University of Medicine and Pharmacy Târgu Mureş
Doctoral School

Thesis summary:
The importance of molecular biology testing (PCR) in management of patients with chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is a clonal disease of the hematopoietic stem cell manifested through the expansion of one or more myeloid cell lineages. It is the first human tumor associated with a genetic anomaly. The key event in the development of the disease is the reciprocal translocation between chromosomes 9 and 22 t(9;22)(q34;q11), which leads to placement of the ABL gene from chromosome 9 near the BCR gene on chromosome 22, resulting in the **BCR-ABL fusion gene;** the latter has different variants and subtypes depending on the breaking points of the two genes; the most frequently encountered variant is the major variant (M-BCR-ABL), occurring in 90% to 95% of CML cases. This hybrid gene encodes a constitutively active **tyrosine-kinas**e responsible for uncontrolled proliferation, decreased apoptosis, impaired adhesion and genomic instability of myeloid cells.

Following clarification of these phenomena, the management of CML changed significantly: the diagnosis is based on detection of the genetic anomalies, gold standard treatment consists of tyrosine kinase inhibitors targeting the molecular substrate of the disease, and therapeutic efficacy is monitored by cytogenetic and molecular biology (PCR) testing.

The thesis consists of three studies.

The major objective of the *first study* was regular monitoring of the M-BCR-ABL expression levels in CML patients using real time quantitative PCR (RQ-PCR) performed every six months to monitor efficacy of the administered treatment (2 patients underwent allogeneic hematopoietic stem cell transplantation, 23 patients received drug treatment).

In the *second study* we identified the variants and subtypes of the BCR-ABL fusion gene using single step PCR, and we investigated their influence on the clinical and paraclinical phenotype and the course of the disease.

In the *third study* we measured concomitantly the expression levels of M-BCR-ABL and WT1 using RQ-PCR in CML patients of all clinical phases, and we monitored for changes in these expression levels. Overexpression of the WT1 gene is involved in development of several solid tumors (pulmonary, breast, ovarian, thyroid, gastrointestinal, cutaneous) and malignant hematological conditions (predominantly in acute leukemias). There is a paucity of data regarding its role in CML.

Results:

In case of the regularly monitored 25 patients we performed a total of 130 RQ-PCR tests. The patients were categorized based on the administered treatment:

- The two transplant patients had different courses: a female patient had undetectable transcript levels for four years after the intervention and good clinical course during this time, and a male patient had

increased M-BCR-ABL values within one month from transplantation with relapse and unfavorable course of the disease.

- 23 patients received drug treatment: hydroxyurea/interferon initially, followed by tyrosine kinase inhibitor. Analyzing the expression dynamics of M-BCR-ABL according to the administered treatment we observed that there is only a minimal or completely missing decrease under hydroxyurea treatment, while following administration of standard dose imatinib 13 patients showed optimal therapeutic response (decreased, and sometimes undetectable expression). In case of 10 patients M-BCR-ABL values were stable or increased, prompting a change in therapy (either increased imatinib doses, or initiation of dasatinib, a second generation tyrosine kinase inhibitor), which led to favorable responses in chronic phase patients..

In case of 31 patients we managed to identify the *BCR-ABL gene variant* using single step PCR. All patients expressed the major variant of the oncogene, and the distribution of the two subtypes was similar: 15 patients had the b2a2 subtype, and 16 patients the b3a2 subtype. Comparing different clinical and biological parameters (sex, age, presence of splenomegaly, leukocyte count, hemoglobin, hematocrit values, platelet count) we demonstrated statistically significant differences between ***platelet counts*** and ***hematocrit values***: patients with subtype b3a2 typically have enhanced thrombocytosis and lower hematocrit values than b2a2 subtype patients.

We could not demonstrate statistically significant differences in the course of the disease according to the subtypes of M-BCR-ABL gene, but b3a2 patients seem to show a tendency for a better prognosis: higher rate of survival, more patients showing decreases of M-BCR-ABL expression levels after drug treatment (7 versus 4).

In most of the CML patients (20) we identified *WT1 expression* during follow-up, and in case of two patients the transcript was invariably undetectable for the whole duration of the study. All returned WT1 values were smaller than M-BCR-ABL by 2-3 log units, with the exception of two tests. Analyzing all results returned from the 73 concomitant WT1 and M-BCR-ABL expression testing we demonstrated significant ($p < 0.0001$) positive correlation (Spearman correlation factor 0.59; confidence interval: 0.4192-0.7302).

In case of two chronic phase patients the last tests showed an exclusive increase of WT1 expression; it is undecided whether this anticipates a clinical or a molecular event.

Conclusions:

PCR testing plays a key role in the modern and adequate management of CML patients. Due to its increased sensitivity, ***RQ-PCR*** is the optimal method for evaluation of the minimal residual disease in transplanted patients, as well evaluation of therapeutic response to tyrosine kinase inhibitors. Through regular (every six months) testing, patients with ***suboptimal response*** to imatinib can be detected in a timely manner, which allows an early therapeutic switch to the benefit of the patient.

The majority of CML patients show ***WT1 gene overexpression***, with similar dynamics to M-BCR-ABL most of the cases. The influence of discordant WT1 and M-BCR-ABL gene expression values on the course of the disease requires further investigation.

Keywords: chronic myeloid leukemia, BCR-ABL gene, PCR testing, WT1 gene