Author's response to reviews

Title: Prognostic implication of intratumoral metabolic heterogeneity in invasive ductal carcinoma of the breast

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Author's response to reviews: see over
May 19th, 2014
Professor Stephen P. Povoski
Section Editor, *BMC Cancer*

Dear Professor Povoski,

We thank you and the reviewers of *BMC Cancer* for taking time to review our article. We have carefully read the comments and tried to respond to the best of our abilities. We hope that the following revisions are acceptable.

**Referee: 1**

**Reviewer’s report:**
You mention that post-surgical radiation was given “when indicated.” Can you expound on this a bit more? Did you use specific guidelines, and if so, which ones?

Thank you for the comment. Radiation therapy was given to the patients according to the NCCN Guidelines and Recommendations for breast cancer treatment of the Korean Breast Cancer Society. We have added this information to the Methods section, under the subheading “Patients.”

Measurement of tumor heterogeneity is the main topic of your paper, but I remain confused if you have measured the heterogeneity of each FDG avid lesion or just of the primary tumor. It would be helpful to have this better addressed.

Thank you for your suggestion. The heterogeneity factor (HF) was measured only in the primary tumor. The description of measuring the HF has been edited for clarification.

**18F-FDG PET/CT acquisition and image analysis**

All patients fasted for at least 6 hours, and the blood glucose levels were checked before the administration of 18F-FDG. The blood glucose concentration was managed at less than 150 mg/dL in all subjects. Patients with elevated blood glucose levels had their examinations rescheduled. Approximately 8.1 megaBecquerels (MBq) of 18F-FDG per kilogram of body weight was injected intravenously, and the patients rested for 1 h before acquisition of the PET/CT images. PET/CT scans were performed using one of two PET/CT systems (Reveal RT-HiREZ®, CTI Molecular Imaging, Knoxville, TN, USA) (Discovery STE®, GE Healthcare, Milwaukee, WI, USA). Following a low-dose CT scan without contrast from the skull vertex to the knee, with the patient in the
supine position and breathing quietly, a PET scan with a maximum spatial resolution of 6.5 mm (Reveal PET/CT) or 5.5 mm (Discovery PET/CT) were obtained from the skull vertex to the knees, at 3 min per bed position. The images obtained by the Reveal PET/CT and Discovery PET/CT scanners were reconstructed with a 128 × 128 matrix, an ordered-subset expectation maximum iterative reconstruction algorithm (4 iterations, 8 subsets), a Gaussian filter of 5.0 mm, and a slice thickness of either 3.0 mm (Reveal PET/CT) or 3.27 mm (Discovery PET/CT).

The SUVmax was obtained using the following formula: 
\[
\text{SUVmax} = \frac{\text{maximum activity in the region of interest (mBq/gram)}}{\text{injected dose [mBq]/body weight [grams]}}.
\]
The MTV was determined as the total number of voxels with threshold SUV of \(\geq 40\%\) of the SUVmax in the volume of interest. The TLG was calculated as the MTV multiplied by its SUVmean.

The intratumoral metabolic heterogeneity was represented by the heterogeneity factor (HF) [16], which was determined for each patient as follows: a region of interest (ROI) was manually drawn to fully include the primary tumor and a surrounding region of normal tissue (normal background). The tumor volume was determined with a series of SUV thresholds (e.g., 40%, 50%, 60%, 70%, and 80% of SUVmax) using the semiautomatic software of the workstation (Advantage Workstation 4.3, GE Healthcare). We excluded the values of <40% from the heterogeneity analysis because a previous study reported that the minimal threshold that represents the actual tumor volume was 40% and the values of <40% included too much normal tissue background activity [28]. In addition, values of >80% were also excluded because the volumes were small and the partial volume effect was pronounced [29]. A volume-threshold function of the tumor was acquired by plotting thresholds to volumes. Linear regression analysis was performed and the HF was calculated by finding the derivative \(dV/dT\) of the volume-threshold function for each tumor. Next, the values of the HF were modified into absolute values so that all resulting values were positive; the more positive the factor, the more heterogeneous the tumor. It took about 1 min to obtain the intratumoral metabolic heterogeneity for each lesion.

You mention that it takes 1 minute to take a measurement of heterogeneity. Is this practical (especially if you are measuring multiple lesions)?

Thank you for raising this point. The HF was assessed solely in the primary tumor, and most of the patients had just one primary tumor. In case of multiple lesions in the breast, we measured the HF in the most hypermetabolic lesion. It was measured by using semiautomatic software, and the slope (HF) was rapidly calculated using a statistical program. Therefore, it took about 1 min to obtain the intratumoral metabolic heterogeneity for the primary breast tumor.

Consider referring to “invasive ductal breast cancer” as invasive ductal carcinoma (or IDC).
Might consider listing “Glut” as GLUT. Both instances are published, but GLUT is preferred; also needs updating if you choose to use this suggestion in the abbreviation list.

