Liver INTERNATIONAL

Liver International ISSN 1478-3223

REVIEW

Early diagnosis of liver cancer: an appraisal of international recommendations and future perspectives

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Liver Int. 2016; 36: 166–176. DOI: 10.1111/liv.12965

Abstract

All Societies, AASLD, EASL, APASL and JSH, identify patients with cirrhosis as a target population for surveillance, with minor differences for additional categories of patients, such as chronic hepatitis B and hepatitis C patients with advanced fibrosis. According to AASLD, liver disease related to metabolic diseases including diabetes and obesity is a recognized target of screening, since those conditions have been causally related to HCC. All societies endorse radiological non-invasive techniques as the mainstay for early diagnosis of HCC, but discrepancies exist between Societies on the utilization of contrast-enhanced ultrasound and utilization of serum markers for surveillance and diagnosis of HCC. The diagnostic algorithm of the international societies differ substantially in the anatomic paradigm of EASL and APASL which identify 1 cm size as the starting point for radiological diagnosis of HCC compared to APASL algorithm based on the dynamic pattern of contrast imaging, independently on tumour size. While strengthening prediction in individual patients is expected to improve cost-effectiveness ratios of screening, the benefits of pre-treatment patient stratification by clinical, histological and genetic scores remain uncertain and exclusion of patients with severe co-morbidities and advanced age is still debated.

Keywords

cirrhosis – HCC – miRNA – tumour markers

Substantial progresses have been made in the treatment of hepatocellular carcinoma (HCC), yet early diagnosis through surveillance of patients at risk remains the only hope for a cure (1–4). Unfortunately, only a minority of patients with HCC get the end-point of an early diagnosis, owing to the fact that a majority of patients with chronic liver disease who are at risk of HCC remain undetected. Early diagnosis of HCC is further hampered by the complexity of surveillance programs, which involve more than a single test and is carried out in hospital facilities. Yet tests, recall policies and quality control procedures are standardized and bring along with them significant economic consequences. The term of early diagnosis encompasses more than one category of HCC nodule: a very early HCC is a tumour less than 2 cm in size in a patient with a perfectly compensated cirrhosis (Child-Pugh A) which lacks arterial hypervascularization at contrast imaging and thereby requires histological confirmation (*in situ* or Stage 0 HCC). Early HCC is a single 2–5 cm tumour or up to three tumours, each smaller than 3 cm, arising in a patient with Child Pugh A or B cirrhosis (Stage A HCC) (Table 1) (1, 2). Owing to the well-recognized environmental risk factors and the availability of user friendly screening tests, like abdominal ultrasound (US), surveillance has gained popularity even in the absence of robust evidence that it

Abbreviations

AASLD, American association for the study of liver disease; AFP, Alpha-fetoprotein; APASL, Asian Pacific association for the study of the liver; BMI, body mass index; CEUS, contrast-enhanced ultrasonography; DCP, des-gamma-carboxy-prothrombin; EASL, European association for the study of the liver; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; JSH, Japanese society of hepatology; NAFLD, non-alcoholic fatty liver disease; SVR, sustained virological response; US, ultrasound.

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Handling Editor: Francesco Negro

Received 7 August 2015; Accepted 10 September 2015

Key points box

- Comparison among the main Scientific Societies for the study of the liver are provided.
- Target population proposed for surveillance discussed.
- Cost utility, surveillance algorithm and proposal to improve cost-utility of surveillance are summarized.
- Genetic markers to identify high risk screenees are reported.

Table 1. Barcelona-Clinic Liver Cancer (BCLC) classification

BCLC stage	Performance status	Tumour volume, nodule number and invasiveness	Child-Pugh
0 Very early	0	≤2 cm vaguely nodular	A
A Early	0	Single or three nodes <3 cm each	A&B
B Intermediate	0	Large/multinodular	A&B
C Advanced	1–2	Vascular invasion and/or extrahepatic spread	A&B
D End-stage	3–4	Any of the above	C

reduces liver-related mortality (5). The only randomized controlled study supporting screening of patients with chronic liver disease was in fact conducted in the Shanghai area, however, with a number of potential bias in the way the patients were selected and treated, and how the study was conducted (6).

Thus, the real support for liver cancer screening in patients with chronic liver disease comes from the striking differences in response to therapy among screened populations and patients with incidental tumours that have been outlined by numerous retrospective surveys (7, 8). The International Scientific Societies in Europe (EASL), North America (AASLD), Asia Pacific (APASL) and Japan (JHS) have released recommendations for HCC surveillance, however, with some nuances in the target population and recall policies (1–4).

The target population

All Societies identify patients with cirrhosis as the ideal target for surveillance (1–4). AASLD, EASL and JHS identify non-cirrhotic patients with chronic viral hepatitis as candidates for screening, yet with some differences (1, 2, 4) (Table 2). AASLD recommends screening of Asian males older than 40 years of age and Asian females older than 50 years together with all carriers with a family history of HCC and African/North American blacks older than 20 years, since these patients are at a higher risk of liver cancer as a consequence of early exposure to the hepatitis B virus (HBV) (2). While both the Consensus-Based Clinical Practice Manual and JHS Evidence-Based Practice Guidelines define patients with chronic hepatitis B and C and non-viral cirrhosis as high-risk populations for HCC, patients with cirrhosis as a result of HBV or hepatitis C virus (HCV) are identified as being at super high risk of develop liver cancer (4).

HCV infected patients

EASL recommends surveillance for all patients with a clinically active hepatitis C and bridging fibrosis in addition to those with histological or clinical evidence of cirrhosis, given that these are considered preneoplastic conditions (9, 10). However, diagnosis of bridging fibrosis may not be accurate enough with either a percutaneous liver biopsy or non-invasive techniques like fibrotest and transient elastography (TE) (11). Indeed, while a value of Fibroscan >9.5 KPa is suggestive of severe fibrosis and thus indicates patient surveillance (12), TE results may be influenced by several variables including ALT flares, male gender and increased body mass index (BMI) (13–15). In a study evaluating liver stiffness measurement among two hundred and fifty-four consecutive patients compared to liver biopsy of at least 15 mm specimens, 28 patients (11%) had discordances of at least two stages between TE and histological assessment (16). Liver biopsy is also inaccurate in staging fibrosis because of the heterogeneous distribution of tissues scars in the liver and the moderate reproducibility of readings, given that the biopsy specimen has to be at least 25 mm long to accurately evaluate fibrosis with a semiquantitative score (17). Patients with cirrhosis from HBV or HCV who cleared virus spontaneously or responded to treatment, are likely to have a reduced risk of developing HCC. While this risk reduction has been quantified in approximately 75% in patients with hepatitis C, after achievement of sustained virological response (SVR), however, HCC has been reported to occur even years after treatment completion, at a rate between 0.66 and 1.24 per 100 person years or between 0.6% and 2.5% per year (18, 19). By retrospective scrutiny of two large cohort of patients, patient age and hepatitis severity pretreatment have emerged as predictors of liver cancer development in SVR patients with chronic hepatitis C (20, 21), suggesting that treatment of HCV should be anticipated as soon as possible to prevent HCC. HCC developing in SVR patients may be the likely consequence of the carcinogenic effect of the extensive architectural changes within the liver parenchyma prior to treatment or persistence of transformed liver cells (22).

Owing to the preexisting threat of HCC in patients freeded by HCV, surveillance is considered worth in non-cirrhotic patients who achieved a SVR, even though it may not be cost effective. In this population, the yearly incidence of HCC was 0.15% in a cohort of 1751 patients in Japan (23) and 0.68% in a cohort of 556 patients in Taiwan (24). In a cohort of patients in

Table 2. Surveillance for HCC as recommended by AASLD, APASL, JHS and EASL

Strategy	AASLD 2010 (2)	APASL 2010 (3)	JHS 2011 (4)	EASL 2012 (1)
Target population	Cirrhosis, CHB B NAFLD	Viral cirrhosis	Super-high-risk: Viral cirrhosis High-risk: Chronic viral hepatitis Other than viral cirrhosis	Cirrhosis, CHB HCV F3
Screening modality	Abdominal US	Abdominal US + AFP	Abdominal US + AFP/AFP-L3/PIVKA-II	Abdominal US
Optional CT/MRI	No	Yes	Yes	No
Serum markers	No	Yes	Yes	No
Screening intervals, mo.	6	6	Super-high-risk: 3–4 High-risk: 6	6
Radiological Diagnosis	CT, MRI >1 cm Ø	CE-US, CT, MRI Any size	CE-US, CT, MRI Any size	CT, MRI >1 cm Ø (cirrhosis)

US, ultrasound; AFP, alfafetoprotein; AFP-L3, AFP lectin fraction; PIVKA-II, protein induced by vitamin K antagonist-II; NAFLD, non-alcoholic fatty liver disease; CHB, chronic hepatitis B; HCV F3, Chronic hepatitis C with advanced fibrosis (Metavir classification) (9); CT, computed tomography; MRI, magnetic resonance imaging; CE-US, contrast enhancement ultrasound.

