CASE REPORTS

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Follow-up of childhood chronic myelogenous leukemia with monitoring the BCR-ABL fusion gene expression in peripheral blood

ADRIENNE HORVÁTH[1)], MARIA DESPINA BAGHIU[1)], ZSUZSANNA PAP[2)], CLAUDIA BĂNESCU[3)], CRISTINA OANA MĂRGINEAN[1)], Z. PÁVAI[2)]

[1)]*Department of Pediatrics*
[2)]*Department of Morphopathology*
[3)]*Department of Genetics*
University of Medicine and Pharmacy of Targu Mures

Abstract

Chronic myelogenous leukemia (CML) accounts for 15–20% of adult leukemias but is very rare in children (2%). Fewer than 10% of CML patients are younger than 20 years. CML is a myeloproliferative disorder characterized by the presence of the Philadelphia chromosome or the BCR-ABL fusion oncogene. The objective of this paper is to present the monitoring of imatinib therapy in two children with CML by the BCR-ABL fusion gene expression assessment from peripheral blood with quantitative real-time polymerase chain reaction (PCR) method. *Patients and Methods*: The 18 and six months follow-up of the patients included clinical examination, routine laboratory tests, bone marrow aspirate investigation including cytogenetic tests and the major BCR-ABL fusion gene expression measurement with qRT-PCR method from the peripheral blood. *Results*: Patient No. 1 diagnosed with chronic phase CML showed excellent adherence to daily 400 mg imatinib treatment and achieved complete hematologic (CHR) and cytogenetic response (CCR) by three months and major molecular response (MMR) by 12 months, with lack of side effects due to imatinib. Patient No. 2 experienced severe hematologic toxicity, which necessitated temporary withdrawal of the drug. Transient non-compliance together with imatinib dose reduction has driven to treatment failure. In this case, mutational analysis is warranted. *Conclusions*: BCR-ABL fusion gene expression level measurement from peripheral blood with qRT-PCR method is an excellent tool in the follow-up of CML patients.

Keywords: chronic myelogenous leukemia, major molecular response, BCR-ABL fusion oncogene expression.

Introduction

Chronic myelogenous leukemia (CML) accounts for 15–20% of adult leukemias but is very rare in children (2%). Fewer than 10% of CML patients are younger than 20 years [1, 2]. CML is a myeloproliferative disease characterized by the presence of the Philadelphia chromosome or the BCR-ABL fusion oncogene. The natural history of the disease is a continuous progression from the chronic phase to the accelerated and the last brief blast crisis phase. Before the era of the molecular targeted therapy, the prognosis was poor and the therapies were mostly palliative with blood transfusions, irradiation of the spleen, hydroxiurea, busulfan and interferon alpha plus cytosin-arabinoside. Imatinib has changed the outcome of CML, being the first molecular targeted cancer therapy blocking the BCR-ABL tyrosine-kinases in their signaling pathways to proliferation. Second-generation tyrosine-kinase inhibitors (TKIs) (nilotinib, dasatinib, bosutinib) have been approved for the treatment of imatinib resistant or intolerant CML and third generation TKIs are being investigated in clinical trials [3]. However, the only curative treatment for CML is the allogeneic bone marrow transplantation. Recently, treatment efficacy on CML has been monitored by more and more sensitive laboratory tests down to molecular levels. Although the cytogenetic evaluation of the bone marrow is still the "gold standard" in CML monitoring, the measurement of the BCR-ABL transcripts by quantitative real-time polymerase chain reaction (qRT-PCR) from peripheral blood or bone marrow is becoming a more sensitive standard.

The objective of this paper is to present the monitoring of imatinib therapy in two children with CML by means of BCR-ABL gene expression assessment with qRT-PCR from peripheral blood (PB).

Patients and Methods

Two male patients aged 16 and 12 years with CML treated with imatinib were monitored at the Department of Pediatrics, Emergency County Hospital, Târgu Mureş, during a period of 18 and six months respectively (2009–2010) by means of clinical assessment, routine laboratory tests, peripheral blood smear, bone marrow aspirate with the cytogenetic determination of the t(9;22)(q34;q11) reciprocal chromosome translocation and the BCR-ABL fusion gene expression level with qRT-PCR method. Samples of bone marrow from the patients were sent to the Genetic Laboratory of the University of Medicine and Pharmacy of Târgu Mureş for cytogenetic evaluation. Heparinized bone marrow obtained at the time of diagnosis and during imatinib therapy were cultured for 1–3 days in RPMI 1640

