Author's response to reviews

Title: Performance of 5-aminolevulinic acid-based photodynamic diagnosis for radical prostatectomy

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Version: 3 Date: 28 March 2015

Author's response to reviews: see over
March, 28th 2014

Dear Editor-in-Chief,
BMC Urology

We are resubmitting revised manuscript “Performance of 5-aminolevulinic acid-based photodynamic diagnosis for radical prostatectomy”
According to your guidance, we changed some parts and responded to reviewer’s comments as below.

1. We added figure files in the main manuscript.
2. We highlighted all corrections with underlines
3. We modified copyedit English and grammatical errors by Edanz check including title.

Reviewer: Giacomo Maria Pirola

Major Compulsory Revisions

However an interesting theme, the work is really bad written, full of errors that must be corrected.

1. Putting a stomach tube under general anesthesia 3 hours prior to surgery seemsa too invasive procedure,

Firstly, we performed general anesthesia for radical prostatectomy. Secondly, we put a stomach tube and gave ALA solution. Then, operation was started. At 2-3h after start of surgery we performed intraoperative ALA-PDD. So, according to reviewer’s point, we changed 2sentences as bellow.

1. Section of Abstract (method)
Three hours prior to observation, 1 g of 5-ALA dissolved in 50 ml of 5% glucose solution was given orally through a stomach tube.

↓

One gram of ALA solution was given intraoperatively, orally through a stomach tube.

2. Section of Methds
Three hours prior to intraoperative surveillance, 1.0 g of ALA solution was given orally from a stomach tube under general anesthesia.

↓
One gram of ALA solution was given intraoperatively, orally through a stomach tube.

2. **ALA is not demonstrated to be selective absorbed by prostate cancer cells rather than inflammatory ones, and as You said health degeneration make some samples not trustable.**

→ We wanted to demonstrate predominant PpIX accumulation in cancer cells compared to normal cells. So, we changed following sentence in Background section.

Section of Background

The second was to demonstrate the predominant accumulation of PpIX in human prostate cancer cells compared to normal prostate cells by the use of ALA-PDD on the divided surface of excised prostate.

3. **So, I won't feel so sure to base surgical strategy on this finding. The casuistic shows that further studies are needed to ameliorate this technology, still not valid now. Tables should be presented more clearly.**

→ According to reviewer’s point, we changed conclusion.

Section of Discussion

We conclude from this study that intraoperative ALA-PDD is feasible and more appropriate for high-risk patients with highly expected positive surgical margins. In addition, heat degeneration and the linear length of positive surgical margin have crucial influences on ALA-PDD. In the future, a randomized clinical trial for selected cases should be carried out.

↓

This study showed preliminary results of intraoperative ALA-PDD in radical prostatectomy. We conclude that intraoperative ALA-PDD is feasible, but the limitation of this study is clinical stage and heat degeneration. In the future, a randomized trial should be carried out.

**Reviewer: Wael Y Khoder**

Major revisions
the authors are asked to describe thier work in the paper accurately.
1. Please be accurate in description of the methods: PDD was used intraoperatively during prostatectomy, or after? (it is not clear). Describe the open procedures.

We used PDD laparoscope intraoperatively during radical prostatectomy. In open procedure, we put the PDD laparoscope into the surgical field. We added sentence as follow.

Section of Methods
In open radical prostatectomy, we intraoperatively put the PDD laparoscope into the surgical field under general anesthesia and observed surgical margins using both white light mode and blue light mode.

2. Mention your histological work (its results are lightly touched without any informations about it). How had you calculated your statistics? You should mention how you calculated sensitivity and specificity from just one case? Describe the results in more details A)

We calculated diagnostic accuracy from biopsy specimens in divided surface of excised prostate. Sensitivity and specificity of biopsy specimens was calculated by comparison between red fluorescence intensity and pathological results. We already mentioned these results in result section (2. Photodynamic diagnosis in excised whole prostate) as follow.

