Cholangiocellular carcinoma occurrence after HCV eradication therapy: case series and review of the literature

Razvan Cerban1,2*, Adina Croitoru1, Gabriel Becheanu4, Speranta Iacob1,2, Carmen Ester1,2, Mihaela Ghioca3, Mugur Grasu1,4, Radu Dumitru1, Carmen Preda1,2, Madalina Florescu3, Liliana Gheorghe1,2

Author Affiliations
1. Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
2. Center for Digestive Disease and Liver Transplantation, Fundeni Clinical Institute, Bucharest, Romania
3. Department of Oncology, Fundeni Clinical Institute, Bucharest, Romania
4. Pathology Department, Fundeni Clinical Institute, Bucharest, Romania
5. Radiology Department, Fundeni Clinical Institute, Bucharest, Romania
6. Department of Gastroenterology, Sfânta Maria Clinical Hospital, Bucharest, Romania

*Corresponding Author:
Razvan Cerban,
Faculty of Medicine, Carol Davila University of Medicine and Pharmacy,
Bucharest, Romania.
Center for Digestive Disease and Liver Transplantation, Fundeni Clinical Institute,
Bucharest, Romania.
E-mail: claudiu.cerban@umfcd.ro

ABSTRACT
Hepatitis C viral (HCV) treatment has rapidly advanced with the use of direct-acting antivirals (DAA), and many patients achieve sustained virological response (SVR). Although the risk of liver tumors is greatly reduced, there are still patients who achieve SVR but will progress to hepatocellular carcinoma (HCC). HCV infection is also a known risk for cholangiocellular carcinoma (CLC), although it is considered a relative infrequent liver malignancy. We report a series of five cases of CLC in patients that achieved SVR after HCV treatment with DAA. There were three women and two males with a median age of 62 years (range 49 to 77 years). Four patients had liver cirrhosis at the time of their HCV treatment. The interval from achieving SVR until CLC diagnosis varied, ranging from 4 to 36 months (median=12). Three patients presented with advanced disease and had extrahepatic spread at the time of their diagnosis. One patient had a resectable tumor, with no recurrence 4 years later. In one case, the tumor was initially considered an atypical HCC and was treated by radiofrequency ablation. Three years later, she was diagnosed with a large tumor recurrence that was demonstrated to be a CLC on liver biopsy. The last two patients were older males with HCV compensated cirrhosis diagnosed with CLC more than two years after achieving SVR. Palliative chemotherapy was started in both. Only a handful of CLC cases have been reported in HCV patients after SVR. Clinicians should take into account the possible development of an aggressive CLC.

KEYWORDS: hepatitis virus, cholangiocellular carcinoma, sustained virological response.

INTRODUCTION
Infection with hepatitis C virus (HCV) is a significant risk factor for liver cirrhosis and hepatocellular carcinoma (HCC) development. The objective of antiviral therapy in HCV infection is the persistent elimination of HCV RNA, known as sustained virologic response (SVR), SVR described as "undetectable" RNA at 12 weeks after the end of antiviral treatment, has been demonstrated to reduce rates of liver decompensation and lower the risk of liver malignancy in patients infected with HCV [1].

Until a few years ago, interferon and ribavirin were the basis of HCV antiviral treatment. The antiviral effect induced by interferon is thought to be associated with an increased response of the host immune system, but it has limited efficacy and important side effects.

In the last five years, the treatment of HCV infection has greatly advanced with the introduction of new, more efficient agents named direct-acting antivirals (DAAs) [2]. These drugs target several nonstructural proteins of the virus that disrupt replication and stop the infection. Compared to interferon treatment, these new drugs are well tolerated with few, if any, side effects and can also be given in patients with decompensated cirrhosis.

Some patients who achieved SVR after DAA treatment still develop HCC. Cholangiocellular carcinoma (CLC), on the other hand, is considered a relative infrequent liver malignancy.
Although several papers have concentrated on HCC development after achieving SVR, to our knowledge, there have been only three reports published concerning intrahepatic CLC associated with SVR after DAA treatment [3–5]. We report a series of five cases diagnosed with intrahepatic CLC who had previously achieved SVR with DAs.

