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Focal lymphoblastic transformation of chronic myelogenous, leukemia develops into erythroid leukemia: A case report

Wang W *et al*. Lymphoblastic of CML develops into erythroid leukemia

Abstract

BACKGROUND

We present a case of focal lymphoblastic transformation secondary to acute myeloblastic transformation to erythroid leukemia in a patient with chronic myelogenous leukemia (CML) and discuss its mechanism of occurrence and development.

CASE SUMMARY

The presence of the Philadelphia (Ph) chromosome was identified through karyotype analysis, while the *BCR-ABL* fusion gene was detected using quantitative real-time polymerase chain reaction of the peripheral blood sample. Fluorescence *in situ* hybridization was used to detect expression of the *BCR-ABL* gene in the lymphoma. Antigen expression and gene mutations in the primitive cells were detected by flow cytometry. The analysis confirmed the presence of CML along with focal lymphoblastic transformation in T-cell lymphoma.

CONCLUSION

Additionally, the patient was found to have secondary erythroid leukemia, along with multiple new gene mutations and abnormalities in complex karyotypes of chromosomes, which suggest a possible molecular basis for the focal lymphoblastic transformation secondary to myeloblastic transformation.

Key Words: Chronic myelogenous leukemia; Blast crisis; Focal lymphoblastic transformation; Pure erythroid leukemia; Case report

Core Tip: A case of focal lymphoblastic transformation secondary to acute myeloblastic transformation into erythroid leukemia in a chronic myelogenous leukemia patient was presented, and the mechanism of its occurrence and development were discussed, multiple new gene mutations and abnormalities in complex karyotypes of chromosomes were the major reason.

INTRODUCTION

Chronic myelogenous leukemia (CML) is characterized by the presence of the Philadelphia (Ph) chromosome and the *BCR-ABL* fusion gene. The clinical presentation of, CML is often categorized into three phases: Chronic phase, accelerated phase, and blast crisis. Progression to blast crisis substantially worsens the prognosis. The majority (approximately 70%) of acute transformation of CML, his patients develop acute myelogenous leukemia (AML); only a smaller proportion (about 30%) of patients develop acute lymphocytic leukemia. In rare cases, CML may transform into mixed-phenotype acute leukemia. However, to date, there have been no reported cases of CML with blastic lymphoid transformation, followed by blastic myeloid transformation. In this report, we present one such unique case of CML with blastic transformation in extramedullary lymph nodes, followed by secondary myeloblastic transformation that eventually led to pure erythroid leukemia.

CASE PRESENTATION

Chief complaints

Diagnosed CML for over 4 years, with fever for 1 wk.

History of present illness

The patient was a 44-year-old man, diagnosed with a mass on the right side of his neck in June 2016. Initially, the mass was the size of a quail egg, but progressively enlarged. The mass had poor mobility and no tenderness. Subsequently, multiple swollen lymph nodes with similar characteristics were found on both sides of the neck. The patient did

not experience fever, chills, weight loss, night sweats, skin itching, abdominal pain, or abdominal distension. He sought medical attention at the Department of General Surgery at our hospital, laboratory tests revealed a white blood cell (WBC) count of 565.21×10^9/L, absolute neutrophil count of 499.09×10^9/L, hemoglobin level of 47 g/L, and total platelet count of 219×10^9/L. The patient was transferred to our department for further investigation, which included bone marrow (WB) aspiration and *BCR-ABL* fusion gene testing, to confirm the diagnosis of chronic-phase CML. The biopsy of a right neck lymph node, along with the immunohistochemistry results indicated T-cell lymphoblastic lymphoma (T-LBL)/leukemia. Following the biopsy, a fluorescence *in situ* hybridization (FISH) was performed, which showed the presence of the *BCR-ABL* fusion gene. Consequently, the patient was diagnosed with CML concurrent with a blastic focal transformation of extramedullary lymph nodes.

