



CYTOGENETIC CLONAL EVOLUTION IN CHRONIC MYELOID LEUKEMIA: HIGH-ORDER PHILADELPHIA CHROMOSOME DUPLICATION, TRISOMY 8, AND GENETIC COUNSELLING

Pathology

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ABSTRACT

Chronic myeloid leukemia (CML) is a *BCR::ABL1*-positive myeloproliferative neoplasm in which disease progression may occur through molecular resistance, treatment discontinuation, or cytogenetic clonal evolution. Conventional cytogenetics remains clinically valuable because it directly demonstrates the chromosomal architecture of the leukemic clone and identifies additional cytogenetic abnormalities associated with high-risk disease biology. We report a 39-year-old woman with CML who had previously achieved complete cytogenetic response and reported deep molecular response after imatinib therapy, followed by treatment discontinuation. She later presented with pallor, intermittent fever, vomiting, splenomegaly, severe anemia, thrombocytopenia, peripheral blood blasts of 10% & 14% basophils, and bone marrow blasts of 16% with basophilia. GTG-banded metaphase analysis showed re-emergence of a Philadelphia chromosome-positive clone with the karyotype 49,XX,+8,t(9;22)(q34;q11.2),+der(22)t(9;22)(q34;q11.2)x2, indicating trisomy 8 and two additional derivative chromosome 22/Philadelphia chromosome copies. *BCR::ABL1* dual-color dual-fusion FISH demonstrated increased fusion-signal copy number with a pattern consistent with one derivative chromosome 9 and three derivative chromosome 22/Philadelphia chromosomes. These findings confirmed loss of complete cytogenetic response with major-route clonal evolution and high-order Philadelphia chromosome duplication. Under WHO-HAEM5, the findings are best interpreted as CML with high-risk cytogenetic progression after loss of disease control, whereas under ICC 2022, the presence of 10–19% blasts and major-route additional cytogenetic abnormalities supports CML, accelerated phase. This case emphasizes the practical role of cytogenetics and FISH in detecting clinically significant clonal evolution, guiding treatment reassessment, prioritizing molecular testing, and supporting genetic counselling, particularly in resource-limited settings.

KEYWORDS

Chronic Myeloid Leukemia; *Bcr::abl1*; Philadelphia Chromosome Duplication; Trisomy 8; Cytogenetic Clonal Evolution; Additional Cytogenetic Abnormalities; Complete Cytogenetic Response; Accelerated Phase; Fluorescence In Situ Hybridization.

INTRODUCTION

Chronic myeloid leukemia (CML) is a *BCR::ABL1*-positive clonal myeloproliferative neoplasm arising from a hematopoietic stem/progenitor cell and is defined by the Philadelphia chromosome, t(9;22)(q34;q11.2), or its molecular equivalent. The resulting *BCR::ABL1* oncoprotein has constitutive tyrosine kinase activity, leading to deregulated downstream signaling, enhanced myeloid proliferation, altered cellular adhesion, and impaired apoptosis.^{1,2} The introduction of *BCR::ABL1*-directed tyrosine kinase inhibitors (TKIs) has transformed the natural history of CML and markedly improved long-term outcomes. Nevertheless, a subset of patients continues to experience treatment failure, relapse after treatment discontinuation, or disease progression during therapy.^{1,2}

Disease progression in CML is increasingly understood as a dynamic biological process rather than a change defined solely by blast percentage. Under therapeutic selection pressure, the leukemic clone may acquire additional mechanisms of disease persistence or progression, including cytogenetic clonal evolution, additional cytogenetic abnormalities (ACAs), *ABL1* kinase-domain mutations, and other cooperating somatic alterations.^{1,2,8,9} In the current classification era, WHO classification of hematolymphoid tumors (WHO-HAEM5) emphasizes high-risk biological features associated with CML progression, including treatment resistance and clonal evolution, while ICC 2022 retains accelerated-phase criteria based on blast percentage, basophilia, and major-route ACAs in Philadelphia chromosome-positive cells.^{3,4}

