

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: RE : New frontiers in liver ultrasound: from mono to multi parametricity. This is a well written review. Some concerns are listed as following:

(1) Of title and content: "New frontiers in liver ultrasound: from mono to multi parametricity" aims to diagnostic ultrasonography and an integration of diagnosis and treatment based on diagnostic ultrasound system. Nevertheless, High-intensity-focused-ultrasound is different from diagnostic ultrasound and ultrasound mediates drug delivery (or genetic materials) by several aspects, so the reviewers suggested to cut the "High-intensity-focused-ultrasound" and related content off.

Thank you for the comment: as requested, we have deleted the whole part about HIFU

High-Intensity Focused Ultrasound

High-intensity-focused-ultrasound (HIFU) is an emerging technology which uses dedicated transducers to pass through overlying intact skin and to focus ultrasound waves on a precise target volume delivering enough energy to induce thermal effect [8]. HIFU systems can rapidly increase tissue temperature up to 60 °C thus inducing coagulative necrosis in a time span of a few seconds [81].

HIFU and Magnetic Resonance-guided focused ultrasound (MRgFUS) have proven effective as non-invasive, non-surgical ablation modalities for both primary and metastatic cancer involving liver, breast, prostate, kidney, oesophagus, pancreas, brain and bone [8]. Clinical applications of HIFU and MRgFUS also include benign tumor treatment, such as uterine fibroids and non-tumorous diseases, such as pain management, prostate hypertrophy or brain disorders, including essential tremor and Parkinson's disease (Fig. 10) [82-86].

HIFU ablation has been demonstrated to be generally well tolerated for HCC treatment in Child-Pugh A and B cirrhotic patients and selected Child-Pugh C cirrhotic patients [87]. In a retrospective analysis on 275 patients affected by either primary liver cancer (n=80) or metastatic liver disease (n=195), HIFU treatment allowed an objective response rate and a disease control rate of 71.8% and 81.2%, respectively, in patients with primary liver cancer and 63.7% and 83.2% in cases with liver metastases [88]. HIFU can also reduce the dropout rate of liver transplant candidates [89].

(2) In the Abstract: Of "Such assessment is not only subjective, since quantitative data are also available." Rephrase this sentence to make it better.

Response: thank you for the comment: we replaced the sentence: "Such assessment is not only subjective, since quantitative data are also available" with the following sentence:

Modern US techniques allow a quantitative assessment of various liver diseases.

(3) In the Abstract: Of "High frequency ultrasound can also be deeply focused to liver tissue, targeting neoplasms and delivering enough energy to cause coagulative necrosis.". The "High frequency ultrasound" should be "High-intensity-focused-ultrasound".

Response: thank you for the comment. Considering that the Authors were asked to remove the HIFU section, we have consequently removed the following sentence from the abstract

"High frequency ultrasound can also be deeply focused to liver tissue, targeting neoplasms and delivering enough energy to cause coagulative necrosis."

(4) Of "Page 4, down-to-up line 3-1: In this review we will focus on the main topics involving recent advances and modern applications in the field of liver ultrasound.", the word "recent" should be "recent".

Response: thank you for the comment. We have corrected the word "recent" duly.

(5) Of "(kilopascal kPa) or wave speed (m/sec) can be calculated and usually displayed [57]. ", the wordf "(kilopascal kPa)" should be "(kilopascal, kPa)".

Response: thank you for the comment. We have added the comma.

(6) Of "Despite of being operator dependant,", the word "dependant" should be "dependent".

Response: thank you for the comment.

(7) Of References "3 Bartolotta TV, Sidoti Pinto A, Cannella R, Porrello G, Taravella R, Randazzo A, Taibbi A. Focal liver lesions: interobserver and intraobserver agreement of three-dimensional contrast-enhanced ultrasound-assisted volume measurements. Ultrasonography. 2020. [PMID: 33080667 DOI: 10.14366/usg.20025]", it is not appropriate hear, and it had better be rplaced by other one.

Response: thank you for the comment. The cited reference is a paper focusing on 3D Ultrasonography and it seems to fit the main text, providing the reader with up-to-date literature.

