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[Diagnostic Test Accuracy Review]

Contrast-enhanced ultrasound for the diagnosis of hepatocellular carcinoma in adults with chronic liver disease

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ABSTRACT

Background

Hepatocellular carcinoma occurs mostly in people with chronic liver disease. Worldwide, it ranks sixth in terms of incidence of cancer, and fourth in terms of cancer-related deaths. Contrast-enhanced ultrasound (CEUS) is used as an add-on test to confirm the presence of focal liver lesions suspected as hepatocellular carcinoma after prior diagnostic tests such as abdominal ultrasound or measurement of alpha-foetoprotein, or both. According to guidelines, a single contrast-enhanced imaging investigation, with either computed tomography (CT) or magnetic resonance imaging (MRI), may show the typical hepatocellular carcinoma hallmarks in people with cirrhosis, which will be sufficient to diagnose hepatocellular carcinoma. However, a significant number of hepatocellular carcinomas show atypical imaging features, and therefore, are missed at imaging.

Dynamic CEUS images are obtained similarly to CT and MRI images. CEUS differentiates between arterial and portal venous phases, in which sonographic hepatocellular carcinoma hallmarks, such as arterial hyperenhancement and subsequent washout appearance, are investigated. The advantages of CEUS over CT and MRI include real-time imaging, use of contrast agents that do not contain iodine and are not nephrotoxic, and quick image acquisition. Despite the advantages, the use of CEUS in the diagnostic algorithm for HCC remains controversial, with disagreement on relevant guidelines.

There is no clear evidence of the benefit of surveillance programmes in terms of overall survival as the conflicting results can be a consequence of an inaccurate detection, ineffective treatment, or both. Therefore, assessing the diagnostic accuracy of CEUS may clarify whether the absence of benefit could be related to underdiagnosis. Furthermore, an assessment of the accuracy of CEUS for the diagnosis of hepatocellular carcinoma is needed for either diagnosing hepatocellular carcinoma or ruling it out in people with chronic liver disease who are not included in surveillance programmes.

Objectives

1. To assess the diagnostic accuracy of contrast-enhanced ultrasound (CEUS) for the diagnosis of hepatocellular carcinoma of any size and at any stage in adults with chronic liver disease, in a surveillance programme or in a clinical setting.

2. To assess the diagnostic accuracy of CEUS for the diagnosis of resectable hepatocellular carcinoma in people with chronic liver disease and identify potential sources of heterogeneity in the results.

Search methods

We used standard, extensive Cochrane search methods. The last date of search was 5 November 2021.

Selection criteria

We included studies assessing the diagnostic accuracy of CEUS for the diagnosis of hepatocellular carcinoma in adults with chronic liver disease, with cross-sectional designs, using one of the acceptable reference standards, such as pathology of the explanted liver, and histology of resected or biopsied focal liver lesion with at least a six-month follow-up.

Data collection and analysis

We used standard Cochrane methods to screen studies, extract data, and assess the risk of bias and applicability concerns, using the QUADAS-2 checklist. We used the bivariate model and provided estimates of summary sensitivity and specificity. We assessed the certainty of the evidence using GRADE. We presented uncertainty-of-the-accuracy estimates using 95% confidence intervals (CIs).

Main results

We included 23 studies with 6546 participants. Studies were published between 2001 and 2021. We judged all 23 studies at high-risk of bias in at least one domain, and 13/23 studies at high concern for applicability. Most studies used different reference standards to exclude the presence of the target condition. The time interval between the index test and the reference standard was rarely defined. We also had major concerns on their applicability due to the characteristics of the participants.

– CEUS for hepatocellular carcinoma of any size and stage: sensitivity 77.8% (95% CI 69.4% to 84.4%) and specificity 93.8% (95% CI 89.1% to 96.6%) (23 studies, 6546 participants; very low-certainty evidence).

– CEUS for resectable hepatocellular carcinoma: sensitivity 77.5% (95% CI 62.9% to 87.6%) and specificity 92.7% (95% CI 86.8% to 96.1%) (13 studies, 1257 participants; low-certainty evidence).

The observed heterogeneity in the results remains unexplained. The sensitivity analyses, including only studies with clearly prespecified positivity criteria and only studies in which the reference standard results were interpreted with no knowledge of the results about the index test, showed no differences in the results.

Authors' conclusions

We found that by using CEUS, as an add-on test following abdominal ultrasound, to diagnose hepatocellular carcinoma of any size and stage, 22% of people with hepatocellular carcinoma would be missed, and 6% of people without hepatocellular carcinoma would unnecessarily undergo further testing or inappropriate treatment. As to resectable hepatocellular carcinoma, we found that 23% of people with resectable hepatocellular carcinoma would incorrectly be unresected, while 8% of people without hepatocellular carcinoma would undergo further inappropriate testing or treatment. The uncertainty resulting from the high risk of bias of the included studies, heterogeneity, and imprecision of the results and concerns on their applicability limit our ability to draw confident conclusions.

PLAIN LANGUAGE SUMMARY

How accurate are contrast-enhanced ultrasound scans for detecting hepatocellular carcinoma?

Key messages

In adults with chronic liver disease, contrast-enhanced ultrasound (CEUS) can miss diagnosing hepatocellular carcinoma in around 22.2% of people who would not then receive timely or appropriate treatment, and would fail to diagnose hepatocellular carcinoma in 6.2% of people who could receive unnecessary further testing or treatment.

In the subset of people who are able have the hepatocellular carcinoma removed by surgery, CEUS probably misses hepatocellular carcinoma in 22.5% of people who could undergo surgery to remove part of their liver while it would erroneously find cancer in 7.3% of people who would undergo unnecessary further test or surgery.

As there were some problems with the way the studies were conducted, CEUS to detect hepatocellular carcinoma may appear more accurate than it actually is.

Why is improving the diagnosis of hepatocellular carcinoma important?

Hepatocellular carcinoma is cancer originating in the liver. It is sixth in terms of occurrences of cancer and fourth in terms of cancer-related deaths worldwide. It occurs mostly in people with chronic liver disease regardless of the exact cause. People with blood test or ultrasound results that suggest they may have hepatocellular carcinoma may go on to have further tests, such as imaging or a biopsy (where a small piece of the liver is removed and examined). If the cancer is detected early, people may have part of the liver removed or have a liver transplant. In advanced hepatocellular carcinoma, they may need chemotherapy. If hepatocellular carcinoma is missed at

diagnostic testing, people will not receive appropriate treatment. However, incorrectly diagnosing hepatocellular carcinoma when it is not present means that people may undergo unnecessary testing or treatment.

What is contrast-enhanced ultrasound and how can it diagnose hepatocellular carcinoma?

CEUS can detect abnormalities in the liver that might be due to cancer and, using contrast agents, confirm the diagnosis of hepatocellular carcinoma. These contrast agents are safe. CEUS is used in clinical practice to confirm the presence of hepatocellular carcinoma in people in whom suspicion was raised by prior performed abdominal ultrasound or a blood test to measure alpha-foetoprotein.

The role of CEUS in the diagnosis of hepatocellular carcinoma remains controversial between guidelines. Previous systematic reviews have assessed the performance of CEUS in detecting hepatocellular carcinoma but they have included different studies and found different results.

What did we want to find out?

We wanted to find out if CEUS is accurate enough to diagnose hepatocellular carcinoma in adults with chronic liver disease (a progressive deterioration of liver functions for more than six months). We were interested first in hepatocellular carcinoma of any size and severity and, second, in hepatocellular carcinomas that were suitable for surgical removal (resection).

What did we do?

We searched for studies that assessed the accuracy of diagnostic tests of CEUS scans compared to the best available tests to confirm hepatocellular carcinoma in adults with chronic liver disease. The best available test is examination of the liver, or part of it, under a microscope.

What did we find?

We found 23 studies with 6546 adults.

Around 690 (69%) out of 1000 adults with chronic liver disease had a confirmed hepatocellular carcinoma of any size and severity. Considering these 1000 people, CEUS:

– correctly detected liver cancer in 537 people;

– missed liver cancer in 153 people;

– incorrectly detected cancer in 19 people;

– correctly detected no cancer in 291 people.

Around 690 (69%) out of 1000 adults with chronic liver disease had a confirmed hepatocellular carcinoma that could be removed by surgery. Considering these 1000 people, CEUS:

– correctly detected liver cancer in 535 people;

– missed liver cancer in 155 people;

– incorrectly detected cancer in 23 people;

– correctly detected no cancer in 287 people.

What are the limitations of the evidence?

Our confidence in the evidence is limited as the studies used different methods to select study participants and used different reference standards. This means that CEUS scans may be more or less accurate than what the evidence suggests.

How up to date is this evidence?

The evidence is up to date to 5 November 2021.

SUMMARY OF FINDINGS

Summary of findings 1. Diagnostic accuracy of contrast-enhanced ultrasound for the diagnosis of hepatocellular carcinoma

Review question: what is the diagnostic accuracy of CEUS for the diagnosis of HCC in people with chronic liver disease?

Population: adults with chronic liver disease

Setting: clinical setting (secondary or tertiary care setting) or surveillance programmes

Study design: cross-sectional studies

Index test: CEUS

Target condition: HCC of any size, any stage

Reference standards:

- typical characteristics on cross-sectional multiphasic contrast CT or MRI with a follow-up period of ≥ 6 months, to allow the confirmation of an initial negative result of CT or MRI;
- the pathology of the explanted liver in case of transplantation;
- the histology of resected focal liver lesion(s), or the histology of biopsied focal liver lesion(s) with a follow-up period of ≥ 6 months to exclude the presence of focal lesions not detected by the index test.

Limitations in the evidence

- Risk of bias and applicability concerns
- Participant selection: high/unclear risk of bias: 21 studies (91%); high concern for applicability: 13 studies (56%)
- Index tests: high/unclear risk of bias: 7 studies (30%); high concern for applicability: 0 studies (0%)
- Reference standard: high/unclear risk of bias: 17 studies (74%); high concern for applicability: 0 studies (0%)
- Flow and timing: high/unclear risk of bias: 18 studies (78%)
- Overall assessment: high risk of bias: 23 studies (100%); high concern for applicability: 13 studies (56%)

Findings

Implication in a hypothetical cohort of 1000 people with chronic liver disease

Index test	Number of studies (participants)	Sensitivity (95% CI)	Specificity (95% CI)	Prevalence[a]	True positives: people who will receive appropriate treatment (surgery or local ablative therapy or systemic chemotherapy)	False negatives: people who will be misdiagnosed and who will not receive appropriate treatment	True negatives: people who will not undergo inappropriate treatment or unnecessary further testing	False positives: people who will undergo inappropriate treatment or further testing	Certainty of the evidence

CEUS	23 (6546)	77.8% (69.4% to 84.4%)	93.8% (89.1% to 96.6%)	60%	467	133	375	25	**Very low**[b] ⊕⊖⊖⊖
				69%	537	153	291	19	**Very low**[b] ⊕⊖⊖⊖
				80%	622	178	188	12	

CEUS: contrast-enhanced ultrasound; CI: confidence intervals; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging.

[a]For illustration, we chose three values of hepatocellular carcinoma prevalence: 69% as a median derived from our study analysis, 60%, the first quartile, and 80%, the third quartile.

[b]Downgraded three levels for risk of bias, indirectness, and inconsistency. Risk of bias downgraded one level because all studies were at high risk of bias; indirectness downgraded one level as we considered most studies to have high concern regarding applicability, mainly in relation to the population (including disease spectrum); inconsistency downgraded one level as for individual studies' summary sensitivity ranged from 29% to 100% and summary specificity from 63% to 100%, and we could not explain the heterogeneity by study quality or other factors.

Summary of findings 2. Diagnostic accuracy of contrast-enhanced ultrasound for the diagnosis of resectable hepatocellular carcinoma

Review question: what is the diagnostic accuracy of MRI for the diagnosis of hepatocellular carcinoma in people with chronic liver disease?

Population: adults with chronic liver disease

Setting: clinical setting (secondary or tertiary care setting) or surveillance programmes

Study design: cross-sectional studies

Index test: CEUS

Target condition: resectable hepatocellular carcinoma

Reference standards:

- typical characteristics on cross-sectional multiphasic contrast CT or MRI with a follow-up period of ≥ 6 months, to allow the confirmation of an initial negative result of CT or MRI;
- the pathology of the explanted liver in case of transplantation;
- the histology of resected focal liver lesion(s), or the histology of biopsied focal liver lesion(s) with a follow-up period of ≥ 6 months to exclude the presence of focal lesions not detected by the index test.

Limitations in the evidence: risk of bias and applicability concerns

- Participant selection: high/unclear risk of bias: 6 studies (46%); high concern for applicability: 3 studies (21%)
- Index tests: high/unclear risk of bias: 4 studies (28%); high concern for applicability: 0 studies (0%)
- Reference standard: high/unclear risk of bias: 10 studies (77%); high concern for applicability: 0 studies (0%)
- Flow and timing: high/unclear risk of bias: 8 studies (61%)

- Overall assessment: high risk of bias: 13 studies (100%); high concern for applicability: 3 studies (21%)

Findings

Implication in a hypothetical cohort of 1000 people with chronic liver disease

Index test	Number of studies (participants)	Sensitivity (95% CI)	Specificity (95% CI)	Prevalence[a]	True positives: people who will receive appropriate treatment (surgery or local ablative therapy or systemic chemotherapy)	False negatives: people who will be misdiagnosed and not receive appropriate treatment	True negatives: people who will not undergo inappropriate treatment or unnecessary further testing	False positives: people who will undergo inappropriate treatment or further testing	Certainty of the evidence
CEUS	13 (1257)	77.5% (62.9% to 87.6%)	92.7% (86.8% to 96.1%)	60%	465	135	371	29	**Low**[b] ⊕⊕⊖⊖
				69%	535	155	287	23	**Low**[b] ⊕⊕⊖⊖
				80%	620	180	185	15	

CI: confidence intervals; CT: computed tomography; MRI: magnetic resonance imaging.

[a]For illustration, we chose three values of hepatocellular carcinoma prevalence: 69% as a median derived from our study analysis, 60%, the first quartile value, and 80%, the third quartile.

[b]Downgraded by two levels due to risk of bias and inconsistency. Risk of bias downgraded one level because all studies were at high risk of bias; inconsistency downgraded one level as for individual studies' summary sensitivity ranged from 29% to 100% and summary specificity from 69% to 100%, and we could not explain the heterogeneity by study quality or other factors.

BACKGROUND

Hepatocellular carcinoma is the most common primary liver neoplasm. Usually, hepatocellular carcinoma develops in people with chronic liver disease. It represents the fourth most common cause of death from cancer worldwide, with exceedingly high rates in East and South-East Asia, several areas in Africa, and southern Europe (Bertuccio 2017; Bray 2018). Since the early 2000s, hepatocellular carcinoma has been one of the few cancers showing unfavourable trends in several areas of the world including Europe, and North and Latin America (Bralet 2000; Hashim 2016; Ryerson 2016). In Europe and North America, the incidence and mortality rates have increased since the mid-2000s (Bertuccio 2017). Mortality rates are reported to be two- to five-fold higher in Japan, Hong Kong, and Korea than in most European countries. In the Americas, the reported trends are downward (Bertuccio 2017). Most common risk factors include liver cirrhosis, severe liver fibrosis, chronic infections with hepatitis B and C, heavy alcohol intake, smoking, overweight, diabetes, metabolic syndrome, aflatoxins (poisonous carcinogens produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which grow in soil, decaying vegetation, hay, and grains), and non-alcoholic fatty liver disease (Bertuccio 2017; Bosetti 2013; Bosetti 2014; Stanaway 2016; Yang 2011), although cases of hepatocellular carcinoma without known risk factors have been reported (Bralet 2000; Young 2012).

Hepatocellular carcinoma is rare among adolescents, with an incidence of 0.30 to 0.45 people per million per year, and it accounts for less than 1% of all malignant neoplasms among people younger than 20 years (Mann 1990). The reported hepatocellular carcinomas were associated with hepatitis B virus infection or with inherited metabolic disorders, specifically hereditary tyrosinaemia, alpha-1-antitrypsin deficiency, and glycogen storage disease type 1. Only approximately 30% of childhood hepatocellular carcinomas are associated with cirrhosis, and the carcinogenesis and clinical course are considered distinctive (Mogul 2018; Ni 2004; Omata 2017).

Clinically, hepatocellular carcinoma is frequently diagnosed at the late stage because of the absence of specific symptoms, other than those related to chronic liver disease. Less than 20% of patients are eligible for curative treatment including liver resection, transplantation, or ablation due to advanced tumour stage, liver dysfunction, or shortage of liver donors (Davila 2012). Furthermore, curative treatment options are unfeasible in most patients due to severe clinical deterioration at the moment of diagnosis confirmation or the inaccuracy of preoperative clinical evaluation and staging procedure (or both).

Despite the poor initial prognosis (with an overall ratio of mortality to incidence of 0.95) (Ferlay 2019), a five-year survival rate of more than 50% can be achieved if hepatocellular carcinoma is detected at an early stage (Forner 2012). According to the modified Barcelona Clinic Liver Cancer (BCLC) staging system (Forner 2018; Llovet 1999), only people with very early- or early-stage hepatocellular carcinoma are eligible for curative treatment. Therefore, accurate and early diagnosis of hepatocellular carcinoma is of high importance.

Prior to advancements in medical imaging, biopsy and cytological examination of the liver specimen were used to make a definitive diagnosis of hepatocellular carcinoma (Tao 1984). With the development of advanced imaging techniques, hepatocellular carcinoma has become unique among tumours with its characteristics, accurately being detected on imaging, thus reducing the need for invasive biopsy (Forner 2008; LI-RADS 2017; Manini 2014). Currently, biopsy is not preferred for the diagnosis of hepatocellular carcinoma due to concerns about tumour seeding, bleeding, and the rate of false-negative results (Pomfret 2010; Silva 2008). However, it is reserved for lesions with atypical appearance and when imaging results are equivocal (Bruix 2011).

Due to development of microbubble contrast agents, contrast-enhanced ultrasound (CEUS) has gained increasing interest and offers the potential for ultrasound to show enhancement patterns in liver lesions (Niu 2013). Dynamic CEUS images are obtained similarly to contrast-enhanced computed tomography and magnetic resonance imaging (MRI) depending on the time of image acquisition after intravenous contrast injection. The study differentiates arterial and portal venous phases in which sonographic hallmarks for hepatocellular carcinoma (such as arterial hyperenhancement and subsequent washout appearance) are investigated (Chung 2015; LI-RADS 2017). Unlike the contrast agents used in computed tomography and MRI, ultrasound contrast agent is a purely intravascular agent, and, therefore, highly accurate in detecting tumour angiogenesis (Schirner 2004). However, due to the nature of the contrast, CEUS does not depict the hepatocellular carcinoma capsule that is another hallmark in liver lesion characterisation on computed tomography and MRI (LI-RADS 2017). Advantages of CEUS over computer tomography and MRI include real-time imaging, use of contrast agents that do not contain iodine and are not nephrotoxic, possible multiple injections of contrast in the same examination, safety, practicality, no risk of nephrotoxicity, no ionising radiation, and short time of image acquisition. However, CEUS is not recommended for disease staging or assessment of treatment response, and the adequacy of the examination depends on the liver window and expertise of the operator (LI-RADS 2017).

Despite the advantages, the use of CEUS in the diagnostic algorithm for hepatocellular carcinoma remains controversial with disagreement between pertinent guidelines (EASL 2018; Heimbach 2018; Omata 2017). Previous non-Cochrane systematic reviews have assessed the performance of CEUS in detecting hepatocellular carcinoma and they have included different studies and yielded different results (Deng 2016; Huang 2017; Li 2021; Niu 2013; Westwood 2013; Yang 2021; Zhang 2017). These reviews assessed CEUS either as a stand-alone test or compared CEUS with computed tomography or MRI. Most of these are comparative reviews that compare two or more tests (CEUS, computed tomography, MRI) and address a wider question, that is, the diagnosis of any focal liver lesions, not only hepatocellular carcinoma, but also benign tumours and metastases (Westwood 2013), or a narrower question, that is, only small hepatocellular carcinoma, with a diameter less than 2 cm (Deng 2016; Niu 2013). Assessment of methodological quality and definition of inclusion criteria, type of studies, and reference standards are often inconsistent. Furthermore, these reviews did not put the index tests into context and did not clearly define their role. Instead, they compared all the available tests as they were used simultaneously (Huang 2017; Zhang 2017).

The aim of this systematic review and meta-analysis is to determine the accuracy of CEUS using either extracellular or hepatocellular contrast agents for the diagnosis of hepatocellular carcinoma of any size, as well as to identify resectable hepatocellular carcinoma

in people with chronic liver disease, by applying Cochrane methodology.

Target condition being diagnosed

Hepatocellular carcinoma

Hepatocellular carcinoma is the most common primary liver cancer which occurs mostly in people with chronic liver disease. The incidence of hepatocellular carcinoma increases in individuals with chronic hepatitis B and C, alcohol use and non-alcoholic fatty liver disease, and people with liver cirrhosis of various aetiology (Bruix 2011). There is no definite threshold in the definition of lesion size, although the literature tends to classify lesions with a diameter of 2 cm or less as small (Choi 2014; Hussain 2002; Park 2017). The histological diagnosis of hepatocellular carcinoma poses many challenges, particularly when dealing with liver biopsy specimens, because of the heterogeneity of genetic and histopathological characteristics of hepatocellular carcinomas and occasional difficulties confirming hepatocellular differentiation. Primary liver tumours should be considered as a continuum with typical hepatocellular and cholangiocarcinoma at the two ends and a whole range of tumours showing both hepatocellular and cholangiocellular differentiation with or without an associated progenitor/stem cell component in the middle. Characterisation of combined (or mixed) hepatocellular–cholangiocarcinoma can be very challenging. In advanced-stage chronic liver disease, the main challenge for the histopathologist is still to differentiate between hepatocellular carcinoma and its precursors, large regenerative and dysplastic nodules. The transition from dysplastic nodule to hepatocellular carcinoma is thought to be associated with a change in the lesional vascular supply, from a dual porta-arterial to a predominantly arterial due to neo-angiogenesis (i.e. the growth of new vessels from the existing vasculature) (Quaglia 2018).

In clinical practice and according to pertinent guidelines, multiphasic computed tomography or MRI with intravascular contrast application allow for a highly accurate diagnosis of hepatocellular carcinoma, without an invasive biopsy. The diagnosis of hepatocellular carcinoma is usually based on cross-sectional computed tomography or MRI features: focal liver lesions which show non-rim-like hyperenhancement in the arterial phase, subsequent non-peripheral washout appearance, and capsule appearance (LI-RADS 2018). Liver histology is required only for undefined lesions at computed tomography and MRI (EASL 2018; Heimbach 2018; Omata 2017).

Several staging systems for hepatocellular carcinoma have been proposed and developed; however, there is no globally applicable staging system (Kinoshita 2015). Among different protocols, the modified BCLC staging system has a notable feature of treatment recommendations for each stage based on the best treatment options currently available (Forner 2018; Llovet 1999; Llovet 2003; Llovet 2008). It is comprised of four elements: tumour extension, liver functional reserve, physical status, and cancer-related symptoms. According to BCLC staging, only people with early-stage hepatocellular carcinoma are eligible for curative treatment such as surgical resection or percutaneous treatment.

Orthotopic liver transplantation (OLT) is reserved for people with decompensated cirrhosis and is considered a definite curative treatment for hepatocellular carcinoma. The early experience with OLT for hepatocellular carcinoma in the 1980s included initial poor five-year survival and high recurrence leading to OLT being contraindicated in hepatocellular carcinoma (Yokoyama 1990). In 1996, specific criteria, known as the Milan criteria, were developed for hepatocellular carcinoma patient selection (Mazzaferro 1996). These criteria have been repeatedly validated and their value is considerable (EASL 2018). With their implementation, overall five-year survival of patients after OLT exceeded 70% (Mazzaferro 2011). The criteria for patients eligible for OLT include single hepatocellular carcinoma lesion with diameter of 5 cm or less; or up to three hepatocellular carcinoma lesions, each with diameter of 3 cm or less; no vascular invasion; and no extrahepatic involvement (no metastasis).