Conventional cytogenetic analysis therefore retains a clinically important role in CML, not only at diagnosis but also during suspected

relapse or progression. Cytogenetics directly demonstrates the chromosomal structure of the leukemic clone, identifies persistence or re-emergence of Ph-positive metaphases, and detects ACAs that may not be inferred from *BCR::ABL1* transcript quantification alone.^{1,2} Major-route ACAs, including trisomy 8, additional Philadelphia chromosome, i(17q), and trisomy 19, are recognized markers of cytogenetic clonal evolution and have been associated with inferior response, increased risk of progression, and adverse clinical behavior.^{3,5-7}

We report a case of CML with prior complete cytogenetic response and reported deep molecular response, followed by cytogenetic relapse after treatment discontinuation. The relapsed clone showed trisomy 8 and high-order Philadelphia chromosome duplication with increased *BCR::ABL1* fusion-signal copy number on FISH. This case highlights the diagnostic, prognostic, and counselling value of conventional cytogenetics and *BCR::ABL1* FISH in identifying high-risk clonal evolution, particularly in settings where advanced molecular testing may be delayed or financially inaccessible.

Case Presentation

A 39-year-old woman with a known diagnosis of chronic myeloid leukemia, diagnosed two and a half years earlier, was referred to the cytogenetics laboratory for bone marrow karyotyping and *BCR::ABL1* fluorescence in situ hybridization (FISH) analysis. She had received imatinib mesylate for approximately one year after the initial diagnosis and subsequently discontinued treatment following reported cytogenetic and molecular remission approximately one and a half years before the current presentation. Currently, the patient complained of pallor, intermittent fever, and recurrent episodes of

vomiting for approximately one month. She required hospitalization for blood transfusion support. Physical examination revealed splenomegaly. There was no sternal tenderness or peripheral lymphadenopathy. No bleeding manifestations, pedal edema, skin changes, or goiter were noted. Radiological evaluation did not reveal any additional significant abnormality. Hematological evaluation showed severe anemia with hemoglobin of 5 g/dL, leukocytosis with a total leukocyte count of $20.0 \times 10^3/\mu\text{L}$, and thrombocytopenia with a platelet count of $75 \times 10^3/\mu\text{L}$. Peripheral blood smear examination showed 10% blasts & 14% basophils, while bone marrow evaluation demonstrated 16% blasts with basophilia.

In view of worsening cytopenias, increasing blast percentage, systemic symptoms, and splenomegaly, disease progression was clinically suspected. Cytogenetic evaluation was therefore requested to assess for Ph-positive relapse, loss of cytogenetic response, and acquisition of additional cytogenetic abnormalities. Review of prior records showed that bone marrow karyotyping and *BCR::ABL1* FISH performed approximately one and a half years earlier, after imatinib therapy, had shown no detectable Ph-positive clone, consistent with complete cytogenetic response at that time.

Materials and Methods

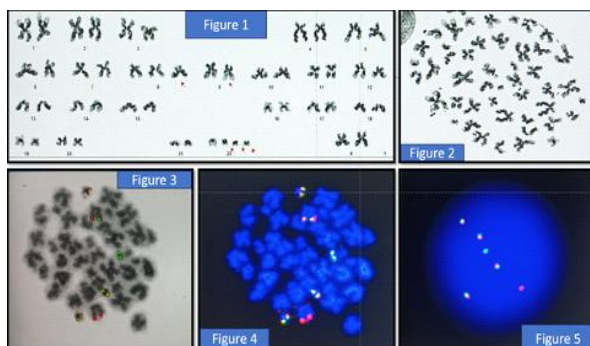
Conventional Cytogenetics

Conventional cytogenetic analysis was performed on bone marrow cells at a resolution of 300–400 bands using GTG banding according to standard protocols. Bone marrow cells were cultured in RPMI-1640 medium supplemented with 20% fetal calf serum, L-glutamine, and antibiotics (penicillin and streptomycin). Unstimulated cultures were incubated for 24 hours at 37°C in a humidified atmosphere with 5% CO₂. Colcemid (10 µg/mL) was added for 45 minutes to arrest cells in metaphase prior to harvesting.¹³ Cells were treated with hypotonic solution (0.075 mol/L KCl) and fixed with methanol: acetic acid (3:1). Slides were prepared and stained using G-banding.¹³ A minimum of 20 metaphases were analyzed using an Olympus BX-63 microscope (Applied Spectral Imaging software, version 8.3.2), and karyotypes were described according to ISCN 2024 recommendations.¹⁴

