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MARROW CHROMOSOME STUDIES IN "PRELEUKEMIA"

Further Correlation with Clinical Course

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New data are provided in a long-term investigation of the prognostic value of marrow chromosome studies in "preleukemic" states. Fifty-one patients have now been studied and followed for at least a year or until death: 26 with the myeloproliferative syndrome, including polycythemia vera; 16 with idiopathic pancytopenia; and 9 "miscellaneous" patients with unexplained anemia, neutropenia, leukocytosis, or thrombocytopenia. No chromosome changes were found in this last group, and no leukemia developed. Sixteen of the 42 patients in the myeloproliferative and pancytopenia groups had a marrow chromosome abnormality when studied, and 9 of these developed rapidly fatal clinical leukemia within 3 months thereafter. The other 7 patients (including 3 in whom the marrow chromosome change may have been induced by ³²P therapy) remained free of leukemia when followed up to several years. Neither the type of chromosome change nor the size of the abnormal cell clone in the marrow was of prognostic value. Four of the 26 patients without chromosome changes in the myeloproliferative and pancytopenia groups developed leukemia, one within a few weeks and the others, 7 to 26 months after study. It is concluded that if a marrow chromosome abnormality is detected in a non-irradiated "preleukemic" patient, the risk of developing clinical leukemia within the next few months is great, and patients who show such progression probably had subclinical leukemia already present in the marrow at the time of study. If, however, frank leukemia does not appear within 3 months, "preleukemic" patients with marrow chromosome abnormalities are perhaps thereafter at no greater risk than comparable patients without such changes.

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FOR SEVERAL YEARS THIS LABORATORY HAS been investigating marrow chromosomes in human "preleukemia." The patients have been individuals in whom a definite clinical diagnosis of leukemia could not be made at the time of the chromosome study, but whose hematologic findings strongly suggested them to be at high risk. Two major groups have been involved: individuals with the so-called myeloproliferative syndrome (e.g., polycythemia vera, myelofibrosis, myeloid metaplasia)

and individuals with idiopathic pancytopenia, usually accompanied by a hypoplastic marrow with many primitive forms.

Previous reports on this series of patients^{3,4} have indicated that individuals with "preleukemia," as so defined, who had in their bone marrow a clone of cells with a chromosome abnormality were much more likely to develop frank leukemia subsequently than those without such changes. However, several exceptions were noted. The present report extends the size of the series as well as the length of follow-up on many patients and suggests modifications in the previous conclusions which may more sharply define the prognostic value of marrow chromosome studies in these cases.

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The chromosome studies and follow-up data in this series were obtained through the cooperation of many physicians, including Dr. Miriam Dalkey, Dr. Manfred Goldwein, Dr. Arlan Gottlieb, Dr. Jeffrey Hartzell, Dr. Scott Murphy, and Dr. Peter White. Expert technical assistance was provided by Janet Finan and Julie Jensen.

MATERIALS AND METHODS

A total of 51 patients has been studied and followed long enough for inclusion in the series. (An additional 10 individuals have had marrow studies but have been excluded because of inadequate follow-up.) The 51 pa-

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tients have been grouped in three diagnostic categories: myeloproliferative syndrome (26), pancytopenia (16), and "miscellaneous" (9).

Among the 26 individuals with myeloproliferative disorders, there have been 9 with polycythemia vera, 1 with myelofibrosis, 1 with "agnogenic myeloid metaplasia," and the remaining 15 with abnormally increased myeloid activity predominating in the marrow. This myeloproliferative group included 15 men and 11 women ranging in age from 37 to 87 years (mean = 64).

In the "pancytopenia" group were 16 patients in whom the clinical picture was characterized by peripheral pancytopenia with marked reduction in all circulating formed elements. In nearly all of these individuals, the bone marrow was hypoplastic, with increased numbers of immature elements, and in no instance could a toxic or immunologic basis for the pancytopenia be determined. These 6 women and 10 men had an age distribution similar to that of the myeloproliferative group (mean = 62 years; range, 45 to 78).