**MATERIAL AND METHODS**

All patients diagnosed with CLC after SVR and treated at our hospital, a tertiary referral center, between 2016 and 2021, were included in this analysis. Demographics (age, gender), initial presentation, location and spread of the tumors, tumor characteristics, treatment and outcome variables were collected.

**RESULTS**

Between 2016 and 2021, five patients were diagnosed with CLC after SVR and treated at our institution. There were three women and two males with a median age of 62 years (range 49 to 77 years). Four patients already had liver cirrhosis at the time of their HCV treatment. Two patients had no symptoms and were diagnosed during routine post-treatment follow-up. In two patients, the primary symptom was right upper quadrant pain, and one patient developed jaundice and pruritus. Three patients presented with advanced disease and had extrahepatic spread at the time of their diagnosis.

The first patient is a 43-year-old woman with compensated HCV cirrhosis who was treated for 6 months in 2016 with the combination of ombitasvir/paritaprevir/ritonavir and dasabuvir plus ribavirin, achieving SVR. Five months later, an abdominal MRI performed for HCC screening demonstrated a large, 8.5 cm heterogeneous, partial necrotic liver tumor, satellite nodules and multiple abdominal adenopathies (Figure 1). Alpha-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) were negative. A liver biopsy was performed and showed poorly differentiated adenocarcinoma. Immunohistochemistry staining was positive for cytokeratin (CK) 7, but negative for CK19 (image unavailable). Considering these findings, a diagnosis of well-differentiated CLC was established. The noncancerous liver tissue was demonstrated to have minimal activity with moderate fibrosis (F3). The patient is alive with no recurrence five years later.

The next patient is a 59-year-old woman with an HCV infection since 2014 who was treated with pegylated interferon plus ribavirin without SVR. She was negative for prior HBV infection, denied alcohol abuse and did not smoke. Physical examination showed no specific findings. Prior to initiating DAA treatment, a transient elastography examination (Fibroscan) revealed F2 fibrosis.

She was started on ombitasvir/paritaprevir/ritonavir and dasabuvir plus ribavirin for 3 months in 2016 and achieved SVR. Routine ultrasonography performed 6 months later revealed a 17 mm hypoechoic lesion in segment VI. A computerized tomography (CT) demonstrated a small tumor in segment VI with arterial phase hyper-enhancement and later phase iso-enhancement (Figure 6). On magnetic resonance imaging (MRI), the tumor had low signal intensity on T1 images, in combination with high signal intensity on T2 and on diffusion-weighted images. A dynamic enhanced Gadolinium-ethoxybenzyl-dihydrileneiamine pentaacetic acid (Gd-EOB-DTPA) MRI demonstrated arterial hypervascularity and hypointensity in the hepatobiliary phase (Figure 7). Considering all the clinical and imaging findings, a diagnosis of atypical HCC was established. The patient refused a liver biopsy and was treated by radiofrequency ablation. The patient followed hepatology appointments from 2017 until 2019 and underwent repeated liver imaging with no tumor recurrence.

She missed all appointments in 2020 due to the COVID-19 pandemic and only came in January 2021 when she was diagnosed with a 5 cm liver tumor, several satellite lesions and abdominal adenopathies (Figure 8). A percutaneous liver biopsy was performed with an aspect of CLC (Figure 9), and chemotherapy treatment was started.

The fourth patient is a 65-year-old male treated for HCV compensated cirrhosis with DAA in 2017 with SVR. Three years later, he was admitted for jaundice. On clinical examination, he was jaundiced and had severe pruritus without abdominal pain. Prior medical history consisted of benign prostatic hyperplasia and arterial hypertension; both controlled with therapy. Family history was significant, with two relatives with cancer, the father with colon cancer and the maternal grandmother with gastric cancer.

The patient’s liver function tests showed abnormal results, with increased total bilirubin of 11.9 mg/dL, alkaline phosphatase of 1328 IU/L, and elevated transaminase levels. The CA19-9 was mildly elevated to 63 U/mL. The endoscopy and colonoscopy were negative. He was negative for hepatitis B and had hepatitis C antibodies with undetectable HCV RNA.