Taiwan, age over 60, GGTP greater than 75 U/L (corresponding to the 75 percentile level of population) and advanced fibrosis [grade 2 and 3 by Knodell and Sheuer (25)] were independently associated with a likelihood of HCC, with an yearly incidence ranging from 0.14% in patients with any of the above factors to 2.8% in patients with two factors, excluding fibrosis.

HBV infected patients

HCC is known to occur in HBV patients who are successfully suppressed by antiviral therapy, including both treatment responders with pre-treatment cirrhotic liver disease (26) as well as in those who sero-converted to anti-HBs and stopped the treatment. In Hong Kong, HCC was detected in seven patients of 298 who cleared serum HBsAg during a mean follow-up of 43 months (27), however, only four patients developed anti-HBs antibodies and six had cirrhosis. Given all these data, since surveillance effectiveness cannot be determined with any certainty, HBV and HCV patients who respond to antiviral therapy should continue screening for HCC.

Several studies support the efficacy of nucleoside/nucleotide-analogues (NUC) in the prevention of HBV-related HCC. However, the more consistent published results to date are with the earlier generation of drugs (lamivudine and adefovir). In a retrospective study of 872 Korean patients vs 699 historical controls, the annual incidence of HCC was 4.1% in the controls, 0.95% in the sustained responders to lamivudine, 2.18% in patients with viral breakthrough and 5.26% in those with suboptimal response (28), with circumstantial evidence that lamivudine had no protective effect in patients with decompensated cirrhosis. A recent cohort study from Greece, in which adefovir was given as rescue therapy in 79 of 109 lamivudine-treated patients without virological remission, confirmed the persistence of HCC risk in cirrhotic patients despite a long-term response to lamivudine (29). Less data are available with the third generation entecavir and tenofovir. All three recently validated clinical risk scores that can accurately estimate the risk of HCC up to 10 years, showed that entecavir was able to significantly reduce HCC incidence in patients with a higher risk (REACH B risk score >12, $P < 0.006$; GAG risk score >82, $P < 0.002$; CU risk score >20, $P < 0.001$).

Kim *et al.* (30) evaluated the incidence of HCC based on the REACH-B risk calculator. During this time, 13 cases of HCC were reported, the 10th which occurred at 3.3 years, at which time the REACH-B model predicted 11.2 cases. Furthermore, beyond that time point, there was a progressive divergence between the predicted and observed number of HCC cases showing a progressive decreased incidence of HCC compared to the predicted risk after long-term therapy with tenofovir.

Family history

According to AASLD (2), surveillance should be expanded to subjects with a family history of HCC. However, the large majority of studies on the association between family history and HCC were carried out in East, whereas robust data on this association in the West is lacking (31). In a case–control study in Italy, subjects with a family history of liver cancer were shown to have a one- to three-fold increase in HCC risk (32). A family history score was proposed to identify subjects with high and low/moderate HCC risk among those with a family history of this cancer, depending on the number of first-degree relatives involved, being the highest risk of cancer in males (odds ratio OR, 3.21; 95% confidence interval CI, 1.13–9.10) with affected parents (OR, 6.08; 95% CI, 1.99–18.62), reaching a 70-fold increase risk in subjects with serum markers of chronic viral hepatitis B or C infection. The combination of a family history of liver cancer and serum

markers of viral hepatitis B or C infection, as compared to subjects without a family history and hepatitis.

