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SEVERAL DIFFERENT CYTOGENETIC CLONES ARISING DURING TREATMENT OF PHILADELPHIA POSITIVE CHRONIC MYELOID LEUKEMIA WITH TYROSINE KINASE INHIBITORS LEADING TO THE PROGRESSION INTO PHILADELPHIA NEGATIVE ACUTE MYELOID LEUKEMIA

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Running title: Different clones in chronic myeloid leukemia
Abstract

Introduction. Additional karyotype abnormalities in Philadelphia-positive (Ph+) clone can emerge during the progression of chronic myeloid leukemia (CML) and are often associated with resistance to treatment with tyrosine kinase inhibitors (TKI). Sometimes, during the TKI treatment, karyotype abnormalities can appear in Philadelphia-negative (Ph-) cells as well, but do not seem to adversely affect the outcome with the exception of chromosome 7 abnormalities. Case report. The patient presented was in a chronic phase of Ph+ CML with highly diverse karyotype abnormalities which appeared in three unrelated clones during the TKIs treatment followed by the evolution of the disease into acute myeloid leukemia (AML). The primary Ph+ clone was revealed during the chronic phase of CML and a therapy with imatinib mesylate was started. After a three year hematologic and cytogenetic remission period, the evolution of the primary clone was noticed. The nilotinib therapy was introduced, leading to a good molecular response and the disappearance/loss of Ph+ clone with additional abnormalities but with the appearance of Ph- clone with trisomy 8. Finally, after 5.5 years of the nilotinib therapy, the Ph- clone with monosomy 7 occurred during the deep molecular response for bcr-abl. At that time, the FISH analysis for trisomy 8 was negative but the rise in blast count was noticed in the bone marrow and the diagnosis of the secondary AML was established soon after. Conclusion. Achievement of the deep molecular response in CML patients does not rule out regular testing of their bone marrow with cytogenetic analysis which is of crucial importance for the detection of the adverse karyotype abnormalities leading to development of the myelodysplastic syndrome and AML.

Key words: chronic myeloid leukemia, tyrosine kinase inhibitors, philadelphia chromosome, treatment resistance, chromosome 7 abnormalities.

Apstrakt

Uvod. Dodatne kariotipske abnormalnosti u Filadelfija-požitivnom (Ph+) klonu se mogu javiti tokom progresije hronične mijeloidne leukemije (CML) i često su povezane sa rezistencijom na terapiju tirozin kinaznim inhibitorima (TKI). Ponekada se tokom terapije TKI kariotipske abnormalnosti javljaju i u Filadelfija-negativnim (Ph-) ćelijama ali ne utiču na progresiju bolesti, izuzev abnormalnosti hromozoma 7. Prikaz bolesnika. Kod bolesnice u hroničnoj fazi CML, tokom lečenja TKI uočene su kariotipske abnormalnosti prisutne u tri nezavisna klon sa evolucijom bolesti u akutnu mijeloidnu leukemiju (AML). Primarni Ph+ klon je otkriven tokom hronične faze CML i započeta je tepija imatinib mesilatom. Nakon tri godine hematološke i citogenetske remisije, uočena je evolucija primarnog klon. Započeta je terapija nilotinibom koja je dovela do molekularnog odgovora i povlačenja Ph+ klon sa dodatnim aberracijama ali i pojavljivanja novog Ph- klon sa trizomijom 8. Nakon 5.5 godina lečenja nilotinibom i postizanja kompletne molekularnog odgovora, uočen je Ph- klon sa monozomijom 7. Fluorescentna in situ hibridizacija (FISH) pokazala je odsustvo trizomije 8 i prisustvo monozomije 7. Istovremeno, registrovan je porast broja blasta u kostnoj srži i ubrzo je postavljena dijagnoza sekundarne AML. Zaključak. Postizanje kompletne molekularnog odgovora
Introduction

The *BCR-ABL* fusion gene which generates the Philadelphia chromosome (Ph) is a sole genetic abnormality in 90% of chronic myeloid leukemia (CML) patients in the chronic phase [1]. With the progression of the disease, additional karyotype changes in the Philadelphia positive (Ph+) clone emerge and are often associated with resistance to imatinib mesylate and/or nilotinib. The resistance can be a consequence of one of the numerous mutations in the tyrosine kinase domain or some other underlying mechanism, and is usually overwhelmed with some of the novel tyrosine kinase inhibitor (TKI) drugs. Sometimes, during the TKI treatment, karyotype changes in the Philadelphia negative (Ph-) cells can appear [2]. These aberrations, similar to those frequently seen in the myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), include: trisomy 8 (+8), the deletion or monosomy of chromosome 7 (del(7q)/-7) and nulisomy of Y (-Y). However, chromosome abnormalities in a Ph- clone do not seem to adversely affect the outcome with the exception of the chromosome 7 abnormalities [1]. Monosomy 7 and del(7q) require frequent bone marrow follow-up as several case reports indicate the development of the MDS and subsequent AML [3-7].

