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CORRESPONDENCE

CASE REPORT

Hypereosinophilic syndrome or chronic eosinophilic leukemia: report of a case with a lytic bone lesion

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Some cases of hypereosinophilic syndrome and myeloproliferative disorders exhibit common features and thus pose diagnostic and therapeutic problems. We describe a 68-year-old patient who presented with such features and developed lytic lesion in the tibia. Based on our case and a review of literature we suggest that cases like ours should be classified and treated as chronic eosinophilic leukemia (a myeloproliferative disorder) rather than as a hypereosinophilic syndrome or as an atypical chronic myeloid leukemia.
Keywords: hypereosinophilic syndrome; chronic myeloid leukemia; chronic eosinophilic leukemia

Introduction

In some patients diagnostic confusion between hypereosinophilic syndrome (HES) and myeloproliferative syndromes like chronic myeloid leukemia (CML) with prominent eosinophilia can occur because of the overlapping features.[1,2] HES is an idiopathic leukoproliferative disorder characterized by sustained eosinophilia ($>1.5 \times 10^9$/l) without an apparent etiology or disease association and with evidence of organ involvement.[3,4] There is no specific diagnostic test for HES. Lytic bone lesions have not been described in HES. We describe a patient with hypereosinophilia who developed lytic bone lesions 4 months after initial diagnosis and was treated as a myeloproliferative syndrome (chronic eosinophilic leukemia) rather than as a hypereosinophilic syndrome.

Case report

A 68-year-old symphony clarinettist presented in January 1996 with a 6 month history of lethargy, weight loss, anorexia and pruritic skin nodules. Three months prior to presentation he suffered from an episode of acute gout. On examination he looked well with enlarged liver and spleen, 2 and 4 cm below the costal margin (BCM), respectively. There were skin nodules measuring about 1 cm in diameter on the upper chest. There was no lymphadenopathy. Laboratory investigations revealed Hb 103 g/l, platelets 169×10^9/l and WBC 42.1×10^9/l, with neutrophils 19.0×10^9/l, eosinophils 16.8×10^9/l, monocytes 1.3×10^9/l and lymphocytes 3.8×10^9/l. In the blood film there were a few myelocytes and metamyelocytes indicating myeloid left shift. In addition, some eosinophils were hypogranulated. The liver function tests, renal function and LDH were normal. Bone marrow examination revealed hypercellular marrow with panhyperplasia which was predominantly myeloid. About half the granulocytic cells were eosinophils and eosinophilic precursors. Trephine biopsy confirmed the nearly 100% marrow cellularity and moderately diffuse reticulin fibrosis. Cytogenetic studies on peripheral blood cells and bone marrow aspirate revealed a normal male karyotype with no evidence of Ph chromosome. Bcr-abl rearrangement was not detectable by polymerase chain reaction studies. X-rays of pelvis, shoulder, skull and chest were unremarkable. Biopsy of a skin nodule revealed granulocytic infiltrate with prominent eosinophilia and a small focal area of excess of blasts. The patient was treated with allopurinol and hydroxyurea. After an initial response to hydroxyurea, there was progression of the eosinophilia, splenomegaly and cutaneous nodules.

Four months after the initial presentation, he developed severe pain below the left knee. There was swelling and tenderness in the corresponding superomedial aspect of the left tibia. Spleen had enlarged further to 6 cm BCM but the skin lesions were stable. The WBC was 50.7×10^9/l with 17.2×10^9/l eosinophils, Hb 111 g/l and platelets 122×10^9/l. Radiological examination revealed extensive erosion of medial condyle and upper shaft of the left tibia with an adjacent extraosseous soft tissue mass. Magnetic resonance imaging (MRI) revealed diffuse extension of the myeloid tissue into the medullary cavities of the femur and destructive lesion of the left medial tibial condyle (Figure 1). He underwent internal bone fixation and biopsy on 25 April 1996. The bone biopsy revealed an extensive myeloid infiltrate with marked eosinophilia. In focal areas there were collections of immature cells (? blasts) and no megakaryocytes were recognized. Immunohistological studies revealed the immature blastic cells to be positive with antiglycophorin C antibody (ab) and therefore most likely erythroblasts. The blast cells did not stain with antimyeloperoxidase, CD61 (for megakaryoblasts), anti-B and T cell antibodies or the lysozyme. Bone marrow examination from the iliac crest at this time revealed no change in morphology and in particular there was no evidence of blastic transformation. Repeat cytogenetic and molecular studies also revealed no chromosomal abnormalities or bcr-abl rearrangement, respectively. He was treated with irradiation to the left tibia. Subsequently he was treated with three courses of mitozantrone and cytarabine which resulted in partial response. A trial of interferon therapy failed because of poor patient tolerance. His last treatment was a 4-day course of cytosine arabinoside. Twelve months after initial presentation, he continues to be reasonably well with WBC of 10.0×10^9/l with nearly 50% eosinophils.

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Discussion

The features of our case, ie marked eosinophilia, splenomegaly and skin nodules are characteristic of HES. However, lytic bone lesions have not been described in this syndrome. In two previous studies[1,2] it was noted that about half the patients of HES exhibited features common to myeloproliferative disorders. The myelofibrosis and increased numbers of mature and immature neutrophils in addition to the mature and immature eosinophils which were seen in our case are features which are more like a myeloproliferative syndrome. The term chronic eosinophilic leukemia[5] (CEL) has been proposed for this category of patients. CEL is a rare and controversial entity. Its distinction from HES and CML can be difficult. These patients have clinical and laboratory features like CML but like our case lack Ph chromosome or its molecular surrogate, the bcr-abl rearrangement. Cases in the literature with Ph1 chromosome[6] or bcr-abl rearrangement are best regarded as CML with marked eosinophilia. The *in vitro* growth pattern of CEL cases is also similar to that seen in CML further supporting the hypothesis that these cases are a myeloproliferative disorder.[7] Lytic bone lesions which were present in our case and can occur in blastic phase of CML, have not been described in HES. The cellular infiltrate in the lytic areas of the bone in our case consisted predominantly of eosinophilic myeloid cells. The focal areas of excess of blasts could represent areas of blastic transformation. Expansion of hemopoiesis in the femoral marrow was demonstrated on MRI and radionuclide scanning. This however is unlikely to produce lytic bone lesions which are more likely the result of localized blastic transformation of CEL, analogous to that seen in CML. Lytic bone lesions due to leukemia infiltrates have also been described in acute eosinophilic leukemia[8] but such a label would be inappropriate for our case because there was never an excess of blasts in the peripheral blood or the bone marrow. On the other hand, the label of chronic eosinophilic leukemia would be appropriate to our case despite the fact that no cytogenetic abnormalities were demonstrated on repeat examinations. It has been suggested very recently[9] that in such cases a presumptive diagnosis of eosinophilic leukemia is reasonable. Kueck *et al*[10] have reported a case very similar to ours and used a similar label in preference to hypereosinophilic syndrome to describe a condition which represents a primary neoplastic eosinophilic proliferation.

In summary, we describe a patient with hypereosinophilia who developed lytic bone lesions 4 months after initial presentation. It would be inappropriate to label our case as a hypereosinophilic syndrome with lytic bone lesions which has not been previously described. Such cases should be classified and treated as chronic eosinophilic leukemia which is a primary neoplastic eosinophilic proliferation, analogous to CML but without the Ph chromosome or evidence of bcr-abl rearrangement.

Addendum

Since submitting this manuscript the patient's disease has undergone unequivocal myeloid blastic transformation in the bone marrow and blood. Cytogenetic analysis at this timepoint reveals trisomies of chromosomes 8 and 6. These findings further support the conclusion reached in this case report.

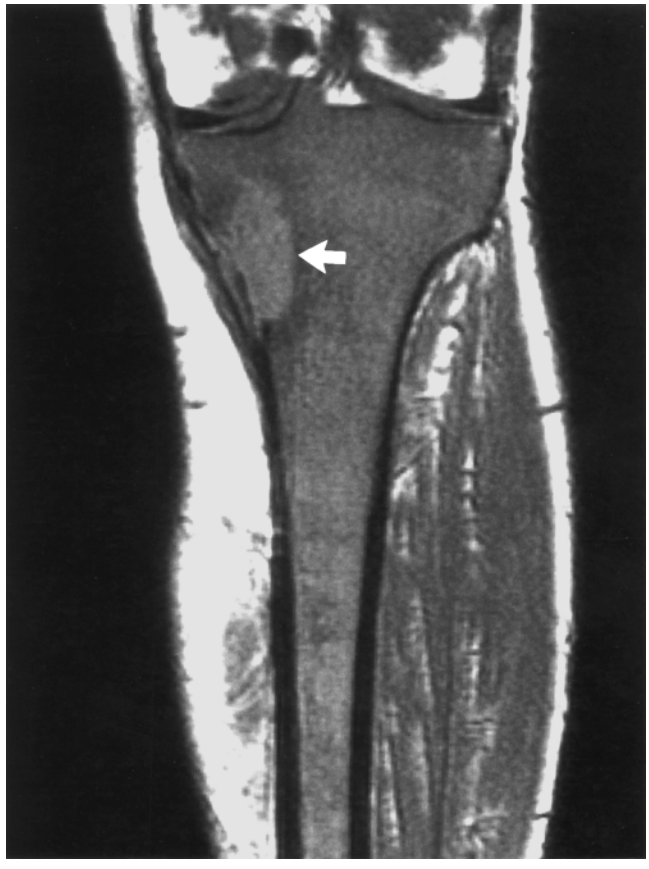

Figure 1 Magnetic resonance image of upper end of tibia showing lytic bone lesion.

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CASE REPORT

Emergence of new clonal abnormalities following interferon-alpha induced complete cytogenetic response in patients with chronic myeloid leukemia: report of three cases

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New treatments which may change the course of a disease, or which have potential carcinogenicity, may result in the development of new cytogenetic or clinical disorders. Three patients with Philadelphia chromosome-positive (Ph-positive) chronic myeloid leukemia (CML) who developed new cytogenetic abnormalities after achieving a cytogenetic complete remission (CR) of their Ph-positive disease with interferon alpha (IFN-α) based therapy are described. Patient 1 developed chromosomal abnormalities involving chromosomes 5 (5q13–34) and later 7 (monosomy 7) 60 months after the start of therapy and 20 months after IFN-α was discontinued. A myelodysplastic syndrome was noted 83 months from the start of therapy. Patient 2 developed a myeloproliferative syndrome with 18p11 chromosomal abnormalities 90 months after the start of the therapy and 60 months after IFN-α was discontinued. Patient 3 developed a chromosome 11 abnormality (11q21–23) 23 months after the start of therapy, without hematological manifestations. All three patients remain in cytogenetic CR of Ph-positive disease with the hypermetaphase fluorescent *in situ* hybridization and polymerase chain reaction studies for BCR/ABL showing minimal residual disease. The emergence of new cytogenetic or clinical disorders in patients with CML on IFN-α therapy needs to be monitored. These findings may be related to changing the natural course of CML, to therapy, or to the emergence of suppressed clones in a stem cell disorder.
Keywords: chronic myelogenous leukemia; clonal evolution; interferon-alpha; myelodysplasia; cytogenetic response

Introduction

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder characterized by the presence, in 90% of patients, of a chromosomal abnormality, t(9;22), called Philadelphia chromosome (Ph). Cytogenetic clonal evolution in CML is considered a manifestation of disease progression to a more accelerated form. Its development is noted in 30 to 50% of patients before the onset of blastic phase, and is associated with other manifestations of disease acceleration.[1–3] The presence of clonal evolution at diagnosis has been associated with a poor prognosis.[4–6] Clonal evolution during the course of CML may have variable prognostic implications.[7]

Treatment of CML with interferon-alpha (IFN-α) has resulted in complete hematologic response (CHR) rates of 70 to 80%, and cytogenetic response rates of 50% which are major and durable in 25 to 30%.[8] The latter patients have had the most favorable outcome with IFN-α therapy.[8–10]

In this report, we describe three patients with Ph-positive CML who achieved complete cytogenetic response (cytogenetic CR) on IFN-α treatment, but who developed new clones with cytogenetic abnormalities unrelated to the Ph chromosome, while still remaining in cytogenetic remission. The implications of these findings in relation to (1) changing the natural course of CML, and (2) to IFN-α therapy are discussed.

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Patients and methods

Patient 1

A 72-year-old male was diagnosed to have CML in February 1989. Cytogenetic studies showed 23/25 cells with t(9;22)(q34;q11) and 15p+. The patient received recombinant IFN-α 5 million units (mu)/m^2/day, and achieved a complete hematological remission (CHR) after 2 months, and a cytogenetic complete remission (CR) after 6 months of therapy. He continued in cytogenetic CR until July 1992, when IFN-α therapy was discontinued because of durable cytogenetic response, and moderate symptoms of Raynaud's phenomenon in the lower extremities. He remained in cytogenetic CR off therapy until March 1994 when his platelet count dropped to $83 \times 10^3/\mu$l. Cytogenetic studies on a bone marrow aspirate showed 23 diploid metaphases and two metaphases with deletion in chromosome 5(del(5)(q13q34) (Table 1). Repeat cytogenetic studies in August 1994 and in October 1995 showed diploid metaphases, and improved platelet counts. In February 1996, the Hgb dropped to 11.7 g/dl, the WBC was $3.1 \times 10^3/\mu$l with 1% blasts, and the platelets decreased to 49 $\times 10^3/\mu$l. Bone marrow aspirate showed 3% blasts and cytogenetic studies showed again the del(5q) clone with evidence of clonal evolution: 44,XY,del(1)(q32), del(5)(q13q34), –7,–12,–13,add(20)(p13),+mar. In April 1996, his Hgb fell to 9.9 g/dl, and platelets to $14 \times 10^3/\mu$l; the WBC count was $4.6 \times 10^3/\mu$l with a differential count showing an increase in the blasts to 18%. The bone marrow had <5% blasts (Table 1). Hypermetaphase fluorescent *in situ* hybridization (FISH) studies[11] done in April 1996 on 500 metaphases showed no cells with Ph abnormality (0%). Reverse transcription polymerase chain reaction (RT/PCR) study for BCR/ABL was positive. Repeat RT/PCR study on 17 July 1996 was negative. This RT-PCR was performed using a modified previously described technique.[12] Briefly, 5 μg of total RNA were reverse transcribed using Superscript II RT kit (Gibco BRL) as recommended by the manufacturer. RT/PCR was performed using the following fluorescent primers 5'ACCGGCATGTTCCGGGACAAAAG'-3', 5'-TGTTGACTGGCGTGATGTAGTTGCTTGG-3' to amplify the fusion transcript. The first primer was used with the following fluorescent primer 5'-ATTCGCTGACCATCAATAAG-3' to amplify the BCR transcript as an internal control.

Table 1 Characteristics and course of patient 1

Date	*Months since start of Rx*	*Rx IFN-α % dose*	*WBC (×10³/μl)*	*Platelets (×10³/μl)*	*Hgb (g/dl)*	*%Ph+ met (No. of met) in BM*	*Other cytogenetic abnormalities*	*Comments*
2/21/89	0	5 mu/m²/d	55.1	160	15.1	90 (25)	23/25 15p+	
9/6/89	6	100%	5.6	153	14.7	0 (5)		
2/7/90	11	100%	2.8	106	12.7	0 (20)		IFN-α reduced by 50% due to low counts
7/10/90	16	50%	4.1	80	11.9	0 (20)		
7/8/92	40	50%	6.0	109	14.0	0 (15)		IFN-α discontinued (Raynaud's +2)
10/14/93	55	None	5.1	137	16.2	0 (20)		
3/10/94	60	None	8.1	83	17.2	0 (25)	2/25 5q13q34	
8/24/94	66	None	6.0	119	15.6	0 (20)	diploid	
8/4/95	77	None	5.3	122	16.0	0 (20)	diploid	
2/13/96	83	None	3.1	49	11.7	0 (25)	25/25: 44,XY, del (1)(q32), del(5)(q13q34)–7, –12, –13, add (20)(p13), +mar	
4/25/96	86	None	4.6	14	9.9	0 (20)	17/20: same 3/20: diploid	18% blasts in PB, <5% blasts in bone marrow

Rx, treatment; IFN-α, interferon alpha; WBC, white blood cell count; Hgb, hemoglobin; Ph+, Philadelphia positive; met, metaphases; BM, bone marrow; mu, million units.

Multiplex with the internal control was performed side by side with a separate PCR for only the fusion transcript. In each experiment a reagent mix without DNA, positive control and negative control were included. To prevent carry-over contamination, we used Perkin Elmer (Norwalk, CT, USA) GeneAmp PCR carry-over Prevention kit, in each PCR reaction dUTP substituted for dTTP and each PCR reaction mix was treated with uracil-*N*-glycosidase (UNG) prior to amplification as recommended by the manufacturer. The PCR products were resolved on ABI 373 sequencer and Genescan software for quantitation (Applied Biosystems, Foster City, CA, USA). Southern analysis was performed on the initial presenting samples and showed rearrangement in the BCR typical for the t(9;22) translocation.

The patient became symptomatic later, requiring transfusions support with packed red blood cells and platelets every 1–2 weeks, and is alive as of December 1996. Marrow studies in October 1996 showed transformation to acute myeloid leukemia, still without Ph-chromosome abnormality detectable.

Patient 2

A 62-year-old female was found to have splenomegaly on routine physical examination in March 1984. Her CBC showed a WBC of $45.3 \times 10^3/\mu l$, a Hgb of 11.5 g/dl and a platelet count of $580 \times 10^3/\mu l$. The smear and the bone marrow were consistent with CML and the cytogenetic studies on a bone marrow aspirate showed t(9;22)(q34;q11) in all metaphases. The patient was treated with IFN-α 5 mu/m² daily, achieving a CHR after 2 months, and a cytogenetic CR after 13 months of therapy. She was continued on therapy for 18 more months (total treatment duration 31 months) when IFN-α was discontinued due to the development of Coombs-positive autoimmune hemolytic anemia; she also had concomitant autoimmune-mediated hypothyroidism which was treated with replacement therapy. She remained in cytogenetic CR after IFN-α was discontinued. Sixty-one months after IFN-A was discontinued, cytogenetic studies on a bone marrow aspirate showed 23/25 diploid cells and two with 46XX, 18p+. CBC showed a WBC was $8.3 \times 10^3/\mu l$, a platelet count of $196 \times 10^3/\mu l$ and a Hgb of 9.6 g/dl. Her WBC slowly rose to $32.7 \times 10^3/\mu l$ over the next 3 years. Her spleen was palpable 5 to 6 cm below the costal margin and continued to enlarge up to 17 cm below the costal margin 36 months later, when a splenectomy was performed. Since then, her WBC has been controlled with hydroxyurea. Cytogenetic studies on splenic tissue did not show the Ph abnormality, but showed 3/28 cells with 45XX,–6 (Table 2). Hypermetaphase FISH studies on marrow in February and December 1995 showed Ph-positive metaphases, respectively 5/500 (1%) and 2/374 (0.5%).

Patient 3

A 59-year-old white male was found to have an elevated WBC on a routine medical evaluation in November 1993. He was referred to our institution where his CBC showed a WBC of $78.1 \times 10^3/\mu l$, platelet count of $210 \times 10^3/\mu l$ and a Hgb of 13.7 g/dl. The WBC differential, peripheral blood smear and bone marrow aspiration were consistent with the diagnosis of CML. Bone marrow cytogenetic studies showed 100% Ph-positive metaphases. He was started on IFN-α 5 mu/m²/day and ara-C 10 mg/day subcutaneously, achieving a cytogenetic CR after 6 months of treatment. A 50% reduction in the dose of ara-C was necessitated by cytopenia. In August 1995, he developed hypothyroidism, requiring thyroid hormone replacement therapy. In November 1995, bone marrow cytogenetic studies showed 17 diploid cells and three cells with deletion in chromosome 11 (11q21–23). His counts remained within acceptable limits and the treatment has been continued since then (Table 3). Cytogenetic studies in May 1996 showed disappearance of the clonal evolution, and hypermetaphase FISH studies showed 2/400 Ph-positive cells (0.5%).

