Author's response to reviews

Title: PIpelle Prospective ENDOmetrial carcinoma (PIPENDO) study, pre-operative recognition of high risk endometrial carcinoma: a multicentre prospective cohort study

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Author's response to reviews: see over
Ms. Cherry Battad  
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BioMed Central  
236 Gray's Inn Road  
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Nijmegen, 16 February 2015

Dear Ms. Battad,

Thank you for considering the manuscript “PIpelle Prospective ENDOmetrial carcinoma (PIPENDO) study, pre-operative recognition of high risk endometrial carcinoma: a multicentre prospective cohort study”. We have read the comments with interest, and we have tried to adjust the manuscript accordingly.

Included you find an adjusted draft of the manuscript. We have marked out the adjustments that have been made in the revised manuscript.

We look forward to your decision on the manuscript.

On behalf of the other authors, yours sincerely,

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Our reply to the comments of the reviewers is summarized below:

Reviewer 1 (Soslow):

1. **Background:** The authors make reference to Bokhman's model that accounts for 2 types of endometrial cancer. This model is now more than 30 years old. I suggest a review of the recent TCGA report on the genomic landscape of endometrial cancer.

We do agree that the recently published data of the TCGA Research Network should be mentioned in addition to the historical classification of Bokhman. We adjusted our manuscript.

Page 5 line 114-117. “However, recently published data of The Cancer Genome Atlas (TCGA) Research Network, identified four subgroups of EC based on molecular classifiers such as TP53, PTEN and microsatellite instability [1]. This supports the need for adjusting the currently used classification.”

2. **IHC analysis:** Why have the authors chosen NOT to use p16 and the DNA mismatch repair proteins as part of their IHC panel?

We tried to be selective for the immunohistochemical markers we will use in this study. We thank you for the suggestion for the use of p16 and mismatch repair proteins. Since the aim of this study is to select a limited panel of the most accurate biomarkers that can be used in daily practice for preoperative diagnosis of EC, we will add only MLH1 as a marker for microsatellite instability (MSI). There is more frequent loss of MLH1 expression than MSH2, and loss of MLH1 expression is highly correlated with loss of PMS2 expression [2]. Loss of MLH1 expression is also associated with less aggressive clinical behaviour and longer survival [3]. Loss of p16 is correlated with high FIGO stage and serous and clear cell histological subtype [4]. We accept the addition of these markers (MLH1 and p16) and we will adapt the study protocol. We adjusted our manuscript.

Page 3 line 65; page 10 line 240; page 21 Table 1.

3. **Methodology:**

a. I don’t understand how cervical cytology and comorbidity status will be accounted for in the analysis

Based on previous findings within our research group, the presence of endometrial cells in cervical smears of postmenopausal women was significantly associated with serous histology, and in patients with endometrioid type endometrial cancer with advanced stage [5]. We will determine whether the presence of endometrial cells in preoperative cytology is still valuable when IHC is added to conventional preoperative histological classification. With respect to comorbidity, several studies demonstrated that the presence of comorbidity in EC patients significantly reduces outcome and survival, and hence is an
important confounding factor that needs to be incorporated in the analysis [6]. Our hypothesis is that incorporation of comorbidity and abnormal cervical cytology attributes to an improved risk classification. We adjusted this part in our methodology section.

Page 9 line 217-219. “Based on the present comorbidities, all patients were assigned a comorbidity score based on the Age-Adjusted Comorbidity-index as described by Charlson et al. [7], with EC being excluded from the scoring.”

Page 9 line 205-208. “To determine whether additional immunohistochemical analysis on endometrial biopsies could predict recurrence and disease free survival. Additionally, to determine whether incorporation of abnormal cervical cytology and comorbidity attributes to an improved risk classification.”

Page 10 line 259-260. “For the secondary objective we will also include abnormal cervical cytology and the Age-Adjusted Comorbidity-index as factors in univariate and multivariate analysis.”

b. There is no information about criteria for histological classification, tissue fixation and processing, performance of immunohistochemical stains and interpretation of the stains, or quality assurance. This information is absolutely critical

We do agree that criteria for histological classification are important for final interpretation of the data, and we have added our guidelines for classification and standard operating procedures for tissue processing.

Page 10 line 247-253. “Pre-operative samples will be evaluated on the amount of tissue (quantitatively and qualitatively), the presence of hyperplasia, atypia, endometrial intraepithelial carcinoma (EIC), invasive growth, background endometrium, tumour percentage and tumour type and grade. IHC staining will be performed on formalin-fixed, paraffin-embedded tissue of the pre-operative samples. IHC staining will be graded semiquantitatively by considering the percentage and intensity of the staining. A staining index will be calculated as the product of staining intensity and staining area.”

Reviewer 2 (Zeimet):

This contribution is not a classic original article with communication of scientific results but is a presentation of the study design of an ongoing trial. The most critical issue of this study is that the authors don’t take into account that the one or the other of the evaluated IHC markers are not homogenously expressed in the tumours and staining may be focal. That is especially true when endometrial biopsies obtained by Pipelle are investigated. For some included markers these concerns were raised and confirmed with regard to false negative results when tissue arrays were used instead of full sections. This issue should be more carefully considered and measures should be
included in the study design to avoid false negative evaluation for markers heterogeneously distributed in the tumour.

We do agree that due to tumour heterogeneity and focal staining patterns, IHC on endometrial biopsy may not always be representative for the whole tumour. Yet our clinical challenge is to select high risk tumours preoperatively on a limited amount of material. We added these limitations in our discussion section.

Page 13 line 329-334. “Due to tumour heterogeneity and focal staining patterns, IHC on endometrial biopsies may not always be representative for the whole tumour. Most studies on IHC in endometrial carcinomas were performed on hysterectomy specimens. Yet, our clinical challenge is to select high risk tumour preoperatively on a limited amount of material. Our study design represents daily practice and with this study we will determine whether additional IHC analysis could predict final histology.”

With regard to the included specimens authors don’t give clear insights into the real number of biopsies and curettage specimens included in the study. That should be done!

We added a table with the clinicopathological characteristics in the manuscript.

Page 12 line 310; Page 22 Table 2. “Clinicopathological characteristics are shown in Table 2.”

The possible difference in the predictive value between Pipelle biopsies and classical curettage is one critical study endpoint which is missing in my opinion.

We do agree that the predictive value between Pipelle biopsies and classical curettage is an interesting endpoint. Huang et al. reported that preoperative endometrial sampling with Pipelle and curettage show comparable sensitivity for detecting high grade ECs [8]. The predictive value between Pipelle biopsies and curettage might become different when IHC is applied, and hence influence outcome. To date there are no studies on the influence of IHC on the difference in predictive value between Pipelle and curettage. By using IHC the difference in the amount of material collected by Pipelle or curettage might be relevant. We added this in our discussion section.

Page 13 line 334-339. “Huang et al. reported comparable sensitivity for detecting high grade EC with Pipelle versus curettage [8]. The predictive value between endometrial biopsies and curettage might be different when IHC is applied, and hence influence outcome. To date, there are no studies on the influence of IHC on the difference in predictive value between biopsy and curettage. By using IHC the difference in the amount of material collected by biopsy and curettage might become relevant.”
I am not sure that the inclusion of co-morbidities into the calculations is really beneficial for the study and in the end may raise more questions than giving reliable answers.

In several studies the presence of comorbidity in patients with EC was demonstrated to significantly reduce outcome and survival and hence is an important confounding factor that needs to be incorporated in the analysis [6]. Our hypothesis is that incorporation of comorbidity attributes to an improved risk classification. We adjusted this part in our methodology section.

Page 10 line 259-260. “For the secondary objective we will also include abnormal cervical cytology and the Age-Adjusted Comorbidity-index as factors in univariate and multivariate analysis.”

In this context the issue of selection bias is the most important and the most hindering. Authors refer to literature (25 and 28) which is not very conclusive. The work of Boll et al. is based on overall survival and not on EC-specific survival. Robbins’ work was unable to reveal an independent effect of comorbidity score on EC specific survival in multivariate analysis.

Furthermore, the relation between the IHC approach of the study and comorbidities as such is not very obvious.

We do agree that this part might be confusing to the reader. The study of Robbins et al. showed only minimal impact on recurrence-free survival and disease–specific survival in patients with early-stage EC. However, comorbidity score is a significant predictor of overall survival. Furthermore, Liao et al. reported in a systematic review and meta-analysis a significant increase in the risk of EC-specific mortality among women with diabetes (pooled RR 1.32 [95%CI 1.10-1.60; p=0.003]) [9].

Page 8 line 182-183. “There is also a significant increase in the risk of EC-specific mortality among women with diabetes [9].”

Page 9 line 205-208. “To determine whether additional immunohistochemical analysis on endometrial biopsies could predict recurrence and disease free survival. Additionally, to determine whether incorporation of abnormal cervical cytology and comorbidity attributes to an improved risk classification.”

At page 7 lines 141-142: I would recommend to write that all the mentioned biomarker (not only L1CAM) are lacking validation and are based on single studies!

We adjusted the manuscript according the suggestion.

Page 7 line 159-160. “However, all the mentioned biomarkers are lacking validation on pre-operative histological samples and are based on singles studies. Further research has to validate these promising results.”
I would highly suggest that authors should at least include a Table with all the patient characteristics, as patients are already included in the trial since Dec. 2013. This could be very informative for the reader.

We added a table with the clinicopathological characteristics in the manuscript.

Page 12 line 310; Page 22 Table 2. “Clinicopathological characteristics are shown in Table 2.”

Reviewer 3 (Tanner):

The authors present a thoughtfully designed research protocol evaluating preoperative predictors of high risk endometrial carcinoma. While many of the tests are not entirely novel, a comprehensive evaluation of their utility in tandem is unique. The manuscript is well written, concise, and strongly supports the rationale behind the panel of markers they have selected. Publication of their research protocol is of value to literature as it will herald the results of their final analysis and provide improved awareness of the utility of markers they have selected.

Major Compulsory Revisions: none

Minor Essential Revisions: The authors repeatedly switch between present and past tense throughout the manuscript when referring to specimen acquisition and processing. As this manuscript represents an interim report, I would recommend keeping the acquisition as past tense and the processing/analysis as future tense. Otherwise, it is somewhat unclear about what has already occurred.

We adjusted the manuscript to improve readability and made changes with respect to the past and future parts of the study.

References