(8) Of "At CEUS, the main feature indicating a benign lesion is a sustained and prolonged contrast-enhancement in the portal-venous (i.e., 45-120 seconds after contrast injection) and late phases (i.e., 120 seconds up to 4-6 minutes after contrast injection) (Fig. 3) [25,26]." The starting of portal-venous has been addressed as "30-45s" in [Dietrich CF, Nolsøe CP, Barr RG, et al. Guidelines and Good Clinical Practice Recommendations for Contrast Enhanced Ultrasound (CEUS) in the Liver - Update 2020 - WFUMB in Cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS. Aktualisierte Leitlinien und Empfehlungen für die gute klinische Praxis für CEUS der Leber. Ultraschall Med. 2020;41(5):562-585. doi:10.1055/a-1177-0530].

Response: thank you for the comment. We have updated the timing of the portal-venous phase according to the provided reference

portal-venous (i.e., 30-45 to 120 seconds after contrast injection)

Dietrich CF, Nolsøe CP, Barr RG, et al. Guidelines and Good Clinical Practice Recommendations for Contrast Enhanced Ultrasound (CEUS) in the Liver - Update 2020 - WFUMB in Cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS. Aktualisierte Leitlinien und Empfehlungen für die gute klinische Praxis für CEUS der Leber. Ultraschall Med. 2020;41(5):562-585. doi:10.1055/a-1177-0530

(9) Of "CEUS has also proved useful in the guidance, response assessment and detection of complications of interventional procedures [49-51]." It seems that it should be "CEUS has also been proved useful..."

Response: thank you for the comment. We have made changes duly.

(10) Of "US beam may be focused to directly ablate tumoral tissue or adequately modulated to deliver drugs and genetic material locally." . the word "material" should be "materials".

Response: thank you for the comment. We have made changes duly.

(11) Of "Mastering this complex multiparametric technique is nowadays a cultural and practical challenge to be fully taken up in order to provide our patients the best care option in various liver diseases. ". This sentence had better be rewritten.

Response: thank you for the comment. We have made changes as follows:

We have changed the sentence:

Mastering this complex multiparametric technique is nowadays a cultural and practical challenge to be fully taken up in order to provide our patients the best care option in various liver diseases.

to

Mastering this complex and multiparametric technique is a cultural challenge. Nevertheless, this challenge needs to be fully taken up in order to provide our patients the best care option in various liver diseases.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors:

1. The expansion of FLL(part of Doppler technique, last paragraph),CEUS(part of Contrast-enhanced US, 1st paragraph) and AC(part of Attenuation Imaging, 3rd paragraph) should be written.

Response: thank you for the comment. We have made changes as requested:

2. Punctuation marks should be added in part of B-Mode, 2nd paragraph(when high-pressure US waves (>0.5 MPa) travel through tissues, ...), part of Contrast-enhanced US, 2nd paragraph(In a multicenter study of 23,188 patients,...), part of Contrast-enhanced US, 4th paragraph from bottom(In cirrhotic patients,..).

Response: thank you for the comment. We have made changes as requested:

Reviewer #3:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (High priority)

Specific Comments to Authors:

1. The expansion of FLL should be written (5th page, 3rd line).

Response: thank you for the comment. We have made changes as requested:

2. The expansion of CEUS should be written (5th page, 4rd line).

Response: thank you for the comment. We have made changes as requested:

3. It is sufficient to use only (HCC) on page 7, line 3 (abbreviation explained on page 4). 4. On page 10, at line 2, the (AC) abbreviation for attenuation coefficient should be made one sentence before.

Response: thank you for the comment. We have made changes as requested:

Reviewer #4:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors:

I think the author should compare ultrasound with CT and MRI more specifically.

Response: Thank you for the comment. We added the following sentences, with pertaining references under the heading **Contrast-enhanced US**:

A systematic review compared the accuracy of CEUS, CT and MRI for the differentiation of malignant and benign liver lesions. The reported sensitivities were 88% (95% CI 87% to 90%), 90% (95% CI 88% to 92%) and 86% (95% CI 83% to 88%), respectively, and corresponding specificities were 81% (95% CI 79% to 84%), 77% (95% CI 71% to 82%) and 81% (95% CI 76% to 85%) [Guang Y, Xie L, Ding H, Cai A, Huang Y. *Diagnosis value of focal liver lesions with SonoVue®-enhanced ultrasound compared with contrast-enhanced computed tomography and contrast-enhanced MRI: a meta-analysis.* J Cancer Res Clin Oncol. 2011 Nov;137(11):1595-605. doi: 10.1007/s00432-011-1035-8. Epub 2011 Aug 18. PMID: 21850382].

