

Original Article

Chronic neutrophilic leukemia with JAK2 V617F mutation: a case report

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Abstract: Chronic neutrophilic leukemia (CNL) is a rare disease grouped by World Health Organization under the broader category of chronic myeloproliferative diseases. It is a diagnosis of exclusion in patients with sustained mature neutrophilia and splenomegaly with no evidence of other myeloproliferative diseases or reactive neutrophilia. JAK2V617F mutation has been described in classical myeloproliferative diseases, but its association with CNL has been reported in only a few cases. This study presented the case of a 64-year-old male diagnosed with CNL in August 2013. When the patient was 61 years old, his routine blood test showed a white blood cell count of $28.8 \times 10^9/L$ and neutrophil-lymphocyte ratio of 85%; however, the patient did not have any discomfort. During the next 3 years, his routine blood test showed a white blood cell count between $25 \times 10^9/L$ and $34 \times 10^9/L$; neutrophil-lymphocyte ratio was more than 80%, and hemoglobin and platelet count were in the normal range. The patient felt fatigued for 3 months. All systems appeared normal on examination, with the exception of splenomegaly. A complete blood count showed hemoglobin at 191 g/L and a leukocyte count of $25.24 \times 10^9/L$, with 90% neutrophils. The platelet count was $289 \times 10^9/L$. Bone marrow morphology showed primitive cells (1%), neutrophilic myelocytes (30%), and mature neutrophils (50%). The level of neutrophil alkaline phosphatase was 300 U/L. Molecular genetic analysis showed no *BCR/ABL* gene fusion, but 100% *JAK2 V617F* gene mutations. Chromosome analysis of the patient's bone marrow cells showed 46XY [5]/45XY-4 [2]. The patient was treated with hydroxyurea for 3 years and followed up for almost 5 years; the patient had a good quality of life.

Keywords: Chronic myeloproliferative disease, chronic neutrophilic leukemia, JAK2 V617F mutation

Introduction

Chronic neutrophilic leukemia (CNL), a rare and special type of leukemia, is characterized by persistent neutrophilia in the peripheral blood, hepatosplenomegaly, myeloid hyperplasia in bone marrow, and absence of the Philadelphia chromosome or *BCR/ABL* gene fusion [1]. It has been classified as atypical bone marrow proliferative disease (aMPD) by World Health Organization [2]. CNL was commonly found with an average age of onset of 62.5 years, and the survival periods of patients ranged between 6 months and 20 years (the median survival period of 30 months) with the 5-year survival rate of 28% [3].

JAK2 belongs to Janus kinase (JAK) family, a class of nonreceptor tyrosine kinases. JAK2 is an intracellular tyrosine protein kinase composed of 1132 amino acids with a molecular

weight of 13.493 kDa; its gene is located on chromosome 9p24 [4].

A mutation of V617F (a change of valine to phenylalanine at the 617 position) in JAK2 was found to be implicated in polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) by Baxter in 2005 [5]; subsequently, it was also found in other myeloproliferative disorders, but not in chronic myeloid leukemia, reactive bone marrow hyperplasia, lymphomas, and solid tumors. Therefore, CNL concurrent with the JAK2 V617F mutation was rare. This study described one such case as follows:

Case report

The patient was a 64-year-old man with the major complaint of fatigue and weight loss for more than 3 years, who was admitted to the

