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DOMENIUL MEDICINĂ

Neoplastic risk in patients with chronic HCV infection treated with Direct Acting Antivirals

SUMMARY

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1. Hepatitis Virus C Infection

Diagnosed in 1989, hepatitis C virus (HCV) remains an important global health problem, being responsible for about 1.75 million of new infections and 399000 deaths annually [1]. According to the World Health Organization (WHO), in 2015, the global prevalence of HCV infection was 1.0% [1], with the highest percent (>2%) being reported within the Mediterranean region. During 20 years of chronic HCV infection, about 20-30% of the patients will develop cirrhosis [2], while hepatic decompensation is estimated to affect 2.8-11.7% of the cirrhotic patients [3]. Globally, regarding the number of HCV infections, Romania stands among the first 20 countries, the general prevalence among the adult population being very high - 3.3%, with a predominance of genotype 1b [4]. Parenteral transmission is the most effective means of propagating the virus, a particular category being intravenous drug users. Vertical transmission represents the major cause of HCV infection among the paediatric population [5]. Hepatitis C virus is has hepatic tropism and is a part of the Flaviviridae family and Hepacivirus genus. The viral particle is spheric and heterogeneous, having a diameter between 40 and 80 nm. The viral elements are represented by the envelope glycoproteins (E1 and E2 heterodimers), a lipid membrane, the nucleocapsid, and the single-stranded RNA genome [6]. HCV is a non-cytopathic virus, that inserts itself within the hepatocyte in order to replicate, thus inducing hepatocyte necrosis, as well as several other phenomena (hepatic steatosis, oxidative stress, insulin resistance) [7]. The extrahepatic manifestations of HCV infection are extremely important and a significant percent of patients (almost 76%) develop at least one extrahepatic manifestation during the course of infection [8]. HCV infection evaluation includes anti-HCV antibodies testing, as well as HCV-RNA detection. Evaluating hepatic fibrosis is a key element in the management of HCV infected patients and it can be made by either FibroScan(®) (transient elastography), or by calculating several fibrosis scores, such as APRI (AST to platelet ratio index) and FIB-4 (Fibrosis-4) [9]. Hepatic biopsy is not routinely performed in HCV infected patients [10]. Antiviral treatment is now recommended to all infected subjects, either naïve or therapeutically experienced. The main goal is HCV-RNA clearance, predicted by the sustained virologic response (SVR). Choosing the best therapeutic regimen for each patient must take into consideration the viral genotype, potential drug-drug interactions, as well as some patient characteristics (such as presence of cirrhosis, previous HCV treatment, renal function).

2. Hepatocellular carcinoma

Approximately 75% of the primary hepatic tumours are hepatocellular carcinomas (HCC) [11]. It is estimated that, by 2025, the annual incidence of HCC will reach 1 million individuals [12], with an important increase of HCC cases due to metabolic hepatopathies [13]. HCC screening is essential among patients considered to be at risk [14] and is performed with both imagistic investigations and tumour markers, such as alpha-fetoprotein (AFP) [15].

Direct-acting antivirals (DAAs) are used to obtain viral clearance, but these agents are not able of curing the hepatic disease itself, once it has progressed to cirrhosis or advanced fibrosis. Thus, the risk for complications persists after achieving SVR. Moreover, the risk of HCC occurrence in patients with HCV chronic infection, treated with DAAs is a controversial matter.

3. Working hypothesis and general objectives

DAAs marked the beginning of a new era in HCV management. However, the relationship between DAAs and HCC has been the subject of numerous controversies, some authors reporting a rapid tumoral growth and a high incidence of HCC following Interferon-free treatment. On the other hand, various other researchers found no correlation between HCC and the use of DAAs. The work hypothesis is centred around the possibility of establishing a statistic connection between DAAs and HCC development, by taking into consideration the incidence of this pathology among the observed population, as well as several biological and imagistic characteristics of the tumour.

The aim of the study is to evaluate the risk of hepatic cancer among HCV infected patients, that received treatment with DAAs, while analysing the occurrence and pattern of the tumour, as well as biological and imagistic parameters and risk factors associated with HCC. The general objectives are:

- Describing demographic particularities of HCV-infected patients that were treated with DAAs;
- Evaluating biological and imagistic parameters (conventional ultrasonography, contrast-enhanced ultrasonography, computerized tomography, magnetic resonance imaging, transient elastography), used for liver assessment both at 3 and 12 months after SVR;
- Evaluating liver function severity scores both at 3 and 12 months after SVR;
- Evaluating biological and imagistic parameters determined in the presence of hepatocellular carcinoma and corelating these parameters with the degree of hepatic dysfunction, as well as other biological and metabolic abnormalities;

- Evaluating the scores used for HCC assessment and corelating these scores with the degree of hepatic dysfunction, as well as other biological and metabolic abnormalities;
- Evaluating the therapeutic management applied in patients diagnosed with hepatocellular carcinoma and establishing correlation between different HCC treatment strategies and biological and imagistic findings;
- Evaluating the mortality among the patients diagnosed with HCC, in correlation with tumour characteristics and management.