medium supplemented with 20% fetal calf serum, 1% L-glutamine, 50 ng/mL penicillin/streptomycin without mitogens. After incubation, the cells were exposed to Colcemid (10 µg/mL), followed by hypotonic treatment (0.075M KCl), and were fixed with a mixture of methanol and glacial acetic acid (3:1). Chromosomes were spread on cold, wet slides. We used Giemsa staining (GTG staining) technique. Karyotype was interpreted according to International System for Human Cytogenetic Nomenclature (ISCN) recommendation [4]. Analysis was carried out using a BX51 Olympus microscope and images captured with an automated image analysis system (Cytovision, Applied Imaging). Two ml peripheral blood samples in EDTA tube tests were sent to the Molecular Biology Laboratory of the University of Medicine and Pharmacy of Târgu Mureş, for RT-PCR analysis. RNA extraction was performed using QIAmp RNA Blood Mini Kit 50 (QIAGEN Cat. No. 52304) according the supplier's instructions, and cDNS transcription with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems Cat. No. 4374966) according the supplier's instructions. We studied the b3-a2 and b2-a2 BCR/ABL fusion gene using the primers and protocols recommended by the *Europe against Cancer Program* [5]. The RQ-PCR reaction was performed on an ABI 7500 Real Time PCR instrument (Applied Biosystem), using 5-µL cDNA and TaqMan® Universal PCR Master Mix (Applied Biosystem) in 25-µL end volume. All reactions are made in triplicate. The ABL gene was used as endogenous control, and also were used known positive and negative control samples. We performed relative quantification.

Results

Case No. 1

The 16-year-old boy is found to have a massive splenomegaly during a routine medical visit, but he seeks no further medical care. After six months, he presents with fatigue, abdominal pain and pallor. On physical examination his skin is pale, no lymph nodes are palpable, his cardio-respiratory functions are stable (arterial blood pressure 118/68 mmHg, heart rate 68/min.), in his abdomen giant splenomegaly to the lower iliac fossa and crossing the midline and moderate hepatomegaly are evident. The laboratory analyses show very high white blood cell (WBC) count 424 000/mm^3, Hgb 10.4 g%, Htc 25%, platelets 348 000/mm^3, reticulocytes 46‰. The peripheral blood picture: myeloblasts 1%, promyelocytes 2%, myelocytes 2%, metamyelocytes 12%, band 9%, segmented 36%, eosinophils 2%, basophils 5%, monocytes 1%, lymphocytes 1%, erythroblasts 2/100 leukocytes, macrocytes of 9 µm. The leukocyte alkaline phosphatase activity is 0. The bone marrow is hypercellular with 1% myeloblasts, a hyperplastic myeloid line and basophilia. Cytogenetic examination of the bone marrow revealed 33% Ph$^+$ cells. Molecular biological examination from the peripheral blood shows the 140% expression of BCR-ABL gene in comparison with the control ABL gene. Blood chemistry is with high lactate dehydrogenase (LDH) level (2105 U/L). The ultrasonographically measured size of the liver was 188/85 mm and of the spleen 280/116 mm. Eye fundus examination showed hyperemic papillae, peripapillar hemorrhage and dilated, sinuous retinal vessels. The chronic phase CML diagnosis was made with an intermediate Sokal and Hasford score (0.94, respectively 1142). Initial treatment included hydroxyurea and supportive measures with hydration, alkalinisation, allopurinol, diuretics, followed shortly by the introduction of imatinib mesylate (Glivec) therapy in dose of 400 mg/day. After two weeks of Glivec therapy, WBC count lowered to 34 000/mm^3, Hgb level was 8.8 g%, Htc 27.3%, thrombocytes 179 000/mm^3, LDH 355 U/L, the size of the spleen was decreasing. The details of the 18 months follow-up are shown in Table 1 and Figure 1.

Table 1 – ***A 18 months monitoring of a 16-year-old CML patient treated with daily 400 mg imatinib***

	Diagnosis	3 months	6 months	12 months	18 months
WBC [G/L]	424	3.03	3.92	3.25	3.57
Hgb [g/dL]	10.7	11.3	11.5	12.7	13
Htc [%]	26.6	35.5	35.2	38.9	40.6
PLT [G/L]	348	78	100	145	153
Basophils PB [%]	5	2	1	0.3	1
Blasts BM [%]	1			0.5	1
Cytogenetics BM. Ph$^+$ cells [%]	35		0	0	
BCR-ABL gene expression PB [%]	140	1.41		0.0004	0.0002
Ultrasonographic spleen size [mm]	280/116	190/51	157/50	125/34	
Sokal score	0.94				
Hasford score	1142				
Response to treatment		No HR	CHR, CCR	CHR, CCR, MMR	CHR, MMR

WBC – white blood cells; Hgb – hemoglobin; Htc – hematocrit; PLT – platelets; PB – peripheral blood; BM – bone marrow; HR – hematological response; CHR – complete hematological response; CCR – complete cytogenetic response; MMR – major molecular response.