Index test(s)

CEUS is an advanced form of ultrasound examination in which images are acquired using intravenously injected microbubble contrast agent with optimised technology required for contrast visualisation. Contrast agent particles are small bubbles similar in size to red blood cells. These microbubbles contain low soluble gases encapsulated into a biocompatible membrane which may have variable composition of lipids, proteins, biopolymers, or a combination of these.

Like in any other contrast-based imaging procedure, the CEUS examination consists of a bolus administration of contrast media through a superficial peripheral vein. Due to their extremely small size, the microbubbles pass through the pulmonary circulation and then disseminate into the systemic circulation through the arterial bloodstream. The contrast agent remains in the bloodstream for four to five minutes. There is also a parenchymal phase at the level of the liver and spleen because the contrast agent is captured by the reticuloendothelial system or it becomes adherent to the hepatic sinusoid (or both). The gas used for CEUS is eliminated through the airways 10 to 15 minutes after administration, while the substances that form the membrane are eliminated through the kidneys or metabolised by the liver. The use of CEUS in the examination of the liver has special features due to its double vascularity: through the portal vein (two-thirds) and through the arterial system (one-third). The sequence of blood entering the liver is first arterial (10 to 40 seconds), portal (40 to 120 seconds), and then late venous (greater than 120 seconds). This vascular discrimination (similar to the one obtained by contrast computed tomography or MRI) allows the collection of information regarding the circulatory system of a tumour (types of feeding vessels, tumour circulatory volume). The presence of arteriovenous communications is characteristic for the neoplastic circulation and in CEUS is expressed by the washout process. This phenomenon begins at the end of the arterial phase or during the venous phase (or both), it is persistent, and is characteristic for neoplastic processes in 90% of cases. Studies that correlate the washout speed of the tumour with its aggressiveness exist, attributing CEUS a prognostic value (Bhayana 2010; Boozari 2011; Jang 2007; Liu 2007).

Type of contrast agents

The first-generation contrast medium, such as *Levovist* (Bayer Schering Pharma, Berlin, Germany), consisted of air (99.9%) and palmic acid (0.1%) contained within a shell of galactose microparticles. As this medium was found to be unstable, it was replaced by second-generation contrast media such as SonoVue (Bracco, Milano, Italy), Definity (marketed in North America as Luminity by Lantheus Medical Imaging, North Billerica, MA,

USA), Optison (GE Healthcare, Princeton, NJ, USA), and Sonazoid (GE Healthcare, Oslo, Norway) (Chung 2015). SonoVue consists of sulphur hexafluoride contained within a phospholipid shell. Sulphur hexafluoride is an inert molecule that does not interact with any other molecules in the body. Blood-pool agents (e.g. SonoVue, Levovist), and combined blood-pool and Kupffer cell contrast agents (e.g. Sonazoid (perfluorobutane) that also provide the Kupffer-phase images in CEUS are analysed separately. The 2017 version of LI-RADS (Liver Imaging Reporting And Data System) CEUS criteria apply only to blood-pool agents, and not to the blood-pool and Kupffer cell contrast agents. Therefore, no criteria exist for the Kupffer phase of the examination (LI-RADS 2017).

The characteristic feature of a blood-pool/Kupffer cell agent (e.g. Sonazoid) is the accumulation in the reticuloendothelial system such as in the liver and spleen. This unique feature of Sonazoid allows the visualisation and interpretation of liver parenchyma during the postvascular phase (i.e. Kupffer phase).

The imaging in the Kupffer phase is stable from 10 to 120 seconds after contrast injection, and tolerable for multiple scanning. Malignant hepatic tumours contain few or no Kupffer cells, which can be seen as a perfusion defect in the Kupffer phase.

Positivity criteria

Positivity criteria for hepatocellular carcinoma are based on arterial hyperenhancement and subsequent washout appearance.

On CEUS examination using blood-pool agents (e.g. SonoVue), the typical appearance of hepatocellular carcinoma is characterised by accelerated uptake during the arterial phase (hyperenhancement), contrast washout during the portal venous phase, and a hypoechoic appearance in the delayed phase. The washout speed is conditioned by the degree of cellular differentiation of the tumour: the lower the differentiation, the faster the washout (Bhayana 2010; Boozari 2011).

The typical hallmarks for hepatocellular carcinoma at CEUS differ slightly to those of computed tomography/MRI; for CEUS, hallmarks are arterial hyperenhancement followed by late (more than 60 seconds) washout of a mild degree (Vogel 2018; Vogel 2019). This definition improves the capacity of CEUS to identify malignant lesions such as intrahepatic cholangiocarcinoma, which are often not identified as definitively malignant by computer tomography and MRI, using conventional vascular criteria. These new CEUS criteria for hepatocellular carcinoma have already been adopted by the Italian Association for the Study of the Liver (AISF) and by the American Association for the Study of the Liver (AASLD) (AISF 2013; EASL 2018; Kim 2017).

Differential diagnosis between intrahepatic cholangiocarcinoma and hepatocellular carcinoma in people with chronic liver disease or liver cirrhosis is a controversial issue. AASLD guidelines from 2011 removed CEUS from the diagnostic procedure for hepatocellular carcinoma due to the possibility of false-positive diagnosis of hepatocellular carcinoma in people with intrahepatic cholangiocarcinoma (Bruix 2011). The decision by the AASLD was based on an article stating that 47.6% of intrahepatic cholangiocarcinoma showed homogeneous intense enhancement in the arterial phase and washout in the delayed phase on CEUS; findings that were not distinguishable from hepatocellular carcinoma (Vilana 2010). However, further studies have shown that the enhancement pattern is somewhat different between the two tumours because hepatocellular carcinoma is more likely to appear as homogeneous or heterogeneous hyperenhancement, whereas intrahepatic cholangiocarcinoma often presents with peripheral rim-like enhancement or heterogeneous hypoenhancement in the arterial phase (Chen 2010). In the quantitative analysis with the time–intensity curve, intrahepatic cholangiocarcinoma showed a more rapid and marked washout than hepatocellular carcinoma, although there was significant overlap between the two (Kong 2014). Regarding the size of a suspected liver lesion, intrahepatic cholangiocarcinoma smaller than 3 cm is more likely to show homogeneous hyperenhancement in the arterial phase with delayed washout, a finding also typical of hepatocellular carcinoma (Chen 2010). Therefore, careful interpretation of CEUS is needed in smaller nodules developing in the setting of chronic hepatitis or cirrhosis (or both).

Clinical pathway

CEUS is a technique developed in Asia and Europe where its use is more widespread than in north America. The role of CEUS in the diagnostic pathway for the non-invasive diagnosis of hepatocellular carcinoma is not well defined, and recommendations concerning its use vary according to different clinical guidelines.

The two possible diagnostic pathways, illustrated in Figure 1 and Figure 2, are accepted and recommended by the Asian Pacific Association for the Study of the Liver (APASL) and by the European Association for the Study of Liver Disease (EASL) guidelines (EASL 2018; Omata 2017). On the contrary, the AASLD does not recommend the use of CEUS and claims the need of further studies (Heimbach 2018).

Figure 1. Flow diagram of the diagnostic pathway for the diagnosis of hepatocellular carcinoma, with contrast-enhanced ultrasound used after ultrasound or alpha-foetoprotein as add-on test after clinical assessment and abdominal ultrasound. OLT: orthotopic liver transplantation.

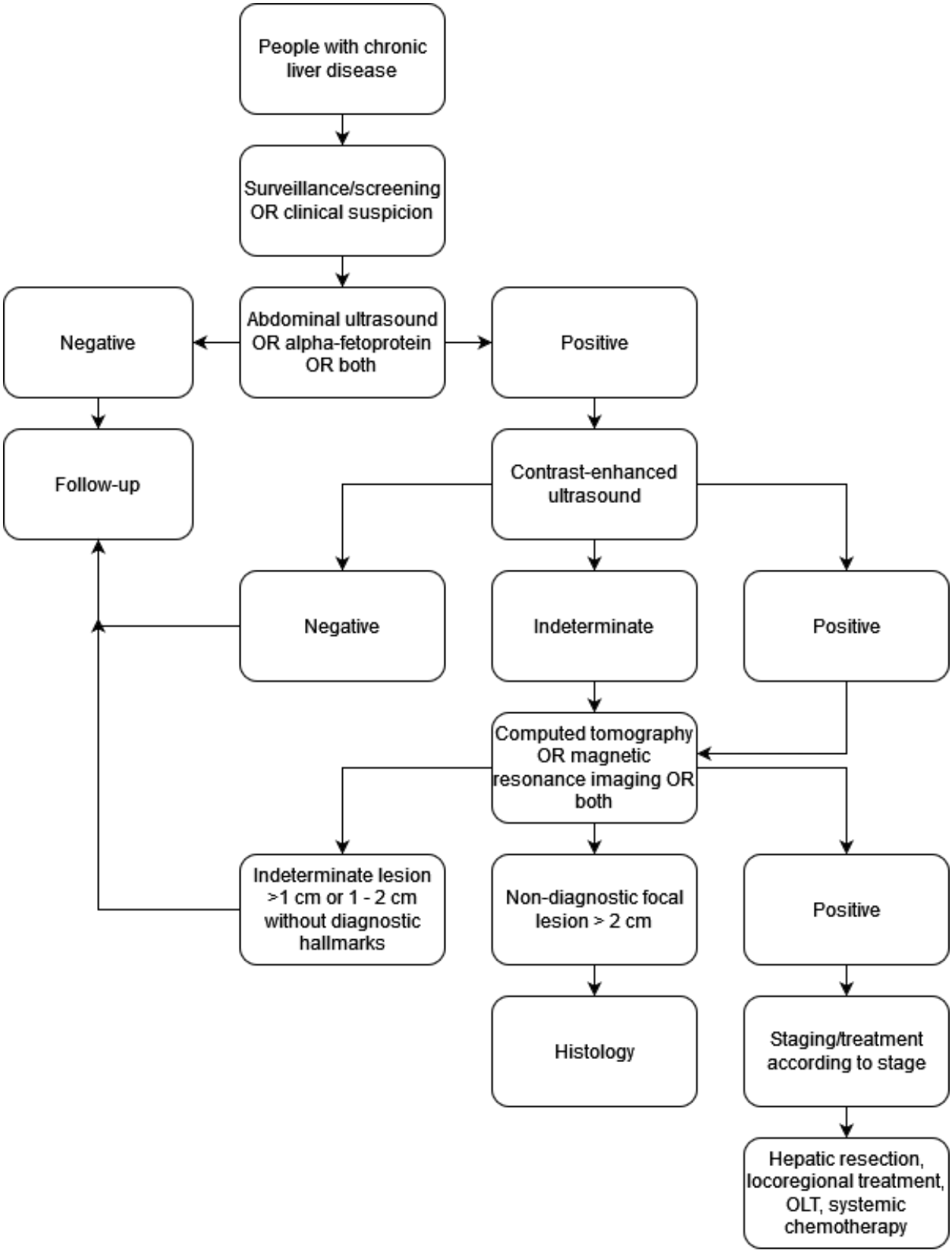
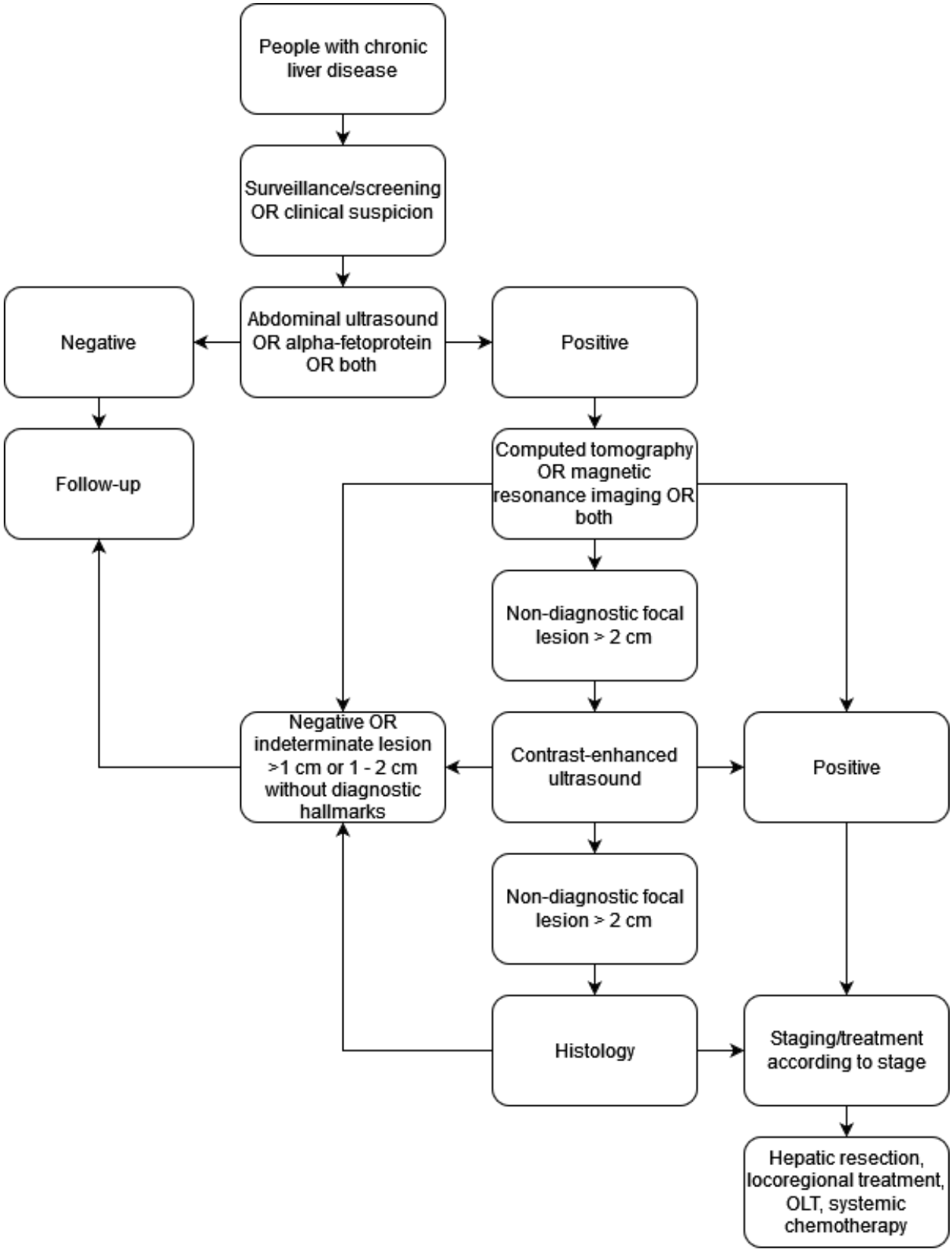


Figure 2. Flow diagram of the diagnostic pathway for the diagnosis of hepatocellular carcinoma, with contrast-enhanced ultrasound used as an add-on test after computed tomography or magnetic resonance imaging. OLT: orthotopic liver transplantation.



Prior test(s)

CEUS is performed after clinical assessment and abdominal ultrasound with the detection of a focal lesion suspected for hepatocellular carcinoma. For surveillance purposes, non-contrast abdominal ultrasound is recommended as a first-line imaging modality in people at risk for detection of hepatocellular carcinoma (EASL 2018; Heimbach 2018; Omata 2017). CEUS is also used as a diagnostic tool in people with clinical suspicion of hepatocellular carcinoma for detecting liver lesions. Furthermore, in the case of non-diagnostic results of computed tomography or MRI, CEUS can be used to reduce the need of histology. Alpha-foetoprotein can also be used prior to CEUS to assess the malignancy of a focal liver lesion.

Role of index test(s)

Proposed roles of CEUS are the following.

- Add-on test after clinical assessment and abdominal ultrasound and before further complex and expensive imaging techniques (computed tomography and MRI)

CEUS is used to further assess and characterise focal liver lesions detected with ultrasound, suspected for hepatocellular carcinoma either in surveillance programmes or in hospital settings in people with clinical suspicion.

Based on the CEUS findings, if the lesion has no clear features of hepatocellular carcinoma, unnecessary further examinations will be reduced. However, if the lesion has a malignant potential, further work-up (computed tomography or MRI) is warranted. In the case of false-positive results, patients have to undergo needless computed tomography or MRI testing; false-negative results have more severe consequences as hepatocellular carcinomas go undetected, especially early-stage hepatocellular carcinomas that are eligible for curative treatment.

- Add-on test after imaging techniques (computed tomography and MRI)

CEUS is also used in the case of non-diagnostic results of computed tomography or MRI. Further testing with liver biopsy is performed only in the case of non-diagnostic results. In this case, false-positive results are associated with surgical or medical treatment with a wrong indication, whereas false-negative results imply the missed detection of potentially curable early-stage hepatocellular carcinoma.

Alternative test(s)

Contrast-enhanced multiphasic multidetector computed tomography and contrast-enhanced MRI have been established as relevant non-invasive modalities for detection and evaluation of liver lesions (Lee 2012; O'Neill 2015). The ability to detect hepatocellular carcinoma rests on characterising the enhancement patterns in arterial, portal venous, and subsequent phases relative to the surrounding liver tissue. The differences in blood flow and extracellular volume between hepatocellular carcinoma and normal liver tissue lead to main radiological hallmarks. These are represented by an homogeneous (non-rim-like) arterial phase hyperenhancement suggesting tumoural neo-angiogenesis and subsequent non-peripheral washout with enhancing capsule in later phases, suggesting the presence of arteriovenous communications (Choi 2014; Hennedige 2012; LI-RADS 2017; Shah 2014). Computed tomography is a commonly used modality for diagnosing hepatocellular carcinoma due to its short acquisition time and high spatial resolution. However, MRI offers several beneficial features such as absence of X-ray radiation and combination of various sequences (multiphasic T1- and T2-weighted sequences, diffusion-weighted imaging, and apparent diffusion coefficient) in combination with the use of extracellular or hepatocellular (or both) gadolinium-based contrast agent (Arif-Tiwari 2014; Roberts 2018).

Two Cochrane systematic reviews recently published by our group assessed the role of computed tomography (Nadarevic 2021a) and MRI (Nadarevic 2021b) for the diagnosis of hepatocellular carcinoma in adults with chronic liver disease.

Rationale

Hepatocellular carcinoma is currently detected by liver ultrasound in people with normal or high alpha-foetoprotein during surveillance programmes for chronic liver disease. Following ultrasound, the diagnosis is usually confirmed with high levels of alpha-foetoprotein with or without CEUS, computed tomography, or MRI. Computed tomography and MRI are also appropriate for staging.

This systematic review represents a part of our series of systematic reviews about the diagnostic accuracy of the most commonly used modalities for diagnosing hepatocellular carcinoma in people with chronic liver disease. The first review aimed at assessing the diagnostic accuracy of ultrasound and AFP levels which are used as triage tests in the surveillance of hepatocellular carcinoma (Colli 2021). A further systematic review assessed computed tomography as a third-line imaging modality in characterising focal liver lesions (Nadarevic 2021a), and another systematic review assessed the diagnostic accuracy of MRI in hepatocellular carcinoma diagnosis (Nadarevic 2021b). The present review aims to assess the role of CEUS for the diagnosis of hepatocellular carcinoma either as an add-on test before computed tomography or MRI, or as an add-on test after computed tomography or MRI. In both cases, the index test, ensuring an adequate accuracy, can be useful to reduce further testing.

OBJECTIVES

To assess the diagnostic accuracy of contrast-enhanced ultrasound (CEUS) for the diagnosis of hepatocellular carcinoma of any size and at any stage in adults with chronic liver disease, in a surveillance programme or in a clinical setting.

Secondary objectives

To assess the diagnostic accuracy of CEUS for the diagnosis of resectable hepatocellular carcinoma in people with chronic liver disease. The definition of resectable hepatocellular carcinoma is a neoplasm amenable to surgical radical resection according to the Milan criteria, and according to current guidelines, it is a single lesion with a maximum diameter of less than 5 cm, or fewer than three lesions with a maximum diameter of 3 cm (EASL 2018; Heimbach 2018; Mazzaferro 1996; Omata 2017).

To identify potential sources of heterogeneity in the results, we investigated the effects of the following: inclusion of participants

with viral (hepatitis B surface viral and hepatitis C viral) aetiology; inclusion of participants without cirrhosis; study location; different role of CEUS in the diagnostic clinical pathway; different hepatocellular carcinoma stage; different reference standard; mean hepatocellular carcinoma diameter; prevalence of the target condition; and type of contrast media.

The use of LI-RADS classification as CEUS positivity criteria compared to studies using other definitions of positivity criteria has been added as post-hoc analysis.

METHODS

Criteria for considering studies for this review

Types of studies

We included studies that, irrespective of publication status (abstracts or full text) and language, had evaluated the diagnostic accuracy of CEUS for the diagnosis of hepatocellular carcinoma in people with chronic liver disease. In these studies, all participants should have undergone one of the acceptable reference standards (see Reference standards).

We considered studies of cross-sectional design including participants with clinical or sonographical suspicion of hepatocellular carcinoma. We excluded studies of case-control design that compared people with known hepatocellular carcinoma to matched controls. Case-control studies, in particularly those that use multiple-group design, are considered at high risk of bias and likely to inflate accuracy estimates (Bossuyt 2022; Colli 2014). We also excluded studies that analysed data only per lesion, rather than per participant, unless we received participant data from study authors.

Participants

We included men and women aged 18 years and older with chronic liver disease, irrespective of aetiology, severity of disease, and duration of illness, with suspicion of having hepatocellular carcinoma based on the results of ultrasound, computed tomography, or MRI. The review focused on diagnostic questions related to people with a first diagnosis of hepatocellular carcinoma.

People with previous diagnosis and treatment of hepatocellular carcinoma make up a distinct group, for which the diagnosis or natural history of hepatocellular carcinoma were modified. These people are not the focus of this review, and, therefore, we excluded studies that included such participants unless they represented less than 5% of all the included participants, or if investigators had presented data in a way that allowed us to isolate this group of participants from the remaining included participants.

Index tests

Contrast-enhanced ultrasound (CEUS) for detection of hepatocellular carcinoma in people with chronic liver disease.

CEUS is considered definitely positive with the following features.

- For blood-pool contrast agents (e.g. SonoVue, Levovist) when hyperenhancement in arterial phase and late washout (60 seconds or greater) features are detected (Kono 2017; LI-RADS 2017). Hyperenhancement should not be rim-like or peripheral discontinuous.
- For blood-pool and Kupffer contrast agent (e.g. Sonazoid) when abundant tumour vessels appearing as basket-like or irregular branched shapes from the periphery to the centre of the lesion, dense tumour staining in the early vascular phase, fast washout in the late vascular phase, and complete Kupffer defect are detected (Kudo 2008).

The results are dichotomous: positive if all the criteria are present, and non-diagnostic/negative if at least one criterion is absent.

Target conditions

- Hepatocellular carcinoma of any size and at any stage.
- Resectable hepatocellular carcinoma (see Secondary objectives above).

Reference standards

Accepted reference standard for the diagnosis of hepatocellular carcinoma included the following

For studies assessing the role of CEUS as an add-on test after ultrasound and before computed tomography or MRI, we accepted one of the following:

- typical characteristics on cross-sectional multiphasic contrast computed tomography or MRI with a follow-up period of at least six months, to allow the confirmation of an initial negative result of computed tomography or MRI;
- the pathology of the explanted liver in case of transplantation;
- the histology of resected focal liver lesion(s), or the histology of resected or biopsied focal liver lesion(s) and a follow-up period of at least six months to exclude the presence of focal lesions not detected by the index test;
- a combination of histology and imaging techniques (histology plus computed tomography or MRI).

