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François Durand
Hepatology, Hospital Beaujon, Clichy, France

Pere Ginès*
Liver Unit, Hospital Clinic, University of Barcelona, IDIBAPS,CIBEReHD, IRSIN, Barcelona, Spain

*Corresponding author.
E-mail address: pgines@clinic.ub.es

Faouzi Saliba
AP-HP Hôpital Paul Brousse, Centre Hépato-Biliaire, Villejuif, France

Javier Fernández
Liver Unit, Hospital Clinic, University of Barcelona, IDIBAPS, CIBEReHD, IRSIN, Barcelona, Spain

Contrast enhanced ultrasound for the diagnosis of hepatocellular carcinoma (HCC): Comments on AASLD guidelines

To the Editor:

Contrast enhanced ultrasound (CEUS) has been introduced 10 years ago for liver imaging in many European and Asian countries, but FDA approval in the US is still lacking [1]. The excellent value of CEUS has been established by numerous prospective studies including the German DEGUM-Study with over 1000 patients [2] and the respective French multicentric study [3]. CEUS was proven able to detect and characterize liver tumours in clinical routine within at least the same accuracy range as contrast enhanced computed tomography (CECT) and contrast enhanced magnetic resonance imaging (CEMRI) [4,5].

Therefore, CEUS has been introduced into important guidelines and recommendations, like those from the American Association for the Study of Liver Diseases (AASLD) 2005 [6], the Asian Pacific Association for the Study of the Liver (APASL) [7], the Japanese Society of Hepatology [8] and the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines 2004 [9], 2008 [10], and WFUMB-EFSUMB guidelines 2012 (in preparation). However, CEUS has been eliminated from the diagnostic flow chart of nodules in cirrhosis in the updated AASLD guidelines 2011 [11]. This removal raised controversial discussion and was not well received in Europe and Asia. This is therefore the issue to which the present commentary refers.

There are two reasons for which CEUS has been eliminated from the AASLD guidelines. 1. "*Contrast-enhanced US may offer false positive HCC diagnosis in patients with cholangiocarcinoma and thus, has been dropped from the diagnostic techniques*"

Intrahepatic cholangiocellular carcinoma (ICC) is a rare tumour in liver cirrhosis (about 1–3% of newly developed tumors) [12,13] but the incidence appears raising [14]. In a retrospective series of 21 patients with histologically confirmed ICC on cirrhosis collected between 2003 and 2009, the Barcelona Clinic Liver Cancer (BCLC) group found that ten ICC had the same CEUS enhancement pattern considered diagnostic for HCC, consisting in homogeneous arterial hyperenhancement followed by wash-out [15]. At variance, these tumours showed hyperintense enhancement in the arterial phase but lacked wash-out at MRI, failing to show the typical HCC pattern, thus prompting biopsy. The difference in the enhancement patterns is probably related to the different pharmacokinetics of contrast agents used for US (strictly intravascular) and MRI (extravascular space diffusion of Gadolinium which transiently binds to fibrous tissue, explaining enhancement in ICC in the late phase) [16,17]. The valuable observation of the BCLC group on the potential risk of false positive diagnosis of HCC in cirrhosis by CEUS has to be fully acknowledged and kept in high consideration. However, the quality of the study and the clinical consequences of this possible risk do not seem to justify the complete removal of CEUS from the imaging armamentarium. The study included a relatively low number of patients recruited over a long time of six years (approximately only 1–2/year at risk of misdiagnosis by CEUS), which outlines the rarity of ICC, and had a non-prospective and non-controlled study design. This means that an incorporation bias could have occurred. In other words, it cannot be excluded that some ICC were erroneously diagnosed as HCC by MRI. MRI was, in fact, the diagnostic reference standard, at least for nodules >2 cm, with no possibility to detect false positive cases for HCC. Furthermore, no information was reported about the behaviour of ICC at CT [15]. Nonetheless, CT was maintained as capable of establishing a diagnosis of HCC, despite it may show the typical pattern of HCC also in primary liver lymphoma [18], an entity which in some series of HCV-related cirrhosis was reported to occur even more frequently than cholangiocarcinoma [19]. Consequently, applying a required positive predictive values (PPV) of 100% for accepting a technique as diagnostic for HCC (CEUS was eliminated despite estimated positive predictive value >95%) could make not only CEUS, but possibly also CT unacceptable, and a large prospective trial of histologically confirmed nodules would be required to demonstrate how accurate MRI is. It is worth reminding, however, that in one prospective trial with histology as reference standard for all patients, a total specificity of the arterial wash-in with venous wash-out pattern for HCC in cirrhosis was reported [20], but the number of cases could have been too low to confirm 100% PPV.

