



**Review Article**

**A Concise Review on the Frequency, Major Risk Factors and Surveillance of Hepatocellular Carcinoma (HCC) in  $\beta$ -Thalassemias: Past, Present and Future Perspectives and the ICET-A Experience**

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**Abstract.** Due to the recent alarming increase in the incidence of hepatocellular carcinoma (HCC) in thalassemias, the present report reviews briefly the frequency, the major risk factors, and the surveillance of HCC in  $\beta$ -thalassemias. Over the past 33 years, 153 cases of HCC were reported in patients with thalassemia, mainly in Italy and Greece. Among HCV-infected patients, additional factors promoting the development of HCC included: advanced age, male sex, chronic hepatitis B (CHB) co-infection, and iron overload. For early diagnosis of HCC, sequential ultrasound screening is recommended especially for thalassemia patients with chronic hepatitis C (CHC), which coincides with (one or more) additional risk factors for HCC. Here we report also the preliminary data from thalassemic patients, above the age of 30 years, followed in 13 ICET-A centers. The total number of enrolled patients was 1,327 (males: 624 and 703 females). The prevalence of HCC in thalassemia major patients [characterized by transfusion-dependency (TDT)] and thalassemia intermedia [characterized by nontransfusion dependency (NTDT)] was

**1.66 % and 1.96 % , respectively. The lowest age at diagnosis of HCC was 36 years for TDT and 47 years for NTDT patients. We hope that this review can be used to develop more refined and prospective analyses of HCC magnitude and risk in patients with thalassemia and to define specific international guidelines to support clinicians for early diagnosis and treatment of HCC in thalassemic patients.**

**Keywords:** Hepatocellular carcinoma; Thalassemias; Risk factors; Surveillance.

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**Introduction.** Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, representing approximately 90% of all cases of primary liver cancer occurring usually in the setting of chronic liver disease and cirrhosis. Chronic hepatitis C (CHC), chronic hepatitis B (CHB) and hereditary hemochromatosis may directly lead to HCC, while HCC related to other underlying liver diseases is linked to the development of cirrhosis.<sup>1</sup> The global age distribution of HCC varies by region, sex, and aetiology. Rates of liver cancer among persons of the same ethnicity may vary by geographic location.<sup>2-5</sup>

Environmental and acquired individual factors, such as excessive alcohol consumption, fatty liver disease, non-alcoholic steatohepatitis, cigarette smoking, occupational exposure to chemicals such as pesticides, and contamination of foodstuff with aflatoxins (a group of mycotoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*), can increase the risk of developing liver cancer.<sup>1</sup>

HCC incidence rises after the age of 45 to 50 and becomes almost consistently high after 65 years.<sup>3</sup> HCC is rare among adolescents and accounts for less than 1% of all malignant neoplasms in this age group.<sup>4</sup> The male-to-female ratio in HCC incidence is lower than 2 below 25 years, increases from 25 to 29 years, and peaks at the age of 50-54 years up to 5.40 (95% CI: 5.02, 5.82).<sup>5</sup>

The guidelines for the management of HCC suggested by the American Association for the Study of Liver (AASLD) and the European Association for the Study of Liver (EASL), recommend the implementation of surveillance by a combination of abdominal ultrasound (US), computed tomography (CT) and/or magnetic resonance imaging (MRI). These can improve the prognosis thanks to the detection of smaller tumors.<sup>6,7</sup> Guidelines for HCC in thalassemias are lacking.

The treatment of HCC requires a multidisciplinary approach involving hepatologists, surgeons, oncologists, radiologists, and other disciplines. Multiple options for treatment are available, depending on the tumor stage, patient's performance status and liver function reserve; they include radiofrequency or microwave ablation, liver resection, or transplantation. For patients who are not candidates of curative treatments, locoregional therapies such as trans-arterial chemoembolization (TACE), trans-arterial radioembolization (TARE), and stereotactic body radiation (SBRT) can improve survival and quality of life. Liver transplantation is another radical therapy for selected patients with HCC.<sup>8</sup>

Sorafenib, a multi-kinase vascular endothelial growth factor (VEGF) inhibitor, is the most widely used systemic chemotherapy approved as a first-line agent for unresectable or advanced HCC.<sup>8</sup>

However, patients with HCC are often diagnosed in advanced stages, and even after complete HCC tumor resection or ablation, the carcinogenic tissue microenvironment in the remnant liver can give rise to recurrent de novo HCC tumors, which progress into incurable, advanced-stage disease in the majority of patients.<sup>9</sup>

The recent alarming increase in the incidence of HCC in thalassemias, prompted us to overview the most common risk factors involved in the development of HCC, to briefly describe the frequency and the clinical and diagnostic characteristics, and to discuss the recommendations for HCC surveillance in high-risk patients with  $\beta$ -thalassemias. The preliminary data of the International Network of Clinicians for Endocrinopathies in Thalassemia and Adolescent Medicine (ICET-A) on HCC survey in thalassemia major (transfusion-dependent thalassemia:TDT) and intermedia patients (transfusion-dependent thalassemia: NTDT) are also presented.

**Frequency of HCC in the General Population and in Thalassemias.** The global distribution of HCC varies by region, gender, and aetiology. The disease burden is highest in areas with endemic HBV infection (where HBsAg prevalence is 8% or more), such as in sub-Saharan Africa and Eastern Asia, with an HCC incidence rate of over 20 per 100,000 individuals. In Egypt, hepatocellular carcinoma (HCC) is the second most common cancer in men and the 6th most common cancer in women. Italy, Spain, and Greece have intermediate incidence rates of 10-20 per 100,000 individuals, while North and South America have a relatively low incidence (< 5 per 100,000 individuals).<sup>10</sup>

Kourakli et al.<sup>11</sup> analyzed all cases of malignant neoplastic disorders, occurring in 3,652 Greek thalassemic patients, diagnosed between 1985 and 2018. A total of 165 cases of malignant disorders were identified, (84 males and 81 females) with a median age at diagnosis of 45 years (range 9-73 years). The dominant malignancy was hepatocellular carcinoma, diagnosed in 63 patients, followed by thyroid cancer (17 cases), non-Hodgkin's lymphoma (13 cases), and renal cell carcinoma (10 cases). There was a strong positive association between HCV infection and hepatocellular carcinoma.

The literature review showed that HCC in  $\beta$ -thalassemias had been reported in Italy (85 cases), UK (2 cases), Lebanon (2 cases), and Iran (1 case), in addition to Greece (63 cases).<sup>12-23</sup>

In a prospective study, based on ultrasound screening of 105 Italian thalassemic patients, over 18 years of age, Mancuso et al.<sup>16</sup> found a 2% incidence of HCC during a 1-year observation period. Risk factors were present in seventy-two patients: iron overload in 72, HCV infection in 46, HBV infection in 2 and cirrhosis in 10 patients.

