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Case Report

Oncology Section

Novel Cytogenetic Aberrations in a Patient of Chronic Myeloid Leukemia with Blast Crisis

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ABSTRACT

Chronic myeloid leukaemia (CML) is a clonal haematological disease which is characterized by a diagnostic karyotypic abnormality t (9;22)(q34;q11) called as Philadelphia (Ph) chromosome. Occurrence of additional chromosomal abnormalities besides the Ph chromosome is defined as clonal evolution (CE) and considered to be a marker of disease progression. A 67-year-old male who was initially evaluated at a private hospital where a diagnosis of acute promyelocytic leukaemia was made on bone marrow aspirate with ambiguous RT-PCR report referred to our centre for further evaluation and treatment. On conventional karyotyping, Ph chromosome along with translocations t(5;13)(q12;p13), t(15;20)(q22;p13) and monosomy 13 was observed in all 20 metaphases. A final diagnosis of CML-myeloid blast crisis with complex cytogenetics was made. Patient succumbed to death within one month of initiation of imatinib therapy.

Keywords: Complex cytogenetics, t(5;13)(q12;p13), t(15;20)(q22;p13), Monosomy 13

CASE REPORT

A 67-year-old male came to Medical Oncology Department of All India Institute of Medical Sciences, New Delhi with chief complaints of generalized weakness, progressive pallor, breathlessness on exertion and syncope for 2 months. Patient was a known case of coronary artery disease. He had undergone coronary angioplasty 6 months before the presentation. He was initially evaluated at a private hospital where a diagnosis of acute promyelocytic leukaemia was made on bone marrow aspirate. RT-PCR report was ambiguous for acute promyelocytic leukaemia. Patient was referred to our centre for further evaluation and treatment. The haemoglobin was 85g/L, total leukocyte count $1.5X10^6$/L and total platelet count $18X10^6$/L. Peripheral smear showed pancytopenia and 40% blasts. Bone marrow aspirate smears showed predominantly myeloid series of cells with 20% blasts and many promyelocytes. There was no basophilia. Flow cytometric evaluation of bone marrow showed 20% blasts which were immunopositive for CD45, CD34, cMPO, CD13, CD33, HLA-DR, CD15,CD36 and CD64; negative for cCD3, CD4, CD5, CD7, CD8, CD10, CD14, CD19, CD22, CD117, cCD79a and CD123. A repeat RT-PCR reaction for PML-RARα transcript was negative. On conventional karyotyping, Philadelphia chromosome along with translocations t(5;13)(q12;p13), t(15;20)(q22;p13) and monosomy 13 was observed in all 20 metaphases. A final diagnosis of CML-myeloid blast crisis with comlex cytogenetics was made. With underlying heart disease, advanced age and poor performance status (PS4), imatinib therapy alone was considered instead of standard acute myeloid leukaemia like induction therapy. Patients' family did not give consent for additional special investigations like fluorescent in situ hybridization, array comparative genomic hybridization (CGH) to explore these novel translocations (as karyotyping can reveals balanced translocations, whereas additional atypical unbalanced sub-chromosomal aberrations and the cryptic/microscopic aberrations can be picked up by CGH). Patient succumbed to death within one month of initiation of imatinib therapy.

DISCUSSION

In CML progression to advanced phase is accompanied by a severe block in differentiation and apoptosis. BCR-ABL is directly or indirectly responsible for progressive genomic instability or epigenetic changes, which occur at the CML stem cell level and/or in later CML progenitor cells. The degree of genomic instability is proportional to the level of BCR-ABL kinase activity. CML stem cells are the least vulnerable to ABL-targeted therapy and may serve as reservoirs for occult CML progression. Together, these phenomena conspire to bring about an acquired loss of hematopoietic cell differentiation, resulting in a highly aggressive, acute leukemia. The aberrant cellular activities of BCR-ABL including increased proliferation through the activation of *ras*, increased transcriptional activity via STAT recruitment, decreases in apoptosis through activation of PI(3)K/AKT12 and changes in adhesion binding to actin with phosphorylation to cytoskeletal proteins contribute to progression of chronic phase to advance phase [1]. Occurrence of additional chromosomal abnormalities besides the Ph chromosome is defined as clonal evolution (CE) and considered to be a marker of disease progression. It reflects the genetic instability of the highly proliferative CML progenitors. Secondary chromosomal abnormalities are non-random and include an additional Ph- chromosome, trisomy 8, 9, 19, 20 or 21; isochromosome 17; monosomy 7 and deletion of the Y chromosome [2-5]. These chromosomal aberrations are designated as major-route additional chromosomal aberrations which include frequently observed abnormalities and minor-route additional chromosomal aberrations which include rarely observed aberrations such as t(3;12), t(4;6), t(2;16), and t(1;21). Though clonal evolution (CE) can occur in any phase of CML, its frequency increases with advancing stage rising from 30% in accelerated phase to 80% in blast crisis [2,3]. Better to omit this sentence as similar sentence is mentioned above in the discussion part. All chromosomes are involved in CML-BC, however chromosomes 17, 2, 8, 16 involvements are the most frequent [6].

