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Occurrence of *BCR-ABL1*-Positive Chronic Myeloid Leukemia following Essential Thrombocythemia

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Myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell diseases. MPNs are divided into *BCR-ABL1*-positive chronic myeloid leukemia (CML) and Philadelphia (Ph)-negative MPN. Classical Ph(–) MPN comprises essential thrombocythemia (ET), polycythemia vera, and primary myelofibrosis (PMF). Though CML and ET are quite distinct in their initial presentation and clinical courses, some CML and ET cases have overlapping clinical features. Moreover, coexistence of ET and CML may occur. As treatment for CML and ET is distinct, tyrosine kinase inhibitors have revolutionized the therapy for CML. Recently, molecular targeted therapy with JAK2 inhibitors demonstrated promising results for treating Ph(–) MPNs [1, 2]. Clinicians should be more aware that both disorders may coexist and need different therapies. Detection of *BCR-ABL1*, *JAK2*V617F, or *MPL* mutations are now recommended by WHO diagnostic algorithms to classify the subtypes of CML and ET [3]. According to these criteria, presence of the Ph chromosome and/or the *BCR-ABL1* fusion gene is mandatory for the diagnosis of CML, whereas 50–60% of ET patients carry *JAK2*V617F or *MPL* mutations. Here, we report a case of coexistence of ET and CML. The patient initially presented with typical ET with extreme thrombocytosis, mild leukocytosis, and grade 1 myelofibrosis without the *BCR-ABL1* transcript, which subsequently emerged. Following imatinib therapy for CML, which was diagnosed 2 years later, the clinicohematological features of ET reappeared while the patient had a good molecular response to the *BCR-ABL1* fusion transcript.

A 41-year-old male patient was referred to the hematological outpatient department because of abnormal blood counts. The patient had been well until he was detected to have mild microcytic anemia and thrombocytosis in a routine health examination in September 2004. He was a non-smoker and denied a prior history of bleeding tendency or thrombotic complications. He had no hepatosplenomegaly. His initial platelet count was $1{,}794 \times 10^9$/l, and his white blood cell (WBC) count was 12.4×10^9/l with a differential count of segments 67%, lymphocytes 27%, eosinophils 1.0%, monocytes 3.5%, myelocytes 1.0%, and atypical lymphocytes 0.5%. Hemoglobin was 13.1 g/dl with an MCV of 76.8 fl. Bone marrow aspiration yielded scanty particles that contained only numerous platelet aggregate masses. Prussian blue iron stain of the marrow smear showed increased marrow iron stores (grade 4); no bone marrow cells were available for cytogenetic analysis. Biopsy showed hypercellularity (80%) with myeloid and megakaryocytic hyperplasia and an M/E ratio of 9/1. Aggregates and clusters of megakaryocytes were found to be about 25–60/HPF; there was no increase in blasts or

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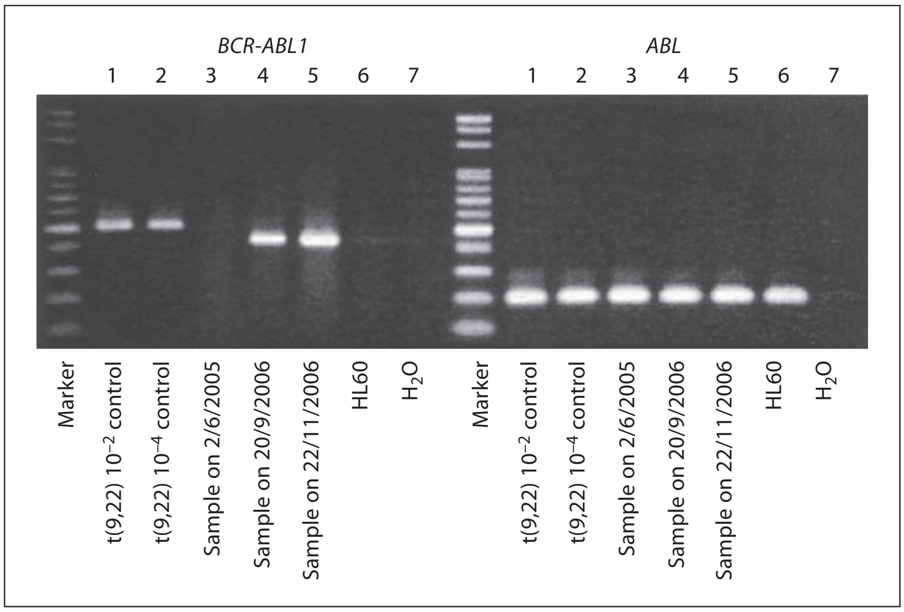

Fig. 1. RT-PCR analysis of the *BCR-ABL1* fusion transcript. Lanes 1 and 2 represent the positive control; lanes 3, 4, 5 display patient samples at different time points showing negativity for the *BCR-ABL1* fusion transcript on June 2, 2005, and positivity for the b2a2-*BCR-ABL1* transcript on September 20, 2006, and November 22, 2006. Lanes in the right panel represent the *ABL1* housekeeping gene for RNA integrity control.

