Reviewer's report

Title: High incidence of microsatellite instability and loss of heterozygosity in three loci in breast cancer patients receiving chemotherapy: a prospective study

Version: 1 Date: 4 April 2012

Reviewer: Katarina Bartuma

Reviewer's report:

This review focuses on the side effects of chemotherapy, including secondary tumors and breast cancer recurrences in relation to the mismatch repair pathway in breast cancer patients. While this is a relevant and unusual focus, the paper would benefit from extensive revision. This applies particularly to the need for a clear structure and development of the sections mentioned below.

Major compulsory revisions

Abstract:
1. Second line: "instability (MSI), loss of heterozygosity (LOH), and mismatch repair (MMR) and associations"

The above sentence makes it sound as if the immunostaining for MMR protein is a functional study. The sentence could be rephrased: "instability (MSI), loss of heterozygosity (LOH), loss of MMR protein expression and associations..."

Introduction
2. The introduction does not present an adequate background and framework for the proposed study. It is somewhat disjointed, and multiple statements are made with no citation to support them. The research question, as stated, is quite vague.

Materials and methods
3. MSI-low: A discussion of the meaning of MSI-low would be interesting and what the different MSI markers indicate. In general microsatellites in the text should be continuously referred to as:

Microsatellite high: MSI-H
Microsatellite low: MSI-L
Microsatellite stable: MSS

4. On what grounds were markers used for determining MSI status? The NCI panel of markers proposed has standardized the MSI determination process [1]. However, it was acknowledged that dinucleotide microsatellites, of which there are three in the NCI panel, are less sensitive and specific than mononucleotide repeats for the determination of MSI status [2].

5. Several statements lack a reference in the background section: e.g.
"Assessing changes at the genetic level in patients receiving chemotherapy is possible using several markers, and one of the most reliable choices is microsatellite markers."

6. Page 5: "In the present study, we used fragment analysis techniques on a significantly larger and unique cohort compared to earlier studies." (references are lacking)

7. Page 6: "In many cases, this regimen was followed by docetaxel." Please specify the number of cases treated with docetaxel.

8. Why was a 12 week interval chosen? There should be clarification of this point and/or a reference.

9. How many patients developed tumor recurrences, how many developed secondary tumors and what kind of tumors? Which treatment had they been on? Please specify this clearly (e.g. in a table). It is not clear whether the secondary tumors and recurrences underwent any kind of analysis. Was MSI or IHC performed on any of these tumors? Were they linked to the breast cancers? When did they recur? This section is very unclear and needs to be developed.

10. Page 7: This method section should be divided into "DNA extraction" and "LOH and MSI analysis" in two separate sections.

Results

11. The results should be discussed more specifically, especially why the MSI mutations are higher at the first analysis point (22.8%) and then lower (5.7%).

Association of changes with chemotherapy regimen

12. Why did some breast cancer patients receive lower conc of FEC regimen? Where their tumors smaller and might this have been why they developed less recurrences, please discuss this further.

13. Altogether 11 patients received other therapies than the mainstream FEC and AC. This group of patients should be excluded as it does not add any further information to the study (these groups are too small to draw any conclusions).

Discussion

14. The discussion section needs to be developed. An essential part of study is missing: limitations of the study where authors usually bring up the limitations that are related to their own study and should be considered in the context of discussing their results.

Generally

15. In conclusion, the concern of scientific accuracy is relevant and the entire manuscript should be written more carefully (scientific precision), the references used should be updated and the references should be added following above recommendations.
16. The writing of the paper is not acceptable with several misspellings and inaccurate formulations and needs to be professionally edited. Further, the word spacing is incorrect in several parts of the paper.

17. A major concern in the study is how the authors could conclude that the tumors affecting the breast cancer patients are due to chemotherapy. This following information would clarify the paper: had the patients undergone screening for Lynch syndrome/ BRCA or other hereditary syndromes linked to an elevated risk of cancer (and the majority of Lynch syndrome associated tumors will be MSI).


Level of interest: An article whose findings are important to those with closely related research interests

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

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.