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Variant translocation with a deletion of derivative (9q) in a case of Philadelphia chromosome positive (Ph+) essential thrombocythemia (ET), a variant of Chronic Myelogenous Leukemia (CML) with a poor prognosis

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Abstract
Patients presenting with thrombocytosis require thorough clinical and laboratory evaluation to determine whether they suffer from essential thrombocythemia or another myeloproliferative disorder. This distinction becomes increasingly relevant as targeted agents become available to treat specific myeloproliferative diseases. Cytogenetic testing plays a major role in this analysis. This study presents a patient with Philadelphia chromosome positive (Ph+) thrombocytosis and a cryptic der(9q)t(5;9)t(9;22) not found by conventional cytogenetics, whose disease progressed within 2 years to typical myeloblastic crisis of CML. It discusses the entity of Ph+ ET, the utility of molecular cytogenetic testing in the diagnosis of this unusual disease entity and the importance of cytogenetic testing in the prognosis of ET.

Keywords: *Variant Philadelphia chromosome, essential thrombocythemia, der(9q), chronic myelogenous leukemia*

Introduction

Essential thrombocythemia (ET) is a myeloproliferative disorder presenting with elevated platelets, similar to chronic myelogenous leukemia (CML). The significant overlap in clinical presentation between CML and ET has led to the wide acceptance that cytogenetic and molecular analyses are appropriate in all patients with unexplained thrombocytosis, although controversies still remain regarding the appropriate classification of *BCR-ABL* fusion positive cases presenting as ET [1]. Whether these patients are ultimately classified as CML or ET with a *BCR-ABL* fusion is less important than the separation of the 'BCR/ABL-positive ET' group of patients from those with 'BCR/ABL-negative ET', as the natural history of these two disease entities is distinct [1–3]. A recent review of Ph+ ET noted that 10 of 16 cases terminated in a disease similar to CML blast crisis [2] and median survival was noted to be 5 – 7 years [1,3], intermediate between CML and ET [4]. As has clearly been described in previous case reports and case series, more than half of BCR/ABL-positive ET patients will progress to the blast phase of CML [1,2] which has important implications for treatment and follow-up of this group of patients. In patients in whom only PCR positivity for BCR/ABL is found, the disease may not differ from PCR negative ET [5–7]. However, the diagnosis of Ph+ ET with cytogenetic positive BCR-ABL can be regarded as an early manifestation of the chronic stable phase of CML and usually represents a variant of chronic phase CML [2,3,8]. Cases described as Ph+ ET have normal WBC counts, often a normal LAP score, rare splenomegaly and may have thrombotic complications similar to Ph-negative ET, unlike most patients with chronic phase CML and thrombocytosis. The megakaryocytes in Ph+ ET and Ph+ thrombocythemia associated with CML are smaller than normal and typically have hypolobulated nuclei and this contrasts with the findings of clustered mature and enlarged megakaryocytes in Ph-negative ET [8]. These clinical differences highlight the importance of cytogenetic testing in patients who present with ET, even though Ph+ ET is rare, as prognosis may be significantly altered by this additional diagnosis. Specific therapies directed against the ABL kinase may be appropriate for patients with Ph+ ET with potential relevance for overall prognosis and survival.

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A verified stepwise diagnostic testing algorithm for the exclusion of the BCR/ABL gene fusion products in ET has yet to be devised. Similar to CML, multiple diagnostic modalities may be employed to exclude the BCR/ABL gene fusion product, including traditional karyotype analysis, reverse transcription-polymerase chain reaction (RT-PCR) and single fusion or double fusion probe fluorescence *in situ* hybridisation (FISH). Recently, FISH capable of identifying deletions in the derivative chromosome 9 has received attention because such deletions are a powerful and independent marker for poor prognosis in CML [9–12], even with the addition of imatinib to the treatment regimen [13]. This study reports a case of Ph + ET in a patient with a poor prognosis der(9) deletion whose disease terminated in a myeloblastic crisis. The case illustrates the importance of evaluating patients with thrombocytosis for BCR/ABL and reinforces the concept that Ph + ET has the potential to evolve clinically in a fashion more similar to CML than Ph − ET.

