Marrow Fibrosis Associated with a Philadelphia Chromosome

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ABSTRACT: *Three patients had marked marrow fibrosis and an apparent Philadelphia (Ph) chromosome. Hematologic, cytogenetic, and molecular studies demonstrated the heterogeneity of such cases, including the first example of clinically typical myelofibrosis (MF) associated with a bcr gene rearrangement characteristic of chronic myelogenous leukemia (CML).*

INTRODUCTION

Many cases of Philadelphia chromosome-positive (Ph^-) chronic myelogenous leukemia (CML) have some increase in reticulin fibers in the bone marrow (BM), and some also have increased deposition of collagen, particularly late in the clinical course [1]. Rarely, however, does a patient with Ph^- disease have the clinical and hematologic picture of typical myelofibrosis (MF), as currently defined [2–5]. We report two such patients and a third Ph^- patient with sufficient marrow fibrosis to confuse the initial diagnosis.

CASE REPORTS

Case 1 (P.W.)

A 45-year old woman was noted to be anemic in February 1989 during hospitalization for a herniated disc. Subsequent studies showed a hemoglobin level of 8.8 g/dl, a white blood cell (WBC) count of 8.8×10^9/L, and a platelet count of 987×10^9/L. The peripheral blood (PB) smear showed nucleated red blood cells (RBC), teardrop forms, schistocytes, and a few immature leukocytes. Platelets were increased, with megakaryocyte fragments. A BM biopsy showed marked increase in fibrosis, megakaryocytic hyperplasia, and markedly increased reticulin. The leukocyte alkaline phosphatase (LAP) level was 6 (normal = 27–67). A clinical diagnosis of MF was made. Cytogenetic and molecular genetic studies were made of the BM at that time.

She was entered on an MF protocol involving treatment with interferon (IFN) daily, resulting in marked improvement in hemoglobin and platelet levels. In April 1990, she received a BM transplant from a sibling and currently (July 1991) has no evidence of her primary disease.

Case 2 (S.S.)

A 61-year-old man was first studied in London in December 1986 because he had dyspnea on exertion and ankle edema. He had a hemoglobin level of 8.4 g/dl, a WBC count of 16×10^9/L, and a platelet count of $1{,}500 \times 10^9$/L. The PB smear showed basophilia, anisocytosis, and teardrop forms. Bone marrow biopsy showed panmyelosis, islands of blasts, and increased reticulin fibers, considered to represent "agnogenic myeloid metaplasia with myelofibrosis." The LAP was not determined. During the next $2\frac{1}{2}$ years, he was treated at several institutions in the United Kingdom and the United States, requiring increasingly frequent transfusions. The clinical and hematologic diagnosis of MF was confirmed on at least two subsequent occasions by repeat BM examination, including one in September 1989, when cytogenetic and molecular genetic studies were also performed. The clinical course remained generally unchanged, with supportive therapy and without progression to acute leukemia, until the patient's death owing to cardiac complications in February 1991.

Case 3 (J.F.)

A 55-year-old woman was studied in December 1989 because of fatigue, weight loss, and lower-leg edema. Splenomegaly was noted. Her hemoglobin was 7.6 g/dl, and the WBC count was 93×10^9/L (28% segments, 14% bands, 4% lymphocytes, 1% monocytes, 7% eosinophils, 13% basophils, 9% atypical lymphocytes, 7% metamyelocytes, 14% myelocytes, 2% promyelocytes, and 1% blasts). The platelet count was 613×10^9/L. The PB smear showed nucleated RBC, with occasional teardropping and large platelet forms. The LAP was 26. Bone marrow biopsy showed greatly increased numbers of megakaryocytes and areas of marked fibrosis alternating with other areas of marked hyperplasia, with nests of cells that appeared to be

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blasts. Chromosome studies were made of the BM in January 1990, and the patient was then treated with hydroxyurea, with normalization of her WBC and platelet counts. Since February 1990, she has been participating in a long-term protocol involving treatment with IFN, and her disease has been stable. Repeat chromosome studies were performed in May, August, and November 1990, with molecular studies also performed on the latter specimen.

MATERIALS AND METHODS

For chromosome studies, standard G-banded preparations were made from BM or PB specimens cultured for 24 hours without mitogen [6].

To determine the involvement of the *bcr* locus, DNA from cases 1 and 3 was hybridized with both a partial cDNA probe [7] and a genomic probe (Oncogene Science) for the *bcr* major breakpoint cluster region [8]. For case 2, RNA was extracted from glass slide smears of BM, and PCR amplification studies for *bcr-abl* chimeric mRNA were performed, as described previously [9]. Controls included a known positive CML specimen, which showed evidence of *bcr-abl* mRNA; appropriate negative controls; and analysis of the patient sample for c-*abl* mRNA, which demonstrated that intact mRNA had indeed been isolated from the glass slide specimen.

