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Case Report

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Co-expression of the CBFβ-MYH11 and BCR-ABL fusion genes in chronic myeloid leukaemia

Coexistenţa genelor de fuziune CBFβ-MYH11 şi BCR-ABL în leucemia mieloidă cronică

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Abstract

The coexistence of t(9;22) and inv(16) has been described in a very limited number of cases of CML, de novo or therapy-related AML. We report a patient with CML who presented both inversion of chromosome 16 and Philadelphia chromosome and evolved towards the blast phase under treatment with Imatinib. Laboratory diagnosis and monitoring was made by flow cytometry, conventional cytogenetics and molecular genetics techniques. The inv(16), detected by karyotyping in the Philadelphia chromosome positive clone at the moment of the blast transformation, was retrospectively assessed by means of real-time PCR, and was proved to have been present since diagnosis. The bone marrow biopsy performed in the blast phase of CML confirmed the presence of blasts belonging to the myeloid lineage, with indications of monocytic differentiation, frequently associated with inv(16). Moreover, the case also associated a F359V tyrosine kinase domain mutation, resulting in intermediate resistance to Imatinib and Nilotinib, which imposed therapy-switch to Dasatinib. In our case the evolution was progressive, followed by death due to lack of response to tyrosine kinase inhibitors, 18 months after diagnosis. The coexistence of t(9;22) and inv(16) in CML seems to be associated with an aggressive clinical evolution and resistance to tyrosine kinase inhibitor therapy. Due to the very small number of cases described in literature, therapeutic decisions are still difficult for patients displaying these abnormalities.

***Keywords**: chronic myeloid leukaemia, Philadelphia chromosome, inv(16), tyrosine kinase domain mutation*

Rezumat

Coexistenţa t(9;22) şi a inv(16) a fost descrisă într-un număr limitat de cazuri de LMC, LAM de novo sau LAM post chimioterapie. Raportăm un pacient cu LMC care a prezentat atât inversie de 16 cât şi cromozom Philadelphia şi care a evoluat spre criză blastică sub tratament cu Imatinib. Diagnosicul de laborator şi monitorizarea s-a realizat prin citometrie în flux, citogenetică convenţională şi tehnici de genetică moleculară. Inv(16), detectată prin cariotipare în clona Philadelphia pozitivă la momentul transformării blastice, a fost evaluată retrospectiv

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prin metoda real-time PCR, şi s-a dovedit a fi fost prezentă încă de la diagnostic. Biopsia de măduvă osoasă, efectuată în faza blastică a LMC, a confirmat prezenţa blaştilor aparţinând liniei mieloide, cu indicii de diferenţiere monocitoidă, frecvent asociată cu inv (16). De asemenea, cazul a asociat şi mutaţia F359V în domeniul kinazic al ABL, care determină rezistenţă intermediară la Imatinib şi Nilotinib, ceea ce a impus schimbarea terapiei cu Dasatinib. În cazul prezentat evoluţia a fost progresivă, urmată de deces ca urmare a lipsei de răspuns la inhibitorii de tirozin kinază, la 18 luni de la diagnosticare. Coexistenţa t(9; 22) şi inv(16) în LMC pare a fi asociată cu o evoluţie clinică agresivă şi rezistenţă la terapia cu inhibitori de tirozin kinază. Având în vedere numărul foarte mic de cazuri descrise în literatura de specialitate, deciziile terapeutice în cazul pacienţilor care prezintă aceste anomalii sunt încă dificile.

***Cuvinte cheie**: leucemie mieloidă cronică, cromozom Philadelphia, inv(16), mutaţii în domeniul kinazic al ABL*

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Introduction

The inversion of chromosome 16 - inv(16) - leads to fusion of the core binding factor β (CBFβ) gene at 16q22 with the smooth muscle myosin heavy chain (MYH11) gene at 16p13 region, leading to the formation of the CBFβ-MYH11 fusion gene, coding for a chimeric protein which inhibits the differentiation of hematopoietic cells by altering transcriptional regulation (1, 2). Inv(16)(p13;q22) or the variant t(16;16)(p13;q22) is found in approximately 10-12% of acute myeloblastic leukaemia (AML) cases and is frequently associated with myelomonocytic differentiation and/or abnormal eosinophils (AML-M4Eo, according to the French-American-British classification, or AMML Eo, according to the World Health Organization classification).

