De novo acute B-cell acute Lymphoblastic Leukemia with BCL2/IGH and BCR/ABL1 rearrangements

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ABSTRACT

T(14;18)(q32,q21) and t(9;22)(q34;q11) translocations, leading to BCL2/IGH and BCR/ABL1 rearrangements, respectively, are common genetic aberrations in hematological malignancies. Particularly, t(14;18)(q32;q21) is the genetic hallmark of follicular lymphoma, while t(9;22)(q34;q11) is commonly rearranged in acute lymphoid leukemia (ALL) and chronic myeloid leukemia. Nevertheless, their association has never been described. We report the first case of acute lymphoid leukemia (ALL) in which both BCL2/IGH and BCR/ABL1 rearrangements were present. The patient presented with pre-B ALL, achieved molecular complete remission with intensified chemotherapy, then reinforced with autologous stem cell transplantation, relapsed after a few months, and unfortunately died 17 months after diagnosis. Of note, only BCL2/IGH but not BCR/ABL1 was detected at relapses.

Key words: B-acute lymphoid leukemia, BCR/ABL1, t(14;18)(q32,q21), BCL2, Philadelphia chromosome, apoptosis, Imatinib, targeted therapy

Introduction

The t(14;18)(q32;q21) translocation is the most common translocation in B-cell malignancies; in particular, it is found in about 90% of follicular lymphomas, being the chromosomal hallmark of this tumor, and in about 20-25% of diffuse large B-cell lymphomas(1–4). Only a few cases of de novo B-acute lymphoid leukemia (B-ALL) carrying t(14;18)(q32;q21) have been described(5–13). Most of these cases presented with additional chromosomal abnormalities, often involving band 8q24 and/or MYC rearrangement and had a very aggressive clinical course(5,6,8,9,12). Central nervous system (CNS) involvement seems to be a frequent event, despite of adequate prophylaxis. The association between t(14;18)(q32;q21) and BCR/ABL1 rearrangement has
never been described in ALL. We report on a de novo B-ALL carrying both t(14;18)(q32;q21) with BCL2/IGH fusion and BCR/ABL1 rearrangement.

**Methods**

**Cytogenetics**

Short term cultures from bone marrow samples were performed at diagnosis and during the follow-up. Metaphases were analyzed after G-banding with Wright’s stain. Karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN 1995)(14–16).

**FISH**

FISH was performed on fixed cells. Directly labeled BCR and ABL probes (Vysis, Inc), producing a split of red signal when ABL is involved in genetic rearrangements. FISH data were collected with a fluorescence microscope (E 1000, Nikon Instruments) equipped with a CCD camera and Genikon software (Nikon Instruments). Two hundred nuclei/cells were analyzed for each experiment.

**Molecular evaluation of BCL2/IGH rearrangement**

Molecular evaluation was based on nested PCR(17). Mononuclear cells from BM and PB samples were obtained by Ficoll-Hypaque density gradient centrifugation. Genomic DNA was isolated from mononuclear cells using the QiAamp DNA mini kit (Qiagen, Hilden, Germany)(18). DNA integrity was assessed by amplifying a 510 bp fragment of the Beta-globin gene. Samples positive for Beta-globin were then investigated for the BCL2/IGH rearrangement using a nested PCR specific for MBR and mcr breakpoints. The first round of amplification was done using 1 microg of genomic DNA and the following primers: 5’–CAGCCTTGAAACATTGTAGG–3’ (forward, for MBR), 5’– CGTGCTGTACCACTCCTG–3’ (forward, for mcr) and 5’– ACCAGGGTTCGGCCA–3’ (reverse, for the JH consensus region). An initial denaturation step of 5 min at 95°C was followed by 30 cycles (denaturation: 40 sec at 95°C; annealing: 30 sec at 60°C, and extension: 30 sec at 72°C). The RNA solution was then incubated for 42 min at 45°C in a 20 L reaction mixture containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 5.5 mM MgCl2, 1 mM of each deoxyribonucleotide, 20 U of RNAsin (Pharmacia, Upsala, Sweden), 25 microM random hexamers (Pharmacia, Upssala, Sweden), 10 mM of DTT (Pharmacia, Upssala, Sweden), and 100U of MoMLV reverse transcriptase (BRL, Bethesda, MD). After incubation, cDNA solution was diluted 1:5 to 50 microL final volume. The cDNA integrity was assessed by amplifying a 296 bp fragment of the ABL1 gene. Samples positive for ABL1 were then investigated for the BCR/ABL1 rearrangement by qualitative PCR. Five microLs of cDNA were PCR-amplified using the following set of primers: EA500 5’ TGTGATTATAGCCTAAGACCCGGAG 3’, and R112 5’ TTGTCTGTTCCGGAGGCCACC 3’. Thirty-five cycles of PCR were performed as follows: denaturation (30 sec at 96°C), annealing (30 sec at 60°C), and extension (30 sec at 72°C). Samples were tested twice, and both positive and negative controls were included in all experiments.

