Cytogenetic Studies and Their Prognostic Significance in Agnogenic Myeloid Metaplasia: A Report on 47 Cases

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Cytogenetic analysis was performed in 47 newly diagnosed patients with agnogenic myeloid metaplasia (AMM); 32 had a normal karyotype (68%, group I), whereas 15 had clonal abnormalities (32%, group II). The most frequent abnormal findings were a 20q- deletion in six cases (either alone or within complex anomalies), interstitial 13q- deletion in three cases (and monosomy 13 in one case), and acquired trisomy 21 or 21p+ in three cases. Four cases exhibited complex aberrations involving several chromosomes, sometimes with a mosaicism. In two patients with an initial abnormal karyotype, further cytogenetic analysis during the disease course showed the appearance of additional clonal anomalies, and particularly of a probable Philadelphia (Ph^1) variant in one case. Treatment was essentially supportive. Survival was significantly shorter in group II (median, 30 months) compared with group I (median, not reached at 6 years; $P = .015$). In univariate analysis, other parameters significantly associated with a poor prognosis ($P < .05$) were higher age, anemia, and increased percentage of circulating blasts. However, in a multivariate analysis, only cytogenetic abnormalities and age retained their independent prognostic value.

AGNOGENIC MYELOID METAPLASIA (AMM) is a myeloproliferative syndrome (MPS) characterized by hepatosplenic myeloid metaplasia and bone marrow fibrosis. It is regarded as a clonal hematopoietic stem cell disorder associated with reactive myelofibrosis.[1,2]

Only a limited number of cytogenetic studies have been reported in AMM and they usually only included a small number of patients.[3-15] In most reports, the chronology of chromosome analysis in relation to the disease course and/or the previous chemotherapy was not clearly mentioned; some series included cases of acute myelofibrosis, post-polycythemic myelofibrosis, or undifferentiated MPS, which plainly differ from typical AMM. Except in one study,[3] the prognostic value of chromosome findings has not been well delineated.

From 1980 to 1985, we studied prospective blood and/or bone marrow (BM) karyotypes in 47 untreated patients with AMM; the patients were followed up from 1 to 6 years (median, 42 months) in order to determine the prognostic value of cytogenetic abnormalities.

PATIENTS AND METHODS

Patients. Our 47 patients fulfilled the Polycythemia vera study group (PVSG) criteria for AMM,[16,17] which were splenomegaly, red cell poikilocytosis, leukoerythroblastosis, absence of monocytosis, BM fibrosis without any identifiable cause, and lack of the Philadelphia (Ph^1) chromosome. Patients with post-polycythemic myelofibrosis or acute (malignant) myelofibrosis, as described by Lewis and Szur[18] and recently redefined by Bearman et al,[19] were excluded. Thirty-three patients (70%) received supportive care only. Fourteen patients (30%) received palliative chemotherapy with low-dose busulfan or hydroxyurea, during short periods of time, that did not exceed a few months and were always late in the disease course, because high leukocytosis and/or massive splenomegaly had developed. Only one patient was splenectomized late in the disease course, because of massive splenomegaly and repetitive anemia.

Chromosome analysis. Karyotype was performed at diagnosis in all cases. Four patients had further cytogenetic evaluations during their disease course. Metaphase chromosomes were obtained after 24-hour culture from BM aspirate and/or unstimulated peripheral blood (PB) leukocytes; the latter is especially interesting in AMM where an increased number of circulating myeloid progenitors is present.[20] Chromosomes were examined with conventional Giemsa stain and by R-banding technique (RHG); in some cases, further analysis using a G-banding technique (GTG) was carried out. Chromosome examination was made in all but six cases from at least ten metaphases photographed and analyzed in detail. Chromosomes were identified according to the International System for Human Cytogenetic Nomenclature (ISCN) and an anomaly was considered as clonal when present in at least three cells.[21]

Other parameters assessed. In each patient, the following clinical and hematological data were recorded at diagnosis and evaluated for their prognostic significance: age, sex, spleen size, liver size, PB parameters including hemoglobin concentration, WBC count, percentage of circulating blasts, platelet and reticulocyte count, and myelofibrosis staging at BM biopsy.

Statistical methods. Statistical analysis was achieved in February 1987. The overall survival curve was plotted according to the actuarial method.[22] Patients were separated into two risk groups depending on the presence of clonal abnormalities; actuarial survival curves of these two groups were compared using the log-rank test. A univariate analysis was carried out by introducing each initial variable (karyotype and the other parameters evaluated) separately into a Cox model,[23] and subsequently, a multivariate analysis was performed with the significant parameters.

RESULTS

Cytogenetic findings. Thirty-two patients (68%, group I) had normal karyotypes and 15 (32%, group II) showed at diagnosis clonal acquired karyotypic abnormalities, either in all cells (11 cases) or coexisting with normal metaphases (four cases). As seen in Table 1 and Fig 1, the most frequent abnormality in group II was a deletion of part of the long arm

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Table 1. Initial Abnormal Karyotypes in 15 of 47 Patients With AMM

Patient No.	Sample	No. of Cells	Karyotype*
1	BM	3	46,XY,del(20)(q11)
2	PB + BM	13	46,XY,del(20)(q11)
3	PB	9	46,XY,del(20)(q11)
4	BM	20	46,XY[1]/46,XY,del(20)(q11) [19]
5	BM	13	46,XX,del(13)(q13q21)
6	BM	4	46,XY,del(13)(q13q21)
7	PB + BM	27	46,XX[5]/46,XX,del(13)(q13q21) [22]
8	BM	15	46,XX,del(5)(q12q23)
9	BM	8	46,XY[3]/47,XY,+21 [5]
10	BM	21	46,XY[17]/47,XY,+C [4]
11	BM	8	46,XX,21p+,t(1;21)(p11;q11)
12	PB + BM	7	46,XX,del(5)(p14),del(8)(q23),del(13)(q13q21)
13	PB + BM	14	48,XX,+8,+19,del(20)(q11)
14	PB	15	46,XX,t(1;7)(p31;p22),t(10;20)(q26;q11)
15	BM	17	46,XX,−7,+21 [4]/46,XX,−13,+21 [3]/47,XX,−7,+21,+?18 [5]/47,XX,−7,+9,+21 [4]/47,XX,−13,+21,+?9 [1]/variations.

*Sign [ ] means the number of mitoses showing the same formula when several karyotypes were found in a single patient.

of chromosome 20 (del 20q-); this was found in six cases, either alone or within complex anomalies. An interstitial 13q- deletion was present in three patients and monosomy 13 was also noted in one case. Other findings included anomalies of chromosome 21: acquired trisomy 21 or 21p+ (three cases); deletions of short or long arm of chromosome 5 (two cases); monosomy 7 (one case) and t(1;7) (one case); trisomy 8 (one case). Four subjects exhibited complex aberrations involving several chromosomes, sometimes with a mosaicism.

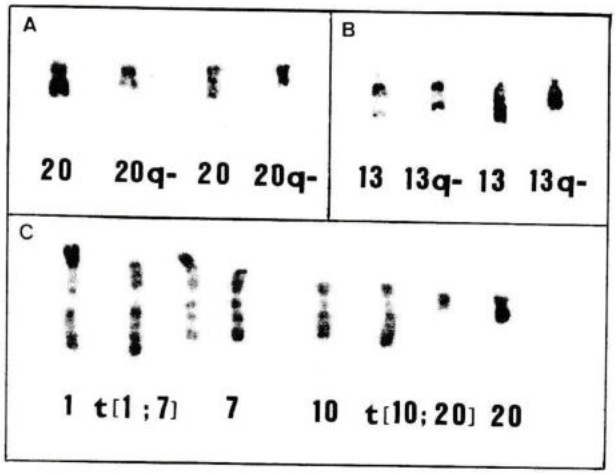

Fig 1. Partial karyotypes showing chromosomal abnormalities. (A) Patient no. 2, R-banded; G-banded: del (20)(q11). (B) Patient no. 7, R-banded; G-banded: del (13)(q13 q21). (C) Patient no. 14, R-banded: t(1;7)(p31;p22), t(10;20)(q26;q11). These are two distinct translocations.

Table 2. Serial Cytogenetic Analyses in Four Cases of AMM

Patient No.	Date	Sample	No. of Cells	Karyotype*
3	1982	PB	9	46,XY,del(20)(q11)
	1983	PB+BM	19	46,XY,del(20)(q11)
5	1981	PB	13	46,XX,del(13)(q13q21)
	1983	PB	6	46,XX,del(13)(q13q21)
9	1983	BM	8	46,XY [3]/47,XY,+21 [5]
	1985	PB	30	46,XY,−7,+21 [28]/47,XY,+21 [2]
12	1982	PB+BM	7	46,XX,del(5)(p14),del(8)(q23),del(13)(q13q21)
	1983	PB+BM	41	46,XX,t(3;14;?9;22)(p14;q32;?q34;q11) [36]/46,XX,del(5)(p14),del(8)(q23),del(13)(q13q21) [5]

*Sign [ ] means the number of mitoses showing the same formula when several karyotypes were found in a single patient.

Chromosome studies were repeated after 1 or 2 years of follow-up in four patients who had abnormal findings initially (Table 2). In the first two cases, the subsequent analysis was performed systemically and the karyotype remained unchanged. Conversely, in the other two patients, the second karyotype was determined because their hematological condition was deteriorating, and these two patients had received no previous cytotoxic therapy; in both cases, additional clonal abnormalities were noted. Patient no. 12 is particularly interesting since a clone with a complex translocation involving at least three chromosomes, 3, 14, and 22, appeared 20 months after diagnosis, coexisting with the first abnormal clone. Although this rearrangement could be a Ph^1 variant, no involvement of chromosome 9 was apparent. This patient initially had typical features of AMM but, at the moment of the second karyotype, leukocytosis was rapidly growing and reached 150×10^9/L. Chemotherapy with hydroxyurea was started, but rapidly stopped because of development of an irreversible pancytopenia; death occurred a few months later.

Prognostic factors. Median survival rate of the 47 cases was 42 months (Fig 2). The main clinical and hematological findings at diagnosis were identical between group I and group II (Table 3). As seen in Fig 2, survival was significantly shorter in group II (median, 30 months), than in group I (median, not reached at 6 years; $P = .015$). However, in patients belonging to group II, no correlation was found between the type of cytogenetic abnormalities and survival.

The progression to overt acute nonlymphoblastic leukemia (ANLL) was seen in one patient of group II but none in group I. Additionally, two patients of group II, but none of group I, experienced a subacute course with rapid onset of severe cytopenias and short survival, although they could be distinguished from acute myelofibrosis.[18,19,24] Among the four patients who belonged to group II and had further cytogenetic studies during the course of their illness (Table 2), the two who showed additional anomalies had a poor outcome after the second karyotype; one (patient no. 9) died 6 months later, after progression to ANLL, and the other (patient no. 12) died of irreversible pancytopenia 8 months

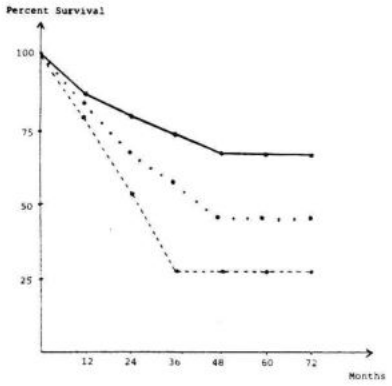

Fig 2. Compared survival curves of the two groups according to initially normal or abnormal karyotype. (——) Normal karyotype group I (n = 32); (----) abnormal karyotype group II (n = 15); (·····) overall survival (n = 47)

after treatment by hydroxyurea (see previous text). The other two patients (patients no. 3 and 5), whose karyotype was unchanged, had a much longer survival (one died 24 months later and the other is still alive after 40 months).

The univariate analysis showed that in our patients, the only parameters significantly associated ($P < .05$) with a poor prognosis were higher age, low hemoglobin level, increased percentage of PB blast cells, and cytogenetic abnormalities.

Table 4 shows that in the multivariate analysis, cytogenetic abnormalities and age retained their unfavorable prognostic significance and were probably independent, whereas low hemoglobin level lost its prognostic influence and could be correlated with the other two parameters. The percentage of circulating blasts could not be included in this multivariate analysis for mathematical reasons.

DISCUSSION

Reports on cytogenetic analysis in AMM and especially prospective studies are scarce and their results heterogeneous. Comparison between our results and previous findings is difficult to perform, since most published series included, in addition to AMM, cases of secondary myelofibrosis or other MPS. Depending on the reports, the proportion of abnormal karyotypes varies from 30% to 75%.[3-6,10,11,13-15] In Table 5, the most frequent cytogenetic abnormalities reported in the literature concerning AMM are summarized; the reports have been analyzed in detail, in order to exclude, when possible, the cases of other related MPS. Data include Miller et al's series[11] and their review of 36 published cases, our 15 patients with clonal abnormalities, and five other series remarkable by the frequence of a particular anomaly.[3,4,10,14,15]

The high incidence of the 20q- deletion in our report is to be noted as it is superior to the frequency reported in the

Table 3. Main Clinical and Hematologic Data at Diagnosis in 47 Patients With AMM

	Group I: Patients With Normal Karyotype (%)	Group II: Patients With Abnormal Karyotype (%)
Total	32	15
Age		
<60	13 (41)	6 (40)
>60	19 (59)	9 (60)
Sex		
M	16 (50)	7 (47)
F	16 (50)	8 (53)
Splenomegaly (cm)		
<10	28 (88)	13 (87)
>10	4 (12)	2 (13)
Hemoglobin (g/dL)		
<12	18 (56)	9 (60)
>12	14 (44)	6 (40)
Leukocytes ($\times 10^9$/L)		
<4	0	1 (7)
4-10	6 (19)	3 (20)
>10	26 (81)	11 (73)
Blast cells (%)		
≤2	28 (88)	12 (80)
>2	4 (12)	3 (20)
Platelets ($\times 10^9$/L)		
<150	7 (22)	4 (27)
>150	25 (78)	11 (73)
Bone marrow biopsy		
Stage I	11 (35)	6 (40)
Stage II	15 (47)	6 (40)
Stage III	3 (9)	2 (13)
ND	3 (9)	1 (7)

Table 4. Prognostic Value in the Multivariate Analysis

Variable	*P* Value	Significance Level
Age	.0095	.0056
Karyotype	.014	
Hemoglobin	.11	

Table 5. Common Cytogenetic Abnormalities in AMM

	Present Report (15 Cases)	Miller et al[11] (11 Cases + Review of 36 Cases)	Other Series
+1q	0	17%	
−5/5q−	13%	12%	
6p−	0	6%	Michaux et al[10] 45% (5/11)
−7/7q−	7%	11%	Smadja et al[14] 83% (5/6)
+8	7%	21%	Besa et al[3] 50% (4/8)
+9	7%	9%	
−13/13q−	27%	4%	Borgström et al[4] 50% (3/6)
20q−	40%	6%	
+21	13%	9%	Whang-Peng et al[15] 14% (2/14)

literature.[7,8] This anomaly, also recently found in some cases of myelodysplastic syndromes,[25,26] had been regarded as relatively frequent in post-polycythemic myelofibrosis.[11] It has been recently demonstrated as an interstitial deletion since the protooncogene c-src1, normally located on the most distal band of 20q (20q12-13), is still present on the rearranged chromosome.[27] Frequency of 13q- deletion must also be emphasized; it is an interstitial deletion too, generally involving bands q12 to q22.[4] This point was confirmed by combined R and G banding studies in our cases.

Acquired trisomy 21 was less frequent; in the literature, it is often associated with acute megakaryocytic proliferations[28] or malignant myelosclerosis.[15] On the other hand, such blood disorders are encountered in patients with Down's syndrome.[29] Trisomy 8, reported in various myeloproliferative disorders, was found in only one of our cases; when reported in AMM, it is generally related to acute myelofibrosis or appears late in the evolution.[13,24] We found no alterations of chromosome 6 and only one anomaly involving chromosome 1, in contrast with most previously published reports.[3,6,10-13,15,30] The translocation t(1;7) (p31;p22), that we noticed in one of our patients, is quite different from the cases of t(1;7) previously described,[9] that gave a picture of monosomy 7.

The differences between our patients and published data are probably due to the miscellaneous nature of many series, combined with the fact that they sometimes included patients treated before karyotype examination. However, since all these aberrations have been reported in various myeloproliferative disorders, it appears that nonspecific chromosomal abnormalities are common in AMM and express the clonal involvement of myeloid stem cells. Delayed appearance of a possible Ph^1 chromosome variant was noted during the course of initially typical AMM in one of our cases; such an evolution is quite unusual and, to our knowledge, had only once been reported in detail.[31]

In most reports, no prognostic significance of cytogenetic findings in AMM was found.[11-13,15] However, according to Besa et al,[3] chromosome analysis was the best predictor of response to androgens (ie, patients with normal karyotype responded better); in their study, good response to androgens was correlated with longer survival. For Whang-Peng et al,[15] chromosomal abnormalities in AMM had little prognostic value, but a change in karyotype might herald the terminal phase of the disease with subsequent development of acute leukemia. Thus, among the four patients of our series in whom a second cytogenetic examination was performed, the two with additional abnormalities had a rapidly fatal outcome. Therefore, additional aberrations probably express further evolution of the pathological clones or the emergence of new ones.

In our series, cytogenetic findings obviously have strong prognostic value on survival. The short survival of patients with abnormal karyotypes may have been due, at least in part, to the fact that subacute courses and acute transformation were only seen in that group. Although, in univariate analysis, three other factors (age, hemoglobin level, and percentage of circulating blasts) were found to have prognostic value; the multivariate analysis showed that karyotype and age were the most important parameters to consider and were probably not correlated. These findings will have to be confirmed on larger groups of patients.

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