Ten years later, Borgna-Pignatti et al. performed a survey of patients followed in 55 Italian centers. The authors collected data from 4,248 patients with thalassemia major and 1,607 patients with thalassemia intermedia. Since their last report,<sup>14</sup> 62 new cases have been identified, of whom 52% with thalassemia major and 45% with thalassemia intermedia and 2 (3%) with compound sickle cell / $\beta$ -thalassaemia. The cumulative incidence reported at diagnosis of HCC was 1.02% and 1.74%, respectively.<sup>22</sup> The mean age at diagnosis of HCC in patients with TDT was 48 years (33–56 years) and in patients with thalassemia intermedia 47 years (36–72 years); 37 patients (60%) were males and 25 (40%) females.<sup>22</sup>

**The ICET-A Experience.** In June 2019, to ascertain the frequency of HCC in patients with thalassemias, the Coordinator (VDS) of ICET-A invited 13 centers of the network to take part in a study and collected information on all patients they followed for HCC. An

*ad hoc* questionnaire, prepared by VDS in accordance with the Declaration of Helsinki (<http://www.wma.net>), was distributed by mail to participating centers. The deadline for sending the requested data was 2 months. The exclusion criteria were: patients with sickle cell disease, and patients already included in other previous publications.

Considering that the youngest patient reported in the literature was 36 years old, we included in the study, only the patients with  $\beta$ -thalassemia above the age of 30 years with  $\beta$ -thalassemias, followed in the participating centers. In detail, the required data were: date of birth, type of haemoglobinopathy, serology for HBV, HCV, detection of HCV-RNA, levels of serum ferritin at diagnosis and chelation therapy, the presence of obesity, alcohol abuse, smoking, and associated clinical complications were also included. In addition, symptoms at onset and clinical course of patients with HCC were reported. Liver iron concentration, measured by magnetic resonance imaging (MRI), was also included.

The demographic details of TDT and NTDT patients, above the age of 30 years, who developed HCC in 13 thalassemia centers from 10 different countries, are presented in **table 1**.

The total number of enrolled patients with TDT and NTDT was 1,327 (males: 624 and 703 females).

17 patients (mean age: 44.3 years) diagnosed with HCC had TDT (11 males and 6 females), and 6 patients (mean age: 56.8 years) had NTDT (5 males and 1 female). The total prevalence of HCC in this subgroup of patients (> 30 year) was 1.66 % for TDT group and 1.96 % for NTDT. The lowest age at diagnosis of HCC in TDT patients was 36 years and 47 years in NTDT patients, and the highest was 59 and 65, respectively.

The mean age in the group of patients with HCC was higher compared to the patients' group without HCC (TDT and NTDT: 41.0 and 45.7 years vs. 35.5 and 39.2 years, respectively).

Full data was available in 15 out of 23 patients with thalassemias and HCC (**Tables 2-4**). The presence of obesity, alcohol abuse, or smoking, was absent or very uncommon. The value of  $\alpha$ FP at diagnosis was raised (> 10 ng/mL) in 9 out of 12 patients for whom it was available; 8 out of 14 patients (57.1%) were clinically symptomatic (**Tables 2-4**). None was positive for HBV surface antigen (HBsAg), while one patient showed evidence of past HBV infection, being HBsAg negative but positive for the antibodies against the HBV core antigen (HBcAb). Interestingly, all Italian patients diagnosed with HCC had genotype 1b. At the time of survey 6 out of 23 patients with thalassemias are alive.

*Comments.* In the general population, the risk for HCC is known to be age dependent; HCC is rarely seen during the first 4 decades of life, except in populations where HBV infection is hyperendemic. The age range

**Table 1.** Demographic details of TDT and NTDT patients with hepatocellular carcinoma (HCC), above the age of 30 years, in 13 thalassemia centers from 10 different countries.

Country	Number of patients with TDT, mean age and sex (Males/Females)	Number of patients with NTDT, mean age and sex (Males/Females)	Total number of patients with TDT and NTDT (Males/Females)	Number of patients diagnosed with HCC and age at diagnosis (Males/Females)	Prevalence Subtotal and Total in Males and Females with HCC
<b>Bulgaria</b>	17 Mean age: 37 yr. (M:7/F:10)	4 Mean age: 39 yr. (M:0/F:4)	21 (M:7/F:14)	none	<b>None</b>
<b>Cyprus</b>	215 Mean age: 46 yr. (M:104/F:111)	29 Mean age: 53 yr. (M:13/F:16)	244 (M:117/F:127)	<b>5 males</b> NTDT:47,50,60, 63 and 65 years <b>1 female</b> TDT:37 years	<b>Subtotal: 2.4%</b> <b>Males: 4.2%</b> <b>Females: 0.7%</b>
<b>Greece</b>	296 Mean age: 39.3 yr. (M:147/F:149)	70 Mean age: 42 yr. (M:33/F:37)	366 (M:180/F:186)	<b>4 males</b> TDT: 39,43,46, and 52 years <b>4 females</b> TDT: 43, 45 and 46 years NTDT: 56 years	<b>Subtotal: 2.1%</b> <b>Males: 2.2%</b> <b>Females 2.1%</b>
<b>India</b>	12 Mean age: 35.5 yr. (M:7/F: 5)	2 Mean age: 45.5 yr. (M:1/F:1)	14 (M:8/F:6)	none	<b>None</b>
<b>Iran</b>	192 Mean age: 36.1 yr. (M:79/F:113)	100 Mean age: 36.1 yr. (M:46/F:54)	292 (M:125/F:167)	none	<b>None</b>
<b>Italy</b>	177 Mean age: 44.2 yr. (M:81/F:96)	60 Mean age:49.5 yr. (M:31/F:29)	237 (M:112/F:125)	<b>5 males</b> TDT:38,41,42, 52 and 59 years <b>2 females</b> TDT: 44 and 49 years	<b>Subtotal: 2.9%</b> <b>Males: 4.4%</b> <b>Females: 1.6 %</b>
<b>Kingdom of Saudi Arabia</b>	30 Mean age: 36 yr. (M:21/F:9)	3 Mean age: 44 yr. (M:2/F:1)	33 (M:23/F:9)	none	<b>None</b>
<b>Oman</b>	51 Mean age: 34.5 yr. (M:19/F:32)	18 Mean age: 38.3 yr. (M:6/F:12)	69 (M:25/F:44)	<b>2 males</b> TDT:36 and 42 years	<b>Subtotal: 2.8%</b> <b>Males: 8%</b> <b>Females: 0 %</b>
<b>Qatar</b>	14 Mean age: 32 yr. (M:6/F:8)	6 Mean age:31 yr. (M:4/F:2)	20 (M:10/F:10)	none	<b>None</b>
<b>Turkey</b>	18 Mean age: 36.8 yr. (M:11/F:7)	13 Mean age: 41 yr. (M:6/F:7)	31 (M:17/F:14)	none	<b>None</b>
<b>Total</b>	<b>1022</b> <b>(M:482/F:540)</b>	<b>305</b> <b>(M:143/F:163)</b>	<b>1327</b> <b>(M:624/F:703)</b>	<b>Total: TDT</b> (M:11/ F:6) <b>Total: NTDT</b> (M:5/F:1)	<b>Total: 1.73%</b> <b>Males: 2.5%</b> <b>Females: 0.9%</b>

at diagnosis of HCC was 63–65 years in Europe and North America, with rare occurrence before the age of 40 years.<sup>3-5</sup>

In our patients with TDT, the mean age at diagnosis of HCC was 44.3 years, younger than that reported in patients without thalassaemia, suggesting the concomitance of other risk factors, namely hemosiderosis, implicated in the development of HCC. Similar results were also reported in 63 Greek thalassemia patients with HCC (median age: 45 years).<sup>11</sup>

Comparing our data with the results of a previous publication,<sup>22</sup> it emerges that our subgroup of NTDT patients with HCC were older (56.8 vs.47 years). However, our series consisted of 6 NTDT patients vs.