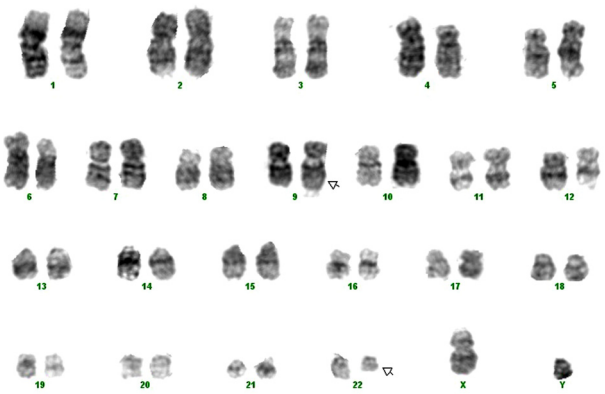

Figure 1 – ***The Ph chromosome t (9,22)(q34q11) from the bone marrow sample of the patient.***

Major cytogenetic and partial hematologic responses were achieved after three months of daily 400 mg imatinib therapy, whereas major molecular response appeared in 12 months time, with a higher than 3-log reduction in BCR-ABL gene expression level and is currently persisting (Figure 2).

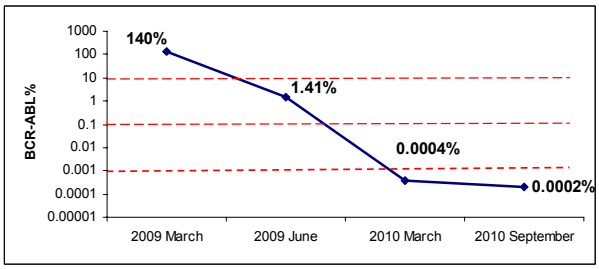

Figure 2 – ***The achievement of major molecular response in Case No. 1, by 12 months under imatinib treatment.***

No side effects were noticed due to imatinib. Family members were examined for human leukocyte antigen compatibility, regarding possible bone marrow transplantation, but no compatible donor was found. The patient is currently on daily 400 mg imatinib therapy with excellent adherence to therapy and maintaining major molecular responses. The monitoring plan consists in monthly clinical and routine laboratory assessment, molecular tests from PB for BCR-ABL gene expression evaluation at six months interval and yearly bone marrow aspirate examination for morphology, basophilia and cytogenetics (Table 2).

Table 2 – ***Cytogenetic examination from bone marrow aspirate of the therapy adherent patient***

	March 2010	June 2010	December 2010
Sample	BM	BM	BM
No. of examined metaphases	6	10	10
No. of metaphases with Ph^+ chromosome	2 (33%)	0	0

BM – bone marrow.

Case No. 2

The 12-year-old boy presented with fatigue, sweating, abdominal pain and fever. The physical examination revealed a prepubertal boy with severe pallor, giant splenomegaly and moderate hepatomegaly, no palpable lymph nodes, grade 3/6 systolic murmur in the left parasternal area. The initial hemogram revealed a high white blood cell count (WBC 365 000/mm^3), anemia (RBC 1.46 G/L, Hgb 5.2 g/dl, Htc 14.1%, MCV 96.6 fL), with normal platelets (193 000/mm^3). Peripheral blood smear showed a leukoerythroblastic picture with basophilia: myeloblasts 2%, promyelocytes 1%, myelocytes 38%, metamyelocytes 16%, band 10%, segmented 25%, eosinophils 2%, basophils 3%, monocytes 1%, lymphocytes 2%, erythroblasts 2/100 leukocytes, normochromia. Bone marrow morphology showed hypercellularity of the granulocytic cells, 2% myeloblasts, moderate basophilia, scarcely represented red blood cells and megakaryocytes. Cytogenetic examination was not performed at diagnosis. QRT-PCR examination from peripheral blood for BCR-ABL transcript detection was positive for the major gene (p210) above 100% and the minor gene (p190): 0.01%. LDH was elevated (2010 U/L).

Eye fundus examination showed papilla edema, sinuous and dilated blood vessels, and multiple hemorrhages. Abdominal CT scan revealed hepatomegaly (200 mm diameter in the right hepatic lobe) and splenomegaly (273×109 mm), retroperitoneal lymph nodes of 20 mm diameter. Ultrasonographically measured spleen size was 252/86 mm and hepar 188/62.3 mm.

Cardiologic assessment revealed good systolic-diastolic function of the ventricles, ostium secundum type minor atrial septal defect. His initial risk scores were in the intermediate range, according to both Sokal's and Hasford's (Sokal score 1.16 and Hasford score 1453.3).

