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Case report

A case of chronic myeloid leukemia with the m-bcr (p190) molecular rearrangement identified during treatment

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Introduction

Chronic myeloid leukemia (CML) is a hematological malignancy in which a high percentage of patients cytogenetically express the Philadelphia chromosome. This chromosome results from the reciprocal translocation, t(9;22)(q34;q11.2). During this exchange, the *Abelson* gene (*abl*) on chromosome 9 and the *breakpoint cluster region* gene (*bcr*) on chromosome 22 generate the breakpoint cluster region-Abelson murine leukemia (BCR-ABL) fusion gene. The most frequent breakpoint on chromosome 22, located between exons 12 and 16, is called the major breakpoint cluster region (M-bcr) and with its counterpart in exon 2 of chromosome 9 results in the b2a2 and b3a2 transcripts that code for a 210 kDa protein. Less frequently, a second rearrangement, minor breakpoint cluster region (m-bcr), occurs between the first intron of the *bcr* gene and exon 2 of the *abl* gene producing the e1a2 transcript that codes for a 190 kDa protein.[1–4] This fusion transcript occurs in 1–2% of CML patients as a sole rearrangement with the first three cases being described in 1990.[5–7] These patients may have a particular clinical condition between CML and chronic myelomonocytic leukemia (CMML). The third type of transcript is e19a2 which occurs between exons 19 and 20 of the *bcr* gene and exon 2 of the *abl* gene. This is called the micro breakpoint cluster region (μ-bcr) coding for a 230 kDa protein. Among other unusual transcripts are e6a2, e2a2, e1a3, b2a3 and b3a3.[1,2] The objective of this article is to describe a case of CML with a minor p190 molecular rearrangement causing monocytosis in peripheral blood, dysplastic changes in the bone marrow and lack of response to treatment with tyrosine kinase inhibitors.

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Case report

A 65-year-old female had a history of type 2 diabetes mellitus, and a diagnosis of CML made in August 2011 at another institution. At the time of diagnosis, the patient presented splenomegaly, with a white blood cell count (WBC) of 380×10^9/L, hemoglobin (Hb) level of 8.8 g/dL and platelet count of 560×10^9/L. The bone marrow aspirate showed marked granulocytic hyperplasia and a cytogenetic test of 20 metaphases exhibited a t(9;22)(q34;q11) in the entire sample. At that time, neither molecular biology analyses nor fluorescent *in situ* hybridization (FISH) was performed. Therapy with hydroxyurea was prescribed, and in September 2011 the administration of imatinib (400 mg/day) was begun. In October 2011, the red blood count was normal, the WBC was 7.3×10^9/L, Hb was 9.2 g/dL and platelet count was 230×10^9/L; at this time, the patient reported hair loss and edema. In November 2011, imatinib therapy was stopped due to pancytopenia (WBC: 2×10^9/L). In December 2011, the patient was referred to our hospital: she was under no treatment and presented mild splenomegaly. In January 2012, her WBC was 11.9×10^9/L with monocytosis (49% neutrophils, 2% basophils, 28% lymphocytes and 21% monocytes), Hb level was 11.57 g/dL and platelet count was 192×10^9/L. A bone marrow aspirate was performed. It revealed marked bone marrow hyperplasia, and dysplastic changes in the myeloid as well as in the erythroid series.

Cytogenetics showed 46,XX, t(9;22)(q34;q11) in 20 metaphases, and the FISH analysis confirmed the BCR-ABL fusion in 100% of the sample. A nested reverse transcription polymerase chain reaction (RT-PCR)[4] of the bone marrow was positive for the m-bcr rearrangement with a 381 base pair (bp) product in the sample obtained in January 2012. No bands for the M-bcr rearrangement were found. The material from the patient and the control sample were high quality according to the amplification product of the 300 bp *abl* gene. This result was confirmed by sequence analysis. A RT-PCR was performed in order to quantify the number of copies of the m-bcr BCR-ABL transcript. A set of primers and a TaqMan probe (Applied Biosystems) were used for the e1a2 transcript and for the *abl* control gene; negative controls were also included in the assays.[8] Nanogen molecular standards (Nanogen Advanced Diagnostics S.p.A., Corso Torino, Italy) were employed for the calibration curve. The assays were carried out in an Applied 7500 real time PCR system (Applied Biosystems Life). The BCR-ABL and ABL transcripts were analyzed in duplicate, and the number of BCR-ABL transcripts was normalized by the expression of ABL. The copy number variation of the e1a2 transcript in the patient's sample was 94.4%.

The patient restarted imatinib (400 mg/day). Three months later, a bone marrow aspirate showed bone marrow hyperplasia with mild megaloblastic changes and absence of response according to cytogenetics and FISH. The copy number variation of the e1a2 transcript by RQ-PCR was 76.1%. These samples were analyzed to detect if the patient presented mutations in the ABL kinase domain; all were negative.

The administration of imatinib was increased to 800 mg/day without achieving response. In June 2012, imatinib was replaced by dasatinib (100 mg/day) due to the lack of response. Three months later, a new evaluation of the patient revealed a better cytogenetic response. However, after a year of treatment the results of a FISH analysis showed a positive BCR-ABL fusion gene in 85% of the sample. Thus, the treatment was changed to nilotinib 400 mg/12 h. Under this medication, which is ongoing, the pancytopenia persists.