NAFLD

Based on several case reports and cohort studies, patients with non alcoholic fatty liver disease (NAFLD) and non alcoholic steato-hepatitis (NASH) are considered to be at risk of developing liver cancer, too (2). In a prospective observational study in Taiwan, extreme obesity, i.e. BMI > 30 kg/m^2, was independently associated with a two-fold risk (RR 2.36; 95% CI 0.91–6.17) in people without hepatitis infections, after adjusting for other metabolic components (33). Diabetes was associated to an increased risk of HCC in patients with HCV infection (RR 3.52; 95% CI 1.29–9.24) and in HBV carriers (RR 2.27; 95% CI 1.10–4.66), and reach a 100-fold higher in HBV or HCV carriers with both obesity and diabetes, indicating synergistic effects between metabolic factors and viral hepatitis. NAFLD was therefore identified by AASLD as a target for screening for liver cancer, since it *per se* was found to be associated with a more than a 10-fold increase in HCC prevalence in United Kingdom, where NAFLD accounted for one-third of all cases of HCC detected in 2010 whereas in the same time period, metabolic risk factors were present in two-thirds of all cases, irrespective of associated etiologies (34). As expected, patients with NAFLD associated HCC were older than non-NAFLD patients with a liver cancer (71 years vs 67 years; $P < 0.001$) and less often identified by surveillance. This notwithstanding, survival in NAFLD patients was similar than in other etiologies, probably reflecting a significantly higher rate of incidental presentation (38%) and a lower prevalence of cirrhosis (77%). In patients referred for liver transplant evaluation at Cleveland Clinic in Ohio, older age at diagnosis of cirrhosis and any alcohol consumption were independently associated with the development of HCC, suggesting that alcohol intake, even in socially accepted amounts, may potentially increase the risk of HCC development both in NASH- and HCV-cirrhotic patients (35). This and other studies contributed to the increased recognition NASH being a significant cause of both cirrhosis and HCC, with many patients however progressing to liver cancer without histological evidence of advanced fibrosis or cirrhosis (36,37).

The inclusion of NAFLD among target populations for screening poses an issue of cost effectiveness since NAFLD identifies a broad set of patients ranging from those with simple hepatic steatosis to those with full-blown cirrhosis, not to mention such competing risks for deaths as cardiovascular accidents. Owing to the clinical heterogeneity of NAFLD population, AASLD decided that surveillance of these patients is worth yet not likely to be cost effective. It should be kept in mind that surveillance of NAFLD patients may also result in biased radiological diagnosis of HCC, since contrast imaging is advocated to diagnose HCC in patients with cirrhosis and chronic active hepatitis B, only.

The surveillance algorithm

Abdominal ultrasound (US) is the most accurate and widely used test for HCC surveillance owing to the absence of risks, non-invasiveness, moderate cost and wide acceptability (38–41). A small HCC < 3 cm in size, on US may take several appearances, none of which is specific: the small HCC may be hyperechogenic, because of enrichment with fatty tumour cells, hypoechoic or with a 'target-like' appearance. The diagnostic sensitivity of US is satisfactory, ranging between 65% and 80%, with a specificity greater than 90% when used as a screening test (42). Based on tumour volume doubling time (43), 6 months have been selected by most Societies as the ideal interval of screening with US (1–3). Yet, JSH (4) recommends a 3 or 4-month interval of screening in 'very-high-risk population' like men with viral cirrhosis or chronic viral hepatitis, aged patients and those with a history of alcohol abuse. While the intensified screening clearly aims to identify HCC at the smallest possible size, the effectiveness of this policy is largely questioned, since an impact on survival was never reported (Table 2).

A recent nationwide cohort study performed in Taiwan confirmed that a shorter ultrasonography screening interval is independently associated with higher chance to receive curative therapy after HCC diagnosis. Compared with the 6-months screened cohort, the adjusted hazards ratios of chance to receive curative therapy for the 24-months, 36-months and never screened cohorts were 0.73 (95% CI 0.69–0.78), 0.65 (95% CI 0.60–0.70) and 0.47 (95% CI 0.45–0.50) (all $P < 0.001$) respectively. However, the chance to receive curative therapy is not statistically different between the 6-months and 12-months cohorts (44).

In a study prevalently including patients with alcoholic cirrhosis randomly allocated to standard (every 6 months) vs intensified (every 3 months) intervals of screening, the shortened surveillance schedule did not significantly increase detection of small (≤3 cm) tumours (79% vs 70%), applicability of curative treatments (62% vs 58%) and 5-year survival rate (85% vs 86%). Conversely, the short surveillance schedule led to a greater cumulative incidence of small nodules that proved non-malignant during the follow-up, leading to increased cost, to achieve a final diagnosis, i.e. with a negative cost-utility ratio (45).

