

Relato de caso/Case report

Expression of p190 *BCR-ABL* fusion gene in a patient with chronic myeloid leukemia

Expressão do rearranjo gênico BCR-ABL com ponto de quebra na região menor do gene BCR em um paciente com leucemia mielóide crônica

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A minority of chronic myeloid leukemia cases have breakpoints in the minor cluster region (*m-bcr*) of the *BCR-ABL* gene. We report on a patient with Ph-positive and *m-bcr* breakpoint at diagnosis. She was treated with hydroxyurea and interferon-alpha. Two years later, she developed a lymphoid blast crisis and died shortly after. We discuss herein the different forms of the *BCR-ABL* oncogene, its products, and the possible influence of them on the clinical outcome of patients with the disease. Rev. bras. hematol. hemoter. 2003;25(3):173-176.

Key words: *BCR-ABL; chronic myeloid leukemia; Ph chromosome; p190, p210, p230, FISH, RT-PCR.*

Introduction

Chronic myeloid leukemia (CML) is a stem cell malignant disorder¹ characterized by the presence of a translocation between the long arms of chromosome 9 and 22, the Philadelphia chromosome (Ph) and its resultant fusion message.^{2,3} The molecular consequence of t(9;22)(q34;q11) is the formation of two hybrid genes: *BCR-ABL* on the Ph chromosome and *ABL-BCR* on 9q+. The *BCR-ABL* fusion oncogene, the product of t(9;22), exists in three principal forms that arise from distinct breakpoints in the *BCR* gene on chromosome 22. One fusion involves breaks within the major breakpoints cluster region (*M-bcr*) of *BCR*, which includes introns 13 and 14 and leads to the production of an 8.5 kb transcript coding for a 210-kDa (p210) protein. The other fusion gene is the

result of breaks within the first intron of *BCR*, the minor breakpoint cluster region (*m-bcr*) and leads to the expression of a smaller 7.5 kb transcript, which codes to a 190-kDa (p190) protein. Rarely, a breakpoint in the micro breakpoint cluster region (*μ-bcr*) leads to the expression of a large transcript, which codes to a 230-kDa (p230) protein.^{4,5,6,7} In the vast majority of CML patients, as well as in 30% of adult acute lymphoblastic leukemia (ALL) and 20% of childhood chromosome Ph positive ALL, the breakpoint in the *BCR* gene are found within the *M-bcr* region.⁶ Otherwise, in two thirds of ALL cases and in rare cases of CML, the breakpoint in *BCR* gene is found within the *m-bcr* coding a p190-kDa protein.^{5,7} Recently, the World Health Organization (WHO), in conjunction with the European Association of Hemopathology and the Society for Hemopathology, published a new

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classification for hematopoietic and lymphoid neoplasms⁸. The WHO classification of the chronic myeloproliferative diseases recognizes seven entities, and CML is defined not only by its classic morphology and clinical features but also by the presence of chromosome Ph or the *BCR-ABL* fusion gene. There have been very few reports of Ph-positive CML cases with breakpoints outside the *M-bcr* region. We describe a case of Ph-positive with *m-bcr* breakpoint that presented as classical chronic phase CML.

Case report

A 62-year-old woman was admitted in June 2000 with leukocytosis. She had a palpable spleen, but liver and lymph nodes were not palpable. Her hemoglobin level was 15.0 g/dl, platelets count $363.0 \times 10^9/l$ and white blood cell count $73.0 \times 10^9/l$ with 60% neutrophils, 3% eosinophils, 2% basophils, 5% lymphocytes, 7% monocytes and 0% blasts cells. The bone marrow aspirate was hypercellular with granulocytic hyperplasia. Cytogenetic analysis (Figure 1) showed in all 20 cell metaphases analyzed: 46, XX, t(9;22)(q34;q11). Fluorescence *in situ* hybridization (FISH) was performed on interphase nuclei obtained at diagnosis, using a Vysis® ES *BCR-ABL* probe (Downers Grove, IL, USA). This probe showed one fusion signal (*ABL-BCR*) on the derivative chromosome 9 and the other fusion signal (*BCR-ABL*) on the translocated derivative chromosome 22 (Figure 2), indicating a breakpoint within the *m-bcr* region of the *BCR* gene. This finding was confirmed by reverse transcriptase-polymerase

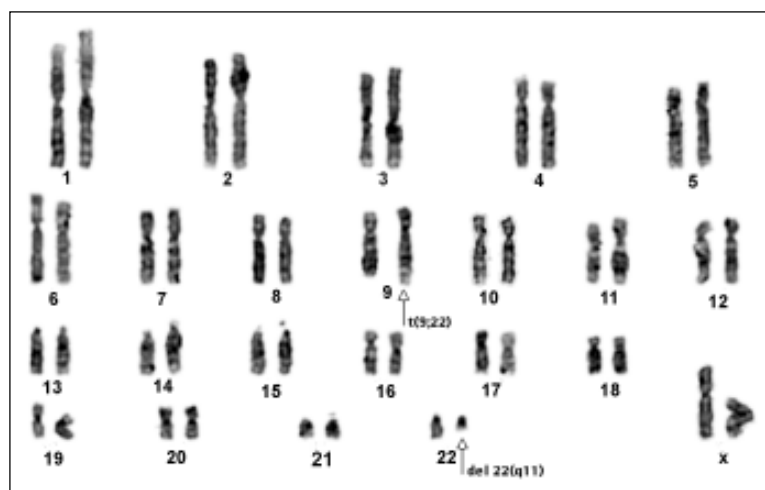


Fig. 1- G-banding karyotype showing 46, XX, t(9;22) (q34;q11)



