Author’s response to reviews

Title: Multi-parametric effect in predicting tumor histological grade by using Susceptibility Weighted Magnetic Resonance Imaging in tongue squamous cell carcinoma

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Reviewer reports:

Ivan Tham (Reviewer 1): A nice data set on SWI in oral SCC.

From what I gather, only a single axial slice per patient was used for SWI analysis. This could lead to sampling bias, and is also not fully utilising the available data.

Suggest added SWI analysis of other slices containing tumour.

Re: Thanks for your great comments. We did the ITSS counting and degree evaluation according to methods described by Park (2010, BJR). They selected imaging slice that visually showed the maximum frequency of the ITSS within a tumor. Some other studies using the methods by Kim et.al, degree of the ITSS was evaluated on each section of the enhancing portion of the tumor on a scale of 1–3 as proposed by Kim et al. For validation, we used methods described by Kim et.al to do ITSS counting and degree evaluation and similar results were obtained. Mean ITSS score was 1.03±0.76 for low-grade and 1.98±0.83 for high-grade, respectively (p<0.05). Since similar
results obtained, we have not include the new analysis results in the revised manuscript. Thanks again for your comments.

Michiel W.M. van den Brekel (Reviewer 2): Summary: The article describes a new method, susceptibility weighted MRI (SWI), which can be applied to predict the histological grade in oral tongue squamous cell carcinomas (OTSCCs). A prospective study cohort of 30 patients are included for analysis, and six parameters are extracted from the SWI by two independent experienced radiologists. Univariate analysis showed three predictive parameters, namely ITSSs, ITSS-score and ITSS ratio. ITSSs seems the best and is combined with the other parameters in the multiparametric analysis. This showed an improvement of the ROC analysis when the parameters ITSSs and Tumor thickness are combined.

Feedback:

For this article the novel technique SWI is used. However, since this sequence is not applied in routine imaging. Not everyone is familiar with SWI, so a few parameters to give the reader more sense of this sequence might help understand some considerations. For example, the scan time. What is the additional scan time for the patient compared with the routine MRI by using this sequence.

Re: Thanks for the comments. Normally, it will take additional 3:40 minutes scanning time for SWI sequence. Details have been shown in MM section of revised manuscript.

SWI images are obtained by multiplying the magnitude image with the phase mask. However, some studies only used the magnitude or phase image. Define which image you are using in your research.

Re: Susceptibility-weighted imaging (SWI) is a technique that enhances image contrast by using the susceptibility differences between tissues. SWI images were generated by the combination of magnitude and phase images with some technical processing. Although phase images could provide susceptibility information, its severe background somehow limited its application. In our study, SWI images were used for analysis. We have defined it in the MM section in the revised manuscript.

Tumor thickness is measured by multiplying the number of axial T2WI slices where the lesion was visible by the slice thickness. The method is a commonly used method, however, this method results in a underestimation of the tumor thickness since the slice thickness are 4 mm. This is a limitation of your research.
Re: Thanks for your comments. We have mentioned it as one of the limitations in the discussion section of the revised manuscript.

Also when predict a histological grade using MRI, you have to consider the difference in scale between these modalities. The MRI scale is 4 mm, while histological grading is assessed on a scale which is much smaller. Are you do a correction for this scale? If not, mention in the discussion section as a limitation or a challenge.

Re: Thanks for comments. We only analyzed the correlation between those two parameters, but not do correction for scale. We have mentioned it as a limitation in the discussion section of the revised manuscript.

ITSSs are low signal intensity and a fine linear or dot-like structures. Which underlying histopathological principles are the result of the low signal intensity? Tumor necrosis can also show a log signal intensity, is this included or excluded in the ITSSs?

Re: Thanks for comments. SWI could maximize the sensitivity to susceptibility effects including hemorrhage, blood by-products or even calcium. Tumor necrosis was differentiated as areas with hyper-intense signal on T2-weighted images and the interior non-enhancing part of an enhancing lesion on contrast-enhanced axial images. Although tumor necrosis can show similar low signal intensities on SWI, it has been excluded in the ITSSs. Details have been described in MM section in the revised manuscript.