Non-invasive markers

Cost-utility ratio of surveillance could be implemented if any serum marker of HCC had an incremental diagnostic and predictive power, yet this is not the case. In the West, these markers have been dismissed in the

surveillance for HCC. The diagnostic and predictive value of alpha-fetoprotein (AFP) is influenced by the size and aggressiveness of HCC, and etiology and activity of the underlying liver disease (46–52). Moreover, the performance of AFP according to antiviral treatment response is still lacking. Setting the prevalence of HCC at 50%, the positive predictive value (PPV) of serum AFP with a cut-off value of 20 ng/ml is 84.6%, but for HCC prevalence rates similar to those seen in most liver clinics, i.e., about 5%, the PPV of an AFP with a cut-off value of 20 ng/ml drops to 41.5%, only. When a cut-off of 400 ng/ml is used, the PPV is 60%, only (53). By the same token, Western societies ruled that the semi-annual combination of US+AFP brings no added value for the early diagnosis of HCC compared to US alone, as it increases the sensitivity for early HCC by 6% in parallel with increased rates of false positive results (54–58). Owing to such limitations, serum AFP is no longer considered for screening (and diagnosis) by EASL-EORTC and AASLD. The AFP assay still holds a place in the surveillance algorithm of APASL (3) and JSH (4). APASL recommends AFP values >200 ng/ml to be used for HCC diagnosis because such relatively high threshold value the risk of false positive cases are minimized, however, without specifying whether a diagnosis of HCC can be accepted without demonstration of the typical contrast pattern at imaging. JSH (4) recommends the use of the imaging techniques in combination with serum des-gamma-carboxy-prothrombin (DCP), an abnormal prothrombin protein elaborated by the neoplastic liver cells and a fucosylated variant of AFP (AFP-L3). Most hepatologists in the West, however, are reluctant to adopt DCP for both screening and diagnosis of HCC owing to its low accuracy in detecting small HCC nodules (59, 60).

Accuracy of alphafetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) in the early diagnosis of HCC was assessed among 1031 patients randomized in the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) Trial, in a case-control study. Neither DCP nor AFP were found to be optimal since diagnosis of early HCC was triggered by surveillance ultrasound in 14, doubling of AFP in 5, and combination of tests in five patients (61).

With respect to AFP utilization, no data are reported in its sensitivity and specificity among patients with virological response to nucleoside/nucleotide antiviral therapy in HBV related cirrhotic patients, as well as in HCV related cirrhotic patients with SVR to antiviral therapy. Expected to increase in number thanks to the increasing number of SVR in HCV infected patients.

The recall policy

Any new nodule identified at screening or during surveillance as well as pre-existing nodule increasing in size or changing the echo-pattern should be regarded as malignant unless otherwise demonstrated (1, 2). AASLD and EASL-EORTC proposed an algorithm to be activated whenever US shows an abnormal result, i.e. a nodule in the liver. Noteworthy, a majority of liver nodules in cirrhosis detected during screening are smaller than 1 cm and non-malignant, resulting in increased surveillance costs without clinical benefits (42). Both AASLD and EASL-EORT recommend patients with less than 1 cm nodule detected during US surveillance to be strictly followed up with US every 4 months for the first year and every 6 months thereafter, until an increase in size of the nodule occurs, allowing a suitable diagnosis with either non-invasive techniques like CT scan and/or magnetic resonance imaging (MRI) or biopsy criteria (1, 2). In a cirrhotic patient, a nodule showing the typical vascular pattern of contrast enhancement by CT/MRI, i.e. a vascular enhancement as compared to the surrounding parenchima in the early arterial phase (wash-in), followed by a rapid release in the portal/venous and/or delayed phases of the exam (wash-out), can be regarded as a HCC, with no need for histological confirmation (62–66). In the absence of such a typical pattern at the first imaging procedure, an alternative imaging technique needs to be performed, leaving a echo-guided FNB necessary for nodules with an atypical vascular pattern, only. When selecting the most adequate imaging technique to be performed first, it should be considered that MRI has the highest sensitivity to detect the typical contrast pattern in smaller HCC (<2 cm) and that it can provide additional diagnostic information in the 'hepato-biliary phase'. MRI therefore helps the diagnosis of malignancy even in the absence of a typical wash-in as it often occurs in smallest tumours (67–72). More controversial is the place of contrast-enhanced ultrasonography (CEUS) in the recall policy, as it started being used for characterization of liver nodules some 10 years ago. APASL (3) and JSH recommend CEUS to be included together with CT and MRI as a first choice technique for the non-invasive diagnosis of HCC. This is not the recommendation of AASLD and EASL given that CEUS was shown to misdiagnose intrahepatic cholangiocarcinoma (ICC) in cirrhotic patients (73, 74) and may lead to an increase in economic costs (74). Whereas APASL (3) and JSH (4) retain CEUS to diagnose hypovascular HCC in cirrhosis, recently CEUS was associated to false negative results in small HCC that are identified by CT or MRI (75, 76).

