A 45-yr-old female presented with a rapidly enlarging bony tumor that was eventually identified as a Philadelphia chromosome (Ph')-positive myeloproliferative disorder with extramedullary blastic transformation. This transformation occurred in the absence of demonstrable chronic or acute leukemic phase. She had no history of a chronic or prodromal illness in spite of a bone marrow biopsy showing myelofibrosis and liver biopsy documenting extramedullary hematopoiesis. This case represents a unique constellation of features of the myeloproliferative syndrome in which the diagnosis was obscure until special stains of the bony tumor and cytogenetic studies were performed.

Various clinical entities have been unified pathophysiologically under the term myeloproliferative syndromes. A variety of hypoplastic, hyperplastic, metaplastic, dysplastic, and neoplastic disorders have been detailed and many characteristic transitions have been defined. While all of these disorders evolve from a derangement in control of a pluripotential stem cell, evidence is accumulating that the manifestations require individualized therapeutic measures. Thus until the molecular events that affect the stem cell are understood and modifiable the clinician must be aware of the unusual patterns that myeloproliferative syndromes may take.

Chronic myelogenous leukemia (CML) is a myeloproliferative syndrome in which a majority of patients conform to a well-delineated clinical picture and course. The presence of a cytogenetic marker in this disease, the Philadelphia chromosome (Ph'), facilitates the identification of variations from the norm. This report describes an unusual constellation of findings in a patient with a Ph'-positive myeloproliferative disorder simulating a primary tumor of bone.

Materials and Methods

Fresh tissue was processed for routine light and electron microscopy (LM, EM) as well as for LM and EM histochemistry. The naphthol-ASD-chloroacetate esterase reaction for neutrophilic myeloid cells was performed according to the method of Leder and the myeloperoxidase reaction was performed on imprints and frozen tissue sections according to the method of Kaplow. For the EM demonstration of endogenous peroxidase activity, tissue slices were incubated in 0.01% H2O2 and 0.5 mg/ml 3,3'-diaminobenzidine (DAB) in Tris-HCl buffer pH 7.6 at 20°C for 15 min; this tissue was washed in buffer and then fixed in 2.5% glutaraldehyde in Sorenson's phosphate buffer, osmicated (1% OsO4 in H2O), and processed routinely. Fresh tumor was fixed, osmicated, and processed in parallel for routine EM.

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CASE REPORT

A.B. was a 45-yr-old housewife and mother of four who developed right knee tenderness, stiffness, and swelling 2 mo prior to her NCI admission. She had no chills, sweats, fevers, weight loss, or any other prodromal symptoms. She had undergone yearly physical examinations and blood counts without any abnormality noted. At the appearance of right knee symptoms her white blood cell count was 11,700/mm$^3$ and Hb 13.5 g/dl. The differential count showed 72% polys, 3% bands, 20% lymphs, 5% monocytes. No red blood cell abnormalities were noted. Her personal physician aspirated the knee joint and treated her with oral cephalaxin for 3 wk without response. The synovial fluid was sterile and free of inflammatory cells or crystals. Two weeks prior to admission she underwent an open biopsy of the large distal right femur mass, which had enlarged markedly and radiographically eroded cortical bone with marked periosteal elevation. Blood studies at the time of the biopsy after 5 wk of knee swelling revealed a white blood cell count of 11,200/mm$^3$ with 67% polymorphonuclear leukocytes, 1% bands, 25% lymphocytes, 3% basophils, 3% eosinophils, and 2% monocytes. No immature white cells were seen, the spleen was not palpable, and the patient was afebrile. The tissue obtained at open biopsy was felt by the local pathologist to be synovial sarcoma, and the patient was referred to the NCI.

Slides of the tumor mass were reviewed at the NCI and were interpreted as showing a diffuse histiocytic (large-cell) lymphoma by the Rappaport classification$^3$ (Fig. 1). The tumor was composed of sheets of poorly cohesive cells that infiltrated fat and fibroconnective tissue. The cells were large (diameter 23-25 μm) with vesicular nuclei bounded by prominent nuclear membranes and one or two large central nucleoli. They had a moderate amount of distinct amphophilic cytoplasm without discernible granularity.

Physical examination on admission 8 wk after symptoms appeared showed a healthy-appearing middle-aged female with normal blood pressure and pulse and who was afebrile. She had no adenopathy, her chest was clear, and her heart had a soft systolic ejection murmur. The liver had a 15-cm total span measured 8 cm right of the midline and was firm and nontender. The

Fig. 1. Biopsy of popliteal mass. Tumor is composed of poorly cohesive blast cells with vesicular nuclei, prominent central nucleoli, and rims of well-defined cytoplasm. Mitotic figure at upper right. H & E. × 630.
spleen tip was palpable. There was a large soft tissue mass above the right knee. The neurologic examination revealed diffuse hyperreflexia without clonus.