The Philadelphia chromosome represents the distinctive cytogenetic marker of chronic myeloid leukaemia (CML). It results from a balanced translocation between chromosomes 9 and 22, with the formation of the BCR-ABL fusion gene coding for a chimeric protein with tyrosine kinase activity. Philadelphia chromosome is also present in 25% of the acute lymphoblastic leukaemia adult patients, as well as in 1% of the de novo AML patients (3).

The co-existence of t(9;22) and inv(16) was reported in a very limited number of cases with CML (especially in the CML blast crisis), de novo AML, and in a few cases with therapy-related AML as a side effect of cytostatic therapy (4-11). Therefore the ability to differentiate between the two types of diseases (CML and AML) that can concomitantly associate these two chromosomal rearrangements remains essential, in order to accurately evaluate the patients' evolution and prognosis and in order to elaborate appropriate treatment plans.

In this study we present a CML case displaying both inv(16) and t(9;22) during the evolution of the disease, we analyse particularities of the disease's evolution and treatment and compare them with similar reported cases.

Materials and methods

Conventional cytogenetics

Bone marrow specimens were cultured for 48 hours (Chromosome Kit M, EuroClone) and then processed according to the manufacturer's specifications using methanol/acetic acid fixation. After slide pre-treatment (1 hour at 80°C) and G banding (trypsin, Wright-Giemsa staining), 20 metaphases were examined with a Nikon Eclipse E6000 microscope and karyotyped with the CytoVision software (Applied Imaging USA).

RNA extraction

Total RNA was extracted from $2x10^7$ nucleated cells of peripheral blood. Erythrocytes were lysed on ice, using Red Blood Cell Lysis solution (Promega Inc, Madison, WI, USA) and the RNA extraction was performed using 1 mL of guanidine thiocyanate reagent (RNAzol® RT, Sigma-Aldrich, US) according to the manufacturer's specifications. Dilutions were prepared down to 500 ng/µl.

Reverse transcription

Reverse transcription was performed on 2 µg of RNA (4 µl of 500 ng/µl RNA solution) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific), according to the manufacturer's specifications. cDNA was diluted to 1/5 with 80 µl nuclease free water for a concentration of 20 ng/µl (100 µl final volume).

Quantitative assessment of BCR-ABL P210 expression

5 µl cDNA (equivalent of 100 ng ARN) were amplified using Translocation Kit t(9;22)/M-BCR-RQ (Experteam, Italy), according to the manufacturer's specifications, on the LightCycler 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany).

The expression of BCR-ABL transcript was appreciated by calculating a ratio between the number of copies of the interest gene and a ABL reference gene. Samples with Ct bigger than 28 for the ABL gene were excluded from analysis.

Identification of the CBFβ-MYH11 fusion gene expression

In order to qualitatively identify the CBFβ-MYH11 transcript, amplification was performed with a forward primer on the CBFβ gene (CBFβ – CATTAGCACAACAGGCCTTTGA) and three reverse primers on the MYH11 gene (MYH11-1 - AGGGCCCGCTTGGACTT, MYH11-2 - CCTCGTTAAGCATCCCTGTGA, MYH11-3 – TCTTTCTCCAGCGTCTGCTTAT) (12) using GoTaq MasterMix (Promega Inc, Madison, WI, USA). The PCR protocol included a 3 minutes initial denaturation at 95°C, followed by 35 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 60°C and 60 seconds elongation at 72°C, with a final elongation of 10 minutes at 72ºC.

Samples were visualized by electrophoresis in 2% agarose gel stained with ethidium bromide, using a Syngene G:Box Chemi imaging system and the GeneSnap software.