28 reported in the multicentre Italian study.<sup>22</sup>

The distribution of HCC varies significantly according to geographic location, and it is more common in middle and low income countries than in developed ones.

It is also interesting to note that no case of HCC in patients with thalassaemias was reported in Bulgaria, India, Kingdom of Saudi Arabia, Qatar, (total number of patients:105 patients), and in a large group of patients (292 patients; 125 males and 167 females) followed in a single center in Iran, although the prevalence of HCV Ab and HCV RNA positivity reported in a previous study was 42.8% and 29.9%, respectively.<sup>24</sup> However, the mean age of patients followed in these centers was lower compared to

**Table 2.** Summary of clinical, laboratory and diagnostic data in thalassemic patients with hepatocellular carcinoma (HCC) in Italy.

Variables	Italy	Italy	Italy	Italy	Italy
Sex	Male	Male	Male	Male	Female
TDT / NTDT	TDT	TDT	TDT	TDT	TDT
Serological markers of hepatitis B	HBsAb pos HBsAg neg HBcAb neg	HBsAg neg HBcAb pos	HBsAb pos HBsAg neg HBcAb neg	HBsAb pos HBcAb pos HBsAg neg HBcAb neg	HBsAb pos HBcAb pos HBsAg neg HBcAb neg
HCV RNA positivity (yes/no) Genotype	Yes Unknown	Yes 1b	Yes 1b	Yes 1b	Yes 1b
Treatment of hepatitis: INF- RBV or DAAs regimen	No	DAAs	Peg-IFN	NA	IFN $\alpha$
Responder to treatment (yes/no) SVR (yes/no)	- -	Yes Yes	Yes No	- -	No .
Obesity (yes/no) Alcohol abuse (yes/no) Smoking (yes/no)	No No No	No No No	No No No	No No No	No No No
$\alpha$ FP level (ng/ml) at diagnosis of HCC	NA	4.1	19.8	15	18
Symptoms at diagnosis of HCC	Absent	Absent	Absent	Absent	Abdominal pain, weight loss
Liver Cancer (BCLC) grading system classification	B	A	A	C	B
Chelation therapy (drug) LIC value (mg/Fe g/dw) SF at the diagnosis of HCC (ng/ml)	DFO NA 808	DFX 9 240	DFX 5 186	DFX 9 2600	DFO 13 2086
<b>Complications:</b> Cirrhosis: yes/no Diabetes: yes/no Hypothyroidism: yes/no Hypogonadism: yes/no Hypoparathyroidism: yes/no	Yes No Yes Yes- NoT Yes	Yes No No Yes - HRT No	Yes No No Yes - NoT No	No No Yes -HRT No No	Yes Yes No SA-HRT No

**Abbreviations:** Transfusion-Dependent Thalassemia (TDT); Nontransfusion Dependent Thalassemia (NTDT); Interferon (INF); ribavirin (RBV); Direct Acting Antivirals (DAAs); Sustained Virological Response (SVR); Not Available (NA); serum  $\alpha$ -fetoprotein ( $\alpha$ FP); Liver Cancer (BCLC) grading system classification: very early or early stage (0-A), intermediate stage (B) and advanced or terminal stage (C-D); Liver Iron Concentration (LIC); Serum ferritin (SF); No Treatment (NoT); Hormone Replacement Therapy (HRT); Secondary Amenorrhea (SA); desferrioxamine (DFO); deferasirox (DFX).

patients with HCC followed in Cyprus, Greece, Italy, and Oman.

In this group of patients with HCC, obesity, NAFLD (assessed by liver ultrasound), or alcoholic abuse was not reported, and NIDDM was diagnosed only in a male TDT patient from Oman (**Tables 2-4**).

Globally, the rate of males with HCC was higher than of females. This is probably due to two main reasons: (a) the effects of estrogen may suppress the inflammatory process mediated by interleukin-6 (IL-6) in women, reducing hepatic injury and the compensatory proliferation of hepatocytes,<sup>25,26</sup> and (b) the effects of testosterone may increase signalling androgen receptor in men, promoting proliferation of hepatocytes.<sup>27-29</sup>

However, no HCCs have been reported up to now in association with currently used transdermal, subcutaneous, or intramuscular testosterone (T) formulations in hypogonadal thalassemic patients.<sup>30,31</sup> Nevertheless, because of the virtual "hepatotoxicity" of T preparations, monitoring of liver function is necessary.

On the other hand, the presence of hypogonadism in female thalassemic patients could be a potential

additional risk factor of HCC. This hypothesis is supported by a pooled analysis of data from 11 prospective US studies.<sup>32</sup> The authors found that oophorectomy was a significant risk factor for HCC.<sup>32</sup>

However, the role of estrogen-progesterone replacement therapy (HRT) in hypogonadal patients remains controversial. The present evidence suggests both a carcinogenic and protective effect of HRT on liver function.<sup>32-34</sup> Further studies on the indications, duration, and safety of different HRT formulations used in hypogonadal females with thalassemia are required to solve this intriguing puzzle.

In summary, the pivotal role for the evaluation of risk factors of HCC in patients with thalassemias seems to be advanced age, male sex, viral liver infections, and prolonged iron overload.

Further studies on the indications, duration, and safety of different HRT formulations used in hypogonadal patients with thalassemias are required.

Finally, HCC seems to be more common in patients with TDT than NTDT. One possible explanation is that patients with NTDT have a milder progression of iron overloading and a lower incidence of chronic viral liver infections.

**Table 3.** Summary of clinical, laboratory and diagnostic data in thalassemic patients with hepatocellular carcinoma (HCC) in Italy, Oman and Greece.

Variables	Italy	Italy	Oman	Oman	Greece
Sex	Female	Male	Male	Male	Female
TDT / NTDT	TDT	TDT	TDT	TDT	TDT
Serological markers of hepatitis B	HBsAb pos HBsAg neg HBcAb neg	HBsAb pos HBsAg neg HBcAb neg	HBsAb pos HBsAg neg HBcAb neg	HBsAb pos HBsAg neg HBcAb neg	HBsAb pos HBsAg neg HBcAb neg
HCV RNA positivity (yes/no) Genotype	Yes 1b	Yes 1b	Yes 2b	Yes 1a	Yes 1b
Treatment of hepatitis: INF- RBV or DAAs regimen	NA	Peg- IFN+RBV	NA	Peg-IFN + RBV	DAAs
Responder to treatment (yes/no) SVR (yes/no)	NA	Yes Yes	NA	Yes Yes	Yes NA
Obesity (yes/no) Alcohol abuse (yes/no) Smoking (yes/no)	No No No	No No No	No No Moderate	No No No	NA NA NA
$\alpha$ FP level (ng/ml) at diagnosis of HCC	32	34	NA	NA	NA
Symptoms at diagnosis of HCC	None; Liver US showed multiple nodules	None; Liver US showed multiple nodules	Abdominal pain and distension. Liver US showed multiple nodules	Abdominal pain and distension. Liver US showed multiple nodules	NA
Liver Cancer (BCLC) grading system classification	A	B	C	C	NA
Chelation therapy (drug) LIC value (mg/Fe g/dw) SF at the diagnosis of HCC (ng/ml)	DFO - 1850	DFO 3.2 1670	DFO-DFP-DFX >20 4574	DFP 1 279	DFO 3.2 1098
<b>Complications:</b> Cirrhosis: yes/no Diabetes: yes/no Hypothyroidism: yes/no Hypogonadism: yes/no Hypoparathyroidism: yes/no	No No No SA No	No No No Yes -HRT No	Yes (mild) No No Yes-HRT Yes	Yes NIDDM No Yes Reversed No	NA Yes NA No No

