A Rare Case of Pericentric Inversion, Inv(9)(p13q34) of the Der(9)t(9;22)(q34;q11) in A Patient with Chronic Myeloid Leukemia – A Case Report

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ABSTRACT

As we know, the Philadelphia chromosome (Ph) is a highly specific marker for chronic myeloid leukemia (CML). This hematological disease is characterised by the formation of the BCR/ABL1 fusion gene, usually with typical translocation pattern including 9q34 and 22q11. In this paper we describe a 55 years old female patient with typical clinical and haematological signs of CML and a chromosome 9 differing from that which normally participates in translocation t(9;22). The karyotype of this Ph positive patient is characterised by pericentric inv(9)(p13q34) of the der(9)t(9;22)(q34;q11). Reverse transcriptase-polymerase chain reaction revealed a e14a2 type of BCR/ABL1 fusion transcript. As a consequence of this unusual translocation, FISH also found the separation of the ABL1/BCR1 fusion gene on chromosome 9. On reviewing the literature, to date only 10 Ph-positive leukemia patients have been noticed to have pericentric inversion inv(9)(p22q34)der(9)t(9;22)(q34;q11). No one case has been described with pericentric inversion inv(9)(p13q34) of the der(9)t(9;22)(q34;q11). This indicate that pericentric inv(9)(p13q34) of the der(9)t(9;22)(q34;q11) is a novel, rare, chromosomal abnormality in Ph-positive CML.

Keywords: Chromosome 9, CML, Ph chromosome, pericentric inversion.

Submitted: November 15, 2021

Published: December 13, 2021

ISSN: 2593-8339

DOI: 10.24018/ejmed.2021.3.6.1144

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I. INTRODUCTION

It has long been known that chronic myeloid leukemia (CML) is a hyperproliferation of all circulating cell lines in the blood except lymphocytes. It is diagnosed by the presence of the BCR-ABL1 fusion gene, which is most often associated with the translocation t(9;22)(q34;q11). This chromosomal rearrangement leads to the well-known BCR-ABL1 fusion, formed by the combination of two normal ABL1 and BCR genes which encodes a 210-Kd (P210) fusion protein. This protein has tyrosine kinase activity and cause inhibition of apoptosis resulting in excessive white blood cells production [1]. Cytogenetic and molecular anatomy showed that BCR and ABL1 genes are located normally at the chromosomes 22 and 9. By rearranging the chromosome segments in the translocation t(9;22)(q34;q11), the BCR gene from chromosome 22 will be fused to the ABL1 gene derived from chromosome 9 resulting in the formation of the BCR-ABL1 fusion gene [2], [3]. On the other hand, as a result of

reciprocal chromosomes translocation, terminally at the long arm of chromosome 9, ABL1-BCR will be formed [2]. The BCR-ABL1 fusion gene and its expression product, play a key role in the neoplastic transformation of the Philadelphia chromosome (Ph) positive clone. In addition to the knowledge that ABL1 enzyme activity is a fundamental factor in the creation and maintenance of the neoplastic phenotype, little is known of the reciprocal chimeric ABL-BCR1 gene, formed at chromosome 9q [3].

Despite the major breakthrough in the knowledge of the molecular events underlying the t(9;22) translocation, still no consistent data have been found on the correlation between those two chimeric genes, and what happens in case there is no ABL1-BCR fusion during and after chromosomes translocation process.

II. CASE REPORT

In December 2020, the 55-old female patient with pronounced leukocytosis was hospitalised at the Department of Hematology, University Clinical Center Tuzla, Bosnia and Herzegovina. Initial blood cells revealed: white blood cells count (WBC) 120.000/L, hemoglobin (HB) 144 g/L, erythrocytes (RBC) $4.8 \times 10^{12}/L$, and platelets (PLT) $320 \times 10^9/L$. A differential white blood count revealed an increased number of monocytes, eosinophils, basophils, metamyelocytes and promyelocytes. Physical examination showed palpable spleen enlargement. For the establishment of diagnosis histopathologic, flow cytometry, cytogenetic, fluorescent in situ hybridization (FISH) and molecular analyses were performed.

Histopathologic examination of bone marrow biopsy cylinder showed about 98.0% cellularity, noticeable maturation, significantly suppressed erythroid lines, where it was present after immunohistochemical staining about 2.0% of cells positive for glycophorin A, while the rest are made up of all cell forms of myeloid lineage positive for myeloperoxidase (MPO). Coloring for CD34 was displayed 2.0% myeloblasts. Megakaryocytes were mostly hypolobular, more chromatic nuclei, on average represented by 7/1HPF. Out of the other cellular composition, rare small, non-neoplastic CD3+ T and CD20+ B lymphocytes were founded. Using Jones methenamine silver (JMS) histochemical stain was not shown the multiplication of the reticular network of the bone marrow.

A flow cytometry test revealed the most dominant population in the bone marrow aspirate consists of granular myeloid cells that represent 90.0% of the cell composition of the sample. In this population, 86.0% of the cells express the CD16 + maturation marker. Erythroid precursors (low SS, CD71 + and CD45-) represent 3.0% of sample cells.

No cells with SS/45 blast characteristics were found in a proportion greater than 5.0%.

Conventional cytogenetic analysis was performed on bone marrow cell culture, directly and over-night. Chromosomes were prepared and GTG banded (G-bands by trypsin and Giemsa), according to the standard laboratory procedure. An abnormal female karyotype with der(9)t(9;22)(q34;q11.1) inv(9)(p13q34) was found in all 20 examined metaphases (Fig. 1).

Fig. 1. GTG-banding revealed a karyotype 46,XX, der(9)t(9;22)(q34;q11.1) inv(9)(p13q34). Derivative chromosomes 9 and 22 are indicated by arrows.

We also analysed 200 metaphase and interphase cells nuclei using fluorescent in situ hybridisation (FISH) technique, with specific *BCR-ABL1* translocation probe (Vysis, Downers Grove, IL, USA). Dual-color FISH analysis demonstrated the presence of an amplification of *BCR-ABL1* rearrangement at locus 22q11, but absence of the amplification of *ABL1-BCR* sequence at locus 9q34. An interphase nuclei FISH signal pattern was also atypical (2R2G1F) (Fig. 2a, 2b).

Fig. 2. a. BCR/ABL1 D-FISH probe (Vysis) showing a split green and red signals from BCR and ABL1 within the derivative chromosome 9 (red and green arrows). There is only 1 fusion signal located at der(22), one green signal at normal chromosome 22, and one red signal at normal chromosome 9. b. A Representative interphase cell with hybridisation LSI BCR-ABL1 D FISH probe showing one fusion BCR-ABL1 signal at der(22) and two separate green and red signals which corresponds hybridization LSI BCR-ABL1 fluorescently labeled probe with ABL1 and BCR sequence at derivative chromosome 9. There are also one red and one green signal at the normal chromosomes 9 and 22.

Qualitative and quantitative real time polymerase chain reaction assays were utilized for the identification and measurement of *BCR-ABL1* fusion gene. RNA was extracted from 2×10^7 leukocytes using Trizol RNA isolation method, according to [4]. Complementary DNA (cDNA) was synthesized using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, CA, USA), in accordance with the manufacturer's instructions. RT-PCR was performed according to standard in house laboratory procedure. The PCR analyze revealed a e14a2 type of *BCR-ABL1* fusion transcript.

On the basis of all performed laboratory tests, the patient was diagnosed to have chronic myeloid leukemia (CML), and imatinib therapy was initiated at a dose of 400 mg/day.

In order to assess the molecular response to therapy and determine the *BCR-ABL1* copy number (minimal residual disease), quantitative analyses of RNA was carried out using a Light Cycler 2.0 device (Roche diagnostics, Mannheim, Germany). For the *BCR-ABL1* amplification we used Roche Master mix (LC Fast Start DNA MasterPlus hybridisation probes, Roche diagnostics, Mannheim, Germany), containing Ipsogen primers (BCR-ABL M-bcr Fusion Kit, Ipsogen/Qiagene, Marseille, France). All results were calculated with conversion factor (CF) and expressed according to the international scale (International harmonization of Scale-IS) [5], [6]. The *BCR-ABL1/ABL1* ratio was 74.35% (IS) at diagnosis. Five months later, on the first QRT-PCR evaluation, the *BCR-ABL1/ABL1* ratio was decreased to 1.34% (IS). The third follow-up, in August 2021 (eight months) showed the presence of *BCR-ABL1* gene as a percentage of 0.15% (IS). At the time of last follow-up, in November 2021, (<12 months) the *BCR-ABL1/ABL1* ratio was 0.036% which corresponds to major molecular response–MMR (3-log reduction from a baseline) [5].