atypical megakaryocyte morphology. Reticulin content showed minimal increase in small focal areas. No *BCR-ABL1* fusion transcript was detected in peripheral blood by reverse transcriptase polymerase chain reaction (RT-PCR). The serum ferritin level was 82.3 ng/ml. Hemoglobin electrophoresis and Gap-PCR followed by sequencing identified that the patient is a carrier of α-thalassemia-2 of the 3.7 deletion type. ET was diagnosed based on sustained excessive platelet counts (>1,200 × 10^9/l) without the *BCR-ABL1* fusion transcript and no apparent causes of secondary thrombocytosis. No specific treatment was prescribed.

The *BCR-ABL1* fusion transcript (b2a2 subtype) was first detected in peripheral blood on September 20, 2006, when the WBC count was 12.6 × 10^9/l and platelets were 996 × 10^9/l. RT-PCR assay again confirmed the absence of *BCR-ABL1* in the earlier sample freshly frozen on June 2, 2005, and presence in the subsequent sample on November 22, 2006 (fig. 1). Mutation analyses for *JAK2*V617F and *MPL*W515K/L showed wild-type results. Bone marrow biopsy in December 2006 showed a marked increase in reticulin fibrosis. Cytogenetic analysis of peripheral blood showed 46XY, t(9;22)(q34;q11) [22/25] and interphase FISH analysis showed that 86% of cells in the peripheral blood were positive for Ph chromosome. The diagnosis of *BCR-ABL1*-positive CML was made. The patient had splenomegaly of 6 cm palpable below the left costal margin. Treatment with imatinib mesylate (IM) 400 mg/day was initiated. The *BCR-ABL1* level was monitored thereafter by real-time quantitative PCR (RQ-PCR). Our laboratory-specific conversion factor for the *BCR-ABL1* International Scale (IS) was 1.32, which was calculated by the reference laboratory in Adelaide, Australia. The *BCR-ABL1* levels related to blood counts and treatment are shown in figure 2.

In April 2007, the *BCR-ABL1* level gradually decreased to 41.3% IS. The spleen became nonpalpable, indicating an initial response to IM. However, the platelet level rose again from 740 × 10^9/l to 1,968 × 10^9/l two months after IM treatment. IM was increased to 800 mg/day on April 12, 2007, when the platelet count was 1,572 × 10^9/l. However, the platelet count increased further to a level of 2,058 × 10^9/l when the *BCR-ABL1* level continued to decline to 2.5% IS. Interphase FISH analysis showed no Ph chromosome on July 12, 2007. The coexistence of ET was reconsidered then, for which hydroxyurea was prescribed and anagrelide was added in October 2007 in addition to IM therapy. The platelet count decreased after treatment, with a transient increase for 3 weeks, while the *BCR-ABL1* level showed a continuous decrease in the following months. The patient achieved a major molecular response (0.051% IS) on November 8, 2007, and IM was reduced to 400 mg/day on December 27, 2007. Bone marrow biopsy showed absence of myelofibrosis in April 2008. The patient has been in complete hematological response for ET and complete molecular response for CML since November 12, 2009. He has been kept on medication for ET and CML, including IM, anagrelide, and hydroxyurea, until now.