To evaluate the expression of the CBFβ-MYH11 transcript throughout the evolution of the disease, a SYBR Green quantitative PCR amplification was performed on a LightCycler 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany). ABL gene was separately amplified as an internal control. For quantification we used standards from Ipsogen and the CBFβ and MYH11-1 primer sets, also used in the qualitative determination during diagnosis.

Immunophenotyping by Flow Cytometry

After detection of inv(16) and the occurrence of blasts in peripheral blood, we evaluated by flow cytometry, from a peripheral blood sample, the immunophenotypic expression of the following markers: CD3 (surface and intercellular), CD4, CD8, CD13/33, CD45, CD34, CD117, CD64, CD56, HLA/DR, CD36, CD15, CD16, CD11b, CD123, CD66c, CD61, CD41a, CD7, CD2, CD20, IgM, TdT, CD79a and myeloperoxidase (MPO). Specimen processing was performed according to the lyse-wash protocol: incubation of cells with monoclonal antibodies (15 minutes) at room temperature was followed by red blood cell lysis (FacsLysys, BD Biosciences, San Jose, USA) for 10 minutes and washing with phosphate-buffered saline solution. Precursors were gated based on their CD45 versus right-angle light scatter specific distibution.

Data were collected using a BD FACSAria III instrument (BD Biosciences, San Jose, USA). Data analysis was performed with the FACS Diva software, version 6.1.2 (BD Biosciences, USA).

Ethics

The study protocol was approved by the Ethics Committee of the Regional Institute of Oncology Iasi and informed consent was signed by the patient.

Results

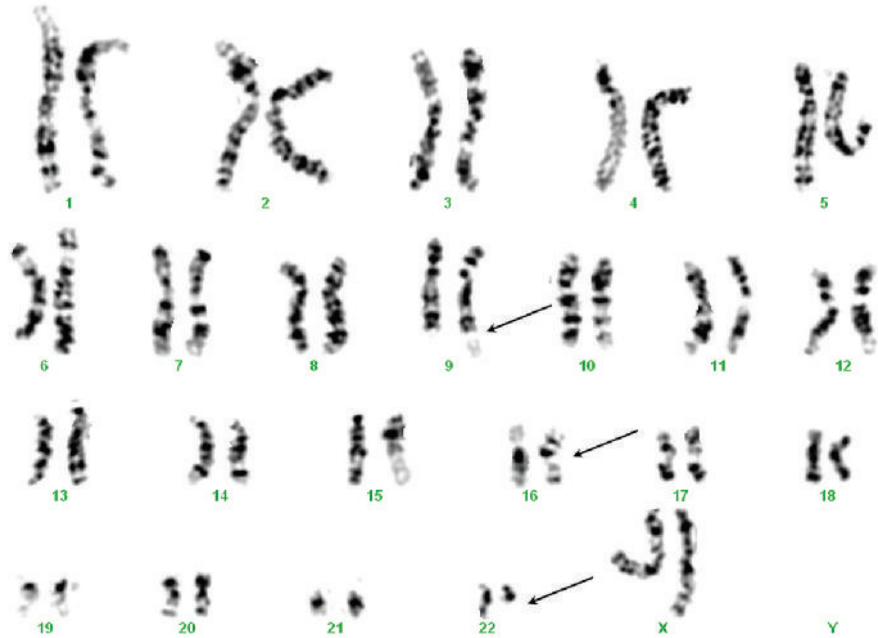
Case report

A 41 year old female patient, without significant personal medical history, was investigated in April 2013 at the Hemato-Oncology Unit of the Regional Institute of Oncology Iasi for profound physical asthenia, headaches and fatigue, which started a month earlier. Initial hematological parameters showed: anaemia (Hb = 8.7 g%), hyperleukocytosis (WBC = 233.350/mmc) and normal platelets (Pl = 326.000/mmc). The differential blood count revealed 2% blasts, 2% promyelocytes, 11% myelocytes, 7% metamyelocytes, 16% band cells, 47% segmented neutrophils, 7% eosinophils, 2% basophils, 6% monocytes, 10% lymphocytes. Abdominal ultrasound revealed splenomegaly (165/83 mm). No medullar morphology modifications were detected at bone marrow examination.