Oral therapy with imatinib mesylate was initiated, which resulted in the disappearance of the mass, without recurrence. The patient received follow-up treatment for CML in the outpatient department. However, the patient was not adherent to the treatment regimen and frequently self-discontinued the medication. Additionally, regular monitoring of the fusion gene levels was not done, and molecular remission was not achieved with the treatment.

In September 2019, visible abdominal distension and splenomegaly were noted, accompanied by incomplete intestinal obstruction. The patient had recurrent fever and was diagnosed with a lung infection. Supportive treatment was initiated in the form of fasting, gastrointestinal decompression, enema, and parenteral nutrition, along with anti-infective medications such as sulperazone, imipenem, cilastatin, and voriconazole The patient's symptoms improved. However, subsequent re-examination revealed accelerated phase CML. Therefore, dasatinib was initiated, which was followed by prolonged pancytopenia lasting for a month. Dasatinib was discontinued, but the pancytopenia persisted; therefore, a blast crisis was considered.

Results of routine blood tests showed a WBC count of 0.43×10^9/L, hemoglobin level of 69 g/L, and total platelet count of 83×10^9/L. Since the onset of the illness, the

patient had poor spirits, average physical strength, fair appetite, normal sleep, no significant weight changes, and normal bowel movements.

History of past illness

No special.

Personal and family history

No special.

Physical examination

On physical examination, body temperature was 36.4 ℃; pulse rate, 103 beats/min, respiratory rate, 20 breaths/min; and blood pressure, 105/76 mmHg. The heart rate was 103 beats/min, with a regular rhythm and no pathological murmurs. The patient was conscious and pale, without superficial lymphadenopathy or tenderness. Slight tenderness of the sternum was noted. Clear breath sounds were auscultated in both lungs without dry or wet rales. Abdominal bulging and splenomegaly were detected, with measurements along the midclavicular, midabdominal, and third abdominal lines being 28 cm, 35 cm, and +5 cm, respectively. Palpation revealed a hard texture with no obvious tenderness, and a palpable spleen notch. There was no swelling in the lower limbs.

Laboratory examinations

Findings of BM examination on February 19, 2016: Significant proliferation of nucleated BM cells was noted. The ratio of granulocytes to red blood cells (RBC) was 31:1, indicating significant granulocytic hyperplasia. Primitive granulocytes accounted for 3% of the cells. An increase in the proportion of promyelocytes and metamyelocytes, as well as the basophil cell ratio, was noted. Erythroid hyperplasia was suppressed, and the lymphocyte ratio was decreased. The monocyte ratio was normal. Megakaryocytes were greater than 500, with noticeable megakaryocyte presence. The results of the

peripheral blood smear showed an extremely high total WBC count and a predominance of neutrophils with left shift. Primitive granulocytes accounted for 3% of the cells, with an increased proportion of basophils.

Findings of BM examination on August 24, 2020: Significant BM hyperplasia was noted, with the percentages of myelocytes (M) and erythrocytes (E) being 22.0%,and 72.0%, respectively. The granulocyte to RBC ratio was 0.31:1, indicating a reduction in the extent of granulocytic hyperplasia. Primordial granulocytes accounted for 7.5% of the cells (34.1% of the nucleated erythroid cells). Cells of various sizes with increased plasma content were detected, with dark blue plasma and possible purple-red granules. The nuclei were round and loosely distributed, with 0-2 nucleoli per nucleus. A predominance of basophils was noted. Marked erythroid hyperplasia was observed, with an increased proportion of young RBCs at different stage, with large cell bodies. The monocyte counts as well as the proportions of binuclear, malformed nucleus and multinucleated young RBCs and lymphocytes were normal. More than 1000 megakaryocytes were detected in the smear, with the absence of platelets. Examination of the peripheral blood smear showed a normal total WBC count. Primitive cells accounted for 2.0% of the cells, with an increased expression of basophils. Four nucleated RBCs per 100 WBCs were noted, with noticeable primordial RBCs, along with various sizes of mature RBCs with deformities such as fragmentation, stripped, and Cabot rings, among others. Platelet counts were increased. The findings were indicative of the transformation of CML into acute leukemia.

