Author’s response to reviews

Title: Real time contrast enhanced ultrasonography in detection of liver metastases from gastrointestinal cancer.

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Version: 2 Date: 30 April 2007

Author’s response to reviews: see over
To
Prof. Iratxe Puebla
Senior Assistant Editor
BMC Cancer

Re: MS:
Real time contrast enhanced ultrasonography in the detection of liver metastasis from gastrointestinal cancers.
Fabio Piscaglia, Francesco Corradi, Mikaela Mancini, Francesco Giangregorio, Stefano Tamberi, Giampaolo Ugelini, Bruno Cola, Fabio Fornari, Alberto Bazzocchi, Roberto Righini and Luigi Bolondi

Dear Editor,

We paid great attention to the criticism raised by the reviewers to our manuscript, which led to the decision to offer the possibility of resubmitting a revised form to BMC Cancer. We appreciated the work of the reviewers, whose suggestions were helpful to improve the manuscript. Here below please find our point-by-point reply to the Reviewers criticisms.

As regards the English writing the text has been revised by a British professional who works as main consultant for English writing for “Digestive and Liver Disease”, a gastroenterology scientific journal and is now submitted to BMC in the final corrected version.

Yours sincerely.

Fabio Piscaglia

Reply to Reviewer Chris J Harvey.
General.
As regards the English writing the text had already been corrected by a British professional who works as main consultant for English writing for “Digestive and Liver Disease”, a gastroenterology scientific journal. We resubmitted to her the present revised version of the manuscript and she carefully reviewed the English language of the article, which is now submitted to BMC in its final version.

Several sections were underlined since the manuscript had already been submitted to BMC Cancer and the previous managing editor accepted to allow us to resubmit the manuscript, but finally was considered as a completely new submission. At the time of revision we intended that all changes had to be underlined.

Major Compulsory Revisions
1. The 120 patients were consecutive, as observed in the two Ultrasound Units participating in the study, whereas we are not sure that the patients were all the consecutive cases observed in some of the referring units (since some of them used to refer patients also to other Ultrasound Units not participating in the study). Briefly the patients are most likely not to be consecutive in the surgical Units, but only as observed in the Ultrasound units). The fact that the patients were consecutively enrolled as investigated in the US study units is now specified in the Methods section (pag 5)
2. The study was prospectively carried out, this is now better specified in the text (page 5).
3. Two US systems were used since once center was equipped with both types of instrumentations during the study period. However, both types of instrumentations were from the same manufacturer (already specified at page 6) and utilized the same imaging software (CnTI software, Esaote, Genoa, Italy), making possible differences related to the equipments negligible. This is now more clearly specified in the text (page 6).
4. The criteria used to identify and characterize lesions either in general or particularly in case of metastatic disease, were agreed by the various operators and were the ones accepted universally. They correspond, in fact, to those described in the European guidelines published in 2004 (reference n° 22), which one of the centers (the Bologna one) contributed to write. The contrast pattern to diagnose a metastatic lesion was already reported at page 6, where it is also specified that the criteria we adopted were those included in the European guidelines for the use of ultrasound contrast agents.
5. CEUS imaging was performed until global marked decrease in contrast signal intensity or until complete contrast disappearance, which on average corresponded to 3-6 minutes after contrast injection. This is now specified at page 6.
6. Repeat injections were allowed, but eventually carried out in a very limited number of cases, usually related to improper contrast injection (extravasion of the contrast from the vein, due to malposition of the infusion needle). Therefore, it did not seem necessary to report such details, since it appeared already self explanatory that in such situations a repeat injection is necessary. Repeat injections due to insufficient liver visualization at first injection were extremely rare and usually did not improve visualization, so were practically never used.
7. The segmental distribution was recorded in all 3 modes. The agreement in lesion location was always consistent in conventional US and CEUS mode, since the same operator performed both techniques and the imaging approach was the same. When a discrepancy was recorded in the location of a lesion between CT and US mode(s), the CT films and US images/clips were retrospectively reviewed to assess whether the same lesion was visualized by the various modalities, but described in different segments or the two imaging modalities had visualized two separate lesions. This working modality has been now detailed in the methods section (page 7).
8. Judgement on presence, number and location of metastases was made directly at the time of the examination and not after blind review of digitally recorded examinations. This is now clearly specified in the methods section (page 6) and addressed as a limitation of the study in the discussion section (page 12).
9. US readers were blind to CT data (moreover in several cases US was performed before CT) and viceversa for CT; CEUS readers were, instead, not blind to conventional US findings, according to the study protocol. This is all specified in the text, now (page 6).
10. The use of multiple CT scanners is a limitation of the study but well reflects the everyday clinical setting, to which the study protocol tried to adhere. Such limitation is now acknowledged in the discussion (page 12).
11. A few hemangiomas and symple cysts were found in the study subjects, according to the expected prevalence of these lesions in the general population. However, simple cysts are clearly identified and characterized by conventional ultrasonography and hemangiomas are also well characterized by the combined contributions of conventional US and contrast enhanced examinations (either CT or US). The fact that these lesions could have been present, but are not reported in the present manuscript is aimed at not confounding the reader’s attention from the main topic of this work. This specification is now reported in the methods section.
12. The size of the smallest lesions detected by CT and US was similar (7-8 mm). The size of the smallest lesion reported by CT, CEUS and US is now reported at page 9.
13. A few patients (<8%) were suboptimally explorable by ultrasonography (both at
conventional US and CEUS), due to liver location and/or liver echogenicity (bright/fatty liver limiting the study of the deepest portions). This is now reported at page 8.

