Reviewer's report

Title: Surgery Time Interval and Molecular Subtype May Influence Ki67 Change after Core Needle Biopsy in Breast Cancer Patients

Version: 2 Date: 28 January 2015

Reviewer: Linda Sofie Lindström

Reviewer's report:

This is an interesting and potentially important manuscript on time from core biopsy to surgery in relation to tumor proliferation. However, please see my major concerns as listed below.

Major Compulsory Revisions

1. Patient cohort definition

   The patient inclusion is unclear. “Consecutive breast cancer patients with paired CNB samples and SRS.”

   a) When were the patients diagnosed and which patients had paired samples (unclear even after reading your previous article in BMC Cancer 2013)?

   b) Were the samples taken because of study enrolment including selected patients (and tumor characteristics) or was this done for most patients at that time? The tumor characteristics (Table 1) points at more aggressive breast cancer disease than in a general adjuvant breast cancer cohort. This needs to be described in detail in the Patient and Methods section.

   c) It’s unclear to the reader what analysis/ analyses were done retrospectively. If the immunohistochemical analyses for ER/PR/HER2 and Ki67 were redone by you, please clarify this along with the year(s) this was done and other relevant details (including antibodies used etc). Helpful for the reader to have all the information about the marker assessment in the same paragraph.

   d) Did time to surgery depend on initial core biopsy tumor characteristic or patient characteristics? It would be interesting to see a table with time to surgery categorized versus initial (core biopsy) tumor characteristics as well as patient characteristics (as found in Table 1).

   e) Age selection? Percentage of women below 45 or 40? Helpful with an age cutoff at 40 or 45 in the table to understand the age distribution in the patient cohort.

2. Time to surgery.

   Most patients were operated within a few days. However, a smaller proportion of the patients waited much longer (up to 37 days). It’s important to understand how the surgery interval is associated with proliferation change especially for the
patients with a longer time to surgery. Are patients with long time to surgery impacting the results the most?

a) How were the categories for the time to surgery defined?
The categories should either be chosen due to a general accepted definition (i.e. most studies categorize in this way or the categorization is biologically reasonable), or alternatively a statistical definition (generally from the distribution).

A clear explanation and reasoning behind the current categorisation of time to surgery is needed, alternatively if this is not clear my recommendation is to re-categorize the time to surgery from a statistically reasonable point of view (using the distribution of time to surgery categorizing by quartiles for example).

b) First, to understand the distribution of time to surgery and proliferation, it would be interesting to see a graph of the included patients’ tumor proliferation (as a continuous variable of Ki-67 percentage) at core biopsy and at surgery (as two different graphs) versus patients’ time to surgery (as a continuous variable), i.e. time to surgery as X and proliferation as Y.

c) Second, it would be interesting to see a graph of the included patients’ tumor proliferation change between core biopsy and surgery (as a continuous variable of Ki-67 percentage) versus patients’ time to surgery (as a continuous variable) i.e. time to surgery as X and proliferation as Y.

Given the large variability of Ki-67 it’s vital for the authors to describe the details of their assessment of Ki-67. It should be clear to the reader how the core biopsy/surgery specimen was handled for the complete process including handling (time to fixate and how the specimen was stored contrasting core biopsy and surgery specimen), antibody/ protocol used and how the pathologist scoring was done (for instance number of cells scored for the core biopsy versus the surgery specimen including whether hot spots were considered and if so what strategy was chosen). In addition, the core biopsy selection (representative regions or hot spots) is very important, especially when investigating proliferation change as done in this work, and should therefore be described.

4. Statistical methods need to be revised and clarified.
The statistical methods need to be clarified, tests (why used and in what way helpful to answer the hypothesis) and assumptions described (normality assumption etc checked) and variable inclusion reasoned for.

It’s very important for the reader to be able to follow between the Methods section, the Results section and the Tables. What finding was the result of which test and what does this mean.

For instance, the use of ANOVA/multivariate ANOVA/subgroup test need to be described together with why and what the results actually say about the data. What variables were included in the specific analyses and why?
Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

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