RESULTS

As indicated in Table 1 and Fig. 1, all BM cells from case 1 had the karyotype 46,XX,t(9;22)(q34;q11),del(20)(q12). The molecular studies showed a single rearranged *bcr* allele with *Eco*RI, *Bam*HI, and *Bgl*II enzymes. The pattern suggested that the breakpoint was located in the F2 or F3 region of Shtalrid [10], toward the 3′ end of the major breakpoint region of typical CML.

In case 2, although the cytogenetic abnormalities involved the same three chromosomes as in case 1, with an apparent Ph chromosome, there was not actually a t(9;22). As indicated in Table 1 and Fig. 2, the karyotype was interpreted as 46,XX,t(20;22)(p13;q11),del(9)(q34). All metaphases examined were abnormal, and approximately one third were polyploid, with the same chromosome aberrations. The possibility of a "masked" *bcr-abl* translocation was considered, but the PCR amplification studies provided no evidence of *bcr-abl* chimeric mRNA.

In case 3, the original chromosome study, performed at another institution, demonstrated a Ph chromosome, and our subsequent three studies of BM and PB, summarized in Table 1, confirmed the karyotype of the neoplastic cells as 46,XX,t(9;22)(q34;q11). All metaphases were abnormal and, as with case 2, a proportion were polyploid, consistent with the observed megakaryocytosis and thrombocytosis. As in case 1, molecular studies for *bcr* involvement showed a single rearranged *bcr* allele with both the *Bam*HI and *Bgl*II enzymes. In this case, the pattern indicated that the breakpoint was located in the F1 region, toward the 5′ end of the major breakpoint region.

DISCUSSION

These three cases illustrate a variety of ways in which severe marrow fibrosis may be associated with a Ph chromosome. The first case represents the rare circumstance in which the karyotypic abnormalities in a patient with typical MF include the characteristic t(9;22) usually associated with CML. Only one such case is listed in the 1988 Mitelman catalogue [4], and a few others have been described in earlier articles [2, 3]. None have been previously studied for *bcr* rearrangement. The fibrous proliferation in MF is not part of the preneoplastic or neoplastic clone, but is apparently stimulated by products of the abnormal hemic cells, particularly platelet-derived growth factor (PDGF) [5, 11, 12]. This helps to explain the coexistence of megakaryocytosis and marrow fibrosis in this case, as well as in case 3, although it is not known what genetic alteration, if any, in the neoplastic cells, other than the *bcr-abl* translocation contributes to this phenomenon. In case 1, the del(20q) abnormality may have contributed to the specific phenotype. However, although this alteration does occur nonrandomly in MF, it has also been associated with a variety of other myeloproliferative and myelodysplastic disorders [4, 5, 13–16], as well as with some cases of Ph$^-$ CML without significant MF [17, 18]. There has been recent speculation that this del(20)(q13) abnormality might specifically involve the *hck* gene (C. Willman, personal communication), which maps to this region and codes for a tyrosine kinase, but this has not been confirmed, and in this particular case no rearrangement of the *hck* gene was detected on standard Southern blots (K. Huebner, personal communication).

The second case was also considered typical MF when studied clinically and hematologically at a number of medical centers in the United States and the United King-

Table 1 Cytogenetic and molecular findings

Patient	Date	Specimen	No. of metaphases	Karyotype	*bcr-abl* Rearrangement
1 (P. W.)	2/1989	Marrow	25	46,XX,t(9;22)(q34;q11),del(20)(q12)	Positive
2 (S. S.)	9/1989	Marrow	30[a]	46,XY,9(20;22)(p13;q11),del(9)(q34)	Negative
3 (J. F.)	6/1990	Marrow	25[a]	46,XX,t(9;22)(q34;q11)	
	9/1990	Marrow	12	46,XX,t(9;22)(q34;q11)	
	12/1990	Blood	25[a]	46,XX,t(9;22)(q34;q11)	Positive

[a] Ten to 30% tetraploid.