CEUS is not deemed to completely replace CT or MRI but, depending on the clinical setting, CEUS could be included in the diagnostic algorithm either as a replacement or as a triage step to reduce the use of CT and MRI [Westwood M, Joore M, Grutters J, et al *Contrast-enhanced ultrasound using SonoVue® (sulphur hexafluoride microbubbles) compared with contrast-enhanced computed tomography and contrast-enhanced magnetic resonance imaging for the characterisation of focal liver lesions and detection of liver metastases: a systematic review and cost-effectiveness analysis.* Health Technol Assess. 2013; 17(16):1-243. [PMID: 23611316 DOI: 10.3310/hta17160]].

We also added the following sentence

A multi-center study by Lu and coworkers, aimed at comparing CEUS and contrast enhanced CT or MRI in monitoring percutaneous thermal ablation procedure in 151 patients with HCC, has found out that specificity and accuracy values of CEUS in detecting tumor vascularity were 98.2% and 96.6%, respectively [Lu MD, Yu XL, Li AH, Jiang TA, Chen MH, Zhao BZ, Zhou XD, Wang JR. *Comparison of contrast enhanced ultrasound and contrast enhanced CT or MRI in monitoring percutaneous thermal ablation procedure in patients with hepatocellular carcinoma: a multi-center study in China*. *Ultrasound Med Biol*. 2007 Nov;33(11):1736-49. doi: 10.1016/j.ultrasmedbio.2007.05.004. Epub 2007 Jul 16. PMID: 17629608].

We also added the following sentence under the heading **Elastosonography**:

Another prospective study conducted on 104 patients Magnetic Resonance Elastography detected any fibrosis (stage 1 or more) with an area under the receiver operating characteristic curve (AUROC) of 0.82 (95% confidence interval [CI], 0.74-0.91), which was significantly higher than that of TE (AUROC, 0.67; 95% CI, 0.56-0.78) [Park CC, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, Hooker J, Sy E, Savides MT, Alquiraish MH, Valasek MA, Rizo E, Richards L, Brenner D, Sirlin CB, Loomba R. *Magnetic Resonance Elastography vs Transient Elastography in Detection of Fibrosis and Noninvasive Measurement of Steatosis in Patients With Biopsy-Proven Nonalcoholic Fatty Liver Disease*. *Gastroenterology*. 2017 Feb;152(3):598-607.e2. doi: 10.1053/j.gastro.2016.10.026. Epub 2016 Oct 27. PMID: 27911262; PMCID: PMC5285304.]

We also added the following sentence under the heading **Attenuation Imaging**:

Of note, Magnetic resonance (MRI-PDFF) is another valuable tool to quantify liver steatosis. In a study, MRI-PDFF detected any steatosis with an AUROC of 0.99 (95% CI, 0.98-1.00), which was significantly higher than that of CAP (AUROC, 0.85; 95% CI, 0.75-0.96) [Park CC, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, Hooker J, Sy E, Savides MT, Alquiraish MH, Valasek MA, Rizo E, Richards L, Brenner D, Sirlin CB, Loomba R. *Magnetic Resonance Elastography vs Transient Elastography in Detection of Fibrosis and Noninvasive Measurement of Steatosis in Patients With Biopsy-Proven Nonalcoholic Fatty Liver Disease. Gastroenterology.* 2017 Feb;152(3):598-607.e2. doi: 10.1053/j.gastro.2016.10.026. Epub 2016 Oct 27. PMID: 27911262; PMCID: PMC5285304.]

Special comments from the editor: The similarity index of revised manuscript is 40%, when cross-checked with iThenticate.

Thank you for the comment. We have changed the main text accordingly.

The sentence

More recently, newer Doppler-based techniques have been introduced which can separate slow or small-vessel flow signals from clutter artifacts, including superb microvascular imaging (SMI, Canon Medical Systems, Otawara, Japan), microflow imaging (MFI, Philips Healthcare, Best, The Netherlands), and microvascular flow imaging (MVFI, MV-Flow™, Samsung Medison Co., Ltd., Seoul, Korea).

Has been changed to

More recently, newer Doppler-based techniques have been introduced which can separate slow or small-vessel flow signals from clutter artifacts, including superb microvascular

imaging (SMI), microflow imaging (MFI), and microvascular flow imaging (MVFI, MV-Flow™) [4].