The patient's bone marrow karyotype indicated a chromosomal mosaicism: 46,XX /46,XX,t(9;22)(q34;q11) with the presence of Philadelphia chromosome in 9 of 20 analysed metaphases. Using Real Time PCR for the evaluation of the BCR-ABL transcript we identified the major transcript p210 (b3a2), with a BCR-ABL/ABL ratio of 10% at the moment of diagnosis. The JAK2V617F mutation was not identified. The patient was diagnosed with chronic phase CML and cytoreductive therapy was started with Hydroxyurea (HUR) 500 mg x 4/day with a favourable evolution. The patient was included into the intermediate Sokal risk. The rapid decrease (within a month) of WBC to 38.000/mmc determined the decrease of HUR dosing to 1000 mg/day alternatively with 500 mg/day. In May 2013 therapy with Imatinib (IM) 600 mg was started. The haematological response was obtained after four weeks of treatment with IM and after two months the re-evaluation of BCR-ABL transcript indicated the achievement of major molecular response (ratio BCR-ABL/ABL of 0.05%). After six months of treatment the patient experienced intense pain in the lumbosacral spine region. The MRI pelvic examination revealed an infiltrative aspect in the retrospinal muscle tissue L4-S1. The loss of haematological response was noticed along with an evolution towards blast phase (BP), molecularly objectivised by the increase of BCR-ABL transcript up to 98%. The cytogenetic examination performed at that date revealed the coexistence of the Philadelphia chromosome and an inv(16): 46,XX,t(9,22)(q34;q11.2),inv(16)(p13;q22)[17]/46,XX,t(9,22)(q34;q11.2)[2]/46,XX[1] (Figure 1). This aspect suggests the acquisition of inv(16) by the BCR-ABL positive clone, the double mutant subclone becoming dominant.

Qualitative evaluation of the CBFβ-MYH11 fusion gene indicates a rare transcript version, the E type fusion (Figure 2). Evaluation of the PML-RARα and AML1-ETO fusion genes was negative.

The re-evaluation of previous samples in the attempt to identify the exact moment of inv(16) acquisition revealed that the transcript of the fusion gene was present even since the diagnosis and evolved at the same time with that of the BCR-ABL fusion gene (Table 1). The CBFβ-MYH11 expression was quantified using ABL as reference gene. The evolution of the cytogenetic and molecular abnormalities is summarized in Supplemental material 1.



46,XX,t(9,22)(q34;q11.2),(inv(16)(p13q22)

Figure 1. G-banded karyotype of bone marrow cells showing the coexistence of the Philadelphia chromosome and the pericentric inv (16).