Fluorescence In Situ Hybridization

BCR::ABL1 fluorescence in situ hybridization was performed on interphase nuclei and available metaphase spreads using the ZytoLight® SPEC *BCR/ABL1* Dual Color Dual Fusion Probe (PL68; ZytoVision GmbH, Germany). The probe targets the *BCR* gene region at 22q11.22–q11.23 using ZyGreen-labelled polynucleotides and the *ABL1* gene region at 9q34.11–q34.13 using ZyOrange-labelled polynucleotides. Hybridization, post-hybridization washes, counterstaining, and signal evaluation were performed according to the manufacturer's instructions. A minimum of 200 interphase nuclei were evaluated. Metaphase FISH was used to correlate the fusion-signal pattern with the chromosomal location of derivative chromosomes.

RESULTS



Cytogenetic Findings

Figure 1-

GTG-banded metaphase analysis revealed the following abnormal karyotype:

49,XX,+8,t(9;22)(q34;q11.2),+der(22)t(9;22)(q34;q11.2)x2[20]

All analyzed metaphases demonstrated the Philadelphia chromosome together with trisomy 8 and two additional derivative chromosome 22 copies. These findings are consistent with **cytogenetic clonal evolution and high-order Philadelphia chromosome duplication**,

both recognized as adverse biological features in CML^{2,7}. The presence of two additional derivative chromosome 22 copies, in addition to the primary Philadelphia chromosome, indicates a **total of three Philadelphia chromosomes**, consistent with advanced cytogenetic clonal evolution.

Figure 2. Representative Metaphase Spread

Representative unarranged GTG-banded metaphase spread showing an abnormal hyperdiploid clone with 49 chromosomes. The spread corresponds to the karyotypic abnormalities shown in Figure 1, including trisomy 8 and multiple Philadelphia chromosomes/additional derivative chromosome 22 copies.

Figure 3. Metaphase FISH (Brightfield Overlay)

Metaphase *BCR::ABL1* dual-color dual-fusion FISH with brightfield overlay demonstrates multiple *BCR::ABL1* fusion signals on derivative chromosomes. The signal pattern is consistent with one standard t(9;22) rearrangement and two additional derivative chromosome 22 copies, confirming structural duplication of the Philadelphia chromosome.

ish der(9)t(9;22)(q34;q11.2)(BCRx5,ABL1x5)(BCR con ABL1x4)

Figure 4. Metaphase FISH (Fluorescence Image)-

ish t(9;22)(q34;q11.2)(BCRx5,ABL1x5)(BCR con ABL1x4)

Metaphase *BCR::ABL1* dual-color dual-fusion FISH using the ZytoLight® SPEC *BCR/ABL1* probe demonstrated four fusion signals. In correlation with GTG-banded metaphase analysis, this pattern is consistent with one derivative chromosome 9 and three derivative chromosome 22 (Philadelphia chromosome) copies.

Figure 5. Interphase FISH Demonstrating Increased Fusion Signal Copy Number

Representative interphase nucleus showing:

nuc ish(BCRx5,ABL1x5)(BCR con ABL1x4)[200]

Interphase *BCR::ABL1* dual-color dual-fusion FISH using the ZytoLight® SPEC *BCR/ABL1* probe showed increased fusion-signal copy number. Correlation with metaphase cytogenetics supports high-order Philadelphia chromosome duplication.

One normal chromosome 9 (*ABL1*), One normal chromosome 22 (*BCR*), Three derivative 22 chromosomes. One derivative 9. Total 5 *BCR* and 5 *ABL1* loci. Signal pattern confirms triple Philadelphia chromosomes with increased *BCR::ABL1* gene dosage, supporting cytogenetic clonal evolution.