The following sentence:

These new, third-generation Doppler-based techniques enable the depiction of slow blood flow at a high spatial resolution and frame rate by using advanced clutter suppression, thus improving sensitivity and accuracy of Doppler US in the detection of vascularity in liver tumors with a safe, inexpensive and readily available modality (Figure 2) [4].

Has been changed to:

These new, third-generation Doppler-based techniques enable the depiction of slow flowing blood at very high spatial resolution and frame rate by using advanced clutter suppression, thus improving the sensitivity of Doppler US in the assessment of vascularity in hepatic tumors with a safe, inexpensive and readily available modality (Figure 2) [4].

CONTRAST-ENHANCED US

In the late 1990s, the introduction of intravenously injected microbubble-based contrast agents, along with contrast-specific gray-scale US techniques, has enabled ultrasonography to depict not only macro-vascularity but also microcirculation (i.e., vessels as thin as 40 μm) [15]. At the beginning of the 2000s, the development of gas bubbles with a radius ranging from 1 to 10 μm , presenting flexible shells (e.g., phospholipids) which are filled with low-solubility gases (e.g., perfluoropropane, perfluorocarbon, or sulfur hexafluoride) has led to a full real time contrast enhanced US (CEUS) examination [16].

Has been changed to

By the late 1990s, the availability of intravenously injected microbubble-based contrast agents and the development of contrast-specific US techniques, has enabled ultrasonography to depict not only macro-vascularity but also micro-vessels as thin as 40 μm [15]. At the beginning of the 2000s, the development of microbubbles with flexible shells (e.g., phospholipids) and filled with low-solubility gases such as perfluoropropane,

perfluorocarbon, or sulfur hexafluoride, has led to a full real time contrast enhanced US (CEUS) examination [16].

The following text has been changed

CEUS is unique in that it allows non-invasive assessment of liver perfusion in real time throughout the vascular phase, without the use of ionizing radiation, potentially nephrotoxic contrast agents or costly and not widespread equipment, such as CT or MRI. Of note, when injected intravenously, microbubble-based contrast agents pass through the pulmonary filter and remain within the intravascular space acting as purely vascular tracers (blood-pool agents), although one of them exhibits an additional Kupffer phase [17]. CEUS is safe and well tolerated: it can be used even in patients with hepatic or renal failure, renal obstruction, or chronic obstructive pulmonary disease and there is no need to perform laboratory tests of renal function in advance. In a multicenter study of 23,188 patients, a serious adverse event rate of 0.0086% was reported, with no deaths and a life threatening anaphylactoid reaction rate of less than 0.002% [18].

To

CEUS allows to assess liver perfusion non-invasively and in real time, without the need of ionizing radiation, potentially nephrotoxic contrast agents or costly and not widespread equipment, such as CT or MRI. Of note, microbubble-based contrast agents present a radius ranging from 1 to 10 μm , so they can pass through the pulmonary filter but they do not exit the vascular space, acting as purely vascular tracers (blood-pool agents), although one of them exhibits an additional Kupffer phase [17]. CEUS is safe and well tolerated: it can be performed in patients with hepatic or renal failure. Renal obstruction or chronic obstructive pulmonary disease are not a contraindication and laboratory tests of renal function are no necessary in advance. In a study encompassing 23,188 patients, the overall reporting rate of serious adverse event was 0.0086%. In the same study no deaths were reported and the life-threatening anaphylactoid reaction rate was less than 0.002% [18].

The following text

Currently, CEUS is included as a part of the suggested diagnostic work-up of FLLs, including incidental masses detected in non-oncologic non-cirrhotic patients, suspect metastases in oncologic patients and HCC in cirrhotic patients, resulting in better patient management and cost-effective delivery of therapy [5, 19].

Has been changed to

Nowadays, CEUS is suggested as useful tool for diagnostic work-up of FLLs, including incidental masses detected in non-oncologic non-cirrhotic patients, suspect metastases in oncologic patients and HCC in cirrhotic patients, aiming at optimizing patient management and at cost-effective therapy delivering [5, 19].

and

A recent meta-analysis showed that pooled sensitivity, specificity, diagnostic odds ratio, positive likelihood ratio, negative likelihood ratio, and area under the curve for CEUS in the characterization of FLLs were 92%, 87%, 104.20, 7.38, 0.09, and 0.9665, respectively [20].

to

A recent meta-analysis focusing on the role of CEUS in the characterization of FLLs showed pooled sensitivity, specificity, diagnostic odds ratio, positive and negative likelihood ratio and area under the curve of 92%, 87%, 104.20, 7.38, 0.09, and 0.9665, respectively [20].