III. DISCUSSION

The female patient we describe here, presenting *BCR-ABL1*-positive typical CML with characteristic karyotype abnormality der(9)t(9;22)(q34;q11)inv(9)(p13q34). On reviewing the literature, it was found a very similar pericentric inv(9)(p22q34) of the derivative chromosome 9, resulted from a standard translocation t(9;22)(q34;q11.2) [7], [8].

Pan and the group of authors from China [7] described four cases with this very rare chromosome aberration in a population of 2,200 Philadelphia chromosome (Ph)-positive leukemia patients. The three of them are CML patients in chronic phase and one with acute myeloid leukemia (AML). Those patients had two malignant independent clones, one with translocation t(9;22)(q34;q11.2) and another with der(9)t(9;22)(q34;q11.2)inv(9)(p22q34). The last-one chromosomal rearrangement resulted in the separation of the ABL1/BCR fusion gene. FISH analysis they performed, in the AML patient, also revealed a deletion of partial sequence of BCR on der(9)t(9;22)(q34;q11.2)inv(9)(p22q34).

Another group of authors also described the same chromosomal aberration in four cases with AML and CML associated with poor prognosis [8].

The patient we present here, carrier of pericentric inversion inv(9)(p13q34) of the der(9)t(9;22)(q34;q11), with described morphological, histochemical, immunohistochemical and molecular analysis support a complete bone marrow infiltration by myeloproliferative neoplasm which corresponds to chronic myeloid leukemia. Fluorescence in situ hybridization analysis demonstrated the presence of an unusual signal pattern of hybridization *BCR-ABL1* probe [9]. We found only one fusion signal at der(22), while at the derivative chromosome 9, instead fusion orange signal we expected, separate red and green signals were noticed (Fig. 2a,2b). It has been observed that the red signal is at the long arm of derivative chromosome 9 (9q34), while the green one

is at its short arm. It unequivocally proves that the separation of the *ABL1-BCR* fusion gene, we noticed at 9q, has occur as a consequence of the chromosome 9 inversion.

Variation in FISH signal patterns include the loss of sequences proximal 9q and distal 22q breakpoint. In the Ph positive population, about 71.9% cases displayed the typical D-FISH signal pattern, while 10.4 % of them showed atypical FISH signal patterns [9].

In a study conducted by Pan and co-workers [7], patients received hydroxyurea, combined chemotherapy and imatinib. Despite the treatment, three patients, including one with AML and two with CML died (median survival time 25 months). Only one patient with CML remained alive for 5.5 months. Inversion inv(9)(p22q34) found in this patients, correlate with poor prognosis. The presented patient initially started to receive tyrosine kinase inhibitor imatinib in daily dose 400mg. In the first three months of follow-up, a hematologic response was achieved. Eleven months after therapy was started, November 2021, the *BCR-ABL1* ratio was 0.036% (IS) which corresponds to major molecular response –MMR (3-log reduction from the standardized baseline), which is an ideal response to the tyrosine kinase inhibitor treatment [6].

Early molecular response (EMR) achievement improves the disease burden and the overall survival (OS) and progression free survival (PFS) in the patients with chronic myeloid leukemia. There is also a lower transformation rate to accelerated/blast phase of CML [10].

If we compare molecular with cytogenetic reports, there is no negative outcome implies that inv(9)(p13q34) of the der(9)t(9;22)(q34;q11.2) has an unfavorable impact on the disease prognosis [11].

IV. CONCLUSION

In conclusion, we present a case diagnosed to have chronic myeloid leukemia with very rare chromosomal abnormality. To our knowledge, this is the first observation of the pericentric inversion inv(9)(p13q34) of the der(9)t(9;22)(q34;q11.2) associated with Philadelphia translocation, reported in Bosnia and Herzegovina and internationally. This case also illustrates, the importance of cytogenetic, FISH and molecular testing in patients with unusual chromosomal changes. There is a need for further continue molecular monitoring, especially in terms of the disease prognosis.

CONFLICT OF INTEREST

Authors declare that they don't have any conflict of interest.

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