Computed tomography (CT) scan revealed a large liver tumor, hilar adenopathies that cause biliary obstruction and multiple metastatic spinal lesions (Figure 10). Percutaneous biliary drainage was performed with successful internalization with endoscopic retrograde cholangiopancreatography (ERCP). A biopsy of the liver lesion established the diagnosis of undifferentiated CLC. Tumor cells were negative for CK-7, CK-20 and hepatocyte antigen and positive only for CK MNF 116 (Figure 11). Palliative treatment with a combination of gemcitabine and cisplatin was started. The patient deteriorated and decided to stop chemotherapy and enroll in hospice care. He died 7 months later.

The last patient, a 78-year-old male with a prior medical history of coronary artery disease and total gastrectomy for gastric cancer, presented with advanced disease and had extrahepatic spread at the time of achieving SVR. The patient followed hepatology appointments from 2017 until 2019 and underwent repeated liver imaging with no tumor recurrence.

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Figure 1. Abdomen MRI revealing a large lesion 8.4×9 cm heterogeneous right hepatic mass with central necrosis and satellite nodules.

Figure 2. A – Hematoxylin and eosin staining of liver biopsy, demonstrating moderately differentiated adenocarcinoma with very poorly organized glandular structures. B – Immunohistochemistry of liver biopsy, demonstrating positive CK7 staining.

Figure 3. CT abdomen revealing a 3 cm lesion in the left liver lobe A – arterial phase; B – venous phase; C – portal phase.
Figure 4. Macroscopically, a single whitish nodular lesion, measuring 4×3.5 cm, with regular margin and capsule.

Figure 5. Hematoxylin and eosin staining, tumor cells, with the formation of small and irregularly tortuous glands in the tumor tissues. The noncancerous hepatic tissue was classified as having minimal activity with moderate fibrosis (F3).
lymphoma and adjuvant chemotherapy, presented for HCV treatment in 2016. He was previously treated with pegylated interferon plus ribavirin but did not achieve SVR. He was negative for hepatitis B surface antigen and positive for HBV core antibody and HBV surface antibody, with undetectable HBV DNA. Before starting the DAA treatment, transient elastography (FibroScan) revealed F4 fibrosis. The patient was treated with sofosbuvir/ledipasvir for 12 weeks, achieving SVR. He was followed up regularly and underwent imaging studies every 6 months. Approximately 2 years after the completion of treatment, he was evaluated for HCC surveillance and discovered to have an elevated CA 19-9 (700 UI/mL), negative AFP and liver function tests within normal limits. He had performed a CT scan 6 months prior that showed no liver tumors. He underwent an abdominal and pelvis CT (Figure 12) which showed multiple heterogeneous small liver masses. A CT scan-guided liver mass biopsy was performed and demonstrated adenocarcinoma with immunohistochemical staining positive for CK7 and negative for CDX2 and CK20. Histologic pictures are not available. Diagnosis of intrahepatic CLC was established based on radiology.
studies, histology, and immunohistochemical staining. The patient started palliative chemotherapy with gemcitabine and cisplatin. Patients’ data are summarised in Table 1.

**DISCUSSION**

Cholangiocellular carcinoma (CHC) is the second most frequent primary malignant liver tumor after hepatocellular carcinoma (HCC), with a variable incidence between 5% and 25%. It can be classified into two groups, intrahepatic CLC and extrahepatic CLC [6].

Cholangiocellular carcinoma was initially characterized by Steiner and Higginson in 1959. Its origin was considered to be the canals of Hering, which are located between the interlobular ducts and bile canaliculus [7].

Globally, intrahepatic CLC is known to have a wide incidence variability and geographically distinct risk factors. Europe and North America have historically been considered low incidence areas for CLC, but the number of cases has steadily risen in the last decades [8].

Besides widely recognized risk factors (primary sclerosing cholangitis, hepatolithiasis and liver fluke infestation) that promote CLC development, different studies have assessed the relationship between viral hepatitis infections and this type of tumor. HBV and HCV infections and heavy alcohol consumption are known risk factors for HCC, which is a fast-rising cause of cancer-related death worldwide [9]. While their roles as risk factors in CLC development are not clearly established, in recent years, several studies have linked HCV infection and liver cirrhosis with the development of CLC [10, 11].