The text

The majority of clinical US systems use second harmonic echoes for THI image formation.

Advantages of THI include improved signal-to-noise ratio and reduced artifacts produced by side lobes, grating lobes, and reverberation (Fig. 1) [2].

to

Clinical US systems usually employ second harmonic echoes in order to generate THI images. Among the advantages of THI, a better signal-to-noise ratio and less artifacts deriving from side and grating lobes as well as reverberation are included (Fig. 1) [2].

The sentence

Another issue related to the clinical use of US Elastography is that cut-off values for fibrosis staging vary across US systems from different vendors.

To

Another issue related to the clinical use of US Elastography is the inconsistency of the cut-off values suggested by various manufacturers for the staging of liver fibrosis.

The paragraph

Recently, to overcome this issue, a consensus panel has proposed a vendor-neutral “rule of four” (5, 9, 13, 17 kPa) for the ARFI techniques for viral etiologies and NAFLD: Liver stiffness of 5 kPa (1.3 m/sec) or less has high probability of being normal; liver stiffness less than 9 kPa (1.7 m/sec), in the absence of other known clinical signs, rules out compensated advanced chronic liver disease (cACLD); values between 9 kPa (1.7 m/sec) and 13 kPa (2.1 m/sec) are suggestive of cACLD but may need further test for confirmation; and values greater than 13 kPa (2.1 m/sec) are highly suggestive of cACLD. There is a probability of clinically significant portal hypertension with liver stiffness values greater than 17 kPa (2.4 m/sec) [42]. More in general, a liver stiffness value of less than 7 kPa (1.5 m/sec) (pSWE and 2D SWE) can help rule out significant fibrosis [42].

To

Recently, to overcome this issue, a consensus panel has proposed a manufacturer-neutral “rule of four” with values of 5, 9, 13, 17 kPa for ARFI assessment of liver fibrosis of viral etiologies and NAFLD. Liver stiffness ≤ 5 kPa (1.3 m/sec) has high probability of being normal; liver stiffness < 9 kPa (1.7 m/sec), without other known clinical signs, rules out compensated advanced chronic liver disease (cACLD); values between 9 kPa (1.7 m/sec) and 13 kPa (2.1 m/sec) are suggestive of cACLD (further test may be needed for confirmation); and values > 13 kPa (2.1 m/sec) are highly suggestive of cACLD. Clinically

significant portal hypertension may be present with liver stiffness values $>$ than 17 kPa (2.4 m/sec) [42]. More in general, liver stiffness values $<$ 7 kPa (1.5 m/sec) (pSWE and 2D SWE) indicate the absence of significant fibrosis [42].

The sentence

For patients with biopsy-proven NAFLD, 2D SWE, MRE and TE exhibited comparable and very good to excellent diagnostic accuracy for advanced fibrosis and comparable but lower accuracy for significant fibrosis [43].

To

In a recent study, 2D SWE, MRE and TE showed comparable and very good to excellent diagnostic accuracy for advanced fibrosis and comparable but lower accuracy for significant fibrosis in patients with biopsy-proven NAFLD [43].

The paragraph

Since its introduction in clinical settings, three-dimensional Ultrasound (3D-US) has been widely used in gynecology, obstetrics, and cardiology, and in recent years, its use in liver imaging has also gained interest [50]. Nevertheless, volume rendering techniques cast "rays" through the 3D voxel-based volume and project the resulting image onto a 2D plane. In this situation, suboptimal results are obtained when there is not enough contrast between the examined structure and the adjacent tissues, such as in liver parenchyma [50]. The injection of microbubble-based contrast agents allows 3D-CEUS to overcome this limitation, paving the way for new applications of ultrasonography of the liver.

Has been reworded as it follows

Three-dimensional Ultrasound (3D-US) has been found useful in obstetrics, gynecology and cardiology, [50]. More recently, 3D-US has also gained interest for liver assessment [50]. Of note, 3D-US techniques cast "rays" through the 3D voxel-based volume: the resulting image is projected onto a two-dimensional plane. When the contrast between the

examined structure and the adjacent tissues is not wide enough, such as it occurs in liver parenchyma, the results are suboptimal [50]. The administration of microbubble-based contrast agents may overcome this limitation, allowing 3D-CEUS to indicate the way for new applications in hepatic ultrasonography.