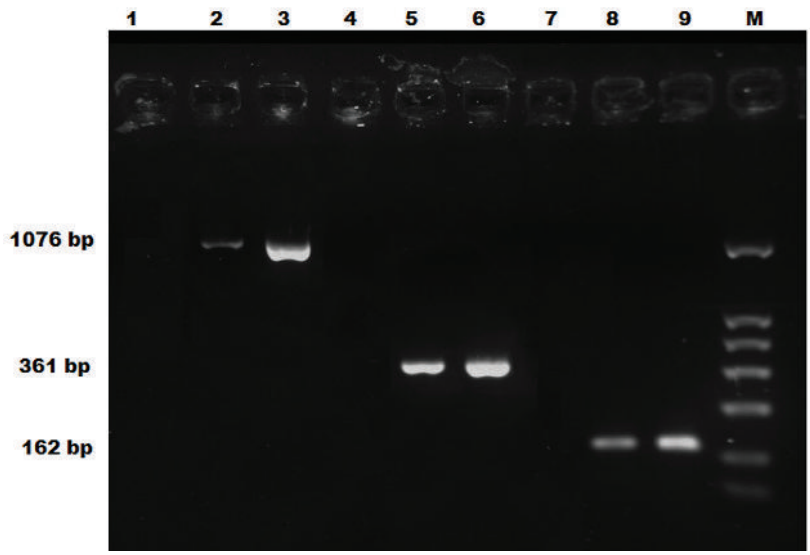


Figure 2. Detection of the CBFβ-MYH11 transcripts by PCR amplification. Lanes 1, 4 and 7 – no template control; lanes 2, 5 and 8 –positive control; lanes 3,6 and 9 - patient sample demonstrating a type E fusion using primers pairs CBFβ-MYH11-1, CBFβ-MYH11-2 and CBFβ-MYH11-3, respectively; lane M - molecular weight standard.

Table I. The main laboratory features of the patient throughout the evolution of the disease

Date	CBFβ-MYH11/ ABL	BCR-ABL1/ ABL	Karyotype	Diagnostic
09.04.2013	0.9%	10%	46,XX/46,XX,t(9,22)(q34;q11.2)	CML-CP
28.08.2013	0.03%	0.05%	46,XX	CML-CP
29.10.2013	0.05%	6.4%	46,XX/46,XX,t(9,22)(q34;q11.2)	CML-CP
03.12.2013	0.58%	97%	46,XX,t(9,22)(q34;q11.2), inv(16)(p13q22)/ 46,XX,t(9,22) (q34;q11.2)/46,XX	CML-BP
17.12.2013	1.79%	98%	-	CML-BP
17.01.2014	0%	3%	-	CML-CP
25.06.2014	0%	0.8%	46,XX	CML-CP
3.09.2014	0.1%	45%	46,XX,t(9,22)(q34;q11.2),(inv(16) (p13q22)/46,XX	CML-BP

CML - chronic myeloid leukaemia; CP- chronic phase, BP- blast phase

The composition of the malignant myeloid clone was immunophenotypically confirmed. The presence of a mixture (70%) of large-sized myeloid - monocytoid precursors presenting the phenotype: CD13/33+ MPO+int CD64-/+(25.7%) CD45+low CD34+/- (68.8%) CD117+/-(71%) CD56-/+ (45.7%), HLA/DR+/- (61.4%) was identified in a peripheral blood sample with 31.480 WBC/mmc. Expression of other investigated markers was negative. The same sample contained 5.5% lymphocytes (3.6% T lymphocytes, 0.9% NK lymphocytes, 1% B lymphocytes), 8% monocytes, 16% granulocytes in various differentiation stages (Figure 3). These immunophenotypic characteristics were suggestive for myelomonocytic differentiation frequently associated with inv(16). In December 2013, the patient was diagnosed with blast phase CML (CML-BP) with myelomonocytic blasts and induction chemotherapy with Cytarabine and Idarubicin (3/7-type induction regimen) was started. BCR-ABL tyrosine kinase domain mutation analysis in peripheral blood revealed the presence of the T1057G mutation, corresponding to the F359V phenotype – a mutation resulting in resistance to IM and consequently Dasatinib therapy was also added to the therapeutic scheme.

In January 2014 complete remission was obtained and the stem cell allogeneic transplantation from sibling donor was indicated. A severe infectious complication has delayed the allotransplant. Six months after starting the treatment (June 2014), a complete cytogenetic response was obtained, as well as a decrease of the transcript level to 0.8%. The patient displayed in evolution the persistence of the pre-sacral lesion (possibly granulocytic sarcoma that could not be observed through biopsy). Since July 2014 the patient has suffered from recurring pleurisy interpreted as a side effect of the Dasatinib treatment. The evolution was progressive, with a new acute episode in October 2014, followed by death due to disease progression and lack of response to tyrosine kinase inhibitors and chemotherapy, 18 months after diagnosis.