Fig. 2 - Interphase fluorescence *in situ* hybridization analysis in chronic myeloid leukemia patient, using specific probes for *ABL* (●) and *BCR* (●) loci (ES *BCR-ABL*, Vysis, Downers Grove, IL, USA), exhibiting an abnormal nucleus with a fusion *BCR-ABL* gene with breakpoint within the minor region of the *BCR* gene (two yellow signals)

chain reaction analysis (RT-PCR) (Figure 3). The patient was treated with hydroxyurea and interferon-alpha (IFN- α), achieving a hematological response and minimal cytogenetic response (83% Ph-positive chromosome in 24 cell metaphases analyzed). IFN- α was interrupted in March 2002 due to side effects. In September 2002, bone marrow aspirates showed infiltration by 95% blast cells that were negative for the cytochemical stains Sudan Black and peroxidase. Immunophenotyping (positivity for CD10, CD19, HLA-DR and TdT antibodies) demonstrated the B-cell precursor nature of the blasts. STI 571 treatment was added with no evidence of response. The patient died three months later.

Discussion

Ph-positive CML with *BCR-ABL* rearrangement in the *m-bcr* region appears to be a very rare disease. To our knowledge, there have been very few cases reported. The common feature of CML cases described with breakpoints in *m-bcr* region is an absolute and relative monocytosis.⁹ The clinical features and cell morphology initially

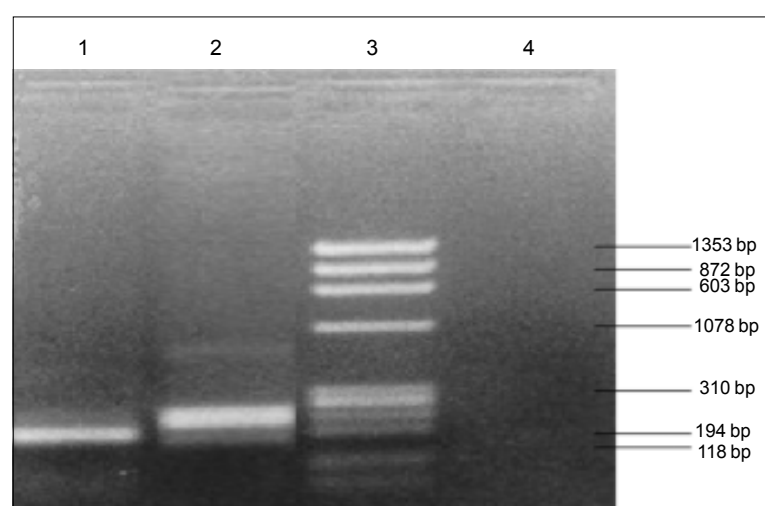


Fig. 3 – Ethidium bromide-stained 2% agarose gel showing reverse transcriptase-polymerase chain reaction products. Lane 1: patient reported with chronic myeloid leukemia, lane 2: positive control (patient with acute lymphoid leukemia), lane 3, molecular weight markers fX 174RF, and lane 4, negative control (H_2O)

suspected the diagnosis of CML in our patient. Her clinical evolution was typical of CML and she had no monocytosis. The clear association of different forms of the *BCR-ABL* oncogene with distinct types of leukemia requires a biological explanation.¹⁰ The m-*bcr* breakpoint might be frequent in B lymphoid progenitor cells but uncommon in hematopoietic stem cells that explains the rarity of p190 *BCR-ABL* rearrangements. In CML patients, the Ph chromosome is present in myeloid, erythroid, megakaryocytic and B lymphoid cells, confirming that the translocation between chromosome 9 and 22 took place in a very early multipotential cell.¹⁰ Shaoguang LI et al (1999) used a mouse bone marrow transduction/transplantation system for a direct comparison of the leukemogenic properties of the three principal forms of *BCR-ABL*. They found no significant difference in the ability of the three oncogenes to induce a CML-like myeloproliferative syndrome, but observed that p190 *BCR-ABL* had increased potency for induction of B lymphoid leukemia.¹⁰ Some reports suggested that the p190 *BCR-ABL* CML results in a more aggressive disease,⁵ but this issue is controversial. The fact of our patient's survival was no longer than 30 months gives support to the postulate that m-*bcr* CML determines a more rapid outcome for patients with CML. Therefore, we suggest that m-*bcr* rearrangements should be analyzed in all CML cases. In addition, further studies are required to evaluate

whether p190 protein characterizes a particular subtype of CML or a novel entity.

Resumo

A leucemia mielóide crônica (LMC) é uma doença mieloproliferativa clonal e caracteriza-se pela presença da translocação cromossômica entre os braços longos dos cromossomos 9 e 22, o denominado cromossomo Ph. Esta translocação determina a fusão dos genes *BCR* e *ABL*. Os diferentes pontos de quebra no gene *BCR* determinam a síntese de proteínas com diferentes pesos moleculares pelo gene *BCR-ABL*. Nós relatamos o caso de uma paciente portadora de LMC com ponto de quebra cromossômico na região menor do gene *BCR*. Foi tratada com hidroxiuréia e interferon alfa. Dois anos após o diagnóstico desenvolveu crise blástica linfóide e evoluiu rapidamente para o óbito. Nós discutimos nesta apresentação as diferentes formas do gene *BCR-ABL* e seus produtos e a possível influência dos mesmos na evolução clínica dos pacientes com a doença. Rev. bras. hematol. hemoter. 2003;25(3):173-176.

Palavras-chave: *BCR-ABL*; leucemia mielóide crônica; cromossomo Ph, p190, p210, p230, FISH, RT-PCR.

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