

# Acute Myeloid Leukemia Secondary to Chronic Lymphocytic Leukemia After Prolonged Chlorambucil Therapy: A Case Report

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**Background:** This study aimed to improve the understanding of acute myeloid leukemia (AML) secondary to chronic lymphocytic leukemia (CLL), and to explore the sequence of occurrence and clonal origin of the two diseases.

**Case Report:** We reported a case of a 71-year-old man with a history of CLL. The patient was administered with chlorambucil for 19 years and was admitted to our hospital due to fever. Then he was subjected with routine blood tests, bone marrow smear examination, flow cytometric immunophenotyping and cytogenetic analysis. A final diagnosis of AML-M2 secondary to CLL with -Y,del(4q),del(5q),-7,add(12p),der(17),der(18),-22,+mar was made. After rejecting the therapy with Azacitidine combined with B-cell lymphoma-2 (Bcl-2) inhibitor, the patient died of pulmonary infection.

**Conclusion:** This case highlights the rare occurrence of AML secondary to CLL after prolonged chlorambucil therapy and the poor prognosis of such cases, underscoring the importance of enhanced assessment of these patients.

**Keywords:** acute myeloid leukemia, chronic lymphocytic leukemia, Bcl-2 inhibitor, Azacitidine

## Introduction

Chronic lymphocytic leukemia (CLL) is an indolent B-cell lymphoproliferative neoplasm that mainly occurs in adults, with favorable 5-year survival rate.<sup>1</sup> CLL is usually related to second primary solid malignancies such as bronchus/lung carcinoma and melanoma,<sup>2</sup> but its relationship with hematologic malignancy is uncommon. It has been proposed that most of the associations between acute myeloid leukemia (AML) and CLL are treatment-related and have unfavorable karyotypes.<sup>3</sup> DeFilipp et al<sup>4</sup> have described a white male who arises AML with del(7q) four years after diagnosis of CLL, with complete remission of both disorders after transplantation. However, Wang et al found that a patient was diagnosed as secondary AML after treatment of CLL with Venetoclax monotherapy.<sup>5</sup> In a large cohort study of CLL patients, 30 out of 1269 cases (2.4%) were found to have developed a secondary bone marrow disorder, most commonly myelodysplastic syndrome/AML, accounting for 76.7% of cases.<sup>6</sup> AML has been reported as the second malignancy in CLL patients, with most cases occurring after exposure to alkylating agents commonly used in CLL treatment.<sup>3</sup> Chlorambucil is a DNA alkylating agent based on nitrogen mustard, and is often used as a first-line treatment for CLL in clinical practice.<sup>7</sup> However, there are few reports on the development of secondary AML in CLL patients after long-term treatment with chlorambucil.

We hereby described the progression of secondary AML in a CLL patient after 19 years of administration with chlorambucil.

## Case Presentation

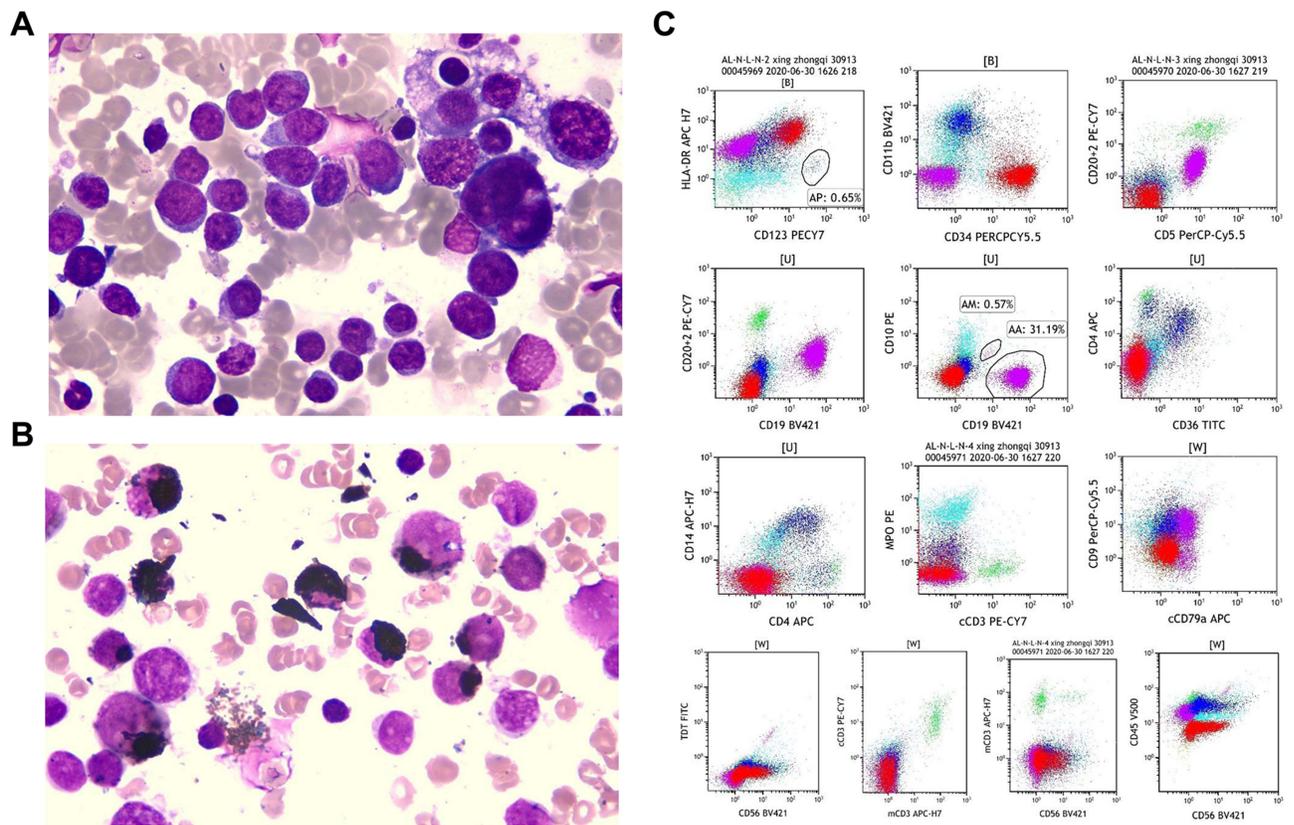
A 71-year-old man who suffered from CLL was admitted to our hospital on June 23th, 2020 due to fever. In 2001, he was admitted to a hospital due to submaxillary mass. On physical examination, multiple enlarged lymph nodes of the bilateral

