Abstract

With a continuous development of molecular-biology methods more attention has been paid to molecular detection of minimal residual diseases in solid tumors. In our study we focused on detection of MRD in neuroblastoma. Neuroblastoma is one of the *peripheral* neuroblastic *tumors* (pNTs) that accounts approximately for*10 percent of all* childhood cancers. The question raised however not answered until this day is whether evidence of MRD in bone marrow may be used as independent prognostic factor in diagnosis of neuroblastoma. Furthermore, it is important to establish what kind of testing technique should be used and what values to look at. There exist various methodologies in detection of MRD evidence in neuroblastoma. These methods differ in cost and complexity, but mainly some of them are more specific and sensitive than the other. Cancer cells may be detected in the blood as well as in the bone marrow. Very often it is the bone marrow that is affected by the metastasis in neuroblastoma, therefore 85% of all high risk neuroblastomas show positive results in the standard cytomorphology tests of bone marrow. Low numbers of cancer cells in bone marrow or peripheral blood (especially during or after the end of treatment) are below the standard values of detection limit in most of the classic methodologies used: cytomorphology testing, imaging techniques (MRI, CT, UZ), isothopic exchange methods (MIBG). Because of this reason we chose more sensitive techniques in our study: real-time reverse transcription polymerase chain reaction, flow cytometry and imunocytochemistry. Then these methods were compared with a cytomorphological examination demonstrating 1 tumor cell between 200 bone marow cells.

Our study is aimed retrospectively in prospective examinations of samples from patients with neuroblastoma. The study included 46 patients from whom 132 bone marrow samples were obtained.

We used retrospective prospective study design to asses bone marrow in patients diagnosed with neuroblastoma. The samples were taken from two locations and several samples were assessedat different timepoints of patient's treatment. We compared the results between each others and the amount of gene expression at by using QRT-PCR. In our case we wanted to prove the evidnece of expression of three mRNA genes *TH*, *PHOX2B* and *DCX* which are normally expressed in neuroblastoma, but are not expressed by bone marrow. We expected similiar

senisitivity that is known by RT/PCR also to be found in imunocytochemistry. We proved that antigen GD2 was present on the cells of neuroblastoma however was not present in bone marrow cells.

The sensitivity of diagnosis by flow cytometry depended on the technique which was being used and also on the number of diagnostic characters in combination. In our study we used combination of $CD45^{-}/CD81^{+}/CD56^{+}$. In this procedure we are able to achieve a tumor cell sensitivity of 1 to 100-1000 bone marow cells. The sensitivity and specificity these investigations as already validated in *in vitro* models. We compared these examinations with cytomorpholoical examination. In our study we have shown that QRT-PCR and immunocytochemistry are more sensitive techniques for MRD detection than flow cytometry and cytomorphology.

The results could be helpful in establishment of suitable method and in understanding and in understandinh diagnostic character of MRD in neuroblastoma. On the basis of our results we tried to establish the diagnostis scheme for diagnosis of MDR in neuroblastoma.

Key words: neuroblastoma, QRT-PCR, flow cytometry, imunocytochemistry, morfology, bone marow, peripheral blood