Thank you. “Glut” has been changed to “GLUT”

Referee: 2

Reviewer’s report:
I am grateful to the authors for their submission. This article relates the tumor metabolic heterogeneity, as imaged by PET/CT, with the clinical behavior of these tumors. The statistical tool used are appropriate and the results obtained are reasonable under the circumstances described by the authors. Related to the submission, I have a number of concerns:

Major compulsory revisions:
• The derivative of the threshold function is not well defined in the abstract, or in the body of the paper. I urge the authors to reformulate it, precisely indicating what its component quantities mean, and what its units of measurements are. Also TLG is reported without units. Units should be clearly indicated. Also, because the function is defined as negative leads to the situation where lower values reflect more heterogeneity. (Discretionary revision: Would authors consider modifying the function to reflect the absolute change in volume with changing in glycolysis threshold? This way, values would be positive, and higher values would reflect higher tumor heterogeneity.)

Thank you for the comment. To make the section more comprehensible to readers, we reformulated the explanation for measuring the HF. The derivative of the volume-threshold function is calculated as the slope from linear regression.

18F-FDG PET/CT acquisition and image analysis
All patients fasted for at least 6 hours, and the blood glucose levels were checked before the administration of 18F-FDG. The blood glucose concentration was managed at less than 150 mg/dL in all subjects. Patients with elevated blood glucose levels had their examinations rescheduled. Approximately 8.1 megaBecquerels (MBq) of 18F-FDG per kilogram of body weight was injected intravenously, and the patients rested for 1 h before acquisition of the PET/CT images. PET/CT scans were performed using one of two PET/CT systems (Reveal
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Some journals report TLG in grams, but generally, TLG is not expressed in units. Incidentally, we changed the decimal point of the value of TLG; that is, we divided all TLG values by \( 10^3 \), because we calculated TLG by multiplying MTV in units of cubic millimeters (m\( \text{m}^3 \)) by its SUVmean at first. Therefore, to simplify, we recalculated the TLG by multiplying MTV in units of cubic centimeters (cm\( \text{m}^3 \)) by its SUVmean.
Thank you for your suggestion. We have modified the function to reflect the absolute change in volume with changing SUV thresholds, according to your suggestion. The HF is now positive, and the higher the HF, the more heterogeneous the tumor.

• It is not clear to me how the 40% and the 80% values were reached. Could the authors present a detailed explanation?

Thank you for the comment. We excluded values < 40% from the heterogeneity analysis, because a previous study reported that the minimal threshold that represents the actual tumor volume was 40% and values < 40% represent too much normal tissue background activity [28]. In addition, values > 80% were also excluded, because the volumes were small and the partial volume effect was pronounced [29].

• Due to limited PET resolution, PET would only be able to identify variations at a macroscopic scale. Therefore microscopic variability in tumor metabolism cannot be supported as an explanation/motivation for the findings. Authors need to focus on mechanisms of tumor heterogeneity that explain findings at the spatial scale of their observations.

Thank you for pointing this out; we hope we can clarify it. In a previous study (Lau et al. Clin Radiol 2004, 59:487–98), large GISTs showed heterogeneous enhancement on contrast-enhanced CT. Focal areas of low attenuation on CT in small GISTs represented various pathological conditions, including solid tumor, hemorrhage, hemorrhage with necrosis, cystic degeneration, fluid in ulcers, and fibrous septa (Kim et al. Clin Radiol 2005, 60:384–8). Recently, Watabe et al. reported heterogeneous F-18 FDG uptake in solid portions of the GISTs, despite homogeneous density on contrast-enhanced CT (Watabe et al. Ann Nucl Med. 2012, 26:222–7). Similarly, in our study, many of the tumors had focal low density areas on CT with corresponding $^{18}$F-FDG uptake, and some of the primary breast tumors showed heterogeneous $^{18}$F-FDG uptake with homogeneous attenuation on CT.

Fig. 1 a 2D-ROI area and threshold tumor area showing over b 30, c 50, and d 70% of the 2D-SUVmax. (percent area = 98.3, 68.5, and 28.6%, respectively) (Watabe et al. Ann Nucl Med 2012, 26:222–7)
Second, macroscopic areas of tumor necrosis will increase the observed tumor metabolic heterogeneity. Necrotic tumors are biologically more aggressive. Do authors consider the possibility of the signal they identified simply reflecting necrosis in the tumor?

That is true. Tumor necrosis is one of the known mechanisms of heterogeneous FDG uptake (Henriksson et al. Br J Radiol 2012, 85:e694–701). It has also been postulated that increased heterogeneity within tumors may be associated with differences in tumor cellularity, proliferation, angiogenesis, and hypoxia (Tixier et al. J Nucl Med 2011, 52:369–78), (Ganeshan et al. Radiology 2013, 266:326–36), known factors that independently have been associated with more aggressive behavior, poorer response to treatment, and worse prognosis.

Minor concern:
Throughout the paper, authors report their quantities using 4 significant digits. 2 would suffice, in my opinion.

Thank you. Corrections have been made according to the suggestion.

Quality of written English: Needs some language corrections before being published

In accordance with this comment, the manuscript has been checked and revised by a professional native English-speaking editor.

We deeply appreciate the careful reviews and hope that this revision is acceptable for your journal.

Sincerely,

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