His further physical and laboratory evolution is presented in Table 3.

Table 3 – ***A 9-month monitoring of a 12-year-old CML patient on imatinib therapy with hematologic toxicity and non-compliance***

	Diagnosis February 2010	31 March 2010	26 April 2010	11 May 2010	13 July 2010	October 2010	November 2010
WBC [G/L]	366		3.55	3,92	5.47		147
Hgb [g/dL]	5.2		6.9	10	12.3		8.8
Htc [%]	14.1		21.3	28.6	36		24.2
PLT [G/L]	193		13	107	109		101
Basophils PB [%]	3		4	1	2		3
Blasts BM [%]	2		–	–	–		2
Cytogenetics BM Ph^+ cells [%]	–		–	–	–		–
BCR-ABL gene expression PB [%]	100		–	–	7.03	23	–
Ultrasonographic spleen size [mm]	252/86		185/62	202/50.4	145/56		155/62
Sokal score	1.16						
Hasford score	1453.3						
Treatment	Hydroxyurea	Imatinib 400 mg	Stop	Imatinib 200 mg	Imatinib 400 mg		Imatinib 400 mg
Response	No		Hematologic toxicity		Non-adherence	Failure	Failure

WBC – white blood cells; Hgb – hemoglobin; Htc – hematocrit; PLT – platelets; PB – peripheral blood; BM – bone marrow.

During the first month after diagnosis until molecular biology tests turned out to be positive for increased BCR-ABL gene expression, the patient was treated with hydroxyurea without any clinical or laboratory response. Imatinib 400 mg/day had been administered for one month, when severe hematologic

adverse effects developed and the drug needed to be discontinued for two weeks. A lower dose imatinib (200 mg/day) was used for seven weeks, followed by a gradual increase of the dose back to 400 mg/day again. After three months of standard dose imatinib (400 mg/day) the patient still presented moderate palpatory splenomegaly, his blood cell counts were in normal range but the peripheral blood the BCR-ABL gene expression level rose from the earlier 7.03% to 23%. This warning laboratory sign ended up in hematologic relapse at six months after diagnosis. The patient has only achieved a partial hematologic response at four months, because of a mild persistent splenomegaly beside a normal blood cell count. Bone marrow aspirate morphology was carried out at the loss of hematologic response, which showed a myeloid proliferation, 2% myeloblasts and basophilia. The cause of unfavorable treatment response revealed after repeated anamnesis was inadequate drug-adherence and inappropriate parental care. The patient was hospitalized and imatinib treatment was reintroduced under close clinical supervision (Figure 3).

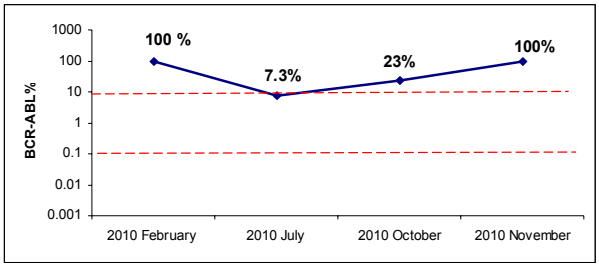

Figure 3 – ***The development of the BCR-ABL gene expression in the peripheral blood in Case No. 2, under imatinib treatment but with severe hematologic toxicity and non-adherence to treatment.***

Discussion

Pathomechanism of CML

Chronic myelogenous leukemia (CML) arises from neoplastic transformation of a primitive progenitor cell that retains the ability to further maturation and differentiation. Fast cell proliferation and longer life span of transformed cells cause the accumulation of a large number of leukocytes of all maturation stages in the peripheral blood. The abnormal BCR-ABL fusion gene encodes a continuously activated BCR-ABL fusion protein, which is a cytokine independent tyrosine-kinase that catalyses a phosphorilation process important in signal transduction to increase cell proliferation and decrease apoptosis [6, 7]. Multiple molecular events enables the natural evolution of CML from the chronic phase to the accelerated phase (AP) and blast phase (BP), respectively are the causes of tyrosine kinase inhibitor resistance. It has been shown that in CML (alike breast cancer, acute myelogenous leukemia, brain tumors, colon cancer, pancreatic cancer) there exist cancer stem cells with self-renewal capacity and resistance to therapies, leading to disease progression. Quiescent BCR-ABL_1 positive hematopoietic stem cells (HSC) account for about 0.5% of $CD34^+$ cell population and unlike their progeny, are refractory to chemo-therapeutic agents, radiation and BCR-ABL tyrosine-kinase inhibitors (TKI_s), leading to persistent residual disease [8]. Several studies suggested that CML stem cell insensitivity to TKI_s is due to their BCR-ABL kinase domain mutations that are present from the beginning, even before initiation of TKI therapy [9, 10].