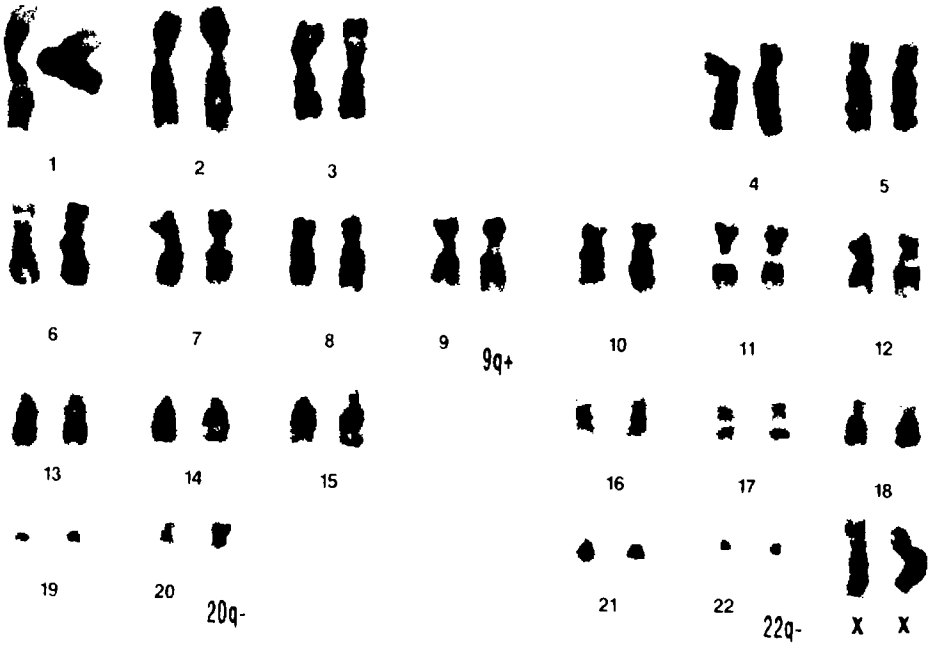
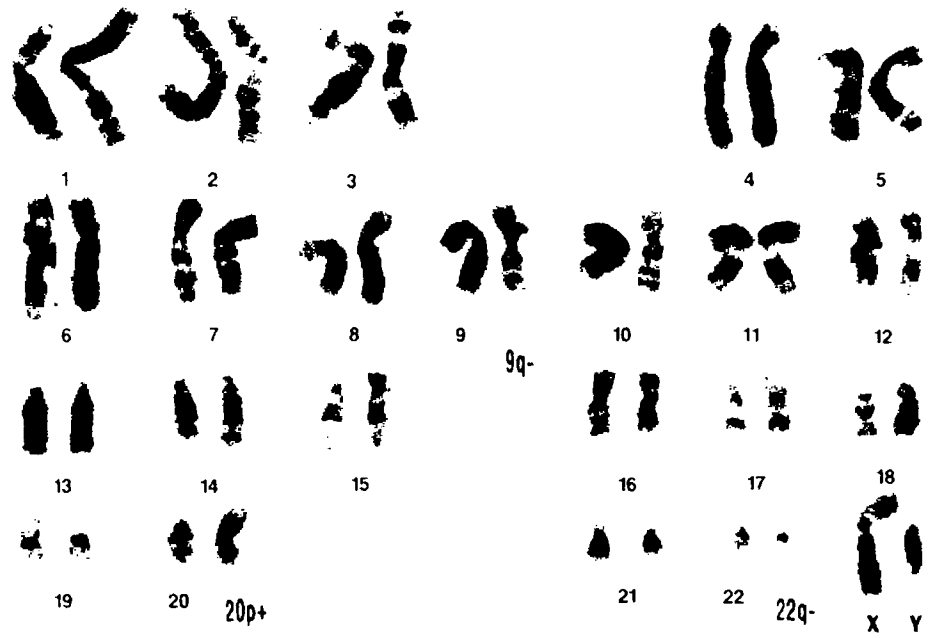


Figure 1 Representative karyotype from marrow of patient 1 (P.W.): 46,XX,t(9;22)(q34;q11),del(20)(q12).

dom. The karyotype also appeared initially to contain a Ph chromosome. However, both cytogenetic and molecular analysis indicated that there was not a *bcr-abl* translocation, but instead an unusual t(20;22)(p13;q11), as well as a small deletion from the terminal portion of the long arm of one chromosome 9. The t(20;22) has not previously been described in myeloproliferative or myelodysplastic disorders, but there have been at least two reported cases of Ph' CML with translocations involving the short arm of chromosome 20, although neither was confirmed at the molecular level as having a *bcr* rearrangement. In one instance, the translocation was described as t(9;20;22)(q34;p11–13;q11) [19], and in the other as simply t(20;22)(p12;q11) [20]. Because of the overall rarity in hemic disorders of these rearrangements involving 20p, speculating about their significance is difficult.

Figure 2 Representative karyotype from marrow of patient 2 (S.S.): 46,XY,t(20;22)(p13;q11),del(9)(q34).



The third case exemplifies a somewhat more common entity in the spectrum of disorders that range from typical CML with minor degrees of marrow fibrosis and/or megakaryocytosis to Ph$^+$ cases initially appearing as typical essential thrombocythemia (ET) with marked increase in megakaryocytes and platelets and with some degree of marrow fibrosis [21, 22]. This particular case was unusual in that the severity of the fibrosis complicated the original diagnosis, raising a question of whether the patient should be considered, and managed, as having MF or CML. These three cases indicate the need for careful evaluation, including appropriate cytogenetic and molecular studies, of all patients who initially have severe marrow fibrosis, as treatment options are considered.

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