4. General methodology

I conducted a cohort prospective observational study, which included 535 patients treated with DAAs for HCV chronic infection between May 2016 and December 2017, within the Internal Medicine Department at Fundeni Clinical Institute. The treatment was conducted according to the national protocol existent at the time:

- patients with advanced fibrosis and compensated cirrhosis received antiviral treatment with ombitasvir/paritaprevir/ritonavir and dasabuvir (Viekirax/ Exviera);
- patients with decompensated cirrhosis received antiviral treatment with ledipasvir/ sofosbuvir (Harvoni).

The local Ethical Committee approved the study. An informed written consent was taken from all the participants and all their records were confidential. We included in the study patients older than 18 years-old, with personal history of HCV chronic infection, who achieved sustained virologic response through direct acting antivirals. The following situations represent exclusion criteria:

- death during DAAs treatment;
- hepatitis B virus or human immunodeficiency virus (HIV) co-infection;
- concomitant or previous malignant processes;
- non-viral hepatopathy, autoimmune liver disorders, infectious disorders, heart failure.

Data was achieved through anamnesis, clinical examination, biological parameters, imagistic investigations and various scores (used either for hepatic function or fibrosis assessment) – all these findings being attentively collected both at 3 and 12 months after SVR. For statistical analysis, the soft IBM SPSS Statistics for Windows, version 26.0. Armonk, NY: IBM Corp was used.

5. Results

The medium age among the study group was 56,94 years, with a predominance of females (Figures 5.1 and 5.2).

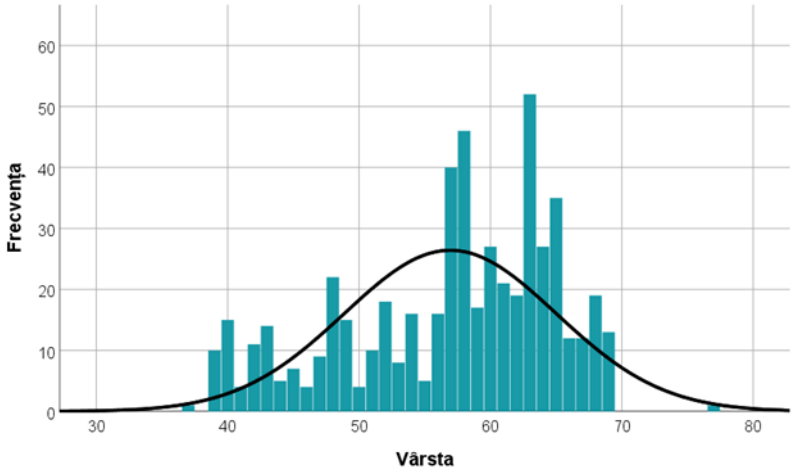

Figure 5.1. Age distribution of the study group

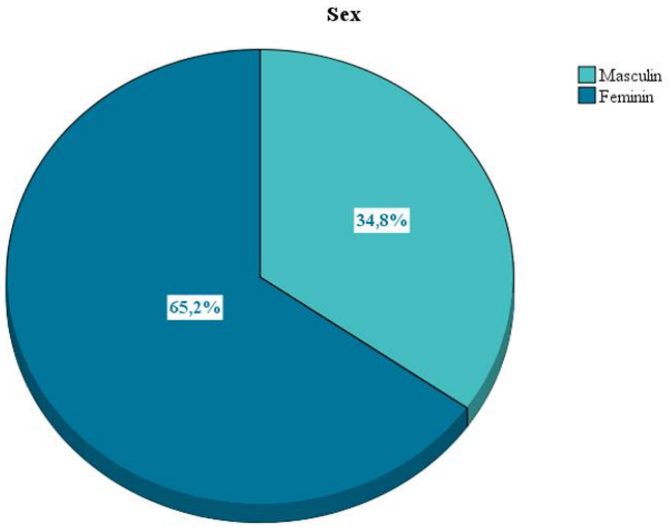

Figure 5.2. Sex distribution of the study group

Patients were also analysed according to previous use of HCV treatment (Figure 5.3).

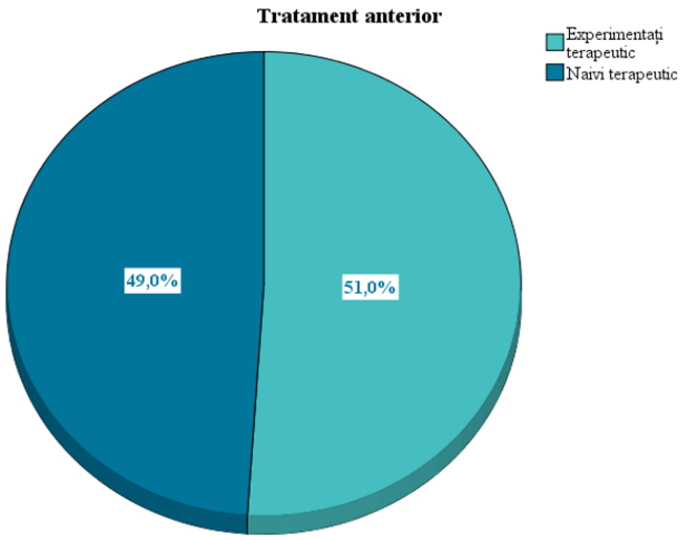

Figure 5.3. Distribution of the study group according to previous antiviral treatment

Three months after achieving SVR, more than half of the patients presented with cirrhosis – most of them having compensated Child A cirrhosis (Figure 5.4).