jaw bone were touched with nontender and of medium in quality, among which the largest lymph node was about  $1.0 \times 1.5 \text{ cm}^2$ . Routine blood results showed white blood cell (WBC) of  $148.52 \times 10^9/\text{L}$ , lymphocyte ratio of 81.5%, hemoglobin of 101 g/L and platelet of  $68 \times 10^9/\text{L}$ . Bone marrow smear revealed active hyperplasia of nucleated cells in bone marrow and increased proportion of lymphocytes, including mature lymphocytes (91%) and immature lymphocytes (2.5%). Likewise, peripheral blood films also suggested significantly augmented proportion of lymphocytes, with 91% of mature lymphocytes and 3% of immature lymphocytes, which was consistent with the bone marrow smear of CLL. Subsequently, the patient was subjected to bone marrow biopsy. Results indicated active bone marrow hyperplasia and nodular or patchy proliferation of mature lymphocytes, without granulocyte, erythrocyte and megakaryocyte, as well as germinal center and macrocell transformation. Flow cytometric immunophenotyping showed CD5(+) $81\%$ , CD19(+) $70\%$ , CD20(+) $25\%$  and CD22(+) $8\%$ . Finally, the patient was diagnosed with CLL at Binet C stage and was given 4 courses of combination therapy of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). After discharge, he received daily oral chlorambucil 2 mg for a long time, and the disease was controlled.