Discussion

We report here a CML case that evolved towards BP under treatment with tyrosine kinase inhibitors and for whom the inv(16)(p13q22) detected at the moment of blast transformation

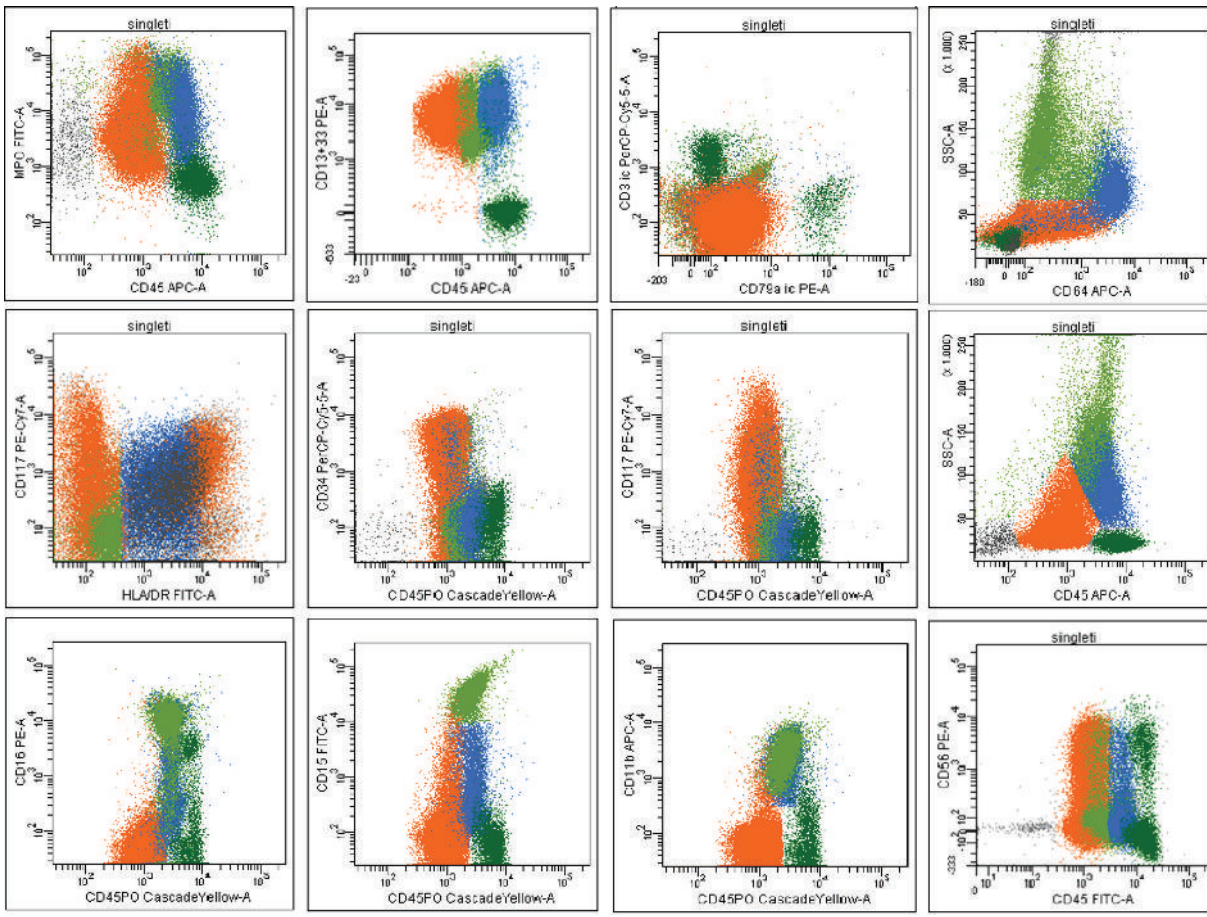


Figure 3. The immunophenotypic profile identified by flow cytometry in the patient's peripheral blood at the moment of blast crisis occurrence: 70% myelomonocytoid precursors (orange), 32% having aberrant CD56 expression. Other cellular populations used as internal control were identified: lymphocytes (dark green), monocytes (blue), pro-monocytes (violet), and granulocytes (light green).

was proved to have been present since diagnosis. Moreover, this case also associated a BCR-ABL kinase domain mutation.

