ABSTRACT

Introduction: The presence of circulating tumor cells (CTC) in the peripheral blood has been associated with worse prognosis and early relapse in breast cancer patients. CTC determination in the peripheral blood has been considered as a liquid biopsy. The aim of this project was to analyze the presence of CTC followed by their molecular characterization with the potential use not only as a new biomarker for real-time monitoring of therapy efficacy but also as a suitable tool for patient's stratification and individualization of treatment for breast cancer.

Methods: A total of 54 patients with diagnosed early breast cancer were enrolled into a prospective study. Ten millilitres of peripheral blood were sequentially collected to test for the presence and characterization of CTC during the follow-up of patients. CTC isolation and detection was performed by AdnaTest BreastCancer™ (AdnaGen AG, Germany), which is based on the detection of EpCAM, HER2 and MUC1 specific transcripts in enriched CTC-lysates. cDNA from isolated CTC has been further used for newly optimized qPCR assays for breast tumor and therapy resistance associated genes: TOP1, TOP2A, CSTD, ST6GAL, KRT19 and reference gene actin. qPCR results have been analyzed by Genex software (MultiD Analysis).

Results: 195 blood samples have been analyzed in total. We detected CTC before therapy initiation in 31 % of patients. Only 10 % of patients remained CTC positive after the completion of therapy. We have successfully designed and optimized qPCR assays for genes TOP1, TOP2A, CSTD, ST6GAL, KRT19 and reference gene actin. Our data indicated noticeable heterogeneity of expression profile of detected CTC for all studied genes. The most expressed gene in CTC was MUC1 (70%), HER2 (52%) and EpCAM (35%). For others genes the detection rate was lower than 20%. HER2 status comparison between the primary tumor and CTC shown mismatch in 20% of patients. We found no significant correlation between CTC and individual clinico-pathologic characteristic of the primary tumor.

Conclusions: We have confirmed practicability of our methodological approaches for the detection and characterization of CTC in the peripheral blood of breast cancer patients. Information based on the CTC presence and expression profiles could provide clinicians additional support for therapy management.

Key words: circulating tumor cells; breast cancer; quantitative; polymerase chain reaction; immunomagnetic separation; gene expression; characterization; detection.