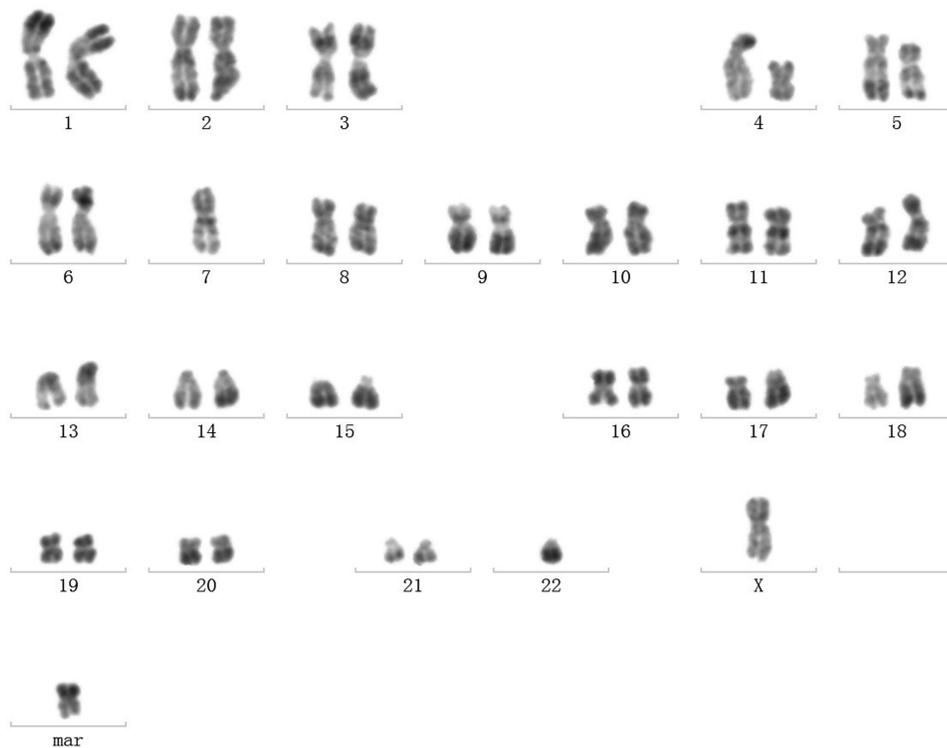
On May 20th, 2020, the patient revisited the hospital. The bone marrow smear still conformed to CLL, and the bone marrow biopsy indicated that tumor cells were scattered or clustered (10–20%). Flow cytometric immunophenotyping performed on the peripheral blood showed that abnormal cell population accounted for 87.16% of nuclear cells, expressing CD19, CD23, CD5, CD200 and CD22, partially expressed CD20, CD123 and CD11c, and weakly expressing CD81 and CD43, suggesting CLL/small lymphocytic lymphoma (CLL/SLL) phenotype. The karyotype of the chromosome was 45,X,-Y[4]/47,XY,+12[6]/46,XY[15]. Fluorescence in situ hybridization (FISH) showed negative IGH/BCL2, ATM/CEP11, RB-1, CCND1/IGH, CEP12 and P53/CE P17. The second-generation sequencing showed that Y220C and I195F mutations in exon 6 of TP53 mutation were positive, with a mutation rate of 4.1% and 1.5%, respectively. Somatically mutated monoclonal IGHV4-34 gene rearrangement was demonstrated, with 6.7% of mutation rate. Taken together, these findings indicated that this patient was diagnosed with RaiIII-phase CLL at Binet stage C, with IgHV and TP53 mutation. Oral ibrutinib tablet 420 mg was given once a day. No increase in hemoglobin was observed in blood routine monitoring during the oral administration of ibrutinib, and intermittent transfusion for the treatment was conducted in our hospital.

On June 23th, 2020, the patient was referred to our hospital when he presented with fever, fatigue and anorexia. On admission, the physical examination revealed anemia, a body temperature of  $37.9^\circ\text{C}$ , pulse rate of 102/min, breathing rate of 20 times/min and blood pressure of 120/67 mmHg, without obvious abnormalities in the heart and lungs. There were enlarged lymph nodes in the bilateral neck and groin, with the most being  $2 \times 3 \text{ cm}^2$ , as well as splenomegaly (line A and B of 5 cm, line A and C of 6 cm, line D and V of  $-6 \text{ cm}$ ) with small-medium in size, nontender and pitting edema of lower limbs. Routine blood results showed WBC of  $59.16 \times 10^9/\text{L}$ , lymphocyte count of  $121.04 \times 10^9/\text{L}$ , hemoglobin of 89 g/L and neutrophil granulocyte of  $17.78 \times 10^9/\text{L}$ . Bone marrow smear indicated active bone marrow hyperplasia, and we observed 46.5% of protogranulocytes (small and unequal in size and some cytoplasm containing a few azurophilic granules), many basophilic erythroblast, megakaryocytes and small megakaryocytes, but little immature lymphocytes (Figure 1A). The positive rate of POX staining was about 65%, indicating CLL accompanied by AML-M2 (Figure 1B). As shown in Figure 1C, abnormal myeloid primordial cells were found in the specimens, accounting for 32.86% of the nuclear cells, which was in line with AML phenotype. In addition to expressing myeloid antigens, CD56-positive lymphocytes were also noticed, which could be used as a monitoring indicator for minor residual disease. A cluster of abnormal B lymphocytes with small forward scatter (FSC) intensity and side scatter (SSC) intensity was observed in the specimen of the patients, and the phenotype of the cluster of cells was consistent with CLL/SLL. Moreover, the patient's karyotype was 44, X,-Y,del(5)(q31),-7add(12)(p13)der(17)der(18), 22,+mar ([8]/44,idem,del(4)(q22)[12] (Figure 2).

On the basis of the data above, a final diagnosis of AML-M2 secondary to CLL was made, with -Y,del(4q),del(5q),-7, add(12p),der(17),der(18),-22,+mar. Azacitidine combined with B-cell lymphoma 2 (Bcl-2) inhibitor was recommended for the treatment, which was rejected by his family, and the patient died of pulmonary infection on October 17, 2020. This study followed the principles of the Declaration of Helsinki, and informed consent was obtained from the patient's family.