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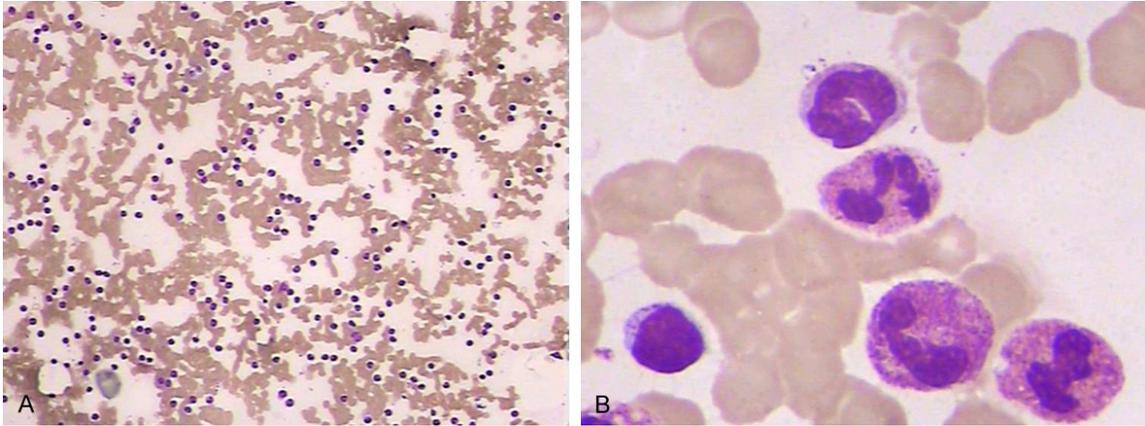


Figure 1. Bone marrow morphology: bone marrow hyperplasia obviously active, containing 1.5% bone marrow blasts, 2.5% promyelocytes, and 30% neutrophilic myelocytes. Cell chemical dyeing NAP was positive with a score of 300. (A) $\times 10$, (B) $\times 100$.

Zhongshan Hospital of Xiamen University, in January 2014.

Medical history

The patient felt slightly fatigued without any other discomfort 3 years ago. The blood cell count showed a white blood cell count of $26.80 \times 10^9/L$ with neutrophils 90%, hemoglobin 150 g/L, and platelet count $135 \times 10^9/L$; however, the patient did not receive any treatment. The fatigue remained with 10% weight loss 2 years ago; blood cell count showed a white blood cell count of $30.2 \times 10^9/L$ with neutrophils 88%, hemoglobin 162 g/L, and platelet count $210 \times 10^9/L$. Yet the patient did not receive any treatment until 1 month ago; he felt aggravated fatigue with 20% weight loss; and blood cell count showed a white blood cell count of $25.24 \times 10^9/L$ with neutrophils 90%, hemoglobin 191 g/L, red blood cell count $6.33 \times 10^{12}/L$, and platelet count $289 \times 10^9/L$.

Physical examination

The physical examination of the patient indicated purplish red face, cyanotic lips, and conjunctival congestion. The spleen was detected below the ribs with the measurements of 7 cm on line I, 9 cm on line II, and -1 cm on line III. The texture of the spleen was moderate with a smooth surface by palpation and no tenderness. The liver was not palpably detected.

Laboratory examination

There was marked hyperplasia of megakaryocytes, with the obvious active proliferation of

myeloid cells, containing 1.5% bone marrow blasts, 2.5% promyelocytes, and 30% neutrophilic myelocytes. The cell morphology remained normal. Neutrophil alkaline phosphatase was positive with a score of 300 (**Figure 1**). The screening for leukemia fusion genes (including *MLL-AF9*, *PML-RAR α* , *AML1-ETO*, *MLL-AF4*, *TEL-AML1*, *E2A-PBX1*, *MLL-ENL*, *BCR-ABL1*, and *SIL-TAL1*) was negative. The *BCR/ABL* gene fusion (P190, P210, P230) was negative, and JAK2 V617F mutation was 100% positive (**Figure 2**).

Chromosome analysis of the patient's bone marrow cells showed 46XY [5]/45XY-4 [2]. Other positive findings of serum tests included Erythropoietin (EPO) 2.90 IU/L, lactate dehydrogenase 305.0 U/L, and uric acid 459.7 $\mu\text{mol}/L$.

