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Brief communication

JAK2 mutation in a patient with CLL with coexistent myeloproliferative neoplasm (MPN)

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ABSTRACT

JAK2 mutation has not been described in patients with chronic lymphocytic leukemia (CLL). We found JAK2 mutation in a patient with CLL and coexisting myeloproliferative neoplasm (MPN). In this patient, we demonstrated the presence of the JAK2 mutation in $CD34^+$ progenitor cells, myeloid lineage cells, megakaryocytes, B lymphocytes but not in T lymphocytes. This case represents the first case report of JAK2 mutation in CLL and may also suggest that, JAK2 mutation most likely represents a secondary event from primary gene mutations involving the primitive stem cells which give rise to MPN and CLL. Furthermore, in this case, we believe that we are the first to demonstrate that JAK2 mutation in myeloid and B lymphoid cells but not T lymphocytes in a case of coexisting CLL and MPN.

1. Introduction

The coexistence of chronic lymphocytic leukemia (CLL) and myeloproliferative neoplasm (MPN) is very rare and only a few cases were reported [1–5]. It has been suggested that impaired immune surveillance in CLL patients might contribute to increased risk of neoplastic proliferation causing second malignancies [1]. The coexistence of these two clonal hematological disorders in a patient raises the possibility that both are derived from the same pluripotent stem cell or they are purely coincidental. It is well established that the neoplastic cell in chronic myeloid leukemia is pluripotent and can give rise to myeloid and lymphoid cells [6]. A Janus Kinase 2 (JAK2) V617F mutation is present in the higher percentage of patients with myeloproliferative disorders (MPD) and may be the primary causative lesion, but has been reported as absent in CLL [7,8]. Here we report a case of an 80-year-old female who had both MPN with JAK2 mutation and CLL. To determine the presence JAK2 mutation in various cell lines we analyzed this mutation status in stem cells, myeloid cell lineages and lymphoid cells.

2. Case report

An 80-year-old Caucasian female was referred by primary care physician for a persistent leukocytosis in 2001. Patient was asymptomatic. No history of fever and weight loss. Physical examination was normal. Hemoglobin was 13.4 g/dl, hematocrit 42.7%, and platelet count was 525,000/μl. WBC count was 42,500/μl with 60% neutrophils, 23% lymphocytes, 5% monocytes, eosinophils 1% and 10% bands. The peripheral smear, shown in Fig. 1 and flow cytometry of peripheral blood cells, shown in Table 1 revealed a mixture of well differentiated lymphocytes, suggesting the presence of CLL cells with predominence of neutrophils and increased platelet count. Total lymphocyte count was 9795/μl which is suggestive of CLL and not benign lymphocytosis. Gated $CD45^+$ cells (Table 2) showed that $CD5^+$ (95%), $CD19^+$ (58.7%), $CD20^+$ (57.7%), $CD23^+$ (77%), $CD10^+$ (0.7%), consistent with the diagnosis of CLL. Bone marrow biopsy report revealed a hyperproliferative marrow with M:E ratio of 4:1. Nodules of small lymphocytes were seen in bone marrow. Megakaryocytes were increased and atypical with moderate increased in reticulin fibers. Flow cytometric analysis of bone marrow aspirate had monoclonal kappa B-cell population with co expression of $CD5^+$, $CD23^+$ consistent with chronic lymphocytic leukemia. Computerized tomography (CT) of abdomen and pelvis were with in normal limits. A diagnosis of CLL with associated chronic myeloproliferative disorder was made. Cytogenetics were normal. Patient was observed for 3 years without any treatment. After 3 years of follow-up her WBC increased to 76,400 with 42% lymphocytes and 52% neutrophils. Platelet counts increased to 1,000,000/μl. Repeat bone marrow aspirate was similar to previous bone marrow examination. BCR/ABL translocation was negative by RT-PCR analysis and by FISH. CD38 was negative and ZAP 70 was positive. The JAK2 mutation analysis by PCR was positive for the JAK2 V617F point mutation. Patient was started on hydroxyurea 1 g BID orally and her WBC decreased to 50,000/μl, with 39.7% lymphocytes and platelet count decreased to 243,000/μl and she remains asymptomatic. Patient had two coexisting diseases even before treatment with hydroxyurea ruling out the possibility of treatment associated disease. To determine the origin of JAK2 mutation we studied this mutation status in stem cells, myeloid cell lineages and lymphoid cells.