How to improve cost-utility of surveillance

While the clinical benefits of screening for HCC are intuitive, the economic consequences of surveillance strategies of at risk patients are poorly appreciated. This is the consequence of the lack of randomized trials evaluating moderators of treatment outcome like compliance, heterogeneity of liver disease and treatment effectiveness that, in addition to tumour incidence, impact the cost-utility ratio of surveillance. According

to both EASL (1) and AASLD (2), screening is worth in selected populations like hepatitis C patients with ≥1.5% incidence of HCC, hepatitis B patients with >0.2% incidence and, in general, patients with cirrhosis with >2.5% incidence. These groups include patients with Child-Pugh A and B cirrhosis and Child-Pugh C patients listed to liver transplantation. For non-cirrhotic patients with active hepatitis B, surveillance can also be beneficial as the estimated incidence rates of HCC in these subjects in Europe/North America range from 0.1% to 0.4%/year peaking 2.2% in subjects with compensated cirrhosis (1).

A recent review of observational studies found that HCC screening was associated with detection of earlier stage HCC and improved survival (77). A Cochrane review found insufficient evidence for screening but focused only on studies of patients with hepatitis B and did not examine observational studies (78). Finally, a systematic review of cost-effectiveness modelling studies, most of which were based on assumptions from the literature, concluded that periodic surveillance with ultrasonography was probably cost-effective when the annual incidence of HCC was higher than 1.5%, although annual surveillance may be more cost-effective than semiannual surveillance in populations with annual HCC risk of 1.5–3.5% (79).

Markov models

In the absence of randomized controlled trials, cost-effectiveness analysis of HCC surveillance is mainly based on Markov models that focus on variables different from country to country in the frame of epidemiological and interventional assumptions which do not necessarily reflect real life practice (80). For example, the review and economic analysis published by Thompson Coon (81) modelled a population with a diagnosis of compensated cirrhosis eligible to enter a surveillance program. A combination of AFP testing and US at 6-monthly intervals, more than triples the number of people with operable HCC tumours at time of diagnosis, and almost halves mortality rates from HCC, owing to the identification of over 10 times as many small (<2 cm) HCC tumours and over twice as many medium-sized tumours (between 2 and 5 cm in diameter). Consequently, based on the assumptions used in the model, more tumours were suitable for surgical intervention with an increase in the percentage of liver transplantations performed for known HCC, as opposed to decompensated cirrhosis, from 8% to 28%, compared with no surveillance. A cost-utility analysis done in parallel indicates that adding US to 6 month AFP surveillance leads to a cost-utility ratio of US $ 60 000 for QALY gained with evidence for greater cost-effectiveness in patients with hepatitis B related cirrhosis, owing to the younger age at diagnosis of cirrhosis.

Besides the limitations mentioned above, cost-effectiveness evaluation of a surveillance program is limited also because mainly based on the experience of a single radiological centre. Specificity and sensitivity of US can substantially vary depending on the expertise of the operator involved. In parallel, CT scan and MRI can be considered more cost-effective than US in settings with poor radiological training in detecting liver nodules or inadequate machinery quality. Further attenuating the credibility of modelling is the *a priori* decision to measure cost-utility ratios at less than US$ 50 000 for quality-adjusted life year (QALY) saved, an assumption that may conflict with policies of equitability while being influenced by the worldwide trends of economy (82).

Finally, the missed evaluation of added costs, related to the detection of nodules that cannot be otherwise characterized through CT scan or MRI application has to be considered, as in the prospective study performed by Sangiovanni *et al.* (62) whereby CT scan and MRI, 19% more nodules were identified compared with US, including three HCC nodules and 10 non-characterized nodules, whose further management undoubtedly implies additional costs.