Furthermore, it is worth pointing out that, in ICC, the pattern of CEUS would anyway suggest a diagnosis of malignancy, for which it is approximately totally specific [20], whereas the pattern of MRI in case of ICC would not be diagnostic for malignancy (wash-in not followed by wash-out). This is an important point, since biopsy is not always technically feasible and it shows only moderate sensitivity for malignancy in very small nodules (1–2 cm) [21].

Detailed analysis of the patterns reported in the study by which guidelines were modified [15] could be of help in better

understanding the role of CEUS. Eleven patients showed rim enhancement in the arterial phase, a pattern which is not typical for HCC and tends to suggest ICC (or metastasis), whatever contrast imaging technique is used [22–25], whereas 10 showed homogeneous enhancement, consistent with HCC. However, half of the latter (5 out of 10) showed early wash-out at CEUS (namely before 60 s) [15], which is no longer totally typical of HCC. Even in poorly differentiated HCC, median time of onset of wash-out was reported to be 2 min and it is longer in well differentiated HCC [26]. It is worth pointing out that the possibility to grade the intensity of the wash-out in the late venous phase applies to all contrast imaging techniques, whereas the possibility to establish an accurate temporal pattern of enhancement belongs to CEUS only, due to its real time modality [10,27,28]. To summarize, a true false positive diagnosis of HCC instead of ICC would have been established in only 5 cases in expert hands, out of the hundreds probably seen in the 6-year study period at BCLC.

Briefly, the strength of the BCLC study on ICC is not so convincing to completely remove CEUS from the recall strategy, as it instead happened [11].

We acknowledge, however, the great value of MRI (and CT), and their capacity of overview of the liver. Combining these properties and the new size threshold of only 1 cm of liver nodules to establish a diagnosis of HCC in case of typical pattern [11], a cost-effective approach would recommend to start either with MRI or CT. However, in cases lacking arterial hyperenhancement, it would seem preferable to the authors to use CEUS before biopsy. Such approach would allow theoretically rescuing a few possible cases which show a typical malignant pattern to establish the diagnosis of HCC. Indeed, absence of arterial hyperenhancement at MRI was not found in any ICC of the 21 cases described in the BCLC study [15]. This approach would probably rescue a small number of cases from biopsy.

Moreover, the guidelines do not take into consideration the not negligible number of cases in which MRI or CT are contraindicated or are carried out suboptimally due to insufficient patient cooperation, especially in presence of motion artefacts. In these cases, even a bioptic sampling could be technically suboptimal. Thus, having a real time imaging modality, such as CEUS, at least in second line after MRI or CT, to establish a diagnosis of HCC, would provide great benefit for the patient management.

In conclusion, ICC is a rare primary liver tumour in liver cirrhosis. CEUS depicts ICC with either a peripherally located rim sign or homogeneous hyperenhancement and with pronounced hypoenhancement in the portal venous phase, whereas MRI, after an arterial pattern similar to CEUS, does not often show tumour wash-out, especially along the periphery. The MRI findings have to be confirmed in larger and prospective studies as well.

2. "*Contrast enhanced ultrasound is not available in the USA, so these results are not entirely applicable to a North American population*"

Finally, we want to highlight that CEUS had been removed from the latest AASLD guidelines also because UCA are not available in USA. This is a conclusive argument, but it conflicts with the ambition of AASLD guidelines to be applied in other continents, rather than in America only. In this case, the limiting factor is restricted to USA, where indeed phase III trials in liver CEUS are ongoing. Furthermore, the guidelines suggest MRI, but in many countries worldwide, with a high incidence of HCC, the availability of MRI is close to exceptional, making CEUS, which is cheaper and much easier to implement, a powerful tool for daily routine.