The paragraph

CEUS has been proved to improve sensitivity compared to unenhanced US for the detection of liver metastases, providing similar diagnostic performance to other imaging modalities (CT and MRI) for the detection of liver metastases

To

For the detection of liver metastases, CEUS has been proved to improve sensitivity compared to unenhanced US and to provide a diagnostic performance comparable to that of CT and MRI

The sentence

In particular, CEUS showed a range of 77.5%-100% was found for sensitivity and 76.7%-97.6% for specificity in the detection of focal liver lesions vs. CT [17].

Has been changed to

In particular, in the detection of FLLs CEUS showed sensitivity and specificity values of 77.5%-100% and 76.7%-97.6%, respectively, when compared to CT [17].

The sentence

In cirrhotic patients, CEUS allows to assess noninvasively the contrast enhancement patterns of HCC, without the use of ionizing radiation and with a much higher temporal resolution than CT and MRI [5].

Has been changed to

In cirrhotic patients, CEUS allows to detect in real time the typical contrast-enhancement behavior of HCC, without using ionizing radiation and at a higher temporal resolution than CT and MRI [5].

The sentence

Several international guidelines currently recommend CEUS as an adjunct tool in the imaging work-up of HCC, such as the LI-RADS lexicon of the American College of Radiology [31].

Has been changed to

Several international guidelines currently recommend CEUS as a useful imaging modality for the radiological work-up of HCC, such as the LI-RADS lexicon of the American College of Radiology [31].

The paragraph

A systematic review compared the accuracy of CEUS, CT and MRI for the differentiation of malignant and benign liver lesions. The reported sensitivities were 88% (95%CI 87% to 90%), 90% (95%CI 88% to 92%) and 86% (95%CI 83% to 88%), respectively, and corresponding specificities were 81% (95%CI 79% to 84%), 77% (95%CI 71% to 82%) and 81% (95%CI 76% to 85%) [32]. CEUS is not deemed to completely replace CT or MRI but, depending on the clinical setting, CEUS could be included in the diagnostic algorithm either as a replacement or as a triage step to reduce the use of CT and MRI. [28]

Has been changed to

A systematic comparison of the accuracy of CEUS, CT and MRI in the characterization of FLLs, reported sensitivities values of 88% (95%CI 87% to 90%), 90% (95%CI 88% to 92%) and 86% (95%CI 83% to 88%), respectively, with specificities values of 81% (95%CI 79% to 84%), 77% (95%CI 71% to 82%) and 81% (95%CI 76% to 85%) [32]. CEUS is not deemed to completely replace CT or MRI but, depending on the clinical setting, CEUS could reduce the use of CT and MRI. [28]

The sentence

CEUS has also been proved useful in the guidance, response assessment and detection of complications of interventional procedures [33].

Has been changed to

CEUS may play a role in the guidance, response assessment and detection of complications of interventional procedures [33].

The sentence

A multi-center study by Lu and coworkers, aimed at comparing CEUS and contrast enhanced CT or MRI in monitoring percutaneous thermal ablation procedure in 151 patients with HCC, has found out that specificity and accuracy values of CEUS in detecting tumor vascularity were 98.2% and 96.6%, respectively [34].

Has been changed to

A multi-center study by Lu and coworkers, aimed at comparing CEUS with CT or MRI in detecting tumor vascularity after thermal ablation procedure in 151 HCC patients, found out specificity and accuracy values for CEUS of 98.2% and 96.6%, respectively [34].

The sentence

In particular, intraprocedural use of CEUS has been shown having a relevant clinical impact, reducing the number of re-treatments and the related costs per patient [35].

Has been changed to

In particular, the use of CEUS during the procedure has been reported to have a significant clinical impact, reducing the need for re-treatments and increasing the cost-effectiveness of the therapy [35].

ATTENUATION IMAGING

The paragraph

CAP measurements rely on the degree of ultrasound echoes attenuation through the liver and results are therefore expressed in acoustic energy attenuation in decibel/meter (dB/m). CAP software is embedded into the transient elastography device (FibroScan, Echosens, France), and CAP is measured simultaneously to liver stiffness [45].