Discussion

The case described was referred to our institution with a diagnosis of CML and under no treatment due to pancytopenia. On admission, the patient's bone marrow was reexamined and the cytogenetic analysis showed Philadelphia chromosomes in all of the metaphases analyzed; this result was confirmed by FISH, which revealed a BCR-ABL fusion gene in 100% of the sample, and by RT-PCR, which identified an m-bcr BCR-ABL mutation with an e1a2 transcript that encodes for a 190 kDa protein. Subsequently, the transcript was quantified by RQ-PCR and a BCR-ABL/ABL ratio of 94.4% was obtained. At the time of diagnosis, most CML patients have an M-bcr BCR-ABL molecular rearrangement corresponding to the b2a2 or b3a2 transcripts. In a large series of patients with CML, the frequency of the m-bcr e1a2 p190 molecular rearrangement was 1–2%.[9,10] The coexistence of M-bcr and m-bcr rearrangements, apparently due to alternative splicing, has also been described.

The red blood count performed at our institution revealed absolute and relative monocytosis with granulocytic hyperplasia and dysplasia of the bone marrow. Several authors report BCR-ABL p190 CML cases with monocytosis, sharing some features with myelodysplastic/myeloproliferative neoplasms (MDS/MPN) due to the differentiation of both lineages, granulocytes and monocytes. Even though the unique feature of the BCR-ABL p190 CML seems to be monocytosis without splenomegaly,[11,12] this entity can also present without monocytosis, with basophilia or with splenomegaly. It seems that there is no characteristic pattern in these cases; it can only be confirmed that some patients present monocytosis at diagnosis or during the course of the disease, and others, with or without splenomegaly, have no monocytosis. In the current case it is not exactly known if the patient had monocytosis at the time of diagnosis or not, but she did present with this feature when she was admitted to our institution.

The prognosis of patients with the BCR-ABL p190 molecular rearrangement in CML is controversial. There are doubts as to whether this rearrangement has a poor prognosis independent of the therapy used,[9,10] or whether patients are candidates for bone marrow transplantation in the case of advanced disease. Twenty-three articles reporting on p190 CML cases were found in the literature with some authors proposing bone marrow transplant in cases of disease progression.[9] The patient herein reported had a rapid response to dasatinib therapy, but according to FISH analysis, after a year of treatment she again presented a positive BCR-ABL fusion gene, which resulted in a change of treatment due to the lack of response. Although the follow-up in this case is short, it can be seen that the BCR-ABL p190 molecular rearrangement is difficult to treat as has already been reported in the literature.

BCR-ABL p190 CML may have unique clinical and biological features. Identifying these patients at the time of diagnosis would allow the optimization of treatment.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood. 1996;88(7):2375–84.
2. Melo JV. BCR-ABL gene variants. Baillieres Clin Haematol. 1997;10(2):203–22.
3. Kurzrock R, Gutterman JU, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemias. N Engl J Med. 1988;319(15):990–8.
4. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia. 1999;13(12):1901–28.
5. Sawyers CL, Timson L, Kawasaki ES, Clark SS, Witte ON, Champlin R. Molecular relapse in chronic myelogenous leukemia patients after bone marrow transplantation detected by polymerase chain reaction. Proc Natl Acad Sci U S A. 1990;87(2):563–7.
6. Selleri L, von Lindern M, Hermans A, Meijer D, Torelli G, Grosveld G. Chronic myeloid leukemia may be associated with several *bcr-ab1* transcripts including the acute lymphoid leukemia-type 7 kb transcript. Blood. 1990;75(5): 1146–53.
7. Zaccaria A, Tasslnarl A, Testoni N, Lauria F, Tura S, Algeri R, et al. Alternative *bcr/abl* transcripts in chronic myeloid leukemia. Blood. 1990;76(8):1663–8.
8. Gabert J, Beillard E, van der Velden VH, Bi W, Grimwade D, Pallisqaard N, et al. 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): 2318–57.
9. Verma D, Kantarjian HM, Jones D, Luthra R, Borthakur G, Verstovsek S, et al. Chronic myeloid leukemia (CML) with P190 BCR-ABL: analysis of characteristics, outcomes, and prognostic significance. Blood. 2009;114(11):2232–5.
10. Pardanani A, Tefferi A, Litzow MR, Zent C, Hogan WJ, McClure RF, et al. Chronic myeloid leukemia with p190BCR-ABL: prevalence, morphology, tyrosine kinase inhibitor response, and kinase domain mutation analysis. Blood. 2009;114(16):3502–3.
11. Melo JV, Myint H, Galton DA, Goldman JM. P190BCR-ABL chronic myeloid leukaemia: the missing link with chronic myelomonocytic leukaemia? Leukemia. 1994;8(1): 208–11.
12. Ohsaka A, Shiina S, Kobayashi M, Kudo H, Kawaguchi R. Philadelphia chromosome-positive chronic myeloid leukemia expressing p190(BCR-ABL). Intern Med. 2002;41(12):1183–7.