Author's response to reviews

Title: Incidence of chemotherapy-induced amenorrhea associated with epirubicin, docetaxel and navelbine in younger breast cancer patients

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Author's response to reviews: see over
Dear editor:

Thank you very much for your letter dated on March 25, 2010 in which you sent us the Referees’ Reports on our manuscript. We would also like to thank the referees and editor for their valuable comments and suggestions.

The concerns raised by the referees are responded as follows.

I hope the manuscript has been improved satisfactorily and that it will be accepted for publication in your journal.

With best wishes.

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Response to Reviewers and editor

Reviewer 1 Referee Mikkel Rosendahl:

In the manuscript “Incidence of chemotherapy-induced amenorrhea associated with epirubicin, docetaxel and navelbine in younger breast cancer patients” the authors analyse retrospectively in 170 patients the self-reported incidence of cessation of menses for at least three months. The authors use this as a marker of amenorrhea. They find that the risk of amenorrhoea is associated with increased age, administration of cyclophosphamid and tamoxifen. Overall the paper is very well written in clear and concise English and has the appropriate length. Nevertheless the study seems to have been written without basic insight in ovarian physiology. There are some concerns with the setup and the conclusions:

Response: We are grateful for the positive comments on our manuscript by the reviewer and have revised our manuscript according to reviewer’s suggestion point by point. The changes in our revised manuscript were marked in red.

Major compulsory revisions and general remarks:

1. Amenorrhea and prognosis:

It is not clear whether amenorrhea itself has an impact on prognosis or whether it is a marker of higher bio-availability causing both ovarian destruction and tumour-cell destruction. See Rosendahl et al. Eur J Cancer, 2009 for references.

Response: Thank you very much for pointing these out. We agreed with the comments about the relationship between amenorrhea and prognosis raised by the reviewer and added the sentence “but it is not clear whether CIA itself has an impact on prognosis or whether it is a marker of higher bio-availability causing both ovarian destruction and tumour-cell destruction” and reference 12 [Rosendahl, Eur J Cancer 2009] in Page 8, Line 15-17 according to reviewer’s suggestion.
2. The definition of amenorrhoea:
The authors evaluate “amenorrhoea” 6 and 12 months after initiation of chemotherapy.
Six months since the initiation of chemotherapy is much too soon! Any patient receiving chemotherapy will experience irregular menses – no matter the age. This is merely evidence of chemotherapy-induced destruction of the granulosa cells in the growing follicles resulting in oligo- or amenorrhea. It is NOT evidence of destruction of the ovarian reserve. The author should acknowledge this.

Response: We are appreciated for the critical and valuable suggestions for the definition of amenorrhoea. We noticed there are four main different definitions for CIA in the literature. (1) CIA was defined as an amenorrhea arising during the first year following the beginning of chemotherapy (Vanhuysse et al, 2005. Ann Oncol) (2) CIA was defined as menses disappeared for at least 12 months from the first treatment cycle (Pérez-Fidalgo et al, 2009. Breast Cancer Res Treat); (3) CIA was defined as the cessation of menses for at least 3 consecutive months from the point of breast cancer diagnosis(Di Cosimo S, et al. 2004. Ann Oncol; Han et al, 2009. Breast Cancer Res Treat); (4) CIA was defined as the cessation of menses for 12 consecutive months after the end of chemotherapy (Fornier et al, 2005. Cancer). We chose the third definition of CIA in the previous manuscript. However, as the reviewer mentioned, most patients receiving chemotherapy will experience irregular menses and some women may experience a return of menses during the first year after chemotherapy. amenorrhea 6 months after initiation of chemotherapy may be not the evidence of destruction of the ovarian reserve. We realized the fourth definition of CIA is the best one, just as the reviewer’s suggestion. So we changed the definition of CIA as the cessation of menses for 12 consecutive months after the end of chemotherapy (Page 5, line 1 - 2). Accordingly, the data was reanalyzed, and the results and discussion were also changed (Page 7; Table 2 and 3; Page 9 line 10 – 12, 17-18 and last line). Although the main conclusion was not changed in the revised manuscript, the new definition of CIA made our data more reasonable. Thanks again for reviewer’s excellent advice.
3. Administration of tamoxifen:
Tamoxifen has various effects in different tissues. In the endometrium is an agonist, in the breast tissue an antagonist. In the ovaries, tamoxifen results in follicular stimulation and – like clomiphene citrate – can be used to stimulate the ovaries for IVF (See Oktay, Fertil Steril). So – YES – tamoxifen will cause both increase in FSH and oligo-amenorrhoea but also an increase in estradiol! These changes are physiological and simply evidence of multiple follicles resulting in anovulation. Tamoxifen is NOT responsible for destruction of primordial follicles and the interpretation – that tamoxifen causes premature ovarian failure is incorrect. Anti Müllerian Hormone can be used to evaluate the pool of resting primordial follicles in the ovaries. In a follow-up study (Rosendahl et al, Hum Reprod 2008) the authors found no difference in AMH between users and non-users of tamoxifen suggesting that only growing follicles were influenced by this. It may be responsible for the amenorrhoea but this is physiological and unrelated to the ovarian prognosis. It would be like suggesting that amenorrhoea during breastfeeding causes premature ovarian failure. The authors are suggested to include these reflections in the manuscript.

