Reviewer's report

Title: Digital imaging in the immunohistochemical evaluation of the proliferation markers Ki67, MCM2 and Geminin, in early breast cancer and their prognostic value

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Reviewer: Anna-Maria Tokes

Reviewer's report:

Comments to the Authors

This is a very interesting topic. Congratulations on your effort in assembling a cohort with long patients follow up data and performing immunohistochemistry for an array of markers.

Major Compulsory Revisions:

1. In my opinion and according to my experience the cores of 0.6 mm are really small and even if three cores were taken these hardly represent the characteristics of the primary tissues/tumours

2. Were the ER and HER2 immunohistochemistry reperformed on TMA slides or the original data were used? If the data were collected from the original files the same antibodies were applied during the analysed period or different? Please mention the antibodies used for ER and HER2 IHC.

3. The authors have used a mixed bag of cases which includes ER positive, Her2 positive and triple negative cases. First of all the Table nr. 1 it seems interesting. Probably it is not acceptable to consider the cases with not known data as a part of 100%. For example considering the expression of HER2 the authors have found the followings “Positive 26 (8.4%), Negative 129 (41.7%) and Not known 154(49.8%)”. How the authors should know that from the 154 not known cases how many are in reality HER2 positive or HER2 negative. I would strongly suggest to recalculate the data considering the percentages only in the cases with known data. According to this calculation in the case of HER2 IHC approximately 80% of the cases are going to be HER2 negative instead of 41.7%.

4. Have the authors compared for example the Ki67 expression observed on the whole, original tissues with that observed on TMAs. This should be important especially considering the relatively low expression of the Ki67 observed on TMAs. At least in some cases I would suggest to compare the expression of Ki67, MCM2 and geminin expression of whole tissues with that of TMAs.

5. How many of the cases were of LUMA subtypes known to express Ki67 at low level?

6. Analysing proliferation markers and their prognostic value is difficult given the
heterogeneity of breast carcinomas and the different treatment modalities used. As an idea it would be interesting to divide the cases in the known subtypes and analyse if there are significant differences especially in the geminin expression between different subgroups.

Minor suggestions:
The p values have to be given uniformly and it is not essential to give 5-6 decimals.

Recommendation: major revision

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests