

LONGITUDINAL BONE MARROW CHROMOSOME STUDIES IN POTENTIAL LEUKEMIC MYELOID DISORDERS

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The purpose of this study was to answer two questions: 1. Are patients with potentially leukemic myeloid disorders who develop leukemia necessarily preceded by marrow chromosome abnormalities? and 2. Do all that have such abnormalities develop acute leukemia? Direct bone marrow chromosome studies were performed in 18 patients with pancytopenia and in 10 with several unusual myeloproliferative disorders. The former have been followed between 6 months and 13 years; all had normal karyotypes and no evidence of leukemia. In the miscellaneous group, follow-up has varied between 6 months and 5 years, six patients had assorted chromosome abnormalities, including two with a clonal evolution. Three have died without evidence of leukemia, one has developed a questionable form of chronic eosinophilic leukemia and two are alive without leukemic transformation. It is concluded that the presence of bone marrow chromosome abnormalities in such patients does not necessarily mean that transformation to acute leukemia is imminent.

IT HAS BEEN ESTABLISHED THAT APPROXIMATELY 50% of patients with acute leukemia have bone marrow chromosome abnormalities.¹⁵ Their significance in the development of the disease is uncertain as they are usually found after the diagnosis has been established. A method to investigate their importance may be afforded by the study of certain hematologic syndromes which have the tendency to terminate in acute leukemia and that, in addition, have a variable frequency of bone marrow chromosome abnormalities.¹⁷ Several such investigations have been published,^{2-9,11-13,16} and there seems to be agreement in that abnormal stemlines may occur prior to the blast transformation characteristic of acute leukemia. The frequency of such transformations is difficult to assess; in the combined experience of the majority of the investigators who publish sufficient data to answer this question

(Table 1), we arrived at a figure of 46%. The largest series is that of Nowell⁹ who finds a frequency of 56%, and states that the risk of developing clinical leukemia is high within 3 months after the demonstration of bone marrow chromosome abnormalities, while after this period, the patients have probably no more risk than similar individuals without such abnormalities.

In this report, we present the results obtained during a longitudinal study of 28 patients with potentially leukemic myeloid disorders. We were interested in finding data relating to two main questions: 1. Are the patients who develop leukemia necessarily preceded by the presence of marrow chromosome abnormalities?, and 2. Do all who have such abnormalities develop acute leukemia?

MATERIALS AND METHODS

The clinical material was divided in two main groups: pancytopenia suggestive of acquired aplastic anemia (18 patients) and miscellaneous myeloproliferative syndromes (10 cases).

The criterion for diagnosis of the former was the presence of peripheral blood pancyto-

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TABLE 1. Compilation of Patients with Assorted Myeloproliferative Syndromes and Bone Marrow Chromosome Abnormalities

Reference	No. patients	Developed acute leukemia (no.)	
		Yes	No
Freireich et al. ²	3	2	1
Sandberg et al. ¹²	1	0	1
Rowley et al. ¹¹	3	0	3
Kemp ⁴	1	0	1
Winkelstein et al. ¹⁶	1	0	1
Jackson and Higgins ^{5*}	1	0	1
Krøgh-Jensen ⁷	2	1	1
Krøgh-Jensen and Pihlip ⁸	2	1	1
DeLaChapelle et al. ²	1	0	1
Teasdale et al. ¹³	3	2	1
Humbert et al. ^{4*}	1	1	0
Nowell ⁹	16	9	7
TOTAL	35	16	19

* Cytogenetic studies performed in peripheral blood without phytohemagglutinin.

penia, variable bone marrow cellularity. In 11 patients, it was markedly hypocellular, in 4 it was normal, and, in the rest, it was considered hypercellular.