DISCUSSION

This case highlights the continuing clinical value of conventional cytogenetics in chronic myeloid leukemia (CML), particularly when disease progression is suspected after an initial response to tyrosine kinase inhibitor (TKI) therapy. Although quantitative *BCR::ABL1* reverse-transcription polymerase chain reaction remains the standard method for longitudinal molecular monitoring, cytogenetic analysis provides a complementary and non-substitutable layer of information by directly demonstrating the chromosomal architecture of the leukemic clone, identifying Philadelphia chromosome-positive metaphases, and detecting additional cytogenetic abnormalities (ACAs) that indicate clonal evolution.^{1,2}

Cytogenetic response in CML is assessed by the percentage of Philadelphia chromosome-positive metaphases in bone marrow conventional cytogenetic analysis. Complete cytogenetic response is defined as 0% Ph-positive metaphases, partial cytogenetic response as 1–35% Ph-positive metaphases, minor cytogenetic response as 36–65% Ph-positive metaphases, minimal cytogenetic response as 66–95% Ph-positive metaphases, and no cytogenetic response as >95% Ph-positive metaphases. Major cytogenetic response includes both complete and partial cytogenetic responses, corresponding to 0–35% Ph-positive metaphases. Earlier ELN recommendations used these cytogenetic response categories for treatment assessment, whereas contemporary monitoring relies primarily on quantitative *BCR::ABL1* RT-PCR; nevertheless, conventional cytogenetics remains important in suspected relapse or progression because it can document re-emergence of Ph-positive metaphases and detect additional cytogenetic abnormalities.^{1,2,15}

In our case, prior marrow cytogenetic analysis and *BCR::ABL1* fluorescence in situ hybridization showed no detectable Philadelphia chromosome-positive clone, consistent with complete cytogenetic

response. The current marrow analysis demonstrated re-emergence of a Ph-positive clone with trisomy 8 and two additional derivative chromosome 22/Philadelphia chromosome copies:

49,XX,+8,t(9;22)(q34;q11.2),+der(22)t(9;22)(q34;q11.2)x2[20]

This karyotype represents loss of complete cytogenetic response with cytogenetic relapse. Importantly, the relapse was not limited to recurrence of the original Ph-positive clone; it was accompanied by acquisition of major-route cytogenetic abnormalities, namely trisomy 8 and additional Philadelphia chromosomes. The presence of two extra der(22)t(9;22) copies, in addition to the original derivative chromosome 22, indicates a total of three Philadelphia chromosomes, consistent with high-order Philadelphia chromosome duplication.

Metaphase and interphase *BCR::ABL1* dual-color dual-fusion FISH further supported this interpretation. The representative signal pattern, ish/nuc ish(ABL1x5,BCRx5)(ABL1 con BCRx4), is consistent with one derivative chromosome 9, three derivative chromosome 22/Philadelphia chromosomes, one normal chromosome 9, and one normal chromosome 22. When correlated with GTG-banded metaphase analysis, this confirms increased *BCR::ABL1* fusion-signal copy number due to structural duplication of the Philadelphia chromosome. This should be described as increased fusion-signal copy number rather than molecular *BCR::ABL1* amplification, unless true genomic amplification is confirmed by an independent copy-number assay.¹

Cytogenetic clonal evolution in CML refers to the acquisition of chromosomal abnormalities in addition to t(9;22)(q34;q11.2). Major-route ACAs, including trisomy 8, additional Philadelphia chromosome, i(17q), and trisomy 19, have been associated with disease progression and adverse clinical behavior.^{3,7} The coexistence of trisomy 8 and high-order Philadelphia chromosome duplication in our patient therefore represents biologically significant clonal progression rather than simple cytogenetic recurrence. This distinction is clinically important because relapse of the original Ph-positive clone indicates loss of disease control, whereas relapse with major-route ACAs identifies an evolved leukemic subclone requiring urgent reassessment.

Classification and prognostic relevance- The classification implications requires careful distinction between WHO-HAEM5 and ICC 2022. WHO-HAEM5 no longer requires accelerated phase as a separate CML category. Instead, it emphasizes high-risk features associated with progression, including treatment resistance, *ABL1* kinase-domain mutations, additional cytogenetic abnormalities, and evolution toward blast phase.³ In the present case, the findings are therefore best described under WHO-HAEM5 as CML with treatment-emergent high-risk cytogenetic progression, provided blast-phase criteria are not met.

By contrast, ICC 2022 retains accelerated-phase CML as a diagnostic category. Under ICC 2022, 10–19% blasts in blood or bone marrow, peripheral blood basophils $\geq 20\%$, or major-route ACAs in Ph-positive cells support accelerated phase.¹ Therefore, the presence of 16% marrow blasts along with basophilia together with major-route cytogenetic clonal evolution supports classification as CML, accelerated phase according to ICC 2022. From a practical clinical perspective, both systems identify the case as high-risk CML progression during therapy. This warrants urgent evaluation of treatment response, compliance, molecular burden, and resistance mechanisms.