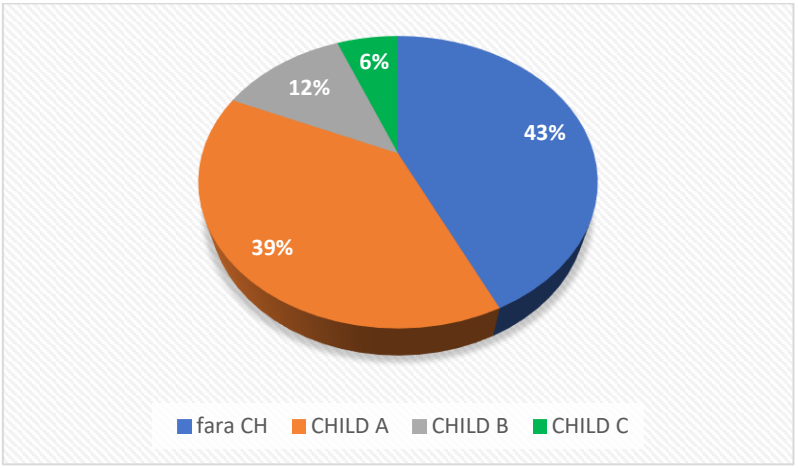

Figure 5.4. Distribution of the study group according to hepatic function at 3 months after SVR

The presence of diabetes mellitus (DM) was also evaluated after achieving SVR, revealing that 11% of the total number of subjects were diabetic: 3,7% had DM treated with oral antidiabetics, 6% had insulin-dependent DM and 1,3% had diet-controlled diabetes. Thyroid function was also taken into consideration, resulting in 2,2% of the patients having autoimmune thyroiditis, while 4.5% had hypothyroidism. Also, 47 patients (8.8%) were diagnosed with metabolic syndrome (Figure 5.5).

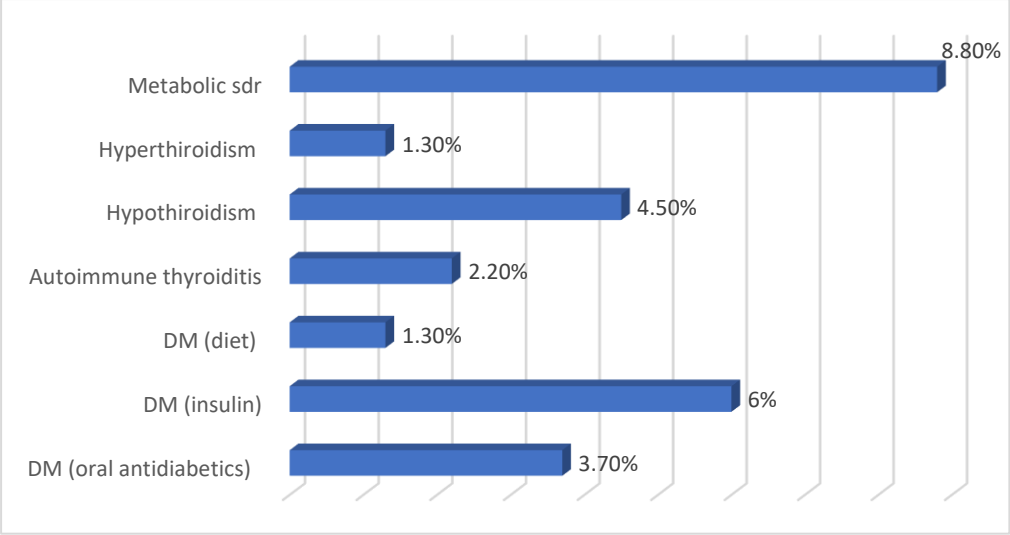

Figure 5.5. The presence of comorbidities at 3 months after SVR

Three months after SVR, all patients were evaluated with conventional ultrasonography. For three of the 535 patients (0,6%), ultrasonography (US) revealed the presence of intrahepatic masses, thus further investigations were necessary. Contrast-enhanced ultrasonography (CEUS) and computerized tomography (CT) were performed for these patients, revealing that no patient had HCC at 3 months after SVR. Table 5.1 shows the diagnostic means that were applied among the study group at 3 months after SVR.

Table 5.1. Pathologies and diagnostic methods applied at 3 months after SVR

Parameters	N=535
Ultrasonography	535 (100%)
Splenic hilum venous dilation	294 (55%)
Hepatomegaly	315 (58,9%)
Homogeneous liver at US	221 (41,3%)
Non-Homogeneous liver at US	314 (58,7%)
HCC at US	0 (0%)
Hepatic mass <1cm at US	0 (0%)
Hepatic mass 1-2 cm at US	3 (0,6%)
Hepatic mass >2cm at US	0 (0%)
CEUS	3 (0,6%)
Regenerative nodule at CEUS	2 (0,4%)
Dysplastic nodule at CEUS	1 (0,2%)