14. Same as point 13.
15. The text has been amended according to the appropriate reviewer’s indication.
16. We accepted the reviewer’s suggestion and incorporated the data in table 2.
17. Table 2 legend appears now complete.
18. Possible causes of CEUS false negative results were fatty liver in one case and location in the dome of the liver in a further case (this is suggested now at page 8), whereas in a further case of a small superficial lesion a specific cause could not been identified.
19. The characteristics of the lesions at conventional US were not reported because there was no relationship with the CEUS appearance and conventional US appearance had already been extensively reported in the past. Therefore, we believe that on the one hand no significant additional information is provided by these data and on the other hand these data could cause interpretation problems in the reader not expert in imaging, since we woule have needed to report findings of hypoechoic, isoechoic, hyperechoic lesions and so on, which could be confused with the CEUS appearance by a non-expert. As regards the arterial characteristics, these were not available for a certain number of lesions, according to the scanning modalities. CT, in fact, was performed without the arterial phase in most patients, according to accepted protocols for metastasis detection. As regards CEUS, this technique is well suited to investigate the arterial phase of a single or a few known focal lesions of the liver, but is not apt to adequately scan the whole liver during the short time of the arterial phase. Therefore the CEUS pattern during the arterial phase was not available for all lesions (especially it was missing for smallest lesions). Indeed, the late phase is the relevant one for metastasis detection. We added, however, a figure, showing two possible arterial patterns.
20. Biopsy could have been necessary on individual mets to decide on whether to proceed to laparotomy or not. Therefore, patients with already an indication to surgery (for instance because with 1-3 ascertained lesions) did not require biopsy for this reason. In a few dubious cases MRI (table 2) was used as a reference to decide whether to proceed to surgery, which was subsequently carried out, confirming the lesions as metastasis at pathological examination of the surgically resected lesions. In no cases biopsy was critical to decide whether to start chemotherapy or not, but this could be a potential use of biopsy. Only in one case a patient was confirmed as metastatic by biopsy, before hepatic surgical resection.
21. We added a figure with aspects of metastases also during the arterial phase, in order to clarify such CEUS aspects.

Reply to Reviewer Kazushi Numata.
We corrected the misspelling in the table legend.

Reply to Reviewer Edward Leen.