For studies assessing the role of CEUS as an add-on test after non-diagnostic computed tomography or MRI results, we accepted either of the following:

- the pathology of the explanted liver in case of transplantation;
- the histology of resected focal liver lesion(s), or the histology of resected or biopsied focal liver lesion(s) and a follow-up period of at least six months to exclude the presence of focal lesions not detected by the index test.

All the accepted reference standards are currently used. The pathology of the explanted liver can be regarded as perfectly accurate, but it is obviously possible only when all the included participants undergo liver transplantation; therefore, the setting does not correspond to the clinical question as only people with advanced and decompensated liver disease can be candidates for OLT. Computed tomography and MRI are not perfectly accurate as the pathology of the explanted liver, but their accuracy is considered sufficient to guide further clinical decisions; moreover, an appropriate follow-up is required to confirm a negative result. The histology of resected specimen or of lesion biopsy may have false-negative results and requires follow-up to exclude the presence of hepatocellular carcinoma undetected by CEUS. In order to minimise verification bias, we included only studies in which all participants underwent one of the acceptable reference standards; however, using histology, a differential verification is unavoidable

and an appropriate follow-up is required to confirm their negative results. We evaluated different reference standards as possible sources of heterogeneity.

Search methods for identification of studies

Electronic searches

We searched The Cochrane Hepato-Biliary Group Controlled Trials Register and The Cochrane Hepato-Biliary Group Diagnostic Test of Accuracy Studies Register. The Cochrane Hepato-Biliary Group Information Specialist searched both internally via the Cochrane Register of Studies Web on 5 November 2021. We also searched the Cochrane Library (2021, Issue 11), MEDLINE Ovid (1946 to 5 November 2021), Embase Ovid (1974 to 5 November 2021), LILACS (Bireme; 1982 to 5 November 2021), Science Citation Index – Expanded (1900 to 5 November 2021), and Conference Proceedings Citation Index – Science (1990 to 5 November 2021). The latter two were searched simultaneously through Web of Science. We applied no time, language, or document type restrictions.

Appendix 1 gives the search strategies with the date ranges of the searches.

Searching other resources

We manually searched articles retrieved from electronic databases and relevant review articles for additional studies. We sought information on unpublished studies by contacting experts in the field. In addition, we handsearched abstract books from meetings of the AASLD, EASL, and APASL held over the 10 years prior to the search. We also searched other types of grey literature in the System for Information on Grey Literature in Europe 'OpenGrey' (www.opengrey.eu/; date of search 5 November 2021).

Data collection and analysis

We followed available guidelines as provided in the *Cochrane Handbook for Diagnostic Test of Accuracy Reviews* (Macaskill 2022).

Selection of studies

We used Covidence to manage the selection of studies (Covidence 2019). Two review authors (MF and CM) independently scrutinised titles and abstracts identified by the electronic literature searches to identify potentially eligible studies. We selected any citation, identified by either of the two review authors, as potentially eligible for full-text review. The same review authors independently assessed full-text papers for study eligibility, using predefined inclusion and exclusion criteria. We resolved any discrepancies by discussion. We recorded all studies excluded after full-text assessment and their reasons for exclusion in the Characteristics of excluded studies table, and we illustrated the study selection process using a PRISMA diagram (McInnes 2018; Figure 3).

Figure 3. Study flow diagram. Date of search 5 November 2021

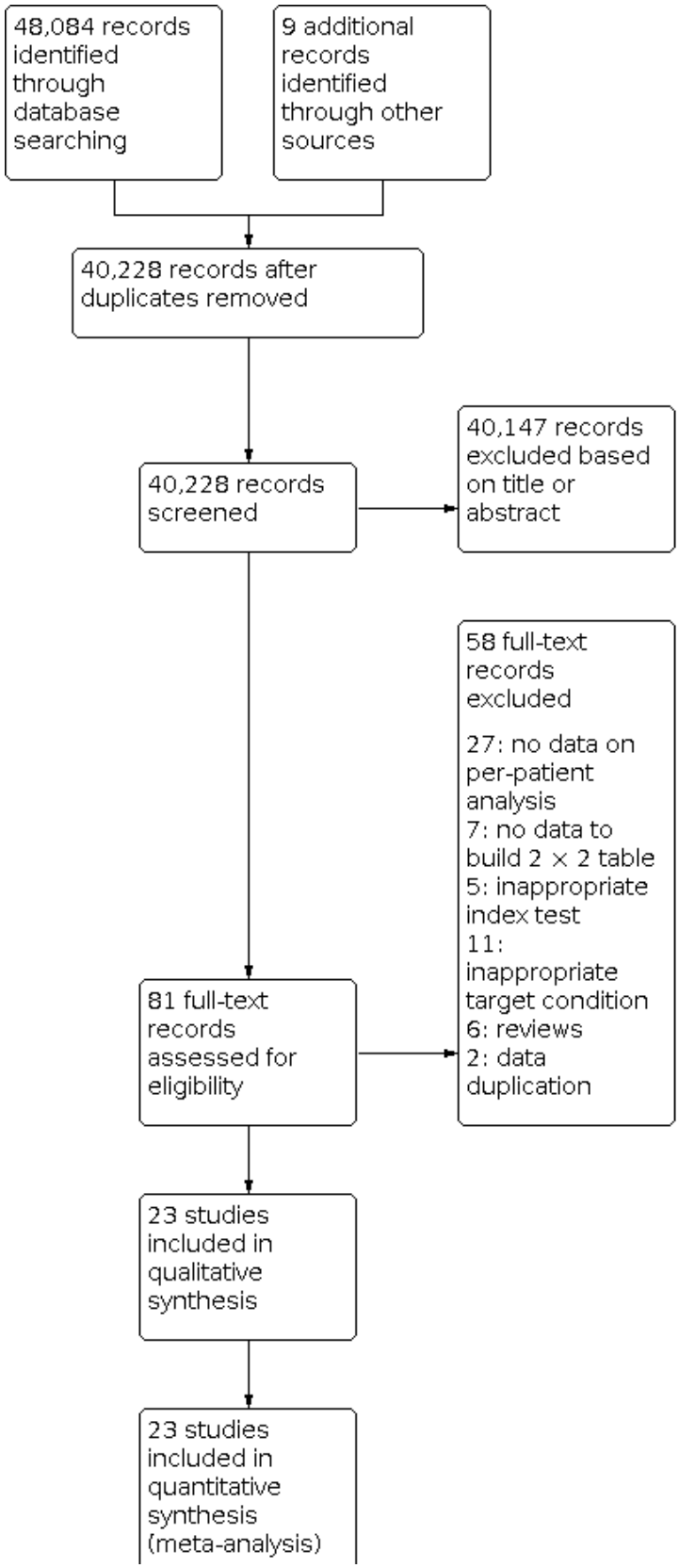
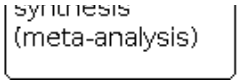


Figure 3. (Continued)



Where studies had multiple publications, we collated the reports of the same study so that each study, rather than each report, was the unit of interest for the review, and such studies had a single identifier with multiple references.

Data extraction and management

Two review authors (MF and CM), working as a pair, completed a piloted data extraction form for each included study. Each review author independently extracted study data. In cases of discordance, we reached consensus through discussion.

We retrieved the following data and reported them in the Characteristics of included studies table:

- general information: title, journal, year, publication type, study characteristics and design, and direction of data collection (prospective versus retrospective);
- sample size: number of participants meeting the criteria and total number of participants assessed;
- baseline characteristics: baseline diagnosis, age, sex, race, presence of cirrhosis, and mean diameter of hepatocellular carcinoma;
- index test with predefined positivity criteria and its role in the clinical pathway;
- reference standard tests;
- numbers of true-positive, true-negative, false-positive, and false-negative findings. We extracted these data for the two target conditions (hepatocellular carcinoma of any size, stage, and resectable hepatocellular carcinoma);
- information needed to assess bias risk and certainty of evidence;
- study correspondence author and email/address.

We summarised the data from each study in 2 × 2 tables (true positive, false positive, false negative, true negative), according to the index tests considered, and we entered the data into Review Manager 5 (Review Manager 2020).

Missing data

We contacted primary authors by email to ask for missing data needed to complete the 2 × 2 tables. If we received no reply, we sent a second email after two weeks. If no reply was received, we excluded the study in question for missing data.

Assessment of methodological quality

Two review authors (MF and CM) independently assessed the risk of bias of included studies and applicability of their results using QUADAS-2 (revised tool for quality assessment of diagnostic accuracy studies) (Whiting 2011). In cases of discordance, we reached a consensus through discussion. We addressed aspects of study quality involving the participant spectrum, index tests, target conditions, reference standards, and flow and timing. We classified a study as having high risk of bias if at least one of the domains of QUADAS-2 was at high risk of bias. This assessment concerned the primary objective (i.e. the diagnostic accuracy for hepatocellular carcinoma of any size and at any stage). We also performed an assessment for the secondary objective (i.e. the diagnostic accuracy for resectable hepatocellular carcinoma), using the same signalling questions as for the primary objective.

Statistical analysis and data synthesis

First, we performed a graphical descriptive analysis of the included studies. We presented forest plots (sensitivity and specificity separately, with their 95% confidence intervals (CIs)), and we provided a graphical representation of studies in the receiver operating characteristic (ROC) space (sensitivity against 1 – specificity). Second, we performed the meta-analyses using the bivariate model and provided estimates of summary sensitivity and specificity. We used the summary estimates obtained from the fitted models to calculate summary estimates of positive (LR+) and negative (LR–) likelihood ratios.

We anticipated that ultrasound visualisation and hence contrast disposition can often be suboptimal due to patient characteristics; hence, lack of reporting the number of non-evaluable results or excluding non-evaluable exams from analyses could overestimate the accuracy of CEUS. The clinical consequence of non-evaluable results is the need of further testing (computed tomography, MRI, or biopsy). Including non-evaluable results and considering them as false positives and false negatives seems to summarise the diagnostic accuracy and true clinical potential most adequately. Hence, in case of non-evaluable index test results, we planned to analyse data according to the intention-to-diagnose principle (Schuetz 2012), also described as the worst-case scenario (Cohen 2016). However, we had insufficient data to carry out this analysis (see Differences between protocol and review).

We performed all statistical analyses using SAS statistical software, release 9.4 (SAS Institute Inc, Cary, NC, USA) and macro METADAS (Macaskill 2022).

Investigations of heterogeneity

We investigated the effects of the following potential sources of heterogeneity:

- date of study publication;
- inclusion of participants without cirrhosis: studies including 10% or greater participants without cirrhosis compared to studies including less than 10% participants without cirrhosis (categorical covariate);
- study location (population differences): studies conducted in Americas compared to Europe compared to Asia (categorical covariate);
- different role of CEUS in the diagnostic clinical pathway: studies using CEUS after ultrasound compared to studies using CEUS after computed tomography and MRI (categorical covariate);
- different hepatocellular carcinoma stage: studies with 20% or greater of resectable hepatocellular carcinoma compared to studies with less than 20% of resectable hepatocellular carcinoma (categorical covariate);

- different liver cirrhosis aetiology: hepatitis C or hepatitis B virus-associated cirrhosis compared to all other aetiologies (categorical covariate);
- different reference standard: studies using the pathology of the explanted liver compared to liver biopsy compared to other reference standards (categorical covariate);
- mean diameter of the cancer (continuous covariate);
- prevalence of the target condition (continuous covariate);
- type of contrast media: blood-pool versus blood-pool/Kupffer cell (categorical covariate).

We chose the above listed variables for the following reasons. Due to advancements in technology and change in diagnostic criteria, we considered the date of study publication. The proportion of participants without cirrhosis is relevant because hepatocellular carcinoma in the absence of cirrhosis has different computed tomography characteristics, prognosis, and treatment. In epidemiological studies, this proportion is usually less than 10% (Forner 2018). The epidemiological, radiological, and clinical characteristics of hepatocellular carcinoma differ in Asia and western countries. The hepatocellular carcinoma prevalence in included studies can change according to selection and epidemiology. Assessing the accuracy of CEUS in a different role in the diagnostic pathway implies different participant characteristics and reference standards, and different results are to be expected. The proportion of resectable hepatocellular carcinoma found in the studies reflects different epidemiology and participant selection. The clinical and radiological characteristics of hepatocellular carcinoma vary according to the aetiology of the underlying liver disease, mainly in the case of chronic hepatitis C virus or hepatitis B virus infection compared to other aetiologies. The accuracy of CEUS may vary according to the reference standard used, the diameter of the neoplastic lesion, the type of contrast used, and the definition of positivity criteria. In addition, as the accuracy of CEUS may vary according to the different definition of positivity criteria, we investigated the effect of the use of LI-RADS classification compared to studies using other definitions of positivity criteria in a post hoc analysis.

We estimated effects by adding covariates to the bivariate model. We assessed the statistical significance of the covariate effect using the log-likelihood ratio test for comparison of models with and without the covariate term. We considered P values less than 0.05 as two-sided and statistically significant.

Sensitivity analyses

We assessed effects of risk of bias of included studies on diagnostic accuracy by performing a sensitivity analysis from which we excluded studies with the following characteristics:

- studies classified as at high risk of bias, that is, studies having high risk of bias in at least one of the domains of QUADAS-2 (Appendix 2). In addition, we defined the following signalling questions as most relevant, and assessed them in separate sensitivity analyses, excluding studies with 'No' or 'Unclear' answers.
  - “Were the positivity criteria defined?”
  - "Were the reference standard results interpreted without the knowledge of the results of the index test?"
  - "Were participants with non-evaluable result of the index test included and analysed according to the intention-to-diagnose principle (non-evaluable results considered as false)?"

We conducted a sensitivity analysis in excluding studies published only in abstract or letter form.

Assessment of reporting bias

We did not test for publication bias due to the lack of validated methods for diagnostic test accuracy reviews.

Summary of findings table and certainty of the evidence

We prepared two summary of findings tables, using the GRADE assessment (GRADEpro GDT). A summary of findings table in a diagnostic test accuracy review requires presentation of the following information: the question (population, setting, index test(s), role and purpose of the test(s), reference standard; the accuracy estimates (sensitivity and specificity); available data, certainty of evidence, and practical implications such as prevalence estimates or normalised frequencies (e.g. true positives, true negatives, false positives, and false negatives, in a hypothetical population (Schünemann 2020a; Schünemann 2020b). In our review, we presented the accuracy estimates for 1. hepatocellular carcinoma of any size and at any stage; and for 2. resectable hepatocellular carcinoma. We also presented the estimates with their 95% CIs.

As recommended, we rated the certainty of evidence as high (not downgraded), moderate (downgraded by one level), low (downgraded by two levels), or very low (downgraded by more than two levels) based on five domains: risk of bias, indirectness, inconsistency, imprecision, and publication bias (Balshem 2011; GRADEpro GDT; Schünemann 2008; Schünemann 2016; Schünemann 2020a; Schünemann 2020b).

The certainty of evidence starts as high when there are high-quality observational studies (cross-sectional or cohort studies) that enrolled participants with diagnostic uncertainty. When we found a reason for downgrading, we used our judgement to classify the reason as either serious (downgraded by one level) or very serious (downgraded by two levels).

We applied the GRADE judgements for the GRADE domains as following:

- risk of bias: we used QUADAS-2 to assess risk of bias;
- indirectness: for concerns of applicability we identified important differences between the populations studied (e.g. the spectrum of disease), the setting, and the index test;
- inconsistency: we carried out prespecified analyses to investigate potential sources of heterogeneity, and we downgraded the evidence when we could not explain inconsistency in the accuracy estimates;
- imprecision: we considered the CIs of sensitivity and specificity estimates and the unexplained heterogeneity of the results;
- publication bias: we did not evaluate publication bias as such validated methods for diagnostic test accuracy reviews are lacking.

We justified all decisions to downgrade the certainty of evidence of studies using the footnotes of each table. We made comments to aid reader's understanding of the review where necessary.

RESULTS

Results of the search

We identified 48,084 records by searching the following databases on 5 November 2021: The Cochrane Hepato-Biliary Group Controlled Trials Register (703 records), The Cochrane Hepato-Biliary Group Diagnostic Test of Accuracy Studies Register (13), the Cochrane Library (284 reviews, 80 protocols, 4479 Cochrane Central Register of Controlled Trials), MEDLINE Ovid (21,759), Embase Ovid (15,173), LILACS (196), and Science Citation Index – Expanded with Conference Proceedings Citation Index – Science (5397). We retrieved nine additional records through handsearching. After exclusion of 7864 duplicates, 40,228 records remained for possible eligibility. After reading the title and the abstract of these records, we excluded 40,147 records, as they did not meet the inclusion criteria. We retrieved full texts of 81 studies and excluded 58 studies for various reasons (see Characteristics of excluded studies table). Of the 58 excluded studies, we indicated those studies that would have fulfilled the inclusion criteria if they had provided data for the 2 × 2 table. Hence, our review includes 23 records reporting data on 23 studies with 6546 participants (de Sio 2014; Di Carlo 2012; Ding 2021; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Hwang 2021; Kan 2010; Kang 2020; Kudo 2019; Li 2019; Sangiovanni 2010; Schellhaas 2017; Shin 2015; Sporea 2019; Strobel 2021; Sugimoto 2020; Tan 2020; Terzi 2018; Wang 2006; Zuo 2021; Figure 3). Two of the included studies were published in an abstract format only (Di Carlo 2012; Giorgio 2010).

Our inclusion criteria had no language restrictions, which resulted in retrieving three full-text study articles published in non-English languages (Chen 2005; Strunk 2005; Zeng 2006). Two members of the hospital staff who speak German and Chinese translated these. None of these studies fulfilled the inclusion criteria.

Nine studies did not report the 2 × 2 table directly, and we could not obtain estimates based on the available data. We asked the authors of these studies to provide data, and in four cases they answered and provided the requested data (de Sio 2014; Sangiovanni 2010; Sporea 2019; Terzi 2018).

Methodological quality of included studies

We reported in detail the results of the quality assessment of the included studies in the Characteristics of included studies table and we summarised this information in Figure 4 and Figure 5.

Figure 4. Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies.

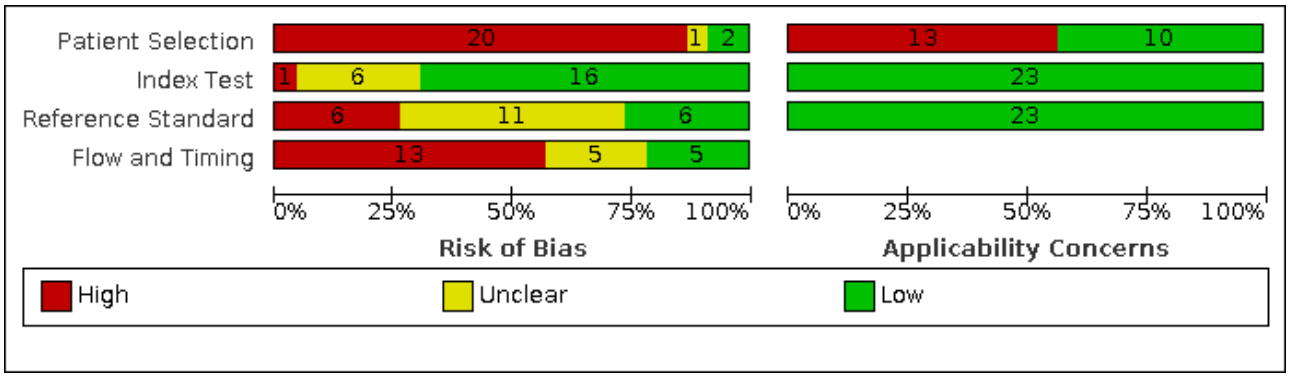
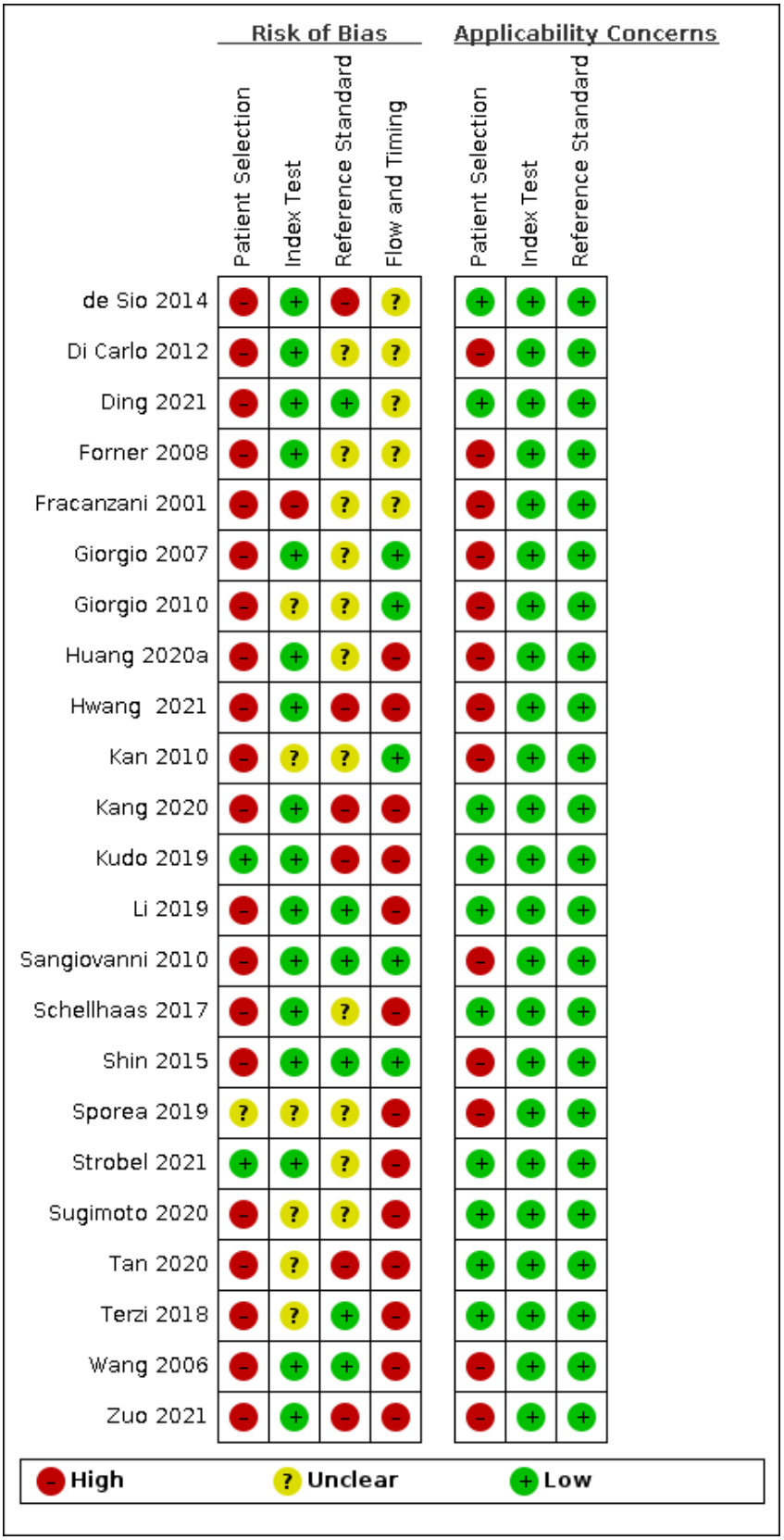


Figure 5. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study.



Patient selection

Risk of bias

Hepatocellular carcinoma of any size and stage: two studies were at low risk of bias for this domain (Kudo 2019; Strobel 2021). We judged one study at unclear risk of bias because there was no information on participants selection (Sporea 2019).