HCC at CEUS	0 (0%)
Portal vein thrombosis at CEUS	0 (0%)
CT	3 (0,6%)
HCC at CT	0 (0%)
MRI	0 (0%)
oesophageal varices	294 (55%)
Ascites	
- No ascites	465 (86,9%)
- Ascites grade 1	29 (5,4%)
- Ascites grade 2	24 (4,5%)
- Ascites grade 3	17 (3,2%)
Hepato-renal syndrome	6 (1,1%)
Hepatic encephalopathy (HE)	
- No encephalopathy	479 (89,5%)
- Hepatic encephalopathy grade 1	25 (4,7%)
- Hepatic encephalopathy grade 2	29 (5,4%)
- Hepatic encephalopathy grade 3	2 (0,4%)
DM (oral antidiabetics)	20 (3,7%)
DM (insulin-dependent)	32 (6%)
DM (diet)	7 (1,3%)
Autoimmune thyroiditis	12 (2,2%)
Hypothiroidism	24 (4,5%)
Hyperthiroidism	7 (1,3%)
Metabolic syndrome	47 (8,8%)

One year after obtaining SVR, 47 cases of HCC were diagnosed, using both imagistic criteria and tumour markers (Figure 5.6).

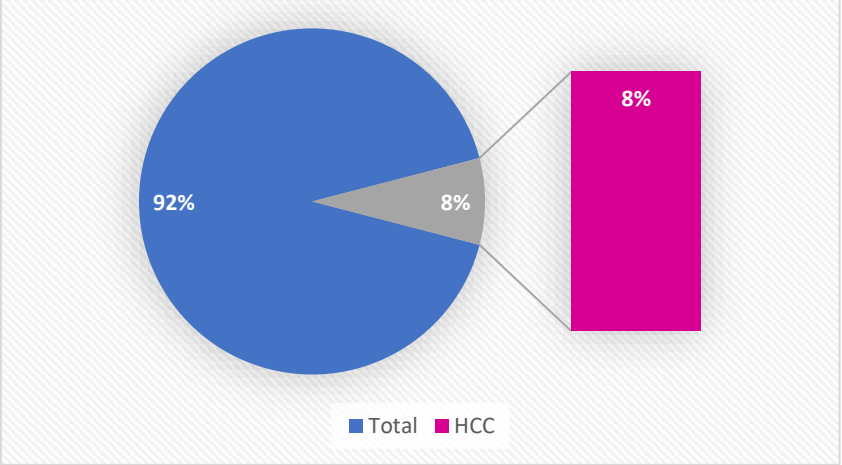

Figure 5.6. HCC distribution within the study group

Computerized tomography revealed the predominance of solitary masses in more than half of the cases (51.06%). Also, most of the patients (over 87%) presented with visible lymphadenopathies, while HCC metastases and portal vein thrombosis (PVT) were relatively rare (Table 5.2).

Table 5.2. HCC characteristics at 12 months after SVR

Parameters	N=47
Nodules	
- 1	24 (51.06%)
- 2	14 (29.78%)
- 3	9 (19.14%)
Lymphadenopathies	41 (87.23%)
Metastases	3 (6.38%)
Portal vein thrombosis	5 (10.63%)
Localisation	
- Segment 2	8 (17.02%)
- Segment 3	7 (14.89%)
- Segment 4	5 (10.63%)
- Segment 5	11 (23.40%)
- Segment 6	2 (4.25%)
- Segment 7	5 (10.63%)
- Segment 8	9 (19.14%)

Maximum tumour diameter (cm)	4.44±1.74

Calculating Tumour Burden Score (TBS) showed mainly medium burden, while the use of Seven Eleven Criteria (SEC) resulted in minimum burden for most patients (Figures 5.7 and 5.8).

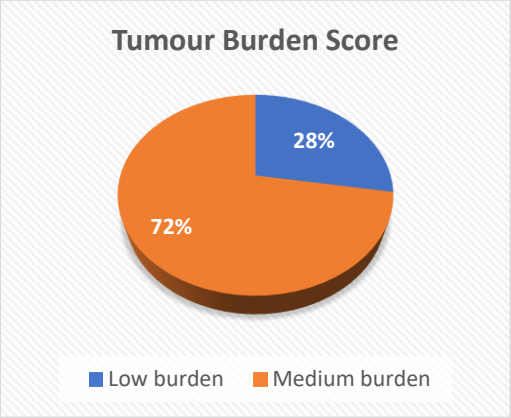

Figure 5.7. Tumour Burden Score

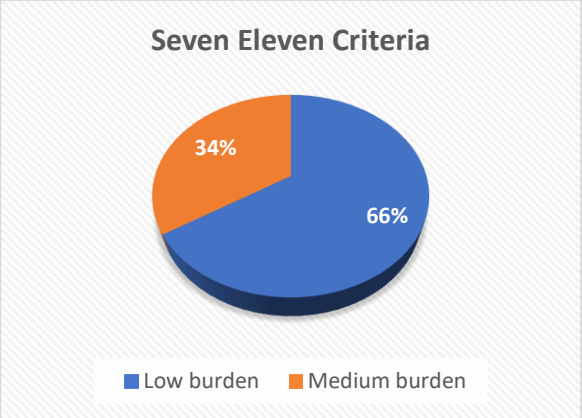

Figure 5.8. Seven Eleven Criteria

Hepatic function assessment among the 47 patients diagnosed with HCC revealed mostly cases of decompensated cirrhosis - 55% (Figure 5.9). According to the BCLC classification, the most prevalent stages were C, D and B (Figure 5.10).

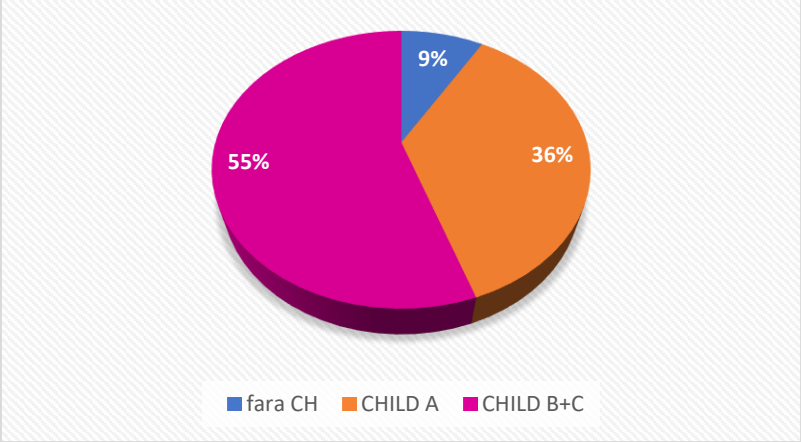

Figure 5.9. Distribution of HCC patients according to the liver function assessment (at 12 months after SVR)

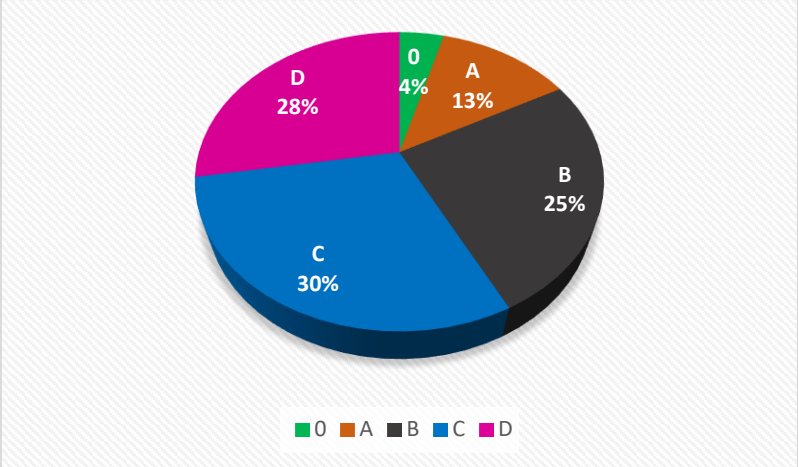

Figure 5.10. Distribution of HCC patients according to the BCLC classification (at 12 months after SVR)

According to the BCLC stage at the time of HCC diagnosis, most of the patients received either systemic therapy of best supportive care. Trans-arterial chemoembolization (TACE) was performed initially in more that 25% of the patients.

Statistical analysis of the collected data showed the existence of important correlations between HCC and the following parameters: aminotransferases, albumin, alkaline phosphatase, total bilirubin, platelets, sodium, cholesterol, CK, CK-MB, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), serum ammonium, spleen dimensions, portal vein diameter, fibrosis degree (either expressed by FibroScan, APRI or FIB-4), MELD and Child scores, insulin-dependent DM, metabolic syndrome, hypothyroidism. Serum level of alpha-fetoprotein significantly correlated not only with the presence of hepatic masses, but also with the number of nodules, maximum tumour diameter and tumour burden scores (TBS and SEC). A statistical association between TBS and SEC was only present for patients considered ”low burden”. Moreover, both TBS and SEC scores correlated very well with the BCLC stage (Figures 5.11 and 5.12).

