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Original Article

DIFFERENCES IN THE COURSE OF HEPATOCELLULAR CARCINOMA DEPENDING ON THE DAA TREATMENT

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ABSTRACT

Background and aims: 58 million people live with chronic hepatitis C virus (HCV) infection which can lead to liver cirrhosis and hepatocellular carcinoma (HCC). Currently, a non-interferon therapy based on direct-acting antivirals (DAA) is the standard treatment of HCV infection. This study aimed to assess the occurrence of HCC after treatment with DAA and evaluate whether the course of the disease and liver function is different in patients treated with DAA compared to those who did not receive such therapy. **Materials and methods:** We studied the population of adult patients from the 2015-2021 period and analyzed the demographic and clinical data, including alcohol abuse, liver enzymes, Child-Pugh and model for end-stage liver disease scores, imaging tests, liver biopsy and elastography, if performed. The Mann-Whitney U and the McNemar's tests were used. The p-value was set at 0.05. **Results:** The study included n=34 patients with HCC. In this group n=22 patients (61%) were diagnosed with a chronic HCV infection and n=11 (50%) were receiving DAA treatment. Patients who were receiving DAA developed multiple HCC significantly less often compared to patients not treated with DAA (45.5% vs. 69.6%). We also observed a significantly less common occurrence of portal vein thrombosis (8.7% vs. 18.2%) and distant metastases (0.0% vs. 13.0%) in patients with HCC receiving DAA. Patients with HCC who underwent DAA therapy had significantly lower levels of serum liver enzymes in comparison with patients diagnosed with HCC and not receiving DAA. **Conclusions:** HCC occurring in patients treated with DAA was more often associated with lymphadenopathy, multiple tumors, portal vein thrombosis and distant metastases probably due to restricted treatment qualification criteria. DAA treatment led to a decrease in the inflammatory activity in the liver. It is important to adjust qualification protocols so that all patients suffering from chronic HCV infection could receive the treatment as soon as possible.

KEYWORDS: hepatocellular carcinoma, HCV, direct-acting antivirals, liver cirrhosis, liver tumor.

1. Introduction

According to the World Health Organization (WHO) around 58 million people live with chronic hepatitis C virus (HCV) infection. HCV is a bloodborne virus, transmitted mostly by injecting drugs with unsterile needles, inadequate sterilization of medical equipment, unscreened blood transfusions and sometimes vertically and during sexual intercourse [1].

It is estimated that approximately 15-45% of HCV-infected individuals have the capacity to spontaneously clear HCV viraemia [2]. In the remaining population, it advances to a chronic infection which means that HCV viraemia is persistent for over 6 months [3]. Chronic HCV infection and inflammation may lead to the development of potentially life-threatening outcomes including liver cirrhosis and hepatocellular carcinoma (HCC) [1]. HCC is a widely occurring disease and is currently the fifth most common cancer worldwide [4]. Liver cirrhosis is considered one of the most common risk factors of HCC [5].

The aim of HCV treatment is to reach sustained virological response (SVR). SVR is defined as a non-detectable HCV RNA in the patient's serum 3 months after the end of the therapy [6]. The treatment underwent a revolutionary change since the introduction of direct-acting antivirals (DAA). Previously, pegylated interferon (PEG-INF) in combination with ribavirin was commonly used in the treatment of an HCV infection and the successfulness of this therapy depended on the HCV

genotype [7]. SVR rates in patients with genotype 1 were approximately 42-46%, whereas in patients with non-type 1 genotype SVR reached 76-82% [8]. DAA paved the way to the increase in SVR rates to over 90% [9].

DAA agents consist of three main groups, depending on their target of action: NS3/4A inhibitors, NS5A protease inhibitors and NS5B nucleoside and non-nucleoside polymerase inhibitors [10]. The first DAA drugs, which were boceprevir and telaprevir, were approved in combination with PEG-IFN-alpha and ribavirin for the treatment of a chronic HCV genotype 1 infection in 2011. Soon the non-interferon DAA-based regimens became the standard HCV infection therapy [11]. In Poland, the new therapeutic options became available in 2015 [12].

As the new treatment began to be widely available, the researchers took notice of HCC cases among patients undergoing DAA therapy [13]. It is now suggested that the virus eradication reduces the risk of HCC but does not eliminate it completely. This is especially concerning for patients with liver cirrhosis or previous HCC history. The etiology of that phenomenon is multidirectional and can depend on various factors [14]. In the available literature, reports of early occurrence of HCC after the DAA treatment can be found [15,16]. Therefore, patients undergoing successful HCV treatment should be monitored for the contingency of HCC occurrence [17].

This study aimed to assess the occurrence of HCC after DAA treatment and evaluate whether the course of HCC and liver function differ among the population of patients treated with DAA and those who did not receive such therapy.