We judged 20 studies at high risk of bias (de Sio 2014; Di Carlo 2012; Ding 2021; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Hwang 2021; Kan 2010; Kang 2020; Li 2019; Sangiovanni 2010; Schellhaas 2017; Shin 2015; Sugimoto 2020; Tan 2020; Terzi 2018; Wang 2006; Zuo 2021): three due to non-consecutive participant enrolment (de Sio 2014; Kan 2010; Schellhaas 2017), nine due to the inclusion of participants based on the availability of test results (Ding 2021; Fracanzani 2001; Huang 2020a; Kang 2020; Li 2019; Sugimoto 2020; Tan 2020; Terzi 2018; Zuo 2021), and 11 due to the inappropriate exclusion criteria such as exclusion based on lesion diameter (Di Carlo 2012; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Hwang 2021; Kan 2010; Sangiovanni 2010; Shin 2015; Wang 2006).

Resectable hepatocellular carcinoma: we judged six studies at low risk of bias (Forner 2008; Giorgio 2007; Giorgio 2010; Hwang 2021; Kudo 2019; Sangiovanni 2010), six studies at high risk of bias (Fracanzani 2001; Huang 2020a; Kan 2010; Shin 2015; Sugimoto 2020; Wang 2006), and one at unclear risk (Di Carlo 2012).

Applicability

Hepatocellular carcinoma of any size and stage: we judged 10 studies at low concern for applicability (de Sio 2014; Ding 2021; Kang 2020; Kudo 2019; Li 2019; Schellhaas 2017; Strobel 2021; Sugimoto 2020; Tan 2020; Terzi 2018). The remaining 13 studies were at high concern for applicability: one study enrolled 64.7% of participants without chronic liver disease (Sporea 2019), 10 studies excluded participants based on lesion diameter (Di Carlo 2012; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Hwang 2021; Sangiovanni 2010; Shin 2015; Wang 2006), one study selected participants based on ultrasound and computed tomography findings (Zuo 2021), and one study excluded participants on both the basis of lesion diameter and ultrasound and computed tomography findings (Kan 2010).

Resectable hepatocellular carcinoma*:* we judged 10 studies at low concern for applicability as they correctly included only participants with chronic liver disease and nodules with diameter less than 3 cm (Di Carlo 2012; Forner 2008; Giorgio 2007; Giorgio 2010; Huang 2020a; Kudo 2019; Sangiovanni 2010; Shin 2015; Sugimoto 2020; Wang 2006). We judged three studies at high concern for applicability as the participants included in these studies did not match the review question (e.g. the participants were selected based on other test results) (Fracanzani 2001; Hwang 2021; Kan 2010).

Index test

Risk of bias

Hepatocellular carcinoma of any size and stage: we judged 16 studies at low risk of bias that clearly predefined CEUS positivity criteria and blinding to reference standard results (de Sio 2014; Di Carlo 2012; Ding 2021; Forner 2008; Giorgio 2007; Huang 2020a; Hwang 2021; Kang 2020; Kudo 2019; Li 2019; Sangiovanni 2010; Schellhaas 2017; Shin 2015; Strobel 2021; Wang 2006; Zuo 2021). Six studies were at unclear risk of bias (Giorgio 2010; Kan 2010; Sporea 2019; Sugimoto 2020; Tan 2020; Terzi 2018) due to unclear blinding to reference standard results. One study was at high risk of bias as details regarding the index test were not given (Fracanzani 2001).

Resectable hepatocellular carcinoma: we judged nine studies at low risk of bias (Di Carlo 2012; Forner 2008; Giorgio 2007; Huang 2020a; Hwang 2021; Kudo 2019; Sangiovanni 2010; Shin 2015; Wang 2006), one at high risk of bias (Fracanzani 2001), and three at unclear risk of bias (Giorgio 2010; Kan 2010; Sugimoto 2020).

Applicability

Hepatocellular carcinoma of any size and stage: we judged all studies at low concern as regards applicability in this domain.

Resectable hepatocellular carcinoma*:* we judged all studies at low concern as regards applicability in this domain.

Reference standard

Risk of bias

Hepatocellular carcinoma of any size and stage: we judged six studies at low risk of bias (Ding 2021; Li 2019; Sangiovanni 2010; Shin 2015; Terzi 2018; Wang 2006), 11 studies at unclear risk of bias (Di Carlo 2012; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Kan 2010; Schellhaas 2017; Sporea 2019; Strobel 2021; Sugimoto 2020), and six studies at high risk of bias (de Sio 2014; Hwang 2021; Kang 2020; Kudo 2019; Tan 2020; Zuo 2021) regarding the reference standard domain.

In 10 studies, the reference standard was histology in all participants (de Sio 2014; Di Carlo 2012; Ding 2021; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Li 2019; Sangiovanni 2010; Shin 2015), in 12 studies, the reference standard was a combination of the following options: histology in a subset of participants and imaging in another (Huang 2020a; Hwang 2021; Kang 2020; Kudo 2019; Schellhaas 2017; Sporea 2019; Strobel 2021; Sugimoto 2020; Tan 2020; Terzi 2018; Wang 2006; Zuo 2021), and in one study the reference standard was dynamic-computed tomography (Kan 2010).

Five studies interpreted the reference standard results without the knowledge of the results of the index test (Ding 2021; Li 2019; Sangiovanni 2010; Terzi 2018; Wang 2006).

Resectable hepatocellular carcinoma*:* we judged three studies at low risk of bias (Sangiovanni 2010; Shin 2015; Wang 2006), two studies at high risk of bias (Hwang 2021; Kudo 2019), and the remaining eight studies at unclear risk of bias (Di Carlo 2012; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Kan 2010; Sugimoto 2020).

Applicability

Hepatocellular carcinoma of any size and stage: we judged all studies at low concern as regards applicability in the reference standard domain.

Resectable hepatocellular carcinoma: we judged all studies at low concern as regards applicability in this domain.

Flow and timing

Risk of bias

Carcinoma of any size and stage: we judged five studies at low risk of bias (Giorgio 2007; Giorgio 2010; Kan 2010; Sangiovanni 2010; Shin 2015), five studies at unclear risk of bias (de Sio 2014; Di Carlo 2012; Ding 2021; Forner 2008; Fracanzani 2001), and 13 studies at high risk of bias (Huang 2020a; Hwang 2021; Kang 2020; Kudo 2019; Li 2019; Schellhaas 2017; Sporea 2019; Strobel 2021; Sugimoto 2020; Tan 2020; Terzi 2018; Wang 2006; Zuo 2021). The reason for judging 12 studies at high risk of bias was that not all participants underwent the same reference standard (Huang 2020a; Hwang 2021; Li 2019; Kang 2020; Kudo 2019; Schellhaas 2017; Sporea 2019; Strobel 2021; Sugimoto 2020; Tan 2020; Terzi 2018; Zuo 2021). The remaining study was at high risk of bias because some participants were excluded from the analyses (Wang 2006).

Resectable hepatocellular carcinoma*:* we judged at five studies at low risk of bias (Giorgio 2007; Giorgio 2010; Kan 2010; Sangiovanni 2010; Shin 2015), five at high risk of bias (Huang 2020a; Hwang 2021; Kudo 2019; Sugimoto 2020; Wang 2006), and three at unclear risk (Di Carlo 2012; Forner 2008; Fracanzani 2001).

Overall assessment

Hepatocellular carcinoma of any size and stage: we judged all the included studies at high risk of bias for at least one domain and 13 studies at high concern for applicability (Di Carlo 2012; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Hwang 2021; Kan 2010; Sangiovanni 2010; Shin 2015; Sporea 2019; Wang 2006; Zuo 2021).

Resectable hepatocellular carcinoma: we judged 12 studies at high or unclear risk of bias for at least one domain (Di Carlo 2012; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Hwang 2021; Kan 2010; Kudo 2019; Shin 2015; Sugimoto 2020; Wang 2006), and three studies at high concern for applicability (Fracanzani 2001; Hwang 2021; Kan 2010).

Findings

Twenty-three studies with 6546 participants provided data for assessing CEUS for the diagnosis of hepatocellular carcinoma. The studies were published from 2001 to 2021. The median prevalence of the target disease was 69% (interquartile range 60% to 80%; range 9% to 92%). The median age of the participants was 63 years (interquartile range 57 to 66 years; range 52 to 71 years) and the median proportion of men was 71% (interquartile range 65% to 58%; range 44% to 85%).

Table 1 shows the characteristics of participants, study location settings, index test, contrast media, hepatocellular carcinoma diameter, and reference standard in the included studies. Ten studies used Liver Imaging Reporting and Data System (LI-RADS, Contrast-Enhanced UltraSound Liver Imaging Reporting and Data System) that is a standardised system for technique, interpretation, reporting, and data collection for CEUS examinations in people at risk of developing hepatocellular carcinoma, and 13 studies used other classifications (AASLD, EASL, Esculap (Erlanger Synopsis for Contrast-enhanced Ultrasound for Liver lesion Assessment in Patients at risk); EFSUMB (European Federation of Societies for Ultrasound in Medicine and Biology); KCLA-NCC, (Korean Liver Cancer Association and National Cancer Center)). No studies reported the number of examinations not performed due to contraindications. Two studies reported the presence of adverse effects (Giorgio 2010; Kudo 2019). In Giorgio 2010, there were no adverse effects observed, and in Kudo 2019, one participant experienced a grade-1 rash attributed to Sonazoid. No other studies reported adverse effects to CEUS. Only one study reported the number of uninterpretable index test results: two non-evaluable cases (Kang 2020).

Eight studies reported no information about the authors' possible conflicts of interest (Di Carlo 2012; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Kan 2010; Li 2019; Tan 2020; Wang 2006), two studies reported possible conflict of interest (Kudo 2019; Terzi 2018), and 13 reported no possible conflict (de Sio 2014; Ding 2021; Forner 2008; Huang 2020a; Hwang 2021; Kang 2020; Sangiovanni 2010; Schellhaas 2017; Shin 2015; Sporea 2019; Strobel 2021; Sugimoto 2020; Zuo 2021).

The included studies used three different reference standards; 10 studies used biopsy (de Sio 2014; Di Carlo 2012; Ding 2021; Forner 2008; Giorgio 2007; Giorgio 2010; Li 2019; Sangiovanni 2010; Schellhaas 2017; Shin 2015), 12 used a combined reference standard (biopsy plus imaging) (Fracanzani 2001; Huang 2020a; Hwang 2021; Kang 2020; Kudo 2019; Sporea 2019; Strobel 2021; Sugimoto 2020; Tan 2020; Terzi 2018; Wang 2006; Zuo 2021), and one used computed tomography (Kan 2010).

The forest plot of sensitivity and specificity with their 95% CIs is represented in Figure 6. Considering hepatocellular carcinoma at any size and stage, the reported sensitivity in the 23 studies ranged from 29% to 100% and the specificity ranged from 63% to 100%. Summary sensitivity was 77.8% (95% CI 69.4% to 84.4%), and summary specificity 93.8% (95% CI 89.1% to 96.6%), LR+ was 12.6 (95% CI 7.15 to 22.2) and LR– was 0.23 (95% CI 0.16 to 0.33). Figure 7 shows a graphical representation of studies in the ROC space (sensitivity against 1 – specificity); the prediction region was considerably wide suggesting heterogeneity between studies.

Figure 6. Forest plots of sensitivity and specificity of contrast-enhanced ultrasound (CEUS) for detection of hepatocellular carcinoma of any size and stage against different reference standards in 23 studies in alphabetical order. Reference standards were the histology of resected focal liver lesions or the histology of biopsied focal liver lesions with a follow-up period of at least six months, or typical characteristics on cross-sectional multiphasic contrast computed tomography (CT) or magnetic resonance imaging (MRI) with a follow-up period of at least six months, to allow the confirmation of an initial negative result of CT or MRI; values between square brackets are the 95% confidence intervals (CIs) of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line). FN: false negative; FP: false positive; TN: true negative; TP: true positive

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
de Sio 2014	220	0	10	18	0.96 [0.92, 0.98]	1.00 [0.81, 1.00]
Di Carlo 2012	31	2	29	27	0.52 [0.38, 0.65]	0.93 [0.77, 0.99]
Ding 2021	144	2	79	39	0.65 [0.58, 0.71]	0.95 [0.83, 0.99]
Forner 2008	31	2	29	27	0.52 [0.38, 0.65]	0.93 [0.77, 0.99]
Fracanzani 2001	19	6	1	15	0.95 [0.75, 1.00]	0.71 [0.48, 0.89]
Giorgio 2007	39	2	9	23	0.81 [0.67, 0.91]	0.92 [0.74, 0.99]
Giorgio 2010	22	1	3	10	0.88 [0.69, 0.97]	0.91 [0.59, 1.00]
Huang 2020a	76	2	28	69	0.73 [0.63, 0.81]	0.97 [0.90, 1.00]
Hwang 2021	66	10	23	23	0.74 [0.64, 0.83]	0.70 [0.51, 0.84]
Kan 2010	66	2	2	9	0.97 [0.90, 1.00]	0.82 [0.48, 0.98]
Kang 2020	48	0	31	24	0.61 [0.49, 0.72]	1.00 [0.86, 1.00]
Kudo 2019	28	11	0	270	1.00 [0.88, 1.00]	0.96 [0.93, 0.98]
Li 2019	723	52	90	213	0.89 [0.87, 0.91]	0.80 [0.75, 0.85]
Sangiovanni 2010	12	0	30	22	0.29 [0.16, 0.45]	1.00 [0.85, 1.00]
Schellhaas 2017	63	4	24	9	0.72 [0.62, 0.81]	0.69 [0.39, 0.91]
Shin 2015	28	0	9	9	0.76 [0.59, 0.88]	1.00 [0.66, 1.00]
Sporea 2019	525	21	160	1356	0.77 [0.73, 0.80]	0.98 [0.98, 0.99]
Strobel 2021	280	34	98	58	0.74 [0.69, 0.78]	0.63 [0.52, 0.73]
Sugimoto 2020	45	3	19	37	0.70 [0.58, 0.81]	0.93 [0.80, 0.98]
Tan 2020	32	1	4	8	0.89 [0.74, 0.97]	0.89 [0.52, 1.00]
Terzi 2018	342	0	231	133	0.60 [0.56, 0.64]	1.00 [0.97, 1.00]
Wang 2006	10	1	8	11	0.56 [0.31, 0.78]	0.92 [0.62, 1.00]
Zuo 2021	80	7	19	107	0.81 [0.72, 0.88]	0.94 [0.88, 0.97]

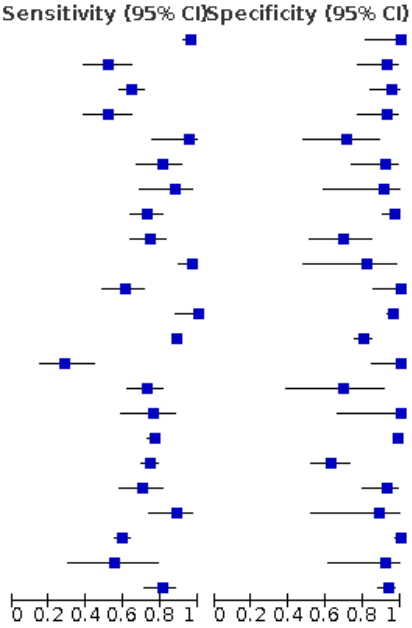
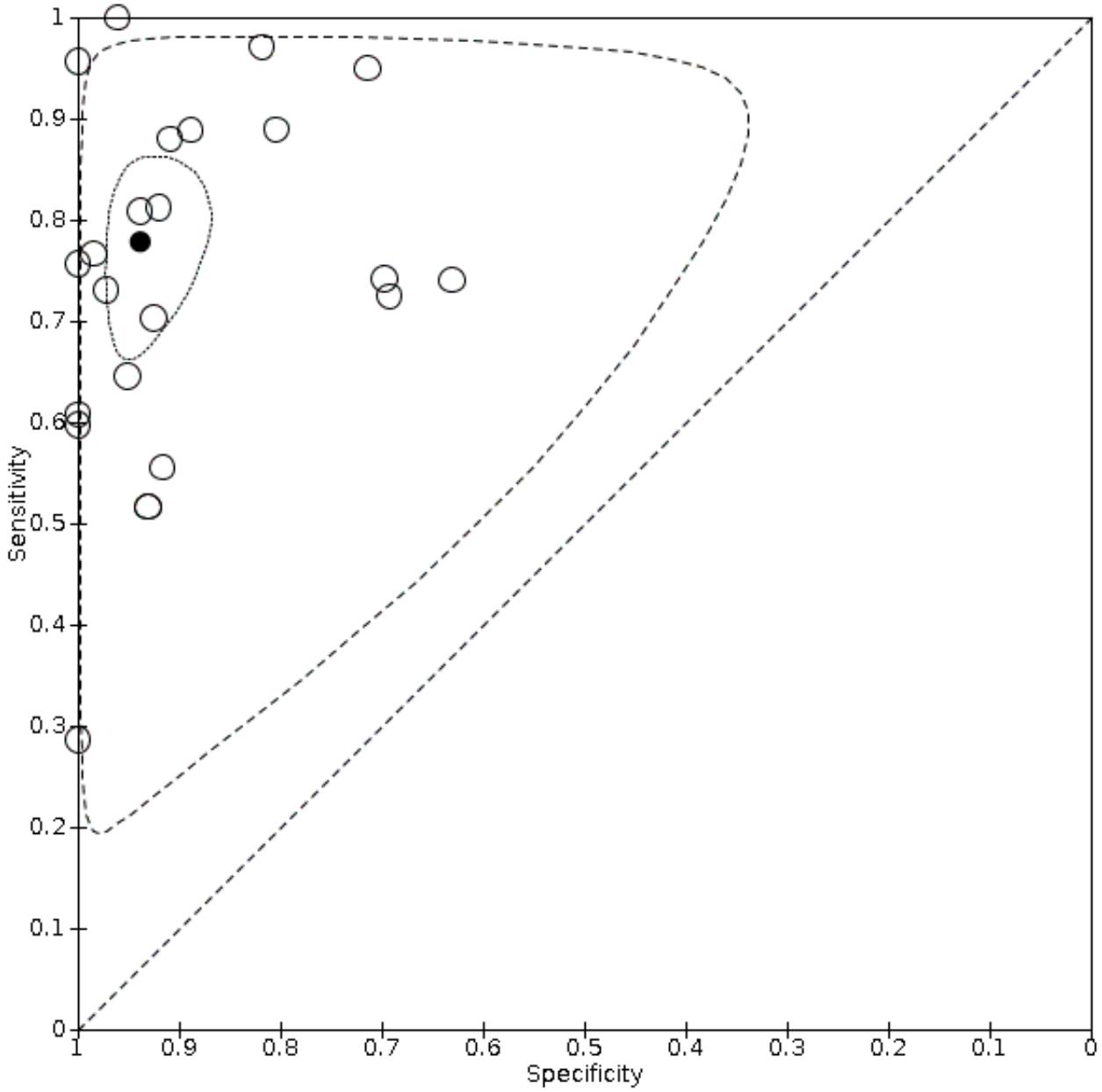


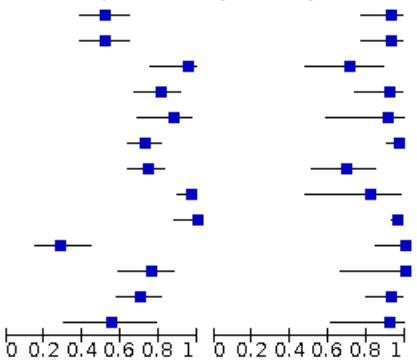
Figure 7. Summary receiver operating characteristic (ROC) comparing in 23 studies CEUS and different reference standards. Reference standards were: the pathology of the explanted liver in case of transplantation; the histology of resected focal liver lesions, or the histology of biopsied focal liver lesion(s) with a follow-up period of at least six months, typical characteristics on cross-sectional multiphasic contrast computed tomography or magnetic resonance imaging with a follow-up period of at least six months. Each individual study is represented by a circle. The solid circle (summary point) represents the summary estimate of sensitivity and specificity. The dotted region around the summary point is the 95% confidence region while the dashed region is the 95% prediction region.



We assessed, as a secondary objective, the diagnostic accuracy for resectable hepatocellular carcinoma. We found 13 studies including 1257 participants with resectable hepatocellular carcinoma (Di Carlo 2012; Forner 2008; Fracanzani 2001; Giorgio 2007; Giorgio 2010; Huang 2020a; Hwang 2021; Kan 2010; Kudo 2019; Sangiovanni 2010; Shin 2015; Sugimoto 2020; Wang 2006). The reported sensitivity ranged from 29% to 100% and the specificity from 70% to 100%. Summary sensitivity was 77.5% (95% CI 62.9% to 87.6%) and summary specificity 92.7% (95% CI 86.8% to 96.1%), LR+ was 10.60 (95% CI 5.9 to 19.0) and LR– was 0.24 (95% CI 0.14 to 0.42). Figure 8 shows the forest plot of sensitivity and specificity with their 95% CIs.

Figure 8. Forest plots of sensitivity and specificity of contrast-enhanced ultrasound (CEUS) for detection of resectable hepatocellular carcinoma against different reference standards in 13 studies in alphabetical order. Reference standards were the histology of resected focal liver lesions or the histology of biopsied focal liver lesions with a follow-up period of at least six months, or typical characteristics on cross-sectional multiphasic contrast computed tomography (CT) or magnetic resonance imaging (MRI) with a follow-up period of at least six months, to allow the confirmation of an initial negative result of CT or MRI; values between square brackets are the 95% confidence intervals (CIs) of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line). FN: false negative; FP: false positive; TN: true negative; TP: true positive

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Di Carlo 2012	31	2	29	27	0.52 [0.38, 0.65]	0.93 [0.77, 0.99]
Forner 2008	31	2	29	27	0.52 [0.38, 0.65]	0.93 [0.77, 0.99]
Fracanzani 2001	19	6	1	15	0.95 [0.75, 1.00]	0.71 [0.48, 0.89]
Giorgio 2007	39	2	9	23	0.81 [0.67, 0.91]	0.92 [0.74, 0.99]
Giorgio 2010	22	1	3	10	0.88 [0.69, 0.97]	0.91 [0.59, 1.00]
Huang 2020a	76	2	28	69	0.73 [0.63, 0.81]	0.97 [0.90, 1.00]
Hwang 2021	66	10	23	23	0.74 [0.64, 0.83]	0.70 [0.51, 0.84]
Kan 2010	66	2	2	9	0.97 [0.90, 1.00]	0.82 [0.48, 0.98]
Kudo 2019	28	11	0	270	1.00 [0.88, 1.00]	0.96 [0.93, 0.98]
Sangiovanni 2010	12	0	30	22	0.29 [0.16, 0.45]	1.00 [0.85, 1.00]
Shin 2015	28	0	9	9	0.76 [0.59, 0.88]	1.00 [0.66, 1.00]
Sugimoto 2020	45	3	19	37	0.70 [0.58, 0.81]	0.93 [0.80, 0.98]
Wang 2006	10	1	8	11	0.56 [0.31, 0.78]	0.92 [0.62, 1.00]



Heterogeneity analysis

We investigated the heterogeneity for all the predefined potential sources (Secondary objectives). Table 2 shows the comparisons of the different predefined subgroups.

Sensitivity analysis

When considering the 22 studies that clearly predefined the positivity criteria and excluding the one study without a clear definition (Fracanzani 2001), we obtained a summary sensitivity of 77.3% (95% CI 68.6% to 84.2%) and specificity of 93.9% (95% CI 89.0% to 96.7%).

When considering the five studies in which the reference standard results were interpreted without the knowledge of the results of the index test (Ding 2021; Li 2019; Sangiovanni 2010; Terzi 2018; Wang 2006), we obtained a summary sensitivity of 63.9% (95% CI 43.0% to 80.6%) and a specificity of 98.1% (95% CI 89.9% to 99.7%).

When considering only the studies published as full texts and excluding the two studies published only as abstracts (Di Carlo 2012; Giorgio 2010), we obtained a summary sensitivity of 78.3% (95% CI 69.6% to 85.0%) and a specificity of 94.0% (95% CI 88.8% to 96.9%).