The quantitative measurement is performed three times and the mean value is recorded and used for analysis. As reader, the mean value can be still a random value, if there is a high range in the three quantitative measurements of the radiologists. To give the reader more feeling of this mean value and strengthen your research, I propose to do a statistical analysis to prove the variability between the three measurements. This can be done by an intraclass correlation coefficient. Furthermore, knowledge about the time interval between those three measurements might also give more information to the reader. If the time interval is too short between the measurements, the results can be biased by knowledge bias. If so, this must be described in the discussion section. In addition, this method of manually delineating and measuring the parameters is a time-invasive principle.

Re: Thanks for good comments. To strengthen our research, we did intra-class correlation coefficient for quantitative parameters including tumor thickness, tumor volume and ITSSs. Intra-class correlation coefficient for tumor thickness, tumor volume and ITSSs was 0.99, 0.98, 0.99, respectively, showing almost perfect reproducibility for above variables. Furthermore, the
interval time among three times measurement was one week we think is enough for overcome the knowledge bias.

Points of attention

Figure 1B: There is a discrepancy between the number of arrowheads in the normal (n=3) and enlarged image (n=4).

Re: we have corrected in the revised manuscript.

Figure 2B: In the enlarged box, the second highest two arrowheads are pointing to the same low signal intensity spot. Is this spot counted twice since there are two arrows? Or are these two spots?

Re: We apologized for the confusion. Actually, the two arrowheads you mentioned pointed to two adjacent spots. Although they are very close, they are two separate spots, so we counted them for twice.

Figure 3: In my opinion, this image has no added value to the article.

Re: Thanks for comments. We have deleted fig 3 in the revised manuscript.

Figure 5: In figure 4 the AUC values are shown in the figure. In figure 5 the different AUC values for the univariate and multivariate analysis are shown. However, this is only visual. The notation of the AUC values (similar as in figure 4) can strengthen this image.

Re: thanks for the comments. We have added the notation of AUC values in the revised figs.

Table 1: The unit of the ITSSs ([#]) is missing and an explanation of the ITSS score is missing in the caption of the table.

Re: thanks for the comments. We have added unit of ITSSs and caption of ITSS score in the revised tables.

Typo’s

Background section: "Intratumoral various characteristics such as ….. in brain tumors such as gliomas [20,21]" Start with capital letter

Image processing and analysis section: "Intratumoral calcifications or macrohemorrhages ………… into account. ITSS score was ….. more than 10 ITSSs [29]". Dot to separate the sentence in two sentences
"Tumor thickness was measured by counting numbers of axial T2w1 slice where .... Slice thickness." Turn around: 'number' instead of 'numbers' and 'slices' instead of 'slice'.

Re: Above typo’s have been corrected in the revised manuscript.

SWI results section: "Of the 30 tumors, ITSSs were seen in 25 (83.3%) cases." This number has to be 23 with a percentage of 76%

Pre- and post-contrast SWI section: "Representative images showed in .... delineated on CE-SWI (fig 7)." The sentence is not fluent. It is hard to understand the meaning of the sentence, even after reading multiple times. Furthermore, I think in the sentence "tumor lesion was with obvious enhancement", the word "was" is redundant.

Re: Above typo’s have been corrected in the revised manuscript.

For sentences, our meaning was that CE-SWI axial SWI shows the internal structure more clearly than non-contrast SWI. The sentence has been modified in the revised manuscript.

Discussion section: " ITSSs only yielded an area .... (rCBV) performed best." 2 small typo's. Firstly it has to be 'glioblastomas' instead of 'globlastomas'. Secondly, the abbreviation (rCBV) is used before the explanation of this abbreviation. So maybe you can slide this definition forward.

Re: All typo’s have been corrected in the revised manuscript.

Figure 4: In the text and table we can read an AUC value range of (0.613-0.916), while the figure shows an AUC range of (0.603-0.916).

Figures: The abbreviation ITSSs brings some confusion. In the text, we can read always ITSSs. While in the figures ITSS is always used.

Re: Thanks for comments. We apologized for the typo mistakes and confusion brought to you. We have corrected the AUC range of (0.61-0.92) in all places including manuscript, figs and tables.

We also corrected "ITSS" to “ITSSs” in the figures.
Max Witjes (Reviewer 3): This paper describes the pre operative use of MRI, in particular, SWI to predict the tumor histological grade in oral squamous cell carcinoma by means of comparing intratumoral susceptibility signal intensities to histology.