Findings of BM examination on October 07, 2020: BM hyperplasia was reduced, with the percentages of myelocytes to erythrocytes being 11.0% and 76.0%, respectively (M:E = 0.14:1). A marked reduction in granulocytic hyperplasia was noted. Erythroid hyperplasia was still noticeable, with predominance of middle and late stages of young RBCs, which were findings consistent with the results of the blood smear. Lymphocyte proportions and monocyte counts remained normal. Only seven megakaryocytes were detected in the film, and platelets were rare. No parasites were detected in the blood smear. The results of the peripheral blood smear showed a reduced total WBC value,

with a predominance of mature lymphocytes. An increased ratio of eosinophils, along with various sizes of RBCs, including fragmented cells with shapes resembling helmets, Cabot rings, *etc*. Platelets were hardly detected.

Findings of BM examination on October 16, 2020: BM hyperplasia, with the percentages of M and E being 3.5% and 90.0%respectively. The granulocyte to RBC ratio was 0.04:1, indicating reduced granulocytic hyperplasia. Marked erythroid hyperplasia was observed, with an increased proportion of RBCs in all stages of development. Malformed nuclei with budding nuclear and multinucleated megaloblasts were detected. Mature RBCs of various sizes were observed, with nuclear stippling, polychromatic appearance, and helmet-shaped forms. Basophils were detectable. The ratio of lymphocytes to monocytes was normal. Forty-three megakaryocytes were found in the film, with platelets being rare. On the basis of these findings of BM smear examination, transformation of CML into acute erythroid leukemia (AEL) was considered as the diagnosis. However, immunophenotyping and related genetic testing were required to conclusively establish the diagnosis (Figure 1).

Findings of BM examination on August 20, 2020: Results for the *BCR-ABL1* (p210) fusion gene test were positive. The copy number of *BCR-ABL1* and *ABL1* were 149082.6 and 127119.0, respectively. The ratio of *BCR-ABL1/ABL1* was 117.28, and the ratio of IS *BCR-ABL1/ABL* was 77.40 (Figure 2). Karyotype analysis on August 21, 2020: Karyotype analysis revealed 47XY,+X,t(9;22)(q34;q11.2)[6]/49,idem,+1,der(1;7)(q10;q10),+7,+8[2]/49,idem,+1,der(1;7),+8,+10[2] (Figure 3).

Flow cytometry (FC) immunofluorescence analysis on August 25, 2020: FC of the examined specimens revealed the absence of the immunophenotypic abnormalities related to acute leukemia and high-risk myelodysplastic syndrome in the examined specimens. There was no noticeable occurrence of CD34+ cells, and the relative proportion of granulocytes was reduced. No significant changes were observed in the expression levels of immunophenotypes CD13, CD15, CD16, and CD11b. T cells accounted for 77.5% of lymphocytes, with a CD4:CD8 ratio of 0.95 and no noticeable

abnormalities. Natural killer (NK) cells accounted for 6.9% of lymphocytes, with no noticeable abnormalities. Mature B lymphocytes accounted for 2.5% of lymphocytes, with a dominance of polyclonal B cells.

FC immunofluorescence analysis on October 8, 2020: The FC results of the examined specimens did not show evidence of the immunophenotypic abnormalities associated with acute leukemia, non-Hodgkin lymphoma (NHL), or high-risk myelodysplastic syndrome. There was a significant increase in the relative proportion of nucleated RBCs. The proportion of CD34+ cells in nuclear cells was approximately 0.24%. No noticeable abnormal immunophenotypes were observed. The analysis also showed a reduced relative proportion of granulocytes and significant changes in the expression levels of the immunophenotypes CD13, CD15, and CD16. The relative proportion of lymphocytes was within the normal range. T cells accounted for 88.68% of lymphocytes, with a CD4:CD8 ratio of 0.69 and no noticeable abnormalities. NK cells accounted for 5.00% of lymphocytes, with no noticeable abnormalities. Mature B lymphocytes accounted for 1.96% of lymphocytes. A significant increase in the relative proportion of nucleated RBCs was noted, accounting for approximately 55.41% of the total RBCs (Figure 4).