1. This point was already raised by another radiologist in the former submission of the manuscript. We replied to this issue to the Assistant Editor obtaining approval from the Editor to resubmit our manuscript and we report the same argument here. For clarity we refer to a recently published manuscript on the same topic (reference n° 17). We agree that the criticism of dr. Leen is appropriate from a merely radiological approach. However, we tried to emphasize that our study stem from a clinical approach and this was a main reason for submitting it to BMC Cancer rather than to a radiologic journal.
Any clinician would classify as a metastasis a lesion confirmed to be consistent with metastasis, at two imaging techniques (for instance CT and CEUS), in a patient with gastrointestinal cancer. CT
and CEUS both have definitively established diagnostic criteria for metastasis. Therefore each of them is considered definitively diagnostic, when typical, in the daily clinical practice. I think that a surgeon would proceed to surgery on the basis of CT alone if he/she is familiar with CT imaging and he/she does not request further MRI confirmation to characterize a certain lesion. The same happens for CEUS, in cases in which the surgeon (or the oncologist for chemotherapy) is familiar with this methodology. Therefore, in a clinical study the consistent findings of both techniques was held as diagnostic. For discrepant cases a further technique was used. This approach for the reference methods is similar to that of Quaia and coworkers (ref n° 17), who compared conventional US to CEUS and to CT. They state (page 1602) that “different reference standards were employed to confirm liver metastases. In 95 patients reference standard was …CT… combined to follow-up data obtained by multimodality imaging modalities… An increase in lesions diameter >50% or number documented at follow up were considered as diagnostic criteria for liver metastasis ”. In the quoted article, CT was considered as the reference standard. In our investigation, in 56 patients with 104 metastatic lesions, detected by CEUS, the lesion was confirmed at CT and subsequent follow-up. In the work of Quaia, there were several cases in which CT was not conclusive or did not detect the lesions revealed by CEUS. In these cases, CT was not the reference and the final diagnosis was achieved by Magnetic Resonance or intra-operative ultrasound. This was done also in our study and is now reported in table 2. For those lesions which were identified by only one technique in our study (in other words, 18 lesions detected by CEUS without CT confirmation and 10 lesions revealed only by CT) the confirmation came from surgical inspection and pathological examination or an increase in size (clearly by multimodality imaging, although not specified) in the follow up. This is the same approach as that used by Quaia and coworkers.

2. We added the Negative Predictive Value in the Results section. The specificity, accuracy and PPV could be added to sensitivity and NPV (which is already reported), by calculating them from the data reported in the manuscript. We chose, however, not to report the three latter values, since, in our opinion, this is not the appropriate setting for calculating the accuracy of a technique. In fact, to assess the accuracy, a sort of prerequisite is that the analysis is performed in patients in which multiple lesions are to be characterized and possibly not only in oncologic patients, so that a certain risk of false positive results is forecast (false positive results contribute to the correct assessment of PPV and accuracy). In the present case, instead, the study is just focused on the sensitivity of the techniques under investigation and these techniques themselves are used to establish the diagnosis and hence to determine the total number of lesions. Therefore the probability of having false positive cases is extremely reduced. Thence, the results could be unbalanced in favor of an excess of accuracy, specificity and PPV. We believe that this could mislead a reader who does not go in depth in the manuscript and we preferred therefore not to report specificity, accuracy and PPV. We reported, instead, the NPV since this test is related to false negative results or, in other terms to sensitivity and helps the reader to immediately catch the value of a technique in the setting of metastasis detection. We point out, again, however, that all the data reported in the text are enough for the reader to calculate also the other tests.

3. The modality to exclude patients with more than 4 lesions was conventional US, as already stated at page 5, line 5.

4. The Reviewer is right. Gastric cancer is relatively different from a biological point of view and was initially included in the study protocol only according to a organ concept (“intrabdominal digestive tract”) and due to the fact that some authors advocate a thorough resection of liver metastasis also for gastric cancer when metastatic disease is limited to a few lesions only in the liver, like for colonic cancer. The rate of patients with liver metastasis from gastric cancer was similar to that of large bowel cancer. For these reasons we decided to leave the study population as
it was, although limiting the study to patients with colonic cancer, as intended by the reviewer, has also a very strong and clear rationale.

5. Cysts and haemangioma are not mentioned in the results.

Minor revision (see also general comments to the review of dr. Harvey).