Treatment

The patient was treated with hydroxyurea, with the dosage adjusted according to the blood cell counts. The patient was reviewed regularly at the outpatient department, and the disease stabilized. The blood cell count showed that the white blood cell count ranged between $9 \times 10^9/L$ and $15 \times 10^9/L$ with neutrophils 60%, hemoglobin ranged between 140 and 160 g/L, and platelet count ranged between $210 \times 10^9/L$ and $300 \times 10^9/L$. The examinations were repeated every 4 or 6 weeks, and the JAK2 V617F mutation was persistent during the entire treatment period. Yet *BCR/ABL* gene fusion was never detected.

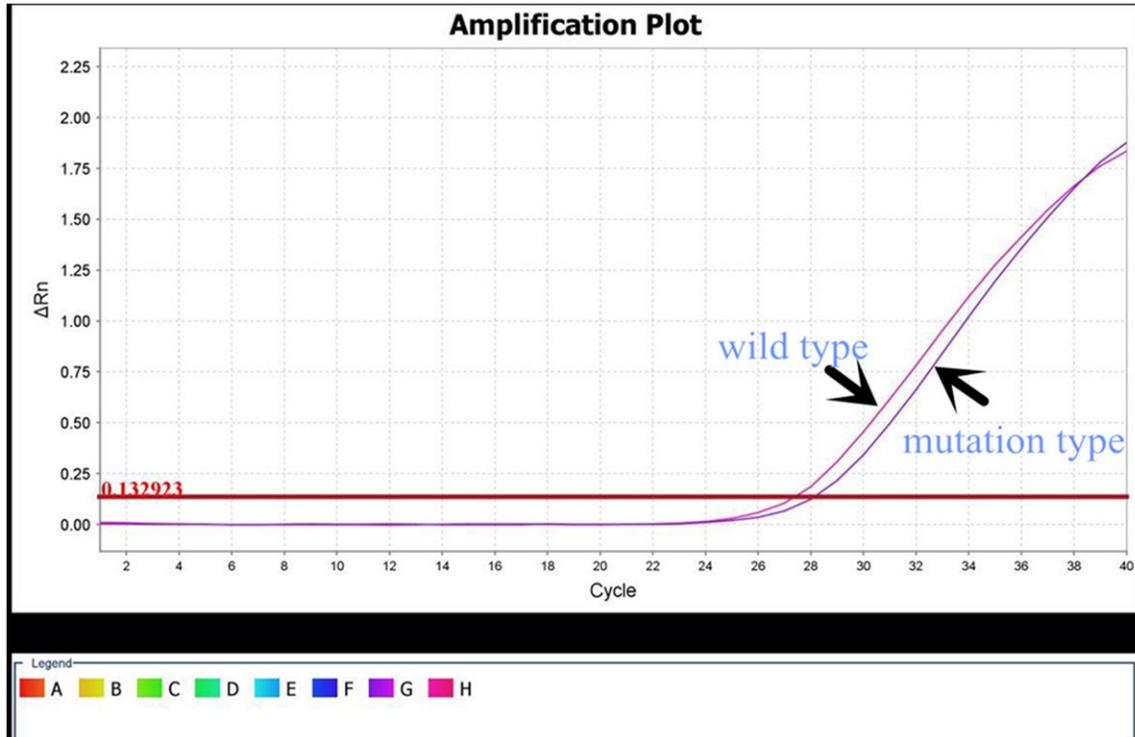


Figure 2. Jak2 V617F mutation type (purple curve) 100% mutations, red curve for the wild type positive control.

Discussion

CNL is a clonal myeloid disorder that might originate from pluripotent hematopoietic stem cells (HSCs) or derive from the progenitor cells of neutrophils. The disease has a chronic onset without subjective symptoms at an early stage, except the syndrome of hypermetabolism with symptoms such as occasional fatigue, fever, and weight loss. The diagnosis of CNL requires excluding reactive neutrophilic granulocytosis and bone marrow proliferative diseases with characteristic features such as bone marrow dysplasia, reticulin positive, and presence of the Philadelphia chromosome or *BCR/ABL* gene fusion. In the present case, the patient had persistently elevated neutrophils more than $20 \times 10^9/L$, splenomegaly, absence of *BCR/ABL* gene fusion, and obvious myelodysplasia with neutrophilic myelocytes or progenitor cells as the major type. All features fulfilled the diagnosis of CNL.