Summary of findings tables

The main results are shown in the Summary of findings 1 and Summary of findings 2.

For the primary objective, we judged the certainty of evidence as very low. We downgraded it three levels for risk of bias, indirectness, and inconsistency. We downgraded the risk of bias one level because all studies were at high risk of bias; indirectness one level as we considered most studies to have concern regarding applicability, mainly in relation to the population (including disease spectrum); inconsistency one level as sensitivity for individual studies ranged from 29% to 100% and specificity from 63% to 100%, and we could not explain the heterogeneity with study quality or other factors.

For the secondary objective, we judged the certainty of evidence as low. We downgraded it two levels for risk of bias and inconsistency. We downgraded the risk of bias by one level because all studies were at high risk of bias; inconsistency one level as sensitivity for individual studies ranged from 29% to 100% and specificity from 69% to 100%, and we could not explain the heterogeneity with study quality or other factors.

DISCUSSION

Summary of main results

The main objective of this review was to assess the diagnostic accuracy of CEUS for the diagnosis of hepatocellular carcinoma of any size and at any stage in people with chronic liver disease.

We included 23 studies that assessed 6546 participants; 15 studies were conducted on people with clinical suspicion of having hepatocellular carcinoma and eight studies were conducted in the context of a surveillance programme. The prevalence of hepatocellular carcinoma varied widely in all the included studies, from 9% to 93%. Among the 10 studies that used biopsy as the reference standard, it is important to define the alternative diagnosis as they reflect the population in study (de Sio 2014; Di Carlo 2012; Ding 2021; Forner 2008; Giorgio 2007; Giorgio 2010; Li 2019; Sangiovanni 2010; Schellhaas 2017; Shin 2015).

The main hallmarks of hepatocellular carcinoma on a CEUS examination are hyperenhancement in the arterial phase, and washout in portal-venous and delayed phases. However, around 40% of hepatocellular carcinomas present with atypical morphological features (Bolondi 2005). This significant number of atypical hepatocellular carcinomas may influence

sensitivity; therefore, operators should be acquainted with these atypical appearances to correctly interpret CEUS findings. Another issue is the presence of hepatocellular carcinoma mimickers, such as intrahepatic cholangiocarcinoma, combined hepatocellular carcinoma–cholangiocarcinoma, arterioportal shunt, and haemangioma in cirrhotic liver (Lee 2012).

In most included studies, the alternative diagnosis seemed homogeneous, showing similar numbers and types of alternative diagnoses, encompassing regenerative and dysplastic nodules, and more rarely haemangioma, cholangiocarcinoma, and focal nodular hyperplasia. In four studies, the prevalence of benign tumours was higher than in the other studies exceeding 20% of the final diagnoses (Sporea 2019; Tan 2020; Wang 2006; Zuo 2021). In the present review, the prevalence of cholangiocarcinoma ranged from 0% to around 6%.

All 23 included studies provided data for meta-analysis. We used the bivariate model, and, for the diagnosis of hepatocellular carcinoma at any size and stage, we obtained the following summary estimates: sensitivity 77.8% (95% CI 69.4% to 84.4%), specificity 93.8% (95% CI 89.1% to 96.6%). Our secondary objective was to assess the diagnostic accuracy of CEUS for the diagnosis of resectable hepatocellular carcinoma. Thirteen of the 23 studies included only participants with hepatocellular carcinoma amenable for surgical resection, and for the diagnosis of resectable hepatocellular carcinoma, the summary estimate of sensitivity was 77.5% (95% CI 62.9% to 87.6%) and specificity 92.7% (95% CI 86.8% to 96.1%), Hence, the sensitivity and specificity were very similar for both any size hepatocellular carcinoma and resectable hepatocellular carcinoma. The mean reported diameter ranged from 1.4 cm to 5.2 cm and, at least within these limits, CEUS showed the same accuracy for the detection of lesions with different diameters.

We judged all 23 included studies at high risk of bias in at least one domain, and we assessed the results of 13 studies of resectable hepatocellular carcinoma at high concern for applicability.

We summarised the main results of the analyses in Summary of findings 1 and Summary of findings 2, assuming three different prevalence values (60%, 69%, and 80%). We chose these values because 69% was the median value of hepatocellular carcinoma prevalence in the 23 studies, 60% was the first quartile value, and 80% was the third quartile value.

For participants with hepatocellular carcinoma at any size and stage, we assumed the following consequences of test results.

- People with true-positive results: those people with hepatocellular carcinoma and positive test results will receive the appropriate treatment (liver transplantation, surgery, local ablative therapy, or systemic chemotherapy).
- People with true-negative results: those people without hepatocellular carcinoma and negative test results will not undergo inappropriate treatment or unnecessary further testing.
- People with false-negative results: those people with hepatocellular carcinoma and negative test result will be misdiagnosed, will not receive the appropriate treatment, and might be detected later as having more-severe hepatocellular carcinoma.
- People with false-positive results: those people without hepatocellular carcinoma and positive test results will undergo further testing and possibly an inappropriate treatment.

For a hypothetical cohort of 1000 people with **hepatocellular carcinoma at any size and stage**:

- when the hepatocellular carcinoma prevalence is 69% (the median value in the included studies), we can expect 153 false-negative and 19 false-positive results;
- when the prevalence is 60% (i.e. lower, the first quartile), we can expect 133 false-negative and 25 false-positive results.
- when the prevalence is 80% (i.e. higher, the third quartile), we can expect 178 false-negative and 12 false-positive results.

We judged the certainty of evidence to be very low; we downgraded the evidence by three levels because of risk of bias, indirectness, and inconsistency.

For a hypothetical cohort of 1000 people with **resectable hepatocellular carcinoma**:

- when the prevalence is 69%, we can expect 155 false-negative and 23 false-positive results;
- when the prevalence is 60%, we can expect 135 false-negative and 29 false-positive results;
- when the prevalence is 80%, we can expect 180 false-negative and 15 false-positive results.

We judged the certainty of evidence to be low; we downgraded the evidence by two levels because of high risk of bias and inconsistency.

In Table 3, we have shown the post-test probability of having hepatocellular carcinoma in case of positive or negative results of the index test, assuming different values of pretest probability, chosen based on hepatocellular carcinoma prevalence distribution in the 23 included studies (median, first and third quartiles, and minimum and maximum values).

Strengths and weaknesses of the review

This review included 23 studies with 6546 participants, covering a time span of 20 years, from 2001 to 2021. Twelve studies were conducted in Asia and 11 in Europe. We found no studies conducted in America, Africa, or Australia. The studies performed in Asia included 2604 participants, and those in Europe included 3942 participants. An overall quality assessment of the studies showed methodological flaws. We judged all studies at high risk of bias mainly because of non-consecutive participants enrolment, inclusion of participants based on availability of tests' results, inappropriate exclusion criteria, and the use of different reference standards. The choice of the reference standard represents a major issue for all the studies, and we have to admit that none is perfect. The three reference standards were the histology of biopsied focal lesions with adequate follow-up (10 studies), a mix of histology of biopsied focal lesions, imaging techniques, and follow-up (12 studies), and computed tomography (one study). Several studies did not use a single reference standard on all the participants, but instead they used an alternative reference standard with some participants (differential verification) (Naaktgeboren 2013).

In contrast to our Cochrane Reviews that assessed the accuracy of computed tomography and MRI for hepatocellular carcinoma (Nadarevic 2021a; Nadarevic 2021b), none of the studies included in the present review used pathology of the explanted liver as a reference standard. Pathology of the explanted liver is considered a near-perfect reference standard.

Only 8/23 studies reported the time span between the index test and the reference standard. In one study the index test and the reference standard were performed at the same time (Shin 2015). In the remaining seven studies (Giorgio 2007; Giorgio 2010; Huang 2020a; Kan 2010; Kudo 2019; Sangiovanni 2010; Tan 2020), this time ranged from 0 to 60 days, which is an acceptable timeframe considering that the approximate hepatocellular carcinoma volume doubling time is four to five months, with a range of 2.2 to 11.3 months (Nathani 2021). Fifteen studies reported no data on this matter.

We found no studies reporting the number of examinations not performed because of contraindications and only one study reporting the number of uninterpretable index test results (Kang 2020). In another study, uninterpretable results of the index test was an exclusion criterion and the number of participants excluded on this basis was not reported (Kan 2010). In the process of visual interpretation of CEUS examinations, like for the other imaging techniques, it is impossible for the operator to make a definitive diagnosis of hepatocellular carcinoma. This can be primarily related to difficulty with visualisation or with categorisation due to the absence of morphological criteria needed for a definite diagnosis (LI-RADS 2018; Wilson 2018). The lack of information on non-evaluable results is a very critical issue preventing correct accuracy estimates. Even simple ultrasound examination may be associated with frequent technical failure and with uninterpretable results: interferences due to extrinsic factors such as interposed bowel, ribs, lung, or ascites, as well as patient factors such as obesity or inability to comply with breathing instructions, severe steatosis, or severe parenchymal heterogeneity from advanced cirrhosis may impair visualisation of the liver (Rodgers 2019). Up to 14% ultrasound examinations were retrospectively judged as inadequate, and only 66.5% as definitely adequate in a study of quality of ultrasound examination in a hepatocellular carcinoma surveillance programme with people with liver cirrhosis (Simmons 2017). Regarding CEUS, which requires a proper ultrasound visualisation of the liver, no data is so far available as regards the overall rate of uninterpretable results, but considering findings reported for ultrasound examinations without contrast, we can expect at least a similar figure for CEUS.

Strengths and weaknesses of the review process

Search strategy

Our search strategies returned a significant number of studies that were performed in various geographical areas, with high and low prevalence of chronic liver disease and hepatocellular carcinoma. Manually searching the references of the included studies and previous narrative and systematic reviews identified nine additional studies, of which three were ultimately included in the final analysis (Kan 2010; Kudo 2019; Zuo 2021). We applied no language restrictions in the inclusion criteria, which resulted in retrieving full-text articles of two studies published in non-English languages, neither of which we included in the final analysis (Strunk 2005; Zeng 2006). We requested further information from study authors regarding 10 studies (de Sio 2014; Sangiovanni 2010; Sporea 2019; Terzi 2018; Guo 2022; Leoni 2010; Martie 2011; Palmieri 2015; Sirli 2010; Zheng 2020): four provided the requested information (de Sio 2014; Sangiovanni 2010; Sporea 2019; Terzi 2018). We are confident that the search strategies designed for the various databases resulted in the detection of the most eligible studies, with a low probability of not detecting relevant studies.

Quality assessment and data extraction

We consider our attempts to reduce subjectivity in our judgements to minimise errors and miscalculations in data extraction to be the strength of this review. Two review authors independently assessed the risk of bias of the included studies and applicability of their results using the QUADAS-2 tool. We extracted data using a prepiloted data extraction form. In case of disagreement, we reached consensus through discussion. Disagreements were most frequent for the two QUADAS-2 domains patient selection (eight studies) and reference standard (four studies). All agreements were reached through discussion between two review authors, and the conclusions were discussed and approved by a third review author. For data extraction, most of the discordances were due to miscalculations and typographical errors, which were easily resolved. The same review authors assessed the certainty of evidence using the GRADE approach, and the level of agreement was high.

Review analysis

We performed meta-analyses using the bivariate model, as the results of the index test were reported as dichotomous (positive or negative), with no explicit threshold. We recognise that implicit thresholds cannot be excluded. None of the studies included fewer than 30 participants. The estimates of sensitivity ranged from 29% to 100% and those of specificity from 63% to 100%.

Inconsistency of the overall results, visualised in Figure 7 by the prediction region largely wider than the confidence region, was not explained by any of the subgroup analyses performed. Different geographic areas, prevalence of viral aetiology, severity of the underlying disease (prevalence of cirrhosis), use of different contrast types, differences in the choice of reference standards, clear definition of positivity criteria, and use of LI-RADS positivity criteria seem unable to explain the observed inconsistencies. Some of our planned investigations were not carried out because of the lack of data (MELD (Model for End-Stage Liver Disease) score, Child-Pugh classification of severity of cirrhosis). Furthermore, we were able to investigate only the characteristics that could be assessed at study level whereas participants' factors or hepatocellular carcinoma characteristics were only assessed by aggregate statistics with the inherent risk of ecological bias. Therefore, some important relationships, such as the one with hepatocellular carcinoma volume, could have been missed. In addition, many of the included studies did not report data on the covariates of interest.

We excluded studies that reported only per-lesion analyses and included only the studies with per-patient analyses. Per-patient and per-lesion analyses represent two different approaches to diagnostic accuracy assessment, and their choice depends on the type of clinical or scientific questions, and requires different and appropriate statistical methodologies. In the present review, we aimed to assess the accuracy of CEUS for the diagnosis of hepatocellular carcinoma. Consequently, we chose to include the

studies that evaluated the ability of CEUS to detect hepatocellular carcinoma at any size and any stage, therefore applying a per-patient approach. Otherwise, per-lesion analysis is properly performed to assess accuracy in detecting multiple lesions on a single image, providing information that is relevant for hepatocellular carcinoma staging. Studies planning per-lesion analysis require a different methodological approach and cannot be pooled with studies using a per-patient approach (Chang 2006; Zwinderman 2008). Furthermore, the inclusion criteria of studies planning per-lesion analysis are quite different and do not match our review question. They usually exclude people with chronic liver disease and suspected hepatocellular carcinoma, and include people with known focal liver lesions, encompassing hepatocellular carcinomas, cholangiocarcinomas, benign liver tumours, and even metastases from abdominal or extra-abdominal primary cancers (Li 2021; Niu 2013).

All but one study (Fracanzani 2001) reported a clear definition of diagnostic criteria, and we tried to explore the effect on the diagnostic accuracy estimates of different positivity criteria, traditional perfusion compared to LI-RADS criteria. However, 10/23 studies used LI-RADS positivity criteria and we were unable to find any differences with those not using such criteria.

We were also unable to estimate the effect of uninterpretable results as only one study reported the frequency of technical failures.

The sensitivity analysis shows that the obtained results are arguably robust with no variation, after including only the studies that clearly prespecified the positivity criteria, including only those in which the reference standard results were interpreted without the knowledge of the results of the index test, and including only the studies published in full text.

Comparison with previous research

We found five non-Cochrane systematic reviews that assessed the accuracy of CEUS for detection of hepatocellular carcinoma (Deng 2016; Huang 2017; Li 2021; Niu 2013; Yang 2021).

The main characteristics of these reviews are summarised in Table 4. Three reviews focused on the diagnostic accuracy of CEUS alone (Deng 2016; Niu 2013; Yang 2021), and two on the diagnosis of CEUS compared to other techniques, MRI, or computed tomography (Huang 2017; Li 2021).

None of these reviews performed a per-patient analysis: two performed per-lesion analysis and the others included mixed studies (some performing per-patient others per-lesion analysis). The summary sensitivity and specificity of CEUS for detection of hepatocellular carcinoma ranged from 75% to 90%, and 86% to 91%.

The results of these reviews are in accordance with our present results, despite methodological differences and the number of included studies.

We additionally evaluated all the primary studies included in these systematic reviews and assessed them for inclusion in our analysis.

Applicability of findings to the review question

Using the QUADAS-2 tool, we assessed the applicability of the results of the included studies. We judged 10 studies at low concern for applicability and downgraded by one level the certainty of evidence for indirectness in the other 13 studies. Fourteen studies selected participants based on focal lesion diameter. As our aim was to assess the CEUS accuracy in people with the whole spectrum of liver disease severity (i.e. without any exclusion for severity of liver disease or hepatocellular carcinoma volume), the percentage of studies included in the present review and judged with concerns regarding applicability is lower than in our computed tomography and MRI Cochrane Reviews (Nadarevic 2021a; Nadarevic 2021b). Regarding the alternative diagnoses of hepatocellular carcinoma which actually reflect the population selection, the prevalence of benign tumours was higher in four studies compared to other studies, exceeding 20% of the final diagnoses (Sporea 2019; Tan 2020; Wang 2006; Zuo 2021), suggesting the selection and inclusion of participants without chronic liver disease.

For the accuracy estimates for our secondary objective, that is, CEUS for resectable hepatocellular carcinoma, we judged 10/13 studies at low concern for applicability. Such studies correctly included people with small liver nodules (i.e. with a diameter smaller than 3 cm).

AUTHORS' CONCLUSIONS

Implications for practice

Hepatocellular carcinoma is a frequent complication of chronic liver disease. The detection of a tumour amenable to surgical resection, thermal ablation, or liver transplantation can improve the prognosis, which is severe in the absence of indications to radical treatment. Being the third leading cause of death from cancer worldwide, accurate tests are needed to diagnose hepatocellular carcinoma. In the clinical pathway for hepatocellular carcinoma diagnosis in people with chronic liver disease, contrast-enhanced ultrasound (CEUS) is currently the second step after ultrasound and alpha-foetoprotein or the combination of the two, its main role being confirming the presence of the disease. Depending on the diagnostic pathway, CEUS can be performed either before magnetic resonance imaging (MRI), or computed tomography, or after these two techniques in case of inconclusive results of the former ones. As an ideal diagnostic test, CEUS should ensure a low proportion of false-negative results because people with undetected hepatocellular carcinoma cannot receive proper treatment. People with false-positive results are exposed to unnecessary further diagnostic work-up and possible invasive treatment. The estimated summary sensitivity and specificity derived from our analysis suggest that 22% of people with hepatocellular carcinoma would be missed, and 6% of people without hepatocellular carcinoma would be unnecessarily further tested or treated.

An important piece of clinical information, which is meaningful for further patient work-up, is the possibility of surgical resection. Ideally, CEUS should ensure a low proportion of false-negative results because people with false-negative results will not undergo surgical resection, and people with false-positive results will undergo inappropriate surgical resection. Based on our results, 23% of people with hepatocellular carcinoma would be incorrectly classified as without any hepatocellular carcinoma and would

incorrectly not be resected, while 8% of people with non-resectable hepatocellular carcinoma will undergo inappropriate surgery.

The CEUS accuracy estimates can only indirectly be compared with the results of computed tomography and MRI as obtained in the recent systematic reviews (computed tomography: sensitivity 77.5%, 95% CI 70.9% to 82.9%; specificity 91.3%, 95% CI 86.5% to 94.5%; Nadarevic 2021a; MRI: sensitivity 84.3%, 95% CI 77.6% to 89.3%; specificity 92.9%, 95% CI 88.3% to 95.9%; Nadarevic 2021b).

Interestingly, CEUS diagnostic accuracy for the detection of resectable tumours (the accuracy estimates for our secondary objective) is similar to that previously reported for computed tomography and MRI, indicating that CEUS maintains a good diagnostic accuracy also for small tumours (less than 3 cm). However, only the direct comparison on the same participants can support the choice between these techniques. Such a choice would also depend on their availability, costs, and risks. Overall, caution is needed in interpreting our review results as we judged all the studies at high risk of bias, and most of them with high concern regarding their applicability, mainly due to the patient selection and reference standard domains.

Implications for research

The currently available evidence on the diagnostic accuracy of CEUS for the diagnosis of hepatocellular carcinoma is not conclusive. Therefore, more high-quality primary studies are needed. With the introduction of LI-RADS criteria, the results of CEUS studies should no longer be dichotomised, allowing the assessment of also inconclusive and probable findings. Apart from typical hepatocellular carcinoma appearances, atypical features of hepatocellular carcinoma should also be taken into account. Further studies using LI-RADS positivity criteria will possibly document an improvement of the technique's accuracy.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

de Sio 2014

Study characteristics	
Patient Sampling	Study enrolled 282 participants (197 men, 85 women; mean age 67 years; age range 28–79 years) with a 'de novo' diagnosis of single (165; 58%) and multiple (117; 42%) FLLs. In this review, after obtaining data from the authors, we included the results of 248 participants with a single FLL.

de Sio 2014 *(Continued)*

Patient characteristics and setting	Adults with diagnosis of chronic liver disease undergoing ultrasound evaluation. 197 men and 85 women, mean age 67 (SD 7) years. Underlying aetiology of cirrhosis was: hepatitis C (220 participants; 78%), hepatitis B virus (31; 11%), alcoholic cirrhosis (11; 4%), alcoholic plus hepatitis C virus (7; 2.5%), hepatitis C virus plus hepatitis B (10; 3.5%), cryptogenetic cirrhosis (2), and primary biliary cirrhosis (1).
Index tests	CEUS examination started and continued for 5 min after injection of ultrasound contrast agent SonoVue (Bracco SpA, Milan, Italy), a sulphur hexafluoride-filled microbubble covered by a phospholipid shell. Contrast injection procedure and observation of its haemodynamic behavior conducted during arterial, portal, and late-vascular phases. CEUS considered conclusive if studied lesion showed a contrastographic pattern so typical as to be classified as HCC or as a lesion other than HCC, according to EFSUMB guidelines. Washout defined as 'early' when started within 60 s, according to Italian Association for the Study of the Liver guidelines.
Target condition and reference standard(s)	If the participant presented multiple focal lesions, those showing a typical enhancement pattern at CEUS were considered optimal to obtain a sample for pathology. For all lesions (primarily studied at CEUS), a confirmation of diagnosis was obtained by ultrasound-guided percutaneous biopsy (adopted as the gold standard).
Flow and timing	No details concerning time span between the index test and reference standard.
Comparative	
Notes	Following our request, authors supplied data on 248 participants with a single FLL. Quote: "This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector". Authors declared no COI.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		

de Sio 2014 *(Continued)*

Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Could the reference standard, its conduct, or its interpretation have introduced bias?		High risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Unclear risk	

Di Carlo 2012

Study characteristics	
Patient Sampling	89 people with cirrhosis without prior HCC in whom baseline ultrasound detected a small (≤ 2 cm) solitary lesion underwent CEUS. Thus, authors excluded patients based on lesion diameter.
Patient characteristics and setting	89 people with cirrhosis without prior HCC in whom baseline ultrasound detected a small (≤ 2 cm) solitary lesion underwent CEUS. Excluded people with larger lesions.
Index tests	CEUS enhancement patterns studied during arterial, portal, and late phase. Intense arterial uptake followed by washout in the venous /delayed phase was registered as conclusive for HCC. This is the definition for positivity criteria.

Di Carlo 2012 *(Continued)*

Target condition and reference standard(s)	Fine needle biopsy was performed in all participants to detect HCC.
Flow and timing	No details concerning the time span between the index test and reference standard.
Comparative	
Notes	Only abstract available. No information about COI and funding.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			

Di Carlo 2012 *(Continued)*

Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Unclear risk

Ding 2021

Study characteristics	
Patient Sampling	Retrospective study performed at Tianjin Third Central Hospital enrolled participants at high risk of HCC who underwent CEUS from January 2018 to April 2020.
Patient characteristics and setting	264 participants (264 nodules); 202 men and 62 women; mean age 59.4 years; mean maximum nodule diameter 3.2 cm
Index tests	Sulphur hexafluoride (SF6) microbubble contrast agent (Sono-Vue, Bracco, Milan, Italy) was used as ultrasound contrast. Each nodule was categorised based on CEUS LI-RADS version 2017. Arterial phase hyperenhancement defined as partial (neither rim-like nor peripheral discontinuous) or complete hyperenhancement compared with the surrounding parenchyma. Washout defined as partial or complete hypoenhancement relative to the liver beginning in or after the arterial phase. Early washout occurred within 60 s after injection of contrast agent. A punched-out appearance was defined as marked hypoenhancement of the nodule (resulting in it appearing black).
Target condition and reference standard(s)	Liver histology, including ultrasound-guided biopsy and surgical pathology, served as standard reference.
Flow and timing	No details are given concerning the time span between the index test and reference standard.
Comparative	
Notes	Quote: "The work was supported by the Tianjin Health and Health Committee (Grant Nos. MS20017 and KJ20170). Authors declared no COI.

Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		

Ding 2021 *(Continued)*

Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Unclear risk	

Forner 2008

Study characteristics	
Patient Sampling	Study included people with asymptomatic Chid-Pugh A-B cirrhosis, with no history of HCC, in whom new solitary, well-defined, solid nodule 5–20 mm was detected by screening ultrasound.
Patient characteristics and setting	89 people with liver cirrhosis; median age 65 years; cirrhosis caused by hepatitis C virus infection in 68 (76.4%) with preserved liver function (Child-Pugh class A: 80). Participants selected based on lesion diameter.

Forner 2008 *(Continued)*

Index tests	CEUS performed using contrast coherent imaging (CCI, Siemens) with 4C1 convex array probe. Selected a low mechanical index (0.2) to avoid the microbubbles disruption. Performed CEUS explorations after administration of 2.4 mL SonoVue (Bracco, Italy). Bolus repeated if first exploration was not evaluable. Enhancement patterns studied during vascular phase up to 3.5 min, including arterial (0–49 s), portal (50–179 s), and late phase (180 s). Nodules in which both CEUS depicted a conclusive pattern were classified as 'AASLD criteria positive'. Nodules not displaying this coincidental profile were classified as 'AASLD criteria negative'. According to previous definitions, findings of CEUS were defined as hypovascular (no specific contrast enhancement of nodule compared with surrounding liver), suspicious (arterial hypervascularisation regardless of washout), or conclusive (arterial hypervascularisation followed by venous washout). Therefore, nodules classified as suspicious for HCC included those defined as conclusive and those categorised as suggestive but non-conclusive. 3 expert radiologists performed CEUS. ≥ 2 blinded radiologists recorded and reviewed examinations. Categorisation of doubtful explorations achieved by consensus. Lesions defined as: 1. conclusive HCC: nodules showing intense contrast uptake during the arterial phase followed by washout in portal or venous phase (or both); 2. suggestive of HCC, but non-conclusive: nodules showing early enhancement during the hepatic arterial phase without washout in venous phase; 3. dysplastic/regenerative nodules: nodules with no contrast enhancement during the 3 phases; and 4. haemangioma: early centripetal contrast uptake after arterial phase that persists in delayed phases.
Target condition and reference standard(s)	Fine needle liver biopsy performed in all participants using a 20-gauge spinal needle (Yale Spinal BD medical, NJ). Several back and forth passages were done after insertion of needle. When technically feasible because of location and accessibility, a core-biopsy was performed using an 18-gauge needle-biopsy. Blinding unclear.
Flow and timing	No details concerning the time span between the index test and reference standard.
Comparative	
Notes	Quote: "Supported in part by grants of the Instituto de Salud Carlos III (grant PI 05/150 and PI 06/132) and from NIH-NIDDK grant: 1R01DK076986-0. Alejandro Forner is partially supported by a grant of the Instituto de Salud Carlos III (PI 05/645) and a grant of the BBVA foundation. María Varela was supported by Fundación Científica de la Asociación Española de Ayuda contra el Cáncer, Spain. Josep M. Llovet is Professor of Research at Institut Catalá de Recerca Avanzada (ICREA, IDIBAPS, Hospital Clinic)". No potential COI.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High

Forner 2008 *(Continued)*

DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it prespecified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Unclear risk	

Fracanzani 2001

Study characteristics	
Patient Sampling	500 consecutive participants with cirrhosis without history of hepatic lesions enrolled between January 1998 and December 1999 and followed up every 3 months to December 2000 with ultrasound to detect focal lesions. Among 61 participants who developed hepatic focal lesions, 41 underwent CEUS, CT, and liver biopsy. 20 participants were excluded because of multifocal lesions, lesions > 30 mm, and clinical problems not allowing executions of the 3 tests.
Patient characteristics and setting	41 participants (30 men; mean age 62 years) from a population of 500 people attending Liver Units of Policlinico Ca' Granda Milan and who developed focal hepatic lesion during a 3-month surveillance programme. 20 participants were excluded because of multifocal lesions, lesions > 30 mm, and clinical problems not allowing executions of the 3 tests.
Index tests	CEUS. Contrast agent Levovist. Colour Doppler ultrasound visualisation continuously videotaped until enhancement effect disappeared. Participant studied after overnight fasting, supine position, during suspended respiration. No positivity criteria given.
Target condition and reference standard(s)	Reference standard: in all participants fine needle biopsy. In the case of negative biopsy, spiral CT follow-up for ≥ 6 months. CT studies interpreted without knowing CEUS results. Not known if pathologist interpreting liver biopsy was blind to CEUS results.
Flow and timing	No details concerning time span between the index test and reference standard.
Comparative	
Notes	Quote: "Supported by MURST (ex 40%) 2000; MURST 60% 1999, Ricerca Finalizzata ISS 1999(30/12/92 n.502): Storia naturale, terapia e prevenzione delle epatopatie acute e croniche." No information about COI.

Methodological quality			
Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			

Fracanzani 2001 *(Continued)*

Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	No		
Could the conduct or interpretation of the index test have introduced bias?		High risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Unclear risk	

Giorgio 2007

Study characteristics	
Patient Sampling	From a cohort of 584 people with cirrhosis bearing liver masses based on ultrasound findings and referred between September 2003 and June 2004 for further evaluation, 73 consecutive participants each with a single liver nodule ≤ 30 mm were selected. Only exclusion criterion was presence of any heart diseases. They underwent CEUS, MRI, and liver biopsy.
Patient characteristics and setting	73 participants with FLLs (49 men and 24 women; mean age 63 years; age range 40–84 years), of which 52 with nodules 11–30 mm in size. Participants were selected based on lesion diameter.

Giorgio 2007 *(Continued)*

	Diagnosis of liver cirrhosis was based on liver biopsy findings in 58/73 participants and on clinical data in the remaining 15 participants. 46 participants were in Child-Pugh class A and 27 in class B.
Index tests	CEUS contrast agent: SonoVue. Contrast enhancement pattern determined by evaluating the behaviour of the hepatic lesion throughout the sonographic examination after intravenous injection of contrast agent. Whole vascular phase was recorded. Appearance of focal areas of hyperechogenicity, which are related to hypervascularity, in the nodule seen on baseline precontrast grey-scale imaging was carefully searched and timed. Washout estimated as a change from a hyperechoic lesion relative to the surrounding liver to an isoechoic or hypoechoic lesion relative to the surrounding liver at any vascular phase.
Target condition and reference standard(s)	All lesions were histologically confirmed after both imaging studies. Diagnosis of HCC on liver biopsy according to International Working Party criteria.
Flow and timing	CEUS and dynamic contrast-enhanced MRI performed in all participants on consecutive days. Biopsies were performed in all participants on the day after both imaging studies, in order to avoid any interference with vascularity assessment.
Comparative	
Notes	No information about COI and funding.

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern

Giorgio 2007 *(Continued)*

DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Giorgio 2010

Study characteristics	
Patient Sampling	From February to October 2009, 36 consecutive participants with cirrhosis with a single nodule ≤ 2 cm (range 9–20 mm) that was newly emerged during ultrasound surveillance, underwent CEUS immediately after conventional ultrasound.
Patient characteristics and setting	36 participants; age range 49–64 years; 29 Child-Pugh class A, 7 class B; 25 hepatitis C virus, 9 hepatitis B virus. Participants selected based on lesion diameter.
Index tests	CEUS with low mechanical index and SonoVue intravenous injection
Target condition and reference standard(s)	Diagnosis of HCC on liver biopsy according to International Consensus on small nodular lesions in cirrhotic liver (Hepatology 2009), 6 participants had low-grade dysplastic nodule, 5 participants had high-grade dysplastic nodule, 14 participants had early HCC, and 11 participants had overt HCC on histology.
Flow and timing	All participants underwent ultrasound-guided percutaneous biopsy of the nodule within 2–7 days after CEUS.
Comparative	
Notes	Published in abstract form.

Giorgio 2010 *(Continued)*

	No information about COI and funding

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Unclear		
Were positivity criteria clearly defined?	Unclear		
Could the conduct or interpretation of the index test have introduced bias?		Unclear risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Huang 2020a

Study characteristics	
Patient Sampling	Between January 2015 and February 2018, consecutive participants at risk for HCC presenting with untreated liver nodules ≤ 20 mm were enrolled in this retrospective double-reader study. 172 participants with risk factors for HCC who presented with untreated liver nodules ≤ 20 mm at initial imaging (screening or diagnostic ultrasound, or contrast-enhanced CT or MRI performed as a part of standard clinical care) were included.
Patient characteristics and setting	Of the 172 participants (mean age 51.8 years), 136 (79.1%) were men with chronic liver disease (95% hepatitis B virus or hepatitis C virus aetiology). Participants selected based on lesion diameter.
Index tests	Conventional precontrast grey-scale and CEUS examinations performed using a ultrasound system (IU22; Philips Medical Solutions, Mountain View, CA) with a C521 MHz convex or L923 MHz linear probe. 2 certified radiologists with > 5 and > 10 years of experience in liver CEUS who were blinded to reference standard results and other imaging test results independently reviewed CEUS examinations in liver nodules and assigned a category according to CEUS LI-RADS (2017 version). If there was no consensus, a blinded expert radiologist (with > 20 years of experience) arbitrated. Used following diagnostic features to characterise each nodule based on CEUS LI-RADS: nodule size; arterial phase enhancement and its pattern; presence, timing, and degree of washout; mosaic and nodule-in-nodule architecture, and tumour in vein, size change at follow-up imaging. Correlation between histological HCC tumour grading and CEUS LI-RADS classification was performed to better understand why small HCC nodules could manifest with different enhancing patterns.
Target condition and reference standard(s)	Target condition represented by HCC nodules ≤ 20 mm in people at risk for HCC. Different reference standards used. All observations with LR-1 classification at contrast-enhanced CT or MRI were considered benign. All lesions with LR-5 classification, a contrast-enhanced CT or MRI were considered to be HCC. Histopathological tissue analysis of 124/175 nodules (70.9%) was obtained, including 114 surgical specimens and 10 ultrasound-guided core biopsies.
Flow and timing	Mean time between CEUS and biopsy or operation was 13 days. Time lag between CEUS and CT or MRI not reported.
Comparative	
Notes	No information available about funding. COI: all authors disclosed no relevant relationships.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		

Huang 2020a *(Continued)*

Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Hwang 2021

Study characteristics	
Patient Sampling	Retrospective study enrolling consecutive participants with risk factors for HCC who presented with untreated liver nodules with diameter ≤ 20 mm at initial imaging (screening or diagnostic ultrasound, or contrast-enhanced CT or MRI performed as a part of standard clinical care) were included. All nodules were visible at baseline ultrasound. Participants with CEUS LR-3 and LR-4 observations without histological diagnosis that remained indeterminate at follow-up imaging were removed from the analysis because of lack of a reference standard.
Patient characteristics and setting	Included participants were selected based on the diameter (≤ 20 mm) of the focal hepatic lesions. Participants with CEUS LR-3 and LR-4 observations without histological diagnosis that remained indeterminate at follow-up imaging were removed from the analysis because of lack of a reference standard.
Index tests	Ultrasound examinations performed using the IU22 ultrasound system (Philips Medical Solutions, Mountain View, CA) with a C521 MHz convex or L923 MHz linear probe. Pulse inversion harmonic imaging and mechanical index < 0.1 were used for CEUS examinations with technical recommendations following the WFUMB EFSUMB and CEUS LI-RADS guidelines. Contrast media was hexafluoride-filled microbubble contrast agent (SonoVue; Bracco, Milan, Italy). The following diagnostic features were used to characterise each nodule based on CEUS LI-RADS: nodule size; arterial phase enhancement and its pattern; presence, timing, and degree of washout; mosaic and nodule-in-nodule architecture; and tumour in vein, size change at follow-up imaging. 2 certified radiologists who were blinded to reference standard results reviewed the CEUS examinations.
Target condition and reference standard(s)	Different reference standards was used: CT, MRI, and histology.
Flow and timing	Flow and timing not detailed.
Comparative	
Notes	COI: authors reported that Kyoung Jeong serves as Editor for *Ultrasonography*, but had no role in the decision to publish this article. All remaining authors declared no COI.

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High

Hwang 2021 *(Continued)*

DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Could the reference standard, its conduct, or its interpretation have introduced bias?		High risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Kan 2010

Study characteristics	
Patient Sampling	Between January and August 2007, 70 participants with chronic liver disease, suspected as HCCs were studied at Ehime Prefectural Central Hospital, Japan. Nodules showing the typical findings of liver haemangioma were excluded.
Patient characteristics and setting	79 nodules in 69 participants with chronic liver disease, suspected as HCCs were studied. The nodules were selected based on the results of B-mode ultrasonography or dynamic CT (or both) conducted be-

Kan 2010 *(Continued)*

	tween January and August 2007. 45 men and 24 women; mean age 71 years.
Index tests	CEUS with erfluorobutane (Sonazoid) (4 µL/kg of bodyweight) used in all examinations, and target lesions were scanned after injection in the arterial and Kupffer phases using a ProSound Alpha-10 (Aloka Co Ltd, Tokyo, Japan). Arterial phase of CEUS imaging identified 10–60 s after Sonazoid injection, and the Kupffer phase 10 min after the injection. ProSound Alpha-10 was set up in the extended pure harmonic detection mode and used with a convex-type probe.
Target condition and reference standard(s)	Target condition was represented by HCC and reference standard was dynamic CT. Nodules were diagnosed as typical HCCs by dynamic CT when they were enhanced in the arterial phase and were revealed as a defect in the portal phase of Dy-CT.
Flow and timing	CEUS performed within 1 month from the dynamic CT examination in all participants.
Comparative	
Notes	No information about funding and COI.

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Unclear risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		

Kan 2010 *(Continued)*

Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear	
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk
Are there concerns that the target condition as defined by the reference standard does not match the question?		Low concern
DOMAIN 4: Flow and Timing		
Was there an appropriate interval between index test and reference standard?	Yes	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		Low risk

Kang 2020

Study characteristics	
Patient Sampling	From November 2018 to August 2019, 107 participants at risk of HCC with treatment-naïve solid hepatic observations (≥ 1 cm) of LR-3/4/5/M during surveillance and performed gadolinium ethoxybenzyl-MRI were prospectively enrolled. Among them, 4 participants were excluded for insufficient diagnosis, referring to an inconclusive histopathologic diagnosis (2), or did not meet the non-invasive diagnostic criteria of HCC (2).
Patient characteristics and setting	103 participants with 103 hepatic observations (mean size 28.2 (SD 24.5) mm). Participants had liver cirrhosis (43.7%, 45/103), hepatitis B infection (65.0%, 67/103), or both (8.7%, 9/103). HCC diagnosed in 76.7% (79/103) of participants. Mean size of observations 28.2 (SD 24.5) mm (range 11–114 mm).
Index tests	CEUS contrast agent: SonoVue. Arterial phase hyperenhancement and washout on CEUS were evaluated. The distinctive washout in CEUS was defined as mild washout 60 s after contrast injection. The diagnostic ability of CEUS for HCC was determined according to the EASL and the KLCA-NCC guidelines. Continuous CEUS images of the target were recorded for the first 60 s after contrast injection followed by intermittent scans every 15 s for 5 min after contrast media administration.
Target condition and reference standard(s)	Diagnosis of HCC (79 cases) was based on pathology or characteristic imaging features. 1 of the 2 experienced pathologists (with > 17 and 19 years' experience in hepatic pathology) made pathological diagnoses. For the radiological diagnosis of HCC, used contrast-enhanced CT findings based on the CT/MRI (LI-RADS 2018). 1 haemangioma was diagnosed by characteristic imaging features on contrast-enhanced CT, which referred to a peripheral globular, centripetal enhancement pattern that remained stable in size during follow-up.
Flow and timing	Flow and timing not detailed. There were 2 non-evaluable cases in CEUS even after real-time CT/MRI fusion due to a poor sonic window.

Kang 2020 *(Continued)*

Comparative	
Notes	No information about funding. Authors reported that they had no potential COI.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Could the reference standard, its conduct, or its interpretation have introduced bias?		High risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			

Kang 2020 *(Continued)*

Was there an appropriate interval between index test and reference standard?	Unclear	
Did all patients receive the same reference standard?	No	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		High risk

Kudo 2019

Study characteristics	
Patient Sampling	Prospective, multicentre, randomised, controlled trial conducted to demonstrate the usefulness of Kupffer phase surveillance in the detection of small HCC compared to B-mode ultrasound. In this systematic review, we included only the arm with CEUS (Sonazoid) surveillance.
Patient characteristics and setting	309 Japanese participants with hepatitis B virus or hepatitis C virus-related liver cirrhosis considered at very high risk for HCC development were enrolled in a surveillance study with Kupffer phase CEUS with Sonazoid surveillance group. 136 men; mean age 66.8 years
Index tests	Contrast type: Sonazoid. In surveillance setting, diagnostic criteria on CEUS included a defect in the Kupffer phase 10–60 min after injection and arterial enhancement following reinjection of Sonazoid at the Kupffer phase. Surveillance consisted of Kupffer phase CEUS every 4 (SD 1) months and CT/MRI every 8 months. Participants in the CEUS group were scanned during the Kupffer (postvascular) phase 10–40 min after Sonazoid injection. Only if the Kupffer defect was depicted, reinjection of Sonazoid was performed to confirm HCC.
Target condition and reference standard(s)	For participants with positive index test, reference standard set as hallmark findings by dynamic CT/MRI at cut-off point; in case of negative index test, further follow-up after the cut-off point was completed.
Flow and timing	Surveillance consisted of B-mode ultrasound or Kupffer phase CEUS every 4 (SD 1) months and CT/MRI every 8 months.
Comparative	
Notes	COI: Masatoshi Kudo received honoraria from Daiichi-Sankyo and GE HealthCare; other authors had no COI.

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			

Kudo 2019 *(Continued)*

Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Could the reference standard, its conduct, or its interpretation have introduced bias?		High risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	No		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Li 2019

Study characteristics			

Li 2019 *(Continued)*

Patient Sampling	Retrospective study. People who underwent CEUS of the liver between January 2014 and December 2017 were consecutively enrolled. Inclusion criteria: FLLs solid and detected by conventional ultrasound with a CEUS examination also available; people with cirrhosis or chronic hepatitis; pathologically confirmed hepatic lesions. Participants with > 1 hepatic lesion (the most visible and accessible lesion) were chosen for study.
Patient characteristics and setting	1366 participants enrolled, including 1097 men and 269 women. Age range 18–90 years, with mean age 52.3 (SD 12.0) years. All participants had history of chronic liver disease, and the aetiologies were as follows: hepatitis B virus (1300 participants), hepatitis C virus (38), alcohol-related liver disease (2), autoimmune hepatitis (4), non-alcoholic steatohepatitis (11), and schistosomiasis infection (11). Among the 1366 participants, 512 (37.5%) had cirrhosis (489 caused by hepatitis B virus, 18 by hepatitis C virus, 1 by autoimmune hepatitis, and 4 by chronic schistosomiasis infection), and the other 854 (62.5%) did not have cirrhosis.
Index tests	CEUS contrast agent: SonoVue. FLLs were classified according to CEUS LI-RADS version 2017.
Target condition and reference standard(s)	All FLLs were confirmed by pathology. Due to uncertain imaging diagnosis or prior to radiofrequency ablation, ultrasound-guided puncture biopsy was performed for 198 FLLs; surgical resection was conducted for the other 1168 FLLs.
Flow and timing	Flow and timing not detailed.
Comparative	
Notes	Research supported by Post-Doctor Research Project, West China Hospital, Sichuan University (NO. 2018HXBH073), and National Natural Science Foundation of China (No. 81701702). No information about COI.

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			

Li 2019 *(Continued)*

Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Sangiovanni 2010

Study characteristics	
Patient Sampling	64 people with a Child-Pugh A or B cirrhosis and a de novo 67 liver nodule (diameter 1–2 cm) detected during ultrasound surveillance were consecutively included and examined. CEUS, CT, MRI, and FNB were diagnostic standards. HCC diagnosed in 44 (66%) participants.
Patient characteristics and setting	Authors requested to supply data the from participants with a single liver lesion and provided data on 64 participants. Participants were selected based on lesion diameter.
Index tests	CEUS performed with up to 2 bolus injections of 2.4 mL of second-generation contrast agent (SonoVue, Bracco, Milan, Italy), having 8 mm microbubbles and stability for 6–8 min. Bolus followed by a 10 mL saline flush. A low mechanical index (< 0.1) was set for

Sangiovanni 2010 *(Continued)*

	CEUS examination. Enhancement patterns studied during the vascular phase for up to 3 min, including the arterial (0–35 s), portal (35–120 s) and late phase (120–180 s). The typical vascular pattern of HCC on CEUS was characterised by hyperenhancing in the arterial phase, followed by washout in the portal/venous phase.
Target condition and reference standard(s)	Diagnostic gold standard was histology through a fine needle biopsy performed within the nodule and the surrounding liver parenchyma. Procedure was repeated in all participants with unsolved histological diagnosis, participants showing similar histological features within and outside the liver nodule. All participants with nodules lacking histological features of malignancy underwent a repeat ultrasound every 3 months and an abdominal CT/MRI every 6 months to assess changes in size and in the vascular pattern of the nodule at imaging. All nodules, either enlarging or showing changes in the vascular pattern, underwent a further FNB. FNB procedure performed using a 21-gauge trenchant needle for microhistology (Biomol, HS Hospital Service, Italy) to examine both intranodule and extranodule liver parenchyma tissue. Diagnosis made according to the International Working Party criteria.
Flow and timing	All participants had examinations with abdominal CT scan, MRI, CEUS, and ultrasound-guided FNB carried out within 2 months from detection of a liver nodule.
Comparative	
Notes	Authors were requested to supply the data from participants with a single liver lesion and provided data on 64 participants with a single FLL. Study supported by grant no. PUR 2008, University of Milan, and by a generous contribution from Dr Aldo Antognozzi. COI: authors declared none.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		

Sangiovanni 2010 *(Continued)*

Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		Low risk	

Schellhaas 2017

Study characteristics	
Patient Sampling	People at risk for HCC with FLL on conventional ultrasound were included. Risk population for HCC defined according to German national guidelines as participants with liver cirrhosis of any origin, chronic hepatitis B virus infection, chronic hepatitis C virus infection with advanced fibrosis, non-alcoholic steatohepatitis or treated HCC. HCC surveillance was the reason for presentation in 44% of participants. In addition, participants were identified when presenting for conventional liver ultrasound or CEUS (or both) including patients undergoing HCC surveillance, symptomatic patients, and patients with incidental FLLs.
Patient characteristics and setting	85 men and 15 women, mean age 61 years (range 42–85 years). 81% had liver cirrhosis; 57% reported hazardous alcohol consumption. HCC surveillance was reason for presentation in 44% of participants. 20% of participants were symptomatic (abdominal pain, ascites, weight loss). Distribution of BCLC stages was A, 52%; B, 31%; C, 16%; and D, 1%.