Epidemiology, clinical assessment of CML

Seventy five percent of patients are diagnosed in the chronic phase, they are mainly adults in their fifties and only 10% of the patients are younger than 20 years [11]. A slightly male predominance prevail (1.4–2.2:1). The clinical presentation includes fatigue, sweating, abdominal pain, weight loss, hyperviscosity syndrome with stroke, priapism or retinal hemorrhage. Initial paraclinical assessment of CML patients includes a complete blood cell count with differential, blood chemistry, leukocyte alkaline phosphatase activity, bone marrow aspirate and biopsy for morphology (percent of blasts, basophils), cytogenetics and molecular biology (RT-PCR, FISH), fibrosis. The chronic phase is characterized by elevated WBC count (>20 G/L), less than 10% blast count in peripheral blood and bone marrow, increased basophils, increased or normal thrombocytes, hypercellular bone marrow with myeloid hyperplasia and fibrosis. Cytogenetics of bone marrow and blood reveals in 95% of cases the presence of Ph chromosome and molecular biology the presence of BCR-ABL fusion gene. The chronic phase lasts for 3–6 years with conventional treatment. In the accelerated phase, beside the bone and abdominal pain, fever and pallor, the blast count rises above 10% in the blood or bone marrow, and basophils exceeds 20% in the peripheral blood. A thrombocytopenia (below 100 G/L) or persistent thrombocytosis (above 1000 G/L) appears along with elevated WBC count. This phase has a median duration of 6–9 months. The blast crisis phase is characterized by transformation to myeloid, lymphoid or hybrid acute leukemia, with bleeding, anemia, infections, adenomegaly, central nervous system dysfunction, increasing blast count (>30% blasts) in blood or bone marrow and decreased platelet count. This phase has a short course of 3–6 months. However, this timely progression refers to natural course or conventional therapy CML and not to CML under TKI treatment.

Treatment options in CML

While the only treatment options in the 1950s were irradiation of the spleen and busulphan, the introduction of the hydroxyurea in 1960 represented a huge leap in the treatment of CML, though only 42% of the patients were alive after five years and fewer than 5% survived beyond 10 years [11]. Interferon-alfa, which modulates the immune response and inhibits cell growth, was introduced in 1983 in the treatment of CML. Although hematological response can be obtained with this drug, cytogenetic and molecular responses are rare and transient. Association with cytarabine has resulted in a higher rate of major cytogenetic response and survival but has also increased toxicity. The stem cell transplantation (SCT) with HLA-matched related or

unrelated donor is currently the only known cure for CML, yet only 18% of the CML patients undergo SCT [11] and transplantation related morbidity and mortality has to be taken into account.

Imatinib mesylate, a tyrosine-kinase inhibitor was the first molecular targeted agent with a well-known mechanism of action within the leukemic cell, introduced in the treatment of CML in 2001. Today imatinib is the standard first-line therapy for all Ph chromosome positive CML patients, in the dose of 400 mg/die for chronic phase (260 mg/m^2/day in children) and 600 mg/day in accelerated or blast crisis phase in adult patients (340 mg/m^2/day in children). Imatinib is better tolerated than chemotherapy, yet adverse effects can occur, like gastrointestinal side effects with nausea, vomiting, diarrhea, abdominal pain, cardiac disorders, liver toxicity, fluid retention, skin rashes, etc. [1, 2, 12, 13]. Some 20–30% of CML patients do not achieve a complete cytogenetic response and others develop resistance or intolerance to imatinib [7, 14].

Second generation BCR-ABL tyrosine kinase inhibitor nilotinib was first approved in chronic or accelerated phase CML patients who were resistant or intolerant to imatinib but from June 2010 it is approved for first-line treatment of patients with Ph$^+$ positive CML in chronic phase. Regarding the side effects, neutropenia and anemia were more frequent with imatinib and thrombocytopenia with nilotinib. The hematologic toxicities appeared in both groups in the first two months of treatment [15]. Dasatinib is approved for treatment in adults with CML in the chronic, accelerated or myeloid or lymphoid blast phase who have resistance or intolerance to prior therapy including imatinib [16]. Nilotinib and dasatinib have the same efficacy in CML treatment.