The hypocellular marrows had pronounced megakaryocytopenia, lymphocytosis, decreased granulocytes, variable normoblasts, and frequent increase in tissue basophils. The normo- and hypercellular marrows were characterized by decreased number of megakaryocytes, normoblastic hyperplasia without abnormal forms, lymphocytosis, increase in tissue basophils, and decreased granulocytes. Increase in immature elements was not seen in any case, and there was definite absence of any cytologic data suggestive of megaloblastic anemia or

malignancy, particularly leukemia. Clinically they had thrombocytopenic purpura, moderate to severe anemia, and absence of liver, spleen, or lymph node enlargement. In 12 patients, no etiologic agent could be ascertained, and, in 6, previous contact with different chemicals such as DDT and chloromycetin was present. The group included 11 males and 7 females, ranging in age from 14 to 73. Most patients had received treatment with either prednisone or anabolic steroids prior to the study.

The miscellaneous group included three patients with primary sideroblastic anemia, one with an unusual type of chronic eosinophilia and visceromegaly, and, in six, we could arrive

TABLE 2. General Data of the Group with Miscellaneous Myeloproliferative Syndromes

Patient no.	Diagnosis	Sex	Age (yrs.)	Time of disease*		Evolution
				Before study	After study	
1	Eosinophilia	M	37	12 yrs.?	58 mos.	Alive, chronic eosinophilic leukemia?
2	Sideroacrestic anemia	F	73	4 yrs.	2 mos.	Dead, bronchopneumonia
3	Myeloproliferative syndrome	M	52	2 mos.	9 mos.	Lost, no evidence of leukemia
4	Myeloproliferative syndrome	F	65	9 mos.	1.5 yrs.	Lost, no evidence of leukemia
5	Myeloproliferative syndrome	M	59	1 yr.	2 yrs.	Dead, bronchopneumonia
6	Sideroacrestic anemia	F	55	6 mos.	8 mos.	Dead, hemorrhage
7	Myeloproliferative syndrome	F	50	2 yrs.	7 mos.	Dead, hemorrhage
8	Myeloproliferative syndrome	F	12	7 mos.	15 mos.	Alive, no evidence of leukemia
9	Myeloproliferative syndrome	F	67	1 yr.	4 mos.	Lost, no evidence of leukemia
10	Sideroacrestic anemia	M	49	4 yrs.	7 mos.	Alive, no evidence of leukemia

* Related to the first cytogenetic investigation.

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at no diagnosis other than atypical myeloproliferative syndrome characterized by variable degrees of anemia and thrombocytopenia, tendency to infections, and hypercellular bone marrow in which the diagnosis of acute leukemia could not be made. No patient had received alkylating agents or any form of ionizing radiation before the cytogenetic studies. The general data of these 10 patients, including the time of observation and their evolution, are shown in Table 2.

Chromosome studies were done by direct bone marrow analysis following the technique of Tjio and Whang.¹⁴ In all cases, an attempt to study 20 metaphases suitable for chromosome counting was made, but this could not be accomplished in all, particularly in the pancytopenic group because either too few cells were present, or those seen were considered inadequate for chromosome count because of poor morphology or not being intact. In the pancytopenic group, photographs of at least two cells were taken for karyotype analysis, while in the miscellaneous group as many karyotypes as possible were performed in each case, in an effort to depict pseudodiploid cells. If the first bone marrow study was abnormal, it was repeated at different intervals, depending on many factors such as availability of the patient, willingness to cooperate, and hematologic indication for bone marrow tap. In addition, peripheral blood culture stimulated with phytohemagglutinin was done in all but one patient with bone marrow abnormalities.

Whenever the first bone marrow study was normal, no particular attempt to repeat it was made in either group, but inadvertently several patients were restudied, and, in one (case 1, Table 2), this study was abnormal and the patient was thereafter closely followed.

RESULTS

The cytogenetic results of the pancytopenic group have been partially reported.¹ For present purpose, it suffices to state that in all patients the chromosome complement was normal, except that in four individuals over 3% of polyploid cells were seen. At present, five patients have died without evidence of acute leukemia, seven are in remission, and six have been lost after a period of over 12 months' observation following cytogenetic analysis, and, in none, at the last consultation, was there any indication of leukemic transformation. Follow-up time in the deceased patients

ranged from 4 months to 13 years, and those in remission have been observed between 1 and 5 years.