Amplified products were visualized on a 2% agarose gel stained with ethidium bromide (19). REF

**Case report**

In July 2020, a 40-years-old woman, presenting only with moderate fatigue, was diagnosed with pre-B ALL, L2 subtype. The peripheral blood count showed: Hb 9.3 g/dl; WBC 17x109/L; PLT 56x109/L. The bone marrow aspirate was hypocellular with 80% of lymphoid blasts. The karyotype was: 46,XX, del(6)(q21q25), t(9;9)(p11;q22), t(14;18)(q32;q21)(10/20). The immuphenotype, assessed by flow cytomtery, was: CD19+, CD22+, TdT+, CD20-, CD3-, CD10-.
The molecular analysis carried out by PCR confirmed a BCL2/IGH rearrangement (mcr breakpoint) but also unveiled a BCR/ABL1 (E1-A2/p190) rearrangement. Thus, FISH analysis was also performed. The probe for BCR/ABL1 dual fusion gene gave two green signals and two red signals as expected from samples not carrying the ABL1 rearrangement. Molecular analysis was then repeated confirming the previous results. We administered a standard induction therapy (doxorubicine, vincristine, L-asparaginase, and prednisone plus imatinib), and an intensified consolidation therapy (idarubicine and high dose cytarabine) obtaining a molecular complete remission (CR). Particularly, neither BCL2/IGH nor BCR/ABL1 rearrangements were detected. Other 2 consolidation courses were then administered (BFM-B regimen, including vincristine, ifosfamide, methothrexate, teniposide, high dose cytarabine, and dexamethasone; and BFM-A regimen, including vincristine, doxorubicine, cyclophosphamide, high dose methothrexate, and dexamethasone) associated with imatinib. Bone marrow harvest and autologous bone marrow transplantation were then performed, lacking a HLA-matched donor. Twelve months after the first documentation of CR, the patient relapsed. The bone marrow aspirate was hypercellular with 90% of leukemic cells. The karyotype was: 46 XX, t(1;5)(p32;q31), del(12)(p11;p13)(14/15); the molecular analysis conducted by PCR showed the BCL2/IGH rearrangement, whereas there was no evidence of the BCR/ABL1 fusion transcript. Salvage therapy with liposomal daunorubicin and intermediate dose cytarabine (23) was then administered, obtaining a second molecular CR (disappearance of BCL2/IGH). Two months later, a second relapse occurred. The karyotype was: 46 XX, t(1;5)(p32;q31), del(12)(p11;p13)(29/30). The molecular analysis showed again only the BCL2/IGH rearrangement, without evidence of the BCR/ABL1 fusion gene. Despite of neuro-meningeal prophylaxis, there was clinical evidence of CNS involvement. Compassionate treatment with campath-1H, 30 mg/dose, for 5 doses, was administered i. v., obtaining a peripheral blood blast clearance, but not a CR. The patients eventually died 17 months after diagnosis due to leukemic progression.

