 patients – 72.8%) in more than half of the patients questioned, while only 25 patients (27.2%) affirm a moderate affectation of the quality of life, no patient describes a mild affectation of his quality of life. The score obtained varied between 20 and 65 points.

The statistical results related to the patients' quality of life for the control lot (L0) and for the study lot (L1) show statistically significant differences related to the severe affectation of the quality of life ($p < 0.001$), in the case of patients who did not receive antiviral therapy, and the mild affectation of the quality of life, valid for the patients who received antiviral therapy ($p < 0.001$). Even in the case of a moderate affectation of the quality of life there are statistic differences between the studied lots ($p < 0.034$).

For the patients of the study lot (L1), before the initiation of the antiviral therapy, the research evidences a predominance of the following pathological states: depression caused by the uncertainty about the future in 73.3% (22) of the patients, anxiety related to the evolution of the hepatic disease in 76.6% (23) of the patients, lack of hope in relation to the future, transposed into isolation, in 66.6% (20) of the patients, loneliness (especially present in the age group 50-59 years), in 33.3% (10) patients, degradation of self-confidence, occurred in 86.6% (26) of the patients.

The results related to the social issues evidence the following aspects: 27 patients (90%) mentioned a certain limitation of their work capacity, while 28 patients (93.3%) specify the lack of satisfaction of their emotional and physical needs.

For the patients of the control lot (L0), the study evidences the predominance of the following pathological states: depression caused by the uncertainty about the future in 73.9% (68) of the patients, anxiety related to the evolution of the hepatic disease in 78.2% (72) of the patients, lack of hope in relation to the future, transposed into isolation, in 66.3% (61) of the patients, loneliness

present in 54.3% (50) of the patients, degradation of self-confidence, occurred in 83.6% (77) of the patients. With regard to the social issues, the following statistical aspects are evidenced: limitation of the work capacity in 81.5% (75) of the patients, lack of satisfaction of the emotional and physical needs in 95.6% (88) of the patients.

Between the two lots, the control lot (L0) and the study lot (L1), there is no evidence of any statistically significant differences ($p < 0.34$), since, for both lots, the questionnaire addressed patients who did not receive any antiviral therapy.

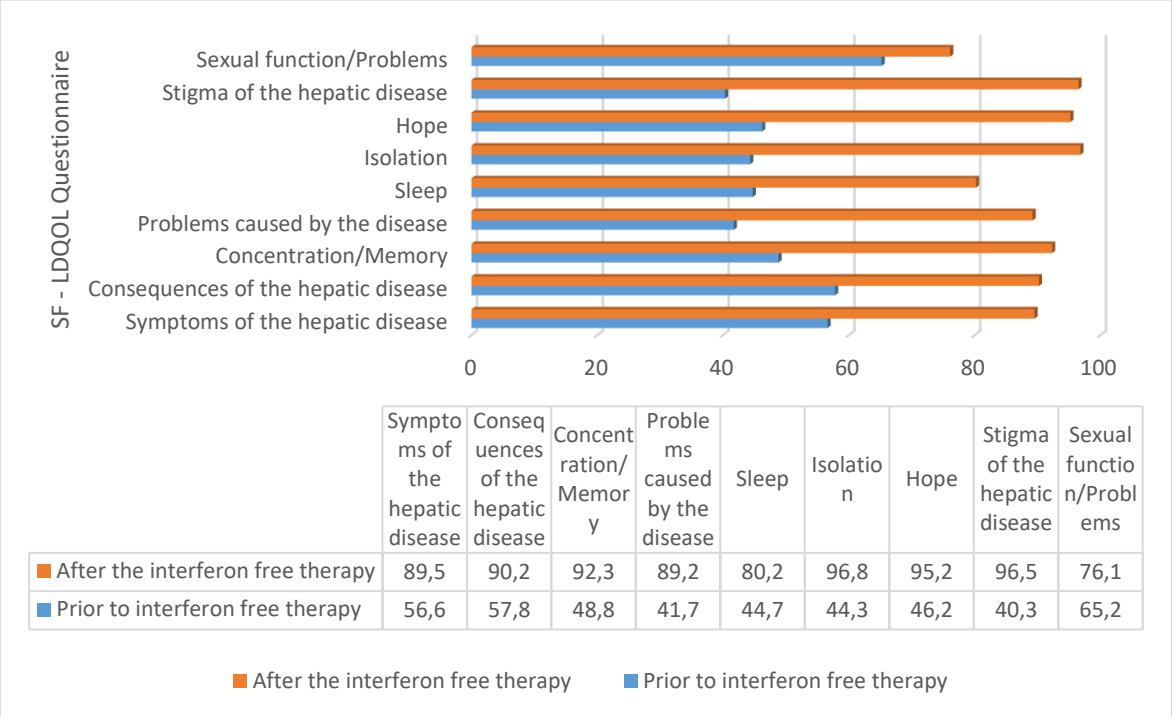
For the patients of the study lot (L1) the post-therapy statistical results evidence the following pathological states: 6.66% (2) patients affirmed depression caused by the uncertainty about the future, 3.33% (1) patient affirms degradation of self-confidence. With regard to the social issues, the following aspects were revealed: 3.33% (1) patient complained from the limitation of the work capacity and 6.66% (2) patients complained for the lack of satisfaction of the emotional and physical needs.