Allogeneic bone marrow transplantation (BMT) is the only known cure for CML. Five-year survival rate is 40–70% if performed in chronic phase, 22–43% in accelerated phase and only 15–20% of patients in blast crisis are alive 2–3 years after BMT. However, access to BMT is limited by a relative lack of HLA-compatible donor and patient age above 55 years, when transplantation-related morbidity and mortality is higher. Overall, only 15–18% of patients are good candidates for BMT. Relapses after allogeneic BMT appear in 10–20% of good candidates and 60–70% of patients in accelerated or blast phase [1, 2]. Allogeneic hematologic stem cell transplantation is recommended in children and adolescents (under 20-year-old), in patients presenting accelerated or blast phase at diagnosis, in TKIs failure and in patients carrying the T315I mutation [17].

Monitoring CML during treatment

Prognosis of CML with imatinib treatment has improved to an 89% overall survival rate at five years. The goal of Glivec therapy is to achieve a complete or major hematological, cytogenetic and molecular response. The complete hematological response (CHR) is defined as maintenance for at least four weeks of normal physical examination, normal WBC count and lack of immature myeloid cells. In CHR the WBC count is under 10 G/Lm^3, platelet count under 450 G/Lm^3 and basophil count in peripheral blood <5% with no immature granulocyte forms (myeloblast, promyelocyte, myelocyte) in peripheral blood. The cytogenetic response may be complete (CCyR – complete cytogenetic response) with elimination of Ph chromosome, partial (PCyR) with the presence of 1–35% Ph$^+$ cells and major cytogenetic response (MCyR) indicating 0–35 Ph$^+$ cells. Molecular response may be complete (CMR) when with RT-PCR there are no detectable BCR-ABL transcripts, major molecular response (MMR) with 3-log reduction from a standardized baseline in the level of BCR-ABL transcripts.

Sokal and the newer Hasford scores were developed for a better prediction of the prognosis. These scores need to be calculated quite at the beginning of the disease, without any previous treatments. The followed criteria are age, spleen size, number of platelets ($\times 10^9$) and myeloblasts in peripheral blood (%). A score under 0.8 indicates low risk CML, a score between 0.8 and 1.2 represents intermediate risk, while a 1.2 or higher score shows high risk. The Hasford score (1998), relies on more numerous criteria, such as age, spleen size, platelet count ($\times 10^9$), blood myeloblasts (%), blood basophils (%) and eosinophils (%). A score result below 780 indicates low risk, a score between 780–1480 points to intermediate risk, while a score above 1480 indicates high-risk CML patients [18, 19].

The most sensitive monitoring method in CML is the detection of the BCR-ABL mRNA with the qRT-PCR technique. Recently international efforts are being made to standardize the molecular tests so that results could be comparable and reproducible. On the international scale (IS), 100% of the BCR-ABL transcript level corresponds to the IRIS standardized baseline transcript level and 0.1% indicates MMR. The BCR-ABL transcript level corresponding to CMR is yet under debate, meaning at least a 4-log reduction ($\leq$0.01%) up to a 5-log reduction ($\leq$0.001) from the standardized international baseline. The primary endpoint of the ENEST1 trial is the achievement of CMR after 18 months of TKI treatment. Even patients with undetectable BCR-ABL transcripts (CMR) can harbor a substantial amount of residual leukemic cells. In recent times, the trend is to use the absolute quantification method, with internationally accredited reference reagents for BCR-ABL. Until now, we have not used this method, but its implementation is in progress [20].

During imatinib therapy, the hematological, cytogenetic and molecular responses are closely monitored. The European LeukemiaNet (ELN) recommends for the management of chronic phase CML treated with imatinib the introduction of the definitions of optimal response, suboptimal response, failure and warnings. The recommended checkpoints are made by 3, 6, 12 and 18 months of treatment. The "optimal response" supposes that the patient gains CHR by three months, at least partial cytogenetic response (PCyR) by six months, CCyR by 12 months and MMR by 18 months. "Failure" means that no HR is achieved by three months, no cytogenetic response (Ph$^+$ cells >95%) after six months

from diagnosis, a less than PCyR is reached (Ph^+ cells >35%) by 12 months and less than CCyR by 18 months, and afterwards loss of CHR or CCyR any time [21]. Our second case report presents a patient with severe hematologic toxicity to imatinib who achieved only a short hematologic response, without molecular response. One of the causes of therapy failure was non-adherence to therapy. Therapy failure or suboptimal responses appear in 20–30% of patients, in these cases compliance checking, mutational analysis investigations are recommended and the dose of imatinib can be raised to a maximum of 800 mg/day or newer drugs like nilotinib and dasatinib can be applied or allogeneic bone marrow transplantation may be indicated. The IRIS study demonstrated the long-term prognostic significance of early molecular response to imatinib in newly diagnosed CML. During a seven-year follow-up, 95% of patients who achieved MMR by 18 months did not progress to advanced phase. Twenty six percent of patients who had been in CCyR but not MMR by 18 months of imatinib treatment, lost the CCyR, compared with 3% of the patients who had achieved both CcyR and MMR by 18 months [22]. The BCR-ABL T315I mutation represents a major mechanism of resistance to tyrosine kinase inhibitors. Medium overall survival in patients with BCR-ABL T315I mutation depends on the disease phase at the time of mutation detection, being 22.4 months for chronic phase, 28.4 months for advanced phase and four months for blast phase in a study conducted in 2009 [23].