The "miscellaneous" group consisted of 9 individuals who were studied because of atypical hematologic findings, but who did not fall into either the myeloproliferative or pancytopenia categories. These patients had either unexplained anemia (including 3 with sideroblastic anemia), leukopenia, thrombocytopenia, or leukocytosis, but they were not considered, on clinical grounds, to represent as clear a risk for leukemia as the other two groups. They covered a wide age range (20 to 80 years), including 3 patients under 30.

Patients in all 3 groups had been treated with various hematinics, steroids, and sex hormones, but none had been exposed to ionizing radiation or other therapeutic agents known to damage human chromosomes except for 3 patients with polycythemia vera who had been treated with ^{32}P .

All bone marrow preparations for chromosome study were made by the direct technique, without culture. In our current procedure, aspirated marrow (0.1-0.2 ml) is immediately incubated in balanced salt solution containing heparin (16 USP units/ml) and colchicine (1 $\mu\text{g}/\text{ml}$) for 30-60 minutes at room temperature. This is followed by hypotonic treatment in 9:1, 0.75% sodium citrate: bovine serum for 15 minutes at room temperature, and then by standard fixation and air-drying techniques.²

A minimum of 25 chromosome counts and 3

karyotype analyses were done on all specimens, and whenever an abnormal clone appeared to be present, additional counts and analyses were done to define its nature and size.

Clinical follow-up data were obtained through referring physicians and hospital records, and repeat bone marrow studies were obtained in several instances. All 51 patients presently included in the series (including 15 not previously reported) have been followed for at least 12 months after the initial chromosome study or until death.

RESULTS

Sixteen of the 51 patients had a clonal chromosome abnormality in the bone marrow. These included 11 of the 26 patients with myeloproliferative disorders (including 3 patients treated with ^{32}P), 5 of the 16 patients with pancytopenia, and none of the 9 cases in the "miscellaneous" group. Data on these 51 patients are summarized in Table 1. (The 10 patients not listed in the table and excluded from the series because of inadequate follow-up included only 1 with a chromosome abnormality.)

As indicated in Table 1, 9 of the 16 patients with chromosome abnormalities promptly developed clinically demonstrable acute or subacute leukemia which consistently failed to respond to therapy; 8 were dead within 3 months and the 9th at 5 months.

Of the 7 patients with chromosome changes who did not develop leukemia, 3 had been treated with ^{32}P for polycythemia vera, and their abnormal clones may well have been radiation-induced.⁶ The remaining 4 non-leukemic patients with chromosome abnormalities include 2 who are still alive after more than 30 months, and 2 who died 24 months and 35 months after study. More details of these cases will be given in conjunction with the discussion of Table 2.

Of the 26 cases of the myeloproliferative syndrome or pancytopenia listed in Table 1 as not showing chromosome abnormalities in the marrow, only 4 have thus far developed frank leukemia. One patient in the pancytopenia group died of acute granulocytic leukemia approximately 1 month after study, one died of erythroleukemia after 11 months, and the other 2 patients, both in the myeloproliferative group, developed subacute granulocytic leukemia in 7 and 26 months, respectively.

TABLE 1. Relation between Marrow Chromosome Findings and Clinical Course in "Preleukemia"

Original diagnosis	Total patients	Dead with leukemia		Alive without leukemia	Dead without leukemia
		1-3 mos.	>3 mos.		
Patients with marrow chromosome abnormality					
Myeloprolif. syndrome:					
P. vera	4*	0	0	2 (30, 72) [†]	2 (5, 11)
Other	7	4	1 (5)	1 (36)	1 (35)
Pancytopenia	5	4	0	0	1 (24)
Patients without marrow chromosome abnormality					
Myeloprolif. syndrome:					
P. vera	5	0	0	3	2 (8, 30)
Other	10	0	2 (7, 26)	3 (15-25)	5 (1, 1, 8-36)
Pancytopenia	11	1	1 (11)	2 (18, 51)	7 (1, 1, 12-28)
Miscellaneous [‡]	9	0	0	7 (12-39)	2 (1, 13)

* Includes 3 patients treated with ³²P.
[†] Length of follow-up (months).
[‡] See text for details.