Examination of kinase domain mutation on October 15, 2020: The mutation sites detected were as follows: Positive results were obtained for E255K, V299L, and T315I mutations. No mutations were detected in M244V, L248V, G259E, Q252H, Y253F, Y253H, E255V, D276G, E279K, F311L, T315A, F317L, F317C, F317I, M351T, E355G, V379I, L384M, L387M, H396R, H396P, E459K, F486S, F359I, F359V, and F359C. On analysis of the gene mutations related to CML progression, no mutations were detected in the genes *ASXL1, MYC, CBL, NRAS, CDKN2A, RUNX1, IDH1, TET2, IDH2, TP53,* and *KRAS* (Figure 5).

Findings of molecular genetic studies of lymph node biopsy sample and immunophenotyping performed in March 2016: In the examined lymph nodes, tumor cells were found to be distributed in a flaky shape. The cells were small and round, with uniform size and round or occasionally irregular nuclei. Additionally, nucleoli and

residual germinal centers were observed. Proliferation of interstitial fibrous vascular was detected. Immunohistochemistry results were as follows: Showed the CD3(+), CD79a(+), CD5(+), Bcl-2(+), CD34(++), TdT(+), CK(-), CD43(++), cyclin D1(-), CD20(-), CD10(-), Bcl-6(-), MPO(-), and Ki-67 approximately 70%. Follicular dendritic cells were CD21(+) and CD23(+).The findings of immunohistochemistry were consistent with T-cell T-LBL/leukemia. The FISH test of the lymph node specimens was positive for the *BCR-ABL* fusion gene in the tumor cells (Figure 6).

Imaging examinations
No special.

FINAL DIAGNOSIS
Focal lymphoblastic transformation of CML develops into erythroid leukemia.

TREATMENT
The patient was admitted to the hospital. Results of BM aspiration test revealed progression into pure erythroid leukemia through blastic transformation bone puncture report. Due to the ineffectiveness of imatinib and dasatinib, a switch was made to flumatinib after hospitalization. Chemotherapy based on omoharringtonine (HHT) was administered, which resulted in certain hematological improvement. The pancytopenia improved, with a significant increase in the total platelet count. However, the CML mutation gene test revealed multiple new gene mutations, including the *T315I* mutation of *BCR-ABL* and abnormalities in multiple karyotypes of chromosomes.

OUTCOME AND FOLLOW-UP
Eventually, treatment with flumatinib proved to be unsuccessful. Therefore, we strongly recommended allogeneic hematopoietic stem cell transplantation as soon as possible.

DISCUSSION

Evidence and mechanism of lymphoblastic transformation based on the lymph node biopsy

The pathology reports of the patient's lymph nodes indicated lymphoblastic leukemia, and accordingly, the involved lymph node tissues were removed. Morphological and immunological assessments of tumor cells revealed the presence of the *BCR-ABL* fusion gene in the FISH test. The results were consistent with CML, and the Ph chromosome was detected. The lymph node mass disappeared after treatment with imatinib, without the need for the chemotherapy that is typically used for acute lymphoblastic leukemia (ALL) or lymphoma. This further confirmed that the patient's lymph node blastoma was of the focal, acute lymphoblastic type induced by the transformation of CML.

