Reviewer’s report

Title: Cervical microbiota in women with cervical intra-epithelial neoplasia, prior to and after local excisional treatment, a Norwegian cohort study.

Version: 0 Date: 30 Sep 2018

Reviewer: James Malone-Lee

Reviewer's report:

I think that this is a good paper with an important message for a developing field. Whilst I think that the data and experiment are sound the report is marred by an over-analysis which is too much for the sampling and does not add anything to the key message, which is interesting in itself without any embellishments. I think that this paper should be published but would suggest that the analysis be simplified in order to allow a better understanding of what is being reported.

Page 8 Line 26/27 "included" should be included

Page 8 Lines 24 to 34

I am not too keen of derived variables or categories, as in this case, because the involve the risk of additional error. They are dealing with biological continua that are not really suitable to categorisation and the categories are inevitably arbitrary.

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Statistical tests

I do not think that they should be using paired t-tests for the between group analyses. I doubt that the data are compliant with the parametric criteria so it would be better for them to stick to non-parametric analysis typically Kruskall Wallis for three group comparison and Mann-Whitney U test for two groups and the Wilcoxon signed-rank test for paired samples.

"We analysed if women that underwent treatment, more often acquired bacteria than lost earlier detected bacteria."

This is difficult to understand.

I think that the statistics are overdone.

There is no point in analysing quantitative differences in species before and after because they are not using reliable quantitative methods. The numbers are also too small for the statistical analysis of the dichotomies presence or absence of individual species.
Thus they are limited to analysing the non-lactobacilli species dispersion - How many different species before and after? - it is as simple as that. Then how many had lactobacilli before and after?

The trouble with using these repeated statistical tests is that they run into troubles with multiplicity and a statistical nightmare. Simplicity is a much better option.

They do not have the statistical power to analyse a subgroup (Aged <46) and should probably limit their contribution to no apparent difference in this small sample.

Results

"The bacteria composition in the LEEP group before and after LEEP as well as in the reference group are described in Supplementary Table 1"

It would make much more sense of have a table describing the species as, number of isolates and % of all isolates for each assessment and for the healthy controls. There is not need for all of the complicated sub-group analysis and categorisation it is too confusing.

The comparison before and after LEEP should stick to differences in lactobacilli isolation and non-lactobacilli dispersion (number of different species). There is not point in going on about the presence of individual microbial species because it does not have any known significance.

"We found no difference in the detection of bacterial species or the occurrence of the constructed broader groups according to HPV DNA positivity after LEEP, neither when analysing all women at six and 12 months (Table 3) nor just women positive for bacteria before treatment"

Adding in these subgroup analyses was not part of the deal and the underlined bit should go. There was no difference between the HPV DNA group - end of story

I think that the cone depth analysis goes too far into another sub group analysis:

"We found no association between the microbiota changes after LEEP and the cone depth" Say no more the subgroup analysis is not statistically sound.

"Comparisons of microbiota in women planned for treatment versus healthy references"

This section should also be simplified - There was a greater non-lactobacilli species dispersion in the patients compared to the controls. That is it and it is an important finding because it is commensurate with data from other groups reporting on UTI - Disease is associated with increased species dispersion.[1-3]

1. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the


Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
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Are the conclusions drawn adequately supported by the data shown?
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