Poor compliance in CML therapy was reported by the ADAGIO study from Belgium performed on 162 patients, out of which only 14% were perfectly compliant and 71% took a suboptimal dose. The six-year probability of MMR was about 90% in patients who had an adherence rate above 90% and about 20% in those with adherence rate under 90%. The term "adherence rate" is defined as the dose taken expressed as percentage of the dose prescribed during the total study duration. Adherence or compliance in CML therapy can be assessed by self-reporting, pill counts, drug plasma levels and microelectronic monitoring systems (MEMS). Non-compliance can be unintentional (forgetting, prescribing error, etc.) or intentional (fear of side effects, temporary illness, socializing, traveling, etc.) [24]. In our patient, the cause of non-compliance was parental neglect and fear of the side effects. The patient has not achieved CHR, nor MMR, by six months of imatinib treatment. In contrast, the compliant patient has achieved CCyR by three months which has been maintained up to the present and MMR by 12 months.

Conclusions

We presented two cases of chronic phase CML in children, on standard dose imatinib treatment, who were monitored by qRT-PCR detection of BCR-ABL transcripts from peripheral blood. One of the patients with excellent compliance achieved early cytogenetic and molecular response, which has maintained during the follow-up period of 18 months in contrast with the other case who presented severe hematologic toxicity to imatinib and non-compliance to therapy with failure to achieve sustained hematologic and molecular response.

Acknowledgements

This paper is partly supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the Contract No. POSDRU/89/1.5/S/60782, and partly supported by Stichting Ondersteuning Medische Hulpverlening en Medisch Onderzoek Nederland.

References

[1] ***, *CML clinical monograph*, http://www.glivec.com/resources/cml-resources.jsp, accessed 29.08.2010.
[2] ***, *About Glivec*, http://www.glivec.com/about-glivec/index.jsp, accessed 29.08.2010.
[3] Sawyers CL, *Even better kinase inhibitors for chronic myeloid leukemia*, N Engl J Med, 2010, 362(24):2314–2315.
[4] Mitelman F, *An international system for human cytogenetic nomenclature*, S. Karger, Basel, 1995.
[5] Gabert J, Beillard E, van der Velden VH, Bi W, Grimwade D, Pallisgaard N, Barbany G, Cazzaniga G, Cayuela JM, Cavé H, Pane F, Aerts JL, De Micheli D, Thirion X, Pradel V, González M, Viehmann S, Malec M, Saglio G, van Dongen JJ, *Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – a Europe Against Cancer Program*, Leukemia, 2003, 17(12):2318–2357.
[6] Jamieson CH, *Chronic myeloid leukemia stem cells*, Hematology Am Soc Hematol Educ Program, 2008:436–442.
[7] Deininger MW, *Milestones and monitoring in patients with CML treated with imatinib*, Hematology Am Soc Hematol Educ Program, 2008:419–426.
[8] Quintás-Cardama A, Cortes J, *Molecular biology of bcr-abl1-positive chronic myeloid leukemia*, Blood, 2009, 113(8):1619–1630.
[9] Hochhaus A, La Rosée P, *Imatinib therapy in chronic myelogenous leukemia: strategies to avoid and overcome resistance*, Leukemia, 2004, 18(8):1321–1331.
[10] Gambacorti-Passerini CB, Gunby RH, Piazza R, Galietta A, Rostagno R, Scapozza L, *Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias*, Lancet Oncol, 2003, 4(2):75–85.
[11] Losonczy H, Molnár L, *Glivec – egy forradalmi terápia a betegek szolgálatában*, IME, 2006, V(6):34–37.
[12] Molnár L, Losonczy H, *Új lehetőség a krónikus myeloid leukaemia kezelésében: specifikus tirozinkináz-inhibitor (STI571)*, Orvosi Hetilap, 2002, 143(42):2379–2384.
[13] Baccarani M, Rosti G, Castagnetti F, Haznedaroglu I, Porkka K, Abruzzese E, Alimena G, Ehrencrona H, Hjorth-Hansen H, Kairisto V, Levato L, Martinelli G, Nagler A, Lanng Nielsen J, Ozbek U, Palandri F, Palmieri F, Pane F, Rege-Cambrin G, Russo D, Specchia G, Testoni N, Weiss-Bjerrum O, Saglio G, Simonsson B, *Comparison of imatinib 400 mg and 800 mg daily in the front-line treatment of high-risk, Philadelphia-positive chronic myeloid leukemia: a European LeukemiaNet Study*, Blood, 2009, 113(19):4497–4504.
[14] Pavlů J, Kew AK, Taylor-Roberts B, Auner HW, Marin D, Olavarria E, Kanfer EJ, MacDonald DH, Milojkovic D, Rahemtulla A, Rezvani K, Goldman JM, Apperley JF, Szydlo RM, *Optimizing patient selection for myeloablative allogeneic hematopoietic cell transplantation in chronic myeloid leukemia in chronic phase*, Blood, 2010, 15(20):4018–4020.
[15] Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, Pasquini R, Clark RE, Hochhaus A, Hughes TP, Gallagher N, Hoenekopp A, Dong M, Haque A, Larson RA, Kantarjian HM; ENESTnd Investigators, *Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia*, N Engl J Med, 2010, 362(24):2251–2259.