2. Materials and Methods

2.1. Patients

This is an observative cohort retrospective study. We studied the population of adult patients from the 2015-2021 period. The inclusion criteria were: hepatocellular carcinoma diagnosis, age >18 years. The exclusion criteria were: another cancer diagnosis and non-specific liver lesions occurrence.

2.2. Assessments

All patients underwent physical examination, laboratory tests, abdominal ultrasound examination, transient elastography or histopathological examination of the liver biopsy and abdominal computed tomography (CT) or magnetic resonance imaging (MRI). We analyzed the tumor size in patients with single and multiple lesions; in patients with multiple lesions the size of the largest tumor was used in the analysis.

We collected epidemiological data: age, gender, comorbidities, alcohol abuse – which was defined as consuming more than 14 units of alcohol a week, and clinical data: serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and alpha-fetoprotein (AFP) concentrations, international normalized ratio (INR), Child-Pugh and model for end-stage liver disease (MELD) scores, imaging tests, liver biopsy and elastography, if performed. In all patients HCV infection and co-infections were identified. In the group undergoing the DAA treatment, the composition of therapy and the response to treatment was evaluated.

2.3. Statistical analysis

The Mann-Whitney *U* test was performed to evaluate the difference in the mean value among quantitative variables. The McNemar's test was used to determine the difference in proportions between the paired data. The *p*-value was set at 0.05. All statistical analyses were performed using Python 3.7 software.

3. Results

3.1. Patients

Our study included n=34 patients (n=26 men, n=8 women), all of whom were diagnosed with HCC. In this group n=22 patients (61%) were diagnosed with a chronic HCV infection (n=17 men, n=5 women), n=2 patients had a chronic HBV infection (n=2 men), n=10 patients had alcohol-related liver disease (n=9 men, n=1 woman). There were n=30 (83%) patients (n=23 men, n=7 women) diagnosed with liver cirrhosis. In the study population n=11 patients (32%) had chronic HCV infection and were receiving DAA treatment (n=9 men, n=2 women) and the rest of the analyzed patients (n=23) were not receiving it (n=17 men, n=6 women), regardless the HCV status. The characteristics of the patients are presented in Table 1.

Table 1. Baseline characteristics of the patients, regardless the HCV status.

Characteristics of the patients	Patients receiving DAA (n=11)	Patients not receiving DAA regardless the HCV status (n=23)	*p*-value
Age mean (min-max) years	62.5 (42-79)	60.4 (36-76)	0.606
ALT mean (min-max) U/L	61.5 (14-240)	100.6 (12-274)	**0.041**
AST mean (min-max) U/L	73.3 (25-195)	158.1 (33-670)	**0.045**
GGT mean (min-max) U/L	118.9 (18-495)	333.7 (22-1315)	**0.027**
ALP mean (min-max) U/L	124.0 (44-289)	334.1 (38-1491)	**0.039**
Total bilirubin mean (min-max) μmol/L	33.4 (8.7-149.4)	69.3 (10.8-409.7)	0.439
INR ratio mean (min-max) score	1.45 (1-2.8)	1.4 (0.9-2.5)	0.740
AFP mean (min-max) IU/mL	1012.7 (2.1-10000.0)	1012.8 (1.04-10000.0)	0.568
Ca 19.9 mean (min-max) U/mL	95.0 (3.0-828.9)	92.7 (3.0-1500.0)	0.736
Hemoglobin mean (min-max) g/dL	12.4 (6.8-15.7)	12.4 (8.3-16.0)	0.685
Platelets mean (min-max) G/L	128.7 (34.0-229.0)	184.6 (52.0-629.0)	0.544
Child-Pugh mean (min-max) score	6.5 (5.0-11.0)	6.6 (5.0-11.0)	0.696
MELD mean (min-max) score	12.0 (3.0-23.0)	12.0 (4.0-32.0)	0.644
Alcohol abuse n (%)	3 (27.3)	7 (30.4)	0.796

In comparison to patients diagnosed with HCC and not receiving DAA treatment, patients with HCC who underwent DAA therapy had significantly lower serum levels of AST, ALT, GGT and ALP. Table 2 presents the differences among basic characteristics in patients with a chronic HCV infection who were and were not receiving the DAA therapy.

Table 2. Baseline characteristics of the patients with a chronic HCV infection.