Screenees at higher risk of HCC

In principle, strengthening prediction in individual patients is expected to improve cost-effectiveness ratios of screening, but the benefits of approaches like pre-treatment patients stratification by clinical, histological and genetic scores, remain uncertain. Following stratification by different risk factors for HCC, mainly hepatitis markers and serum tests of liver disease, a proportion of the resident population in Taiwan was invited to attend a risk score-guided mass abdominal ultrasonography screening between 2008 and 2010. Compared with unscreened population who was not invited, a 31% reduction in HCC mortality was reported among screens, suggesting that stratification of general population by a propensity score might improve cost-effectiveness of mass screening (83). This is also true for patients with hepatitis B and hepatitis C successfully responding to antiviral therapy. HCC risk predictors from untreated Asian patients with chronic HBV infection are also applicable in patients receiving antiviral therapy with NUC. The accuracy of Reach-B, CU_HCC and GAG-HCC has been confirmed in entecavir treated patients in a cohort from Hong Kong (84). In that study, HCC prediction seemed to increase from year 2 of therapy onwards compared with baseline, but it failed to be validated in caucasian patients of European ancestry under entecavir or tenofovir therapy (85). In European patients with treatment suppressed HBV, the HCC risk can more reliably be predicted by the PAGE score which includes age, gender and platelet counts (86). In the setting of hepatitis C, a score combining age, markers of disease severity and alfafetoprotein was able to identify three categories of patients at different level of HCC risk. The proportion of HCC development increased from 1.37% (9/657) in the low-risk group to 9.14% (16/

175) in the intermediate-risk group and 30.77% (12/39) in the high-risk group ($P = 0.001$) (20). On the same line is the possibility to detect group of non-cirrhotic patients at higher risk of developing HCC (24).

Hung *et al.* present another model for assessing HCC risk. They analysed a large number ($n = 12\ 377$) of Taiwanese subjects from three different observational cohorts, most of them HBV infected. They proposed four models. The first only included age, gender and ALT, the second model added history of chronic liver disease, family history of HCC and smoking. The third model incorporated all these variables plus HBsAg data and the fourth model included both HBsAg and anti-HCV data. Models 3 and 4 performed better than those with less information (models 1 and 2) (87).

However, given the level of uncertainty about the applicability of these scores, at least in Europe and North America physicians may just choose to be more inclusive and use the AASLD/EASL guidelines. What is still uncertain is whether a complementary policy to optimize surveillance based on the exclusion of patients with severe co-morbidities who do not fit criteria for curative therapies or on restriction of screening to aged individuals who would not have significant survival benefit if diagnosed with an HCC, is worth.

For patients in which a coarse pattern or obesity limit US performance, a more sensitive screening technique could be proposed, whose utilization could be cost-effective in this subgroup of patients.

A recent retrospective Japanese study assessed that among patients who underwent dynamic CT analysis of a single-nodular HCC, additional evaluation by MR imaging with gadoxetic acid led to the detection of additional HCC nodules in 16% of patients, reduced the risk of disease recurrence and decreased overall mortality. The same could be suggested in the *super-risk* cathegories of patients, after the application of the previously cited clinical scores.

Genetic markers to identify high risk screenees

In principle, a clinical model incorporating a single nucleotide polymorphism (SNP) for a potentially pathogenic gene might increase prediction finalized to pretreatment stratification of patients who are at risk of developing HCC. In HCC, many tumour suppressor genes and oncogenes were identified based on recurrent genetic lesions, including the loss of TP53 (17p13) (88), RB and BRCA2 (13q) (89), amplification of c-myc (8q24) (90) and ERBB2 (17q12-q21) (91).

A 186-gene signature used to predict outcomes of patients with HCC was found to be associated with outcomes of patients with hepatitis C-related early-stage cirrhosis. This gene expression profile was analysed on formalin-fixed needle biopsy specimens from the livers of 216 patients with hepatitis C-related early-stage (Child-Pugh class A) cirrhosis who were prospectively followed up for a median of 10 years in an Italian centre (92).