Table 2 Characteristics and course of patient 2

Date	*Months since start of Rx*	*Rx IFN-α % dose*	*WBC (×10³/μl)*	*Platelets (×10³/μl)*	*Hgb (g/dl)*	*% Ph+ met (No of met) BM*	*Other cytogenetic abnormalities*	*Spleen (size bcm in cms)*	*Comments*
7/16/84	0	5 mu/m²/d 100%	47.7	522	11.3	100 (25)		8	
2/5/85	6	50%	3.0	185	9.2	10 (20)		0	
6/3/85	10	50%	2.6	176	8.4	5 (20)		0	
9/10/85	13	50%	3.1	214	9.4	0 (20)		0	
2/20/87	31	Stop	2.6	214	7.7	0 (20)		0	developed hypothyroidism and AHA
10/2/90	74	None	6.9	255	9.9	0 (20)		0	
4/16/91	81	None	8.1	258	10.2	0 (20)		6	
2/25/92	91	None	8.3	196	9.6	0 (25)	2/25 18p+	5	
8/4/92	96	None	10.7	226	10.8	0 (20)	diploid	5	
1/13/93	101	None	10.4	196	10.4	0 (18)	1/18 18p+	6	
7/19/93	108	None	11.2	166	10.4	0 (20)	diploid	8	
1/17/95	114	None	32.7	145	11.3	0 (20)	1/20 18p11	17	
2/3/95	114						spleen CG: 25/28 diploid; 3/28 45XX,–6		splenectomy hydroxyurea 2 g/day started 2/17/95
12/11/95	124	Hydroxyurea	16	186	10.9	0 (20)	diploid	No spleen	

Rx, treatment; WBC, white blood cell count; Hgb, hemoglobin; Ph+, Philadelphia positive; BM, bone marrow; CG, cytogenetics; IFN-α, interferon alpha; mu, million units; met, metaphases; bcm, below the costal margin; cms, centimeters: d, daily; AHA, autoimmune hemolytic anemia.

Table 3 Characteristics and course of patient 3

Date	*Months since start of Rx*	*Rx IFN-α % dose; Ara-C % dose*	*WBC (×10³/μl)*	*Platelets (×10³/μl)*	*Hgb (g/dl)*	*%Ph+ met (No. met) BM*	*Other cytogenetic abnormalities*	*Comments*
11/17/93	0	IFN-α 5 mu/m²/d Ara-C 10 mg/d	78.1	191	13.2	100 (20)		
2/7/94	2	100%	2.9	85	11.9	insufficient metaphases		
5/9/94	5	100%	2.6	91	12.5	0 (24)		
11/14/94	11	100%	2.2	80	12.0	0 (20)		↓ara-C 50%
3/27/95	16	IFN-α 100%; Ara-C 50%	4.5	115	12.2	0 (20)		
7/17/95	20	IFN-α 100%; Ara-C 50%	3.5	98	13.8	0 (15)		developed hypothyroidism
11/6/95	23	IFN-α 100% Ara-C 50%	3.5	92	14.3	0 (20)	3/20 11q21q23	
5/20/96	30	IFN-α 100%; Ara-C 50%	3.5	100	13.8	0	diploid	

Rx, treatment; IFN-α, Interferon alpha; ara-C, cytosine arabinoside; mu, million units; sq, subcutaneously; WBC, white blood cell count, Hgb, hemoglobin, Ph+, Philadelphia positive; met, metaphases; FISH, fluorescent *in situ* hybridization.

Discussion

In this report, we describe three patients with Ph-positive CML who achieved a durable cytogenetic CR with IFN-α therapy, but developed unrelated new clones, with cytogenetic abnormalities, 5q13q34, 18p11, and 11q21q23 while still in cytogenetic CR of their CML disease. Two of the patients, had been off IFN-α treatment for 36+ and 60+ months, respectively. Both patients had, in addition, a myelodysplastic syndrome in the first case, and myeloproliferative syndrome in the second case, but the bone marrow cytogenetic studies did not show the Ph abnormality. The third patient developed the new cytogenetic abnormality while still on treatment with IFN-α and ara-C which later disappeared, but remains with normal counts on therapy and in Ph cytogenetic CR.

Several possible explanations can be related to the emergence of new clones in patients with CML in cytogenetic CR including: (1) changing the natural history of the disease; (2) treatment-related clonal emergence; and (3) underlying stem cell disease, associated with Ph-positive as well as other abnormalities.

Changing the natural course of a particular disease may unmask previously unknown patterns of disease evolution, which would become evident because of survival prolongation. The question always arises as to whether the new findings are part of the disease process or treatment-induced.