JAK2 is a nonreceptor tyrosine kinase. Guanine (G) is mutated into thymine (T) at 1849 position on chromosome 9 exon 12 of *JAK2* gene, leading to a change of valine to phenylalanine V617F. It results in the over-reaction of bone

marrow to cytokine and abnormal hematopoietic clone formation, finally leading to MPD [6-8]. The JAK2 V617F mutation has been found in 95% patients with PV, and 50%-60% patients with ET and IMF [9, 10]. The presence of JAK2 V617F mutation was included as the main diagnostic factor for MPD in 2008. Moreover, the mutation was also found in other types of bone marrow diseases, such as 60% of refractory anemia with ringed sideroblasts and thrombocytosis, 8% of chronic myelomonocytic leukemia, very few acute myeloid leukemia, and bone marrow hyperplasia syndrome [11-13].

However, very few reports were available on CNL concurrent with JAK2 V617F mutation [14-19]. The age of onset in patients with CNL ranged between 53 and 70 years (64 years on average), with no gender preference (male/female ratio 50:50). No specific symptoms were reported in the two patients; other non-specific symptoms such as fatigue, sweating, weight loss, and ankle pain were described. The chromosomes of patients were normal except of one patient with 46XY inv (9) and 40XY Del-20q12. Hydroxyurea administration was the major therapeutic strategy; only one

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patient underwent allogeneic HSC transplantation. The overall survival periods were more than 1.5 years with the maximum of 8 years till the current follow-up. The patient in the present case was 61 years old at disease onset, without specific symptoms except an increased number of white blood cells and increased percentage of neutrophils in the peripheral blood. Five years after CNL onset, the disease was stable under the treatment with hydroxyurea and interferon.

It was still an open question why JAK2 V617F mutation could be manifested as varied clinical symptoms, including PV, ET, PMF, and CNL. Three hypotheses were proposed as follows:

Hypothesis 1

JAK2 V617F targets different types of cells and hence leads to different diseases. It has been confirmed by X chromosome-linked cloning analysis that PV, ET, and PMF were all clonal diseases, and the JAK2 V617F-targeted cells might be HSC. Since the megakaryocytes, erythrocytes, and granulocytes were affected in ET, PV, and PMF, respectively, it was speculated that the JAK2 V617F-targeted progenitor cells differentiated into different types of cells. For instance, the progenitor cells with JAK2 V617F mutation had preferred differentiation to platelets rather than to erythrocytes or granulocytes, and thrombocytosis was the final outcome. The mature neutrophils might be the major target of AK2 V617F mutation in CNL, and the increased percentage of neutrophils was the eventual phenotype.

Hypothesis 2

The phenotype depends on the tyrosine kinase activity of JAK2 with V617F mutation. JAK2 V617F mutation has been found to be both heterozygous and homozygous, and the homozygous mutation might be caused through mitotic recombination. Therefore, different copy numbers could induce different dose intensities in the JAK2 signaling pathway. A low activity of JAK2 kinase leads to megakaryocytes/ET phenotype, whereas a high activity leads to erythrocytes/PV phenotype; a persistent activity of JAK2 kinase leads to PMF.

Hypothesis 3

It is also called the “double strike” theory. It states that JAK2 V617F mutation is not the only