TABLE 2. Chromosome Data and Clinical Course in 16 "Preleukemic" Patients with Abnormal Marrow Chromosomes

Patient no.	Original diagnosis	Abnormal Clone(s) in Marrow			Clinical course	
		Chrom. no.	Nature of aberration	% abn. cells		
74*	Myeloprolif. syndrome—polycythemia vera	45, 45	C or E-group chromosome missing	95	Dead-CVA-11 mos.	
81*		46	Deleted D	100	No change-72 mos.	
91*		44	D and G missing	50	Dead-CVA-5 mos.	
254		47	Extra C	25, 40	No change-30 mos.	
107		46	Minute replacing F	40	Dead-SAGL [†] -1 mo.	
111	Myeloprolif. syndrome	48	Extra F (abn?) and G	90, 90	Dead-Pneum.-35 mos.	
120		45	1-2 translocation	55	Dead-SAGL-1 mo.	
123		47	Extra D	20	Dead-SAGL-3 mos.	
206		45	C-C translocation	80	Dead-AGL-3 mos.	
245		45	G missing	75	No change-36 mos.	
268		46	C replaced by abn. D	100	Dead-SAGL-5 mos.	
132		Pancytopenia	45	B and C missing, minute, abn. E	90	Dead-SAGL-2 mos.
180			45	Extra E (abn.), 2 C's missing, abn. D	100	Dead-Pneum.-24 mos.
183			44, 45, 88, 90	Same minute in all lines, other changes	95	Dead-"Megakaryocytic myelosis"-2 mos.
265			43	Missing B, C, 2 D's; abn. C	90	Dead-AGL-3 mos.
280		42	Changes in all groups but A (see Fig. 1)	85	Dead-Erythroleukemia-2 mos.	

* Previous ³²P therapy.
 SAGL = Subacute granulocytic leukemia.

In all instances, the leukemia was rapidly fatal, and chromosome preparations were not obtained during the terminal illness.

Fourteen of the remaining 22 patients in these two groups without chromosome abnormalities have subsequently died of complications related to their hematologic disorders. Ten of these patients were followed for 8 months or more prior to death, and in no instance was transition to a frankly leukemic state apparent either clinically or at autopsy. Nor has leukemia been observed in any of the 9 patients in the "miscellaneous" group, although all but one has been followed for more than a year.

The nature of the chromosome abnormality observed in the 16 positive cases is shown in Table 2, as well as its frequency in the bone marrow and the subsequent clinical course in each instance. It is apparent that there is no typical chromosome change associated either with a particular initial diagnosis or with the subsequent development of leukemia in this series. Abnormalities involving all chromosome groups have been observed, without any consistent pattern, although, in general, the cytogenetic alterations have been more extensive in the pancytopenia group than with the myeloproliferative disorders (Fig. 1). Perhaps this greater genetic derangement in the marrow cells contributes to the more severe disturbance in hematopoiesis observed clinically in the pancytopenia patients as compared to the myeloproliferative category.

With respect to the subsequent clinical course of the patients in both groups, the magnitude of the chromosome change or of the abnormal clone itself does not appear to be of great prognostic value. Although some patients with extensive alterations promptly progressed to fatal leukemia (e.g., patient nos. 132, 265, 280), several with very minor changes or a small clone (107, 268, 123) did also; and 2 patients with major rearrangements (180, 111) survived 2 and 3 years without specific therapy and never developed frank leukemia. (The abnormal clone involved 90-100% of the dividing marrow cells in these two cases, including 2 studies a year apart on patient no. 111, and hematologically the marrow findings, even at autopsy, remained highly suggestive of leukemia, but the overt disease never developed.)