**Abbreviations:** Transfusion-Dependent Thalassemia (TDT); Nontransfusion Dependent Thalassemia (NTDT); Interferon (INF); ribavirin (RBV); Direct Acting Antivirals (DAAs); Sustained Virological Response (SVR); Not Available (NA); serum  $\alpha$ -fetoprotein ( $\alpha$ FP); Liver Cancer (BCLC) grading system classification: very early or early stage (0-A), intermediate stage (B) and advanced or terminal stage (C-D); Liver Ultrasound (US); Liver Iron Concentration (LIC); Serum ferritin (SF); No Treatment (NoT); Hormone Replacement Therapy (HRT); Secondary Amenorrhea (SA); Non-Insulin Dependent Diabetes Mellitus (NIDDM); desferrioxamine (DFO); deferiprone (DFP); deferasirox (DFX).

### Major Risk Factors in Thalassemic Patients with HCC.

**HCV and HBV.** In patients with thalassemias, the predominant role of chronic infection with HBV, and mainly with HCV, in the etiology of HCC is well documented.<sup>12,14,17</sup>

A prospective study to evaluate the incidence and aetiology of transfusion-related hepatitis was reported by Lai et al.<sup>35</sup> Seventy-three of 135 thalassemic patients (62.2%) acquired HCV. An extended follow-up (22 to 30 yr) with HCV RNA assessment was available in 52 patients. Of them, 23 (44.2%) cleared the virus. Fibrosis progression was similar in HCV RNA-positive and HCV RNA-negative patients. Liver iron was the only factor associated with fibrosis.

In another series of 233 HCV positive thalassemic patients, 30% were RNA negative. In these patients, from 2 to 20 years after HCV infection, liver fibrosis was minimal, if any. (Kattamis C et al. unpublished data).

Generally, chronic hepatitis C (CHC) is a slowly

progressive disease characterized by persistent hepatic inflammation, leading to the development of cirrhosis in approximately 10-20% of patients over 20-30 years of HCV infection. Overall, once cirrhosis has developed, there is a 1-5% annual risk of HCC and a 3-6% annual risk of hepatic decompensation. The clinical and histopathological disease progress is influenced by several factors, mainly the level of HCV viremia, the virus genotype, the duration of infection, the age at infection, the sex, and the co-existence of active HBV.<sup>36,37</sup>

HCV is classified into 6 major genotypes. Some genotypes have a restricted geographical distribution (genotypes 4-6), while others (genotypes 1-3) are more broadly disseminated. Genotype 1 (subtypes 1a and 1b) is the most prevalent genotype in the world. Genotype 2 is found in clusters in the Mediterranean region; genotype 3 is most prevalent among intravenous drug users and genotype 4 is found mostly in Egypt, while genotypes 5 and 6 are less frequent.<sup>38</sup> The HCV genotypes strongly affect the response to antiviral

**Table 4.** Summary of clinical, laboratory and diagnostic data in thalassemic patients with hepatocellular carcinoma (HCC) in Cyprus.

Variables	Cyprus	Cyprus	Cyprus	Cyprus	Cyprus
Sex	Male	Male	Female	Male	Male
TDT / NTDT	NTDT	NTDT	TDT	NTDT	NTDT
Serological markers of hepatitis B	negative	negative	negative	negative	negative
HCV RNA positivity (yes/no) Genotype	negative	negative	negative	negative	negative
Treatment of hepatitis: IFN -RBV or DAAs regimen	-	-	-	-	-
Responder to treatment (yes/no) SVR (yes/no)	-	-	-	-	-
Obesity (yes/no)	No	No	Yes	No	No
Alcohol abuse (yes/no)	No	No	No	Yes	No
Smoking (yes/no)	No	No	No	Yes	No
$\alpha$ FP level (ng/ml) at diagnosis of HCC	196	82	NA	300	44
Symptoms at diagnosis of HCC	Abdominal pain	Weight loss and cachexia	Abdominal pain and weight loss	Abdominal pain and jaundice	Fever and shortness of breath
Liver Cancer (BCLC) grading system classification	NA	NA	NA	B	B
Chelation therapy (drug)	DFP	DFO-DFP	DFO	DFO-DFP	DFO
LIC value (mg/Fe g/dw)	1.65	10.12	NA	4.28	2.15
SF at the diagnosis of HCC (ng/ml)	215	2855	NA	2982	4126
<b>Complications:</b>					
Chronic hepatitis: yes/no	No	No	No	No	No
Cirrhosis: yes/no	Yes	No	No	Yes	No
Diabetes: yes/no	No	Yes	Yes	No	No
Hypothyroidism: yes/no	No	No	Yes	Yes	Yes
Hypogonadism: yes/no	No	No	Yes- NA	Yes - HRT	No
Hypoparathyroidism:yes/no	No	No	No	No	No

**Abbreviations:** Transfusion-Dependent Thalassemia (TDT); Nontransfusion Dependent Thalassemia (NTDT); Interferon (INF); ribavirin (RBV); Direct Acting Antivirals (DAAs); Sustained Virological Response (SVR); Not Available (NA); serum  $\alpha$ -fetoprotein ( $\alpha$ FP); Liver Cancer (BCLC) grading system classification: very early or early stage (0-A), intermediate stage (B) and advanced or terminal stage (C-D); Liver Iron Concentration (LIC); Serum ferritin (SF); No Treatment (NoT); Hormone Replacement Therapy (HRT); Secondary Amenorrhea (SA); desferrioxamine (DFO); deferiprone (DFP).

treatment.

HCV infection is the main risk factor for liver fibrosis in TDT.<sup>39</sup> An accelerated hepatic fibrosis was observed by Kamal et al. in patients with thalassemia and was attributed to the cumulative effect of HCV induced liver injury and the increased iron overload, particularly in inadequately chelated patients.<sup>40</sup>

Efficient chelation therapy may prevent the development of liver fibrosis in thalassemic patients with CHC.<sup>41</sup> Hepatic siderosis and fibrosis were assessed in 99 TDT patients using transient elastography (TE) and liver iron concentration (LIC) at baseline and after 4 years. At baseline, the overall mean liver stiffness measurement (LSM) was  $7.4 \pm 3.2$  kPa, and the mean LIC, assessed by T2\*MRI, was  $4.81 \pm 3.82$  mg/Fe g/dw. At  $4 \pm 1.5$  years, a significant reduction in LSM ( $6.6 \pm 3.2$  kPa; p: 0.017) and LIC ( $3.65 \pm 3.45$  mg/Fe g/dw; p: 0.001) was observed.<sup>42</sup>

*Comments.* Due to frequent blood transfusion treatment, many patients with thalassemias were infected with HCV, HBV, or both. This applies mostly to patients in their 30s or older, as the risk of viral transmission through blood transfusion was minimized after the

1990s by implementation of blood donor screening in developed and developing countries.