Of the miscellaneous group, chromosome abnormalities were observed in six patients (Figs. 1, 2); details can be seen in Table 3. In four patients, no chromosome abnormalities were found in the first study.

DISCUSSION

Further follow-up time may be needed in both groups before definite conclusions can be made. However, the results obtained in this investigation are apparently contrary to those reported by Nowell,⁹ particularly in the pancytopenic group where, of 16 patients, 5 have bone marrow chromosome abnormalities and 4 of these died of acute leukemia within 3 months of study, while none of our 18 patients had abnormalities or died with evidence of acute leukemia. The reason for the discrepancy probably lies in the fact that the same name is being used for different conditions. In Nowell's group, the bone marrow was hypoplastic and had an "increased number of immature elements," while in ours there was no such finding. In fact, one can't help wondering, as suggested by Trujillo et al.,¹⁵ whether at least some of his cases correspond to "aleukemic" leukemia, in which case neither the high frequency of chromosome abnormalities nor of acute leukemia are surprising. We have seen two patients with pancytopenia, increased immature elements in the bone marrow, and abnormal bone marrow chromosome studies who developed overt acute leukemia, but who were not included in the present study because there were clinical or cytologic data indicative of acute leukemia from the beginning. The cytologic interpretation of the "increased immature elements" in the bone marrow is probably the critical point in this regard.

On the other hand, the results obtained in the miscellaneous group, in which several patients had some type of chromosome abnormality and two (cases 1 and 10, Table 3) had a clonal evolution of the abnormal cell line, are not surprising and several facts merit comment. Patient 5 had only one aneuploid cell, out of 18, and it is difficult to decide whether it is truly abnormal, but we will consider it as such. Patient 1 was normal the first time he was studied and, 2 years later, was shown to have an abnormal stemline characterized by a

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member of the D group having the long arm increased in size in approximately 50%, and which had a clonal evolution and eventually

became the only cell line present. This means that the four cases which were studied only once could have developed an abnormality

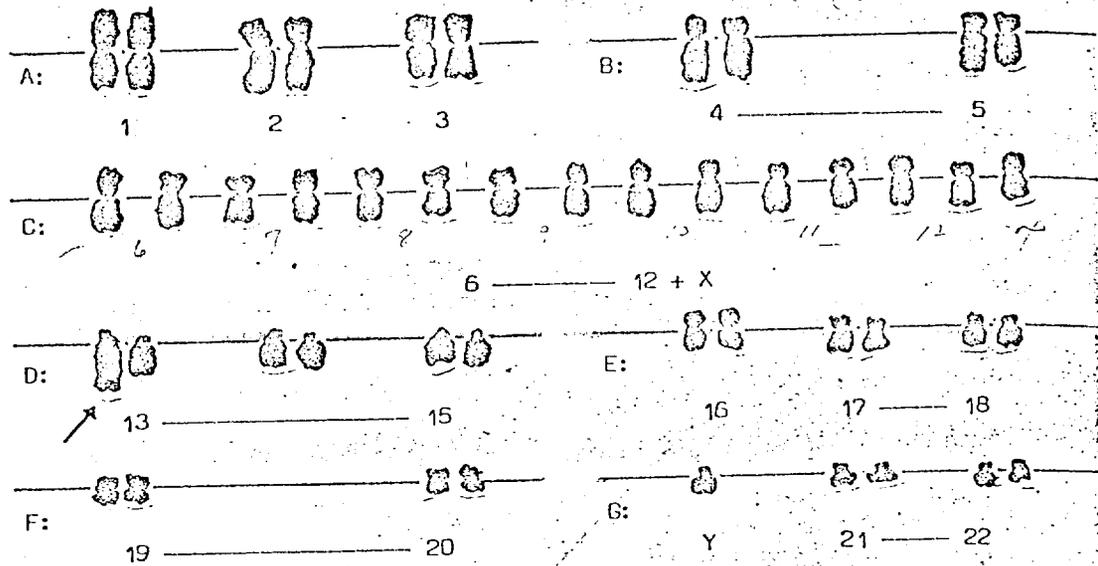


FIG. 1. Representative cell of patient 1. Note the presence of the large "D" chromosome.

which went undetected at a later time and points out the convenience of studying this type of patients in a longitudinal manner.