AASLD guidelines are traditionally not only important for American medical professionals but have impact worldwide. Therefore, the consequences of the revised AASLD guidelines 2011 should be discussed in the light of their impact beyond the USA. CEUS proved to be of great values in several well conducted studies with specific advantages such as extremely high safety profile, being easy repeatable and high temporal resolution and should be, therefore, in the opinion of the authors, part not only of most, but all international HCC guidelines.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Ana Paula Barreiros*
Johannes Gutenberg University of Mainz, Department of Internal Medicine I, 55101 Mainz, Germany
* Corresponding author. *Tel.: +49 6131 17 6071*
E-mail address: barreiro@mail.uni-mainz.de

Fabio Piscaglia
S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

Christoph F. Dietrich
Caritas Krankenhaus, Bad Mergentheim, Germany

Regulatory T cells in autoimmune hepatitis

To the Editor:

We read with interest the paper by Peiseler *et al.*, and the accompanying editorial by Oo and Adams, reporting that in patients with autoimmune hepatitis (AIH), regulatory T cells (T-regs) are '*fully functional and not reduced in frequency*' [1]. These data contrast with our findings of T-reg impairment in AIH, especially during active disease [2–5]. This discrepancy is attributed by the authors of the paper and the editorial to their use of an '*improved*' methodology to define '*true*' T-regs, i.e. $CD4^+CD25^{high}CD127^{low}$ cells.

We wish to raise a few discussion points.

A key finding of Peiseler *et al.* is that '*the frequency of* $CD4^+CD25^{high}CD127^{low}FOXP3^{pos}$ *cells in blood of AIH patients was not reduced compared to healthy subjects*'. These results are similar to our recent data [6] – of which, however, they do not appear to be aware – showing that $CD4^+CD25^{high}CD127^{neg}$ cell numbers are similar in patients with AIH, their first degree relatives and healthy subjects. In contrast to them, we show that these cells, similarly to CD4+CD25high T-regs [2–5], do have a decreased function in patients compared to healthy relatives and controls.

The discrepancy between our findings and those of Peiseler *et al.*, therefore, relates mainly to T-reg function. Methodological approaches are a plausible explanation for this divergence. Both groups use suppression of $CD4^+CD25^{neg}$ cell proliferation as readout of the suppressor function, but we use the more physiological suppressor/effector ratio of 1:8, while, somewhat surprisingly, Peiseler *et al.* use a ratio of 1:1, which is utterly non-physiological. In fact, from the limited information provided in the methodology, it is unclear whether their results are due to a dilution of the $CD4^+CD25^{neg}$ cells more than to suppression.

A few other points: to purify $CD4^+CD25^{high}$ cells, Peiseler *et al.* use a modified technique claimed to yield CD4 cells with high CD25 density. The authors, however, in contrast to us [4], do not provide evidence that their cells are indeed CD25 high. Moreover, they state that the purity of their $CD4^+CD25^{high}$ cells '*was always about 80–90%*', a value lower that what we have consistently reported (>95%).

The authors suggest that the majority of their $CD4^+CD25^+$ cells may represent activated T lymphocytes, whose number mirrors disease activity. This would confirm old – not cited – data from our group [7,8]. We have, however, shown that purified $CD4^+CD25^+$ cells expressing high levels of CD25 have suppressor function [2–5]. The difference between our results and those of Peiseler *et al.* is likely to be due to the different protocol used for the isolation of $CD4^+CD25^{high}$ cells. Moreover, Peiseler *et al.* use a gating technique that yields numbers of T-regs (their Fig. 1B) vastly lower than those reported in the literature [10] and by us when we used markers similar to theirs [6]. No sufficient technical details, however, are provided to retrace their protocol.

The explanation suggested by the authors, that our results are different because we study paediatric AIH patients, might be of relevance, as indeed our patients have paediatric onset AIH and have much more frequently type 2 AIH than their adult cohort. However, results very similar to those obtained in paediatric patients have been reported in adult patients with AIH [9].