

Polycythemia Vera Terminating in Refractory Ascites

Kohtaro Toyama,¹ Takeki Mitsui,¹ Akihiko Yokohama,²
Takayuki Saitoh,^{1,3} Hideki Uchiumi,¹ Hiroshi Handa,¹
Masataka Sakuraya,¹ Hirokazu Murakami,⁴ Yoshihisa Nojima¹
and Norifumi Tsukamoto^{1,3}

A 64-year-old woman, with more than a 20 year history of polycythemia vera (PV), developed portal hypertension, myelofibrosis and extramedullary hematopoiesis accompanied by massive ascites. Portal hypertension resulted not only from infiltration of the liver sinusoids by hematopoietic cells but also from nodular regenerative hyperplasia of the liver. Wright-stained smears of ascites samples consisted of mesothelial cells and macrophages. However, cultures of mononuclear cells from the ascites showed the presence of hematopoietic progenitor cells including megakaryocyte colony formation and burst forming units. The JAK2-V617F mutation was positive in granulocytes. Contrary to other reports, radiation therapy was not effective and severe myelosuppression continued for more than one month. We present the unusual clinical course for this case of PV and discuss the pathophysiology of refractory ascites. (Kitakanto Med J 2012 ; 62 : 159~162)

Key words : polycythemia vera, ascites, extramedullary hematopoiesis, nodular regenerative hyperplasia, JAK2-V617F mutation

Introduction

Polycythemia vera (PV) is one of hematopoietic stem cell disorders characterized by JAK2-V617F mutation with uncontrollable proliferation of hematopoietic cells in the bone marrow, especially of the erythroid lineage. In the later stages of this disease, the development into myelofibrosis (MF) occurs, resulting in insufficient hematopoiesis. After transition into MF, there may be an extreme increase in portal blood pressure accompanied by hepatosplenomegaly.^{1,2} In this stage of PV, ascites can be observed, but excessive and refractory ascites is very rare.^{3,4} We report here on a female patient with PV who developed MF and refractory ascites during late stages of the disease.

Case Report

A 42-year-old woman visited our hospital com-

plaining of redness of her face in 1976. Physical examination revealed ruddy cyanosis on her face and mild splenomegaly. Laboratory data were as follows : hemoglobin (Hb) 18.8g/dl, hematocrit (Ht) 63%, RBC $7.59 \times 10^{12}/l$, reticulocytes 1.9%, white blood cells (WBC) $22.5 \times 10^9/l$ with a normal differential count, platelets (PLT) $186 \times 10^9/l$, leukocyte alkaline phosphatase activity 316 (control 208), blood cell volume 45ml/kg, arterial oxygen saturation 96%, serum total protein (TP) 8.8g/dl, lactate dehydrogenase (LDH) 422 IU/l, and elevated vitamin B12. She was diagnosed as PV and was primarily treated with intermittent phlebotomies, but she did not require phlebotomies in 1988. In 1990, she showed anemia and marked splenomegaly. At this time, she was diagnosed as spent phase of PV. In 1996, she was admitted to our hospital for surgery for an abdominal aneurysm. Esophageal varices and marked hepatosplenomegaly were noted. A liver biopsy specimen

1 Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan 2 Blood Supply Center, Gunma University Hospital, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan 3 Oncology Center, Gunma University Hospital, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan 4 Gunma University Graduate School of Health Sciences, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan

Received : February 24, 2012

Address : NORIFUMI TSUKAMOTO Oncology Center, Gunma University Hospital, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan

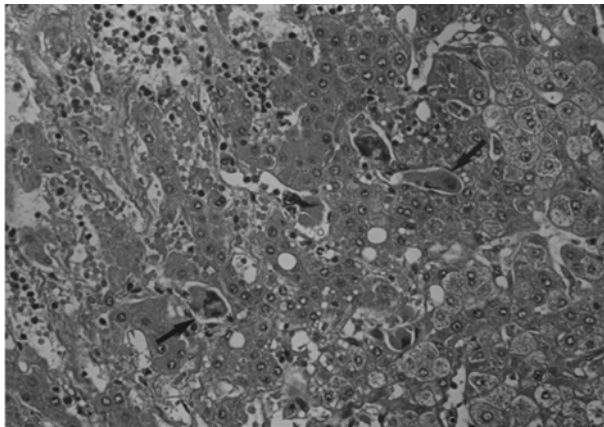


Fig. 1 Liver biopsy showing infiltration by hematopoietic cells. Arrows indicate megakaryocytes.

obtained during the operation revealed megakaryocytes and erythroblasts within the sinusoids. In addition, several nodules composed of enlarged hepatocytes were observed in each hepatic lobule, which were separated by compressed hepatic cells. There was no increase in collagen fiber indicating no evidence of cirrhosis. The microscopic examination was consistent with nodular regenerative hyperplasia (NRH) and extramedullary hematopoiesis. (Fig. 1)