We isolated neutrophils, $CD20^+$ cells, $CD3^+$ cells and $CD34^+$ cells by ficoll/hypaque gradient density method and MACS columns (Miltney). $CD61^+$ were obtained after cultured $CD34^+$ cells in serum-free medium as outlined in Section 4. We did JAK2 mutation analysis in neutrophils, $CD20^+$ B lymphocytes, $CD3^+$ T lymphocytes, $CD34^+$ stem cells and $CD61^+$ megakaryocytes as stated in Section 4. Mutant JAK2 was found in myeloid progenitor cells, megakaryocytes, neutrophils, and B lymphocytes but not in T lymphocytes (Fig. 2). BsaX1 restriction enzyme analysis showed the mutations were heterozygous (Fig. 3).

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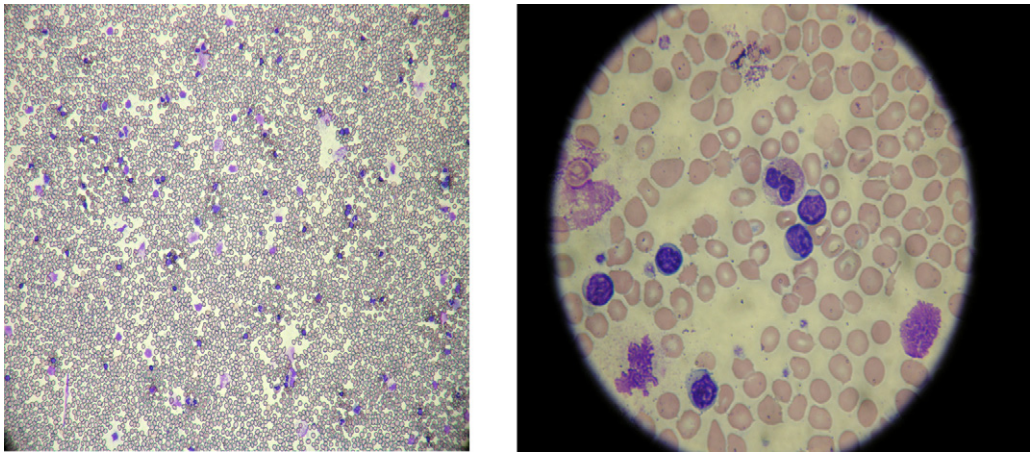

Fig. 1. Peripheral blood smear showing mixture of lymphocytes, neutrophils and smudge cells.

3. Discussion

The coexistence of CLL with MPN is very rare [2]. JAK2 V617F mutation is present in 95% of patients with polycythemia vera (PV), and about 50% of primary myelofibrosis (PMF) and ET and may be the primary causative lesion in MPN. The ratio of mutant to wild type JAK2 may determine the different phenotypic manifestation of MPN [9].