Schellhaas 2017 *(Continued)*

Index tests	EFSUMB guidelines for the characterisation of FLLs following a standardised protocol with low mechanical index and intravenous bolus of 1.5 mL SonoVue (Bracco Imaging GmbH, Konstanz, Germany) followed by a saline flush. Contrast enhancement patterns of FLLs during the arterial, portal venous, and late phase were assessed. Vascular phases defined according to EFSUMB guidelines CEUS-LI-RADS contains 5 categories named LR-1, LR-2, etc, with LR-1 designating definitely benign lesions and LR-5 designating definite HCCs. LR-Tr is used for treated observations. LR-5-V describes a definite tumour in the veins. LR-M is used for lesions 'definitely or probably' malignant, not specific for HCC.
Target condition and reference standard(s)	Histology, contrast-enhanced CT, and contrast-enhanced MRI served as reference standards.
Flow and timing	No details concerning time span between index test and reference standard.
Comparative	
Notes	Acknowledgement to the Society for Gastroenterology in Bavaria (GFGB) for their grant supporting parts of this work. COI: authors reported no COI.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern

Schellhaas 2017 *(Continued)*

DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Shin 2015

Study characteristics	
Patient Sampling	46 people with cirrhosis and a liver nodule < 3 cm showing an atypical or non-coincident typical vascular pattern on 2 dynamic imaging techniques, who underwent liver CEUS and ultrasound-guided liver biopsy, were retrospectively reviewed.
Patient characteristics and setting	46 people with cirrhosis recruited in an internal medicine department from Incheo in Korea. Mean age 58 (SD 9) years; 34 (73.9%) men. Aetiology of liver cirrhosis was hepatitis B virus-associated in 37 participants. Hepatitis C virus-associated in 6 participants, and alcoholism and hepatitis B virus-associated in 3 participants. Participants were selected based on lesion diameter.
Index tests	Enhancement features of CEUS were classified as hypo-, iso-, or hyperenhancement compared with surrounding liver parenchyma and related to vascular phase. Lesions that appeared hypoechoic versus surrounding liver parenchyma were defined as exhibiting hypoenhancement, and lesions that had the same echogenicity as surrounding liver parenchyma were defined as exhibiting isoenhancement. Hyperenhanced lesions were subdivided into homogeneously hyperenhanced and reticularly hyperenhanced. Reticular hyperenhancement defined as a fine network of many hyperenhancing lines filling the nodule against a hypoenhanced background. Homogeneously hyperechoic lesions versus background liver parenchyma defined as exhibiting hyperenhancement.

Shin 2015 *(Continued)*

Target condition and reference standard(s)	Specimens of all liver nodules were obtained by ultrasound-guided biopsy using Tru-cut biopsy needles (ACECUT biopsy needle, TSK). All participants underwent ≥ 2-piece biopsy. Nodular hepatocellular lesions diagnosed according to International Working Party criteria. To eliminate interobserver variation affecting pathological diagnosis of liver nodules, a single experienced hepato-pathologist reviewed all histology slides.
Flow and timing	All biopsies were performed on the same day as CEUS and always after CEUS, to avoid changes in imaging appearance.
Comparative	
Notes	COI: none declared by authors.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	

Shin 2015 *(Continued)*

Are there concerns that the target condition as defined by the reference standard does not match the question?		Low concern
DOMAIN 4: Flow and Timing		
Was there an appropriate interval between index test and reference standard?	Yes	
Did all patients receive the same reference standard?	Yes	
Were all patients included in the analysis?	Yes	
Could the patient flow have introduced bias?		Low risk

Sporea 2019

Study characteristics	
Patient Sampling	Prospective study conducted by the Romanian Society for Ultrasound and Medicine and Biology at 14 Romanian centres over 6 years (February 2011 to April 2017). Included 2062 FLLs assessed by CEUS. Mean size of FLLs 4.5 (SD 3.3) cm. We requested the 2 × 2 table for the diagnosis of HCC on a patient and not for lesion bases.
Patient characteristics and setting	2062 participants (1148 men and 913 women). Mean age 52.4 (SD 7.5) years. 1335 (64.7%) lesions were detected in participants without chronic hepatopathies. An oncological history was present in 16.4% of participants. In 47% of cases, the lesions were incidental findings, and in 1.3%, the indication was inconclusive contrast-enhanced CT or contrast-enhanced MRI.
Index tests	Lesion enhancement pattern at CEUS was assessed and documented during the arterial (until 30 s after the contrast bolus), portal (30–120 s), and late phase (> 120 s). The FLL enhancement pattern was assessed according to the EFSUMB guidelines. All contrast studies performed using SonoVue (Bracco Spa, Milan, Italy) as a contrast agent, dedicated contrast software, and low mechanical index. Amount of contrast used was different, according to the ultrasound machines (1.6 mL or 2.4 mL).
Target condition and reference standard(s)	CEUS diagnosis was compared with the final diagnosis established, based on the reference method (contrast-enhanced CT, contrast-enhanced MRI, or histology).
Flow and timing	No details concerning time span between the index test and reference standard.
Comparative	
Notes	Authors were requested to supply the data from patients with a single liver lesion and provided data on 2062 participants with a single FLL. COI: none to declare.
Methodological quality	

Sporea 2019 *(Continued)*

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Unclear		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Unclear risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Unclear risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Strobel 2021

Study characteristics	
Patient Sampling	470 people with cirrhosis with liver lesions on B-mode ultrasound were recruited prospectively in 43 centres from April 2018 to April 2019, and clinical and imaging data collected. Final diagnosis was HCC in 378 cases (80.4%).
Patient characteristics and setting	Inclusion criteria: age ≥ 18 years, presence of a FLL visible on conventional B-mode ultrasound, and availability of a reference standard. 470 participants with cirrhosis recruited in 43 centers. 389 (82.8%) men and 81 (17.2%) women; mean age 67.1 (SD 10.3) years.
Index tests	All participants underwent conventional liver ultrasound, followed by immediate CEUS. CEUS was performed according to the EFSUMB guidelines. Used a standardised protocol with continuous assessment of the arterial phase until maximum contrast enhancement was reached in the lesion, followed by intermittent scanning with short sweeps through the lesion 1 min, 3 min, and 4–6 min in case of no contrast washout after 3 min. With CEUS LI-RADS, there are 8 categories: CEUS-LR-1 = definitely benign; CEUS-LR- 2 = probably benign; CEUS-LR-3 = intermediate probability of malignancy; CEUS-LR-4 = probably HCC; CEUS-LR-5 = definitely HCC; CEUS-LR-M = probably or definitely malignant, not necessarily HCC; CEUS-LR-TIV = tumour in vein; CEUS-LR-NC = non-categorisable.
Target condition and reference standard(s)	Reference standard for the assessment of diagnostic accuracy of CEUS was histology. If histology was not available, contrast-enhanced MRI or contrast-enhanced CT were accepted as reference standards.
Flow and timing	No details concerning time span between the index test and reference standard.
Comparative	
Notes	Funding Open Access funding enabled and organised by Projekt DEAL. The study has received funding from the German Society for Ultrasound in Medicine (Deutsche Gesellschaft für Ultraschall in der Medizin, DEGUM). COI: authors declared no relationships with any companies whose products or services they used.

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	Yes		
Could the selection of patients have introduced bias?		Low risk	

Strobel 2021 *(Continued)*

Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Sugimoto 2020

Study characteristics	
Patient Sampling	Used a clinical/pathological database to retrospectively identify 430 consecutive participants with risk factors for HCC who presented with untreated liver nodules and who underwent CEUS between March 2017 and April 2020. Inclusion criteria: aged ≥ 20 years, visible liver nodule in baseline ultrasound, availability of a CEUS examination that conformed with CEUS protocol and in-

Sugimoto 2020 *(Continued)*

	cluded vascular phase and Kupfer phase information, and availability of an accepted diagnostic reference standard.
Patient characteristics and setting	104 participants; median age 70 years, interquartile range 54.5–78.0 years; 74 men. Participants selected based on data availability.
Index tests	Pulse inversion harmonic imaging used for CEUS examinations with mechanical index 0.1–0.2 and dynamic range 45 dB. Sonazoid (GE Healthcare) injected as a 0.5-mL bolus into an antecubital vein via a 21-gauge peripheral cannula, followed by a 10-mL saline flush. Images recorded continuously as a cine clip for 60 s. immediately after injection of contrast agent (for evaluation of the vascular phase), after which the scan was frozen. After a waiting period of approximately 10 min from the time of contrast agent injection to permit pooling of the agent in the liver parenchyma, enhancement of the lesion was observed using a sweep scan, and images were recorded (for evaluation of the Kupffer phase or postvascular phase).
Target condition and reference standard(s)	All malignant lesions, including both HCC and non-HCC malignancies, were diagnosed based on the findings of histopathological examination. Reference standard for benign lesions was either histopathological assessment or typical imaging features on dynamic CT or MRI with no change in size over a minimum 1-year follow-up.
Flow and timing	No details concerning time span between the index test and reference standard.
Comparative	
Notes	Authors reported that research received no external funding. COI: authors declared no COIs.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	Yes		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		

Sugimoto 2020 *(Continued)*

Could the conduct or interpretation of the index test have introduced bias?		Unclear risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	Yes		
Could the patient flow have introduced bias?		High risk	

Tan 2020

Study characteristics	
Patient Sampling	Retrospective study. Inclusion criterion was availability of CEUS and paired contrast-enhanced liver protocol CT or MR in the radiology database between July 2010 and April 2017. Exclusion criteria: prior treatment to lesion; contrast-enhanced CEUS and contrast-enhanced CT/MR were not within 3 months of each other; non-diagnostic quality examinations; inadequate clinical, pathological or follow-up to establish a reference standard.
Patient characteristics and setting	Final cohort consisted of 13 women and 32 men; mean age 63.1 years; age range 34–84 years. Aetiology for cirrhosis was alcohol-induced (4 participants), hepatitis B virus (28), hepatitis C virus (6), non-alcoholic steatohepatitis (4), and idiopathic (3).
Index tests	CEUS was performed using 2 ultrasound systems (LOGIQ E9, GE HealthCare, Amersham, UK and Aplio 500, Canon Medical Systems, Otawara, Japan). All participants underwent conventional B-mode ultrasound prior to CEUS to identify the lesion of interest. Lesion size measured on B-mode ultrasound. Characterisation of identified lesion was performed according to the joint WFUMB-ESFUMB CEUS guidelines. Standard protocol used low mechanical index imaging and administration of intra-

Tan 2020 *(Continued)*

	venous bolus of sulphur hexafluoride (SonoVue, Bracco Imaging) (40 participants) or perfluorobutane (Sonazoid, GE HealthCare) (6 participants) microbubbles with standard recommended dosage as per manufacturer recommendations, followed by a saline flush. Cine clips of the entire first minute, followed by periodic short cine-clips thereafter (to prevent early premature inertial cavitation of microbubble) taken over ≤ 5 min, beginning after the injection of the microbubbles. Enhancement patterns of lesion(s) in the arterial, portal venous, and late phases (defined according to the WFUMB-EFSUMB guidelines were reviewed and assessed. Information from the postvascular Kupffer phase for Kupffer-based agent (Sonazoid) was not included in analysis.
Target condition and reference standard(s)	Reference standard for final determinant as to whether the lesion was HCC or not was made at multidisciplinary meetings (26 participants), on histopathology (core biopsies 2, surgical resection 6), or follow-up imaging (12).
Flow and timing	Mean time between contrast-enhanced CT/MR and CEUS studies was 28.1 days.
Comparative	
Notes	No information available about funding and COI.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Unclear risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			

Tan 2020 *(Continued)*

Is the reference standards likely to correctly classify the target condition?	No		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		
Could the reference standard, its conduct, or its interpretation have introduced bias?		High risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Yes		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	No		
Could the patient flow have introduced bias?		High risk	

Terzi 2018

Study characteristics	
Patient Sampling	Retrospective study of people with distinct nodules in cirrhosis submitted to CEUS in 5 Italian centres. 848 people with 1006 liver nodules evaluated between January 2005 and December 2015, were included in final study population. After obtaining data from study authors, we included the results on 706 participants with a single FLL.
Patient characteristics and setting	Inclusion criteria: presence of cirrhosis as identified risk for HCC according to the EASL guidelines; visible nodules investigated by CEUS; availability of CEUS information reporting the arterial phase pattern, timing of onset and degree of washout whenever this feature occurred (either reported on the original report or, whenever this lacked, as assessed retrospectively by the investigator on recorded video clips and images); availability of an accepted diagnostic reference standard, either CT/MR scan or histology; diagnosis performed within 12 weeks from index CEUS; no vascular invasion; and no local nodule relapse on site of previously treated HCC.
Index tests	CEUS with sulphur exafluoride (SonoVue, Bracco, Milan Italy). Arterial phase hyperenhancement defined as a lesion becoming globally or partially hyperechoic (but not with rim or globular peripheral distribution) compared to the surrounding parenchyma in arterial phase. Washout defined when lesion became hypoechoic compared to surrounding parenchyma in portal venous phase. When such washout occurred, it was further classified according to its timing; defined as 'early', if it appeared before 60 s following contrast injection or as 'late' if occurring later, and to its intensity, as 'marked' when the lesion became markedly hypoenhanced or punched out (otherwise defined as 'mild') within 2 min. A rim enhancement pattern (not globular peripheral) in arterial phase categorised the lesion as LR-M, regardless of the venous pattern. Furthermore, marked or early-onset venous washout (or both) classified a lesion as LR-M regardless of the arterial appearance. LR-5 class comprised nodules ≥ 10 mm with arterial phase hyperenhancement (either global or in part) followed by washout appearance that was

Terzi 2018 *(Continued)*

	mild in degree and late in onset. If the same pattern was observed in lesions < 10 mm the category was LR-4 (this definition was adopted by the American College of Radiology consistently with the CT/MRI LI-RADS).
Target condition and reference standard(s)	Diagnostic reference was histology, whenever available (either obtained by percutaneous biopsy or after surgical resection) or a CT/MRI diagnosis of HCC according to the vast majority of guidelines. The combination of arterial and venous phase appearances allowed classification of nodules within the LR-M, LR-3, LR-4, and LR-5 classes according to the LI-RADS scheme.
Flow and timing	No details concerning time span between the index test and reference standard.
Comparative	
Notes	Authors were requested to supply the data from participants with a single liver lesion and they provided data on 706 participants with a single FLL. Funding: no financial support. COI: Iavarone M: Bayer, Gilead Science, Janssen, Abbvie – speaker bureau honoraria; BTG: speaker bureau honoraria and consultant. Wilson SR: Siemens, Philips and Samsung – equipment support; GE and Samsung – speaker honoraria. Piscaglia F: Bayer – speaker bureau and advisory board honoraria; Bracco – speaker bureau honoraria; Esaote – Research contract. Member of the governing board of the International Contrast Ultrasound Society.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			Low concern
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear		
If a threshold was used, was it prespecified?	Yes		
Were positivity criteria clearly defined?	Yes		

Terzi 2018 *(Continued)*

Could the conduct or interpretation of the index test have introduced bias?		Unclear risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	No		
Were all patients included in the analysis?	No		
Could the patient flow have introduced bias?		High risk	

Wang 2006

Study characteristics	
Patient Sampling	Retrospective study of people with cirrhosis with well-defined hepatic nodules 1–2 cm in diameter as determined by ultrasonography.
Patient characteristics and setting	30 people with cirrhosis with 30 small hepatic nodules (1–2 cm) enrolled. Participants selected based on lesion diameter.

Wang 2006 *(Continued)*

Index tests	Contrast agent: Levovist (Schering, Berlin, Germany). Upon completion of Levovist injection, hepatic nodules were studied continuously for 40 s to evaluate arterial phase enhancement. Then there was a delay of 5–6 min before scanning. CEUS performed in the delayed phase with sweep scanning from above to below the hepatic nodules. With surrounding hepatic parenchyma enhancement as a reference, arterial phase enhancement defined as linear contrast enhancement within the hepatic nodule during first 40 s after completion of Levovist. Absence of delayed phase enhancement defined as a contrast filling defect corresponding to the hepatic nodule at sweeping scanning performed 6–7 min after Levovist.
Target condition and reference standard(s)	Reference standard to diagnose HCC was dynamic CT images reviewed by a radiologist who had no knowledge of the final diagnosis CT.
Flow and timing	No details concerning time span between the index test and reference standard.
Comparative	
Notes	No information available about funding and COI.

Methodological quality

Item	**Authors' judgement**	**Risk of bias**	**Applicability concerns**
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			

Wang 2006 *(Continued)*

Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes		
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk	
Are there concerns that the target condition as defined by the reference standard does not match the question?			Low concern
DOMAIN 4: Flow and Timing			
Was there an appropriate interval between index test and reference standard?	Unclear		
Did all patients receive the same reference standard?	Yes		
Were all patients included in the analysis?	No		
Could the patient flow have introduced bias?		High risk	

Zuo 2021

Study characteristics	
Patient Sampling	During this retrospective study, examination of 873 consecutive people with FFLs undergoing CEUS in a tertiary central hospital from January 2017 to May 2020 were reviewed. Exclusion criteria: without contrast-enhanced CT/MRI or biopsy or other data; lost to follow-up; with diffuse HCCs, FLLs of maximal diameter > 8 cm (compromising visualisation); had undergone transcatheter arterial chemoembolisation, radiofrequency ablation, chemotherapy, and radiotherapy; and with poor quality of CEUS for the FLLs.
Patient characteristics and setting	Only participants with available data were included.
Index tests	Contrast agent: sulphur hexafluoride microbubbles (Shanghai Bracco Sine Pharmaceutical Corp Ltd, Shanghai, China). During procedure, 2.4 mL suspension liquid of sulphur hexafluoride microbubbles was administered by bolus injection from the antecubital vein via a 20-gauge catheter, and a flush of 5 mL 0.9% sodium chloride solution. Imaging timer started simultaneously with the injection of microbubbles. Target lesion was imaged and observed for ≥ 5 min. The CEUS imaging and representative images were saved in the ultrasound system and the Picture and Archiving and Communication Systems.
Target condition and reference standard(s)	Different reference standards were used. In particular, reference criteria for participants with FFLs categorised as CEUS LR-1 and LR-2 was contrast-enhanced CT or MRI and FFLs categorised as LR-1 and LR-2 were considered benign. FFLs categorised as CEUS LR-3 and LR-4 were evaluated with imaging follow-up or biopsy. FFLs that did not progress to a higher CEUS LR category at 3 times follow-up in 12 months were considered benign; if FFL increase > 20% in size at follow-up CEUS, further assessment with contrast-enhanced CT or MRI, or biopsy was performed. Inconclusive FFLs that developed to LR-5 at follow-up CEUS and contrast-enhanced CT or MRI were considered to be HCC. Biopsy and histological analysis was used as reference criteria for participants with FFLs of CEUS LR-M. Participants with FFLs of CEUS LR-3 and LR-4 without histo-

Zuo 2021 *(Continued)*

	logical diagnosis that remained inconclusive at follow-up contrast-enhanced CT or MRI were ruled out (counted as exclusion patients). All FFLs categorised LR-5 after contrast-enhanced CT or MRI (or both) assessment were considered to be HCC.
Flow and timing	No details concerning time span between the index test and reference standard.
Comparative	
Notes	Project supported by National Natural Science Foundation of China (Grant No. 81560290). COI: authors declared no COIs.

Methodological quality

Item	Authors' judgement	Risk of bias	Applicability concerns
DOMAIN 1: Patient Selection			
Was a consecutive or random sample of patients enrolled?	No		
Did the study avoid inappropriate exclusions?	No		
Could the selection of patients have introduced bias?		High risk	
Are there concerns that the included patients and setting do not match the review question?			High
DOMAIN 2: Index Test (All tests)			
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes		
If a threshold was used, was it pre-specified?	Yes		
Were positivity criteria clearly defined?	Yes		
Could the conduct or interpretation of the index test have introduced bias?		Low risk	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?			Low concern
DOMAIN 3: Reference Standard			
Is the reference standards likely to correctly classify the target condition?	Yes		
Were the reference standard results interpreted without knowledge of the results of the index tests?	No		

Zuo 2021 *(Continued)*

Could the reference standard, its conduct, or its interpretation have introduced bias?		High risk
Are there concerns that the target condition as defined by the reference standard does not match the question?		Low concern
DOMAIN 4: Flow and Timing		
Was there an appropriate interval between index test and reference standard?	Unclear	
Did all patients receive the same reference standard?	No	
Were all patients included in the analysis?	No	
Could the patient flow have introduced bias?		High risk

BCLC: Barcelona Clinic Liver Cancer; CEUS: contrast-enhanced ultrasound; COI: conflict of interest; CT: computed tomography; EASL: European Association for the Study of the Liver; EFSUMB: European Federation of Societies for Ultrasound in Medicine and Biology; FLL: focal liver lesion; FNB: fine-needle biopsy; HCC: hepatocellular carcinoma; ICC: intrahepatic cholangiocarcinoma; KLCA-NCC: Korean Liver Cancer Association and National Cancer Center; LI-RADS: Liver Imaging Reporting and Data System; min: minute; MRI: magnetic resonance image; SD: standard deviation; s: second; WFUMB: World Federation for Ultrasound in Medicine and Biology.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Amadei 2008	Participant population only cases of HCC.
Arn 2003	Accuracy of conventional ultrasound and other imaging techniques, not CEUS.
Chen 2005	Analysis for lesions, not for participants.
Chen 2006	2 × 2 table not reported directly in study and could not be calculated/extracted based on the data that were available.
Chen 2019	Participants with HCC or cholangiocarcinoma.
Cheng 2021	Study aimed to correlate pathology with CEUS LI-RADS. 2 × 2 table not reported directly in study and could not be calculated/extracted based on the data that were available.
D'Onofrio 2004	Participant population: only cases of HCC.
Dai 2008	Analysis for lesions, not for participants.
Duan 2020	Diagnostic accuracy of an algorithm of which CEUS was part.
Dumitrescu 2013	Analysis for lesions, not for participants.
Esfeh 2020	Index test was standard ultrasound, not CEUS.

Study	Reason for exclusion
Forner 2012	2 × 2 table not reported directly in study and could not be calculated/extracted based on the data that were available.
Gaiani 2004	Analysis for lesions, not for participants.
Geyer 2021	Population selected based on the presence of a CEUS examination. Population was mixed, and it was not clearly stated how many participants had chronic liver disease.
Giangregorio 2010	Participant with previous treatment of HCC.
Giorgio 2004	Participant population only cases of HCC.
Goto 2012	Population of participants with HCC.
Guo 2020	CEUS accuracy for hepatic inflammatory lesions, not HCC.
Guo 2022	Population including only participants with malignant tumours.
Hatanaka 2008	Analysis per lesions, and not for participants.
Huang 2020b	Study reported partial data fully reported in Huang 2020a. Aim was to investigate the possibility and efficacy of differentiating intrahepatic cholangiocarcinoma from HCC.
Inoue 2005	CEUS for prediction of HCC differentiation grade.
Kim 2005	2 × 2 table not reported directly in study and could not be calculated/extracted based on the data that were available.
Kudo 2010	Not matching the review question.
Lee 2012	Index test was contrast computed tomography, i.e. different from the index test in our review.
Lee 2020	Prognostic study.
Lencioni 2008	Review on CEUS for HCC.
Leoni 2010	Analysis for lesions, not for participants.
Li 2021	Review without original data.
Liu 2016	Analysis for lesions, not for participants.
Lv 2021	Analysis for lesions, and not for participants.
Martie 2011	Analysis for lesions, not for participants.
Mita 2010	Analysis for lesions, not for participants.
Motz 2021	2 × 2 table not reported directly in study and could not be calculated/extracted based on the data that were available.
Nicolau 2004a	Participant population only cases of HCC.
Nicolau 2004b	Review on CEUS for HCC.

Study	Reason for exclusion
Palmieri 2015	Analysis for lesions, not for participants.
Pan 2021	Analysis for lesions, not for participants.
Park 2017	Review article.
Pompili 2008	Analysis for lesions, not for participants.
Quaia 2002	Analysis for lesions, not for participants.
Quaia 2009	Analysis for lesions, not for participants.
Rode 2001	Traditional ultrasound, not CEUS.
Sawatzki 2019	Analysis for lesions, not for participants.
Schellhaas 2021	2 × 2 table not reported directly in study and could not be calculated/extracted based on the data that were available.
Sirli 2010	Analysis for lesions, not for participants.
Strunk 2005	Analysis for lesions, not for participants.
Suzuki 2004	Analysis for lesions, not for participants.
Tranquart 2008	Analysis for lesions, not for participants.
Tranquart 2009	Analysis for lesions, not for participants.
Uno 2001	Analysis for lesions, not for participants.
von Herbay 2004	Analysis for lesions, not for participants.
Yang 2021	Systematic review with meta-analysis.
Zeng 2006	Study aimed at assessing the role of CEUS in identifying the actual tumour size and invasion range.
Zeng 2022	Analysis for lesions, not for participants.
Zheng 2020	Analysis for lesions, not for participants.
Zhou 2021	Study aimed at evaluating microvascular invasion in HCC.
Zocco 2010	Analysis for lesions not for participants.