In January 1998, her liver and spleen were palpable in the midclavicular line 7 cm and 15cm beneath the costal margin, respectively. Two months later, ascites and edema emerged without the presence of peripheral lymphadenopathy. Laboratory findings were: Hb 8.9g/dl, Ht 30.4%, RBC $3.11 \times 10^{12}/l$, reticulocytes 1.8%, PLT $77 \times 10^9/l$, WBC $9.2 \times 10^9/l$ with leukoerythroblastic changes. Liver and renal function tests were normal with TP 7.3g/dl, albumin 3.6g/dl, LDH 1116 IU/l, and ammonia level $83 \mu\text{g}/\text{dl}$. Tests for hepatitis B antigen and hepatitis C antibody were negative. Prothrombin time and activated partial thromboplastin time were normal. Bone marrow biopsy showed marked reticulin fibrosis, which was consistent with a diagnosis of myelofibrosis. Bone marrow scintigraphy (^{111}In) showed a remarkably reduced uptake in the vertebra but increased uptakes in both the liver and spleen, indicating the presence of extramedullary hematopoiesis. Computed tomography (CT) showed hepatosplenomegaly with dilated portal and splenic veins and a large quantity of ascites without the evidence of veno-occlusive disease or lymphadenopathy (Fig. 2). Magnetic resonance imaging supported this finding. Laboratory evaluation of the ascites revealed a specific gravity of 1.020, TP 4.5g/dl, and LDH 586 IU/l, which suggested that the ascites was an exudate. She was treated with diuretics, but it had little effect.

In November 1998, as the ascites continued to increase, she received 10 Gy of irradiation to the whole



Fig. 2 CT scan that shows remarkable hepatosplenomegaly and ascites with dilated portal and splenic veins. There was no evidence of veno-occlusive disease.

abdomen. However, there was no reduction in size of the hepatosplenomegaly and the volume of ascites. Pancytopenia required red blood cell and platelet transfusions, which were continued for one month. After that, physical condition was well maintained, but she died of septic shock due to ileus in February 2000.

Materials and Methods

Culture of Ascites

Ascites from the present patient and two other cases with carcinomatous peritonitis due to gastric cancer were obtained with informed consent. Mononuclear cells in the ascites were separated by use of the Ficoll-Hypaque density-gradient centrifugation method. Using MegaCult Kits (Stem Cell Technologies Inc, Vancouver, Canada), 1×10^5 of mononuclear cells were cultured in 5% CO_2 for 12 days in Iscove's medium containing bovine serum albumin (1%), insulin, transferrin, IL-3, IL-6 and thrombopoietin. After fixation with methanol and acetone, cells were stained with GP IIb/IIIa antibody.

For BFU-E analysis, 5×10^5 mononuclear cells were plated in duplicate with 3 IU EPO/ml.

Cell Separation of peripheral blood cells, DNA preparation, and JAK2-V617F mutation analysis

Heparinized peripheral blood sample (20ml) was obtained in 1989 and 1992, and was diluted 1:1 with Hank's balanced salt solution. The sample was subjected to Ficoll-Hypaque density gradient centrifugation at 400g for 30minutes. Granulocytes were isolated from the pellet after the removal of red blood cells by hypotonic lysis.

DNA was extracted from granulocytes and T-cells using a SepaGene DNA extraction kit (Sankyo, Tokyo, Japan). JAK2-V617F mutation status in the granulocytes was investigated using a direct sequencing method. To confirm the JAK2-V617F mutation, allele-specific polymerase chain reaction (AS-PCR)

was performed.⁵

Results

Upon microscopic evaluation, the cells in the ascites were found to be mainly macrophages and mesothelial cells, whereas malignant cells and mature hematopoietic cells were not observed. Cell cultures of the mononuclear cells from ascites revealed the formation of a megakaryocyte colony (CFU-Meg) as well as a burst forming unit (BFU-E) (Fig. 3). On the contrary, neither CFU-Meg nor BFU-E was observed in the ascites obtained from the two patients with carcinomatous peritonitis that resulted from gastric cancer. These results suggest that there were hematopoietic progenitor cells in the ascites of our case.