Preventive measures include early immunisation against the hepatitis B virus and prevention of iron accumulation by intensive use of iron chelation therapy. In non-immune thalassemia patients, the implementation of HBV vaccination started in the early 80s. The development of a vaccine for HCV remains an important goal for global control and eradication of infection.

Globally, chronic hepatitis C progresses to liver fibrosis in 60%-70% of patients, cirrhosis in 10%-20%, and eventually HCC in 1%-5% within two decades of harbouring the virus. The ability of HCV to promote cirrhosis is 10- to 20-fold higher than HBV.<sup>3,6-8</sup>

Therefore, patients in the high-risk category should be offered antiviral therapy, as well as appropriate HCC surveillance. Effective suppression of HBV and HCV replication by antiviral therapy can reduce the risk of HCC development. However, antiviral therapy does not eliminate the HCC risk because of the presence of virus integrated into the host genome.

Interferon-based regimens (Peg-IFN) have been the mainstay of anti-HCV therapy, yielding HCV cure, or sustained virologic response (SVR), in approximately

50% of patients, followed by pegylated interferon and ribavirin (Peg-IFN/RBV) for 24 weeks or 48 weeks, the use of which was limited by adverse effects.<sup>43</sup> The host factors influencing response to treatment include: genetics, particularly interleukin (IL)-28B polymorphisms, race, obesity, insulin resistance, severity of hepatic fibrosis, and the related viral genotype and viral load at initiation of therapy.<sup>44</sup>

The recently developed direct-acting antivirals (DAAs), directly targeting the viral protease, polymerase, or non-structural proteins, enabled interferon-free anti-HCV therapies with a revolutionary improvement of SVR rate, approaching or surpassing 90%.<sup>45,46</sup>

The host factors influencing response to treatment include genetics, particularly interleukin (IL)-28B polymorphisms, race, obesity, insulin resistance, the severity of hepatic fibrosis, and the related viral genotype and viral load at initiation of therapy.<sup>46</sup>

The first generation of DAAs, including boceprevir (BOC) and telaprevir (TVR), was introduced in 2011 and added to the previous regimen of pegylated interferon/RBV (Peg IFN/RBV). In October 2014, the FDA approved the use of ledipasvir (LDV) in combination with sofosbuvir (SOF) for the treatment of chronic HCV genotype 1 hepatitis, which had an efficacy of more than 95%. Both drugs are NS3/4A protease inhibitors and were used in combination with peg-interferons and ribavirin to avoid the emergence of resistant variants. These agents improved SVR rates but did not improve the side-effect profile.<sup>47</sup>

Thus, the use of triple therapy with ombitasvir/paritaprevir/dasabuvir, for the treatment of HCV genotype 1 hepatitis, was introduced in December 2014. In the next two years, several other regimes as DAA - sofosbuvir in combination with ledipasvir (NS5A inhibitor), and combination of ombitasvir (NS5A inhibitor), paritaprevir (NS3/4A protease inhibitor), ritonavir (CYP3A inhibitor) and dasabuvir (non-nucleoside NS5B polymerase inhibitor), and daclatasvir (NS5A inhibitor) were introduced. The latter was approved for the treatment of HCV genotype 3 infection in combination with sofosbuvir.<sup>47</sup>

In January 2016, the FDA approved the combination treatment of grazoprevir/elbasvir (GZR/EBR) with a 95% SVR.<sup>47</sup> for naïve patients or those who failed treatment with genotype 1 or 4, with or without cirrhosis who failed treatment. The approval of sofosbuvir and velpatasvir (NS5A inhibitor) as a fixed dose combination initiated the third generation of DAAs. However, access to therapies remains limited due to the high costs.<sup>47</sup>

The most extensive observational study on DAAs, in patients with hemoglobinopathies and HCV infection, published to date has been reported by Origa et al.<sup>48</sup>

The majority of patients had TDT (114 patients, 82%), 7 patients (5%) had sickle-cell thalassemia, 3 patients (2%) NTDT and 1 patient (0.7%) sickle-cell anemia. The most common HCV genotype was 1b (91, 65.5%). DAA therapy included different basic and in combination treatment regimens. The mean level of serum ferritin was 1,450 ng/mL (range: 103-11,190 ng/mL) and liver iron overload was observed in 51 (37%) patients. 136 patients (97.8%) achieved a response at the end of treatment, and 130 (93.5%) achieved an SVR. Treatment regimens were well tolerated, and no major adverse events or drug-drug interactions were observed. Treatment with DAAs was also associated with a significant reduction of liver enzymes and serum ferritin.<sup>48</sup>

Supportive evidence for the use of DAAs was reported in 61 thalassemic patients (age range: 40–48 years), including some who previously did not respond to antiviral treatment with pegylated IFN and RBV or/and with advanced liver disease due to CHC. The choice of treatment regimen was mainly based on HCV genotype according to published guidelines and also on the chronological availability of HCV agents. The treatment was highly effective (SVR: 90%) and safe.<sup>49</sup>

In another study, the SVR rate to DAAs in patients with chronic HCV and thalassemia was 82%, while in chronic HCV patients without thalassemia it was higher (94.7%), but not statistically significant.<sup>40</sup> Furthermore, SVR rates were significantly higher in patients who were treated with DAAs compared with PEG-IFN based therapy.<sup>40</sup> Early diagnosis and treatment with DAAs were recommended to prevent cirrhosis and HCC, which are relevant causes of mortality in this patients population.<sup>40</sup>

*Iron Overload in TDT and NTDT.* The current treatment of TDT consists of regular transfusions to maintain hemoglobin (Hb) levels of 9 to 10 g/dL. Chronic transfusions lead to iron overload and, then, eventual multiorgan damage if not conveniently treated. Therefore, iron chelation therapy is mandatory to remove iron in excess. Regular subcutaneous (SC) desferrioxamine mesylate (DFO) infusion for the treatment of iron overload was started in 1978 in patients older than 2 years, and since 1995 and 2007, two oral chelators, deferiprone (DFP) and deferasirox (DFX) were respectively available and used.