event in pathogenesis. Another pathogenic event occurs before JAK2 V617F mutation, which is different in different types of disease. One of the evidences was that the JAK2 V617F mutational load in the granulocytes of patients with PV or PMF varied significantly, and the proliferated clonal granulocytes from the patients contained only 2%-25% JAK2 V617F mutation. Similar results were also obtained from clonality analysis with 20q as an autologous clonal marker. All these findings indicated that the first strike caused the clonal proliferation of HSCs, followed by JAK2 V617F mutation as the second strike. Nussenzveig et al [20] found both wild-type JAK2 and mutant JAK2 V617F in the endogenous erythroid colonies (EEC) from some JAK2 V617F-positive patients, indicating that some unknown event before JAK2 mutation not only initiated proliferation of clonal erythroid progenitors but also promoted erythropoietin-independent cell differentiation. Furthermore, Vainchenker et al [21] found that functional loss of epigenetic genes such as *TET2*, *ASXL1*, and *EZH2* might be the pathogenic event prior to JAK2 V617F mutation, although it might be involved at a late stage of disease progression. JAK2 V617F mutation was more often found in PMF, and it was speculated that the mutation could facilitate clonal selection and lead to dominant clones containing JAK2 V617F; the subtypes of MPD could be transformed into various types of leukemia.

Normal chromosome karyotype was reported in 90% patients with CNL, while only a few patients had abnormal clonal karyotype, including +8, +9, +21, del (20q), del (11q), or del (12q) [2]. The patient in the present case report had the partial loss of chromosome 4 without any clear consequence. This abnormality has not been reported yet in patients with CNL. The chromosome abnormality might be the random event prior to JAK2 V617F mutation. Therefore, white blood cells and neutrophils significantly increased at an early stage, and JAK2 V617F mutation resulted in erythroid hyperplasia.

The common therapy for CNL includes hydroxyurea, α -interferon, and induction chemotherapy. All could effectively alleviate the symptoms of CNL. Also, the allogeneic HSC transplantation was considered as a cure for CNL. However, the incidence of transplantation-related death and complications was high [22]. Patients with CNL concurrent with JAK2 V617F mutation usually had a long survival period with a good qual-

ity of life. Therefore, JAK2 V617F was regarded as a potential indicator for good prognosis. JAK2 inhibitor ruxolitinib was used for one patient with CNL concurrent with CSF3R T618I mutations [23], and the dose-dependent efficacy was achieved. The patient in the present case report had a 5-year history since the onset of CNL. He was only treated with hydroxyurea and interferon, and the disease was stable till date. It is presumed that the inhibitor of JAK2 could alter the activity of mutated JAK2, eventually curing the disease.

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Disclosure of conflict of interest

None.