CEUS: contrast-enhanced ultrasound; HCC: hepatocellular carcinoma; LI-RADS: Liver Imaging Reporting And Data System.

DATA

Presented below are all the data for all of the tests entered into the review.

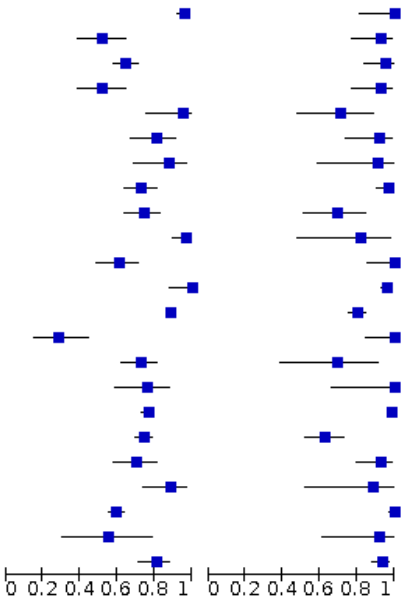
Table Tests. Data tables by test

Test	No. of studies	No. of participants
1 CEUS	23	6546
2 Secondary objective	13	1257

Test 1. CEUS

CEUS

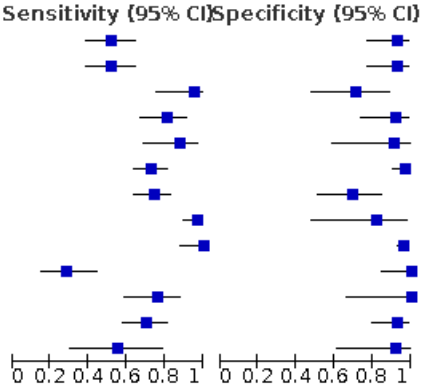
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
de Sio 2014	220	0	10	18	0.96 [0.92, 0.98]	1.00 [0.81, 1.00]
Di Carlo 2012	31	2	29	27	0.52 [0.38, 0.65]	0.93 [0.77, 0.99]
Ding 2021	144	2	79	39	0.65 [0.58, 0.71]	0.95 [0.83, 0.99]
Forner 2008	31	2	29	27	0.52 [0.38, 0.65]	0.93 [0.77, 0.99]
Fracanzani 2001	19	6	1	15	0.95 [0.75, 1.00]	0.71 [0.48, 0.89]
Giorgio 2007	39	2	9	23	0.81 [0.67, 0.91]	0.92 [0.74, 0.99]
Giorgio 2010	22	1	3	10	0.88 [0.69, 0.97]	0.91 [0.59, 1.00]
Huang 2020a	76	2	28	69	0.73 [0.63, 0.81]	0.97 [0.90, 1.00]
Hwang 2021	66	10	23	23	0.74 [0.64, 0.83]	0.70 [0.51, 0.84]
Kan 2010	66	2	2	9	0.97 [0.90, 1.00]	0.82 [0.48, 0.98]
Kang 2020	48	0	31	24	0.61 [0.49, 0.72]	1.00 [0.86, 1.00]
Kudo 2019	28	11	0	270	1.00 [0.88, 1.00]	0.96 [0.93, 0.98]
Li 2019	723	52	90	213	0.89 [0.87, 0.91]	0.80 [0.75, 0.85]
Sangiovanni 2010	12	0	30	22	0.29 [0.16, 0.45]	1.00 [0.85, 1.00]
Schellhaas 2017	63	4	24	9	0.72 [0.62, 0.81]	0.69 [0.39, 0.91]
Shin 2015	28	0	9	9	0.76 [0.59, 0.88]	1.00 [0.66, 1.00]
Sporea 2019	525	21	160	1356	0.77 [0.73, 0.80]	0.98 [0.98, 0.99]
Strobel 2021	280	34	98	58	0.74 [0.69, 0.78]	0.63 [0.52, 0.73]
Sugimoto 2020	45	3	19	37	0.70 [0.58, 0.81]	0.93 [0.80, 0.98]
Tan 2020	32	1	4	8	0.89 [0.74, 0.97]	0.89 [0.52, 1.00]
Terzi 2018	342	0	231	133	0.60 [0.56, 0.64]	1.00 [0.97, 1.00]
Wang 2006	10	1	8	11	0.56 [0.31, 0.78]	0.92 [0.62, 1.00]
Zuo 2021	80	7	19	107	0.81 [0.72, 0.88]	0.94 [0.88, 0.97]



Test 2. Secondary objective

Secondary objective

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Di Carlo 2012	31	2	29	27	0.52 [0.38, 0.65]	0.93 [0.77, 0.99]
Forner 2008	31	2	29	27	0.52 [0.38, 0.65]	0.93 [0.77, 0.99]
Fracanzani 2001	19	6	1	15	0.95 [0.75, 1.00]	0.71 [0.48, 0.89]
Giorgio 2007	39	2	9	23	0.81 [0.67, 0.91]	0.92 [0.74, 0.99]
Giorgio 2010	22	1	3	10	0.88 [0.69, 0.97]	0.91 [0.59, 1.00]
Huang 2020a	76	2	28	69	0.73 [0.63, 0.81]	0.97 [0.90, 1.00]
Hwang 2021	66	10	23	23	0.74 [0.64, 0.83]	0.70 [0.51, 0.84]
Kan 2010	66	2	2	9	0.97 [0.90, 1.00]	0.82 [0.48, 0.98]
Kudo 2019	28	11	0	270	1.00 [0.88, 1.00]	0.96 [0.93, 0.98]
Sangiovanni 2010	12	0	30	22	0.29 [0.16, 0.45]	1.00 [0.85, 1.00]
Shin 2015	28	0	9	9	0.76 [0.59, 0.88]	1.00 [0.66, 1.00]
Sugimoto 2020	45	3	19	37	0.70 [0.58, 0.81]	0.93 [0.80, 0.98]
Wang 2006	10	1	8	11	0.56 [0.31, 0.78]	0.92 [0.62, 1.00]



ADDITIONAL TABLES

Table 1. Studies general findings

Finding	Number of studies	Details		
Cirrhosis	19	Range 37–100%	—	—
Child-Pugh class A	4	Range 61–98%	—	—
Viral aetiology	16	Median 80% (IQ 71–92%)	—	—
Geographic area	23	12 in Asia	11 in Europe	—
Setting	23	15 clinical suspicion of HCC	8 surveillance programme	—
Contrast media	23	17 SonoVue	4 Sonazoid	2 Levovist
HCC median diameter	19	Median 25 mm (IQR 20–32 mm)	—	—
Diagnostic pathway	23	17 CEUS after ultrasound	6 CEUS after CT or MRI	—
Positivity criteria	23	22 clearly defined	1 unclear	—
Positivity criteria	23	10 LI-RADS	13 other criteria	—
Reference standard	23	10 histology	12 different (histology or CT or MRI)	1 CT
Conflict of interest	23	12 no conflict of interest	2 possible conflict of interest	10 no information

CEUS: contrast-enhanced ultrasound; CT: computed tomography; IQ: interquartile; IQR: interquartile range; LI-RADS: Liver Imaging Reporting And Data System; MRI: magnetic resonance imaging; HCC: hepatocellular carcinoma.

Table 2. Heterogeneity and sensitivity analyses for contrast-enhanced ultrasound

Subgroup or sensitivity analysis		Number of studies	Sensitivity (95% CI)	Specificity (95% CI)	P value
All		23	77.8% (69.4% to 84.4%)	93.8% (89.1% to 96.6%)	—
Secondary objective: resectable HCC		13	77.5% (62.9% to 87.6%)	92.7% (86.8% to 96.1%)	—
Sensitivity analyses	Positivity criteria clearly defined	22	77.3% (68.6% to 84.2%)	93.9% (89.0% to 96.7%)	—
	Reference standard results interpreted without knowledge of the results of the index test	5	63.9% (43.0% to 80.6%)	98.1% (89.9% to 99.7%)	—
	Full text	21	78.3% (69.6% to 85.0%)	94.0% (88.8% to 96.9%)	—

Table 2. Heterogeneity and sensitivity analyses for contrast-enhanced ultrasound *(Continued)*

Pathway	Pathway 1	17	76.6% (66.0% to 84.7%)	93.9% (88.4% to 96.8%)	0.950
	Pathway 2	6	80.7% (67.1% to 89.6%)	93.6% (77.2% to 98.5%)	
Country	Europe	11	74.5% (59.8% to 85.1%)	94.8% (85.3% to 98.3%)	0.865
	Asia	12	80.3% (70.8% to 87.2%)	92.9% (87.2% to 96.2%)	
HCC prevalence	HCC prevalence > median (69%)	11	80.8% (70.5% to 88.1%)	93.3% (79.1% to 98.1%)	0.668
	HCC prevalence ≤ median (69%)	12	74.9% (60.9% to 85.1%)	95.3% (91.6% to 97.5%)	
Type of contrast	Type of contrast 1–2	19	74.8% (66.0% to 82.0%)	94.7% (89.6% to 97.4%)	0.364
	Type of contrast 3	4	92.3% (64.7% to 99.0%)	89.5% (76.6% to 96.0%)	
Reference standard	Biopsy	10	74.1% (58.8% to 85.1%)	92.4% (84.9% to 96.3%)	0.563
	Different (histology or CT or MRI)	12	76.1% (68.6% to 82.3%)	94.6% (86.7% to 97.9%)	
	CT	1	97.1% (89.8% to 99.6%)	81.8% (48.2% to 97.7%)	
Viral aetiology[a]	> 80%	8	81.1% (69.7% to 88.9%)	93.1% (87.3% to 96.3%)	0.376
	< 80%	8	79.1% (57.7% to 91.3%)	98.1% (92.0% to 99.6%)	
LI-RADs	Yes	10	75.4% (68.9% to 80.9%)	91.0% (79.7% to 96.3%)	0.340
	No	13	81.0% (65.3% to 90.6%)	95.8% (91.6% to 97.9%)	

CI: confidence interval; CT: computed tomography; LI-RADs: Liver Imaging Reporting And Data System; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging.

[a]Aetiology of the underlying liver disease: prevalence of viral (hepatitis C virus or hepatitis B virus infection) aetiologies. In seven studies, the number of participants with viral aetiology was not reported.

Table 3. Post-test probabilities

Pre-test probabilities		Likelihood ratio	Post-test probabilities
10%	if CEUS positive	12.60	58%
10%	if CEUS negative	0.24	3%
50%	if CEUS positive	12.60	93%
50%	if CEUS negative	0.24	19%
69%	if CEUS positive	12.60	97%
69%	if CEUS negative	0.24	34%

Table 3. Post-test probabilities *(Continued)*

80%	if CEUS positive	12.60	98%
80%	if CEUS negative	0.24	49%
90%	if CEUS positive	12.60	99%
90%	if CEUS negative	0.24	68%

CEUS: contrast-enhanced ultrasound.

Table 4. Other diagnostic reviews on diagnostic accuracy of contrast-enhanced ultrasound (CEUS) for hepatocellular carcinoma

Systematic review	Analysis type	Number of studies included	Number of participants analysed	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Use of bivariate statistical model
Niu 2013	Per lesion	15	908 (1032 nodules)	0.81 (0.78 to 0.85)	0.86 (0.82 to 0.89)	No
Deng 2016	Mixed	16	NR	0.86 (0.79 to 0.91)	0.87 (0.75 to 0.94)	No (random-effects model)
Huang 2017	Per lesion	8	623	0.75 (0.70 to 0.80)	0.91 (0.87 to 0.94)	No (fixed-effect model)
Yang 2021	Mixed	9	2193	0.90 (0.82 to 0.95)	0.97 (0.93 to 0.98)	No
Li 2021	Per lesion	8	4215	0.71 (0.69 to 0.72)	0.88 (0.85 to 0.91)	No (random-effects model)

CI: confidence interval; NR: not reported.

APPENDICES

Appendix 1. Search strategies

Database	Time span	Search strategy
Cochrane Hepato-Biliary Group Controlled Trials Register	5 November 2021	(ultrasound or ultrasonogra* or US or CEUS or sonogra* or echogra* or echotomogra*) AND (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC) AND ((advanc* or chronic) and (liver* or hepat*))
Cochrane Hepato-Biliary Group Diagnostic	5 November 2021	(ultrasound or ultrasonogra* or US or CEUS or sonogra* or echogra* or echotomogra*) AND (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC) AND ((advanc* or chronic) and (liver* or hepat*))

(Continued)

Test of Accuracy Studies Register		
The Cochrane Library	2021, issue 11	#1 MeSH descriptor: [Ultrasonography] explode all trees #2 (ultrasound or ultrasonogra* or US or CEUS or sonogra* or echogra* or echotomogra*) #3 #1 or #2 #4 MeSH descriptor: [Carcinoma, Hepatocellular] explode all trees #5 MeSH descriptor: [Liver Neoplasms] explode all trees #6 (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC) #7 #4 or #5 or #6 #8 ((advanc* or chronic) and (liver* or hepat*)) #9 #3 and #7 and #8
MEDLINE Ovid	1946 to 5 November 2021	1. exp Ultrasonography/ 2. (ultrasound or ultrasonogra* or US or CEUS or sonogra* or echogra* or echotomogra*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] 3. 1 or 2 4. exp Carcinoma, Hepatocellular/ 5. exp Liver Neoplasms/ 6. (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] 7. 4 or 5 or 6 8. ((advanc* or chronic) and (liver* or hepat*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] 9. 3 and 7 and 8
Embase Ovid	1974 to 5 November 2021	1. exp echography/ 2. exp ultrasound/ 3. (ultrasound or ultrasonogra* or US or CEUS or sonogra* or echogra* or echotomogra*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] 4. 1 or 2 or 3 5. exp liver cell carcinoma/

(Continued)

		6. exp liver tumor/ 7. (((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] 8. 5 or 6 or 7 9. ((advanc* or chronic) and (liver* or hepat*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] 10. 4 and 8 and 9
LILACS (Bireme)	1982 to 5 November 2021	((advanc$ or chronic) and (liver$ or hepat$)) [Words] and (((liver or hepato$) and (carcinom$ or cancer$ or neoplasm$ or malign$ or tumo$)) or HCC) [Words] and (ultrasound or ultrasonogra$ or US or CEUS or sonogra$ or echogra$ or echotomogra$) [Words]
Science Citation Index Expanded (Web of Science)	1900 to 5 November 2021	#4 #3 AND #2 AND #1 #3 TS=((advanc* or chronic) and (liver* or hepat*)) #2 TS=(((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC) #1 TS=(ultrasound or ultrasonogra* or US or CEUS or sonogra* or echogra* or echotomogra*)
Conference Proceedings Citation Index – Science (Web of Science)	1990 to 5 November 2021	#4 #3 AND #2 AND #1 #3 TS=((advanc* or chronic) and (liver* or hepat*)) #2 TS=(((liver or hepato*) and (carcinom* or cancer* or neoplasm* or malign* or tumo*)) or HCC) #1 TS=(ultrasound or ultrasonogra* or US or CEUS or sonogra* or echogra* or echotomogra*)

Appendix 2. QUADAS-2

Domain	1. Participant selection	2. Index test	3. Reference standard	4. Flow and timing
Signalling questions and criteria	Q1: "*Was a consecutive or random sample of participants enrolled?*" Yes – if the study reports on a consecutive or a random selection of participants. No – if the study reports on another	Q1: "*Were the index test results interpreted without knowledge of the results of the reference standard?*" Yes – if the study reports that the results of the index test were interpreted without the knowledge of the results of the reference standard.	Q1: "*Is the reference standard likely to correctly classify the target condition?*" Yes – if the reference standard correctly defines the presence/absence of HCC such as pathology of explanted liver in a transplant cohort). No – if other reference tests than pathology of explant-	Q1: "*Was there an appropriate interval between the index test and the reference standard?*" Yes – if the interval between the index test and the reference standard was less than 3 months. No – if the interval was equal or longer than 3 months.

(Continued)

form of selection of participants.

Unclear – if the study does not report on how the participants were enrolled.

Q2: "*Did the study avoid inappropriate exclusions*?"

Yes – if definitions of exclusion criteria are appropriate (i.e. previous surgery or treatment for HCC; people with cholangiocarcinoma) and all exclusions are reported.

No – if exclusion criteria are inappropriate and exclusions are not reported.

Unclear – if the study does not report causes of exclusions.

No – if the study reports that results of the index test were interpreted with the results of the reference standard.

Unclear – if the study does not report information about blinding of the results of the index test and reference standard.

Q2: "*Were positivity criteria clearly defined?"*

Yes – if the study clearly reports positivity criteria (i.e. for blood-pool agents when hyperenhancement in arterial phase and a late washout (≥ 60 seconds) features are detected. For blood-pool/Kupffer cell agent when abundant tumour vessels appearing as basket-like or irregular branched shapes from the periphery to the centre of the lesion, and dense tumour staining in the early vascular phase and fast washout in the late vascular phase, and complete Kupffer defect are detected).

No – if the study does not report the positivity criteria.

ed liver were used, such histology of resected specimen or of focal lesion biopsy.

Q2: "*Were the reference standard results interpreted without the knowledge of the results of the index test?*"

Yes – if the study reports that the results of the reference standard were interpreted without the knowledge of the results of the index test.

No – if the study reports that the results of the reference standard were interpreted with the knowledge of the results of the index test.

Unclear – if the study does not report information about blinding of the results of the reference standard and the index test.

Unclear – if the study does not report the interval between the index test and the reference standard.

Q2: "*Did all participants receive the same reference standard?*"

Yes – if the study has only 1 reference standard for all the participants.

No – if the study has > 1 reference standard.

Unclear – if the study information regarding the use of reference standard are unclear.

Q3: "*Were all participants included in the analysis and analysed according to intention-to-diagnose principle (non-evaluable results considered as false)?"*

Yes – if all enrolled participants were included in the analysis.

No – if any participant was excluded from the analysis for any reason.

Unclear – if the exclusion of participants from the analysis is unclear.

Q4: "*Were participants with non-evaluable result of the index test included and analysed according to intention-to-diagnose principle (non-evaluable results considered as false)?*"

Yes – if participants with non-evaluable results were included and analysed according to intention to diagnose principle.

No – If participants with non-evaluable results were not included and analysed according to in-

(Continued)

				tention-to-diagnose principle.
Risk of bias	*Could the selection of participants have introduced bias?* If we answer 'yes' to all signalling questions, then we judged the risk of bias as 'low'. If we answer 'no' to at ≥ 1 of the signalling questions, then we judged the risk of bias as 'high'. If we answer 'unclear' to all signalling questions, then we judged the risk of bias as 'unclear'. If we answer 'unclear' to ≥ 1 of the signalling questions and to the remaining our answer is 'yes', then we judged the risk of bias as 'unclear'.	*Could the conduct or interpretation of the index test have introduced bias?* If we answer 'yes' to all signalling questions, then we judged the risk of bias as 'low'. If we answer 'no' to ≥ 1 of the signalling questions, then we judged the risk of bias as 'high'. If we answer 'unclear' to all signalling questions, then we judged the risk of bias as 'unclear'. If we answer 'unclear' to ≥ 1 of the signalling questions and to the remaining our answer is 'yes', then we judged the risk of bias as 'unclear'.	*Could the reference standard, its conduct, or its interpretation have introduced bias?* If we answer 'yes' to all signalling questions, then we judged the risk of bias as 'low'. If we answer 'no' to ≥ 1 of the signalling questions, then we judged the risk of bias as 'high'. If we answer 'unclear' to all signalling questions, then we judged the risk of bias as 'unclear'. If we answer 'unclear' to ≥ 1 of the signalling questions and to the remaining our answer is 'yes', then we judged the risk of bias as 'unclear'.	*Could the participant flow have introduced bias?* If we answer 'yes' to all signalling questions, then we judged the risk of bias as 'low'. If we answer 'no' to ≥ 1 of the signalling questions, then we judged the risk of bias as 'high'. If we answer 'unclear' to all signalling questions, then we judged the risk of bias as 'unclear'. If we answer 'unclear' to ≥ 1 of the signalling questions and to the remaining our answer is 'yes', then we judged the risk of bias as 'unclear'.
Concerns about applicability	*Are there concerns that included participants and setting do not match the review question?* Low concern: the participants included in the review represent the participants in whom the test is used in clinical practice (i.e. second-line imaging modality in people with suspected liver lesion). High concern: the participants included in the review differ from the participants in whom the test is used in clinical practice.	*Are there concerns that the index test, its conduct, or interpretation differ from the review question?* Low concern: the index test, its conduct, or its interpretation does not differ from the way it is used in clinical practice. High concern: the index test, its conduct, or its interpretation differs from the way it is used in clinical practice.	*Are there concerns that the target condition as defined by the reference standard does not match the question?* High concern: the definition of the target condition as defined by the reference standard does not match the question (i.e. pathology of the explanted liver is feasible only in the case of liver transplant; the natural history and prognosis of HCC detected in explanted liver might be different). Low concern: the definition of the target condition as defined by the reference standard does match the question, e.g. CT scan or MRI for all included participants.	—

CT: computed tomography; HCC: hepatocellular carcinoma; MRI: magnetic resonance imaging.

HISTORY

Protocol first published: Issue 11, 2019

CONTRIBUTIONS OF AUTHORS

MF: wrote the protocol and performed searches for references, evaluated references for obtaining the full reports, evaluated studies for inclusion, extracted data from studies, assessed the risk of bias, and designed and wrote the final review.

TN: wrote the protocol, performed searches for references, evaluated references for obtaining the full reports, assessed studies for inclusion, extracted data from studies and write the final review.

AC: co-ordinated protocol design, assessed the risk of bias, designed and wrote the final review.

CM: evaluated references for obtaining the full reports, evaluated studies for inclusion, extract data from studies, assessed the risk of bias.

VG: commented on the protocol and critically commented on the final review.

DM: commented on the protocol and critically commented on the final review.

DŠ: critically commented on the protocol, acted as arbiter when review authors could not reach a consensus, and critically commented on the final review.

GC: wrote the protocol, provided statistical expert opinion, performed statistical analyses, and critically commented on the final review.

All authors accepted the review for publication.

DECLARATIONS OF INTEREST

MF: none.

TN: none.

AC: none.

CM: none.

VG: none.

DM: none.

DŠ: none.

GC: none.

SOURCES OF SUPPORT

Internal sources

- No sources of support provided

External sources

- None, Other

  None

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The title was modified by leaving out "advanced" as chronic liver disease better describes the population of interest (i.e. people at risk for hepatocellular carcinoma).

We also performed a methodological quality assessment for the secondary objective (i.e. the diagnostic accuracy for resectable hepatocellular carcinoma), using the same signalling questions as for the primary objective.

We planned to analyse data according to the intention-to-diagnose principle (Schuetz 2012), also described as the worst-case scenario (Cohen 2016) and to classify participants with indeterminate index test results as false-positive if they had a negative reference standard,

or as false-negative if participants had a positive reference standard, but we did not actually carry out this analysis as we found only one study that reported the number of uninterpretable results (Kang 2020).

We added an additional potential source of heterogeneity: the use of LI-RADS classification as a positivity criterion. We recognised that the use of the standardised LI-RADS classification might affect the accuracy estimates of magnetic resonance imaging.

We did not perform the planned comparison of studies published before 2004 to studies published after 2004 as only one study was published before this date (Fracanzani 2001).

INDEX TERMS

Medical Subject Headings (MeSH)

*Carcinoma, Hepatocellular [diagnostic imaging]; Cross-Sectional Studies; *Liver Neoplasms [diagnostic imaging]; Sensitivity and Specificity; Tomography, X-Ray Computed; Ultrasonography

MeSH check words

Adult; Humans