

Letter to the Editor

Multiplex ligation-dependent probe amplification to detect HER2 amplification in breast cancer: New insights in optimal cut-off value *

Dear Sir,

In Volume 31 of *Cellular Oncology* (2009), we published an article titled “HER2-*neu* amplification in breast cancer by multiplex ligation-dependent probe amplification in comparison with immunohistochemistry and *in situ* hybridization” [3]. To analyze our multiplex ligation-dependent probe amplification (MLPA) data we used a cut-off value of 1.5 to discriminate between HER2 non-amplified and low-level amplified patients. This cut-off was at that time empirically established in our lab during routine diagnostic application of MLPA kits for trisomy detection. However, based on recently published data [1,2,4], we now believe that a cut-off value of 1.3 (delta value 0.3) instead of 1.5 is better validated and more closely reflects the amplification status. We therefore re-analyzed our data with 1.3 as a cut-off value.

HER2 amplification status by MLPA was normal in 82% of cases, low level amplified in 7% and high level amplified, as before, in 11% of cases. Of all immunohistochemistry (IHC) negative cases, 95% were MLPA normal, and in the group of IHC 1+ cases, 88% were MLPA normal. In these IHC 0 and 1+ cases, 4% and 10% were MLPA low level amplified, respectively. In the IHC 3+ group there was no change in the percentage of MLPA normal and low-level amplified cases. In the IHC 2+ group discrepancies with MLPA were, as expected, most pronounced: 59% was not amplified, 22% low level amplified and 19% amplified. Overall, there was 87.5% agreement between both techniques, which is slightly lower than with the former 1.5 cut-off value (90%).

Correlation of MLPA with fluorescence *in situ* hybridization (FISH, selected cases) and chromogenic *in situ* hybridization (CISH, consecutive cases) was 73% and 91%, respectively, with corresponding Spearman correlation coefficients of 0.78 and 0.83. None of the

MLPA normal cases was amplified by FISH and 1/248 by CISH. MLPA low level amplified cases were high level amplified by FISH and CISH in 29% and 12% of cases, and low level amplified in 12% and 27% of cases, respectively.

With the new cut-off value (1.3 instead of 1.5), using CISH as gold standard and considering CISH and MLPA low level amplified tumors as amplified, sensitivity of MLPA increased from 90% to 98%, specificity dropped from 97% to 92%, positive predictive value (PPV) dropped from 84% to 70% and negative predictive value (NPV) increased from 98% to 99.6%. When CISH and MLPA low level amplified tumors were considered not amplified, sensitivity, specificity, PPV and NPV remained 90%, 99%, 90% and 99%, respectively. So, using the new cut-off value increases sensitivity and NPV of MLPA but decreases the specificity and PPV, given that all low level amplifications detected by MLPA are considered amplified or, even better, are re-analysed by CISH or FISH.

In conclusion, MLPA is a reliable and cheap high throughput method to detect breast cancer HER2 amplification in small quantities of DNA isolated from paraffin embedded material, and thereby a good alternative for FISH or CISH. Lowering the cut-off value for low level amplification to 1.3 increases the sensitivity of MLPA to detect HER2 amplification in breast cancer, indicating that this new experimentally validated cut-off value indeed improves the value of MLPA as a diagnostic test.

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