Iron overload is known to cause cancer in animal models,<sup>50</sup> and conversely, iron depletion has been reported to suppress tumor growth.<sup>51</sup>

The first mechanism by which free iron is believed to trigger malignant transformation is the generation of reactive oxygen species (ROS), which causes peroxidation of membrane fatty acids and subsequent formation of toxic by-products that impair protein synthesis and disrupt DNA, leading to mutations in tumor suppressor genes (such as p53) and DNA repair

genes.<sup>52,53</sup>

Iron overload may also promote malignant transformation in the liver through the acceleration of fibrosis to cirrhosis by activation of stellate cells and the profibrogenic effects of lipid peroxidation.<sup>54,55</sup>

It also has been suggested that iron overload induces immunologic aberrancies, which may contribute to cancer formation. Growing evidence highlights the negative effect of iron and ferritin on the tumoricidal function of macrophages in mice,<sup>56</sup> in which it was specifically demonstrated that iron overload decreased antibody-mediated and mitogen-stimulated phagocytosis by monocytes and macrophages.<sup>57</sup>

Patients with genetic hemochromatosis were found to be 23 times more likely to have liver cancer than healthy people, with an annual incidence rate of HCC-related cirrhosis of 3%–4%.<sup>58</sup>

Furthermore, a number of case reports and case series have described the development of HCC in patients with thalassemia who were negative for hepatitis B and C testing but had significant hepatic iron overload.<sup>17,21-23</sup>

One NTDT male patient, anti-HCV and HbsAg negative and never transfused, with a previous histological diagnosis of cirrhosis related to secondary hepatic iron overload developed HCC. He was diagnosed at the age of 34 years and was chelated first with deferoxamine and later with deferiprone. Before the diagnosis of HCC, at the age of 73 years, the serum ferritin level was 222 ng/mL.<sup>17</sup> Similar findings were reported by Borgna Pignatti et al.<sup>22</sup> and Maakaron et al.<sup>21,23</sup> in patients with negative serology for HBV and HCV infection.

In a Greek study, HCC was reported in 3 out of 9 NTDT patients with liver fibrosis and siderosis due to the late introduction of iron chelation therapy, and negative history for viral hepatitis.<sup>18</sup> Notably, the incidence of HCC was higher in NTDT compared to TDT (p: 0.032). These findings suggest that the duration of exposure to toxic iron levels may be more important for the development of HCC than the amount of liver iron content.

*Comments.* Due to frequent and regular blood transfusions, many patients with thalassemia are infected with either HCV or HBV. This occurrence applies mostly to patients in their 30s or older because the risk of viral transmission through blood transfusion was minimized after the 1990s (even annihilated) through blood donor screening.

Preliminary data seem to indicate that NTDT patients might be at a higher risk for developing HCC than TDT patients.<sup>17,20,22</sup> One possible explanation is that patients with NTDT have much longer survival compared to TDT patients, which enables them to live long enough to develop HCC. The state of chronic

anemia and hypoxia in NTDT patients, resulting from ineffective erythropoiesis and hemolysis, leads to the expansion of the erythroid marrow and extramedullary hematopoiesis. The chronic ineffective erythropoiesis also triggers increased intestinal iron absorption and deposition in the liver and endocrine glands despite the lack of transfusional iron.<sup>59</sup> Therefore, close surveillance of iron overload via non-invasive quantification of LIC with R2 or T2\* MRI is recommended.

### **Pathophysiology Past and Recent Issues.**

*Transfusion-Associated Immunomodulation.* Increasing evidence suggests that blood transfusions, in addition to iron overload, by inducing chronic antigenic stimulation, have adverse effects on the recipient's immune system, which is an essential mechanism for antiviral immunity and anticancer immune surveillance.<sup>54,55,58</sup> However, to date, the precise role of transfusion-related immunomodulation in the development of HCC in thalassemia has not yet been elucidated, and needs further studies.<sup>60</sup>

*Host genetics factors associated with HCC.* Genetic variability has been discussed as a risk factor for the development of HCC since many patients exposed to known environmental risk factors never develop cirrhosis or HCC.<sup>61</sup> Furthermore, family clustering and incidence differences among different ancestry groups suggest that inherited genetic factors may contribute to HCC risk.<sup>62</sup>

From a search of 1,668 publications, Walker et al.<sup>63</sup> identified 166 relevant studies evaluating the associations of HCC with cirrhosis or fibrosis in HCV-infected patients in 137 different genes. *IFNL3/4*, *TNF- $\alpha$* , and *PNPLA3* genes had the most probable evidence of association.

In summary, host genetics could add discriminatory value to risk prediction tools, allowing better stratification and personalized assessment of optimal long-term management, thereby increasing the efficacy of surveillance programmes.<sup>63</sup>

*Insulin resistance.* Chronic hepatitis C is associated with an increased risk of diabetes mellitus (DM) or insulin resistance (IR).<sup>64,65</sup> IR is associated more frequent in patients with chronic hepatitis C with hepatic steatosis, advanced fibrosis, and HCC.<sup>64</sup> IR may induce the release of free fatty acids (FFA) towards hepatocytes and may cause oxidative stress through the overproduction of ROS, cellular inflammation, and carcinogenesis.

Disturbances of glucose homeostasis, ranging from mild glucose intolerance to overt diabetes mellitus, and hyperinsulinism were reported in young adult patients with thalassemia and have been attributed to iron overload, HCV infection, anemia, and chronic liver

disease.<sup>66,67</sup>

An acute effect of blood transfusion on insulin sensitivity and  $\beta$ -cell function in patients with thalassemia has been reported by Wankanit et al.<sup>68</sup>

*Alcohol and Tobacco.* Alcohol and iron are known prooxidants, and oxidative stress is known to play an essential role in the development of several diseases, including cancer. The metabolism of alcohol, especially through CYP2E1, can lead to the generation of superoxide and hydrogen peroxide. Moreover, hydrogen peroxide can react with ferrous iron ( $\text{Fe}^{2+}$ ) through the Fenton reaction, and generate highly reactive hydroxyl radicals.<sup>69</sup>

Hydroxyl radicals can react with lipid molecules, initiating chain reactions that lead to lipid peroxidation and generation of products, such as acrolein, crotonaldehyde, MDA and 4-HNE; the latter is known to cause mutations of *p53* gene (a tumor suppressor gene), which may initiate the development of HCC.<sup>70</sup>

Tobacco exposure is also a risk factor for HCC. Tobacco smoking is associated with increased plasma levels of inflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$ <sup>71,72</sup> and markers of oxidative stress.<sup>72,73</sup> These mediators can contribute to necro-inflammatory changes in the liver, which in turn may promote the development of HCC.<sup>74</sup>

In brief, prolonged exposure to alcohol and tobacco is expected to promote the development of HCC in an additive and/or synergistic manner. Tobacco smoking may contribute to the initiation and promotion of HCC due to the presence of mutagenic and carcinogenic compounds as well as by promoting oxidative stress via the generation of ROS and depletion of endogenous antioxidants.

Therefore, thalassemic patients should be discouraged from alcohol consumption and tobacco exposure, regardless of the severity of their disease.

*Impact of direct-acting antiviral agents in treated patients.* Several retrospective uncontrolled studies in 2016 reported an increased incidence of *de novo* HCC among patients treated with DAA for HCV infection.<sup>75-77</sup> In a multicentre study of 58 patients in Spain and a single center study of 59 patients in Italy, occurrence or recurrences of HCC after curative therapies in DAA treated patients appeared unusually high (28% and 29%, respectively).<sup>75-77</sup> Although the mechanism underlying this unexpected early HCC recurrence is unknown, it has been reported that HCV eradication by DAA therapy might enhance HCC development or recurrence in patients who have elevated risk for HCC.<sup>78</sup>

In contrast, some other retrospective and prospective cohort studies suggested no significant difference in liver cancer development following DAA therapy.<sup>78-80</sup>

In conclusion, the risk for HCC development is modulated by host features such as gender (male), advanced age, metabolic syndrome, and the severity of the underlying liver disease. The risk for HCC is substantially reduced in patients with CHC who achieve virologic cure, but the risk is not eliminated, and patients should continue surveillance for HCC. Before attempting any interpretation of the discrepancies between the above mentioned DAA reports, long term studies are needed to elucidate this controversial issue.