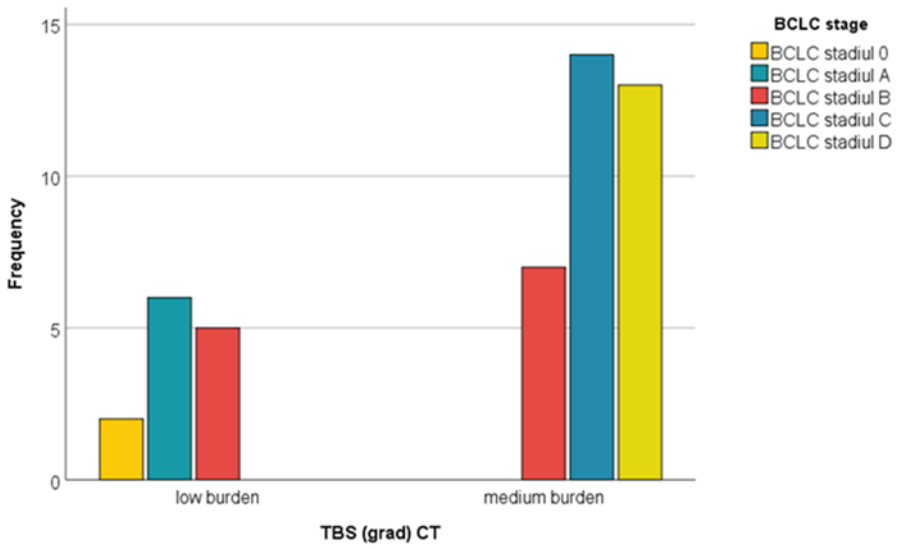

Figure 5.11. Correlation between TBS –BCLC

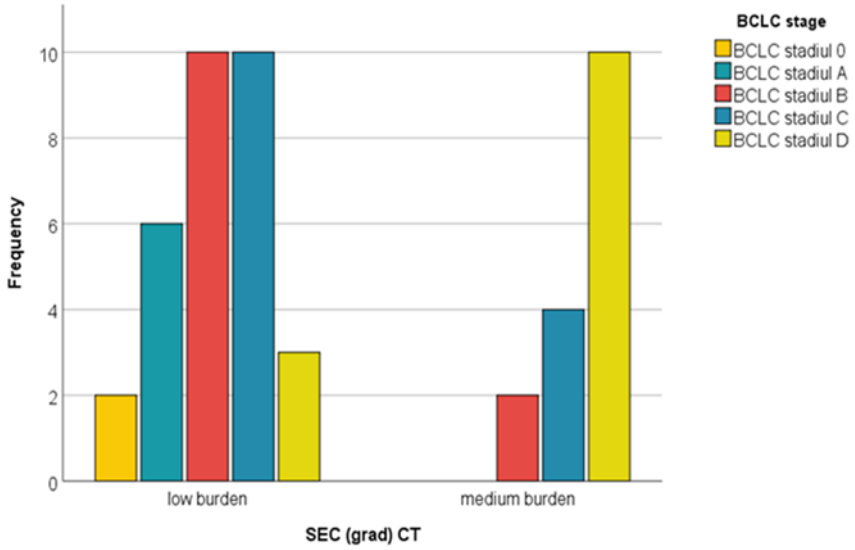

Figure 5.12. Correlation between SEC –BCLC

AFP elevations were associated with a higher prevalence of lymphadenopathies, metastases and portal vein thrombosis. Moreover, after analysing CK and CK-MB levels among the study group, higher values of these two parameters were noted in the presence of hepatocellular carcinoma (Table 5.3). C reactive protein was also significantly different in patients with HCC, irrespectively of the localisation (Table 5.4).

Table 5.3. Corelation between CK – HCC

CK (UI/L)				
	N	Mean	Std dev	p
no HCC	488	88,47	19,967	<0,001
segment 2	8	203,25	8,860	
segment 3	7	188,43	9,658	
segment 4	5	184,60	7,668	
segment 5	11	198,55	14,003	
segment 6	2	202,50	9,192	
segment 7	5	199,20	6,834	
segment 8	9	202,33	37,845	
Total	535	98,03	36,697	

Table 5.4. Corelation between CRP – HCC

CRP (mg/dl)				
	N	Mean	Std dev	p
no HCC	488	2,5045	,46348	<0,001
segment 2	8	6,2500	1,15264	
segment 3	7	4,5857	1,44156	
segment 4	5	4,5800	,87579	
segment 5	11	6,4182	1,82473	
segment 6	2	5,7000	,70711	
segment 7	5	6,4000	,56569	
segment 8	9	5,7333	1,49416	
Total	535	2,7903	1,11291	

Survival among patients with HCC correlated positively with the following parameters: albumin, cholesterol and platelets. On the other hand, between hepatic fibrosis and survival, a negative statistic correlation was expected and observed. Tumour characteristics, tumour burden scores and BCLC stage all correlated with survival in patients diagnosed with HCC. Patients that underwent hepatic resection, radiofrequency ablation (RFA), TACE and liver transplantation had a survival over 48 months, while the subjects with severely impaired hepatic function received systemic therapy/ best supportive care and had a medium survival of 11,32 months.

Among the 47 patients with HCC, 68,1% died on follow-up. Mortality was three times higher in the presence of HCC (Table 5.5).

Table 5.5. Mortality in patients with HCC

Risk			
	Value	95% Confidence Interval	
		Lower	Upper
For cohort death = Nu	3,133	2,064	4,757
N of Valid Cases	535		

Statistical analysis revealed several risk factors associated with mortality in the presence of hepatocellular carcinoma: albumin, total bilirubin, platelets level, INR, CK, CK-MB, AFP, hepatic fibrosis, tumour burden scores. Diet-controlled diabetes mellitus and metabolic syndrome also represented mortality risk factors within this subgroup.

6. Discussion

The use of direct acting antivirals opened a new era in the management of HCV-induced liver disease. However, the relationship between DAAs and HCC has been the subject of numerous controversies, with several authors suggesting that Interferon-free therapy might be responsible for a more rapid tumour growth and a higher incidence of HCC. On the other hand, other researchers established no correlation between HCC occurrence and DAA therapy. Regarding our cohort of 535 people, HCC was only diagnosed in patients with cirrhosis and advanced fibrosis – conditions that are themselves independent risk factors for HCC. Moreover, diabetes mellitus, metabolic syndrome and hypothyroidism were also present within the study group. These pathologies are also involved in HCC development. Several preclinic