Half of the patients with chromosome abnormalities have died without evidence of leukemia and, of the rest, only one (case 1, Table 2) has evidence suggestive of an unusual type

of leukemia. The patient had vague symptoms since 1956, and, in September 1967, was first seen by us. At that time, he had moderate splenomegaly and a CBC revealed 16,200 white cells with 77% eosinophils, mild anemia (Hb. 12.5, Ht. 39%), and normal platelets. Bone marrow tap revealed 43% of eosinophils

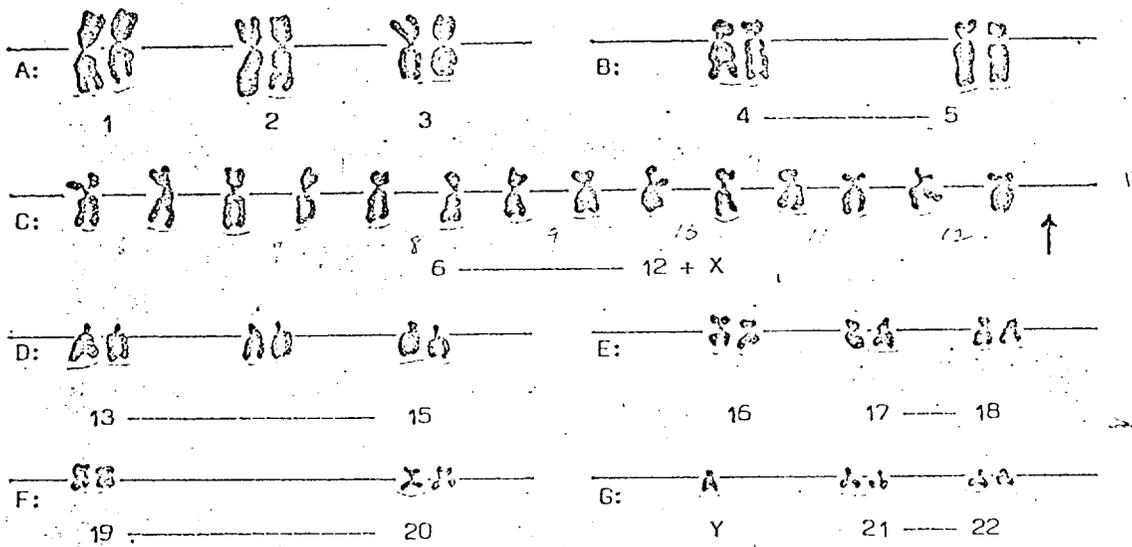


FIG. 2. Representative cell of patient 10. Intact cell with 45 chromosomes, lacking a member of the "C" group.

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TABLE 3. Results of the Cytogenetic Studies Performed in the Miscellaneous Group