*Vitamin D deficiency.* To date, clinical studies have demonstrated that vitamin D deficiency is common, not only in patients with thalassemias,<sup>81</sup> but also in those with HCC. The potential role of vitamin D in HCC has been recently reviewed by Wu et al.<sup>82</sup> and Chiang et al.<sup>83</sup>

Vitamin D is involved in cell proliferation, apoptosis, differentiation, inflammation, invasion and metastasis, angiogenesis and micro-RNA modulation. Although the epidemiologic evidence regarding the association of vitamin D and HCC is still inconclusive, biochemical evidence clearly indicates that HCC cells are responsive to the inhibitory effect of vitamin D and its analogs.<sup>83</sup>

*Nonalcoholic fatty liver disease (NAFLD).* Metabolic risk factors commonly associated with NAFLD or NASH, including diabetes mellitus type II, obesity, and metabolic syndrome, are becoming emerging risk factors for HCC. The pathophysiology of hepatic carcinogenesis in patients with NAFLD-NASH has not been completely elucidated, but initial research suggests that excess fatty acid supply and hepatocellular steatosis elicit increased fatty acid oxidation with subsequent enhanced reactive oxidative stress. This process further promotes the release of proinflammatory cytokines, prooncogenic signals, and epigenetic changes.<sup>84</sup> Genetic and environmental factors, as well as the interaction between them, may be responsible for both the individual susceptibility and the clinical course of NAFLD.

**HCC Surveillance.** The increasing incidence of HCC in advanced age thalassemic populations led to the need to identify early patients at risk for HCC through an efficient program of screening. Nowadays, almost 50% of HCC are diagnosed at an advanced stage with a poor prognosis and a 5-year overall survival rate lower than 10%. In contrast, in patients with early diagnosis, the 5-year survival rate increases to 70%.<sup>85</sup>

Surveillance consists of the periodic application of a diagnostic test to subjects at risk for developing a specific disease. In one study, the risk factors for HCC in thalassemias were defined as follows: increased serum ferritin (at least 2 determinations > 1,000 ng/ml)

or increased LIC >4 mgFe/g dry weight or both; HCV-RNA positivity by polymerase chain reaction and anti-HCV positivity; hepatitis B surface antigen (HBsAg) positivity, or histological diagnosis of cirrhosis.<sup>17</sup>

Currently, the surveillance of HCC in  $\beta$ -thalassemias is achieved by analyzing imaging techniques, abdominal US at 6-12 months frequency, combined with serum  $\alpha$ -fetoprotein (AFP) levels.<sup>15-17,22</sup>

The early detection of HCC by the US is highly dependent on the expertise of the operator and the quality of the equipment. Thus, specialized training for ultrasonographers is recommended. The levels of sensitivity and specificity are also dependent on the size of the tumor, with ultrasound able to detect 80%–95% of tumors 3–5 cm in diameter and 60%–80% of tumors < 1 cm in diameter.<sup>86</sup>

Combining ultrasound and AFP appears to improve the detection rates.<sup>87-89</sup> AFP values may be influenced by non-neoplastic factors, such as viral infections and cirrhosis.<sup>90</sup> At diagnosis of HCC, in thalassemic patients, AFP was within the normal range in 20 out of 45 patients (44.4%) for whom it was available. The median value in TDT patients with HCC was 9.8  $\mu$ g/L (range 1,1–2,000  $\mu$ g/L) while in NTDT it was 12.3  $\mu$ g/L (range:1,5–3,000  $\mu$ g/L)<sup>22</sup> while in a previous study AFP, at the time of diagnosis was  $2,103 \pm 2,067$  kU/L (normal value <16 kU/L); it was within normal levels in 2 out of 22 patients (9.0%).<sup>14</sup>

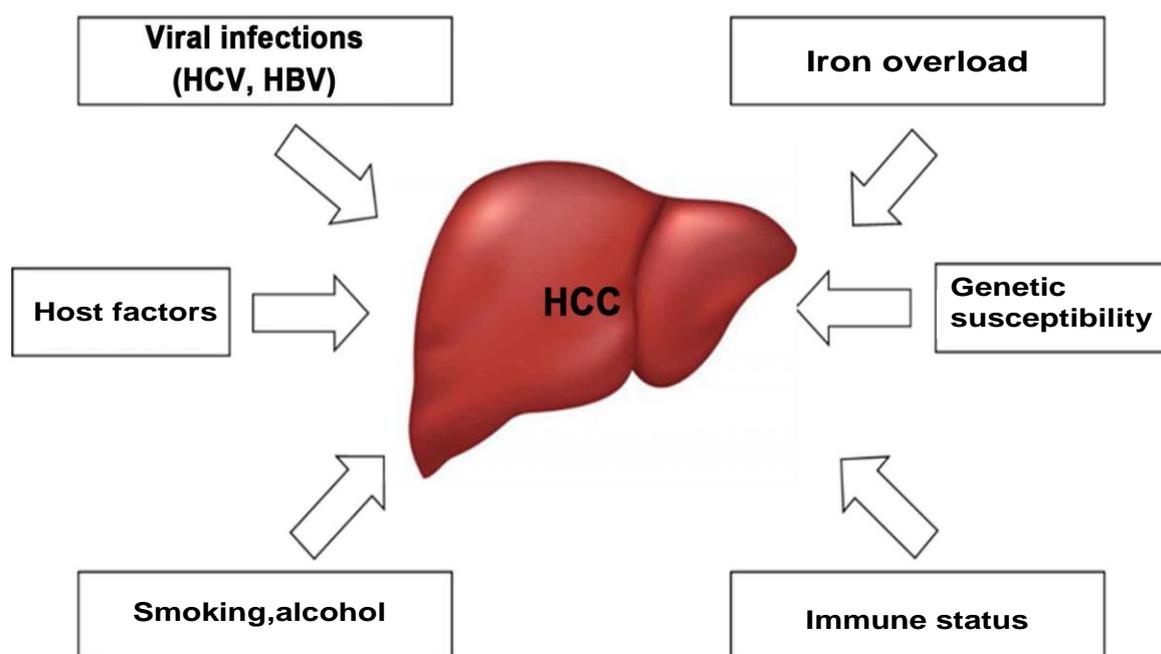
The recent introduction of US contrast-enhanced agents (CEUS) has not proven to increase the ability of the US to detect small HCC tumors.<sup>91</sup> However, CEUS provides an accurate differentiation between benign

and malignant liver nodules, which is critical for adequate management of HCC and is also useful for guidance of local percutaneous therapy of HCC and post procedure monitoring of the therapeutic response.<sup>92</sup>

