Ck 19 as a Predictor for Micrometastasis in Breast Cancer: Study Done on Non-Metastatic Egyptian Female Breast Cancer Patients

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Background

The standard method to detect disseminated epithelial cells (DEC) in bone marrow(BM) is immunocytochemistry (ICC).Several studies demonstrated that the presence of ICC stained cytokeratin 19(CK19) positive cells in BM is associated with a poor prognosis (***Diel et al ,1996; 2000; Singletary et al, 2002;Wiedswang et al ,2003***).The value of this cytological method is limited by its low sensitivity, and is highly dependent on experience of the observer. Since the introduction of molecular based techniques, more sensitive quantitative methods have been developed based on polymerase chain reaction(PCR) methodology. The most commonly used molecular method for the detection of DEC relies on the screening for tumour associated and/or organ specific mRNA expression in cancer cells and on the absence of these gene products in the cells of the host tissue such as BM .The identification of an appropriate target gene is one of the most critical steps in the reverse transcriptase (RT) PCR approach to quantify DEC. *Cytokeratins are widely evaluated as targets for the detection of DEC ,and can be used for the prognostic /predictive evaluation of DEC.*

Aim

To assess the efficacy of CK 19 as indicator for micrometastasis or predictor for relapse in non-metastatic breast cancer patients.

Patients and methods

CK 19 has been estimated in breast cancer patients using both ICC on BM biopsy compared to quantitative real -time PCR (QR –PCR) using *Taqman* fluorescent probes on *ABI prism 7700* instrument (Applied Biosystems).Bone marrow samples were taken from 13 non-metastatic breast cancer female patients with average age of 52.5 yrs(40-65y)and 4 controls matching in age and sex. For exact quantification of gene expression (CK 19) , an endogenous reference (housekeeping gene GAPDH) was used to correct for differences in the amount of total RNA added to the reaction ,for compensation of different RT efficiencies and for compensation of PCR inhibitors in the sample. We used two different calculation methods to quantify our results ; the ΔΔCt method and standard curve using 4 serial dilutions of Raji RNA extracted from a human B cell lymphoma line. Our results were compared to those of ICC using CK-19 antibodies (Ventana no.760-4281 /automated system) on BM biopsy specimens.

Results

CK 19 m RNA could be quantified by QT-PCR in all 4 control samples ,with a mean ratio of 0.35 (considered as cutoff value) using standard curve method. Validation of ΔΔ Ct method was done and proved to be comparable with standard curve results. Eleven out of 13 patients proved to be positive above cutoff value. Two patients(15.3%) had very low CK l9 expression with values below cutoff. Using ICC these 2 patients had borderline results ;and with further follow-up clinically ,no bone or organ metastasis was detected. Another 3 patients (23.1%) out of the 11 were proved to be positive by ICC(intensity of positivity was low) while having high expression of CK19 by PCR. Two of these 3 patients had metastasis in the form of liver deposits.

Conclusion

Estimation of CK 19 by molecular method (QT-PCR) proved to be superior to ICC in the prediction of possible relapse or metastasis in female breast cancer. Further studies with larger number of patients could clarify the prognostic significance of CK 19 in non-metastatic breast cancer patients .Inspite of limited number of patients in our study ,we do recommend to add CK19 QR-PCR to the laboratory tests battery for female breast cancer.