Chronic Neutrophilic Leukemia with V617F JAK2 Mutation

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Abstract Chronic neutrophilic leukemia (CNL) is a rare disease grouped under World health organization classification as chronic myeloproliferative disease. It is a diagnosis of exclusion in patients with sustained mature neutrophilia and splenomegaly with no evidence of other myeloproliferative disease or reactive neutrophilia. V617F JAK 2 mutation has been described in classical myeloproliferative diseases, but its association with CNL has been reported in a few cases. Here in, we describe three cases of CNL with presence of V617F JAK 2 mutation. To distinguish CNL from secondary neutrophilia can be difficult. Detection of the V617F JAK 2 mutation in such scenario can provide a useful diagnostic test to establish the neoplastic nature of the neutrophilia.

Keywords Chronic myeloproliferative disease · Chronic neutrophilic leukemia · V617F JAK 2 mutation

Introduction

Chronic neutrophilic leukemia (CNL) is a rare disease grouped under World health organization (WHO) classification as chronic myeloproliferative neoplasms (MPNs), characterized by sustained peripheral blood neutrophilia, bone marrow hypercellularity due to neutrophilic granulocytic proliferation, and hepatosplenomegaly. It is a diagnosis of exclusion in patients with neutrophilia and splenomegaly and no evidence of other myeloproliferative diseases or reactive neutrophilia. CNL is a disease of elderly with a mean age of diagnosis 62.5 years. The survival of CNL patients is variable, ranging from 6 months to more than 20 years with median survival of 30 months and 5-year survival of 28 % [1]. Transformation to acute leukemia is seen in 20 % of patients. In 2005, the V617F somatic point mutation in the pseudokinase domain of the Janus kinase 2 (JAK 2) gene on chromosome 9p was described in chronic myeloproliferative disorders. This mutation is present in nearly all patients with Polycythemia vera (PV), approximately 50 % of each of those with Essential thrombocythemia (ET), Primary myelofibrosis (PMF), 20 % of atypical myeloproliferative disorder and 0 % of chronic myelogenous leukemia [2, 3]. But it has not been identified in reactive myeloproliferation, lymphoid disorders, or solid tumors. Very few cases of CNL have been reported to harbor JAK 2 mutation [4–8]. Herein, we report three cases of CNL with presence of V617F JAK 2 mutation (Fig. 1).

Case Report

Table 1 shows the main clinical findings, routine laboratory investigations, therapy and outcome of CNL patients with

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Fig. 1 Analysis of V617F JAK 2 mutation using allele specific polymerase chain reaction –463 bp band as a control for DNA quality and quantity, 229 bp for wild-type allele and 279 bp for mutant allele. CNL patients (Pt1, Pt2, and Pt3) strongly exhibit 279 bp and were scored as positive for V617F JAK 2 mutation. L50–50 bp DNA Ladder, NC–Negative control, MutC–Mutant control, WtC–Wild type Control

V617F JAK 2 mutation including the present three cases. In present three cases, there was documented history of neutrophilia since 3 years in first patient, 1.5 years in second patient and 1 year in third patient. In all three present cases peripheral blood smear showed neutrophilia with no increase in eosinophils, basophils, premature granulocytes or myeloblasts. The neutrophils were of normal morphology. Bone marrow examination revealed hypercellular marrow with

neutrophilic granulocytic proliferation as well as erythroid and megakaryocytic proliferation in patient 1 and 2 and only neutrophilic granulocytic proliferation in patient 3. There was no evidence of myeloma or myelodysplastic syndrome. Bone marrow biopsy showed panmyelosis without any increase in reticulin. Megakaryocytes were of normal morphology without any dysplastic features. Neutrophil alkaline phosphatase was elevated in all the cases. Conventional cytogenetic analysis demonstrated a normal karyotype. BCR-ABL transcripts were not detectable on reverse transcriptase polymerase chain reaction (PCR). No cause was established for a secondary neutrophilia and they fulfilled the WHO diagnostic criteria for CNL. Analysis for mutation in V617F JAK2 was done using allele specific PCR, as already described [9]. DNA was extracted from whole blood using Invitrogen Purelink Genomic DNA Mini Kit as per the manufacturer's instructions and quantified using spectrophotometer. Two forward and two reverse primers were used in different combinations to generate three potential PCR products, a 463 bp band as a control for DNA quality and quantity, a band of 229 bp for wild-type allele and a band of 279 bp for mutant allele. All three strongly exhibit 279 bp and were scored as positive for homozygous V617F JAK 2 mutation. The homozygosity was confirmed on sequencing of V617F JAK2 mutation. They were started on hydroxyurea and the WBC count and splenomegaly decreased progressively with continued therapy.

