

Editor-in-Chief

World Journal of Clinical Cases

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Title: GLUT-1 expression in preoperative endoscopic biopsy is helpful for detecting lymph node metastasis on 18F-FDG-PET/CT in colorectal cancer (Manuscript ID: 89690)

Dear Dr. Editor-in-Chief

Thank you very much for your valuable review of our manuscript.

All comments were helpful in revising and improving this report, and we did our best to respectfully follow your detailed advice. Heeding closely to the constructive comments suggested by the Editor and the reviewers, we have addressed and outlined each issue point-by-point in the text below.

We appreciate the thoroughness of the reviewers and hope that these changes adequately address their concerns.

These changes were indicated in red in the revised manuscript.

All of the authors have read and agreed with the revised manuscript.

Again, thank you very much for considering our work for publication in this prestigious journal.

COMMENTS FOR THE AUTHOR:

Reviewer 1 Comments

1. Are there any evidence for the positive criteria determination for GLUT-1, GLUT-3, HK-II, and HIF-1 expressions ?

Thank you for your valuable comments.

When analyzing protein expression through immunohistochemical staining, **the expression is evaluated in several ways** - for example, staining intensity, proportion of stained cells, or a combination of intensity and proportion. **Reviewing the methods used in previous studies, the expression of proteins analyzed in this study was interpreted using various methods rather than one standard criteria.**

In this study, the immunohistochemical stain was performed using a tissue microarray block, so there was a limitation in evaluating the proportion. Therefore, **the expressions of GLUT-1, GLUT-3, Hexokinase-II, and HIF-1 were evaluated based on the intensity, and staining of more than 5% of tumor cells was applied as the minimum proportion criterion.** We already addressed the positive criteria in the section on Materials and methods. Additionally, HIF-1 is a protein expressed in the nucleus, but the criteria were not mentioned in the previous submission. So the criteria for HIF-1 protein was added to the method.

Page 7 (Materials and methods):

“ GLUT-1, GLUT-3, HK-II, and HIF-1 expressions were considered positive when >5% of tumor cells demonstrated cytoplasmic or membranous staining. The immunoreactive score was rated as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong) based on the average staining intensity. A score of 2 or higher indicated positivity (Figure 1). HIF-1 expression was considered positive when >5% of tumor cells demonstrated nuclear staining. ”

2. A scale in all the images in figure 1 would be appreciated.

Thank you for your valuable comments.

All figures are taken at x100 magnification. As you suggested, we added scale in the figure and figure legends, respectively.

Figure 1:

“Figure 1. Immunohistochemical staining for GLUT-1 in colorectal cancer. GLUT-1 expression was demonstrated as cytoplasmic or membranous staining. The score was assessed according to the intensity (0=negative (A), 1=weak (B), 2=moderate (C), 3=strong (D); x100). A score of 2 or higher was considered as positive.”

3. How to define the center and periphery in biopsy samples in this study?

Thank you for your valuable comments.

The core obtained from the center of the tumor close to the mucosa was indicated as the 'tumor center', and the core obtained from the deep invasive front was indicated as the 'periphery'.

As you suggested, we added this information according to your comments in the section on Materials and methods.

Page 6 (Materials and methods):

“ The core obtained from the center of the tumor close to the mucosa was indicated as “tumor center,” and the core obtained from the deep invasive front was designated as “periphery.” .”

4. Is there any evidence that N3 (Table 1, 3-5) was presented in AJCC/UICC staging system?

Thank you for your valuable comments. We wrote it wrong by mistake. We corrected from N3 to N2 in **Table**.

5. Many previous studies indicated a negative correlation of GLUT-1 with SUVmax in various malignancies including gastric cancer, pancreatic cancer, lung cancer; however, the authors detected a positive correlation of them in this study. The underlying reasons should be profoundly discussed.

Thank you for your valuable comments.

Many previous studies not only reported a negative correlation of GLUT-1 with SUVmax but also reported a positive correlation. Therefore, we already mentioned previous discordant findings in various malignancies, including meta-analysis, in the Introduction and Discussion.

The exact reasons for these controversial results are unclear, to date. Therefore, as you suggested, we added an underlying hypothesis to the Discussion.

Page 10 (Discussion):

“ However, to date, the exact reasons underlying these controversial results remains unclear. Probably, the complex glucose metabolism of malignant tumors differ between tumor types and might affect the association between GLUT-1 expression and SUVmax. In addition, GLUT-1 expression is not only specific for tumor cells, as GLUT-1 is also expressed on erythrocytes and immune cells. Moreover, a low burden of tumors and some good differentiated tumor types might reduce GLUT-1 expression, which could be the reason for false-negativity on PET/CT[25]. ”

6. Representative images for GLUT-3, HK-II, and HIF-1 expression could be supplied.

Thank you for your valuable comments. As you suggested we added representative images for GLUT-3, HK-II, and HIF-1 expression as Figure 2.