Case history

A 73-year-old man from the Dominican Republic, with a past medical history significant for hypertension and no known family history of malignancy, presented to his physician in the Dominican Republic in September 2001 when he noticed his toenails and fingernails appeared black. The patient was found to be thrombocythemic with a normal WBC and differential. He was diagnosed with small vessel thromboses. A sternal bone marrow aspiration was performed showing myeloproliferation with megakaryocytic hyperplasia. Cytogenetic analysis revealed a 46,XY,t(14;22)(q23;q13) karyotype. The patient had no splenomegaly and a LAP score was normal. A diagnosis of essential thrombocythemia was made. Hydroxyurea treatment was initiated for control of thrombocytosis. His nail discoloration improved. He continued with a normal CBC and normal differential for 18 months.

The patient had no symptomatic complaints when a routine CBC performed in the spring of 2003 showed a WBC of 32.8×10^3 cells μL^{-1} (16% neutrophils, 34% lymphocytes, 20% monocytes, 30% immature appearing monocytoid cells with basophilic stippling) accompanied by hemoglobin 10.7 g dL^{-1}; hematocrit 33.4%; platelets 262×10^3 μL^{-1}; and mean corpuscular volume (MCV) 113.6 fL. A repeat complete blood count (CBC) 2 weeks later showed the following: WBC 36×10^3 cells μL^{-1} (55% neutrophils, 11% lymphocytes, 11% monocytes, 13% basophils, 1% bands, 9% blasts); hemoglobin 11.0 g dL^{-1}; hematocrit 33.0%; platelets 227×10^3 μL^{-1}. Immunophenotyping of bone marrow aspirate gave the following results: CD13 +, CD33 +, CD34 +, CD45 +, HLA-DR +, CD117 +, consistent with acute myeloblastic leukemia (blast phase of CML).

In July 2003, this patient returned to the US and presented to the medical center. His CBC at that time showed: WBC 52.0×10^3 cells μL^{-1} (25% neutrophils, 4% lymphocytes, 25% monocytes, 15% basophils, 2% eosinophils, 30% blasts); hemoglobin 9.9 g dL^{-1}; hematocrit 31.5%; platelets 218×10^3 μL^{-1}. A repeat bone marrow biopsy showed hypercellularity with myeloproliferation, basophilia and an increase in immature myeloblasts (~50% of nucleated cells). A clinical diagnosis of CML in myeloblastic crisis was made and cytogenetic studies were performed. Systemic chemotherapy was initiated. The patient obtained a hematologic remission and was subsequently treated with imatinib. He relapsed with blastic disease 5 months later and succumbed to complications of his disease.

Materials and methods

Cytogenetic studies

Metaphase preparations were made by standard methods and analyzed on G-banded preparations. Twenty metaphases were analyzed and the karyotype was described by standard ISCN nomenclature. Spectral karyotype (SKY) analysis was performed on metaphase preparations using a human SkyPaint Probe kit obtained from Applied Spectral Imaging (Carlsbad, CA, USA) according to the manufacturer's protocol. SKY images were acquired with a SD300 Spectra cube mounted on a Nikon Eclipse 800 microscope by using a SKY optical filter (Chroma Technology, Brattleboro, VT, USA) and analyzed using SKY View software.

FISH was performed using BCR/ABL dual color, dual fusion (D-FISH) probe combined with an Aqua-labeled ASS probe mapped proximal to ABL at 9q34 (both probes were obtained from VYSIS, Downers Grove, IL, USA). Hybridization was performed using standard methods. Hybridization signals were analyzed on a Nikon Eclipse 600 microscope attached to a CytoVision imaging system (Applied Imaging, Santa Clara, CA, USA).

