Reviewer's report

Title: Clinical Significance of Survivin Expression in Ductal Carcinoma in situ with Microinvasion of the Breast

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Reviewer: FS Al-Joudi

Reviewer's report:

Page 3.

Title of article:
"Clinical Significance of Survivin Expression in Ductal Carcinoma in situ with Microinvasion of the breast"

Title of article: it does not cover the real work performed, which includes many other parameters and findings, other than survivin, like necrosis, and many biological parameters.

Abstract.

Background. This does not include the basic information required to justify the objective of the work, like what survivin and other biologic factor are, and how they have been related to the intended work. The current abstract is merely a display of the usefulness of mammography and a definition of DCIS and DCIS-mi. The issue is NOT mammography. Instead, the actual issue is INVASION, regardless of how it had been detected (whether this invasion had been detected by mammography, by histopathology). It may have been a good idea to include in this background, brief notes on survivin and the other factors.

Methods. It may have been necessary to define the biologic factors intended to be tested.

Results.

Abbreviations have been used: this is usually NOT advisable on first mention. The word Survivin (here and throughout the text) has been written with a (capital S) which is not necessary (unless it if it came in beginning of sentence). In the last line: independent factors affecting invasion: it may be more precise to say (factors associated with invasion) since there is not definite proof for an interplay between the invasion and these factors.

Introduction.

Page 5.

Lines 3 (starting from "Since the introduction of mammography") until line 11 (ending with "the lesion will still be defined as T1 mic") need to be
removed since they are not required: the issue is not to assess diagnostic imaging methods and the history of those.

As explained earlier in comments on abstract, there must have been a display of the relationship between the invasion and the expression of the factors (survivin and others). This display must include the previous reported findings in breast cancer which are available: they try to disclose some relationships between invasiveness and expression, and also prognosis. In addition, a brief explanation of the molecular mechanisms associated with the invasion may also be necessary: all the above is to justify the work performed in this article.

Lines 18-20: â##there is some degree of subjectivityâ#|..until â##with a higher number being worseâ##: this information can be partly used to justify why the work is being performed, so a proper flow of information (which is obviously lacking) is required into the study of biological factors in question.

Lines 21 and 22. . Starting with â##Nevertheless, according to someâ#|.. until Van Buys classification (12)(13): this piece of information can be cancelled or alternatively, transiently mentioned with the preceding lines, especially that no attempt has been made in this work to correlate any findings with any therapeutic protocols or prognostic outcomes.

Page 6.

End of line 4. the â##endometase/matrilysin mention seems to be â##Not very relevantâ##.

Paragraph 2: lines 11-14: the information on survivin expression would be better transferred to the upper paragraph. In this paragraph, the mere mention of survivin (which has already been done in line 10), is enough.

Materials and methods.

Page 7.

Patients. In line 4, it says that informed consents were obtained from patients before surgery. However, many of these tissue samples must have been tissue blocks or formalin-fixed (as some go back to the 1990s): this needs to be re-phrased or â##re-explained.

Line 13: type collagen should be type IV collagenase.

Imunohistochemistry.

- Line 21: pH buffer 6 or 9: it may be necessary to specify, at this stage, where each of these buffers has been used (in which assays). References may also be necessary. In our experience, and regarding survivin in particular, antigen retrieval pH 9 was used for the first time and possibly he only time as in the literature is in the work of â##Al-Joudi et al., Singapore Med J, 2007 ; 48 (7): 607-614â##.
Line 4.: skim milk: it may be better to say :skimmed milkâ##.
Also, it is necessary to specify the concentration of the skimmed milk solution used.
It is also necessary to provide reference (s) for all the work described in the paragraph.

Lines 4 and 5: The word â##Nederlandsâ## (need to be converted into â##Netherlandsâ##).

Line 5-15: the information in this paragraph require a reference (s).

Line 17: the description of temperature is to be corrected into : 40C instead of a small square appearing (here and elsewhere in the text).

Last paragraph: lines 23: antibody recognizing 1-142 amino acids of human Survivin protein: suggest changing it to: antibody raised against the whole length of the human survivin molecule. However, this whole sentence is not very necessary.

Page 9.
Apoptosis. References for the TUNEL method are required.

Evaluation of apoptosis and immunohistochemistry.
It may be better to trim this into â##immunohistochemistryâ## only, and take the first 4 lines starting from â##apoptosis was detected â## until â## defined as the apoptotic indexâ## to the previous section of apoptosis.

The â##gradingâ## or better be â##scoringâ## was assessed by either by number of cells expressing the factor, or by the intensity: the two factors are required in most of the times as explained in previous reports: example for survivin:
- Tanaka et al., Clin Cancer Res, 2000 ; 6 : 127-134.
- Lu et al., Cancer Res, 1998 ; 58 : 1808-12.

Essentially, the scoring considered both, the cellular distribution in tissues, as well the intensity.

We may ask the authors at this stage whether the immunohistochemistry was red microscopically by 2 independent persons, since that may be subjective.

Last two lines: â##Cytoplasmic staining of more â#|. Till Bax: there is a pre-emptive assumption that survivin expression is utterly cytoplasmic: since this cannot be predicted in advance, then it may be better to remove the word â##cytoplasmicâ##, at this stage (until the results show otherwise).