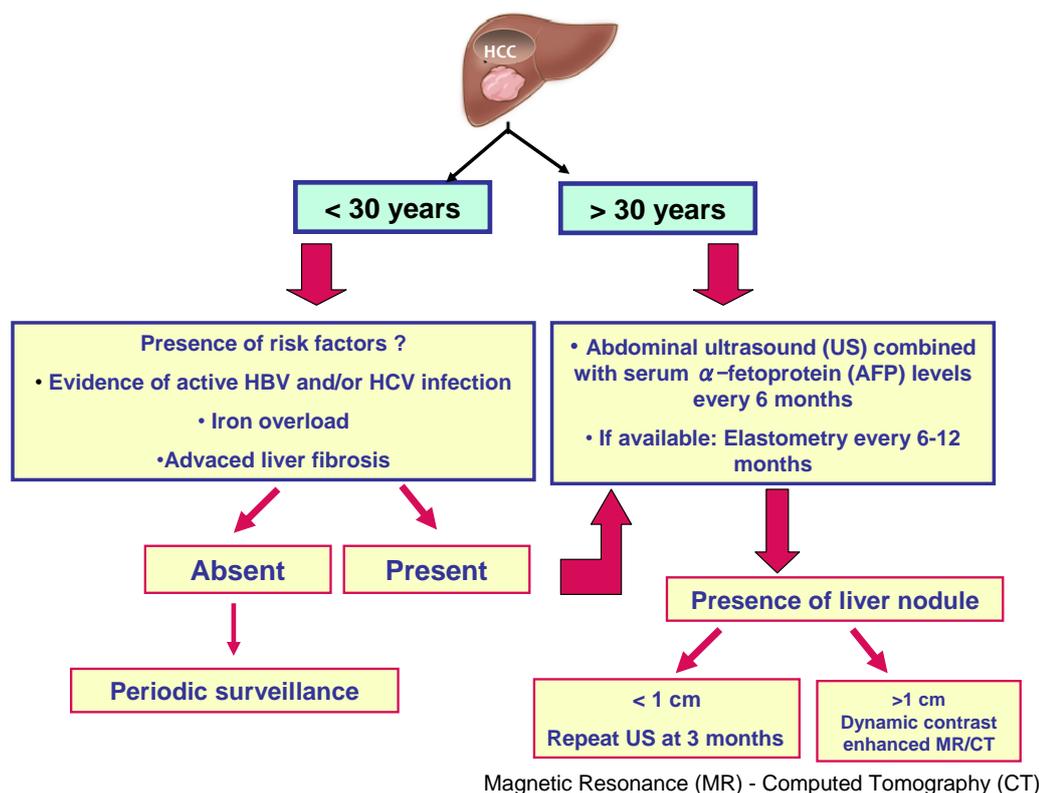
Liver fibrosis is a risk factor for HCC, but at what fibrotic stage, the risk for HCC is increased has been poorly investigated. Nakayama et al. reported that a liver stiffness of more than 12.0 kPa was an independent risk factor for HCC development.<sup>93</sup> Therefore, it could be an additional surveillance factor to be included in the surveillance of patients with chronic liver disease and iron overload.

In conclusion, although the optimal methods of screening and the cost-effectiveness of surveillance for HCC remain to be defined, systematic screening still offers the best hope for early diagnosis, treatment eligibility, and improved survival. Current guidelines advocate the use of abdominal US at 6 months frequency to screen for HCC in high-risk patients because the median doubling time of an HCC lesion is 117 days (range 29 to 398 days).<sup>94,95</sup>

**Conclusions.** A large number of studies have provided evidence that thalassemic patients have a high risk of developing HCC in advanced age. Multicenter international studies for a better evaluation of HCC incidence in different countries are urgently needed. Factors that predispose to HCC, among chronically HCV-infected patients, include advanced age, male sex, viral liver infections, and iron overload (**Figure 1**). However, several aspects remain to be explored further.



**Figure 1.** Risk factors for HCC in thalassemias (From: Sciancalepore et al. J Cancer Ther. 2018; 9:417-437; modified).



**Figure 2.** Flow chart of HCC surveillance in thalassemias in countries with prolonged implementation of HCV screening of donors.

HCC is more frequently diagnosed (at present) in men with TDT in their fourth - fifth decade and older in NTDT<sup>11,22</sup> with long-standing, untreated chronic hepatitis C (76%), irrespective of the content of hepatic iron load, estimated with MRI T2\*.<sup>11</sup> One possible explanation is that patients with NTDT may have a milder process of iron overloading at late age and a lower incidence of chronic viral liver infections, mainly HCV.

HCV is characterized by genetic heterogeneity. Based on the sequence divergence rate, six HCV genotypes and more than 50 subtypes have been identified.<sup>96</sup> The most prevalent HCV subtypes are 1a, 1b, 2a, 2b, and 3a, which are widely distributed globally and account for most HCV infections worldwide.<sup>97</sup> After HCV acute infection an average 50-85% of patients will not clear the virus, and HCV 1b appears to have a high prevalence of HCC.<sup>98</sup>

Interestingly, all 1b HCV genotype-associated neoplasms, reported in the literature, in Greek thalassemic patients were hepatocellular carcinoma (7 cases)<sup>11</sup>: a similar pattern was observed in the Italian patients enrolled in our survey.

The youngest thalassemic patient with HCC was 33 years old. Thus at present, we recommend an abdominal US screening associated with AFP levels estimation in all patients over the age of 30 years with an interval of 6 months. For younger patients there are no specific recommendations.

However, we believe that in these patients,

surveillance should be based on the evidence of active HBV/HCV infections, iron overload, and advanced liver fibrosis (**Figure 2**).

Liver biopsy has been considered the gold standard of staging liver disease in patients chronically infected with HCV.<sup>99</sup> However, due to its invasive nature, sampling error, the potential for adverse events, and high intra- and interobserver variability,<sup>100</sup> noninvasive diagnostic methods have begun to replace the biopsy in many settings.<sup>7</sup>

Transient elastography (TE; e.g., Fibroscan<sup>®</sup>), is an accurate and reproducible method to detect liver fibrosis using ultrasound. It is a promising alternative due to its high accuracy in detecting severe fibrosis and cirrhosis.<sup>101</sup>

According to the TE values, patients are grouped into 3 categories: those with elastography values of  $\leq 7.0$  kPa corresponding to METAVIR stages F0 or F1 (F0: no fibrosis; F1: portal fibrosis without septa); those with elastography values  $> 7\text{kPa} \leq 15\text{kPa}$  who have moderate to severe fibrosis (stages F2 and F3) and are at risk for fibrosis progression and the third group includes patients with high elastography values  $> 15.0$  kPa (METAVIR stage of F4 or some cases of F3) who have a high likelihood of cirrhosis.<sup>102</sup>

Noninvasive, serum biomarker panels, which have also been validated against biopsy, are an attractive alternative for staging patients with chronic HCV infection in low- and middle-income countries.<sup>103</sup>

We hope that this review will stimulate the

development of more refined, prospective analyses of HCC magnitude and of the risk factors in patients with thalassemia. Finally, we believe that specific

international guidelines are urgently needed to support clinicians in the early diagnosis and treatment of thalassaemic patients at risk of HCC development.

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