Patient* no.	Date	Cells counted	Chromosome no.			Karyotypes ¹	Tissue
			45	46	47		
1	10/16/67	17	0	17	0	46,XY(3)	Marrow
	11/12/69	17	0	17	0	46,XY(5); 46,XY,Dq+(3)	Marrow
	10/ 4/71	5	0	5	0	46,XY,Dq+(5)	Marrow
	11/24/71	20	0	20	0	46,XY(5)	Blood
5	11/27/69	18	0	17	1	46,XY(7); 47,XY,E+(1)	Marrow
		20	0	20	0	46,XY(5)	Blood
6	6/ 6/70	38	0	34	4	46,XX,(9); 47,XX,C+(4)	Marrow
	9/ 4/70	36	4	29	1	45,XX,C-(4); 46,XX(5); 47,XX,C+(1)	Marrow
	10/20/70	15	0	15	0	46,XX(3)	Blood
7	9/ 2/70	18	6	12	0	45,XX?,C-(2); 46,XX(1); 46,XX,C-,Mar+(3)	Marrow
8	2/23/71	17	0	16	1	46,XX(2); 46,XX?,C-,Mar+(2); 47,XX,Mar+(1)	Marrow
	5/12/71	3	0	46	0	46,XX(2)	Marrow
	8/ 3/71	3	0	0	3	47,XX,Mar+(2)	Marrow
	8/23/71	20	0	20	0	46,XX(5)	Blood
10	1/ 5/72	22	6	16	0	45,XY,C-(4); 46,XY(2)	Marrow
	1/15/72	20	0	20	0	46,XY,(5)	Blood
	4/ 5/72	16	16	0	0	45,XY,C-(4)	Marrow
	7/26/72	50	46	4	0	45,XY,C-(6); 46,XY(1)	Marrow

* Patient 1 has chronic eosinophilia and is alive at present; patients 5, 7, and 8 had myeloproliferative syndromes--the former two have died and the latter is alive. Patients 6 and 10 had sideroacrestic anemia; no. 6 is dead and no. 10 is alive.

¹ Numbers in parenthesis refer to number of karyotypes performed.

as the only abnormality. Axillary lymph node, liver, and spleen biopsies revealed nonspecific changes and marked eosinophilic reaction. Leukocyte alkaline phosphatases performed on two occasions were of 8 and 10 units, respectively, and numerous immunologic reactions to depict different parasites were all negative. In September 1971, after a car accident, he developed an acute abdomen due to a ruptured spleen which was removed, and histology revealed only marked eosinophilic infiltration with numerous young forms. A blood count performed a few days later showed 95,000 WBC's with 81% eosinophils, and the bone marrow tap demonstrated marked eosinophilia with abundant young forms. Treatment with Myleran (busulfan, Burroughs Wellcome) was started in October 1971 (6 mg/day), and, 2 months later, he had 31,500 white cells with 83% eosinophils. As mentioned, it is difficult to be sure that this patient indeed has an unusual type of chronic eosinophilic leukemia, but it does seem the most likely possibility in view of the low alkaline phosphatase values, absence of pulmonary infiltration characteristic of the hyper-eosinophilic syndrome,¹⁰ and bone marrow clonal chromosome evolution.

Of the four patients without bone marrow

chromosome abnormalities, one has died without evidence of acute leukemia and three, cases 3, 4, and 9, failed to return to the outpatient clinic at various intervals after the cytogenetic study (Table 2). In none were there data suggestive of leukemic transformation. Patients 3 and 9 died at home, as they were discharged from the hospital in critical condition on request. Further precise information has not been possible, but it should be emphasized that follow-up was relatively short and onset of overt acute leukemia in such patients may take several years.

Our data do not answer the question of whether patients with assorted myeloproliferative syndromes who develop acute leukemia are preceded by bone marrow chromosome abnormalities. This will be difficult to answer because one needs a patient in whom leukemic transformation takes place, in whom cytogenetic studies are obtained just prior to the transformation. On the other hand, we present good evidence that their presence does not necessarily mean that leukemic transformation is imminent. This has been observed previously,^{7,11} but it is surprising that no patient in our group developed acute leukemia, as in the experience of other investigators it is a relatively common event (Table 1). This

could be explained as a sampling phenomenon, differences in diagnostic criteria of such cases or, if Nowell's contention that the risk of developing acute leukemia is high only during the first 3 months after the cytogenetic abnormalities appear is correct, our patients except for patient 3 (Table 2) were first studied from the cytogenetic aspect over 6

months after initiation of the disease, and could belong automatically to the low risk group. Patient 10 is particularly surprising as he had a clonal evolution of an abnormal stemline characterized by loss of a chromosome of the C group, an abnormality which seems to be frequent in those cases who develop acute leukemia.⁴

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