The JAK2-V617F was detected in granulocytes of 1989 and 1992. Whether the JAK2-V617F mutation

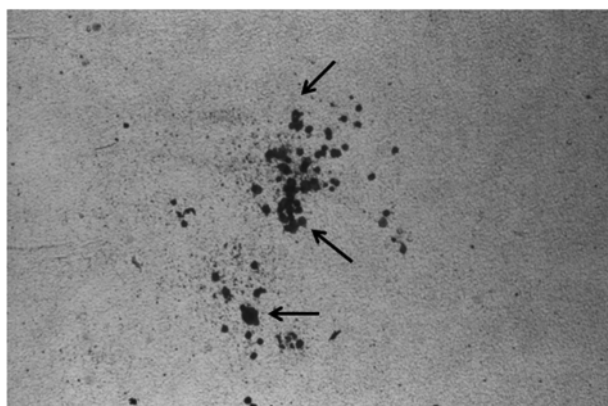


Fig. 3 The IIB/IIIA stain of cultured cells from ascitic fluid. Arrows indicate IIB/IIIA positive cells (CFU-Meg).

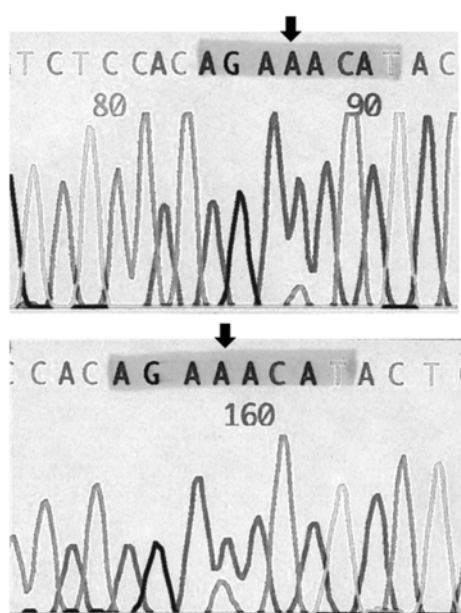


Fig. 4 Results of the JAK2-V617F mutation analysis. The T → A change was noted. A) granulocytes from 1989; B) granulocytes from 1992. Arrows indicate the mutated portion.

was positive or not in megakaryocytes in the present case was matter of interest, however, we were not able to perform the JAK2-V617F analysis because we failed to get enough samples.

Discussion

The importance of the JAK2-V617F mutation has been reported, and its positivity is one of the major criteria for the diagnosis of PV.^{1,5} The present case suggests the JAK2-V617F mutation persisted even after transformation from PV into MF, and importance of JAK2-V617F mutation analysis.

PV is a disorder characterized by excessive proliferation of hematopoietic stem cells, especially erythroid cells in the bone marrow, resulting in an elevated red blood cell mass. The natural course of polycythemia vera involves transition from a plethoric to a spent phase over a period of more than twenty years, and gradual development into myelofibrosis.^{1,2} This process is usually accompanied by extramedullary hematopoiesis in which the liver, spleen and lymph nodes are frequently affected. Ascites accompanied by myelofibrosis is rare, ranging from 2 to 10%, and the volume is usually small because it is controllable through the administration of diuretics.^{3,4}

In the present case, the ascites may have resulted from portal hypertension due to one of several mechanisms. First, it may have resulted from a portal vein or a Budd-Chiari type thrombosis related to the increased clotting tendency of the underlying hematologic disease.^{6,7} However, in our case it was possible to exclude this mechanism based on analysis of data from a CT scan and MRI images. In a second documented mechanism that could apply in this case, nodular regenerative hyperplasia of the liver can lead to an increase in the resistance to portal outflow leading to sinusoidal portal hypertension.⁸⁻¹¹ NRH has been reported to occur in association with congestive heart failure, Felty's syndrome and subacute bacterial endocarditis.^{8,9} Myeloproliferative disorders associated with NRH have rarely been reported.⁸⁻¹¹ The mechanism of NRH of the liver is uncertain but obliterative portal venopathy, especially in small vessels less than 0.2mm in diameter, might play a major role in its pathogenesis. Wanless et al. have suggested that thrombi first form in the portal venous circulation or spleen, with the resulting recanalization bringing about the formation of nodular regenerative hyperplasia. This then leads to the sinusoidal hypertension that may contribute to the emergence of ascites.^{9,11} In a third mechanism, the infiltration of the liver and spleen by hematopoietic cells may also increase portal vein pressure.^{12,13} And finally, splenomegaly itself may be responsible for the increases in blood flow and

portal vein pressure.^{11,13}

As the CFU-Meg and BFU-E were detected in the ascites from our patient, hematopoietic progenitor cells were suspected to present in the ascites of our case.^{13,14} There have been some reports that infiltration of the peritoneum by hematopoietic cells is the major cause of ascites in patients with myelofibrosis.¹⁵⁻¹⁸ Although mature hematopoietic cells were not present, hematopoietic cell infiltration might play a role to cause exudative ascites.¹⁶ In addition, the patient had the past histories of liver biopsies. After a liver biopsy incision, healing takes place and the resulting 'scar' might be weaker than other portions of the organ. Thus, a high portal vein pressure could cause the plasma and hematopoietic cells to infiltrate into the peritoneal space through the 'scar', resulting in massive ascites. However, the precise mechanism of ascites in this case remains uncertain because we were unable to perform an autopsy.