Fifty-five (25%), 101 (47%) and 60 (28%) patients were classified as having poor-, intermediate- and good-prognosis signatures respectively. In multivariable Cox regression modelling, the poor-prognosis signature was significantly associated with death ($P = 0.004$), progression to advanced cirrhosis ($P < 0.001$), and development of HCC ($P = 0.009$). The 10-year rates of survival were 63%, 74% and 85% and the annual incidence of HCC was 5.8%, 2.2% and 1.5% for patients with

Table 3. Down- and up-regulated microRNA in hepatocellular carcinoma and their characteristics

miRs	Targets	Characteristics
Down-regulated		
miR-1	ET1	Proliferation
miRs-7a, -7b, -7c, -7d, -7f-1, -7d	Caspase-3, HMGA2, C-myc, Bcl-xl	Proliferation, apoptosis
miR-101	Mcl-1, SOX-9, EZH2, EED, DNMT3A	Proliferation, apoptosis
miRs-122	Bcl-w, ADAM-1, Wnt-1	Angiogenesis, apoptosis, Metastasis
miR-125a, -125b	MMP11, SIRT7, VEGF-A, LIN28B2, Bcl-2, Mcl-1, Bcl-w	Angiogenesis, apoptosis, metastasis, proliferation
miR-139	c-Fos, Rho-kinase-2	Metastasis
miR-145	IRS1-2, OCT4	Insulin-like growth factor pathway, Stem-like cells tumourigenicity
miR-195	CDK6, E2F3, cyclinD1	Proliferation, apoptosis, tumourigenicity
miR-199a-3p, -199-5p	c-Met, mTOR, PAK4, DDR1, caveolin-2	Proliferation, autophagy, metastasis
miRs-214	HDGF, catenin	Proliferation, angiogenesis, metastasis
Up-regulated		
miR-10a	EphA4, CADM1	EMT, metastasis
miR-21	Pten, RhoB, PDCD4	Drug Resistance, metastasis
miR-221	Bmf, DDIT4, Arnt, CDKN1B/p27, CDKN1C/p57	Angiogenesis, apoptosis, proliferation
miRs-224	Yin Yang1/Raf-1 kinase, NFkB pathways, apoptosis inhibitor-5	Proliferation, apoptosis, metastasis

miRs, microRNAs; EMT, epithelial-mesenchymal transition.

poor-, intermediate- and good-prognosis signatures respectively.

This signature might be used to identify patients with cirrhosis in most need of surveillance and strategies to prevent the development of HCC (92).

Finally, microRNAs (miRNAs), a major class of small non-coding RNAs that regulate the expression of target mRNA transcripts at a post-transcriptional and traslational level (93), are also involved in many cellular processes, such as cell proliferation and differentiation, apoptosis, stem cell maintenance and neuronal patterning (94). Their utilization to optimize early diagnosis could be proposed, although high costs and availability of the tests could limit their application. Table 3 reports the main down- and up-regulated microRNAs in hepatocellular carcinoma, their targets and characteristics.

Conclusions

The implementation of surveillance programs in patients at risk of developing HCC together with advances in imaging techniques and treatment modalities, have significantly improved the prognostic landscape of HCC, worldwide (2). To date, however, even in the resource rich regions, a minority of all HCC patients are diagnosed at early stages, when potentially curative therapies can be offered (95, 96). This coupled with the fact that about 20% of early tumours have a very aggressive behaviour, rapidly spread and leave patients with a small chance of survival, calls for implementation of surveillance programs and identification of patients whose prognosis can only be improved by anticipated diagnosis. Molecular profiling of screenees and development of diagnostic biomarkers could benefit surveillance and treatment of HCC, yet the knowledge of genetic factors influencing the risk of developing HCC are still missing. The identification of subgroups of patients at high risk of HCC, who could benefit from primary or secondary prophylaxis or could be offered more aggressive screening policies merits further investigations. As surveillance for HCC is framed in a program where tests, recall policies and quality control procedures are standardized, all scientific societies acknowledge the benefits of surveillance even in the absence of robust evidence that it reduces liver related mortality. HCC still remains a highly lethal cancer, owing to the lack of effective therapies for advanced patients and biomarkers for early diagnosis. Future efforts should be geared towards removing the barriers to universal surveillance of at risk patients by further improving access to testing.

Acknowledgements

Financial support: No financial support.

Conflicts of interest: The authors do not have any disclosures to report.

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