Has been changed into

CAP measurements rely on the degree of US beam attenuation through the hepatic parenchyma and the energy attenuation is expressed in decibel/meter (dB/m). In clinical practice, CAP is measured simultaneously to liver stiffness with the transient elastography device (FibroScan, Echosens, France) [45].

And the sentences

In a prospective study encompassing 108 patients the attenuation coefficient (AC) from ATI provided good diagnostic performance in detecting the varying degrees of hepatic steatosis. The degree of steatosis was the only significant factor affecting the AC, whereas fibrosis and inflammation were not [48].

Have been changed as follows

In a prospective study encompassing 108 patients the AC values derived from ATI have been shown to present good diagnostic performance in distinguishing the different degrees of hepatic steatosis. Of note, AC values were affected only by the actual degree of steatosis whereas fibrosis and inflammation were not influencing factors [48].

The sentence

In their series, 30 out of 95 Lesions received supplementary ablation immediately during the procedure

Has been changed to

In their series, 30 out of 95 tumors have been re-ablated immediately during the same procedure

DRUG-DELIVERY

The following sentence:

Ultrasound has an ever-increasing role in the delivery of therapeutic agents including genetic material, proteins, and chemotherapeutic agents [62]. Currently, microbubbles are being actively studied not only as ultrasound contrast agents but also for local drug delivery under ultrasound exposure [63]. More elaborated drug delivery carriers have a good perspective for simultaneous imaging and focal therapy of different cancer types, including liver cancer [64].

Has been changed to

Ultrasound may play a promising role as tool for delivering therapeutic agents, such as genetic material, proteins, and chemotherapeutic drugs [62].

Currently, microbubbles are studied not only as ultrasound contrast agents but also as tool for delivering drugs locally under ultrasound exposure [63]. More elaborated drug delivery carriers are studied for both simultaneous imaging and focal therapy in the setting of different cancer types, including hepatic malignancies [64].

A.I. AND RADIOMIC ULTRASOUND ANALYSIS

Radiomics is defined as the quantitative extraction, analysis, and modeling of a large amount of features from medical images in relation to prediction targets, such as clinical end-points, and pathological and genomic features [65]. Radiomics is a complex process that articulates into distinct steps, including: acquisition of images, tumor segmentation, feature extraction, exploratory analysis, and model building [66].

Has been changed to

Radiomics is defined as the process of extraction, analysis, and modeling of a large quantity of features from medical imaging dataset and to correlate this information to prediction parameters, such as clinical end-points, pathological and genomic features [65]. Radiomics is a complex process that can be divided into different phases, including: acquisition of imaging dataset, tumor segmentation, feature extraction, exploratory analysis, and model building [66].

Figure 1 legend from

Images of the gallbladder obtained by using fundamental imaging

To

Gallbladder images acquired with fundamental imaging.

POINT TO POINT RESPONSE

(1) In this review we will focus on the main topics involving recent advances and modern applications in the field of liver ultrasound. » » » » recent ???

- *Thank you for the comment: we have replaced the word “recent” in “recent”*

(2) In the same study no deaths were reported and the life-threatening anaphylactoid reaction rate was less than 0.002% [18]. » » » » no deaths were ???

- *Thank you for the comment: we replaced the sentence “In the same study no deaths were reported and the life-threatening anaphylactoid reaction rate was less than 0.002% [18].” in “In the same study no deaths have been reported and the life-threatening anaphylactoid reaction rate was less than 0.002% [18].”*

(3) as well as reverberation are included (Fig. 1) [2]. » » » » inexpensive and readily available modality (Figure 2) [4]. » » » » is typical of hemangioma (Fig. 3) [21]. » » » » » » » » earlier than 60 s after the contrast injection (Figure 4). » » » » » and mild washout (Figure 5) [29]. >>>> The reviewer suggested the authors to use the same style.

- *Thank you for the comment: we have used the same style in manuscript and we have replaced the word “Fig.” in “Figure”*

(4) CEUS can be very valuable when fatty infiltration of the liver and focal fatty sparing, often induced by chemotherapy, occur in a geographic pattern, in atypical location or shape, thus further worsening US performance both in the detection and the characterization of focal liver lesions [5].