Characteristics of the patients	Patients with HCV infection receiving DAA (n=11)	Patients with HCV infection not receiving DAA (n=11)	*p*-value
Age mean (min-max) years	62.5 (42-79)	63.0 (50-76)	1.000
ALT mean (min-max) U/L	61.5 (14-240)	100.5 (12-274)	0.178
AST mean (min-max) U/L	73.3 (25-195)	136.1 (38-335)	0.131
GGT mean (min-max) U/L	118.9 (18-495)	105.1 (22-182)	0.554
ALP mean (min-max) U/L	124.0 (44-289)	144.1 (38-386)	0.511
Total bilirubin mean (min-max) μmol/L	33.4 (8.7-149.4)	22.4 (13.5-39.3)	0.743
INR mean (min-max) score	1.4 (1-2.8)	1.4 (1.0-2.4)	0.743
AFP mean (min-max) IU/mL	1012.7 (2.1-10000.0)	507.4 (4.18-3600.0)	0.743
Ca 19.9 mean (min-max) U/mL	95.01 (3-828.9)	19.31 (3-95)	0.685
Hemoglobin mean (min-max) g/dL	12.4 (6.8-15.7)	13.6 (10.4-16.0)	0.767
Platelets mean (min-max) G/L	128.7 (34-229)	116.9 (57-198)	0.844
Child-Pugh mean (min-max) score	6.4 (5-11)	5.8 (5-7)	0.861
MELD mean (min-max) score	12 (3-23)	10.8 (6-20)	0.306
Alcohol abuse n (%)	3 (27.3)	3 (27.3)	1.000

We did not observe significant differences in laboratory tests results between patients with both chronic HCV hepatitis and HCC who were and were not receiving DAA therapy. The underlying reason for this might have been the small number of patients in the analyzed groups.

3.2. DAA therapy.

Among n=11 patients treated with DAA, various compositions of the therapy were used. The applied drugs are presented in Table 3.

Table 3. DAA treatment compositions in the studied patients.

DAA treatment composition	n (%)
Ledipasvir + sofosbuvir	3 (27.3)
Sofosbuvir + velpatasvir	1 (9.1)
Ledipasvir + sofosbuvir + ribavirin	3 (27.3)
Sofosbuvir + ribavirin + PEG-IFN	1 (9.1)
Dasabuvir + ombitasvir	2 (18.2)
Sofosbuvir + velpatasvir + ribavirin	1 (9.1)

In n=2 of n=11 patients the treatment was ceased due to a decompensation of liver function. In both patients HCC diagnosis was later confirmed; n=1 patient stopped the treatment after one week and the other one after 10 weeks. All of the n=9 remaining patients (82%) completed the therapy successfully and reached SVR after 12 weeks. Among these patients, HCC was diagnosed 1-6 years after the end of the DAA treatment.

3.3. HCC characteristics

All patients participating in the study underwent abdominal ultrasound examination, followed by a CT or an MRI of the abdomen. We compared the size of the tumor, number of lesions, intraabdominal lymphadenopathy, splenomegaly, portal vein thrombosis (PVT), presence of metastases and liver cirrhosis. The tumor characteristics of the studied patients are presented in Table 4.

Table 4. Tumor characteristics among patients receiving and not receiving DAA treatment, regardless the HCV status.

Characteristics.	Patients receiving DAA (n=11).	Patients not receiving DAA (n=23).	*p*-value
Tumor size mean (min-max) mm	45.18 (7-133)	66.7 (15-172)	0.246
Multiple tumors n (%)	5 (45.5)	16 (69.6)	**0.033**
Lymphadenopathy n (/%)	4 (36.4)	4 (16.4)	0.546
Splenomegaly n (%)	3 (27.3)	6 (26.1)	0.789
PVT n (%)	2 (18.2)	2 (8.7)	**0.035**
Distant metastases n (%)	0 (0.0)	3 (13.0)	**0.033**
Cirrhosis n (%)	10 (90.9)	20 (86.9)	**0.000**

45.5% of the patients who were receiving DAA treatment developed multiple HCC, whereas among patients who were not receiving DAA treatment, multiple HCC were diagnosed in 69.6% and this difference was statistically significant. Another significant difference concerned PVT and distant metastases. PVT was present

in 18.2% of the patients who were receiving and in 8.7% of those who were not receiving DAA treatment. Distant metastases for these groups were detected in 0.0% and 13.0%, respectively. Furthermore, we also compared those characteristics in patients with a chronic HCV infection who were and were not receiving the DAA treatment. The results are presented in Table 5.

Table 5. Tumor characteristics among patients with a chronic HCV infection receiving and not receiving DAA treatment.

Characteristics	Patients with HCV infection receiving DAA (n=11).	Patients with HCV infection not receiving DAA (n=11).	*p*-value
Tumor size mean (min-max) mm	45.18 (7-133)	46.63 (15-160)	0.870
Multiple tumors n (%)	5 (45.5)	7 (63.6)	0.782
Lymphadenopathy n (%)	4 (36.4)	1 (9.1)	**0.034**
Splenomegaly n (%)	3 (27.3)	5 (45.5)	0.405
PVT n (%)	2 (18.2)	0 (0.0)	**0.003**
Distant metastases n (%)	0 (0.0)	0 (0.0)	1.000
Cirrhosis n (%)	10 (90.9)	11 (100.0)	**0.004**

We did not observe similar correlation in the occurrence of multiple tumors and distant metastases in patients with chronic HCV hepatitis. However, we observed that PVT was diagnosed significantly more often among patients receiving DAA therapy compared to those who were not receiving it (18.2% vs. 0.0%). Moreover, abdominal lymphadenopathy was observed significantly more often in the group of patients treated with DAA compared to the other group (36.4% vs. 9.1%).

