Author's response to reviews

Title: Selecting for BRCA1 testing using a combination of homogeneous selection criteria and immunohistochemical characteristics of breast cancers

Authors:

GianMaria Miolo (gmiolo@libero.it)
Vincenzo Canzonieri (vcanzonieri@cro.it)
Clelia De Giacomi (cdegiacomi@cro.it)
Lara Della Puppa (ldellapuppa@cro.it)
Riccardo Dolcetti (rdolcetti@cro.it)
Davide Lombardi (dlombardi@cro.it)
Simona Scalone (sscalone@cro.it)
Andrea Veronesi (averonesi@cro.it)
Alessandra Viel (aviel@cro.it)
Tiziana Perin (tperin@cro.it)

Version: 2 Date: 10 September 2009

Author's response to reviews: see over
Dear Editor,

We are submitting the Revision of paper 2112563705285411 entitled “Selecting for BRCA1 testing using a combination of homogeneous selection criteria and immunohistochemical characteristics of breast cancers.” by GM Miolo et al.

We think the manuscript has been prepared and revised in accordance with BMC requirements. The paper now includes 6 tables.

Here below, are our point-by-point answers to the Editor and Reviewers.

Editor
Source of funding has been introduced in the Acknowledgments section

Reviewer: Maria Worsham
Reply
No reply since the reviewer says that the article is acceptable as it is.

Reviewer: Alison P Klein
Reply
About the BRCA1, BRCA2, BRCA1UV and BRCA2 UV positive cases we have introduced, in the specific tables, some columns. The next to last one and the last one refer to the number of breast and ovarian cancer cases found inside the family with the respective ages. In the preceding three columns we reported the pre-test carrier probabilities for the BRCA1, BRCA2 and BRCA1+BRCA2 associated genes, calculated by the BRCAPRO model of risk.
We have thought that the most practical way to expose in detail the family characteristics and IHC of the BRCA1/2 negative cases was by a new table (Table 4).

Reviewer: Petra Nederlof
Reply

Abstract

In the results the number 93 patients is used, must this be 94?
The overall number of the evaluated cases is 93 and not 94 as previously written in the abstract

Abbreviations are used in the abstract without explanation.
In the abstract, to the abbreviations without explanation, we have added the explanations.

Do the authors mean: primary breast tumors from breast cancer families defined by specific eligibility criteria? If so, what criteria were used?
The eligibility criteria used for the recruitment of the cases to submit to BRCA1 and BRCA2 molecular analysis are reported in Veronesi et al 2005: “Familial breast cancer: characteristics and outcome of BRCA 1-2 positive negative and cases”, (BMC Cancer 2005 5:70). This article is quoted in the methods as bibliographical source [13]. Of the selected cases with this modality have been considered only the female index cases affected with primary breast cancer, excluding the male breast cancer and the ovarian cancer cases. In the methods we have explicitated better, as requested by the reviewer, the used criteria. In the abstract, the sentence reported is now: “The primary breast tumours from 93 FBCs defined by specific eligibility criteria, based on personal and familial tumour history, have been evaluated by Allred’s method”
Title
It is unclear what the “homogeneous selection criteria” are, this is not further specified in the text. One does not “test for BRCA1 mutations” using the criteria and IHC, one “selects” eligible patients for testing. The title is therefore not acceptable and misleading.
Of the patients ascertained through the criteria of selection reported by Veronesi et al [13], only the cases affected with primary breast cancer have been evaluated in this study. The male breast cancer (generally double positive) and ovarian cancer cases have been excluded. This is now better specified in the revised Methods. However the title has been changed into “Selecting for BRCA1 testing using a combination of…..” as suggested.
The study aims to establish on a small number of patients (93 cases), by a posteriori analysis, how many BRCA1 cases we would have lost tightening the BRCA1 molecular analysis to ER- and/or PR-cases only. Also important is to appraise the number of cases that we would not have been submitted to BRCA1 molecular analysis.

