Author’s response to reviews

Title: CLINICAL AND PATHOLOGICAL FACTORS INFLUENCING SURVIVAL IN A LARGE COHORT OF TRIPLE-NEGATIVE BREAST CANCER PATIENTS

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Technical Comments:

Reviewer reports: Nirmala Bhoo-Pathy (Reviewer 1): General comments:

There are minor grammatical errors throughout the manuscript that need to be addressed.

We carefully checked the manuscript and corrected some grammatical errors.

Methods:

1. Authors need to provide information on ER and PR testing, what cut-off values were used; 1% or 10%?

Given that the study included patients diagnosed between 1994 and 2015, it is possible that some patients with ER/ PR positivity may have been included in this study as the change in recommendation for cut-off values by College of American Pathologists (CAP) took place in 2010.
In the Methods (pag. 7, lines 1-3), we specified that we used a cut-off value of 1% for the ER and PR testing and provided a relevant reference. We also indicated that, given that the study included patients diagnosed over almost 20 years in different hospital centers, all surgical specimens of TNBC patients were reviewed independently by three experienced pathologists (pag. 7, lines 7-11).

2. Was information on cause of death available? If yes, it may be worthwhile to also provide the breast cancer specific survival estimates. If information was not available, this needs to be briefly mentioned in the methods section.

Unfortunately, in our cohort electronic cause-of-death certificates were available only for a small proportion women (i.e., those dead at hospital or over most recent calendar years); therefore we could not use this information in our analyses. This was mentioned in the Methods (pag. 8, lines 12-15).

3. How was missing data treated in the multivariable analyses. Was missing indicator method used or was a complete case analysis performed?

In the Methods (pag. 9, lines 6-7), we specified that for multivariable models a complete cases analysis was performed. In the Methods (pag. 9, lines 6-7) and in the Results (pag. 12, lines 3-5), we also mentioned that we conducted a sensitivity analysis using the missing indicator method and this provided quite consistent results.

Results:

1. It may be worthwhile to separate the patients with de novo stage IV disease from the others when describing treatment patterns.

We are sorry, but we did not made any correction in this regard, since it was not clear to us why we should exclude those subjects (they also received some treatments).
2. What is the proportion of patients receiving radiotherapy? How many received post-mastectomy radiotherapy? This information should also be included in the result table.

In Table 1 and in the Results (pag. 10, lines 13-16), we specified the proportion of women who received radiotherapy and post-mastectomy radiotherapy.

3. Information on clinico-pathological features should be presented prior to adjuvant treatment.

Following the reviewer’s suggestion, we moved clinico-pathological features before adjuvant treatment. The information are now combined in Table 1, as suggested by Reviewer 2.

4. KM curves should be censored when the number of patients at risk in the smallest subgroup has reached one third of its original size.

Following the reviewer suggestion, we censored the KM curves at 5 years in Figure 1-2, and Suppl. Fig 1-2.

Discussion

1. Were the associations or lack of association between some relatively new biomarkers and OS/DFS in this study be explained by missing data?

Among the limitations of the study (pag. 16, line 10-11), we indicated that missing clinical and pathological information in some of our patients may have to some extent influenced the associations evaluated.

2. A couple of statements need to be added in the discussion, on whether the differences in ER and PR cut off values between the different calendar periods may have affected study results.

In the Discussion (pag. 16, line 15-17), we further explained that all pathology material was reviewed by three pathologists, in order to standardize the ER, PgR and HER2 immunohistochemical results for TNBC samples.
Michael O"Rorke (Reviewer 2):

The authors have conducted a retrospective cohort study spanning 21 years using clinical records and clinical pathological data from four major oncology centres in Sardinia. Just over 840 patients with triple negative breast cancer (TNBC) were included in the study and the authors report that lymph node ratio (in node positive tumours) and ki-67 may be relevant predictors of overall survival and recurrence in TNBC. The surgical specimens from these women were independently reviewed by 3 pathologists, which lessens the possibility of TNBC misclassification. Overall the study is well written, and the statistical analysis is appropriate, however I have several recommendations for the papers improvement and documentation.