The evolution of CML towards BP is accompanied by clonal evolution with the acquisition of further chromosomal abnormalities, the most common being extra copies of Philadelphia chromosome, trisomy 8 and 19 and isochromosome 17q (13). In myeloid neoplasms, it has been shown that the BCR-ABL rearrangement (class I mutation) can interact with class II mutations (inv(16), t(15;17), t(3;21), t(8;21) and t(7;11)) to cause acute leukaemia. In approximately 0.6% of CML cases, either being present de novo in BP or rapidly progressing towards advanced phases, the Philadelphia chromosome was described in association with CBFβ-MYH11 fusion gene, leading to the formation of a chimera protein, resulting in transcription suppression and deficit in hematopoietic differentiation (14)).

Most cases of accelerated phase or blast crisis CML reported in literature involved the major break point, expressing BCR/ABL1 p210 transcript, while the minor p190 transcript is usually

correlated with AML. However, exceptions from this tendency have been described (15-18). Our patient displayed the b3a2 transcript of BCR/ABL, which resulted in the expression of the p210 fusion protein.

For the CBFβ -MYH11 gene at least 10 different types of transcripts were reported in literature, the most frequently involved being type A (85%), followed by D and E types (5% of cases), the rest of rearrangements occurring quite rarely (19). To the best of our knowledge, our patient is the first reported case of CML associated with inv(16) presenting the E type transcript.

The morphologic traits of CML-BP with inv(16) are similar to those of AML with myelomonocytic differentiation and abnormal eosinophils, making it difficult to discriminate between CML-BP and AML, based on morphological features. In our case, the history of CML-CP and the presence of inv(16) only in Philadelphia positive clone sustain the diagnosis of CML-BP. The bone marrow biopsy performed in CML-BP confirmed the presence of blasts belonging to the myeloid lineage, with indications of monocytic differentiation (morphological traits, immunophenotype) specific to the presence of inv(16), but without abnormal eosinophils or eosinophilia.

The CML cases with inv(16) reported in literature progressed rapidly to BP (15). The average interval from the initial CML diagnosis until the detection of inv(16) and the beginning of BP for patients previously described was of three months (varying between 0 and 173 months) (15). For the patient presented in this study, initially diagnosed with CML with an intermediate Sokal risk, the evolution was rapidly progressive (six months) to CML-BP, probably because the inv(16) was present since diagnosis in a small proportion of the BCR-ABL positive cells. It may be possible that treatment with Imatinib, which acts on BCR-ABL but not on CBFβ-MYH11, had actually selected the double mutant clone, possibly accelerating the acutization process.

The coexistence of t(9;22) and inv(16) in CML seems to indicate an unfavourable prognosis with progression towards BP, with an aggressive clinical evolution and resistance to chemotherapy and tyrosine kinase inhibitors therapy (15, 20). Out of the patients described in literature that were clinically monitored, the vast majority failed to respond to various chemotherapy regimes and clinical remission was attained in rare cases, after bone marrow transplantation or stem cells transplantation (15). Our case also associated a F359V mutation in the BCR-ABL kinase domain, resulting in intermediate resistance to IM and Nilotinib (21). Mutations in the BCR-ABL kinase domain occur in approximately 80% of CML-BP (21). However, in our case, the inv(16) may have contributed to the inefficiency of the IM treatment and involved a distinct pathway of resistance to this type of therapy.

Conclusions

According to our expertise and the cases described in literature so far, inv(16) is a rare event in CML patients and it is associated with a rapidly progressive disease. Our case presented also a F359V tyrosine kinase domain mutation, which worsened clinical prognosis. Due to the very small number of cases described in literature, optimal therapeutic decisions are still difficult for patients displaying these abnormalities.

Acknowledgments

The authors declare that they have no conflict of interest.

Supplemental material is available in the electronic version of this article, on the journal's website: www.rrml.ro

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