JAK2 mutation is somatic and occurs at the level of stem cells and is detected in stem cells and myeloid cells. JAK2 V617F mutation can also occur in erythroid cell lineages in MPD [10]. Levine et al. and Poulain et al. have not detected JAK2 mutation in patients with CLL in their retrospective studies [7,8]. JAK2 mutation was found in a subpopulation of patients in B and T lymphocytes with PV [11]. Hussein et al. reported the absence to JAK2 mutation in CLL cells in his case report with PV and coexistened B-CLL [12]. Henry et al. reported also the absence of V617F mutation in his case with coexistent CLL and ET in lymphoid cells [4]. In contrast to these finding our patient had JAK2 V617F mutation in B lymphocytes but not in T lymphocytes (the over 92% purity of the isolated $CD20^+$ cells and the presence of JAK2 mutation in the $CD20^+$ and not in the $CD3^+$ cells indicates the contamination of myeloid cells or monocytes in the $CD20^+$ cells are unlikely). Therefore, to our knowledge, our case represents the first case report that JAK2 mutation can occur in a patient with CLL. Since primary JAK2 mutations lead to myeloid and not lymphoid malignant phenotype as demonstrated in the mice model [13], JAK2 mutation in this patient is most likely a secondary event to which primary gene mutations occur at the common B lym-

Table 1
Flow cytometric analysis of patient's peripheral blood showing mixture of lymphocytes and neutrophils consistent with the diagnosis of myeloproliferative neoplasm with coexistence CLL.

Gate	% of gated cells
Lymphocytes	38.91
Blasts	0.47
Hematogones	0.25
Monocytes	5.32
Erythroids/plasma cells	0.51
Granulocytes	54.46

Table 2
Flow cytometic analysis of patient's peripheral blood gated for the gated 45^+ cells for lymphocyte markers in demonstrating the lymphocytes carried the B CLL markers as $CD5^+$, $CD23^+$, $CD20^+$ and $CD19^+$.

CD45	Lymphocyte gate	
	%	MFI
CD19	55.30	Mod
CD23	77.36	Bright
CD5	95.36	Bright
CD20	57.2	Mod
CD3	6.43	Bright
CD10	0.06	Mod

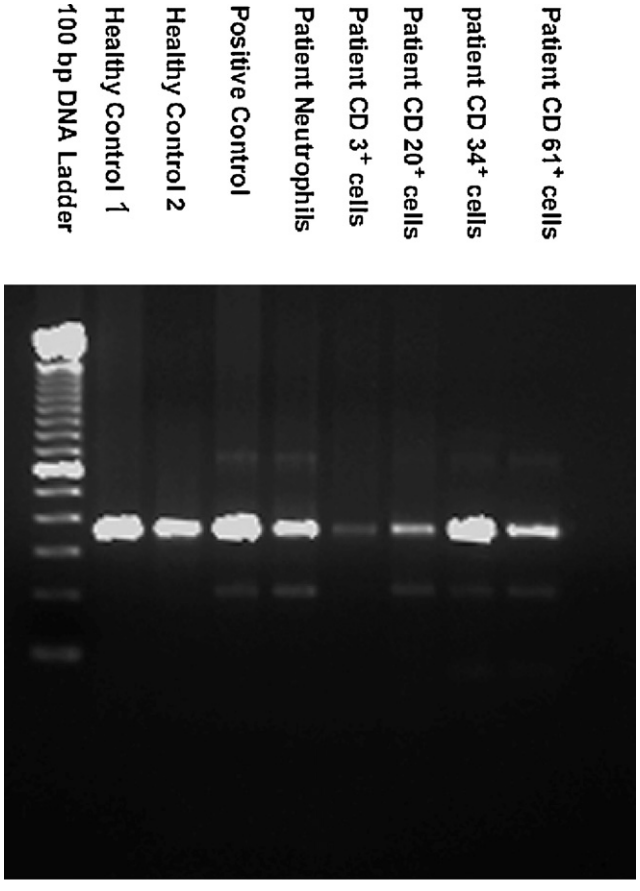

Fig. 2. Screening JAK2 V617F mutation by allele-specific PCR amplification. Genomic DNA from control and patient's neutrophils, T lymphocytes, B lymphocytes, $CD34^+$ cells and $CD61^+$ cells were amplified by allele-specific PCR for JAK2 wild-type fragment (364 bp) and V617F mutant fragment (203 bp). The 203 bp mutation band is present in patient's neutrophils, $CD34^+$ cells, B lymphocytes but not in T lymphocytes.