Response: We are very sorry for lack of insight in ovarian physiology. We are greatly appreciated for the reviewer’s viewpoints. We corrected our point about function of tamoxifen in amenorrhea according to reviewer’s suggestion. (Page 10, Line 9-14)

Minor essential revisions and specific comments:
1. Methods – patients: Had some patients received chemotherapy previously? This is unclear.

Response: All patients included in our study did not received chemotherapy previously, and patients receiving chemotherapy previously were excluded for this study. (Page 4, Line 13-14)

2. Treatment: What were the dose – intensity percentiles of treatment groups? Did all groups receive 100% dose? Reduced dose may affect outcome. How
was this regulated.

Response: Yes, all groups received 100% dose. To reach this dose, all the patients treated with TE regimen were G-CSF supported, and the patients treated with the other two regimens were also G-CSF supported in the case of low white blood cell count (Page 5, Line 16 - 19).

3. treatment, third-last line: “… inhibitor at least 2-year tamoxifen…” Don’t understand it.

Response: We are sorry for this mistake. Patients switched to the aromatase inhibitor after at least 2-year tamoxifen use. (Page 5, last line)

4. Treatment: Follow up time, shortest was 1 year. From initiation of chemo? What was the longest and what was mean or median follow-up?

Response: Thank you for pointing these out. We have added the data about median, shortest and longest follow-up in Page 6, Line 2-3.

5. Statistics: Why was age < 40 / > 40 used. Why a dichotomous variable not a continuous?

Response: We divided all patients into two groups: patients 40 y and younger versus patients older than 40 y. And in the tables, format of \( \leq \) was Times New Roman, and it was not very clear. We modified it in Table 2, 3 and 4.

6. Page 6, results – about age: last line: “overall, these results…” Move to discussion.

Response: We deleted the last sentence.

7. results, last lines of univariate analysis: “…for too little time…” Don’t understand.

Response: We are very sorry for this mistake. This content was already deleted in the revised manuscript since the data of 6 months after initiation of chemotherapy were deleted due to the re-definition of CIA.

**Reviewer 2 Referee Fabio Puglisi**

Response: We are grateful for the points mentioned by the reviewer and have
revised our manuscript according to reviewer’s suggestion. The changes in our revised manuscript were marked in blue.

We added the paper “Determinants of recovery from amenorrhea in premenopausal breast cancer patients receiving adjuvant chemotherapy in the taxane era” in our list of references (Page 15, Line 13 - 15) and comment about it in the discussion. (Page 9, Line 7 - 9)

EDITORIAL REQUESTS:
Thank you for editorial requests, and we added the ethical declaration (Page 4, third-last line to Page 5, first line). In addition, a number of typographical and minor word errors are corrected.