Prognostic significance of CE is heterogeneous and dependent on the time of occurrence, type of cytogenetic aberration, type and time of initiation of therapy but overall it is a poor prognostic factor and represents multistep progression of CML [2,3,7]. Major route additional chromosomal aberrations particularly with complex karyotypic abnormalities are observed to have poor prognostic impact while minor-route additional chromosomal aberrations which occur sporadically might not affect prognosis [3]. Prognostic impact affects time to complete cytogenetic remission (CCR), major molecular remission, Progression-free survival (PFS) and Overall

Survival (OS). Imatinib, a selective inhibitor of the BCR-ABL tyrosine kinase achieved major or complete cytogenetic remissions in 60% of CML patients who were intolerant or refractory to prior IFN therapy and up to 87% in newly diagnosed patient [8] but clonal cytogenetic aberrations play a significant role from the development of imatinib resistance.

The progression of CML from chronic phase (CP) to blast crisis (BC) is frequently associated with non-random secondary chromosomal aberrations [2]. In addition to Ph chromosome, two additional novel chromosomal alterations: t(5;13)(q12;p13) and t(15;20)(q22;p13) were detected in our patient. Initial presentation of disease in blast crisis, existing cardiac condition and old age put patient in more fragile condition to tolerate heavy induction regimen of blast crisis. Cyclin B1 gene, annotated at 5q12, is a member of the cyclin family of proteins and regulates cell cycle through its action on cyclin-dependent kinases [9]. Aberrant cytoplasmic expression of cyclin B1 has been observed in various human malignancies and reported to influence prognosis [10]. The 15q22 locus is the site for PML gene [11], which is a transcription factor as well as tumour suppressor and regulates the p53 response to oncogenic signals. Ito et al., observed high levels of PML expression in CD34+ blasts of CML and reported that patients with low PML expression had better complete cytogenetic and molecular response as compared to those with high PML expression [12]. In our patient, promyelocytes found in bone marrow might be due to high PML expression as a result of translocation t(15;20)(q22;p13). Deletion of chromosome 13 which is a relatively common aberration in myeloid disorders is found in accelerated phase of CML [13]. Common region of deletion is identified at 13q12-14 [14]. Deletions of genetic material from a specific area of chromosome 13 particularly q14, which is a site for miR 15a/16-1 has been strongly linked to the development of haematological malignancies such as chronic lymphocytic leukaemia, acute myeloid leukaemia, myeloproliferative disorders and myelodysplastic syndromes. Gao et al., described miR 15A/16-1 might function as tumour suppressor gene by down regulating WT1 oncogene [15]. The three cytogenetic abnormalities detected in this patient namely, t(5;13)(q12;p13), t(15;20)(q22;p13) and monosomy 13 leading to aberrations in cyclin B1, PML gene and microRNAs could be responsible for such aggressive and chemo-refractory nature of the disease in this case. Treatment of blast crisis depends on the type of blast cells (myeloid/lymphoid). Apart for Imatinib treatment of blast crisis included acute leukemia induction-type treatment and various single agents (ara-C, thioguanine, vincristine/prednisone etc.) as appropriate [16].

Our patient died after one month of hospital discharge, which can be explained by primarily due to probable effect of novel translocations i.e., reduced expression of cyclin B1 and high levels of PML expression along with presence of monosomy 13. Moreover, reduced expression of cyclin B1 might have contributed to the possible imatinib resistance which this patient was offered in view of higher risk involved with standard AML like induction treatment. Additionally diagnosis at advanced stage, old age and cardiac ailment altogether might have contributed significantly to the mortality

CONCLUSION

Novel translocations t(5;13)(q12;p13) and t(15;20)(q22;p13) are categorized under minor-route additional chromosomal aberrations which occur sporadically. Reduced expression of cyclin B1 and high PML expression in this patient might contribute progression of disease and probably to imatinib resistance. This suggests minor-route additional chromosomal aberrations might affect prognosis. Further studies are required to ascertain the expression of cyclin B1 and PML gene in patients of CML-blast crisis to explore its therapeutic significance and prognostic evaluation.

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