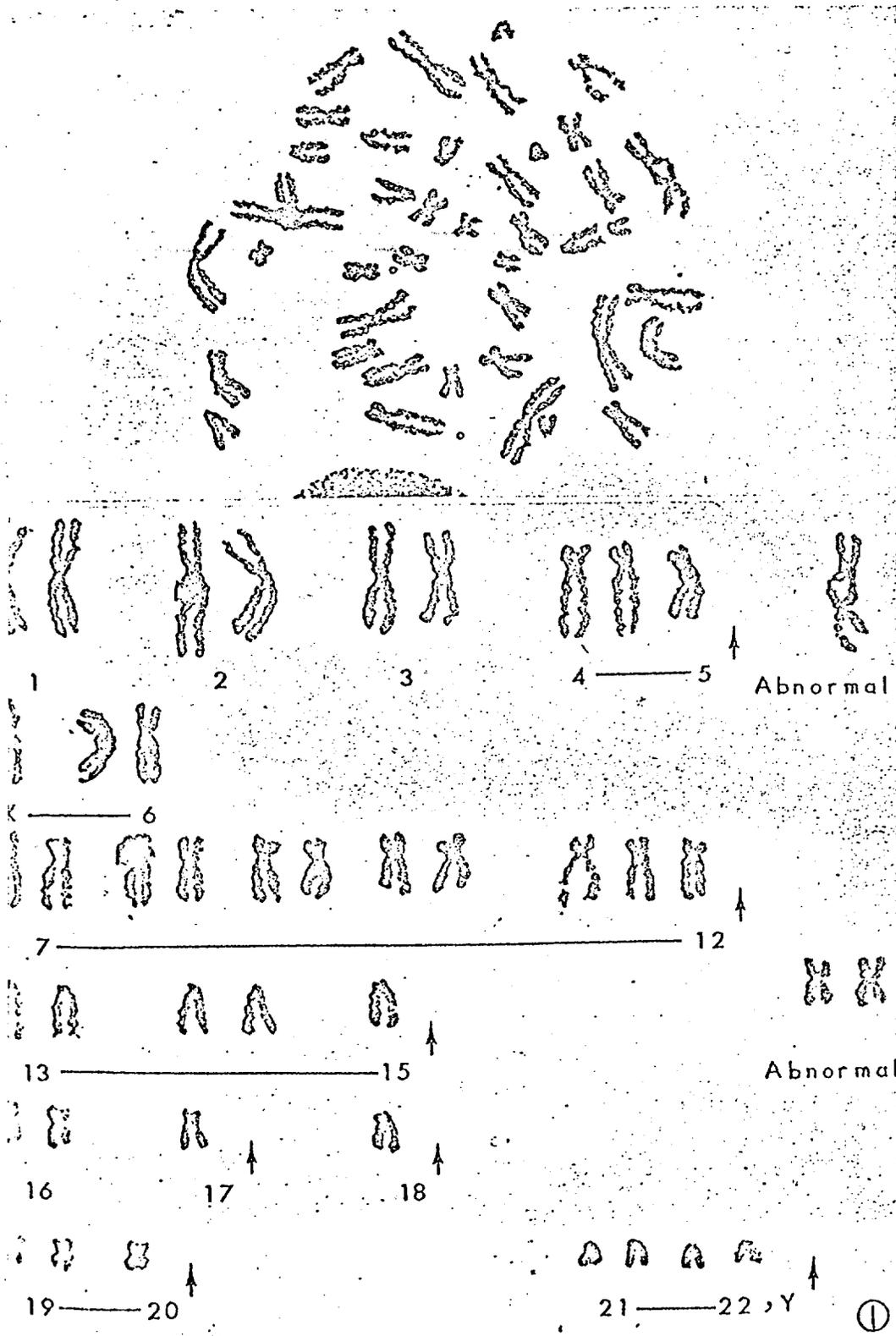
The other 5 patients listed in Table 2 who did not develop leukemia included 3 with polycythemia (nos. 74, 81, 91) whose abnormal clones may well have been induced by the ^{32}P

with which they had been previously treated. Such radiation-induced clones, both in man and experimental animals, have previously been shown to persist and function in the marrow for long periods without resulting in leukemia.⁶ The remaining 2 patients, without known exposure to ionizing radiation, were a 56-year-old woman with polycythemia vera (no. 254) in whom an extra C-group chromosome was present in 25% of the marrow cells at the time of initial diagnosis and in 40% of the cells 2 years later; and a 62-year-old man (no. 245) with unexplained leukocytosis and atypical myeloid hyperplasia in whom 70% of the marrow cells showed the absence of a G-group chromosome. Neither of these patients, 30 and 36 months after initial study, currently shows any indication of transition to frank leukemia.