This results confirm a significant improvement of the quality of life related to the state of health, the antiviral therapy received, which determined a major impact on the quality of life in general.

The analysis of the items of the specific SF-LDQOL questionnaire evidenced some differences in point of quality of life between the study lots and moment of the questioning, before and after the interferon free therapy.

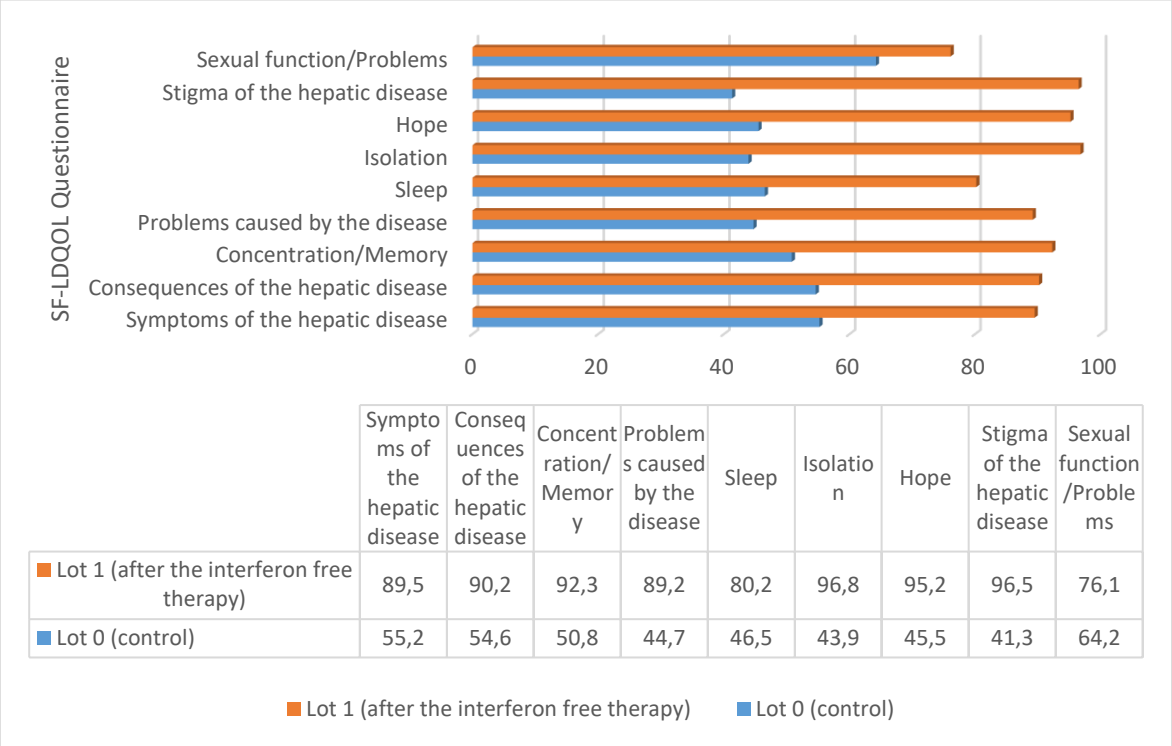
The results obtained from the SF-LDQOL questionnaire for the evaluation of the quality of life evidence a significant increase of all dimensions, after receiving the interferon free therapy, as compared to the moment on initiation of the therapy.

Statistically, according to the respondents, the patients in the study lot L1 (after the interferon free therapy) reveal a significant improvement of the quality of life, resulting from the percentage values of the scales “symptoms of the hepatic disease” (from 56.6% to 89.5%; $p < 0.001$), “consequences of the hepatic disease” (from 57.8% to 90.2%; $p < 0.001$), “concentration and memory” (from 48.8% to 92.3%; $p < 0.001$), “problems caused by the disease” (from 41.7% to 89.2%; $p < 0.001$), “sleep” (from 44.7 to 80.2%; $p < 0.001$), “isolation” (from 44.3% to 96.8%; $p < 0.001$), “stigma of the hepatic disease” (from 40.3 to 96.5; $p < 0.001$) and “sexual function or problems” (from 65.2% to 76.1%; $p < 0.003$).