Results

Cytogenetic studies

Cytogenetic analysis done elsewhere on bone marrow mononuclear cells at initial presentation in 2001 revealed an unequivocal 46, XY,t(14;22)(q23;q13) karyotype. Cytogenetic testing performed in

July 2003 upon presentation to the medical center revealed a 46,XY,t(14;22)(q23;q13)[8]/46,XY, idem,t(13;17)(q12;q22)[9]/47,XY,idem, + 8[3] karyotype (Figure 1(*a*)) and immunohistochemical studies were positive for CD33, CD13, CD117, CD64, CD11c, HLA-DR and MPO antigens. To examine whether this complex karyotype was the result of variant Ph+ chromosome, a spectral karyotype (SKY) analysis was performed (Figure 1(*b*)). A combined G-banding and SKY karyotype of 47,XY, + 8,der(9)t(5;9)(?;q34),t(13;17)(q12;q22), del(14)(q23),ins(22;14)(q13.1;q24q32)[cp25] was derived and present in all metaphases, which provided no direct evidence for the existence of a BCR/ABL gene fusion. A deletion of 14q was recognised, as what was thought to be a translocation of 14;22 by G-banding was discovered to be ins(22;14) by SKY. Based on a high clinical index of suspicion for CML, FISH was also performed using a dual color, dual fusion BCR/ABL probe to look for the presence of a Ph+ chromosome It was observed that 99.5% of the inter-phase cells studied shows a pattern of two orange signals (one with highly reduced signal), one green signal and one fusion signal, consistent with the presence of the *BCR-ABL* gene fusion product (Ph+ chromosome) and deletion of the reciprocal partner. Analysis of metaphase chromosomes showed that the BCR/ABL fusion signal was present on the ins(22;14) chromosome and a reduced spectrum orange signal