DISCUSSION

The present findings are in agreement with the conclusions of our earlier reports on this series of patients.^{3,4} It is clear that "preleukemic" individuals with a marrow chromosome abnormality not attributable to ionizing radiation carry a much greater risk of subsequently developing frank leukemia than do comparable individuals without chromosome changes. The respective figures are 9 of 13 vs. 4 of 26 (excluding the "miscellaneous" group).

The additional cases added to the series in the present report, however, as well as the longer follow-up on earlier cases, have suggested that this general conclusion should be amplified. The 9 individuals with marrow chromosome abnormalities who subsequently developed leukemia all did so within 3 months and died within a few weeks thereafter. None of the other 7 patients in this chromosome-positive group (including 3 who had been treated with ^{32}P) have progressed to leukemia, although 5 have been followed for 2 years or more. Unfortunately, even retrospectively, it has not been possible to identify any specific clinical or hematologic finding present at the time of the chromosome study which would consistently indicate those patients destined to promptly develop frank leukemia. Nevertheless, the time relationships strongly suggest that the 9 patients who did succumb to leukemia within a few months probably had the neoplastic process already well-established in the bone marrow at the time of the chromosome study, even though the clinical diagnosis could not be made.



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Furthermore, it would appear that the 7 chromosome positive patients who did not become leukemic within 3 months after investigation were at no greater risk for developing the disease at a later date than the "preleukemic" patients without chromosome change. Of the 26 myeloproliferative or pancytopenic patients in the latter group, 4 subsequently developed leukemia. One succumbed to an acute granulocytic leukemia within one month, and he probably should be grouped with the 9 early leukemia cases in the chromosome-positive population, representing an already existing leukemic state at the time of study (many acute leukemias never show demonstrable chromosome abnormalities).^{4,5} The other 3 patients developed clinical leukemia 7, 11, and 26 months, respectively, after the initial chromosome study. Thus, if the early leukemias are excluded, as well as four individuals who died within a few weeks without leukemia (Table 1), 3 of 21 chromosome-negative patients developed leukemia more than 3 months after study and none of 7 individuals in the chromosome-positive population.

On the basis of these data, it seems reasonable to modify our previous conclusions along the following lines: A non-irradiated "preleukemic" individual with a marrow chromosome abnormality has a greatly increased probability of developing clinical leukemia within a few months as compared to similar patients without such changes. Those individuals who do develop leukemia within this time probably had incipient leukemia present but not clinically apparent when they were studied, and, unfortunately, the disease is usually not responsive to therapy. If leukemia does not develop within 3 months after the recognition

of a chromosome abnormality in the bone marrow, "preleukemic" patients with chromosome changes probably are not at any greater risk of developing leukemia thereafter than comparable patients without cytogenetic aberrations. In these cases, both with and without chromosome change, conservative therapy would appear to be warranted.

Although no entirely comparable series has been published elsewhere, these conclusions would appear to be in general agreement with those of others who have considered the diagnostic and prognostic usefulness of chromosome studies in these atypical hematologic disorders (e.g., the general review by Sandberg and Hossfeld⁶). Findings which appear to be quite similar have been recently reported by Jensen and Philip,¹ who investigated 35 patients with the same spectrum of "preleukemic" disorders as in our series. They found 5 patients with abnormal stemlines, and of these, 3 died within 3 months, 2 with frank leukemia. The other 2 patients were followed for 28 and 39 months and did not progress to leukemia.

ADDENDUM

Dr. Janet Rowley has kindly reviewed this manuscript and reports similar findings in the large study of "preleukemic" patients which she is preparing for publication. She also suggests that our patient no. 245 should be grouped with the elderly males observed by various workers (e.g., O'Riordan, M. L. et al. *Brit. J. Haemat.* 19:83, 1970) who have a marrow clone missing the Y chromosome, apparently unrelated to hematologic dysfunction. We will attempt to confirm this with fluorescence studies.

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FIG. 1. Metaphase and karyotype of marrow cell from 69-year-old man with unexplained pancytopenia who later developed erythroleukemia. There are 42 chromosomes, with one or two missing (arrows) from all but group A (nos. 1-3) and three abnormal. Similar changes were found in 85% of cells in marrow specimen. Poor technical quality is common with neoplastic material.