	Symptoms of the hepatic disease	Consequences of the hepatic disease	Concentration/ Memory	Problems caused by the disease	Sleep	Isolation	Hope	Stigma of the hepatic disease	Sexual function/Problems
After the interferon free therapy	89,5	90,2	92,3	89,2	80,2	96,8	95,2	96,5	76,1
Prior to interferon free therapy	56,6	57,8	48,8	41,7	44,7	44,3	46,2	40,3	65,2

Figure 65. Graphic representation of the variations of the SF-LDQOL scale for the lot L1 (prior to therapy) and L1 (after the interferon free therapy).



	Symptoms of the hepatic disease	Consequences of the hepatic disease	Concentration/ Memory	Problems caused by the disease	Sleep	Isolation	Hope	Stigma of the hepatic disease	Sexual function /Problems
Lot 1 (after the interferon free therapy)	89,5	90,2	92,3	89,2	80,2	96,8	95,2	96,5	76,1
Lot 0 (control)	55,2	54,6	50,8	44,7	46,5	43,9	45,5	41,3	64,2

Figure 66. Graphic representation of the variations of the SF-LDQOL scale for the lot L0 (control lot) and L1 (after the interferon free therapy).

The statistical results obtained by the numerical analysis of the items of the specific SF-LDQOL questionnaire in the case of the patients in the control lot (L0), who did not receive the

interferon free therapy evidence significant differences for all dimensions, as compared to the study lot (L1) which finished the interferon free therapy; such data result from the percentage values of the scales “symptoms of the hepatic disease” (from 55.2% to 89.5%; $p < 0.001$), “consequences of the hepatic disease” (from 54.6% to 90.2%; $p < 0.001$), “concentration and memory” (from 50.8% to 92.3%; $p < 0.001$), “problems caused by the disease” (from 44.7% to 89.2%; $p < 0.001$), “sleep” (from 46.5 to 80.2%; $p < 0.001$), “isolation” (from 43.9% to 96.8%; $p < 0.001$), “stigma of the hepatic disease” (from 41.3 to 96.5; $p < 0.001$) and “sexual function or problems” (from 64.2% to 76.1%; $p < 0.003$).

Conclusions and Discussion

1. The results obtained at the end of the antiviral therapy for the patients in Lot 1 (30 patients) evidence a significant improvement of their quality of life; only 3 patients (10% ± 5.74) presented a moderate affectation of their quality of life (score: 36-70 points) and 27 patients (90% ± 5.25%) presented a mild affectation of their quality of life (score: 71-100 points), with significant differences ($p < 0.001$) as compared to the pre-therapy period, when 25 patients (83.3% ± 3.63) affirmed a severe affectation of their quality of life (score: 0-35 points) and only 5 patients (16.6% ± 6.67) presented a moderate affectation of their quality of life (score: 36 – 70 points).

2. In the same period, for the lot L0 (control lot, consisting of 92 patients), the evaluation of the quality of life showed a severe affectation (score: 0-35 points) for 67 patients (72.8% ± 5.24), while only 25 patients (27.2% ± 6.99) showed a moderate affectation of their quality of life, the differences being considered as statistically significant ($p < 0.001$).

3. For the patients in the lot L0 (control lot), the results of the study reflect the following pathological aspects: depression caused by the uncertainty about the future (73.9%), anxiety related to the evolution of the hepatic disease (78.2%), lack of hope in relation to the future, transposed into isolation (66.3%), loneliness (54.3%), degradation of self-confidence (83.6%) and, from the social point of view, limitation of the work capacity (81.5%), and lack of satisfaction of the emotional and physical needs (95.6%).

4. For the patients in the lot L1 (study lot), the results of our study reflect the following pathological aspects: depression caused by the uncertainty about the future (73.3%), anxiety related to the evolution of the hepatic disease (76.6%), lack of hope in relation to the future, transposed into isolation (66.6%), loneliness (33.3%), degradation of self-confidence (86.6%) and, from the social point of view, limitation of the work capacity (90%), and lack of satisfaction of the emotional and physical needs (93.3%).

5. Between the two lots L0 and L1, the analysis report did not reveal any statistically significant differences ($p < 0.34$), because, for both lots, the questioning was done with patients that did not receive the interferon free therapy.