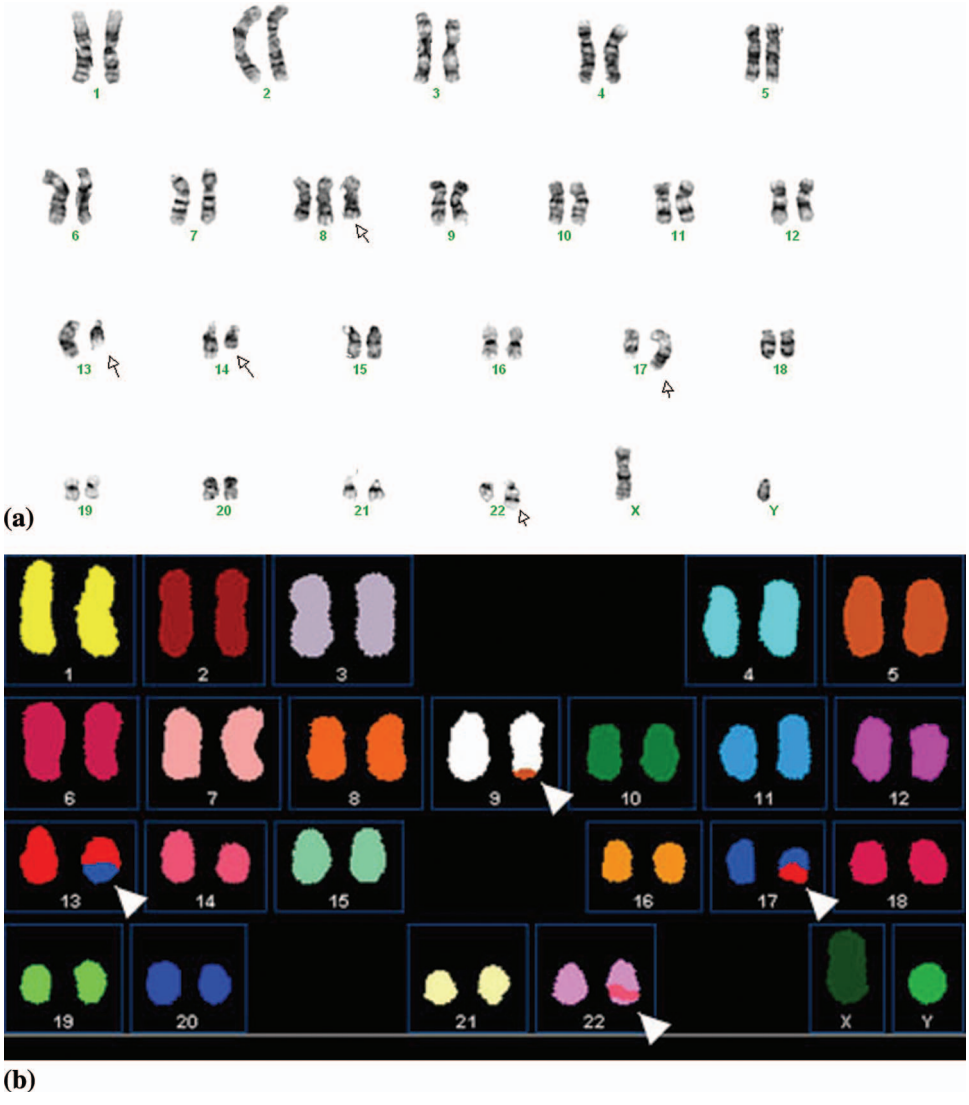

Figure 1. (*a*) A G-banded karyotype showing 46,XY, + 8,t(13;17)(q12;q22),t(14;22)(q23;q13). Arrows indicate abnormal chromosomes. (*b*) A SKY karyotype showing 47,XY, + 8,der(9),t(5;9)(?;q34),t(13;17)(q12;q22),del(14)(q23),ins(22;14)(q13.1;q24q32). Abnormalities are indicated.

on der(9) chromosome. This pattern of signals was suggestive of the presence of BCR/ABL fusion of the t(9;22) and a deletion on the der(9)t(5;9) involving the *3′BCR* sequence,. To confirm the deletion on der(9), FISH was also performed using the 9q (ASS) probe combined with BCR/ABL probe (Figure 2).

The ASS probe was present as a residual signal on the der(9) chromosome, suggesting a large part of the gene is deleted. This result again confirms the presence of a deletion on der(9). All metaphase and interphase cells with *BCR/ABL* fusion showed the same pattern; The experiment was replicated and both times scored by two different investigators. Overall, these results are suggestive of deletion of the most part of ASS gene, 5′ part of the *ABL* gene and the 3′ *BCR* sequence. Based on these results, a diagnosis of CML, with a cryptic *BCR-ABL* fusion and der(9) deletion, presenting with a clinical thrombocythemic onset was made.

RT-PCR for BCR-ABL

To confirm the presence of a standard BCR/ABL translocation, RNA was extracted from the patient's white cells using Triazol^{TM} and 2 μg of patient RNA was reverse transcribed using Superscript M-MLV reverse transcriptase, followed by 2 rounds of PCR using previously published primers for the P210 transcript. The result was positive for the typical chimeric message.

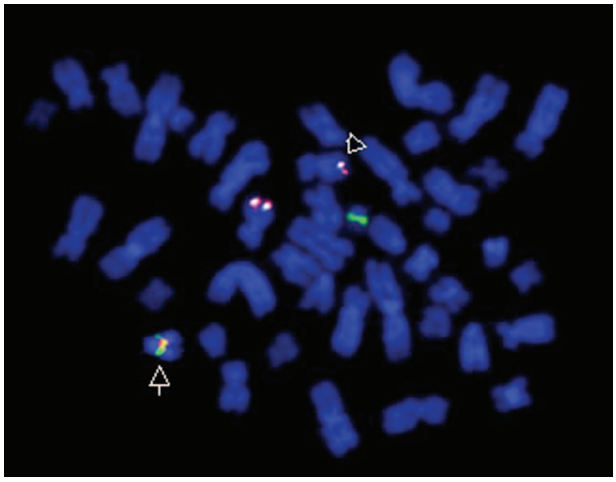

Figure 2. A metaphase showing FISH signals using BCR/ABL dual color, dual fusion probe (BCR, spectrum green; ABL, spectrum orange) combined with ASS probe mapped to 9q34 (Spectrum aqua). Normal chromosome 9 showing an ABL (orange) and ASS (aqua) signals together at 9q34. Normal chromosome 22 showing BCR signal (green). Arrow indicates the presence of BCR/ABL (green and orange) signals together on derivative chromosome 22. Arrowhead indicates the reduced signals of ABL (orange) and ASS (aqua) on der(9) chromosome.