Though the findings in this report are unusual, refractory ascites due to portal hypertension, hematopoietic and progenitor cell infiltration into the peritoneal space may be one of the complications seen in patients during late stage PV.

References

1. Swerdlow SH, Campo E, Harris NL, et al, Eds. WHO classification of tumors of haematopoietic and lymphoid tissues. 2008; IARC, Lyon.
2. Abu-Hilal M, Tawaker J. Portal hypertension secondary to myelofibrosis with myeloid metaplasia: a study of 13 cases. *World J Gastroenterol.* 2009; 15: 3128-33.
3. Picoock JA, Reinhard ED, Justus BW, Mendelsohn RS: A clinical and pathological study of seventy cases of myelofibrosis. *Ann Intern Med* 1962; 57: 73-84.
4. Ward HP, Block MH: The natural history of agnestic myeloid metaplasia (AMM) and a critical evaluation of its relationship with the myeloproliferative syndrome. *Medicine* 1971; 50: 357-420.
5. Toyama K, Karasawa M, Yamane A, et al. JAK2-V617F mutation analysis of granulocytes and platelets from patients with chronic myeloproliferative disorders: advantage of studying platelets. *Br J Haematol* 2007; 139: 64-69.
6. Powell-Jackson PR, Melia W, Canalese J, Pickford RB, Portmann B, Williams R: Budd-Chiari syndrome: etiology, diagnosis and management. *Medicine* 1982; 61: 199-218.
7. Menon KV, Shah V, Kamath PS. The Budd-Chiari syndrome. *N Engl J Med* 2004; 350: 578-585.
8. Shoey J, Weinberg MN, Frenkel EP, Fallis BD: Nodular regenerative hyperplasia of the liver in a case of myelofibrosis with extramedullary hematopoiesis and secondary portal venous hypertension. *Am J Clin Pathol* 1979; 72: 122-125.
9. Wanless IR, Godwin TA, Allen F, Geder A: Nodular regenerative hyperplasia of the liver in hematologic disorders: a possible response to obliterative venopathy. *Medicine* 1980; 59: 367-379.
10. Degott C, Capron JP, Bettan L, Molas G, Berneau D, Potet F, Feldmann G, Benhamou JP: Myeloid metaplasia perisinusoidal fibrosis and nodular regenerative hyperplasia of the liver. *Liver* 1985; 5: 276-281.
11. Al-Mukhaizeem KA, Rosenberg A, Sherker AH. Nodular regenerative hyperplasia of the liver: an under-recognized cause of portal hypertension in hematological disorders. *Am J Hematol.* 2004; 75: 225-230.
12. Roux D, Merlio JP, Quinton H, Lamouliatte H, Balabaud C, Biolac-Sage P: Agnestic myeloid metaplasia, portal hypertension, and sinusoidal abnormalities. *Gastroenterology* 1987; 92: 1067-1072.
13. Loite F, Yeni P, Teilet-Thiebaud F, Barge J, Du Mayne JFD, Flamant Yet al. Ascites revealing peritoneal and hepatic extramedullary hematopoiesis with peliosis in agnogenic myeloid metaplasia: case report and review of the literature. *Am J Med* 1991; 90: 111-117.
14. Kumar NB, Naylor B: Megakaryocytes in pleural and peritoneal fluids: prevalence, significance, morphology and cytohistological correlation. *J Clin Pathol* 1980; 33: 1153-1159.
15. Yotsumoto M, Ishida F, Ito T, Ueno M, Kitano K, Kiyosawa K. Idiopathic myelofibrosis with refractory massive ascites. *Intern Med.* 2003; 42: 525-528.
16. Knobel B, Melamud E, Virag I, Meytes D: Ectopic medullary hematopoiesis as a cause of ascites in agnestic myeloid metaplasia. *Acta Haematol* 1993; 89: 104-107.
17. Leinweber C, Order SE, Calkins AR: Whole-abdominal irradiation for the management of gastrointestinal and abdominal manifestations of agnestic myeloid metaplasia. *Cancer* 1991; 68: 1251-1254.
18. Mishchenko E, Tefferi A. Treatment options for hydroxyurea-refractory disease complications in myeloproliferative neoplasms: JAK2 inhibitors, radiotherapy, splenectomy and transjugular intrahepatic portosystemic shunt. *Eur J Haematol.* 2010; 85: 192-199.