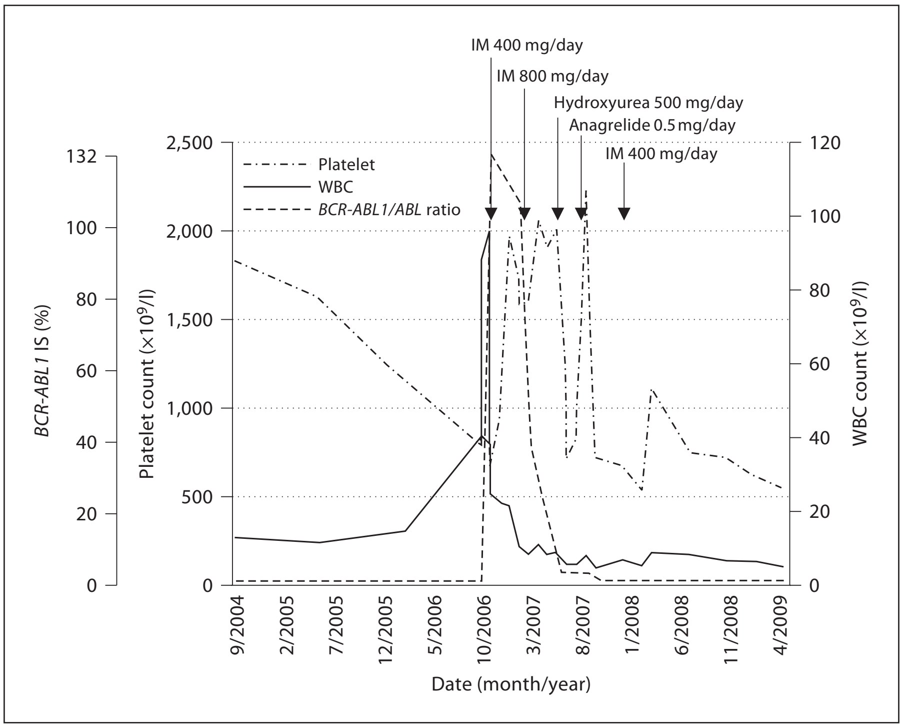

Fig. 2. Levels of *BCR-ABL1* transcript, WBC count, and platelet count during the follow-up period. IM 400 mg/day was initiated in December 2006. Hydroxyurea was added in July 2007 and anagrelide was added in October 2007. The level of *BCR-ABL1* transcript was analyzed by RQ-PCR and expressed in IS (%).

Table 1. Cytogenetic and genetic data of the reported cases with coexistence of ET and CML

BCR-ABL1 in the initial sample			*JAK2*V617 mutation in the initial sample	*MPL*W515 mutation in the initial sample	*BCR-ABL1* after treatment of ET			Reference
RT-PCR	FISH	karyotype			RT-PCR	FISH	karyotype	
+ (b2a2)	+	Ph	+	ND				[4]
ND	ND	No Ph	ND	ND	ND	+	Ph	[5]
ND	ND	No Ph	ND	ND	+	+	Ph	[6]
ND	–	ND	ND	ND	ND	+	Ph	[7]
ND	–	Ph [2/125]	ND	ND	ND	+	Ph	[8]
–	–	ND	–	–	+ (b2a2)	+	Ph	present case

ND = Not done.

A review of the literature showed that a total of 5 cases with coexistence of ET and CML have been reported previously [4–8]; one case had concomitant ET and CML at the initial presentation [4], 3 cases had an initial diagnosis of Ph(–) ET with emergence of a CML clone 6–9 years following hydroxyurea treatment [5–7], the remaining one had had a long history of isolated thrombocytosis before the diagnosis of Ph(–) ET in which rare Ph clones (2/125) were later identified on reanalysis by FISH when the Ph chromosome was detected 4 months later [8]. The current case is unique because we used the most sensitive RT-PCR assay to detect *BCR-ABL1* at the initial presentation whereas RT-PCR assay was only performed in one of the previously reported cases (table 1). The absence of the *BCR-ABL1* fusion gene in the blood samples of our patient at the initial presentation and 9 months later excluded the diagnosis of CML at that time. CML patients might have initial presentations mimicking ET with extreme thrombocytosis, normal WBC, or mild leukocytosis and no other CML features [9, 10]. Given that the *BCR-ABL1* fusion gene might be missed in a significant proportion of patients if they are examined only by conventional cytogenetics [11], presence of the *BCR-ABL1* fusion transcript at the initial presentation could not be completely excluded in some of the reported Ph(–) cases. Moreover, sequential molecular monitoring by RQ-PCR for the *BCR-ABL1* level was performed in our patient following IM therapy. The discrepancy between the molecular response of *BCR-ABL1* and the platelet count provided evidence of the coexistence of ET and CML, namely that the recurrence of extreme thrombocytosis was related not to the CML clone but rather to the ET clone. Therapy directed against both diseases with addition of hydroxyurea and anagrelide to IM which resulted in a further decline of *BCR-ABL1* to an undetectable level and of the platelet count to a normal level also supported our final diagnosis. Finally, our patient did not receive any chemotherapeutic agent before the emergence of the Ph(+) clone, which excluded the possibility of a drug-related leukemogenic effect.

In patients with coexistence of ET and CML, all but one of the cases reported earlier did not have gene mutation analyses for *JAK2*V617F and/or *MPL* [5–8]. Novella et al. [4] illustrated that *BCR-ABL1* rearrangement and *JAK2*V617F mutations in their patients were derived from different clones. Our patient did not harbor the *JAK2*V617F or *MPL* mutation; thus, we were unable to differentiate that CML developed from a normal stem cell or from a stem cell of the ET clone. On the other hand, Stoll et al. [9] described 6 Ph(+) CML presenting with clinical features mimicking ET; whether those cases had a possibility of coexistence of ET and CML was not clear. They were diagnosed and treated in the preimatinib era; no remitted status of CML was achieved and mutations of *JAK2* and *MPL* were not examined.

In summary, the case reported here describes an uncommon condition of coexistence of ET and CML in the same individual; molecular analysis and monitoring confirmed a diagnosis of *BCR-ABL1*-negative ET followed by emergence of a new clone of *BCR-ABL1*-positive CML and then reemergence of the ET clone while CML was remitted on IM. Therapies directed against the two disorders led to a complete hematologic and molecular response. The current case illustrated that two different MPNs may exist and require completely different therapies; they should not be misinterpreted as treatment failure for a single disease when the other one emerges.

Acknowledgments

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