Reviewer's report

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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Reviewer: Susanne Keiding

Reviewer's report:

General
The authors compare measurements of pO2, FDG-PET, FMISO-PET and ultrasoundographic evaluation of tissue blood perfusion and vascularity in cancer noduli in 24 patients (out of totally 32 patients) with cancer in the head and neck region before therapy – and in some of the patients, also CT scans. They use the pO2 measurements as the “gold standard method” to estimate tumour hypoxia and make a number of correlation analyses of the other measurements against 5 sets of pO2 measurements, viz. mean pO2, median pO2, O2-fractions <2.5%, <5%, <10%. In the discussion, they speculate of reasons for outliers of some of the measurements in 4 patients, and conclude that “Each of these approaches is methodologically limited, making evaluation of clinical potential in prospective studies necessary.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. It is not clear to me what the _hypothesis_ of the study was. Did the authors wish to test the clinically relevant hypothesis that non-invasive FDG-PET, FMISO-PET, or the US-measurements can replace the invasive pO2 measurements? If so, say so, and conduct data analysis accordingly. The present data probably will reject such a hypothesis, in agreement with findings in other studies. Then the authors could speculate why this is the case. Among reasons for the 4 “outliers,” the authors mention _tumour tissue heterogeneity_, viz. necrotic tissue, areas with hyperperfusion and the bias of PET measurements of small tumour volumes. Such factors, among other factors, most probably are important biological factors and will bias each of the present measurements. If the authors agree, they could analyze the total material systematically from this point of view?

2. Concerning the statement that the _Eppendorff measurement of pO2_ is an accepted gold standard method to estimate tumour hypoxia, this is a matter of debate. The fact that the volume assessed by the needle measurement is small compared to the total volume of the tumour and tissue heterogeneity, in most tumours, makes the measurements subject to large variations; the measurements are not representative for the whole tumour volume. This question has been examined thoroughly in a recent experimental study in mice tumours comparing pO2 measurements with dual-tracer autoradiography measurements of tissue heterogeneity ([Int J Radiol Oncol Biol Phys 2005; 62:854]), which probably could give the authors better insight to these questions. Furthermore, the authors report the pO2-data in no less than 5 representations; if the authors think this is relevant, they should argue for it and take into consideration co-variations in their statistical analysis. If not, which of the 5 representations do the authors is/are most relevant for the present problems, and why? As mentioned by the authors, a disadvantage of the needle pO2 measurement is that it is confined to superficial tumours accessible to introduction of the needle. However, the authors do not mention the number of superficial tumours and the
> number of deeper tumours found in the patients, how this was defined,
> and in how many of the superficial tumours, the pO2 measurements were
> performed; if not in each of them, why? Furthermore the
> pO2-measurements are difficult to perform and it is a matter of
> judgement of where in a given tumour to perform the measurement. Do
> the authors have any estimates of the observer-effects? The authors
> defined the needle track by CT; how this was done? Simultaneously? In
> each patient? If so, this comprise an interesting procedure with
> possibilities of repeated measurements in regions with different
> CT-signals. Did the authors perform such measurements?
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> 3. Given these concerns, the next question is how valuable the PET
> recordings are? The authors should give data of how many tumours
> (primary and lymph nodes) were seen on the FDG and the FMISO images,
> respectively. And they should give criteria for defining an area with
> increased radioactivity concentrations compared to surrounding tissue
> as a being caused by malignancy by either tracer. How were the
> regions-of-interest defined? why did the authors use rectangular ROIs?
> The authors say that the FMISO-data were retrieved from tumour areas
> defined by the FDG-PET and CT-images. However, they don’t mention how
> the co-registrations were performed. Which computer-algorithms? Which
> landmarks were used? How sensitive was the co-registration procedure
> to e.g. 2-mm misalignment? Without a proper co-registration procedure,
> the definition of FMISO-data is doubtful, especially for tumours
> without FMISO accumulation compared to surrounding tissue.
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> 4. The authors state in the conclusion that studies of the clinical
> usefulness of these measurements are needed. I agree, and would like
> to ask why the authors don’t utilize the present material to perform
> such an analysis? Especially because the present measurements were
> performed before treatment.
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> 5. What is the topic of reference 25, /in press/, with most of the
> authors being the same as in the present manuscript and with nearly
> the same title? Is the patient material and the measurements the same?
> If not, why two papers and not one large and more comprehensive? The
> manuscript should have been enclosed in submission of the present
> manuscript.
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> 6. Minor PET questions: Were the patients fasting and for how many
> hours? Since high blood glucose exerts competitive inhibition on FDG
> uptake and decreases the tumour-surrounding tissue contrast, blood
> glucose concentrations at the time of the FDG PET recordings must be
> reported; also were any of the patients treated with insulin? I assume
> that the PET data were corrected for radioactive decay to the time of
> tracer injection. If so, say so.
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> 7. I’m not an expert on ultrasonography, but would like to ask how
> the ROIs were defined? The authors mention TRI and TPI values, but
> don’t seem to use these values?
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> 8. How was the number of patients determined? Any /a priori/ assessment?
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> 9. In Results, it is not clear how many measurements the data
> reported are based on.
Declaration of competing interests:
I declare that I have no competing interests