



De novo acute myeloid leukemia harboring concomitant t(8;21) (q22;q22);RUNX1::RUNX1T1 and BCR::ABL1 (p190 minor transcript)

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Received: 15 November 2021 / Accepted: 22 July 2022 / Published online: 28 October 2021
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Abstract

De novo AMLs with typical nonrandom chromosomal abnormalities are often associated with specific morphology subtypes. The t(8;21) is one of the most prominent recurrent cytogenetic aberrations (RCA) in AML, frequently associated with AML with maturation, and is characterized as a good prognostic marker. On the contrary, BCR::ABL1 rearrangement is rarely observed in AMLs, without specific morphology, carrying poor prognosis. Its distinction from blastic transformation of chronic myeloid leukemia has been a matter of long debate. The revised WHO classification (2016) recognized AML with BCR::ABL1⁺ as a provisional entity. The occurrence of additional cytogenetic aberrations in AML RCA within the same leukemic clone has been detected, albeit rare cases of BCR::ABL1⁺ were reported, mainly as subclones. Those additional cytogenetic and molecular findings seem to significantly affect patient prognosis. Conventional cytogenetic analysis, fluorescent in situ hybridization (FISH), and polymerase chain reaction (PCR) were applied at presentation and during the follow-up of the patient. We present a 34-year-old male patient with de novo AML harboring concomitant t(8;21) and t(9;22) in a single clone. The presence of both t(8;21) and Philadelphia chromosome (Ph⁺) in the same metaphases but in less than 100% of the analyzed cells, the p190 BCR::ABL transcript type, and absence of splenomegaly support that additional BCR::ABL1⁺ is a part of the main leukemic clone. These findings, accompanied with an encouraging outcome of continuous cytogenetic and molecular remission after induction therapy, support BCR::ABL1 being a secondary genetic event in AML with t(8;21).

Keywords Acute leukemia · Philadelphia chromosome · Translocations · Aberrations

Introduction

In the revised WHO classification (2016), it was agreed that structural chromosome rearrangements (t(8;21) (q22;q22);RUNX1::RUNX1T1 and inversion inv(16) (p13q22)/t(16;16)(p13;q22);CBFB::MYH11) represent “class II mutations” responsible for suppressed and/or altered differentiation as the main leukemic driver genetic event in core binding factor (CBF) leukemias [1, 2].

Those mutations are assumed to cooperate with other “class I mutations” to obtain increased proliferation within the mutated leukemic clone. It is very rare that BCR::ABL1 as a “class I mutation” interacts with “class II mutations” in CBF leukemias, predominantly with CBFB::MYH11 and mainly in the form of subclones [1, 2].

De novo BCR::ABL1 positive (BCR::ABL1⁺) acute myeloid leukemia (AML) was included as a provisional entity in the 2016 revised WHO classification of myeloid

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malignancies and is rare [1, 3]. Its distinction from blast transformation of chronic myeloid leukemia (CML) has been a matter of debate for a long time. Increasing evidence suggests BCR::ABL1⁺ AML is a distinct group, characterized by lack of abnormal blood counts, lack of basophilia, absence of splenomegaly, and no other recurring cytogenetic aberrations present [1–3].

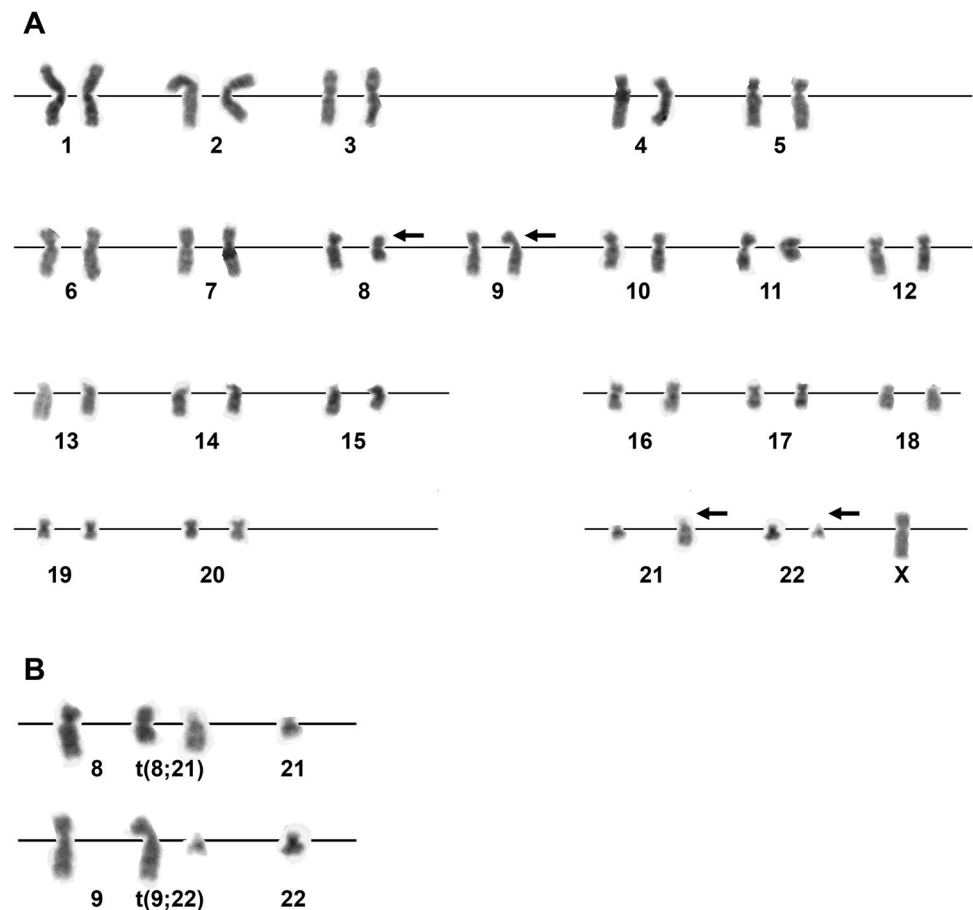
Herein, we report a case of de novo AML which presented with RUNX1::RUNX1 and BCR::ABL1 rearrangements in the same clone. Clinical and molecular characteristics of the patient, as well as the therapy approach, are compared with similar cases and discussed further.