studies established an important connection between thyroid hormones and carcinogenesis. It is also well known that HCV itself is associated with various extrahepatic manifestations, thyroid involvement being one of the most prevalent [16]. Regarding the patients with HCC that were observed, a significant correlation was observed between hypothyroidism and the presence of HCC – this data confirming previous research. Insulin-dependent diabetes mellitus was also correlated with HCC occurrence, as well as with the number of tumour masses. No association was established between the presence of hepatocellular carcinoma and diet-controlled DM/ DM treated with oral antidiabetics. The relationship between DM and HCC was highly investigated by the medical world. Important research regarding this topic was presented in the EPIC study, which concluded that DM is a risk factor for HCC occurrence, with a significantly higher risk for hepatic cancer among insulin-dependent patients [17]. Within the study group, metabolic syndrome was observed in 8.8% of the cases, with a higher frequency among patients diagnosed with HCC (17%) – a fact that suggests the association between these two pathologic entities. Most of the patients with HCC presented with solitary hepatic masses (51%). Moreover, the presence of metastases and portal vein thrombosis among these patients was reduced. The mean maximum tumour diameter was 4.44 cm and no patient presented with high TBS or SEC. These findings may suggest a less aggressive pattern of hepatocellular carcinoma developed after DAAs treatment. Twenty patients had a survival rate over 48 months – a fact that is particular, considering the distribution of hepatic disfunction among patients with HCC (with the predominance of decompensated cirrhosis). All patients diagnosed with hepatic cancer presented elevated AFP levels – that contributed to the diagnosis and post-treatment monitoring of the patients. Moreover, AFP correlated with tumour diameter, as well as with tumour burden. Both TBS and AFP are important prognosis factors in the presence of HCC. The relationship between these two variables allows a stronger prediction of the possible outcome. Literature data observed that TBS and AFP have a synergistic impact on outcomes in these patients [18]. In a recent study [18], patients with similar TBS grades had different evolution, according to the AFP levels. AFP assessment is an important step in the evaluation of HCC-diagnosed patients, especially in the presence of small tumour masses/ metastases. Within the study group, I have observed an important association between high ALF levels and the presence of lymphadenopathies, metastases and PVT. An important correlation was also noted between HCC and CRP, while recent studies suggest the presence of various signalling pathways that can explain the connection between carcinogenesis and inflammatory markers [19]. The presence of hepatocellular carcinoma also correlated well with serum levels of CK and CK-MB – an association that was also suggested by other researchers.

However, further investigation of this matter is necessary [20]. Both tumour burden scores correlated with BCLC classification. A recent study evaluated TBS and BCLC, describing an important number of BCLC-B patients and medium TBS that had a better outcome than individuals with BCLC-A and high TBS [21]. Patients' survival depended on HCC management – the highest survival rates being observed among subjects that benefitted from surgical resection, RFA, TACE and liver transplant. Combining several therapeutic procedures is associated with a better outcome. Mortality evaluation revealed that patients with HCC have a higher mortality rate, up to 3-fold. Several factors were associated with a higher risk of death in patients with HCC: AFP, CK, CK-MB, Albumin, total bilirubin, platelets, hepatic fibrosis, diet-controlled DM, metabolic syndrome, maximum tumour diameter, TBS and SEC.

7. Conclusions

After analysing the data collected, I have made the following observations:

- ✓ There was no statistical correlation established between HCC and DAAs, the incidence of liver cancer within the study group being under 8.8%.
- ✓ Moreover, in patients diagnosed with HCV, HCC was observed exclusively in the presence of cirrhosis and advanced fibrosis.
- ✓ Diabetes mellitus (insulin-dependent), hypothyroidism and metabolic syndrome are important risk factors associated with HCC.
- ✓ After DAAs treatment, the estimated tumour burden among patients with HCC was either low or medium (not high).
- ✓ After DAAs treatment, solitary HCC was predominant.
- ✓ After DAAs treatment, the presence of metastases and portal vein thrombosis was reduced.
- ✓ The levels of CK, CK-MB and inflammatory markers correlated with the presence of HCC.
- ✓ Mortality was three times higher among subjects with HCC in the study group.
- ✓ Several factors are associated with a higher mortality risk, in the presence of HCC: AFP, CK, CK-MB, Albumin, total bilirubin, platelets, hepatic fibrosis, diet-controlled DM, metabolic syndrome, maximum tumour diameter, TBS and SEC.
- ✓ It is important to emphasize that HCC screening (using imagistic methods and tumour markers every six months) should not be stopped after achievement of SVR, as interferon free treatments cure the viral infection, but not the liver disease itself.

Despite the well-established inclusion/ exclusion criteria, the study was limited by the small number of patients diagnosed with HCC at 12 months after SVR. Moreover, this research observed and analysed a cohort that received Interferon-free therapy in 2016-2017, when the available molecules, as well as the eligibility criteria were different from the current ones. The risk of HCC in the absence of DAAs treatment was not taken into consideration – a fact that may represent another limitation of this study.

As a perspective, it would be ideal to continue monitoring patients treated with DAAs, on longer periods of time and at a multicentric level. Creating further prognostic/ diagnostic algorithms able to increase the predictive accuracy is a matter that should be further pursued. The continuous advances in molecular medicine opened a new chapter and the efforts towards the discovery of new HCC biomarkers are deeply encouraged.

References

1. World Health Organization. Web Annex B. WHO estimates of the prevalence and incidence of hepatitis C virus infection by WHO region, 2015. In: Global hepatitis report 2017. https://apps.who.int/iris/bitstream/handle/10665/277005/WHO-CDS-HIV-18.46-eng.pdf (Accessed on May 21, 2019).

2. Alberti A, Chemello L, Benvegnù L. Natural history of hepatitis C. J Hepatol. 1999;31 Suppl 1:17-24. doi: 10.1016/s0168-8278(99)80369-9.