We present a CML patient with highly diverse karyotype abnormalities which appeared in three unrelated clones during the treatment with imatinib mesylate and nilotinib. The patient mentioned developed AML in the end.

Methods

Cytogenetic study and response criteria

The cytogenetic study was performed on unstimulated bone marrow cells using standard technique. The giemsa-banded metaphases were analyzed, and the result was reported by standards of the International System for Human Cytogenetic Nomenclature, 2013. The cytogenetic response was classified according to the standard of the UK Medical Research Council practice as complete (0% Ph+ metaphases), major (1-34% Ph+), partial (35-65% Ph+ metaphases), minor (66-95% Ph+) and no response (95-100% Ph+). The cytogenetic clonal evolution was defined as the presence of any abnormality other than a single Ph chromosome.

*RT-PCR and “nested” RT-PCR analysis*

The total RNA was extracted from peripheral blood cells according to the guanidine thiocyanate phenol-chloroform extraction method [8]. Reverse transcription was performed
on 1μg of total RNA after heating at 65ºC for 15 minutes. A reverse transcription was performed with the 1-Strand cDNA Synthesis Kit for RT-PCR (AMV) (Roche Diagnostics Corporation, Indianapolis, IN, USA) according to the manufacturer’s manual. The amplification was done with slight modifications as described by Moravcova et al [9].

**FISH**

The FISH analysis for centromere regions of chromosomes 7 and 8 (CEP7 and CEP8) was performed on interphase nuclei and metaphase cells according to the manufacturer’s instructions (Vysis/Abbott Laboratories, Des Plaines, IL).

**Results**

**Case report**

A 44-year-old female was diagnosed with CML in the chronic phase in April 2002. Cytogenetic analysis revealed the translocation t(9;22)(q34;q11) (Ph chromosome) in 20/20 mitoses (Fig 1A). The prognostic Sokal and Hasford scores implied a low-risk patient at presentation, without comorbidities or any additional treatment. The antileukemic therapy was started with hydroxyurea and interferon with a subsequent increase in interferon dosage. Regardless of achieving hematological remission, after 6 months of treatment no cytogenetic response was obtained. As she had no available stem cell donor, the imatinib mesylate in standard dose (400mg daily) was started in September 2002. Six months later, she achieved partial cytogenetic response (PCgR) with 53% Ph-negative metaphases and after 12 months, a complete cytogenetic response (CCgR) was achieved, although a b2a2 transcript of the BCR/ABL fusion was detected by “nested” RT-PCR. During the next three years, she remained in a stable chronic phase with CCgR, on 400 mg of imatinib. However, in August 2005, the follow-up cytogenetics revealed complex translocation t(5;6;12) with t(9;22) (Fig 1B) in all analyzed cells, without any clinical or laboratory sign of disease progression. The tyrosine-kinase domain mutations were negative by direct sequencing. The patient was treated with an escalated dosage of imatinib (800 mg daily) since October 2006 without success. She was switched to nilotinib, 800 mg daily, from July 2008 through the “compassionate use program” but due to hematologic and hepatic toxicity, after only a month the nilotinib was reduced to 400 mg. After 6 months of 400 mg nilotinib she achieved CCgR again, but at 12 months the reappearance of Ph+ clone with t(5;6;12) was noticed in 45% of metaphases, suggesting PCgR. Along with the Ph positive cells, a Ph-negative clone with +8 was seen in 10% of mitoses. After the recovery of the blood counts and the hepatic function 6 months later, the patient was escalated to full dosage of nilotinib (800 mg) again. She achieved a major cytogenetic response (MajCgR, 10% of Ph+ with t(5;6;12) clone) at 24 months of nilotinib treatment and finally, after 30 months on nilotinib, she achieved CCgR. During the next 3 years on 800 mg of nilotinib, her follow up showed CCgR and a stable molecular response (MR3), though the Ph-negative clone with +8 was constantly present in 10-30% of metaphases (Fig 1C).

However, during the regular follow up in February 2014 (5.5 years of nilotinib), profound neutropenia without anemia and thrombocytopenia was noticed (Hemoglobin 120 g/L, WBC 2.5x10⁹/L, 76% lymphocytes, 4% blasts, 10% monocytes, Platelets 258x10⁹/L) together with elevated transaminases (ALT 86 U/L). Immediate bone marrow evaluation
revealed dysplastic changes in erythroid and megakaryocyte lineages together with 6% of blasts. The karyotype revealed poor quality of chromosomes but the clonal change with a loss of one chromosome from C group was evident in 60% of mitoses. The FISH analysis with the BCR/ABL probe was negative both for Ph' chromosome and trisomy 8, but the CEP7 probe revealed the monosomy of chromosome 7 in 80% of interphase nuclei.