Page 10: line 1: â##we examined â## had better be changed into: â##the ontra-ductal components for both DCIâ##|..for expressionâ##|â##|..â##.
Lines 2-5: this information does not belong here: it may be better to display in the Discussion of the results.

Statistical Method. (Should be Statistical Methods)
We examined \#. Better be: The statistical tests were utilized to examine the differences \#\#.

Last line: statistical analysis was done\#\# better be changed into\## statistical analyses were performed\#\#..

Page 11. Results
Line 1. The median age was 56 years old (remove the word \##old\##).

Patients characteristics: there was no mention in the Methodologies, as to how the information in this section was collected: some were \##current cases\## and the majority had been old cases for which information had been obtained from their records\#: this may be an extension of the previous section on how the samples were obtained, and may be transferred to the section of patients characteristics in \##Materials and Methods\##.

Histological evaluation.
Line 1-2: the names of who performed what in this work is mentioned later in the Authors\## contribution: it is not necessary here.

Lines 2-4: these must go to the Materials and Methods section.

Page 12.
Line 8: more frequently better be \##more frequent\##.

Line 10: levels of expression of p53, p21 and \##: better refer to (Table 4).

Univariate and multivariate: line 1: Univaraite analyses (not analysis).

Discussion.

Page 13.
Lines 1-2. \## It is very interesting\##\## may be changed to: \##The investigation of the biological features of \##\## may yield some explanations to the progression from \##\##

Line 2: The Invasive focus may be changed to \##invasive tissue\## unless there is general agreement among pathologists to use the word focus.

Line 3: the word \##impossible\## better be \##not possible\## or \##difficult\##.

Lines 7 -23 (end of page): this whole paragraph is a repetition of the \##Results\##: all this is not necessary and can be deleted.
Page 14.
Paragraph 1 (lines 3 to 7): a few references are required to support the information, especially that stating that survivin is related to cell proliferation/differentiation. To date, and to the best of my knowledge, no relationship between survivin and differentiation has been reported.

Paragraph 2: line 8: Previous papers better be changed to "previous reports.
Line 11: However, there has been. Better be changed into the pleural form as such: there have been reports.

Line 15-16: this states that other antibodies against other domains of the survivin molecule be used. The question I raise here is: what other domains are there? The sequence of 142 amino acids represents all the human survivin molecule, meaning that the monoclonal antibodies (or polyclonal antibodies) used have been raised against all he domains available.

Hence, other reasons for the lack of nuclear expression must be sought: these may be related to technical aspects, or to the nature of the proliferating cells at this stage of the cancer.

Line 21: On the other hand: there has been no "previous hand" so better remove that and replace it with "some word like furthermore.

Page 15.
Lines 3-4: the conclusion made here on the role of Bax and Bcl-2 in apoptosis is rather wild: it has not been supported by an explanation especially of the possible molecular activities.

Lines 6-12: the information lack the presence of supporting references.

Line 18: On the other hand better be: "In addition."

Line 19: expression of an apoptotic factor; Expression of the apoptotic factor.

Lines 20-21: the balance is maintained during the progression. Better become: "balance is maintained in DCIS-Mi" because at the stage of detection, the invasion has already taken place, and because it has not been shown or mentioned throughout this work, that both tissues from one patient have been detected together (in one section hat contained both the original DCIS and the DCIS-Mi focus). Furthermore, the balance that the authors mention here can be related, and goes in line with the balance mentioned in the lines 3-4 above, and can be discussed within the same context.

Page 16.
Line 3: survivin is considered. May be changed to: "survivin may prove to be a useful Marker to indicate a difference between DCIS."
Lines 5-6: on the use of adjuvant therapy: it is not clear how this statement can be made, since that has not been displayed in this study. Besides, there were virtually no deaths or cases of poor prognosis, at the time when the therapeutic protocols used were not disclosed. However, this can be discussed in the light of previous reports on the possible role of adjuvant therapies and chemotherapies such as:

-Tamm et al., Cancer Res; 1998; 58 (23): 5315-5320.

Conclusions: this section contains repeated material and can be cancelled, unless it is part of the journal format.

References:
No specific remarks or mistakes.

General conclusions of the reviewer (according to BMC Cancer format):

1- The question posed by the authors is sound, but has not been defined properly. The issue tackled in this work is rather important to the understanding of the underlying mechanisms (or associated mechanisms) of the disease, plus, as the authors state, may have clinical usefulness. The topic has been tackled in one previous report by Barnes et al. (Ref 23). Generally, the Introduction section is required to be extensively revised (or re-written).

2- The methods used are generally appropriate: some remarks have been indicated.

3- The data looks sound. It is properly shown, with representative pictures.

4- Data reporting: appropriate.

5- Discussions and conclusions: these are weakly written: they require major re-writing.

6- Work limitations: the only limitation mentioned is the lack of nuclear staining of survivin, and that has not been discussed properly.

7- Work acknowledgements: there is heavy lack of references which has been highlighted in the text above.

8- The title and abstract require major alterations which have been stated in the text above.

9- The writing is generally weak: the topics covered are not quite relevant to those of the work, namely the biological factors.

Concluding suggestions:

The article cannot be accepted in its present form.

The topic is important, yet I am not sure that, even after re-writing, the article will be raised to the standard of a journal of impact factor of 3.5.
Quality of written English: generally it is good: minor corrections have been suggested.

However, this statement does not abolish the previous remarks on the quality of science displayed.