In a Japanese prospective cohort study in patients with cirrhosis due to HCV infection, 2.3% developed intrahepatic CLC during a mean follow-up period of 7.2 years. The same authors demonstrated that patients with HCV cirrhosis had a 1,000 times higher risk of developing intrahepatic CLC than the general population [12]. A meta-analysis of HCV patients who developed intrahepatic CLC demonstrated a significant association between HCV infection and the incidence of intrahepatic CLC [13]. According to one study, in infected patients with either HBV or HCV, the incidence rate of HCC to intrahepatic CLC is 13.7 to 1 [14]. HCV infection associated with intrahepatic CLC may still be underrated compared to the association with HCC [13]. On the contrary, there are other reports in which more than half of intrahepatic CLC patients had no risk factors associated with chronic liver disease [15, 16].

Because the exact mechanism of CLC development remains largely unclear, different carcinogenic processes in patients with chronic HCV infection have been suggested. It has been demonstrated that the hepatitis C virus can cause injury to cholangiocytes,
Figure 10. CT demonstrating large liver tumor, hilar adenopathies that cause biliary obstruction and multiple metastatic spinal lesions A – arterial phase; V – venous phase; P – Portal phase.

Figure 11. A – Hematoxylin and eosin staining of liver biopsy, demonstrating anaplastic adenocarcinoma; B – Immunohistochemistry of liver biopsy, demonstrating positive for CK MNF 116.

Figure 12. CT scan showing multiple heterogeneous small liver masses A – arterial phase; V – venous phase; P – Portal phase.
leading to a range of inflammatory, proliferative, and degenerative damage. Similar to other pathological conditions that increase the risk for CLC development, long-standing chronic inflammation and regenerative hyperplasia of the bile duct epithelium can induce malignant transformation.

Until recent years, HCV infection treatment associated with pegylated interferon plus ribavirin has been challenging, with significant side effects for patients and low rates of SVR.

A finer understanding of the viral replication mechanism has led to the developing of new treatment agents called direct-acting antivirals (DAAs) that are effective against nonstructural viral proteins. DAAs are now extensively used in clinical practice to treat patients with chronic HCV infection in all stages, from hepatitis to decompensated cirrhosis, with very high SVR rates [17]. The safety of DAA treatments has been demonstrated in clinical trials, with only minor side effects like headache or nausea being reported. Considering their efficacy and tolerability, these drugs have been widely adopted, although long-term outcomes or possible complications are not fully known.

After introducing DAAs in clinical practice, some initial reports showed an apparent increase in de novo rates of HCC in treated patients with SVR. Some authors also linked DAA treatment to an early tumor recurrence after loco-regional therapy in HCC patients [18]. Multiple, mostly retrospective case series comparing HCC recurrence rates to older records in patients with pegylated interferon therapy and also meta-analyses investigating these issues were published. Currently, larger multicenter studies have demonstrated no increased risk of HCC development after DAA therapy [19, 20]. Earlier reports may be explained by the fact that more patients with advanced chronic HCV disease received antiviral than in the past, which increases the general risk for HCC development without any relation to a specific treatment regimen. Until now we have found only three published case reports regarding the occurrence of intrahepatic CLC after DAA treatment [3–5].

We consider that our report is especially significant because none of our patients was known with prior liver malignancy, which demonstrates that these are de novo tumors.

Interestingly, all five cases were intrahepatic CLC, which comprises only 8% of total cholangiocarcinomas [21]. In three cases, the tumors were identified on examinations performed for HCC surveillance. The time interval between the end of the antiviral treatment and the detection of malignancy extended from four to 36 months. In our series, two patients already had distant metastasis when the intrahepatic CLC diagnosis was established, which suggests a very aggressive course.

We consider that five cases of intrahepatic CLC among HCV-treated patients in one center in two years are significant since three cases were diagnosed more than a year after successful DAA treatment.