In our observation n=1 patient who was receiving DAA treatment in the past developed HCC without the diagnosis of liver cirrhosis. There was no such patient in the group of individuals with HCV hepatitis who were not receiving DAA therapy.

4. Discussion

Our findings point that many people with liver disease are at risk of developing HCC, regardless of the etiology of the primary disease. The population of patients with liver cirrhosis is especially susceptible to the development of HCC. HCV infection remains one of the most common causes of liver cirrhosis worldwide [18]. It is estimated that 0.6% of the Polish population is infected with HCV [19]. The introduction of DAA therapy was a milestone in combating HCV which allows to reach SVR, regardless of the HCV genotype, in over 90% of cases [9,20]. As a consequence of DAA therapy becoming more and more available, the WHO Global Health Sector Strategy (GHSS) on viral hepatitis designed a program for the elimination of viral hepatitis as a public health problem by 2030 [21]. Shortly after the introduction of DAA treatment, reports of high HCC incidence after that therapy emerged [22,23]. However, according to the current knowledge, DAA treatment does not increase the risk of HCC development [24,25].

We observed lower serum levels of AST, ALT, GGT and ALP among patients who were receiving DAA therapy in comparison to those who were not receiving the treatment. That finding leads us to the conclusion that DAA therapy may lower the inflammatory activity in the liver. Other studies seem to support this thesis. Cento et al. also observed the lowering of ALT levels and implied that DAA can possibly set the process of „cell-cure" since that treatment regimen provokes liver cells death to a lesser extent than INF-based therapies [26]. In contrast, a study by Liu et al. points out the possibility of the elevation of ALT during the therapy [27]. However, in that study the elevation of transaminases' levels did not lead to the lower efficacy of the treatment. Although in other studies such elevation could possibly result in the premature termination of the therapy [28,29]. Furthermore, a study by Orzechowska et al. confirms the lowering of GGT concentrations after DAA treatment and even suggests that the persistence of high GGT paired with high AFP levels after the treatment may be a risk factor for HCC development [30].

Additionally, our study also showed that patients diagnosed with HCC regardless of the chronic liver disease etiology who were not receiving DAA therapy were more likely to present lymphadenopathy and distant metastases at the point of diagnosis in comparison to patients undergoing the therapy. In contrast, while analyzing only HCV-infected patients, lymphadenopathy and PVT were present at the point of diagnosis significantly more often among patients receiving DAA compared to the patients who were not receiving the treatement. In the available literature there are other studies where similar results were obtained. The researchers from Egypt observed lymphadenopathy and PVT more frequently among patients treated with DAA compared to the patients undergoing other therapy regimens [31]. Moreover, other studies suggest that in DAA-treated patients HCC reaches more advanced stages and its behavior may be more aggressive [32,33]. There are also studies which imply that there are no significant differences in the course of HCC among patients who were and were not treated with DAA [34]. In the studies which report more aggressive course of HCC after DAA therapy, the possible reason may be different qualification criteria for the therapy. During the first years of DAA regimens in Poland not every HCV-infected individual could receive this type of treatment. To undergo the therapy the patient needed to have a history of unsuccessful IFN-based treatment or the minimum of stage 2 fibrosis, with the priority for patients with liver cirrhosis [35]. With the increase in the availability of DAA in Poland, the criteria for the treatment became less rigorous and now it is possible for every patient with chronic HCV infection to receive DAA treatment [36]. Our study included patients hospitalized between 2015 and 2021, therefore for some of them the more restricted criteria for DAA regimen therapy were applied.

5. Conclusions

HCC is prevalent among patients with underlying liver cirrhosis, regardless of the cause of the disease. In our

findings, HCC occurring in patients treated with DAA was more often associated with lymphadenopathy, multiple tumors, PVT and distant metastases. The reason for that was probably restricted qualification criteria for the treatment, especially advanced liver cirrhosis. However, DAA treatment led to a decrease in the inflammatory activity in the liver. Therefore it is important to adjust qualification protocols so that all patients suffering from chronic HCV infection could receive the treatment as soon as possible.

Ethics approval and registration: Ethical approval for this study was obtained from Ethics Committee of the Medical University of Warsaw, Poland (AKBE/118/2021). The study was registered on clinicaltrials.gov with the number NCT05376943.

Data availability: The full study protocol is available upon request.

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