Section of Results
After examination of surgical margins, we set divisions of the excised prostate from 29 cases and biopsied each area by cold-cup with PDD. We obtained 141 biopsied samples in all. Fluorescence positivity was found in 31 samples, while pathological positivity was found in 20 samples (Figure 1b). The overall sensitivity and specificity were 75.0% and 87.3%, respectively (Table 2)

According to reviewer’s point, we changed following sentence from “Examination of excised prostate” to “Statistical analysis” in Methods section.

Section of Methods
Then, the histological results were determined by two pathological specialists without
knowledge of the results of fluorescence intensity. The diagnostic accuracy of the divided surface in excised prostate was calculated on the basis of comparison between fluorescence intensity and pathological results according to general rules for clinical and pathological studies on prostate cancer.

6. Statistical analysis
In this study, we examined the potential utility of PDD technique for diagnostic study. As such, no statistical analysis was performed.

Then, the histological results were determined by two pathological specialists without knowledge of the results of fluorescence intensity.

6. Statistical analysis
The diagnostic accuracy of the divided surface in excised prostate was calculated on the basis of comparison between fluorescence intensity and pathological results according to general rules for clinical and pathological studies on prostate cancer.

3. Clinical results about operative time, blood loss, complications, outcomes, etc.
B) →This study focused on diagnostic accuracy of intraoperative ALA-PDD for radial prostatectomy. So we did not mention about operative, blood loss and so on.

4. Histological workup in details (tumor cells, tumor criteria in relation to PDD, etc. C) your basic research part (or add a reference about this, if already published).

→According to reviewer’s point, we added as our basic research part in reference [25].

Section of Discussion
We have previously shown in vitro accumulation of ALA-mediated PpIX in prostate cancer cell lines [25].

Section of references
enhances 5-aminolevulinic acid-based photodynamic action for prostate cancer.
Photodiagnosis photodyn Ther 2013, 10: 399-409.

5. Discuss your results in comparison to the others in details (eg versus Zaak et al with numbers, outcomes, etc). please avoid unsupported conclusions like (PDD senstivity increases with high tumour grade!!). mention the limitations of the study.

→According to reviewer’s point, we changed 2 following sentence.

1. Section of Discussion
The result of this study showed that one case had a red-fluorescence surgical margin of excised whole prostate and histologically confirmed positive surgical margin of Gleason score 6. In this study, there were few cases of red-fluorescence-positive surgical margin compared with the incidence in previous reports. This may have been due to two factors. First of all, the majority of the patients were in the low-risk group. Adam C et al. reported the feasibility of intraoperative PDD for selected cases [16]. They performed intraoperative PDD for advanced cases greater than pT2c. Thus, we indicated that intraoperative ALA-PDD was more appropriate for high-risk patients with highly expected positive surgical margins. Secondly, heat degeneration using an electric device and linear length of positive surgical margin have crucial influences on ALA-PDD.

↓
The present showed that one case had a red-fluorescence surgical margin of excised whole prostate and histologically confirmed positive surgical margin of Gleason score 6. Heat degeneration using an electric device and linear length of positive surgical margin have a crucial influences on ALA-PDD.

2. Section of Discussion
We conclude from this study that intraoperative ALA-PDD is feasible and more appropriate for high-risk patients with highly expected positive surgical margins. In addition, heat degeneration and the linear length of positive surgical margin have crucial influences on ALA-PDD. In the future, a randomized clinical trial for selected cases should be carried out.

↓
This study showed preliminary results of intraoperative ALA-PDD in radical
prostatectomy. We conclude that intraoperative ALA-PDD is feasible, but the limitation of this study is clinical stage and heat degeneration. In the future, a randomized trial should be carried out.

6. Lastly please improve the article for the language

According to reviewer’s point, we modified copyedit English and grammatical errors by Edanz check including title.

Title: Performance of 5-aminolevulinic acid-based photodynamic diagnosis for radical prostatectomy

We hope that you will find our manuscript suitable for publication in *BMC Urology*.

Best wishes

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