**Discussion**

BCR/ABL1 and BCL2/IGH rearrangements are common molecular abnormalities in B-cell malignancies. In particular, the BCR/ABL1 rearrangement is the most frequent genetic aberration in adult B-ALL (20–22). On the other hand, t(14;18)(q32;q21) with BCL2/IGH rearrangement is the most common abnormality in tumors derived from peripheral B-lymphocytes, whereas it is absolutely rare in B-cell precursor malignancies (24). However, while the biological role of BCR/ABL1 in acute leukemia is at least partially well known (25), the significance of BCL2 in ALL is still largely indefinite. BCL2 overexpression, without BCL2/IGH rearrangement, is frequent in ALL, and does not seem to be associated with a poorer prognosis (26). On the contrary, t(14;18)(q32;q21) and BCL2/IGH rearrangement are a rarity in ALL, but are associated with very aggressive tumors. Morphologically, the described cases are often L3, according to their immunophenotype of mature B-ALL, with Burkitt-like features. Notably, in all cases, complex karyotypes were observed, with almost constant involvement of the 8q24 locus and MYC deregulation (5–13). Sequential emergence of molecular abnormalities has been proposed in these cases, with progression from indolent (BCL2/IGH positive) to aggressive (BCL2/IGH and MYC positive) B-cell tumors (5–13). Therefore, they most likely represented leukemic variants of high-grade B-cell lymphomas with “double hits”. On the clinical ground, most of the patients presented with rapidly worsening general condition, fever, fatigue, night sweat, and weight loss; massive bone marrow and blood involvement, nodal and extra-nodal infiltration were also present. Clinical course was aggressive, with a median overall survival usually below than 12 months (5–13).

To the best of our knowledge, the association between t(14;18)(q32;q21) and BCR/ABL1 rearrangement has not been previously described in ALL. Nevertheless, a case of co-existing

BCR/ABL1 and BCL2/IGH rearrangements was reported in a MDS case (27). Our patient presented with a pre-B ALL, L2 subtype, carrying the t(14;18)(q32;q21) and other additional chromosomal aberrations, such as del(6)(q21;q25) and t(9;9)(p11;p22) but lacking 8q24 involvement; the BCR/ABL1 rearrangement was documented only by molecular analysis. Clinical course was aggressive, with recurrent relapses, CNS involvement, and death within seventeen months. Interestingly, at relapse, the patient presented a different karyotype [t(1,5)(p32;q31), del(12)(p11;p13), quite common as secondary abnormalities], still showing the BCL2/IGH rearrangement. Furthermore, during the clinical history of the patient, other chromosomal aberrations appeared. The relationship between the molecular events, and even a possible sequential appearance cannot be established. No peculiar morphologic or immunophenotypic patterns can be identified, to be easily associated to either one translocation, and the bad prognosis could be conferred by both the main genetic alterations; however, a dominant role of BCL2/IGH should be hypothesized, since it was always present during all disease phases. In this regard, based on the lack of cytogenetic evidence of Philadelphia chromosome we cannot exclude that BCR/ABL1 rearrangement constituted a sub-clonal lesion, cleared out by the more specific targeted therapy (chemotherapy plus imatinib).

Certainly, the treatment of t(14;18)(q32;q21) positive...
ALL remains a major problem, as conventional therapy are scarcely effective. Probably, the highly proliferating phenotype is made highly insensitive to chemotherapy by the antiapoptotic effect of BCL2, as observed in high-grade B-cell lymphomas with double hits.

The present case, besides its unicity, also confirmed the importance of molecular testing after cytogenetic analysis in human leukemia. Future experiences and hopefully trials will be useful to improve the current treatment of t(14;18)(q32;q21) positive ALL by adopting more rationally targeted therapies such as BCL2 inhibitors (e.g. venetoclax), peroxisome proliferator-activated receptor-gamma ligands (28), or others.

References


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