Author's response to reviews

Title: 5-aminolevulinic acid based fluorescence spectroscopy and conventional colposcopy for in vivo detection of cervical premalignancies

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Version: 2
Date: 2 February 2015

Author's response to reviews: see over
Reviewer's report 1.

**Minor essential revisions:**

**Comment 1.**

The aim of the study was a comparative study between conventional colposcopy and 5-aminolevulinic acid based fluorescence spectroscopy. In the title of the paper you cannot find the conventional colposcopy.

Response 1.

The title has been modified.

„5-aminolevulinic acid based fluorescence spectroscopy and conventional colposcopy for in vivo detection of cervical premalignancies“

**Comment 2.**

**How much time did it take for the patient to underwent the spectroscopy including incubation time?**

Response 2.

It took about 3 hours for the patient including first visit (survey, colposcopy, autofluorescence measurements (10-15 min)), about 135 min. intermission (free time) and then the second visit for fluorescence spectroscopy (about 5-7min) and biopsy if needed.

**Comment 3.**

**How did the patient experience the procedure?**

Response 3.

Patients experienced the procedure well, no discomfort sensation (except subjective feeling of warming during fluorescence spectroscopy in few cases was mentioned).

**Comment 4.**

**Darkened room?**

Response 4.

The measurements were performed in the room with the lights off and window blinds closed.
Comment 5.

What about the costs?

Response 5.

The price for 3 % 3 g 5-ALA CREAM (typical dose for one patient) is about 10 €.

Major compulsory revisions

Comment 6.

The methods are not well described or unclear. I miss the results of the referral cytology. High-grade cytology? High percentage of CIN 0+1.

Response 6.

Only patients with suspected high grade changes in cytology were included in this study.

29 % of those cases had histopathological result CIN 0-1.

Each patient had cytological smear (HSIL (37 cases), ASCUS – H (11 cases), colposcopy, fluorescence spectroscopy performed. Every inspected cervix was divided in to four quadrants clockwise (Figure 1), and every quadrant was analyzed as a separate case. In total, 48 patients and 174 cervical quadrants were included in this study. After colposcopy and fluorescence spectroscopy examinations punch biopsy or loop electrosurgical excision of the cervix for histopathological analysis was performed. These tissue samples were diagnosed to be either inflammatory (chronic cervicitis) (CIN 0), mild (CIN1), moderate (CIN2) or severe (CIN3) neoplastic.

Comment 7.

Did they take a new smear in the study? How was the workflow? First colposcopy and when was the spectroscopy performed?

Response 7.

Cytology test was not repeated, we included patients to this study according the cytology result from sending institution.
OUR WORKFLOW:

1. The first visit for the patient:
   patient survey, colposcopy, autofluorescence measurements. 5-ALA cream application on the cervix;
2. **Incubation time** – about 135 min.
3. The second visit for the patient:
   fluorescence spectroscopy measurements, biopsy if needed or conisation (other day).

**Comment 8.**

48 patients means 192 quadrants, 174 quadrants included. 18 missing quadrants? Selection?

**Response 8.**

Data of 18 cervical quadrants was excluded from study,

1. due to absence of histopathological analysis
   (uncertain marking or absence of the quadrants after loop excision or biopsy – 8 cases, coagulation defects - 5 cases)
2. inappropriate fluorescence spectroscopy measurements
   (bleeding from the cervical tissue during the investigation - 4 cases and the device failed during the measurement -1 case).

**Comment 9.**

How many punch biopsies were taken and how much loop excisions performed?

**Response 9.**

Punch biopsies were taken from 15 patients and 33 loop excisions were performed.

**Comment 10.**

Loop excisions without biopsy before? Did they mark the tissue from the loop excision?
Response 10.
Cone from cervix after loop excision was attached with pins to the specially prepared disc, with 3, 6, 9, 12 hour directions marked and then send to pathologist.

Comment 11.
Cervicitis = CIN 0? CIN 0 = normal tissue?

Response 11.
In our study CIN 0 means no neoplasia, chronic cervicitis.
Chronic cervicitis was identified in 23% of all cases.

Comment 12.
How was the histology performed, one pathologist?

Response 12.
The histology was performed by one pathologist.
Comment 13.

Very low CIN 2 numbers?

Response 13.

CIN 2 was identified in 4% of all cases. This could be due to the fact that diagnosis of separate cervical quadrant (per quadrant) and subsequent final diagnosis of the whole cervix (per patient) was concluded according to the highest degree of neoplasia discovered histologically.

Comment 14.

Are the conclusions only for high-grade cytology? In high-grade cytology colposcopy is of small value. In low-grade cytology colposcopy is more important to discriminate low from high-risk lesions.

Response 14.

In this study we analyzed only high grade cytology cases. We agree that the results of the study could give broader approach. We look forward to a continuation of this work, including more patients with low and high grade cytology.

Reviewer's report 2.

Major Compulsory Revisions

Comment 1.

1. Abstract: In general: Please add the aim of the study in the Abstract.

Response 1.

The aim has been added to the Abstract.