For example, prior to the advent of effective therapy with hydroxyurea, busulfan and other agents, most patients with Ph-positive CML (85%) expired from the complications of chronic phase disease. Controlling the chronic phase of CML with these agents resulted in the observation that most patients developed 'acute leukemia' or blastic phase and died of its complications. It was initially thought that the blastic phase may be related to hydroxyurea and busulfan therapy. While the leukemogenic potential of busulfan is established and that of hydroxyurea is questionable, it is now well known that the blastic phase of CML is part of the natural evolution of the disease in most patients, rather than therapy-related. Similarly, patients with hairy cell leukemia treated with effective therapy (IFN-α or nucleoside analogues) were observed to develop second malignancies.[13,14]

With IFN-α therapy, a change in the course of CML was achieved: survival prolongation in the average population, but most importantly among patients achieving major cytogenetic responses. In this report, three patients in the latter group developed new clonal abnormalities, two of them with significant myeloid disorders, myelodysplastic syndrome and myeloproliferative syndrome. Shepherd *et al*[15] reported the development of clonal disease in Ph-negative cells of two patients with CML on IFN-α therapy but who were not completely Ph-negative. The first patient developed trisomy 8 in 30% of Ph-negative metaphases (70% were still Ph-positive) following busulfan-induced aplasia and only 3 weeks of IFN-α therapy. She remains in CR following allogeneic bone marrow transplant (BMT). The second patient also has a minor cytogenetic response (Ph-positive 65%) but showed 5q– in the Ph-negative cells. While not similar to our cases, these two patients illustrate the development of independent clonal abnormalities in CML. Similar case reports have described the appearance of Ph-negative, trisomy 8-carrying clones in patients with Ph-positive CML receiving IFN-α therapy.[16–18] Two previous reports have suggested a possible association between IFN-α therapy and increased frequency of clonal cytogenetic abnormalities. Hild *et al*[19] noted a tendency of uncommon secondary aberrations and frequent cytogenetic clonal abnormalities in chronic phase CML patients treated with IFN-α. Johansson *et al*[20] reported a significantly higher frequency of clonal evolution, of which del(7p) and del(13q) were recurrent, in Ph-positive CML treated with IFN-α or post-BMT compared to those untreated or treated with busulfan or hydroxyurea ($P < 0.001$). However, their study included patients on different studies, patients from their own series as well as from the published literature which could have reported on specific selected patient groups, and patients in different phases of CML. As they pointed out, only one of the 10 patients in their series had clonal evolution in chronic phase, compared with 13 of 22 patients in the published literature. This emphasizes the selective nature of such reports, as well as the importance of defining the denominator from which such patients had been selected. Patients on IFN-α therapy and post-BMT undergo more frequent cytogenetic studies compared to those untreated or on hydrea-busulfan therapy, thus possibly explaining the higher frequency of reported cytogenetic abnormalities.

From the mentioned reports, the major question that arises is whether the cytogenetic abnormalities and hematologic disorders observed are therapy-related or due to changing the natural course of CML. If these complications were due to the effect of therapy, they would also be observed in other solid or hematological disorders treated with IFN-α therapy, and at higher cumulative frequency in patients randomized to IFN-α *vs* hydroxyurea therapy in ongoing randomized trials.[21,22] While such complications have not been reported at a higher frequency in studies of IFN-α therapy in lymphoma, myeloma, or other solid tumors, an analysis of their comparative time-dependent frequency in the CML randomized trials would be important.

Since Ph-positive CML is a stem cell disorder, another possibility is that the selective Ph-suppression obtained with IFN-α therapy may allow the evolution and expansion of other suppressed clones existing simultaneously or arising in time. This hypothesis may also explain the divergent cytogenetic abnormalities observed in our patients, as opposed to consistent particular chromosomal aberrations attributable to therapy.

In summary, this report describes three unusual cases of patients with Ph-positive CML who developed new clonal cytogenetic disorders, two of them with clinical significance, while in cytogenetic CR of Ph-positive disease. These findings stress the importance of long-term monitoring of patients with CML or other cancers who undergo new treatments that alter the previously charted course of their disease, in order to observe potential disease or new treatment-related manifestations.

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LETTER TO THE EDITOR

Highly complex chromosomal abnormalities in plasma cell leukemia as detected by FISH technique

During the last decade, increasing attempts have been made to find nonrandom chromosomal abnormalities to human malignancies. This approach has been most successful for hematological diseases, some of which have well established specific chromosomal markers, such as in CML, ALL and ANLL.[1] Although belonging to this group of neoplasms, cytogenetic studies on multiple myeloma (MM) and plasma cell leukemia (PCL) have been mostly unsuccessful. This is mainly due to the slow plasma cell turnover compared to nonmalignant bone marrow cells, which accounts for the high rate of normal karyotypes found in these two disorders.[2] We report a case of PCL where multiple chromosomal abnormalities were found in the patient's bone marrow. Fluorescence *in situ* hybridization (FISH) technique was employed to unveil the complex nature of the chromosomal abnormalities as routine cytogenetics was an unsuccessful attempt.