Specific comments:

1. Do the author's feel that a mean follow-up of 4.3 years is long enough for outcomes of recurrence or overall survival? If so, can they reference relevant literature to support this? i.e.: paper on patterns of recurrence in TNBC such as Dent et al. Clin Cancer Res. 2007 1;13(15 Pt 1):4429-34.

The mean follow-up of our cohort was enough to study cancer recurrence and survival, since, as we now mentioned in the Introduction (pag. 5, lines 14-15) TNBCs have an increased risk of recurrence and death within 5 years since diagnosis; the suggested reference has been also quoted.

2. I've noticed that the authors are inconsistent with regards their reporting of 95% CIs i.e.: page 10 lines 34 and 41. Personally I'd prefer to read the 95% CIs around the effect estimates, as invariably in this study numbers in the analysis fluctuate depending on the clinical pathological factors being examined.

As suggested, we added the 95% CI estimates around all effect estimates presented in the text.

3. Results, page 9, line 7 - the authors report excluding approx. 27% of patients with no vital status. To assess the potential of selection bias in the remaining cohort, could the authors put a table in supplementary data of these patients' characteristics? I imagine that these patients may
also include a proportion of women with higher stage disease at diagnosis, given that they seem grossly under-represented in Table 2 (i.e.: only 10 patients with stage IV disease?)

We specified in the Results (pag. 9, line 4-5) that the 311 (27%) patients with no information on vital status had also a high proportion of missing for most other variables of interest. Therefore, we believe that a Supplementary Table with the characteristics of these patients would not be of great usefulness.

4. Why not merge Tables 1 and 2 together, why are they separate? Also could the proportion (%) of missing data be presented in italics for ease of reading. Please also correct the titles of the tables to reflect the survival analysis under study - i.e.: Tables 3 and 4 state 'mortality' and 'death' are these OS or DFS? Also the second N(%) columns presumably reflects the number and proportion of events (can this be labelled accordingly?). Also Tables 4 and supplementary Table 2 - what's the difference in these tables? Both refer to mortality, but have different numbers of cases - is this reporting on DFS or OS? Please specify.

As suggested, we merged Table 1 and 2. Moreover, we clarified in the text (pag. 9, line 1) and Tables, that HR for mortality and recurrence were estimated, while corresponding OS and DFS was considered in KM curves (pag. 9, lines 8-9). In the Tables, we specified that N refer to the number of events. For each variable in Table 1, we presented the proportions of missing data indicating them in italics.

5. Results page 10 lines 50-60, this is a very long sentence with lots of results, consider revising.

Since all data are already presented in Table 3, we shortened the sentence in the Results (pag. 11, line 9-13).

6. Results page 11, lines 14-15 seem contradictory to the conclusions being drawn in the abstract and conclusion sections. Ki-67 seems to be predictive of OS presumably in Table 3, but unrelated to recurrence outcomes (available for under 40% of the cohort) in supplementary Table 1. Also on page 11, line 43, the authors refer to '26.7% for stage I' - I think this is a typo and should read stage IV?
We revised the conclusions of the Abstract (pag. 3, line 22-24) and the text (pag. 17, lines 3-5), in order to better reflect the results regarding Ki-6.

7. In relation to statistical analysis - How were the variables for fully adjusted analysis selected? I've noticed that baseline characteristics such as lifestyle/family history and, more importantly co-morbidities, are absent from the adjusted models? Were these not statistically significant? What level of significance/threshold was used to enter covariates in the adjusted models?

We indicated in the Methods (pag. 9, lines 4-6) that the models were adjusted for study center and age at diagnosis, and additionally for clinicopathological factors found to be significantly (p<0.05) associated to mortality or recurrence in the center and age-adjusted analyses. Lifestyle factors and family history were not considered, since information on these variables were missing for many patients.