Reviewer’s report

Title: SISH/CISH or qPCR as alternative techniques to FISH for determination of HER2 amplification status on breast tumors core needle biopsies: A multicenter experience based on 840 cases

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Reviewer: Jane Starczynski

Reviewer’s report:

The determination of HER2 status has focussed on the use of IHC and FISH, to look at protein and gene status respectively. It is recognised that no method is perfect for determining HER2 status or predicting response to therapy. In recent years several alternative approaches have gained acceptance including brightfield methods for evaluation gene status and molecular based approaches including MLPA and PCR. The authors have explored several of these approaches and developed and validated a PCR based method for determining the HER2 status. In parallel they have done a medico-economic study, not presented here. The study centre went through a training period to validate the methodology and the subsequent results show excellent concordance with FISH and their PCR may prove to be a viable alternative.

The study is well thought out and well presented and suitable for publication; however there are a few small areas which need further clarification or could be expanded further. These are not requirements for publication however are worthy of note. These will be addressed first and then corrections and comments that do need remedial action will be listed.

Discretionary revisions

Question 1: It is not entirely clear how the patients were selected, other than based on an expected FISH positivity. The fewest number of patients in the study were in what is probably the most difficult category, the clinically equivocal cases i.e. the HER2 2+ cases, these are the cases that require further investigation by alternate methods and can be challenging. The study may have benefitted from having more cases in this group. This is reflected in sub group analysis where there was a broad range in interpretation amongst the study group. Are the authors planning to look more closely at patients who fall within this sub group?

Question 2: The authors have used a novel PCR technique for evaluating the HER2 status, taking both copy number and HER2/Chromosome 17 ratio into consideration. The determination of chromosome 17 number is based on the average of 5 genes on 17q, alternative FISH approaches have used probes on both arms of 17. In view of the complexity of losses and gains that can be seen on 17 is there any reason for just selecting targets on the q arm? It maybe that this is addressed in the paper by Spyratos F, submitted for publication? This is particularly relevant for some of the more unusual patterns of loss and gain that can be seen, including co-amplification and apparent monosomy.
Question 3: The CISH data adds little to this paper. There are less than half of the cases than in the other methods presented, this could skew the statistics. The authors go on to say that there is a move away from this technique.

Required Actions

P4: Please reference the statement “Therapeutic response to trastuzumab was observed exclusively in patients harbouring HER2 gene amplification”

P4: “…and is also expensive”. Is FISH any more expensive on a cost per test basis that the brightfield alternatives? This statement is equally applicable to both.

P5: The authors talk about SISH, this methodology has evolved into several versions please clarify that all centres were using the two slide method with separate HER2 and CEP 17 and not one of the dual colour versions such as DDISH.

P5: Please review the statement on ASCO/CAP guidelines. The authors state 1.8-2.2 borderline amplification, this is not what ASCO/CAP calls this equivocal, however with patients >2.0 eligible for trials.

P6: Standardize more appropriate word than homogenize.

P9: variable expertise rather than heterogenous, a little misleading when talking about HER2.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare I have no competing interests.