A 68-year-old Caucasian female presented in January 1996 with fatigue, confusion, dehydration, weight loss and low back pain. She was known to have been hypertensive for 4 months. Physical examination was notable for lethargy and the presence of multiple ecchymoses on the upper and lower extremities. The patient was anemic and thrombocytopenic with a hemoglobin level of 6.2 g/dl and a platelet count of 22 000. Her leukocyte count was 12 900 with a shift of the differential to the left. Nucleated red blood cells and several plasma blasts were noted in the peripheral blood. ESR was 140. The patient had an elevated serum calcium of 16 mg/dl and an LDH level of 1417 units/ml. Her renal function was abnormal, with a BUN/creatinine of 36/2.4. The peripheral blood smear showed marked rouleaux formation as well as immature plasma cells and many atypical lymphocytes. Serum protein electrophoresis showed a biclonal IgG/IgA gammopathy and kappa light chains were present in the urine. Biopsy of the bone marrow revealed sheets of atypical plasma cells. The diagnosis was PCL. The patient was treated with melphalan and prednisone, with no response. She expired in March 1996, likely secondary to sepsis. A bone marrow sample was set for cytogenetic evaluation prior to the initiation of chemotherapy.

Cytogenetic analysis was performed from a bone marrow specimen using routine protocol.[3] FISH technique was carried out employing whole chromosome painting probes (WCP) for chromosomes 1, 12, 14, 16, 17 and 19 (Gibco/BRL, Gaithersburg, MD, USA). All procedures were as recommended by the manufacturer. Briefly, slides were air dried for at least 2 days prior to denaturation, then denatured in 70% formamide/2 × SSC at 70°C for 10–15 s each and passed through an ethanol dehydration series. All probes were denatured for 10 min at 70°C prior to hybridization and hybridized at 37°C in a moist chamber overnight. The stringency of the post-hybridization washes was 50% formamide/2 × SSC (pH 7.0), 2 × SSC/0.1% NP-40 at 45°C. The probes were spectrum orange or spectrum green fluorophore labeled and the hybridization included unlabeled competitor human Cot-1 DNA. DAPI/phenylenediamine dihydrochloride was used as a counter stain. The images were captured on an Oncor Imaging system with a three-chip cooled camera (Oncor, Gaithersburg, MD, USA).

A total of 25 metaphases were analyzed: five cells were normal, whereas 20 cells consisted of one hypodiploid clone showing multiple abnormalities (Figure 1). In particular, the most significant finding was an add(14)(q32) which is the most consistent structural rearrangement reported in MM and PCL.[2,4,5,6] It is to be noted that the breakpoint is on band q32 on chromosome 14, where the Ig heavy chain gene locus is located.[1] By FISH technique using whole chromosome painting probes for chromosomes 14 and 16, we were able to identify the donor on 14q as being chromosome 16 in our case (Figure 2). To the best of our knowledge, this is the first report of a t(14;16) in MM or PCL. The importance of such a finding resides in the fact that t(11;14)(q13;q32) has often been identified in MM and PCL,[2,4,5] which may lead to speculation that these two neoplasias have the same molecular basis as B-CLL. However, identification of other donor chromosomes such as chromsomes 1,[6] 3,[6] 6,[6,7] 7,[6] 8[5] and 18[6] and the absence of a breakpoint in the BCL-1 region in MM patients reported by Fielder *et al*,[8] compromise this theory. Moreover, an increasing number of monosomies,[5] deletions[5] and rearrangements[4,5] of chromosome 16 is observed in recent studies on MM and PCL, including t(1;16),[4] but with variable breakpoints. It is to be noted that the VH genes have been localized on that same chromosome.[4] Other hot spots of chromosomal abnormalities in plasma cell malignancies, such as chromosomes 17[2] and 19[4,6] are also found to be implicated in rearrangements in our study. Interestingly, a breakpoint at band q24 of chromosome 12 was noticed in our case as well as in previous studies but with no comments on significance.[5,6] Multiple complex changes of chromosome 1 are also noted in our case. Chromosome 1 is the most commonly implicated in structural abnormalities in MM[2,4,5,6] but the absence of specific breakpoints leads to the hypothesis that these changes could just be secondary events in the evolution of tumor progression. However, trisomy 1q and partial monosomy 1p are recurrent findings[5,6] and are confirmed in our case. Finally, numerical aberrations observed in our patient, such as monosomy 13, 17 and X are consistent, as noted in most previous studies.[2,4,5]

In conclusion, the presence of all these recurrent chromosomal abnormalities has become one more parameter in the process of zeroing in on a specific chromosomal and genetic basis for plasma cell malignancies. Nevertheless, any significance of the t(14;16) present in our patient still remains to be determined by additional cases which should be reported.