**Figure 1** (A) Bone marrow examination. The bone marrow smear showed active hyperplasia. Magnification:  $\times 100$ . (B) Peroxidase stain for bone marrow smear. Magnification:  $\times 100$ . (C) Flow cytometric immunophenotyping of bone marrow.



**Figure 2** Cytogenetic analysis of bone marrow.

## Discussion

CLL patients at an advanced stage may have disease transformation into aggressive lymphoma (Richter's syndrome), which mainly transforms into diffuse large B-cell lymphoma, a few into Hodgkin's lymphoma, and a rare transformation into AML.<sup>8</sup> In this study, the elderly male was administered with long-term chlorambucil orally with a history of CLL for 19 years, and finally developed secondary AML. Flow cytometric immunophenotyping proved the coexistence of CLL and AML cell populations. Besides, he was also accompanied by TP53 mutation and complex karyotype of chromosomes. Combined with the medical history analysis, the AML was confirmed as a second tumor of CLL. Based on the data, we suspected that long-term administration of the alkylating agent chlorambucil in CLL patients may be related to the occurrence of AML. Morrison et al<sup>9</sup> found that the incidence of therapy-related MDS (t-MDS)/therapy-related AML (t-AML) was 0.5% in B-cell CLL patients treated with fludarabine alone, and that the risk was dramatically enhanced with fludarabine combined with chlorambucil, whereas neither t-MDS nor t-AML was found in patients treated with chlorambucil alone. In addition, some case series have reported secondary malignancies, including AML, in CLL patients treated with alkylating agents such as fludarabine.<sup>10,11</sup> It has been reported that the combination of fludarabine and chemotherapy is associated with intermediate risk of therapy-related myelodysplastic syndrome and acute myeloid leukemia (t-MDS/AML).<sup>12</sup> Similarly, Morrison et al<sup>9</sup> have suggested that the combination of alkylating agents and purine analogs may increase the risk of therapy-related myeloid malignancies.

To our knowledge, the clinical manifestations, pathological features, treatment and prognosis of AML and CLL are significantly different. CLL is an indolent disease with a survival of 10–25 years, while AML is an aggressive disease with a short survival time.<sup>12</sup> When these two diseases coexist in the same patient, demethylated drug Azacitidine plus Bcl-2 inhibitor Venetoclax is recommended. Venetoclax, a selective small molecule inhibitor of the anti-apoptotic protein Bcl-2, has synergistic antileukemic activity in combination with hypomethylating agents and Decitabine.<sup>13</sup> Murano et al<sup>14</sup> compared the efficacy of Venetoclax+Rituximab (VR regimen) versus Bendamustine and Rituximab (BR regimen) in the treatment of relapsed and refractory CLL, and found that VR regimen significantly prolonged the PFS in these patients, with good safety.

Monotherapy for relapsed/refractory AML has yielded poor outcomes, but Venetoclax plus Azacitidine has shown synergistic effects in preclinical models.<sup>15</sup> Previous researches have clarified that Azacitidine may modulate cell survival in AML by downregulating the protein expression of Mcl-1, which is a critical anti-apoptotic protein in the pathogenesis of AML and may be the reason of Venetoclax resistance.<sup>16</sup> On this basis, Dinardo et al<sup>17,18</sup> conducted a non-randomized and phase 1b study in which the combination of Venetoclax with either Decitabine or Azacitidine was effective in elderly AML patients who were not eligible for intensive chemotherapy. In the current study, Azacitidine combined with Bcl-2 inhibitor was recommended for the therapy of CLL/AML. Nevertheless, the patient declined this recommendation and survived less than 4 months after being diagnosed with secondary AML, indicating a poor prognosis. Consistent with this finding, in the study by Carney et al,<sup>12</sup> secondary MDS and AML in CLL patients were associated with poor prognosis, with a median survival time of 3.5–11 months after diagnosis.

## Conclusion

While secondary malignancies, including AML, have been reported in patients with CLL, there has been no insight into the development of secondary AML in CLL patients due to long-term chlorambucil use. Therefore, this case report serves as a reminder of the increased risk for these patients and the need for early intervention. Although this is an isolated case report, Azacitidine combined with Bcl-2 inhibitor has shown promising results in clinical studies and may be considered as a potential treatment option for CLL/AML patients. However, further studies are needed to better understand the prevalence, prognosis, and characteristics of these patients.

## Data Sharing Statement

All relevant data and materials are included in the article.

## Statement of Ethics

This study was approved by the ethics committee of the Harison International Peace Hospital Affiliated to Hebei Medical University. This study followed the principles of the Declaration of Helsinki, and informed consent was obtained from the patient's family for publication of this case report and any accompanying images.

## Disclosure

The authors report no conflicts of interest in this work.

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