The Hb was 11.3 g/dl. The white blood cell count was 15,700/mm$^3$ with 49% polymorphonuclear leukocytes, 30% bands, 9% lymphocytes, 2% basophils, 1% eosinophils, 3% monocytes, 3% metamyelocytes, and 3% myelocytes. There were 3 nucleated red blood cells/100 white blood cells. The red cell morphology revealed teardrop cells, basophilic stippling, anisocytosis, and poikilocytosis. The platelet count was 104,000/mm$^3$ with occasional large forms. The peripheral blood picture was felt to be leukoerythroblastic. No blast forms were present.

The chemistry profile showed alkaline phosphatase 198 (normal up to 90), lactate dehydrogenase 1400 (normal up to 340), predominantly fractions 2 and 3, with normal albumin, globulins, transaminases, bilirubin, and immunoglobulins. The urinalysis was benign and the electrocardiogram was normal. Serum calcium was elevated to 5.9 meq/liter.

The chest radiograph showed old granulomatous disease, an abdominal film showed lytic lesions in the pelvis and right femoral neck, and films of the right femur showed lytic lesions of the neck and proximal shaft, with a large luency at the distal femur with periosteal thickening and soft tissue swelling.

A lymphoma staging workup was begun. This included a lymphangiogram, which was abnormal and suggested tumor involving paraaortic and iliac nodes. The lumbar puncture and whole lung tomograms and brain scan were normal. Bilateral iliac crest and sternal bone marrow aspirate attempts yielded no bone marrow elements, and biopsies from each crest showed myelofibrosis. Cytogenetic studies of the material obtained from one of the aspirates showed two analyzable metaphases, one with Ph$^+$ and one without Ph$^+$. Liver biopsy showed extramedullary hematopoiesis (Fig. 2). In marrow scan showed no significant axial skeletal uptake, the tracer localizing homogeneously in the enlarged liver and spleen and the distal right femur. Liver scan showed homogeneous uptake in an enlarged liver, and bone scan showed increased uptake in the right femur, right shoulder, and kidneys, as did a gallium scan. Additional supporting evidence for a diagnosis of myeloproliferative syndrome was an elevated leukocyte alkaline phosphatase score and a B$_{12}$ level greater than 2000 pg/ml.
Fig. 3. Section of popliteal mass stained for naphthol–ASD–chloroacetate esterase. Two positive cells with immature nuclear features are observed. × 800.

Fig. 4. Section of popliteal mass stained for myeloperoxidase. Several cells with granular reaction product are present. × 950.
Extramedullary Blast Transformation

Fig. 5. Electron micrograph of tissue treated for detection of myeloperoxidase. (A) Myelocyte with peroxidase-containing primary granules conspicuous in the cell cytoplasm. Nonreactive secondary granules are rare (arrow), indicating relative immaturity. x 5,500. (B) Immature partially disrupted tumor cell (upper left) with peroxidase-containing primary granules as well as diffuse (endoplasmic reticulum-associated) peroxidase activity. These primitive myeloid cells were more numerous than those in A. Majority of tumor cells were very primitive, as indicated by lack of myeloperoxidase (lower right). x 3700.
Because of the emerging picture of myelofibrosis with myeloid metaplasia and some question about the histologic diagnosis on the first biopsy specimen, the right femur mass was biopsied again. By LM the tumor was similar to that previously described. The esterase reaction showed rare positive tumor cells (Fig. 3). Approximately 10% of the tumor cells were peroxidase positive (Fig. 4). By EM this same population included some obvious granule-containing myelocytes (Fig. 5A). In addition, poorly preserved primitive tumor cells (which composed a majority of the tumor) were also identified. Some of these cells retained granule- and nongranule-associated endogenous peroxidase activity, as seen in primitive myeloid precursor cells (Fig. 5B). The revised pathologic diagnosis was granulocytic sarcoma.

Peripheral blood obtained on the same day as the knee mass biopsy was cultured for 1 and 3 days. In the 1-day unstimulated culture, 55% of the metaphases had 46 chromosomes including one Ph\(^1\) chromosome, 20% had 52-54 chromosomes including one Ph\(^1\) chromosome, and 12% had 70-80 chromosomes including two Ph\(^1\) chromosomes. Banding studies of the Ph\(^1\)-positive cells showed the typical Ph\(^1\) chromosome with the 9q+ translocation: t(9q+;22q-). In the 3-day culture stimulated with phytohemagglutinin, 5% of the cells contained the Ph\(^1\) chromosome, one cell had 51 chromosomes, and three cells had 67 chromosomes. Repeat peripheral blood studies 3 wk later showed that 95% of the cells contained the Ph\(^1\) chromosome in the 1-day unstimulated culture; the chromosome number ranged from 55 to 71, with the majority of cells having 65-67 chromosomes. Unfortunately, no detailed banding analysis was possible in these nearly triploid cells.

The patient was treated with vincristine and prednisone, developed pancytopenia, and had a 6-unit upper gastrointestinal hemorrhage that stopped with platelet transfusion and aggressive antacid therapy. She developed an *Escherichia coli* pneumonia and gram-negative sepsis in spite of intensive three-drug antibiotic therapy, became comatose, and died on the 50th hospital day.