With regard to the mechanism of CML involving lymphocytes, the involvement of T cells in CML is widely believed to be due to a common progenitor cell called CFU-GEMMT, which can differentiate and develop into myeloid cells and T cells in CML patients[1]. Furthermore, CML-Ph-positive T cells have been identified *in vitro* in the BM cells of chronic-phase CML patients, indicating the involvement of the T-cell lineage in CML[2]. A crucial diagnostic criterion for extramedullary T-LBL/ALL secondary to CML is evidence of blast proliferation in extramedullary tissues. Thus, morphological, histochemical staining, and immunophenotypic characteristics of the blasts should align with the features of precursor thymic T cells[3]. Additionally, confirmation of the presence of the Ph or *BCR-ABL* fusion gene through cellular and molecular genetic assessments is necessary to establish the diagnosis. Genetic diagnosis relies on karyotyping to confirm extramedullary lesions (mostly lymph nodes). However, a single positive detection of the Ph chromosome or *BCR-ABL* fusion gene using Southern Blot or quantitative real-time polymerase chain reaction (RT-PCR) analysis cannot be considered a conclusive evidence of tumor cells. This is because CML is a clonal disease of pluripotent hematopoietic stem cells. Normal WBCs, including lymphocytes and myeloid cells, in chronic-phase CML patients can also carry the Ph chromosome or *BCR-ABL* fusion gene, which may be detected when cells of extramedullary lesions are

mixed with some CML clonal cells. On the other hand, true tumor cells such as lymphoma cells may test negative. Ichinohasama *et al*[4] reported two cases of chronic-phase CML patients with secondary Ph(-) NHL. These patients exhibited lymph node enlargement at 33 and 10 mo after the CML diagnosis and pathological examination of the lymph nodes revealed diffuse, large B-cell lymphoma and anaplastic large cell lymphoma, respectively. Southern Blot and RT-PCR analysis confirmed the rearrangement of *BCR-ABL* fusion genes in the BM and lymph node tissues in these two patients. However, FISH testing of the lymph node specimens revealed that the gene fusion signals originated from lymphocytes with normal morphology and karyotype, while the lymphoma cells themselves yielded negative signals. This evidence proved that the diagnosis of Ph(-)NHL secondary to CML in these two cases. Therefore, in CML cases presenting with lymph node tumors, it is crucial to differentiate between CML-induced lymphoblastic transformation and secondary NHL.

In our patient, the diagnosis was CML, with extramedullary T-LBL/ALL transformation, as confirmed by FISH. The presence of primitive cells was initially identified through Giemsa (Uiemsa) staining of the lymph node slides, followed by the FISH test to confirm the presence of *BCR-ABL* fusion signals in the primitive cells. If the FISH test confirms that the tumor cells in the extramedullary lesions carry CML markers, the diagnosis of CML with extramedullary T-LBL transformation can typically be made. In the present case, instead of primary Ph(+)T-ALL, the patient had CML in chronic phase with secondary blastic transformation in the extramedullary lymph nodes, as evident from the clear manifestation of CML in the BM without any signs of T-ALL. Furthermore, FISH analysis of the patient's BM and lymph node biopsy revealed the presence of M-bcr (p210) type breakpoints, whereas Ph(+)ALL is typically associated with the bcr (p190) type breakpoints[5]. This finding further supports the conclusion that the Ph(+)T-ALL in this case was not primary.

Diagnosis of myeloid transformation into erythroid leukemia and its mechanism

The CML patient initially presented with the chronic phase, progressed to the accelerated phase, and eventually developed pure erythroid leukemia, which is consistent with the evolution of CML in the BM[6]. CML can persist in the chronic phase for 3 to 5 years before advancing to the accelerated phase and blast crisis[7]. In this case, the patient entered the accelerated phase after 5 years of treatment, with 15.5% blasts in the BM and a depletion of erythroid cells. Following 3 mo of dasatinib treatment, the patient entered blast crisis. Morphological changes were observed, showing the transition from the absence of erythroid cells during the accelerated phase to erythroid hyperplasia during blast crisis. The hyperplasia predominantly affected erythroid precursor cells and giant multinucleated young RBC, indicating the development of pure erythroid leukemia, which is an uncommon acute transformation in CML. This significant shift in morphological characteristics occurred within 3 mo of acceleration, which is clinically rare.