Discussion

Essential thrombocythemia is a diagnosis of exclusion. It is a clonal myeloproliferative disorder which is limited to the megakaryocytic series. ET has clinical heterogeneity, with many patients having a relatively benign course over many years. It is rare to find chromosomal abnormalities in ET, estimated to be present in only 5–10% of cases by metaphase cytogenetics [14]. The Ph+ chromosome is the most commonly found abnormality and there is considerable evidence that Ph+ET may represent a 'forme fruste' of early chronic phase CML. CML is a chronic myeloproliferative disorder defined by the presence of the Ph+ chromosome, a chromosomal derivative formed by the reciprocal translocation of the long arms of chromosomes 9 and 22. This characteristic translocation takes place between the 5' end of the breakpoint cluster region (*BCR*) gene located at 22q11.2 and the 3' end of the c-ABL proto-oncogene (*ABL1*) located at 9q34, forming a BCR-ABL fusion gene that has a constitutively active Abl tyrosine kinase [15]. The Ph+ chromosome is seen during standard cytogenetic analysis in 85–95% of cases of CML, with the remaining 5–15% of cases showing variant Ph+ chromosomes or cryptic BCR-ABL rearrangements [16–21]. Case reports have described a translocation t(14;22), similar, but not identical to that seen in the patient, as both a simple and complex Philadelphia chromosome variant [22–24]. A number of studies have suggested that these variant translocations have prognostic importance [11,25]. As cytogenetic analysis can miss up to 10% of cases of CML, molecular tests for the BCR-ABL fusion product have been developed for use when a Ph+ chromosome cannot be identified. Southern blotting [26], RT-PCR and FISH [27,28] have all been used to identify the BCR-ABL gene rearrangement.

The patient was found to have a pattern of 2 orange signals, 1 green signal and 1 fusion signal by D-FISH. The solitary green signal and one of the solitary orange probes represent copies of the *BCR* and *ABL* genes on the normal chromosomes 9 and 22, respectively. The fusion probe is consistent with the presence of a *BCR-ABL* fusion gene on the derivative chromosome ins(22;14). The classic t(9;22) normally creates a reciprocal *ABL-BCR* fusion which is seen in the D-FISH assay as a second fusion signal on the derivative chromosome 9 [29,30]. In this patient, the second solitary orange probe seen on der(9) represents the *5′ABL* portion of the reciprocal fusion, without the expected *3′BCR*. The lack of a third green signal suggests that this region of genetic material has been deleted in

the multiple recombination events which formed the cryptic *BCR-ABL* fusion and complex karyotype seen in the patient. The dual fusion FISH probes reduce the false positive rate to almost zero for identifying the reciprocal fusion product [31–33]. However, the use of the dual fusion probe led to the observation that a significant number of patients with clinically apparent CML lacked all or part of the reciprocal fusion product [12]. These studies have revealed that deletions on der(9) are a powerful and independent marker for poor prognosis in CML [9–11]. In the present case the prognostic implications of a derivative chromosome 9 deletion also appear to be negative.

Dual color, dual fusion FISH thus offers both diagnostic and prognostic information in patients with suspected CML. Since survival differences in patients with der(9) deletions appear to be partially abrogated when patients are treated with imatinib [13], the test provides useful treatment information as well. However, the limitation of FISH is that it does not identify the presence of an active bcr-abl tyrosine kinase in the cell. A possible mechanism by which the der(9) deletions may confer a more severe phenotype may be the loss of *ABL-BCR* transcript. Since the FISH probes range from 300–650 kb in size, small, but clinically important, deletions may not be detected with this system. The prognostic relevance of such deletions has not been rigorously studied and published reports indicate that loss of *ABL-BCR* transcript alone does not confer a poor prognosis [34,35]. It is of interest that SKY analysis was unable to identify this complex translocation.

The analysis was confounded by a complex chromosomal rearrangement that lacked the reciprocal fusion product normally found on derivative chromosome 9 which would also have suggested the presence of a derivative *BCR/ABL* rearrangement. Cytogenetic abnormalities in ET are rare and it is believed this is the first report of a patient with Ph + ET, showing a deletion on der(9) with a variant Ph + chromosome. The clinical behavior of the patient in this case report suggests that this syndrome behaves more like CML than ET, in particular with the poor prognosis of those CML patients with der(9) Ph + variants, even with imatinib therapy. Since a functional *BCR-ABL* gene product is theoretically possible with the insertion of less than 350 kB of DNA, more specific identification of any clinically suspected *BCR/ABL* rearrangement in patients with ET must be investigated with molecular techniques such as dual color, dual fusion FISH. The findings in this patient argue for cytogenetic and FISH testing of all patients with suspected ET.

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