The administration of nilotinib was stopped. Two months later, her bone marrow was hypocellular with less dysplasia than at the previous examination but with the rise in blast count (12%). The cytogenetic examination confirmed -7 in all analyzed cells (20/20) and the absence of trisomy 8 (Fig 1D). Real time PCR (RQ-PCR) for bcr-abl revealed deep molecular response, MR4.

Further evaluation after a month, in April 2014, revealed leukemic progression and development of AML (30% of blasts) confirmed by flow cytometry immunophenotype (HLA-DRmed, CD34high, CD117med, CD13high, CD33med, CD7+). She was treated with the antileukemic treatment (3+7 regimen) without success, followed by the „salvage“ protocol FLAG-Ida without achieving any morphological or cytogenetic response. Unfortunately, she died in aplasia during the treatment.

**Discussion**

Our case demonstrates highly diverse karyotype changes appearing one after another in three unrelated clones during the treatment with tyrosine kinase inhibitors. Complex aberrations in the Ph-positive clone emerged during the management with imatinib, while +8 and -7 appeared separately in Ph-negative clones during the nilotinib treatment.

Karyotype changes in Ph+ clone emerged 40 months after the imatinib therapy was started, as the only sign of disease relapse. This distinctive karyotype included complex translocation and the rare event of centromere fission which were previously published [10,11]. Negative search for mutations by Sanger sequencing in the kinase domain further contributed to the complexity of the case.

Only after the introduction of a more potent TKI treatment, nilotinib, the Ph+ clone slowly decreased, but trisomy 8 in the Ph-negative cells appeared. The CCgR was achieved after 30 months on nilotinib, while +8 remained and existed in up to 30% of the analysed cells during the next 2.5 years of follow up.

Nota bene, the Ph-negative clones are less frequent in patients treated with second generation TKIs and after failure of imatinib, due to a higher pressure on leukemic and residual normal hematopoiesis [12]. However, when present, their type and frequency are very similar to those seen in patients on imatinib, as well as their incidence and effect in evolution to MDS/AML [12].

Our patient developed secondary AML after 66 months of the nilotinib treatment. The cytogenetic and FISH analysis revealed -7 in 60% of metaphases and 80% of interphase nuclei, respectively, along with the absence of BCR/ABL and +8. The clone with -7 quickly progressed to 100% of the analyzed cells in two months, while RQ-PCR still showed stable MR4. Despite introducing a high-dose therapy for AML, she died 6 months after the diagnosis of the secondary AML had been established.

Ph-negative clones with -7 were described in the CML cases with high propensity to evolve into MDS/AML [13]. However, there have been rare cases with -7 without disease evolution [4,5,14].

In several studies, factors contributing to the appearance of chromosomal aberrations in the Ph-negative clone have been discussed. The previous cytotoxic treatment [13], the negative
effect of TKIs on DNA repair mechanisms [15-17] or the innate genetic instability in the CML marrow [18] are described as potential causes of the Ph-negative clone appearance. However, among all the abnormalities, only those involving chromosome 7 (del(7q) and/or -7) bear a higher risk of secondary malignancies [5,19]. We can conclude that while the patient was in a stable chronic phase of CML, complex chromosomal aberrations in the Ph-positive cells might reflect a highly unstable genome which could contribute to a further lower sensitivity to a subsequent alternative treatment and thus, negatively affect overall survival.

Other parameters that could lead to the development of MDS/AML are: pretreatment with interferon or hydroxyurea, persistent aberration in the Ph-negative clone and clone size >50% [19]. Unfortunately, our patient had all the negative features mentioned above in developing a secondary malignant disease.

During the treatment with TKIs, it is highly important to reveal biological diversity of Ph-negative clones, which in some patients can lead to disease transformation (clone with -7) while in others do not have the propensity towards secondary hematological malignancy (clone with +8). Minimal investigations should include blood test results (cytopenia), bone marrow morphology (dysplastic changes and blast count) and cytogenetic (evidence of Ph-negative clones and -7). In cases with the additional Ph-negative clones, further evaluation of changes with the FISH and real time PCR analyses are highly recommended.

Conclusion

The evolution of karyotype and the occurrence of diverse clones arising from stem cell level in our patients warrants the need for thorough follow-up and evaluation of all related hematological and biological findings during the treatment with tyrosine kinase inhibitors, including the standard karyotype, although, some study groups tend to omit any bone marrow evaluation in the current monitoring schedule.

Acknowledgment

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References


Title and legends to figures

Karyotypes from a bone marrow metaphase of the patient showing different clones (TT – time of therapy):

**Fig. 1A** 46,XX,t(9;22)(q34;q11) At presentation
**Fig. 1B** 46,XX,t(5;6;12)(q14?q21?q23?),t(9;22)(q34;q11) Imatinib (400 mg), TT 36 m
**Fig. 1C** 47,XX,+8 Nilotinib (800 mg), TT 6 m
**Fig. 1D** 45,XX,-7 Nilotinib (800 mg), TT 66 m

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