On ultrasonography, intrahepatic CLC usually appears as a heterogeneous hypoechoic mass with an imprecise border and without bile duct dilatation. On MRI, CLC usually shows low intensity on T1 images and high intensity on T2 and diffusion-weighted images, but in some cases, it can also have hyper-enhancement of the periphery or the entire lesion in the early phase and hyper-iso-enhancement in the delayed phase. Additionally, intrahepatic CLC, in most cases, demonstrates a defect in the hepatobiliary phase on Gd-EOB-DTPA-enhanced MRI [22]. In our paper, four patients had imaging findings similar to previously described features of intrahepatic CLC. In patient number three the initial imaging diagnosis was not correct. The tumor initially diagnosed as an atypical HCC was, in fact, histologically, a small intrahepatic CLC.

Slowly growing CLC, over several years, have been described in a small number of case reports [23]. In the first two patients, we cannot clearly determine that the intrahepatic CLC had already existed at the time of SVR achievement. However, ultrasonography did not show any suspicious lesion at least 3 months prior to the discovery of the tumor on MRI. This emphasizes that hepatic tumors should be excluded through constant imaging evaluations, even in younger patients achieving SVR.

Histopathological examination of the first three cases consisted mainly of narrow bile duct structures composed of atypical cells, with immunohistochemistry showing tumor cells positive for CK7 and CK19 and negative for hepatocytes markers. The fourth case was an anaplastic carcinoma considered a poorly differentiated cholangiocarcinoma.

Surgical resection is the recommended treatment method but is contraindicated in cases with multiple tumors or if the extrahepatic spread is present [24]. Only one patient in our series was suitable for resection after the initial diagnosis. In our hospital, the dissection of the hilar lymph nodes is done for preoperative diagnosed CLC in cases where there is a suspicion of metastatic involvement. In our case, lymph node dissection was not done since there was no suspicion of being metastatic on either imaging or intraoperatively. Adjuvant chemotherapy could be considered in the future regarding the possibility of regional metastases.

Table 1. Clinical data of patients diagnosed with CLC.

| Age (yr)/gender | Treatment for HCV | Liver disease | Symptoms and signs | Time after SVR (months) | Liver lesions | Metastases | Treatment after diagnosis | Survival |
|----------------|------------------|--------------|-------------------|-----------------------|--------------|-----------|--------------------------|----------|
| Case 1         | 3D+ Riba        | Cirrhosis    | RUQ pain          | 6                     | Multiple     | Peritoneal | Hospice care             | Alive 12 months   |
| Case 2         | 3D+ Riba        | Cirrhosis    | None              | 12                    | None         | None      | Surgery                  | Alive 4 years    |
| Case 3         | Sofosbuvir/ledipasvir | Cirrhosis    | RUQ pain          | 4                     | 1            | None      | RFA Gemcitabine/cisplatin | Dead after 7 months |
| Case 4         | 3D+ Riba        | Cirrhosis    | Jaundice Pruritus | 36                    | 1            | Bone      | Gemcitabine/cisplatin    | Alive 6 months    |
| Case 5         | Sofosbuvir/ledipasvir | Cirrhosis    | Hepatomegaly      | 12                    | Multiple     | Bone      | Gemcitabine/cisplatin    | Alive 12 months   |

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lymph node recurrence. The patient is currently examined by MRI and tumor markers every 6 months. Because there are no signs of recurrence, for the moment, we do not take into account any additional treatments.

Intrahepatic CLC has a poor prognosis in HCV carriers, as previously demonstrated [25]. In our series, all patients except one had a very poor outcome, with a survival of less than a year.

Currently, treating the infection is the simple part of HCV care, as DAA treatment is safe and can cure almost all patients in 8 to 12 weeks. Treating the complications of cirrhosis and especially liver tumors over time can be more problematic. Although there is still no clear explanation for tumor development in patients with SVR after DAA treatment, one theory states that an abrupt reduction of the viral load can cause a reduced intrahepatic immune activation and diminish tumor surveillance [26]. Taking this into account, it can be assumed that an abrupt elimination of HCV can cause the disappearance of the apoptotic effect induced by viral proteins in the host cells. This fact can lead to the unrestricted proliferation of damaged hepatic cells.

An in vitro study that investigated the effect of sofosbuvir and daclatasvir on HCC and CLC-derived cell lines suggested the possible occurrence of off-target effects that can modulate cell proliferation, invasion capability and gene expression [27]. Off-target effects can induce phenotypic changes associated with concomitant variations of gene expression that induce the reduction of malignancy-risk demonstrated in most patients but might also explain some rare instances of particularly aggressive tumors after DAA treatment.