Postmortem examination showed diffuse gram-negative pneumonia with *E. coli* isolated from lung and blood. Subarachnoid hemorrhage was noted over the posterior cerebrum and cerebellum without evidence of herniation.

Thus the patient had a unique Ph\(^1\)-positive myeloproliferative syndrome without evidence of a prodromal illness. She had neither an acute nor chronic clinically evident leukemic phase but at autopsy did have myelofibrosis of the marrow, myeloid metaplasia of the liver and spleen, and leukemic invasion of all vertebral bodies, ribs, both humeri, the right femur and sternum, para-aortic and iliac lymph nodes, both kidneys, the lungs, and the soft tissue of the right knee and popliteal fossa. The bony lesion in the knee was the initial presentation of the illness and resulted in confusing pathologic interpretation until special stains, electron microscopy, and cytogenetic studies were performed.

**DISCUSSION**

CML typically has a chronic phase lasting months to years followed by blast crisis, which is usually terminal. This crisis, in a majority of cases, is morphologically similar to acute myeloblastic leukemia (AML) but clinically is more refractory than de novo AML to antileukemic chemotherapy. In some cases (roughly 10%) the blasts are more characteristic of erythroid precursors, and in about one-third of patients the blasts are morphologically lymphoid and contain lymphoid surface markers and terminal transferase. The ALL-like blast cells characteristically are hypodiploid and more responsive to chemotherapy than AML-like blasts but are less responsive than de novo ALL. About 22% of patients with CML develop myelofibrosis preterminally, and its presence is a harbinger of blast crisis (mean survival 4.9 mo). In addition, there have been case reports of CML terminating in acute basophilic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia (AMML), and acute megakaryoblastic leukemia. In rare cases, the earliest sign of blast transformation of CML has been myeloblastic tumors of lymph nodes, meningeal
leukemia, or destructive bony lesions. In all the previous reported cases of bony lesions, however, there was a documented prodromal chronic phase.

Early reports described CML progressing to diffuse histiocytic lymphoma (reticulum cell sarcoma), but these patients often concurrently developed blast crisis and uniformly had rapid demise. Garfinkle and Bennett documented that one such patient had an extramedullary granulocytic sarcoma and suggested that previously reported cases may have been similar.

The Ph chromosome has been felt to be a specific marker for CML, yet reports continue to appear of Ph-positive states in which typical CML is difficult to document. One patient reportedly had the Ph chromosome in his CLL lymphocytes. Ph-positive myeloid metaplasia has been noted. Several reports of Ph-positive patients presenting with AML and ALL interpreted the presence of the marker as evidence that the chronic phase of CML had been clinically silent. Bloomfield et al. collected data supporting this interpretation. They did cytogenetic studies on 70 adults presenting with acute leukemia: 6 of 15 adults with ALL, 2 of 14 with AMML, and 2 of 41 with AML had the Ph chromosome at presentation without a prodrome suggestive of CML, but those patients having the Ph chromosome were more refractory to therapy than Ph-negative patients with the same histology, behaving in a manner similar to blastic transformation of CML. This suggests that the presence of Ph has prognostic and therapeutic significance. In a previous patient we had the opportunity to observe at the NCI the Ph chromosome was identified in a hematologically normal patient followed over 5 yr before developing blastic transformation. That patient's course confirms that blastic transformation may be the first overt clinical abnormality, while the marrow harbors the clinically inapparent Ph clone for years.

Enzyme histochemical studies were of great value in establishing a correct pathologic diagnosis in this case. Since the majority of the tumor cells were primitive undifferentiated blasts, conventional LM and EM were of equivocal value. Naphthol-ASD-chloroacetate esterase is found in specific secondary neutrophilic granules. Since this enzyme is restricted to the neutrophilic series and is acquired fairly late in maturation (at the promyelocyte state), only rare cells were positive in this predominantly blastic lesion. Such rare cells are sometimes difficult to distinguish from tissue mast cells and infiltrating reactive neutrophils. The peroxidase reaction was positive in a much larger proportion of the tumor cells and, as such, was a more reliable indicator of the correct diagnosis. EM histochemistry was of particular value because of its ability to associate the peroxidase activity with primitive tumor cells. The granule- and endoplasmic reticulum–associated reactivity notably more prominent in this case was a feature of myeloblasts; activity restricted to granules is a feature of more mature myelocytes.

It is apparent that CML has diverse manifestations and courses. Our patient represents a new permutation combining features not previously reported in a single patient. She presented with a destructive bony lesion simulating a primary sarcoma or lymphoma of bone that in fact represented blastic transformation of CML. This lesion was distinguishable from a granulocytic sarcoma (chloroma) presenting in acute leukemia by the presence of myelofibrosis, ex-
tramedullary hematopoiesis, and the Ph\(^1\) chromosome. These features suggest that the patient had a clinically inapparent chronic phase that progressed to myeloid metaplasia and finally blastic transformation. This case underscores the importance of cytogenetic studies in conjunction with EM and enzyme histochemistry in the careful evaluation of patients with hematologic disease.

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Myeloproliferative syndromes: a unique presentation of chronic myelogenous leukemia (CML) as a primary tumor of bone

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