Author's response to reviews

Title: pO2 Polarography, Contrast Enhanced Color Duplex Sonography (CDS), [18F] Fluoromisonidazole and [18F] Fluorodeoxyglucose Positron Emission Tomography: Validated Methods for the Evaluation of Therapy-Relevant Tumor Oxygenation or only Bricks in the Puzzle of Tumor Hypoxia?

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Dear Susanne Keiding

In contrast to your opinion, we believe that we tried to answer the different questions. For better understanding, we marked all changes of our first and second revision. But according to our opinion authors should have the possibility not only to modify the manuscript following the reviewer's ideas but also to justify the own concept.

In the manuscripts we published before, we described and discussed in detail the different new methods, the new parameters and co-variants like hemoglobin values and lymph node size that may influence the results. We examined and discussed whether different parameters of tumor vascularisation or perfusion measured by duplex sonography may influence oxygenation status in a significant way and whether it is possible to detect tumor hypoxia or tumor oxygenation in a direct way by a non invasive methods using FDG and FMISO PET. As many authors before, we used pO2 polarography as a standard measurement for further validation. We also discussed in detail the partly contradictory results in literature. Consequently we cited these papers in our current manuscript avoiding a simple repetition. Reaching a sufficient number of measurements we pooled the data of the different measurements in order to give a critical overview of the different methods, of possible interactions and discussed limitations of the methods using examples or so called outliers. Based on the data acquisition we described again the efforts we made in order to realize reproducible measurements.

"Measurements were performed in one lymph node metastases in each patient. In order to ensure measurements in the same suspected lymph nodes, sonographically examined lymph nodes were marked on diagnostic CT scans or in the case of lymph node conglomerates, the extension of the scanned node was marked on skin. PET examinations were realized in most of the patients within two days (maximum time interval four days) using skin markers and positioning lasers for reproducible data acquisition resulting in a corresponding slice location. No immobilization device was used. Sonographic studies, selection of representative images and parameter calculation as well as polarographic measurement were performed by one person each respectively, resulting in an objective and independent data acquisition."

"The tumor was defined according to the image data of the FDG-PET and the puncture computer tomography (CT) scans. In cases where the tumour was not clearly visible in the FMISO scan, FDG data were used to delineate the malignant lesion and define a region of interest. Rectangular regions of interest depending to the tumor size were placed over the tumor and ipsilateral nuchal muscles in order to calculate..." (page 4)
FMISO tumor to muscle ratios (FMISOT/M)."

(page 6)

Nevertheless, using PET data, it is always an estimation of values that might become more precise by the use of new, high resolution PET-CT-scanners. Possibly the additional use of an algorithm for image fusion could result in a better co-registration but getting experiences with different software for co-registration in the last years, final visual adjustment will enable the best results and it is a matter of debate whether this way or the use of corresponding scans is more objective.

We also described and visualized in Figure 2 and 3 the CT guided pO2 polarography including a very exact probe positioning into the lymph node and enabling an exact determination of the directions and overall length of the needle feed (comparable to the established technique of CT guided biopsy). In case of problems in probe guidance or complex anatomical situations additional scans during measurement were performed.

Technical details and adjustment of the sonography device were used as described before. Standard colour duplex sonographic criteria were used for the diagnosis of metastatic lymph nodes. Extension, size, echogenicity, and the texture of the suspected area were evaluated. In 20 patients a histological examination of lymph nodes was realized, confirming the sonographic diagnosis of malignity. In order to visualize as many vessels as possible a contrast enhancer (Leovist(R), Schering Corp., Germany) was administered. All sonographic studies were recorded in digital video. Representative images were selected for assessment from these video recordings. The maximal systolic phases were used for visualization of tumour vascularisation. Sonographic studies, selection of representative images and parameter calculation were performed by one person each respectively [Gagel B, DiMartino E, Schramm O, Pinkawa M, Piroth M, Demirel C, Maneschi P, Stanzel S, Asadpuor B, Westhofen M, Eble MJ: Contrast-enhanced color duplex sonography (CDS): an alternative for the evaluation of therapy-relevant tumor oxygenation? Strahlenther Onkol, 2006; 10: 604-609.].

Consequently we believe that we acquired data in reproducible and controlled way being accepted by many international reviewers before and even by additional three reviewers for this manuscript.

We analyzed the data in cooperation with Mr. Stanzel (Institute of Medical Statistics, RWTH Aachen). As we wanted to discuss and analyze associations and possible interactions of different factors and even to detect limitations of the different methods by the analysis of outliers, we used scatter plots and Pearson correlation coefficients without threshold based comparison of hypoxic or normoxic tumors, reflecting a straight (direct) way to show associations. Because of the fact that it represents an explorative examination, the aim of the study was not to test predefined hypotheses. Therefore we only conducted descriptive correlation analyses in order to search for potential associations between the parameters. The results of these correlation analyses were interpreted only in an explorative manner.

It is in the nature of research to take different views especially when methods are controversially discussed in literature. In the manuscripts we published before we still discussed these problems in a critical and according to the reviewers' statements in an objective way. In the current manuscript we do not want to give a simple repetition. Pooling the data of the different methods after reaching a sufficient number, we were able to directly compare different clinical methods for determination of tissue oxygenation or different factors influencing tissue oxygenation. Consequently we wanted to focus on the discussion that tumor hypoxia is caused by innumerable, multifactorial, partly contradictory interacting causes and effects complicating detection of therapy relevant hypoxia by the use of clinical examinations (page 12). Using "Evaluation of therapy relevant tumor oxygenation" in the title we used it with a question mark. Why? We wanted to emphasize the necessity of large clinical studies evaluating the different methods for its' prognostic value, that has been realized only for pO2 polarography until now [Nordsmark M, Bentzen SM, Rudat V, Brizel D, Lartigau E, Stadler P, Becker A, Adam M, Molls M, Dunst J, Terris DJ, Overgaard J: Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 2005; 77: 18-24.]. We underlined this necessity by our final statement in the conclusions, in a very critical way, as we believe: "But each of these approaches is methodologically limited. Consequently clinical potential must be substantiated in a prospective study, including uniform treatment modalities in order to be more than only bricks in the puzzle of therapy relevant tumor hypoxia."

According to our statement above we believe that we tried to answer the different questions. If there are misunderstandings or even different opinions we hoped that we could clarify these facts in an objective way. The reviewer process, though critical in nature, should be conducted in a collegial manner indicating personal judgment of the acceptability of a manuscript that has to be finally accepted by the authors. Nevertheless we also believe that the authors should have the possibility not only to modify the manuscript due to one reviewer's ideas but also to justify the own concept. We accept your personal judgement of our manuscript and we are confident that you are also able to accept our point of view.

With kind regards

Bernd Gagel