Dear Mrs. Borthwick,

Thank you very much for your activities concerning our manuscript. Allow me please to inform you of the changes made in the revised version.

Comments by Reviewer Dr. Milde-Langosch

Compulsory ones:

1. Methods - concerning vaginal smears.
   Our revisions:
   a. We have added the following: "Apart from 9 smears (which included 6 samples from healthy women and 3 samples taken from cell cultures which served as controls) the rest 188 materials were paraffin-embedded histological blocks." (see paragraph 1 of the Methods).
   b. We have inserted the word "smears" in table 1 ("Normal epithelium (smears)").
   c. As to the quality of smears, we now state that "The quality of smears turned out to be satisfactory for immunochemical analysis (Fig. 1a and 1d)", - please see in the first paragraph of the Methods section. To confirm this we present additional illustrations: figure 1a is the vaginal smear of a healthy woman, figure 1d is the smear from cervical cell culture HT-3 which we used as one of positive controls (please see about controls below).

2. HPV detection by PCR.
   We present the required table data.
   All 21 squamous cell carcinoma samples were tested for high risk HPV by PCR, 5 of them repeatedly tested by Southern blotting (table 2). All 5 adenocarcinoma samples studied immunochemically were tested for high-risk HPV by means of Hybrid Capture 2 test (enabling to detect but not to discern HPV 16 and HPV 18). Regretfully not all CIN samples underwent such testing due to the tiny size of most of them. Among CINs III 19 samples were tested by PCR (table 2 5 p16INK4a-negative samples are missing out of 24 CINs III studied immunochemically). The lesser part of CINs I and II was tested: 9 out of 51 and 9 out of 32, respectively (additional file is newly given to present these results with CINs I and II).
Corresponding indices (9/51, 9/32, 19/24, 21/21, 5/5) we mention in the revised version of the manuscript (please see Methods, paragraph 4).

We believe that despite the fact that the results of this section of our study are not complete the following trend is confirmed sufficiently: high-risk HPV-positive sample may be p16INK4a-negative or may be stained poorly. Thus 4 HPV-positive CINs I turned out to be poorly stained; 5 CINs II were p16INK4a-negative or stained poorly but were HPV-positive. There were 6 CINs III (3 negative and 3 poorly stained) confirming the given thesis as well.

Minor essential revisions:

3. The term "endocervicoses" is used in Russia as an equivalent for "cervical ectopia". We have made corresponding substitutions both in table 1 and throughout the text.

4. To describe subcellular location of the positive staining (nuclear/cytoplasmic) we have added paragraphs 1 and 2 in the Results section. To stress that subcellular location may vary we also have added new figure 1 c.

We do not know any explanations of the fact that in one and the same cervical cancer sample this protein (normally showing its activity in nuclei) may be detected but in a cytoplasm in the majority of cells and in a number of cells both in nuclei and cytoplasm. As far as our paper is mostly practical we would like to restrict the discussion of the given point with "what we found" and "how to score properly". We found that subcellular location varied, we believe that so far as there is no good explanation of this fact it is worth scoring both "cytoplasmic and nuclear" and "cytoplasmic exceptionally" types.

5. As to the advantages of the antibody E6H4 it was the error of our text in the first version. It was Klaes et al [9] who had tested this antibody among several other commercial antibodies.

We agree with the Reviewer in this point. So now we use in Abstract (page 2, paragraph 1) and in Background (page 4, paragraph 2) the wording "... a highly p16-specific antibody". We also enumerate all the antibodies tested by Klaes et al in the Discussion section (paragraph 5) and use Past Perfect where necessary.

6. In Fig 1, only negative and diffuse staining is shown...

We present one more illustration with both poor (CIN I) and sporadic (cancer in situ) staining (Fig. 1 b). And would you please allow me to mention that the case presented in Fig 2a (it was Fig 1a in the first version) we scored as CIN I, focal staining ?

7. As to the last sentence of the Discussion, we have made the following alterations:

a. We have specified that the sample of uterus body was myometrium (please see table 2 and the text: Methods, 1st paragraph; Results, the last paragraph, Discussion, 1st paragraph).

b. We have presented more information concerning immunohistochemical study by Agoff et al [16] done with endometrium samples (the last paragraph of the Discussion).

8. Positive and negative controls have been described in the Methods section (see paragraphs 7-8 of this section).

9. Page 3, chapter 4: we have changed for the recommended wording "PCR has been applied...".

10. Page 3, chapter 5. We have changed for "...p16INK4a expression is reduced or ceases under such conditions or the protein function may be impaired [18]." (In the first variant of the manuscript it was "p16INK4a expression ceases under such conditions [18].").

11. We would like not to combine these tables (we had tried this variant) because the resulting table becomes rather too bulky.
We are extremely thankful to Dr. Milde-Langosch for evaluating the level of interest of our paper as being an article of importance in its field, as well as for the detailed labor-consuming benevolent review.

Trying to improve our English we have removed several misspellings ("Ukrainian" instead of "Ukranian" on page 1, "aetiological" instead of "ethiological" on page 2, etc.)

Comments by Reviewer Dr. Nozawa

Major ones:

1. We agree with this comment, so our revised wording is more reserved (please see the last sentence of Abstract): "As far as normal cervical epithelium is p16INK4a-negative and the ratio p16INK4a-positive/ p16INK4a-negative samples increases at the advanced stages application of immunohisto-/cytochemical test for p16INK4a may be regarded as a supplementary (optional) test for early diagnostics of cervical cancer.". Similar revisions are made in the end of the Background section as well as in Conclusions.

2. A rather low ratio of positive/negative samples among CINs in our study may be due to some specific features of the patients in Russia and Ukraine. We now mention this possibility in the Discussion section and refer to the study ([26]) in which this problem had been discussed. Please, see in paragraph 7 of the Discussion the following insertion: "The reasons for those discrepancies are not quite clear yet. The population of Russian and Ukrainian patients whose materials were used in the present study was extremely heterogeneous with respect to age, nationality, etc. We cannot exclude that the discrepancies mentioned may be due to these factors as had been discussed earlier [26]."

Minor ones:

1. Is it possible to leave these tables as they are? This comment is a bit against the comment by Dr. Milde-Langosch N 11( about combining tables 1 and 2) which we would not like to do also.

2. Mild dysplasia is a proper term indeed, so we made corresponding changes throughout the whole text.

3. As to endometrial cells, this comment is identical to comment 7 by Dr. Milde-Langosch. We made the required changes (please see above).

We thank very much Dr. Nozawa for the labour of reviewing our manuscript and for the critical remarks done.

With best wishes,

Yours truly,

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