3. Alazawi W, Cunningham M, Dearden J, Foster GR. Systematic review: outcome of compensated cirrhosis due to chronic hepatitis C infection. Aliment Pharmacol Ther. 2010;32:344–355.

4. Streinu-Cercel A. Hepatitis C Burden in Romania 2016-2017 Evaluation 2018 Challenges.https://www.vhpb.org/files/html/Meetings_and_publications/Presentations/BUCH32.pdf (Accessed on March 21, 2022).

5. Benova L, Mohamoud YA, Calvert C, Abu-Raddad LJ. Vertical transmission of hepatitis C virus: systematic review and meta-analysis. Clin Infect Dis. 2014 Sep 15;59(6):765-73. doi: 10.1093/cid/ciu447. Epub 2014 Jun 13.

6. Madan V, Bartenschlager R. Structural and Functional Properties of the Hepatitis C Virus p7 Viroporin. Viruses. 2015 Aug 6;7(8):4461-81. doi: 10.3390/v7082826.

7. Irshad M, Mankotia DS, Irshad K. An insight into the diagnosis and pathogenesis of hepatitis C virus infection. World J Gastroenterol. 2013 Nov 28;19(44):7896-909. doi: 10.3748/wjg.v19.i44.7896.

8. Cacoub P, Comarmond C, Domont F, Savey L, Desbois AC, Saadoun D. Extrahepatic manifestations of chronic hepatitis C virus infection. Ther Adv Infect Dis. 2016 Feb;3(1):3-14. doi: 10.1177/2049936115585942.

9. Itakura J, Kurosaki M, Setoyama H, et al. Applicability of APRI and FIB-4 as a transition indicator of liver fibrosis in patients with chronic viral hepatitis. J Gastroenterol. 2021 May;56(5):470-478. doi: 10.1007/s00535-021-01782-3. Epub 2021 Mar 31.

10. Sebastiani G, Gkouvatsos K, Pantopoulos K. Chronic hepatitis C and liver fibrosis. World J Gastroenterol. 2014 Aug 28;20(32):11033-53. doi: 10.3748/wjg.v20.i32.11033.

11. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of Hepatocellular Carcinoma. Hepatology. 2021 Jan;73 Suppl 1(Suppl 1):4-13. doi: 10.1002/hep.31288. Epub 2020 Nov 24.

12. Global Burden of Disease Liver Cancer Collaboration, Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncol. 2017 Dec 1;3(12):1683-1691. doi: 10.1001/jamaoncol.2017.3055.

13. Valery PC, Laversanne M, Clark PJ, Petrick JL, McGlynn KA, Bray F. Projections of primary liver cancer to 2030 in 30 countries worldwide. Hepatology. 2018 Feb;67(2):600-611. doi: 10.1002/hep.29498. Epub 2017 Dec 23. PMID: 28859220; PMCID: PMC5832532.

14. Kudo M, Izumi N, Kokudo N, et al. Management of Hepatocellular Carcinoma in Japan: Consensus-Based Clinical Practice Guidelines Proposed by the Japan Society of Hepatology (JSH) 2010 Updated Version. Dig Dis 2011;29:339e64

15. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, Waljee AK, Singal AG. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. Gastroenterology. 2018 May;154(6):1706-1718.e1. doi: 10.1053/j.gastro.2018.01.064. Epub 2018 Feb 6. PMID: 29425931; PMCID: PMC5927818.

16. Toma L, Zgura A, Isac T, Mercan-Stanciu A, Dodot M, Iliescu L. The impact of COVID 19 infection on HCV-induced thyroid disease. Acta Endocrinol (Buchar). 2021 Jul-Sep;17(3):372-376. doi: 10.4183/aeb.2021.372.

17. Schlesinger S, Aleksandrova K, Pischon T, et al. Diabetes mellitus, insulin treatment, diabetes duration, and risk of biliary tract cancer and hepatocellular carcinoma in a European cohort. Ann Oncol. 2013 Sep;24(9):2449-55. doi: 10.1093/annonc/mdt204. Epub 2013 May 29.

18. Tsilimigras DI, Hyer JM, Diaz A, et al.: Synergistic impact of alpha-fetoprotein and tumor burden on long-term outcomes following curative-intent resection of hepatocellular carcinoma. Cancers. 2021, 13:747. 10.3390/cancers13040747

19. Chen W, Wang JB, Abnet CC, et al. Association between C-reactive protein, incident liver cancer, and chronic liver disease mortality in the Linxian Nutrition Intervention Trials: a nested case-control study. Cancer Epidemiol Biomarkers Prev. 2015 Feb;24(2):386-92. doi: 10.1158/1055-9965.EPI-14-1038. Epub 2015 Jan 22.

20. Soroida Y, Ohkawa R, Nakagawa H, et al. Increased activity of serum mitochondrial isoenzyme of creatine kinase in hepatocellular carcinoma patients predominantly with recurrence. J Hepatol. 2012 Aug;57(2):330-6. doi: 10.1016/j.jhep.2012.03.012. Epub 2012 Apr 17.

21. Tsilimigras DI, Moris D, Hyer JM, et al. Hepatocellular carcinoma tumour burden score to stratify prognosis after resection. Br J Surg. 2020 Jun;107(7):854-864. doi: 10.1002/bjs.11464. Epub 2020 Feb 14.