6. The SF-36 questionnaire for the analysis of the quality of life evidences an intensely positive correlation between the scales of the generic questionnaire ($r = 0.9862$, $r2 = 0.9726$, $p < 0.0012$) for patients who received the interferon free therapy, as compared to the patients of lot L0, without antiviral therapy, resulting in a high degree of affectation of the general state, of the social life, of the family life, professional activities and interhuman relations.

7. The impact of the antiviral therapy on the quality of life presents a marked boost of the self-confidence, an increase of the expectancy of life, satisfaction and contentedness with regard to the social functioning, the emotional role and mental health of a patient.

8. The dimensions of the quality of life evaluated by the SF-LDQOL questionnaire (symptoms related to the hepatic disease, consequences of the hepatic disease, concentration and memory, the stigma of the hepatic disease, sleep, isolation, expectancy of life, sexual functioning/activity), presented a significant improvement in patients who received interferon free therapy as compared to the patients who were not treated with antiviral therapy, the statistical evaluation revealed significant differences ($p < 0.0001$), with a single mention, namely that no statistical differences were reported with regard to the sexual function ($p < 0.097606$).

9. The antiviral interferon free therapy significantly increases the quality of life of the patients with chronic hepatic diseases demonstrated by the statistically significant improvement of the SF-LDQOL score.

10. The results of our study indicates that the patients who were treated with the antiviral interferon free therapy become aware of the fact that the problems related to the chronic hepatic disease are solvable, which influences the quality of life in a positive way.

GENERAL DISCUSSIONS

- Chronic viral hepatitis C is a disease with important social and economic implications, the therapy with DAA's determines the achievement of a sustained viral response of up to 100% with minimal adverse reactions, which determines an increase of the quality of life of the patients treated with the new therapeutic schemes.

- Romania has a high prevalence of HVC infection, with a general prevalence of 3.23 % within the adult population and with significant differences between the urban and rural areas □283, 284□. In the case of the patients included in our study, we found a predominance of the urban provenance environment. For all three sub-studies, this difference urban/rural may be explained epidemiologically by an awareness of the ways of transmission, by an easier access of the patients to he means of communication, as well as to the possibilities of investigation, diagnosis, and treatment. The access to medical information justified the predominance of young patients from urban areas, diagnosed with the hepatitis C virus, a situation that is ofttern accounted for in the specialized literature as well [283,284].

- Since the main complication in the long term is the hepatic cirrhosis, the degree of liver fibrosis is considered an important marker of the evolution of the chronic hepatic disease. In my study, the distribution of the patients according to the degree of liver fibrosis evidenced a predominance of F3 fibrosis, followed by F4 for the sub-studies I and II, while in the case of the sub-study III, F1 fibrosis was predominant, followed by F1-F2 fibrosis. It is important to underline the fact that, at the moment of the study, the access to free therapy was ensured only for the patients with F3-F4 fibrosis stage.

- From the viewpoint of the distribution of the patients in naïve (with no previous treatment) and experimented (preciously treated with PegINF ± Ribavirin, being null responder or partial responder), the patients in my study lots were: 18 experimented patients (81.2%) for lot A (PegINF ± Ribavirin and Telaprevir), 32 naïve patients (61.5%), respectively 55 patients (60%) in lots B and C. The presence in ever-growing numbers of naïve patients who were not previously subject to antiviral therapy, with and without therapeutic response, may be explained by the increase of the number of patients with HVC diagnosed at a certain stage, but who did not have access to treatment.

- The studies made evidence a prevalence of 99.6% for the genotype 1b in patients ith advanced fibrosis in Romania [263]. Genotyping was in our study for the patients of sub-study I who benefited from the triple therapy with PegINF ± Ribavirin and DAA's (first generation NS3/4A protease inhibitor), which confirmed the data of the studies, namely the frequenct of the genotype 1b.

- The tolerability of the triple therapy was variable, the most frequent adverse reaction being anaemia, in 70.8% of the cases, with a predominance of the moderate form (Hb = 9.99 - 9.9 g/dl), 24% of the patients presented severe anaemia with Hb values = 7.00 – 8.9g/dl, a single patient who had Hb = 7.5 g/dl required the reduction of the Ribavirin dose down to 600 mg/day, as well as transfusion with isogroup isoRh blood; thrombocytopenia is the following adverse reaction present in 58.3% of the patients, with predominance of the moderate form, which did not require a

discontinuance of the therapy; leukopenia was present in 41.6% of the patients with predominance of the moderate form; other adverse reactions, in the order of appearance, were hyperuricemia 33.3%, hypocalcemia 16.6%, mild weight loss 16.6%, anal pruritus 16.6%, and hyperbilirubinemia 12.5%, which did not require a discontinuance of the therapy or modifications of the therapeutic scheme.