As a recent consensus paper clearly demonstrated that liver cirrhosis and HCV infection are both important risk factors for intrahepatic CLC [8], we think that our cases might be explained by the advanced stage of the liver disease at the time of the anti-viral treatment rather than an effect of the DAA treatment itself.

To summarise, we are far from a complete understanding of all the effects of DAA treatment in patients with advanced liver disease and we think this should be investigated in the future. We encourage other clinicians that treat patients with hepatic malignancies after HCV eradication to report similar experiences. We consider that tumor surveillance in all cirrhotic patients who achieve SVR after DAA treatment should be continued even in cases with fibrosis regression. Research on the link between HCV cirrhosis treated with DAA and CLC still remains ill-defined and deserves attention.

CONCLUSION

Intrahepatic cholangiocellular carcinoma seems to be an aggressive malignancy associated with HCV infection. Cirrhotic patients can still develop cholangiocellular carcinoma years after achieving SVR, even in cases with fibrosis regression. Further research on the impact of SVR on all HCV-associated malignancies is needed.

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Conflict of interest

The authors declare no conflict of interest.

Consent to participate

The written and oral consent of all the patients was obtained before the documentation of the cases.

Authorship
RC, LG, and AC contributed to conceptualizing RC and AC contributed to the methodology. RC contributed to writing the original draft. RC, RD, and MG contributed to editing the manuscript. MG, CE, contributed to data curation.