The ideal evaluation of suspected CML progression includes bone marrow morphology, conventional cytogenetics, *BCR::ABL1* FISH where indicated, quantitative *BCR::ABL1* RT-PCR on the International Scale, transcript typing, *ABL1* kinase-domain mutation analysis, and selected myeloid next-generation sequencing in appropriate clinical settings.^{1,2,8,9} However, access to repeated molecular testing is not uniform, especially in financially constrained or resource-limited settings. In such contexts, conventional karyotyping and targeted *BCR::ABL1* FISH remain highly informative because they can answer immediate clinical questions: whether the Ph-positive clone has reappeared, whether complete cytogenetic response has been lost, whether ACAs are present, whether clonal evolution is major-route, and whether urgent treatment reassessment is required.^{1,10} For our patient, it also demonstrated that the relapsed clone had acquired trisomy 8 and high-order Philadelphia chromosome duplication. These findings are sufficient to alert the clinician to high-risk cytogenetic progression even before *ABL1* kinase-domain mutation analysis or broader NGS testing becomes available. However, cytogenetics is not a substitute for molecular monitoring.

Rather, it should be positioned as a practical triage tool that identifies patients who most urgently require quantitative RT-PCR, resistance-directed mutation testing, TKI modification, closer follow-up, or referral to a higher center.^{1,2,10}

A staged testing strategy is therefore scientifically justified. First, conventional karyotyping with *BCR::ABL1* FISH can document Ph-positive relapse, loss of complete cytogenetic response, and cytogenetic clonal evolution. Second, quantitative *BCR::ABL1* RT-PCR on the International Scale should be performed to measure current molecular disease burden and establish a monitoring baseline after therapeutic intervention. Third, *ABL1* kinase-domain mutation analysis should be prioritized when there is loss of response, rising transcript level, accelerated-phase features, or need to select a later-generation TKI. Finally, targeted myeloid NGS may be considered in patients with increasing blasts, persistent cytopenias, unexplained resistance, unusual progression, or transplant consideration.^{1,8,9}

The cytogenetic findings in our case also have direct counselling relevance. Patients may interpret the term “chromosome abnormality” as an inherited defect or familial cancer risk. Counselling must therefore clearly distinguish constitutional chromosomal abnormalities from acquired somatic abnormalities restricted to the leukemic clone.^{11,12}

In our patient, trisomy 8, additional Philadelphia chromosomes, and increased *BCR::ABL1* fusion-signal copy number are acquired somatic abnormalities of the malignant hematopoietic clone. They do not indicate a constitutional chromosomal disorder and do not imply that the patient's children or relatives require routine cytogenetic screening. This explanation is essential to reduce unnecessary anxiety and prevent misinterpretation of the cytogenetic report. Counselling should also explain that these abnormalities are clinically meaningful because they reflect clonal progression of CML. The cytogenetic result provides a visual and biologically understandable explanation for why the clinician may advise closer monitoring, further molecular testing, change of TKI, or referral for advanced care. In financially constrained settings, counselling should include practical test prioritization: cytogenetics has already established relapse with high-risk clonal evolution, while RT-PCR, *ABL1* kinase-domain mutation analysis, and NGS refine molecular burden, resistance mechanism, and treatment selection when feasible.

Thus, cytogenetics serves a dual role in our case. Scientifically, it confirms treatment-emergent clonal evolution with high-risk cytogenetic features. Practically, it provides an accessible framework for clinician communication, patient counselling, and rational prioritization of further testing.

CONCLUSION

Cytogenetics and *BCR::ABL1* FISH confirmed relapse of CML with major-route clonal evolution, characterized by trisomy 8 and high-order Philadelphia chromosome duplication after prior complete cytogenetic response. These findings indicate loss of cytogenetic disease control and high-risk progression. While WHO-HAEM5 supports classification as CML with high-risk cytogenetic progression, ICC 2022 criteria support CML, accelerated phase because of 16% marrow blasts and major-route additional cytogenetic abnormalities. The case highlights the continued value of cytogenetics as an affordable, actionable tool for relapse detection, risk assessment, molecular test prioritization, and Genetic Counselling.

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