- The triple therapy was nevertheless short-lived since its launch until the moment when it was no longer recommended, both by the European Association for the Study of the Liver (EASL), and by the American Association for the Study of Liver Diseases (AASLD) in 2014, mainly due to the apparition of the new molecules of DAA's, extremely effective and safe, for which reason we continued the study in order to prove the effectiveness of the new combined therapies with DAA's, but also to demonstrate the influence of the therapy on the quality of life of patients with chronic hepatic conditions.

- Thus we started the sub-study II, which included patients treated with the new combinations of DAA's extremely effective and safe, and the result was a SVR of 100 %, with minimal adverse reactions.

- We considered it necessary to evaluate the patients from the viewpoint of their quality of life, therefore the sub-study III included two lots, L0 (patients that were evaluated physically and biologically, but who did not benefit from antiviral therapy) and L1 (patients who did benefit from interferon free therapy), with the mention that these patients were evaluated both before the therapy, and after the end of the antiviral therapy.

- The health-related quality of life is a new concept with a multitude of definitions, which incorporates positive and negative aspects of the "well-being" and of the life of an individual. The chronic liver disease has an important impact on the patient's quality of life, the social and demographic characteristics being influenced by the clinical evolution, the rate of complications and the mortality, which increase in direct proportion with the complexity of the hepatic lesions and they are represented by hepatic functional disability (morbidity, invalidity, mortality, social insertion, psycho-somatic development. These indicators, associated with the indicators related to the work conditions (life, work conditions, educational level, state of health of the population), characterizes the life expectancy and the period of survival) [288,293,294].

- The present study has represented a complex evaluation from an observational, clinical, and sociological point of view of the quality of life of patients with chronic liver conditions, being structured on various phases, including establishing the lots of patients, data collection and interpretation. The questionnaires used to evaluate the patients included SF-36 and SF-LDQOL. The phases of CV monitoring and evaluation were carried out at the moment of initiating the antiviral

therapy, at the end of week 12 of treatment (day 85-91), for the patients who benefited from interferon free therapy, and for the lot L0 (control) the evaluation by questionnaires regarding the quality of life was made at the moment when the patient came in for a physical and biological evaluation of his/her chronic hepatic disease.

- The questionnaire SF-36 for the analysis of the quality of life evidences an intensely positive correlation between the scales of the generic questionnaire, for the patients who benefited from interferon free therapy, as compared to the patients in lot L0 (control), the absence of treatment resulting in a high degree of affectation of the general state, of the social life, of the family life, of professional activities and interhuman relationships.

- The dimensions of the quality of life evaluated by the questionnaire SF-LDQOL (symptoms related to the hepatic disease, the consequences of the hepatic disease, concentration and memory, the stigma of the hepatic disease, the sleep, the isolation, the life expectancy, the sexual function/activity) presented a significant improvement in the patients who were subject to interferon free therapy these patients were evaluated before and after treatment), as compared to the patients in lot C (control), who were evaluated, but did not get the treatment because they did not meet the eligibility criteria, which led to statistically significant differences ($p < 0,0001$). This fact translated into the increase of the quality of life of the patients with chronic liver conditions, who benefit from "interferon free" antiviral therapy, while the patients who did not get the treatment affirm a more altered quality of life with regard to all dimensions of the questionnaire SF-LDQOL, with a single exception, namely, with regard to the dimension of sexual function/activity, no significant differences were reported between the two lots L0 and L1 ($p < 0,097$).

- The implications of studying the quality of life of patients with chronic hepatic conditions are relevant, both for patients who benefited from interferon free therapy and for the patients who were not subject to treatment, regarded both in a positive sense for the treated patients, and in the negative sense, with regard to the quality of life of the untreated patients, underlining the need to increase the access of patients to the new therapies and to choose an individualized therapeutic scheme for each patient, considering the effectiveness and the tolerability.

- The chronic infection with hepatitis C virus must be approached as a public health issue both worldwide and at the national level, since it preponderantly affects middle-aged persons, who are socially and professionally active, which determines a special implication from the specialists, which requires an efficient interdisciplinary collaboration with a view to optimize the therapy and to maintain the cost – effectiveness balance.

CONCLUSIONS

1. HCV still remains a public health issue, due to the ever growing number of cases diagnosed and the limited access to therapy for economic reasons.

2. DAA treatment has proved to be extremely effective, with a high healing rate.

3. By an extended access to therapy, Romania has the chance to heal more and more patients, by decreasing the virus reservoir and creating the prerequisites for the eradication of this infection in a near future.

4. The impact of the therapy on the quality of life remains an important factor that may boost the public health policies.

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