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References

- [1] Vardiman J, Hyjek E. World health organization classification, evaluation, and genetics of the myeloproliferative neoplasm variants. *Hematology* 2011; 2011: 250-6.
- [2] Elliott MA, Hanson CA, Dewald GW, Smoley SA, Lasho TL, Tefferi A. WHO-defined chronic neutrophilic leukemia: A long-term analysis of 12 cases and a critical review of the literature. *Leukemia* 2005; 19: 313-7.
- [3] Reilly JT. Chronic neutrophilic leukaemia: a distinct clinical entity? *Br J Haematol* 2002; 116: 10-8.
- [4] Tefferi A. JAK2 Mutations and Clinical Practice in Myeloproliferative Neoplasms. *Cancer J* 2007; 13: 366-71.
- [5] Baxter EJ, Scott LM, Campbell PJ. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders (vol 365, pg 1054, 2005). *Lancet* 2005; 366: 122.
- [6] Saharinen P, Vihinen M, Silvennoinen O. Autoinhibition of Jak2 tyrosine kinase is dependent on specific regions in its pseudokinase domain. *Mol Biol Cell* 2003; 14: 1448-59.
- [7] Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N, Scott MA, Erber WN, Green AR; Cancer Genome Project. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005; 365: 1054-61.
- [8] Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; 352: 1779-90.
- [9] Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, Score J, Seear R, Chase AJ, Grand FH, White H, Zoi C, Loukopoulos D, Terpos E, Vervessou EC, Schultheis B, Emig M, Ernst T, Lengfelder E, Hehlmann R, Hochhaus A, Oscier D, Silver RT, Reiter A, Cross NC. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood* 2005; 106: 2162-8.
- [10] Vizmanos JL, Ormazábal C, Larráyo MJ, Cross NC, Calasanz MJ. JAK2 V617F mutation in classic chronic myeloproliferative diseases: a report on a series of 349 patients. *Leukemia* 2006; 20: 534-5.
- [11] Ceesay MM, Lea NC, Ingram W, Westwood NB, Gäken J, Mohamedali A, Cervera J, Germing U, Gattermann N, Giagounidis A, Garcia-Casado Z, Sanz G, Mufti GJ. The JAK2 V617F mutation is rare in RARS but common in RARS-T. *Leukemia* 2006; 20: 2060-1.
- [12] Levine RL, Loriaux M, Huntly BJ, Loh ML, Beran M, Stoffregen E, Berger R, Clark JJ, Willis SG, Nguyen KT, Flores NJ, Estey E, Gattermann N, Armstrong S, Look AT, Griffin JD, Bernard OA, Heinrich MC, Gilliland DG, Druker B, Deininger MW. The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood* 2005; 106: 3377-9.
- [13] Stijnis C, Kroes WG, Balkassmi S, Marijt EW, Van Rossum AP, Bakker E, Vlasveld LT. No evidence for JAK2(V617F) mutation in monoclonal B cells in 2 patients with polycythaemia vera and concurrent monoclonal B cell disorder. *Acta Haematologica* 2012; 128: 183-6.
- [14] Mc Lornan DP, Percy MJ, Jones AV, Cross NC, Mc Mullin MF. Chronic neutrophilic leukemia with an associated V617F JAK2 tyrosine kinase mutation. *Haematologica* 2012; 90: 1696-7.
- [15] Lea NC, Lim Z, Westwood NB, Arno MJ, Gäken J, Mohamedali A, Mufti GJ. Presence of JAK2 V617F tyrosine kinase mutation as a myeloid-lineage-specific mutation in chronic neutrophilic leukaemia. *Leukemia* 2006; 20: 1324-6.
- [16] Kako S, Ka Y, Sato T, Goyama S, Noda N, Shoda E, Oshima K, Inoue M, Izutsu K, Watanabe T, Motokura T, Chiba S, Fukayama M, Kurokawa M. Early relapse of JAK2 V617F-positive chronic neutrophilic leukemia with central nervous

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- system infiltration after unrelated bone marrow transplantation. *Am J Hematol* 2007; 82: 386-90.
- [17] Thiele J. Philadelphia chromosome-negative chronic myeloproliferative disease. *Am J Clin Pathol* 2009; 132: 261-80.
- [18] Xueya Z, Jingxin P, Jianxin G. Presence of the JAK2 V617F mutation in a patient with chronic neutrophilic leukemia and effective response to interferon α -2b. *Acta Haematologica* 2013; 130: 44-6.
- [19] Jason G, Maxson JE, George TI, Tyner JW. The new genetics of chronic neutrophilic leukemia and atypical CML: implications for diagnosis and treatment. *Blood* 2013; 122: 1707-11.
- [20] Nussenzveig RH, Swierczek SI, Jelinek J, Gaikwad A, Liu E, Verstovsek S, Prchal JF, Prchal JT. Polycythemia vera is not initiated by JAK2(V17F) mutation. *Exp Hematol* 2007; 35: 32-8.
- [21] William V, Isabelle P. TET2 loss, a rescue of JAK2V617F HSCs. *Blood* 2015; 125: 212-3.
- [22] BöhM J, Schaefer HE. Chronic neutrophilic leukaemia: 14 new cases of an uncommon myeloproliferative disease. *J Clin Pathol* 2002; 55: 862-4.
- [23] Lasho TL, Mims A, Elliott MA, Finke C, Pardanani A, Tefferi A. Chronic neutrophilic leukemia with concurrent CSF3R and SETBP1 mutations: single colony clonality studies, in vitro sensitivity to JAK inhibitors and lack of treatment response to ruxolitinib. *Leukemia* 2014; 28: 1363-5.