Figure 2:

“Figure 2. Immunohistochemical staining for GLUT-3, Hexokinase-II, and HIF-1 in colorectal cancer. GLUT-3 and Hexokinase-II expressions were demonstrated as cytoplasmic or membranous staining. (A: GLUT-3 negative, B: GLUT-3 positive, C: Hexokinase-II negative, D: Hexokinase-II positive). HIF-1 expression was demonstrated as

nuclear staining (E: HIF-1 negative, F: HIF-1 positive).

7. Some statements in the paper lack of references supporting, for example: "...Studies have evaluated the correlation between the expression of several proteins..."

Thank you for your valuable comments. As you suggested we added references to sentence in the Introduction and References.

Page 15-16 (References):

14. Yang HJ, Xu WJ, Guan YH, Zhang HW, Ding WQ, Rong L, Qiu ZB, Zhong L. Expression of Glut-1 and HK-II in pancreatic cancer and their impact on prognosis and FDG accumulation. *Transl Oncol* 2016; 9: 583-591. [PMID: 27916293 DOI: 10.1016/j.tranon.2016.08.004]

15. Krzeslak A, Wojcik-Krowiranda K, Forma E, Jozwiak P, Romanowicz H, Bienkiewicz A, Brys M. Expression of GLUT1 and GLUT3 glucose transporters in endometrial and breast cancers. *Pathol Oncol Res* 2012; 18: 721-728. [PMID: 22270867 DOI: 10.1007/s12253-012-9500-5]

16. Krishnamachary B, Berg-Dixon S, Kelly B, Agani F, Feldser D, Ferreira G, Iyer N, LaRusch J, Pak B, Taghavi P, Semenza GL. Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Res* 2003; 63: 1138-1143. [PMID: 12615733]

17. Saigusa S, Toiyama Y, Tanaka K, Okugawa Y, Fujikawa H, Matsushita K, Uchida K, Inoue Y, Kusunoki M. Prognostic significance of glucose transporter-1 (GLUT1) gene expression in rectal cancer after preoperative chemoradiotherapy. *Surg Today* 2012; 42: 460-469. [PMID: 22072148 DOI: 10.1007/s00595-011-0027-2]

25. Baek HJ, Chung JH, Park JH, Zo JI, Cheon GJ, Choi CW, Lim SM, Choi SY, Hong JM, Hong JS. FDG-PET in mediastinal nodal staging of non-small cell lung cancer: correlation of false results with histopathologic finding. *Cancer Res Treat* 2003; 35: 232-238. [PMID: 26680941 DOI: 10.4143/crt.2003.35.3.232]

Reviewer 2 Comments

This is an interesting study on the diagnostic value of GLUT-1 expression for lymph node metastasis in colorectal cancer. Similar results have been reported previously: Yang J, Wen J, Tian T, Lu Z, Wang Y, Wang Z, Wang X, Yang Y. GLUT-1 overexpression as an unfavorable prognostic biomarker in patients with colorectal cancer. *Oncotarget*. 2017 Feb 14;8(7):11788-11796. doi: 10.18632/oncotarget.14352. PMID: 28052033; PMCID: PMC5355304. The authors may look to include more recent work in their discussion: AUTHOR=Kim Tae Hyun, Kwak Yoonjin, Song Changhoon, Lee Hye Seung, Kim Duck-Woo, Oh Heung-Kwon, Kim Jin Won, Lee Keun-Wook, Kang Sung-Bum, Kim Jae-Sung TITLE=GLUT-1 may predict metastases and death in patients with locally advanced rectal cancer JOURNAL=Frontiers in Oncology, VOLUME=13, YEAR=2023
URL=https://www.frontiersin.org/articles/10.3389/fonc.2023.1094480 DOI=10.3389/fonc.2023.1094480
ISSN=2234-943X

Thank you for your valuable comments.

Editorial Office's Comments

I have reviewed the Peer-Review Report, full text of the manuscript, all of which have met the basic publishing requirements of the World Journal of Clinical Cases, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Before its final acceptance, please provide and upload the following important documents:

Biostatistics Review Certificate, a statement affirming that the statistical review of the study was performed by a biomedical statistician;

Institutional Review Board Approval Form or Document, the primary version (PDF) of the Institutional Review Board's official approval, prepared in the official language of the authors' country;

Signed Informed Consent Form(s) or Document(s), the primary version (PDF) of the Informed Consent Form that has been signed by all subjects and investigators of the study, prepared in the official language of the authors' country.

Thank you very much for your valuable review of our manuscript.

All comments helped revise and improve this report, and we did our best to respectfully follow your detailed advice. Heeding closely to the constructive comments suggested by the Editor and the reviewers, we have addressed and outlined each issue point-by-point in the text below.

We can provide an IRB approval form and Biostatistics Review Certificate. However, the requirement for written informed consent was waived due to the retrospective design of the study. So, we mentioned it in the manuscript.

We also added Article Highlights before the reference section.