Approximately 2/3 to 3/4 of acute transformations in CML are myeloid, while 1/3 to 1/4 are lymphoid[8]. Cases of transformation into AEL induced by a myeloid transformation in CML have been rarely reported in the literature. Acute leukemia AML-M6, also known as acute erythroleukemia, was initially described by Di Guglielmo[9], and later Dameshek and Baldini[10] AML-M6 is classified into the phases of simple erythroid hyperplasia, erythroid-granulated mixed hyperplasia, and mononuclear granulated hyperplasia. According to the French-American-British classification system, the diagnostic criteria for acute leukemia require the presence of ≥ 30% blasts. The World Health Organization (WHO) defines acute erythroleukemia as a possible erythroid dysplasia syndrome, excluding cases related to leukemia or erythropoietin treatment. AML-M6 is defined by WHO as acute leukemia with erythroid hyperplasia. Two main types of acute leukemia are recognized, according to the presence or absence of myeloid (granulated) components - erythroid/granulated type of erythroleukemia and pure erythroid leukemia. The erythroid/granulated type of erythroleukemia is characterized by BM erythroid precursor cells that account for ≥ 50% of nuclear cells and primitive granulocytes that account for ≥ 20% of non-erythroid

cells. In contrast, pure erythroid leukemia is defined by immature erythroid hyperplasia accounting for > 80% of BM cells[11]. Our patient's BM shows a proportion of primitive RBC exceeding 90%, confirming the diagnosis of pure erythroid leukemia.

Possibility of poor prognosis

The presence of additional chromosomes, aside from the Ph chromosome, in the accelerated phase or blast crisis is indicative of a poor prognosis. The appearance of extra chromosomes often precedes the clinical blast crisis by 2-4 mo. Common chromosomal aberrations include +8, +19, and i(17q)[12], which primarily occur during myeloblastic transformation. However, other aberrations in acute lymphoblastic transformation have rarely been reported. In the present case, multiple complex extra karyotype changes were identified during the transition to erythroleukemia. The occurrence of these complex karyotype changes may be an important reason for the rapid development of secondary erythroleukemia and may also serve as a high-risk factor for the poor prognosis of AEL[13].

Applied treatment

The patient initially presented with a combination of CML and T-LBL/ALL. Treatment with imatinib led to the disappearance of the extramedullary T-LBL/ALL without any relapse, which indicates the reliable therapeutic effect of imatinib alone in treating CML cases with secondary T-LBL/ALL transformation. This also confirms that T-LBL/ALL can occur secondary to CML. Additionally, in our patient, CML progressed gradually to the accelerated phase and eventually underwent an acute transformation to erythroleukemia. The treatment response to dasatinib, a second-generation tyrosine kinase inhibitor (TKI), was poor, while flumatinib had an even worse effect. Therefore, TKIs can be considered as the main treatment for both the acute lymphoid and acute myeloid transformations of CML, despite their low curative effect. However, secondary TKI resistance remains a challenge for patients with blasts, particularly after the T315I mutation. Bonatinib has been shown to have efficacy in CML with T351i mutation, but

its effectiveness for CML blast crisis with multiple gene mutations and complex karyotypes, still requires more evidence-based medical support. Moreover, it is expensive and requires long-term administration, which may make it difficult for patient to accept. HHT can temporarily control disease progression in the short term, but eventually fails, with an effectiveness rate of only around 30%. Allogeneic hematopoietic stem cell transplantation is considered as the only therapeutic modality useful for the control or even cure of such cases with dual blasts. We intend to continue observing the patient for subsequent treatment response.

CONCLUSION

Thus, this rare case of focal lymphoblastic transformation secondary to acute myeloblastic transformation of CML to erythroid leukemia gives us insight into the clinical presentation, investigative findings, and treatment in such cases.

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