Method
The Allred method should be described in more detail. How is the scoring done?
The Allred method has been described more in detail [18].
In tables 1-3 values are given without any explanation. What does ER score – (2) mean? And +(6)?
In Methods it has been specified that the nuclear staining graded from 0 to 8. The sum between the proportion score (PS) e intensity score (IS) results in a total score that if ≥ 3 is reported as positive. This cut-off has also been reported under the tables.
What does HER2 score – mean? Negative (IHC 0) of “not done”.
In Methods and tables it is specified that a score index of 0, 1, 2 and 3 corresponds respectively to negative, weak, moderate and strong staining intensity.
Which methods/antibodies were used for the ER and PR staining?
In the revised text, it has been reported that ER and PR were determined by IHC using the 6F11 antibody (Ventana) and the 1E2 antibody (Ventana), respectively.

Results
In “Results” we have change the sentence: “Overall, excluding the cancers characterized by double hormonal positive receptorial status, the mutation screening could be performed only in 29 patients” using the one suggested by the reviewer. “Overall, from the 93 cases, 29 tumors did not show a positive staining for both hormone receptors ER and PR; of which 10 showed a BRCA1 mutation.
The IHC score of the mutations or UV carriers are given, but not the results for the other cases. They should be added.
The IHC score and additional information of the BRCA1/2 negative cases has been shown in the new table 4.
According to the tables all BRCA1 mutations found were ER negative. Could use of that criterion not be enough? Why use ER- and/or PR-? Since the results for the remaining tumors are not given, it is unclear what the effect of one marker (ER) would be.
Overall, the ER positive and PR negative cases were 11, of which 2 had BRCA1 UV and 9 were wild type. Excluding these 11 cases the sensibility, specificity, PPV and PNV were 100%, 90%, 55% and 100% respectively. Then to consider only the ER negativity could already be a good IHC criterion considering the good correlation between ER and PR.
Nevertheless in this study we submitted to BRCA1 analysis also the ER-/PR+ cancers, because we wanted to miss the smallest possible number of BRCA1 positive cases. You consider that of these 11 cases, 7 had a negative or weak HER2 expression more compatible with mutation BRCA1.

Table 4/5 are “other cases” all non-BRCA1 mutation carriers?
Yes, we have change “other cases” with non-BRCA1 mutation carriers as recommended by the reviewer. Please, note that after introduction of a new table 4, previous Table 4 and 5 are now Table 5 and 6, respectively.

Conclusion
BRCA1 immunohistochemistry, a rapid and easy performing test…… This suggests the use of an antibody against the BRCA1 protein…. An easy to perform test???
The sentence can be equivocal indeed. We decided to modify it this way: The IHC analysis by Allred’s method, a rapid and easy performing test…..

About 90% of BRCA1 related cancers showed ER negative….. In fact all 10 BRCA1 cases showed ER- staining. Or do the authors discuss the literature?
Yes, since we discuss the literature, we have introduced the bibliographic references.

… with the possible limitation of loosing one rue BRCA1….. The authors mean “miss” But more importantly, there may be a way around this problem in many families. If there are more family members with breast cancer, one could select the case with an ER negative breast tumor. It is very unlikely that all tumors in a BRCA1 mutated family are ER positive.
This remark is absolutely true, but we should not to forget that very often the analysis of the pedigree could be not complete, because a lot of informations can be lost. You should consider that for this article we have had to tighten the analysis only to the cases operated in our center (93 cases), despite the number of the tested subjects was much more greater (800 cases). This because:
1) the Allred test has been applied beginning from 2002
2) the determination of the receptors in the other centers is performed with others methods (fm, etc) and therefore not comparable.

Finally, the dataset is rather small. Given that we already know that BRCA1 tumors are in general ER negative (~90%), one would like to test many more cases (several hundreds) to really get a feel for what one would miss and what the advantage of this approach would be.
Yes, in fact in the conclusions it is specified that the analysis of a larger series of cases is however required to validate the proposed selection approach.