REFERENCES
1. Westbrook RH, Duheko G. Natural history of hepatitis C, J Hepatol. 2014 Nov;61(1 Suppl):S30-68. doi: 10.1016/j.jhep.2014.07.012.
2. Manuc D, Preda CM, Sandra L, Bica C, et al. Significance of Serum Alpha-Fetoprotein Levels in Cases of Compensated Cirrhosis and Hepatitis C Virus without Hepatocellular Carcinoma. J Med Life. 2020 Jan-Mar;13(1):68-74. doi: 10.5521/jml.2019.6076.
3. Osawa M, Saitoh S, Fujiyama S, Kawamura Y, et al. Cholangiocellular Carcinoma in a Young Patient Who Showed Sustained Virological Response after Treatment for Hepatitis C Virus Infection. Intern Med. 2017 Nov 15;56(22):3033-3040. doi: 10.2169/internalmedicine.9021-17.
4. Vakiti A, Cho MH, Lee W, Liang J, et al. Use of direct-acting antivirals for hepatitis C viral infection and association with intrahepatic cholangiocarcinoma: Is there a linkage? J Oncol Pharm Pract. 2019 Oct;25(7):1743-1748. doi: 10.1177/1077552719805908. 12443753:aid-jonp201924920.3.0.co;2-4.
5. Banaz J, Marin JJG, Lamarrce A, Rodriguez PM, et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. Nat Rev Gastroenterol Hepatol. 2020 Sep;17(9):537-548. doi: 10.1038/s41575-020-03160-z.
6. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011 Sep 22;365(12):1116-27. doi: 10.1056/NEJMra1001683.
7. Matsunoto K, Onyamya T, Katsava S, Takeda Y, et al. Hepatitis B and C virus infection is a risk factor for the development of cholangiocarcinoma. Intern Med. 2014;53(7):651-4. doi: 10.2169/internalmedicine.53.1410.
8. Zhou Y, Zhao Y, Li B, Huang J, et al. Hepatitis viruses infection and risk of intrahepatic cholangiocarcinoma: evidence from a meta-analysis. BMC Cancer. 2012 Jul 16;12:289. doi: 10.1186/1471-2407-12-289.
9. Kobayashi M, Ikeda K, Saitoh S, Suzuki F, et al. Incidence of primary cholangiocellular carcinoma of the liver in Japanese patients with hepatitis C virus-related cirrhosis. Cancer. 2000 Jan 1;98(1):2471-7. doi: 10.1002/1097-0142(20000101)98:1<2471::aid-cncr7>3.0.co;2-4.
10. Li H, Hu B, Zhou ZQ, Guan J, et al. Hepatitis C virus infection and the risk of intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma: evidence from a systematic review and meta-analysis of 15 case-control studies. World J Surg Oncol. 2015 Apr 23;13:161. doi: 10.1186/s12957-015-0583-9.
11. Lee CH, Chang CJ, Lin YJ, Yeh CN, et al. Viral hepatitis-associated intrahepatic cholangiocarcinoma shares common disease processes with hepatocellular carcinoma. Br J Cancer. 2009 Jun 2;100(11):1765-70. doi: 10.1038/sj.bjc.6605063.
12. Zhou YM, Yiu ZF, Yang JM, Li B, et al. Risk factors for intrahepatic cholangiocarcinoma: a case-control study in China. World J Gastroenterol. 2008 Jan 28;14(3):403-5. doi: 10.3748/wjg.e1.4.302.
13. Lee TY, Lee SS, Jung SW, Jeon SH, et al. Hepatitis B virus infection and intrahepatic cholangiocarcinoma in Korea: a case-control study. Ann J Gastroenterol. 2000 Jul;105(7):1176-20. doi: 10.1111/j.1572-0840.2000.01796.x.
14. Preda CM, Popescu CP, Baicus C, Voicu EA, et al. Alanine aminotransferase activity in HBV carriers with hepatitis C virus infection. Liver Int. 2018 Apr;38(4):602-610. doi: 10.1111/liv.13550.
15. Reig M, Boix L, Martínez J, Torres F, et al. Liver Cancer Emergence Associated with Antiviral Treatment: An Immune Surveillance Failure? Semin Liver Dis. 2013 Oct;33(4):385-99. doi: 10.1055/s-0034-1375175.
16. Carrat F, Fontaine H, Dorival C, Simony M, et al. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. Lancet. 2019 Apr 6;393(10179):1453-1464. doi: 10.1016/S0140-6736(18)32211-1.
17. Singal AG, Rich NE, Mehta N, Branch A, et al. Direct-Acting Antiviral Therapy Not Associated With Recurrence of Hepatocellular Carcinoma in a Multicenter North American Cohort Study. Gastroenterology. 2019 May;156(5):e1683-e1692. doi: 10.1053/j.gastro.2019.01.027.
18. Blecchaz R, Kamuta M, Roskams T, Goros GJ. Clinical diagnosis and staging of cholangiocarcinoma. Nat Rev Gastroenterol Hepatol. 2011 Aug 2;8(9):513-22. doi: 10.1038/nrgastro.2011.131.
22. Asayama Y, Tajima T, Okamoto D, Nishie A, et al. Imaging of cholangiolocellular carcinoma of the liver. Eur J Radiol. 2010 Jul;75(1):e120-5. doi: 10.1016/j.ejrad.2009.09.010.

23. Koga Y, Nagahama H, Tateyama M, Fukuhaya K, et al. A case of cholangiolocellular carcinoma combined with intrahepatic cholangiocarcinoma diagnosed after 4 years follow-up for hepatic hemangioma. Nihon Shokakibyo Gakkai Zasshi. 2012 Feb;109(2):231-9.

24. Blechacz B. Cholangiocarcinoma: Current Knowledge and New Developments. Gut Liver. 2017 Jan 15;11(1):13-26. doi: 10.5009/gnl15568.

25. Wang Z, Sheng YY, Dong QZ, Qin LX. Hepatitis B virus and hepatitis C virus play different prognostic roles in intrahepatic cholangiocarcinoma: A meta-analysis. World J Gastroenterol. 2016 Mar 14;22(10):3038-51. doi: 10.3748/wjg.v22.i10.3038.

26. Nault JC, Colombo M. Hepatocellular carcinoma and direct acting antiviral treatments: Controversy after the revolution. J Hepatol. 2016 Oct;65(4):663-665. doi: 10.1016/j.jhep.2016.07.004.

27. Giovannini C, Foriari F, Infilo V, Terré D, et al. Direct Antiviral Treatments for Hepatitis C Virus Have Off-Target Effects of Oncologic Relevance in Hepatocellular Carcinoma. Cancers (Basel). 2020 Sep 19;12(9):2674. doi: 10.3390/cancers12092674.