„The aim of this study was to compare topically applied 5-aminolevulinic acid (5-ALA) based fluorescence spectroscopy (FS) in vivo with conventional colposcopy for cervical intraepithelial neoplasia (CIN) detection.“
Comment 2.

1.2 Methods: Most colleagues only read the abstract. Therefore, it is important to make clear what is meant with “abnormal cytology”. Please make this more clear in the abstract.

Response 2.

The term „abnormal cytology“ has been revised.

„The study enrolled 48 patients, referred for colposcopy because of high grade cervical cytology“.

Comment 3.

1.3 Conclusions: There is a difference of 7.2% in specificity between fluorescence spectroscopy and colposcopy (71.4% vs 78.6%), analyzing per patient diagnosis. However, the 95% CI range is 50%. It might be better to only conclude that it is a promising diagnostic tool.

Response 3.

The Conclusions has been rephrased.

„5-ALA based fluorescence spectroscopy is an objective method, requiring a short practice for appropriate fluorescence measurements. Fluorescence spectroscopy provides a potentially promising diagnostic tool with similar accuracy as colposcopy but having the potential advantage of providing objective results.“

Comment 4.

2. Background: The Background section is too long. Please focus on the specific background important for this study.

Response 4.

The Background section was revised and shortened, according to reviewer comments.
Comment 5.

3. Methods: 3.1. Exclusion criteria were inappropriate colposcopy or fluorescence spectroscopy. What were reasons for inappropriate examinations? And what is the percentage of inappropriate fluorescence spectroscopy?

Response 5.
Exclusion criteria were revised and corrected.

„Exclusion criteria were unsatisfactory or absence histopathology analysis, inappropriate colposcopy or fluorescence spectroscopy measurements.“

„Data of 18 cervical quadrants was excluded from study, due to absence of histopathological analysis (uncertain marking or absence of the quadrants after loop excision or biopsy – 8 cases, coagulation defects - 5 cases) and inappropriate fluorescence spectroscopy measurements (bleeding from the cervical tissue during the investigation - 4 cases and the device failed during the measurement -1 case).“

Comment 6.

A total of 48 patients (4x48 = 192) and a total of 174 quadrants were included in this study. Why were 18 quadrants excluded?

Response 6.
This question was explained already (please see Response 5.)

Comment 7.

4. Results: In general: The Results section is not only used for the results of this study, but for much discussion as well. For example: “Relatively…could be explained by… This suggests that…must be better.” Please rephrase this section and use subheadings for the readability.

Response 7.
The Results section was revised and corrected according to reviewers comments. We added some subheadings for more appropriate reading. Some text parts from Results section was moved to Discussion section.

Comment 8.

5. Discussion  In general: see 4. Results.
In general: The authors do not acknowledge any other published work to which they could compare their work.

Response 8.

We added to Discussion section some studies, which investigated sensitized fluorescence diagnostics in cervical neoplasia detection. Only in two studies (P. Hillemanns et al. and Sapoznikova et al.) we found fluorescence spectroscopy method was used, other authors used only fluorescence imaging.


Comment 9.

5.2. Please rephrase paragraph 3: When comparing…desired requirements”

Response 9.

The text has been corrected.
Minor Essential Revisions

Comment 10.

1. Abstract: 1.1. Results: please add percentages for sensitivity in the first paragraph.

Response 10.

Percentages have been added.

Comment 11.

1.2 Conclusions: Please use fluorescence spectroscopy instead of FS or use fluorescence spectroscopy (FS).

Response 11.

The text has been corrected.

Comment 12.

2. Background: 2.1 Paragraph 1: It would be useful to add the cervical cancer incidence rates per 100,000.

Response 12.

Incidence rates have been added.

Comment 13.

2.2 Paragraph 3: The authors should also mention colposcopy with see-and-treat management, and refer to relevant literature (e.g. Bosgraaf et al., Obstetrics and Gynecology 2013).

Response 13.

Relevant studies have been added.


Comment 14.

3. Methods: 3.1. The terms “sensitivity” and “specificity” are common sense. A definition is not necessary. Please also mention in the statistical analysis section: accuracy, PPV and NPV.

Response 14.

The text has been revised and corrected.

Comment 15.

4. Results: 4.1. Please refer to Table 1 in the Results section.

11. Spelling errors (the article needs more language correction than these few points):

- Abstract, Results: remove “a” in “a low and high risk”
- Abstract and further: overdiagnosis instead of over diagnosis
- Abstract and further: 48% instead of 48 %
- Abstract: paragr. 5: “malignant lesion” or “malignancy” instead of malignant
- Methods: remove “years” in the first line and mention first “place” and thereafter “time”.

Response 15.

All those section were corrected according to reviewers comments. Text was proofreaded and corrected by language editor.

ACKNOWLEDGEMENT

The authors wishes to thank respected reviewers for their constructive comments and references. We hope that our corrections will be sufficiently detailed and appropriate.

Sincerely yours,

Rasa Vansevičiūtė,
Jonas Venius,
Simona Letautiene,
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Ričardas Rotomskis.