[16] Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, Moiraghi B, Shen Z, Mayer J, Pasquini R, Nakamae H, Huguet F, Boqué C, Chuah C, Bleickardt E, Bradley-Garelik MB, Zhu C, Szatrowski T, Shapiro D, Baccarani M, *Dasatinib versus imatinib in newly diagnosed chronic-phase myeloid leukemia*, N Engl J Med, 2010, 362(24):2260–2270.

[17] Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J, Cervantes F, Deininger M, Gratwohl A, Guilhot F, Hochhaus A, Horowitz M, Hughes T, Kantarjian H, Larson R, Radich J, Simonsson B, Silver RT, Goldman J, Hehlmann R; European LeukemiaNet, *Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet*, J Clin Oncol, 2009, 27(35):6041–6051.

[18] Rosti G, Trabacchi E, Bassi S, Bonifazi F, de Vivo A, Martinelli G, Alberti D, Fincato G, Saglio G, Baccarani M; Italian Cooperative Study Group on Chronic Myeloid Leukemia, *Risk and early cytogenetic response to imatinib and interferon in chronic myeloid leukemia*, Haematologica, 2003, 88(3):256–259.

[19] Bonifazi F, De Vivo A, Rosti G, Tiribelli M, Russo D, Trabacchi E, Fiacchini M, Montefusco E, Baccarani M, *Testing Sokal's and the new prognostic score for chronic myeloid leukaemia treated with alpha-interferon. Italian Cooperative Study Group on Chronic Myeloid Leukaemia*, Br J Haematol, 2000, 111(2):587–595.

[20] Cross NCP, *Standardisation of molecular monitoring for chronic myeloid leukaemia*, Best Pract Res Clin Haematol, 2009, 22(3):355–365.

[21] Hehlmann R, Hochhaus A, Baccarani M; European LeukemiaNet, *Chronic myeloid leukaemia*, Lancet, 2007, 370(9584):342–350.

[22] Hughes TP, Hochhaus A, Branford S, Müller MC, Kaeda JS, Foroni L, Druker BJ, Guilhot F, Larson RA, O'Brien SG, Rudoltz MS, Mone M, Wehrle E, Modur V, Goldman JM, Radich JP; Iris Investigators, *Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia: an analysis from the International Randomized Study of Interferon versus STI571 (IRIS)*, Blood, 2010, 116(19):3758–3765.

[23] Nicolini FE, Mauro MJ, Martinelli G, Kim DW, Soverini S, Müller MC, Hochhaus A, Cortes J, Chuah C, Dufva IH, Apperley JF, Yagasaki F, Pearson JD, Peter S, Sanz Rodriguez C, Preudhomme C, Giles F, Goldman JM, Zhou W, *Epidemiologic study on survival of chronic myeloid leukemia and Ph$^+$ acute lymphoblastic leukemia patients with BCR-ABL T315I mutation*, Blood, 2009, 114(26):5271–5278.

[24] Marin D, Bazeos A, Mahon FX, Eliasson L, Milojkovic D, Bua M, Apperley JF, Szydlo R, Desai R, Kozlowski K, Paliompeis C, Latham V, Foroni L, Molimard M, Reid A, Rezvani K, de Lavallade H, Guallar C, Goldman J, Khorashad JS, *Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib*, J Clin Oncol, 2010, 28(14):2381–2388.

Corresponding author

Adrienne Horváth, MD, Department of Pediatrics, Emergency County Hospital Târgu Mureş, University of Medicine and Pharmacy of Târgu Mureş, 50 Gheorghe Marinescu Street, 540136 Târgu Mureş, Romania; Phone +40745–593 380, e-mail: adigyer1@yahoo.com

Received: February 1st, 2011

Accepted: June 30th, 2011