The paper is well written and the design seems of a phase 1 trial. The data show that significant difference of ITSS scores between low- and high-grade tumor was observed with an area under ROC curve of 0.84, when a multi-parametric model using combination of ITSSs and tumor thickness was used.

The paper lacks a clear outline of what ITSS are, a statement like: "ITSS are defined as fine linear or dot-like structures with low signal intensity that are not discernable on conventional MR images but are seen within the tumor on magnitude images of SWI"; should be mentioned in the paper.

Re: Thanks for comments. We have mentioned statement in the MM section as following: “ITSSs were defined as low signal intensity and a fine linear or dot-like structure seen within the tumor on SWI, with or without conglomeration.”

The clinical impact of these data are difficult to assess at this point since currently tumor grading in high or low grade oral cancer is not influencing treatment strategies. Histopathological items such as lymph-vascular invasion, invasive front and perineural growth are of much more influence on treatment strategies. Do the authors think that these histological parameters can be identified on MRI? Do the authors foresee that this might be possible using radionomics type of analysis?

Re: Thanks for comments. SWI is currently a relatively new technique on MRI, it had advantages of noninvasively visualizing more internal characteristics in tumors such as hemorrhage, calcification and increased vascularity, which somehow reflected tumor malignant grade and provided more diagnostic information. Therefore, the main purpose of this study was to determine whether SWI associated parameters could help depict more internal characteristics would benefit to tumor evaluation. Although tumor grading is not enough for clinical management, further steps would be proposed on more histopathological items such as lymph-vascular invasion, invasive front and perineural growth by using multi-modalities or multi-parameters from different sequences. Radionmics approaches should be a potentially helpful for providing more tumor detailed information such as tumor heterogeneity.

Would there be a gain if this study had been performed on a 3T MRI?

Re: Thanks for comments. Definitely, better SNR and image contrast for SWI would be obtained on a 3T MRI. However, susceptibility artifacts that deteriorate the quality of SWI will be
enlarged on 3T MRI. Typically in oral cavity, teeth giving rise to susceptibility artifacts would be the major concern. So we conducted current study on a 1.5T MRI scanner.

The authors find a 0.84 correlation, do they think this means that this is sufficient for accurate tumor grading?

Re: Thanks for comments. Routinely, we measured tumor maximal diameter, tumor thickness or tumor volume to access the correlation between those parameters and malignant tumor grade or patient prognosis. Many studies suggested that these parameters were not strong enough to predict progression. Instead of routine measures of tumor thickness or tumor volume, multi-parametric model using combination of ITSSs and tumor thickness yielded a greater accuracy for tumor grading and allowed great differentiation between different histological groups, indicating that parameters obtained from SWI provided more diagnostic information for patients with OTSCC. In our opinion, parameters from multi-sequences or multi-modalities would provide more tumor detailed information and improve better diagnostic accuracy.

In the M&M section the suggestion is raised that patients have been operated without taking a biopsy of the lesion (biopsy was an exclusion item). I assume that a biopsy was taken after MRI to confirm the clinical suspicion of SCC? The authors should explain the diagnostic strategy.

Re: Thanks for comments. As mentioned, SWI is a unique sequence for depicting susceptibility effects including hemorrhage, blood by-products or even calcium. Preoperative biopsy would cause increased hemorrhage which may lead to bias for ITSS evaluation. Therefore, we listed preoperative biopsy as an exclusion item in the study. Normally, biopsy is performed for patients clinically suspected SCC after preoperative MRI scanning. We have also clarified in the revised manuscript.

In table I the characteristics of the cohort are given. Although the authors do not find a difference in tumor volume between high and low grade tumors, the volumes vary greatly. This I find concerning, since a volume of 0.5cm3 is quite small and 50cm3 is quite big. All were low grade tumors. This difference is not seen in the high grade group. Were data of the volume of low/high grade within the groups normally distributed ? Can these very small and large volumes be included ? are they outliers?

Re: Thanks for comments. We have checked our records, the volume in low-grade group varied greatly. Unfortunately, the data of tumor volume were not normal distributed. We have converted the dataset into normal distribution by log conversion. Thus, we have not excluded any value of tumor volume. Still no statistic difference observed between low- and high-grade groups (p=0.5)(Table 2,in revised manuscript). Moreover, we used median value for tumor volume in the revised manuscript.