Clinical history and the results

A 34-year-old male patient was admitted to the hospital in January 2017 with a 2-week history of fever, weakness, and fatigue. Diagnostic workup revealed leukocytosis ($34 \times 10^9/L$), leukemic gap (blasts 84%), and thrombocytopenia ($27 \times 10^9/L$). Splenomegaly and basophilia were absent. Bone marrow analysis revealed myeloperoxidase⁺ myeloblast population with Auer rods (76% of nucleated cells) and the diagnosis of AML with maturation was

suggested. Leukemic population expressed immunophenotypic features typical for early granulocytic differentiation (CD34^{high}, CD117P^{hetero}, HLA-DR^{high}, CD13^{intermed}, CD33^{low}, cMPO^{intermed}, CD15P^{hetero}) with an aberrant expression of CD56⁺ and CD19⁺ antigens. All other investigated antigens associated with B-cell lineage (cyCD79a, CD10, smCD22) as well as T/NK lineage (cyCD3, CD7, CD2, CD16) were negative, ruling out mixed phenotype leukemia. Immunophenotype diagnosis of AML with granulocytic differentiation/CD19⁺CD56⁺ was established and correlation with AML with t(8;21) was suggested, due to aberrant expression of CD19 antigen. Cytogenetics revealed concomitant presence of the t(8;21) and t(9;22) in 18/20 analyzed metaphases (Fig. 1). The karyotype was described as 45,X,-Y,t(8;21)(q22;q22),t(9;22)(q34;q11) [18]/46,XY [2]. Both translocations were confirmed by interphase FISH analysis and RT-PCR, of which the latter detected a p190 BCR::ABL1 fusion transcript. At the same time, RT-PCR analysis for the NPM1 and FLT3 genes was negative. Diagnosis of possible high-risk AML with RUNX1::RUNX1 and BCR::ABL1⁺ was established [4, 5]. The induction chemotherapy “3 + 7” with imatinib (400 mg/day) resulted in cytomorphologic, cytogenetic, and molecular remission. Moreover, no minimal residual disease by flow cytometry

Fig. 1 **a** Karyogram of the patient shows 45,X,-Y,t(8;21)(q22;q22),t(9;22)(q34;q11). Arrows indicate abnormal chromosomes. **b** Partial karyogram showing t(8;21) and t(9;22) in a single clone



was detected in the bone marrow (<0.01% of nucleated cells), nor in the peripheral blood (<0.01% WBC) [4–6]. After completion of consolidation therapy, the patient underwent related sibling allogeneic stem cell transplantation (SCT). He was followed up every 6 months and remained in complete molecular remission for both transcripts, up to current 48 months since diagnosis.

Materials and methods

Cytogenetic analysis

A cytogenetic study was performed on unstimulated bone marrow cells using a standard technique. Giemsa-banded metaphases were analyzed, and the result was reported according to the International System for Human Cytogenomic Nomenclature, 2020 [7].

FISH analysis

FISH analysis for the translocations of t(9;22) and t(8;21) (BCR::ABL1 and RUNX1::RUNX1T1) was performed on at least 100 bone marrow interphase nuclei, according to the manufacturer's instructions (Vysis/Abbott Laboratories, Des Plaines, IL).

PCR analysis

The PCR analysis was performed on bone marrow cells. BCR::ABL1 and RUNX1::RUNX1T1 fusion transcripts were detected using a reverse transcriptase polymerase chain reaction [8]. Mutational analysis was carried out on genomic DNA isolated from mononuclear cell preparations using a QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's recommendation. *NPM1* exon 12 mutations were detected by PCR and the products were purified using a QIAquick PCR Purification Kit (Qiagen) and directly sequenced on a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using NPM1-1112R as previously described [9]. FLT3-ITD mutations were detected using PCR and FLT3-D835 mutations via PCR followed by digestion with EcoRV restriction enzyme as previously described [9–11].

Immunophenotyping by flow cytometry

Immunophenotyping by flow cytometry (BD FACSCalibur, BD CellQuest Pro Software, San Jose, USA) was performed on a native bone marrow specimen (BD Vacutainer tube, K2EDTA) by applying of 4-color panel of commercial monoclonal antibodies and direct immunofluorescent technique, according to proposals of European LeukemiaNet

[12]. Leukemic blast cells were analyzed as a population according to specific CD45 antigen expression pattern and side scatter characteristics. Immunophenotype profile was defined according to the WHO classification [1].

Discussion

De novo AML may show typical nonrandom chromosomal abnormalities often associated with specific morphology subtypes. The t(8;21) is one of the most prominent recurrent aberrations in AML, frequently associated with AML with maturation, and is characterized as a good prognostic marker [5]. Conversely, BCR::ABL1 rearrangement is rarely observed in AMLs, without specific morphology, carrying poor prognosis [1].

We present a case of 34-year-old man with de novo AML harboring concomitant t(8;21), RUNX1::RUNX1T1, and Philadelphia chromosome, BCR::ABL1 (p190 minor transcript), accompanied with the loss of Y chromosome in the same metaphases, defining and confirming a single clone. Beside the aberrant clone, two normal metaphases were present as well.

Bacher et al. described two patients with the diagnosis of de novo AML with t(8;21) and concomitant presence of t(9;22) as subclones at diagnosis with the loss of sex chromosomes in one of them [3]. One can speculate that in those cases, the t(8;21) appeared first, followed by the t(9;22) in subclones as the disease progressed. One patient achieved complete hematologic and molecular remission before stem cell transplant and remained in full molecular remission after 22 months of follow-up. The other patient relapsed after 10 months and after achieving second remission underwent transplant and remained in full donor chimaerism for 70 months of follow-up. When compared to our patient, those patients were of similar age (38 and 41) and had the same morphology subgroup (AML with maturation/former FAB M2), and all three had the p190 BCR::ABL transcript type. However, our case had the concomitant occurrence of t(8;21) and t(9;22) in the same clone.

Similarly, Gupta et al. published an interesting case with morphological presentation corresponding to AML associated with t(8;21), confirmed by immunophenotypic profile. Karyotype showed the presence of t(8;21) and loss of sex chromosome in 55% metaphases and t(1;1) in 5% metaphases. However, there was no detection of Philadelphia chromosome. Further nested PCR analysis confirmed the presence of RUNX1::RUNX1T1 transcripts [13]. After treatment, morphological remission was achieved, but with abundance of dwarf megakaryocytes and mild eosinophilia subsequently leading to full re-evaluation. This confirmed dominant RUNX1::RUNX1T1 transcript, but also the presence of lesser p190 BCR::ABL1 transcript in diagnostic

specimens. Gupta et al. postulated their case as “double hit” leukemia.

Additionally, Najfeld et al. reported a late occurrence of t(9;22), confirmed by FISH and PCR in a second relapse in the patient with AML M2 with t(8;21) [14]. They showed that t(9;22) appeared as a late event together with del(5q) (p13), without previous detection of t(9;22) in karyotype in the first relapse.

Finally, in meta-analysis, Neuendorff et al. analyzed all published cases of AML with BCR::ABL1 mutation present (from 1975 to 2016) and provided deeper analysis on how to discriminate AML cases from other BCR::ABL1⁺ leukemias, mainly the transformation of CML, including additional cytogenetic aberrations and RCA [3]. They reported three already published cases of t(8;21) AML with occurrence of BCR::ABL1⁺ (2.4% or 3/126 cases of AML with BCR::ABL1) [2, 14]. Their algorithm provides basic requirements needed for discrimination between BCR::ABL1⁺ AML and CML in blastic phase, based on the absence of antecedent hematologic abnormality, absence of splenomegaly, no basophilia, less than 100% BCR::ABL1⁺ metaphases, and p190 transcript type, but irrespective of the presence of RCA.

The classification of our patient’s AML may appear controversial. According to the current WHO AML classification criteria, the presence of AML with maturation and specific immunophenotype (e.g., CD19+, CD56+), together with karyotype revealing t(8;21), favors the diagnosis of AML with RCA [1, 15]. However, our case is unique in that our patient had de novo AML harboring concomitant t(8;21) and t(9;22) in a single clone, producing rare mutual leukemogenic interaction. This is different from previously published cases carrying t(9;22) only as subclones [2, 13, 14]. Moreover, our case fits all algorithm features from Neuendorff’s article, but has the t(8;21) [3].

We believe that better understanding of biology in all patients harboring BCR::ABL1 in the future will explain deeper molecular interactions in these rare entities.

Funding All diagnostic procedures and treatment were covered through National Health Insurance Fund of Serbia.

Declarations

Ethical approval Publication of this case was approved by the Ethics Committee of the University Clinical Center of Serbia, No. 410/6, from 15.09.2021. The research was conducted in accordance with the principles of Declaration of Helsinki and in accordance with local statutory requirements.

Consent to participate The patient provided written informed consent.

Consent for publication The patient provided written consent for publication.

Conflict of interest The authors declare no competing interests.

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