Table 1 Laboratory features and treatment outcome of CNL patients with V617F JAK2 mutation

Studies	Age/Sex (years)	Karyotype	Presentation	Hb (g/dl)	TLC ($\times 10^9/L$)	Plt.C ($\times 10^9/L$)	ANC ($\times 10^9/L$)	Treatment	Survival (years)
Steensma et al. [4]	NA	NA	Associated B-cell lymphoma	NA	NA	NA	NA	Hydroxyurea	>2
McLornan et al. [5]	61/M	46XY	Hm + Sm, fatigue, influenza like illness	14.8	54	316	48	Hydroxyurea	>8
Lea et al. [6]	56/F	46XX	Sm, lethargy	14.5	39	259	33.3	Hydroxyurea–Busulphan	>2.5
Kako et al. [7]	46/M	46XY, Inversion 9	Hm + Sm	15.9	23.8	461	NA	Hydroxyurea–Bone marrow transplantation	3.5 (CNS relapse)
Thiele [8]	70/M	40XY, Del-20q12	NA	13.3	49	339	NA	NA	NA
Case1	53/F	46XX	Fatigue, pain abdomen-3years Hm(2 cm) + Sm(4 cm)	13	34	307	31.6	Hydroxyurea	>3
Case2	59/M	46XY	Fatigue, weight loss-2 mos Hm(2 cm)	10.6	29	287	25.5	Hydroxyurea	>1.5
Case3	61/M	46XY	Ankle swelling, weight loss-7years Hm(3 cm) + Sm(7 cm)	14.9	42	248	37	Hydroxyurea	>1.5

F female, M male, Hb hemoglobin, TLC total leucocyte count, Plt.C platelet count, ANC absolute neutrophil count, NA not available, Sm splenomegaly below costal margin, Hm hepatomegaly below costal margin, CNL chronic neutrophilic leukemia, Del deletion, CNS central nervous system

Discussion

The diagnosis of CNL requires exclusion of reactive neutrophilia and other MNPs with absence of dysplasia, reticulin fibrosis, Philadelphia chromosome and bcr-abl transcripts. A variant of bcr-abl fusion protein, p230 is found in few cases but those are considered as neutrophilic-chronic myeloid leukemia [10]. Cytogenetic abnormalities such as trisomy 8, trisomy 9, trisomy 21, deletions 20q, deletion 11q and deletion 12p are seen in 10 % of cases of CNL but none of them are sine qua non of CNL [11]. V617F JAK 2 mutations have been described primarily in PV, ET and PMF and only a handful of CNL cases with JAK 2 mutation have been reported so far (Table.1). JAK2 is a non receptor tyrosine kinase that plays a role in myeloid development by transducing signals from cytokines and growth factor receptors. The protein has two homologous kinase domain-JAK homology (JH) 1, an active tyrosine kinase domain and JH 2, a catalytically inactive pseudokinase domain. The V617F JAK 2 mutation results in valine to phenylalanine substitution at position 617 in the pseudokinase domain, producing a conformational change that interferes with the normal regulatory interaction of the JH 2 domain with functionally active JH 1 domain resulting in a constitutively active cytoplasmic JAK 2 that activates signal transducer and activator of transcription, mitogen activated protein kinase and phosphatidylinositol 3 kinase (PI3K) signaling pathways to promote transformation and proliferation of hematopoietic progenitors [12]. JAK 2 mutation has been identified in a myriad of MNPs and it is not clear why different individuals with V617F JAK 2 mutation show preferential expansion of erythroid, granulocyte, megakaryocyte, monocyte, or eosinophil lineages. This could be due to the constitutional genetic background or secondary acquired changes and thus, JAK 2 mutations, although important contributors in the development of MNPs may not be the sole genetic events responsible for pathogenesis of MPNs. Few cases of CNL with Jak2 mutations have been reported and so far the data shows a favorable impact of JAK2 mutation in CNL (Table.1). Most of the patients had stable clinical course with hydroxyurea/busulphan [4–6]. Kako et al. described a case of V617F JAK2-positive CNL who had progressive disease and developed early systemic relapse and central nervous system infiltration after bone marrow transplantation in the progressive phase after 3 year of initial diagnosis. This patient, however, had an additional cytogenetic abnormality i.e. inversion 9 which may have influenced the disease course [7]. Oral cytoreductive agents including hydroxyurea, busulphan and 6-thioguanine to control hyperleucocytosis, alpha interferon therapy and allogeneic transplantation have all been tried in CNL with variable results. [11] In the current era of targeted drug therapy, specific JAK 2 inhibitors can be considered

for treatment of V617F JAK 2 positive cases of CNL. Since only a few cases of CNL with V617F JAK 2 mutation have been reported so far, it is difficult to establish relationship between JAK 2 mutation, response to specific JAK 2 inhibitors and survival in CNL.

To distinguish myeloproliferative disorders from reactive conditions, particularly CNL from secondary neutrophilia, can be difficult. Detection of the V617F JAK 2 mutation in such scenario can provide a useful diagnostic test to establish the neoplastic nature of the neutrophilia. An analysis of all patients with CNL would be required for evaluation of prevalence and prognostic relevance of the V617F JAK 2 mutation in this disease. This information may further be utilized in diagnosing and treating V617F JAK 2 positive patients of CNL with specific JAK2 inhibitors which are currently being evaluated for treatment for myeloproliferative neoplasms.

Conflict of Interest Nothing to report.

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