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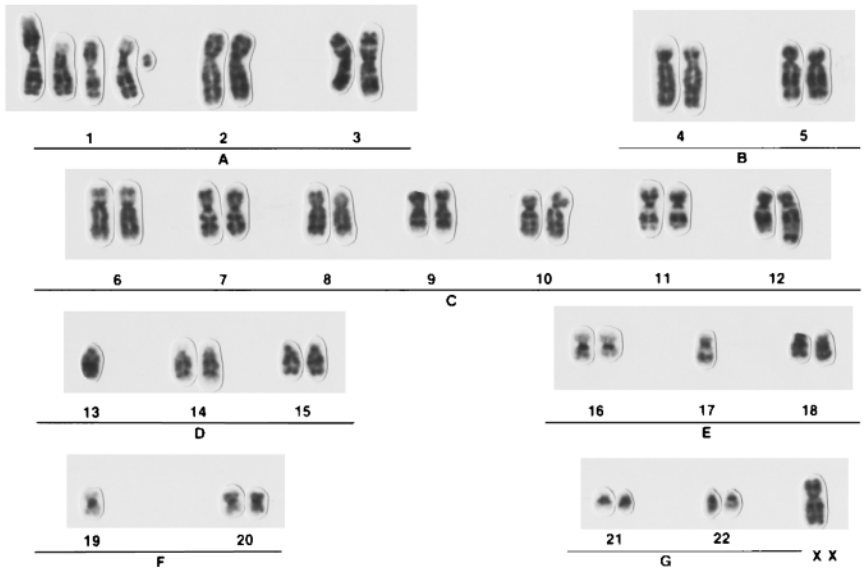

Figure 1 Representative karyotype demonstrating multiple chromosomal abnormalities from the patient.

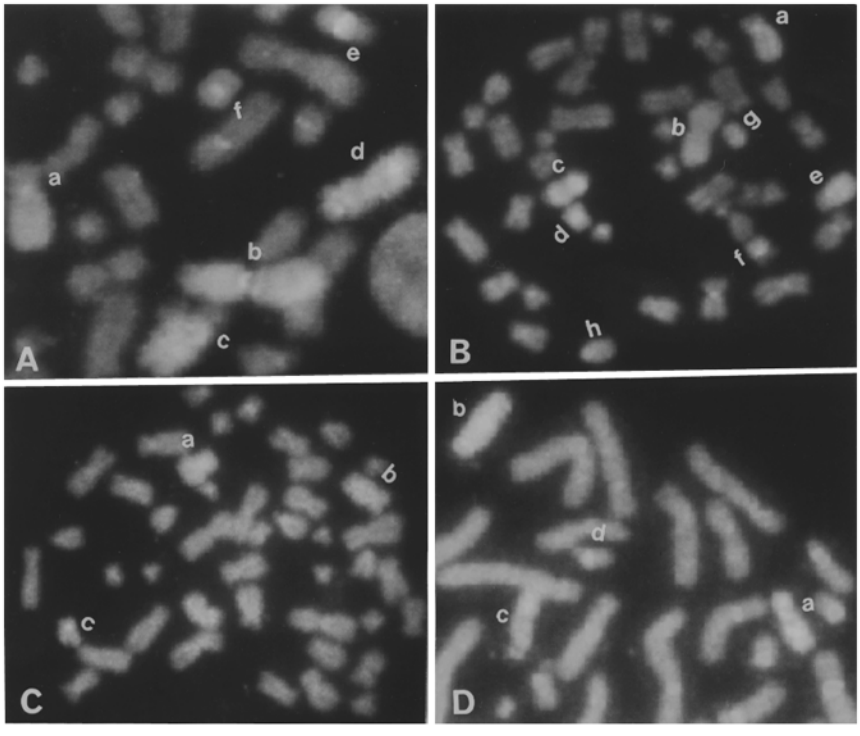

Figure 2 Partial metaphases showing aberrant chromosomes stained by FISH technique. (A) WCP No. 1 and WCP No. 19: (a) del(1)(p?)(WCP 1+); (b) normal No. 1 (WCP 1+); (c) der(1)t(1;16)(p12;q12.1)(WCP 1+); (d) der(1;19)(WCP 1+, WCP 19+); (e) der(16)t(16;19)(WCP 19+); (f) normal No. 19 (WCP 19+). (B) WCP No. 1 and WCP No. 16: (a) del(1)(p?)(WCP 1+); (b) normal No. 1 (WCP 1+); (c) der(1;19)(WCP 1+); (d) normal No. 16(WCP 16+); (e) der(1)t(1;16)(p12;q12.1)(WCP 1+;WPC 16+); (f) der(16)t(16;19)(WCP 16+); (g) der(1)(WPC 1+); (h) der(14)t(14;16)(WCP 16+). (C) WCP No. 12 and WCP No. 17: (a) normal No. 12 (WCP 12+); (b) der(12)t(12;17)(q24.3;q23)(WCP 12+; WPC 17+); (c) normal No. 17(WCP 17+). (D) WCP No. 14 and WCP No. 16: (a) normal No. 16(WCP 16+); (b) der(14)t(14;16)(q32;??)(WCP 14+;WCP16+); (c) normal No. 14(WCP 14+); (d) der(16)t(16;19)(WCP 16+). (Original color photograph can be obtained upon request.)

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