“often induced by chemotherapy” should be deleted, for it’s very common in population with fatty liver. “occur in a geographic pattern” is a well-known usual finding, while other shapes “round and ovoid” are atypical, and are challenging to radiologist in ultrasound. RE: [Wu Size, et al. Focal fatty sparing usually does not arise in preexisting non-alcoholic diffuse homogeneous fatty liver. JUM. 2014; 33(8): 1147-1452. Wu Size, et al.. Characteristics suggestive of focal fatty sparing from liver malignancy on ultrasound in liver screening. Ultrasound Q. 2014; 30(4): 276-281.]

- *Thank you for the comment: we have deleted the sentence “often induced by chemotherapy”*

We have also added the suggested reference

(5) At CEUS, the typical contrast-enhancement pattern of HCC in comparison with liver parenchyma is arterial hyperenhancement followed by late (later than 60 s after contrast injection) and mild washout (Figure 5) [29]. On the other hand, the presence of a marked and early (earlier than 60 s) washout is more typical of non-HCC malignancies, such as intrahepatic cholangiocarcinoma or metastases [30].

Reference with case report of 60s had better be added: Zuo D, et al. Diagnostic performance of intravascular perfusion based contrast-enhanced ultrasound LI-RADS in the evaluation of hepatocellular carcinoma. Clin Hemorheol Microcirc. 2021 Apr 13. doi: 10.3233/CH-211164. Epub ahead of print. PMID: 33867358. ???????

-Thank you for the comment: we have added the suggested reference

(6) Several international guidelines currently recommend CEUS as a useful imaging modality for the radiological work-up of HCC,>>>>" [31]" is not a best reference, and another guideline or recommendation should be added. To indicate Several.

- Thank you for the comment: we have added the following reference

Cassinotto C, Aubé C, Dohan A. Diagnosis of hepatocellular carcinoma: An update on international guidelines. Diagn Interv Imaging. 2017 May;98(5):379-391. doi: 10.1016/j.diii.2017.01.014. Epub 2017 Apr 5. PMID: 28395852.

(7) The reviewer suggested that to add following into the article: >>>>Compared with CT or MRI, CEUS is real-time dynamic enhancement, every second imaging can be read and tracked, so it's able to avoid or markedly reduce imaging information losing. But it is not suitable for simultaneous evaluation of several liver focal lesions, and not suitable for large liver focal lesion of diameter > 10cm.

- Thank you for the comment: as request we have added the sentence.

(8) shearwave elastography (SWE)>>>> shear wave elastography (SWE)

- *Thank you for the comment: we have changed the word “shearwave” to “shear wave”*

(9) Liver stiffness ≤ 5 kPa (1.3 m/sec) has high probability>>>???

- *Thank you for the comment: we have replaced the symbol “ \leq ” in “ $<$ ”*

(10) Recently, to overcome this issue, a consensus panel has proposed a manufacturer-neutral “rule of four” with values of 5, 9, 13, 17 kPa for ARFI assessment of liver fibrosis of viral etiologies and NAFLD. Liver stiffness ≤ 5 kPa (1.3 m/sec) has high probability of being normal; liver stiffness < 9 kPa (1.7 m/sec), without other known clinical signs, rules out compensated advanced chronic liver disease (cACLD); values between 9 kPa (1.7 m/sec) and 13 kPa (2.1 m/sec) are suggestive of cACLD (further test may be needed for confirmation); and values > 13 kPa (2.1 m/sec) are highly suggestive of cACLD.

>>>>>>>Reference should be added.

- *Thank you for the comment: we added reference n. 42*

(11) The reviewer suggested to delete the following sentence: Another prospective study conducted on 104 patients Magnetic Resonance Elastography detected any fibrosis (stage 1 or more) with an area under the receiver operating characteristic curve (AUROC) of 0.82 (95% confidence interval [CI], 0.74-0.91), which was significantly higher than that of TE (AUROC, 0.67; 95%CI, 0.56-0.78) [44]. >>>>> It's not suitable here. It's not need to compare them for this parameter.

???? - *Thank you for the comment: as suggested, we have deleted the sentence “Another prospective study conducted on 104 patients Magnetic Resonance Elastography detected any fibrosis (stage 1 or more) with an area under the receiver operating characteristic curve (AUROC) of 0.82 (95% confidence interval [CI], 0.74-0.91), which was significantly higher than that of TE (AUROC, 0.67; 95%CI, 0.56-0.78) [44].”*