Reviewer’s report

Title: Estrogen receptor alpha (ERα) mRNA copy numbers in immunohistochemically positive-, and negative breast cancer tissues

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Reviewer: Frédérique Spyratos

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General
First, the authors propose QRT-PCR as an alternative to IHC to measure ERα expression in breast cancer biopsies originated from stereotaxic or ultrasound guided methods. For this purpose, they compare QRT-PCR to IHC and older biochemical methods results obtained in surgical samples and not in biopsies. This is not sufficient to validate ER determination by QPCR in biopsies for several reasons: the histological control of the sample analyzed by QPCR is of major importance to ensure it is representative of the lesion and to evaluate the percentage of cancer cells versus normal and stromal cells; this is not mentioned in the paper. Moreover, the sampling method is also of major importance for small biopsies (immediate freezing of the biopsy implying a close coordination between pathologist, radiologist, biologists..., training in the histological control of the frozen biopsy, procedure of qualification of the sample checking quality and quantity of extracted RNA). The authors underestimate all these difficulties and do not mention any histological control of the biopsies. Last comment: IHC can be performed and is performed in small biopsies in a number of institutions to analyze routinely and correctly ERα expression.

Second: the authors compare QPCR to IHC and binding assay, considering that the two last methods correlate perfectly, which is not the case. Discordant results (5 to 18% according to the categories) are not discussed, only false-positivity or negativity is mentioned on page 10, without discussing characteristics of the tumor (histology, % of tumor cells, menopausal status of the patient...) which could explain the observed discrepancies. Moreover, the 21 cases without quantitative assessment of IHC should have been eliminated.

Third: the authors used GAPDH as unique reference gene for QPCR assays while several studies have showed that GAPDH expression was associated with breast cancer cell proliferation and with the aggressiveness of tumours demonstrating that GAPDH gene expression should not be used as a control in breast cancer. (see for example Revillion F et al Glyceraldehyde-3-phosphate dehydrogenase gene expression in human breast cancer. Eur J Cancer. 2000 May;36(8):1038-42). More recently, GAPDH was shown to be down-regulated by chemotherapeutic drugs and bisphosphonates.

QRT-PCR is indeed a cost-effective, quantitative, sensitive and high throughput method but the presented data have not a sufficient level of evidence to sustain the conclusions of the paper and propose the replacement of IHC by QRT-PCR to measure ER expression in breast cancer. It is a pity, because this could probably be demonstrated with much higher standards and to end this paper could be harmful to the clinical implementation of QRT-PCR in that field.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

What next?: Reject because too small an advance to publish
Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests