Cytogenetic Profile of Minimally Differentiated (FAB M0) Acute Myeloid Leukemia: Correlation with Clinicobiologic Findings

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Cytogenetic data were studied in 26 patients with de novo acute myeloid leukemia (AML) with minimal myelodifferentiation, corresponding to the M0 subtype of the French-American-British classification, in correlation with cytogenetic, immunologic, and clinical findings. Clonal abnormalities were detected in 21 cases (80.7%), 12 of which had a complex karyotype. Partial or total monosomy 5q and/or 7q was found, either as the sole aberration or in all abnormal metaphases, in 11 patients; in 8 cases, additional chromosome changes were present, including rearrangements involving 12p12-13 and 2p12-15 seen in 3 cases each. Five patients had trisomy 13 as a possible primary chromosome change; in 5 cases, nonrecurrent chromosome abnormalities were observed. Comparison of these findings with chromosome data from 42 patients with AML-M1 shows that abnormal karyotypes, complex karyotypes, unbalanced chromosome changes (<5/5q− and/or −7/7q− and +13) were observed more frequently in AML-M0 than in AML-M1. Patients with abnormalities of chromosome 5 and/or 7 frequently showed trilineage myelodysplasia and low white blood cell count. Despite their relatively young age, complete remission was achieved in 4 of 11 patients only. Patients with +13 were elderly males with frequent professional exposure to myelotoxic agents. Unlike patients with clonal abnormalities, most AML-M0 patients with normal karyotype showed 1% to 2% peroxidase-positive blast cells at light microscopy and frequently achieved CR. It is concluded that (1) AML-M0 shows a distinct cytogenetic profile, partially recalling that of therapy-related AML, (2) different cytogenetic groups of AML-M0 can be identified showing characteristic clinicobiologic features, and (3) chromosome rearrangements may partially account for the unfavorable outcome frequently observed in these patients.

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MINIMALLY DIFFERENTIATED acute myeloid leukemia (AML) was recognized as a distinct entity as early as 1987 by Lee et al., who described cytologic, immunologic, and clinical features in 10 patients with morphologically undifferentiated leukemia by light microscopy and positivity for the ultrastructural myeloperoxidase (MPO) as well as for myeloid antigens.

Later on, a number of studies confirmed that a significant fraction of leukemias otherwise classified as "undifferentiated" can be shown to be myeloid in nature, when applying sensitive immunologic and electron microscopy techniques.1,2

However, because diagnostic criteria in these series were not uniform, the French-American-British (FAB) Cooperative group recently proposed guidelines for the recognition of this form of leukemia, now referred to as AML-M0.3 The 3% upper cutoff for MPO positivity was set for its distinction from AML-M1, and positive (ie, expression of CD33 and/or CD13 myeloid antigens or ultrastructural MPO) as well as negative criteria (ie, negativity for lymphoid antigens) were put forward to avoid confusing this leukemia with leukemia with stem cell phenotype,4 with lymphoblastic leukemias, and with biphenotypic leukemias.5

Little is known about the clinical and biologic significance of this newly identified subset of AML; however, a low complete remission (CR) rate has recently been described in 15 patients classified according to the FAB proposals, suggesting that a poor prognosis may be associated with this leukemia, possibly because of the convergence of unfavorable cytogenetic and immunologic features.6

In view of the well-established importance of cytogenetic findings in acute leukemias,7 we analyzed the cytogenetic profile in 26 cases of de novo AML-M0, in correlation with cytologic, immunologic, and clinical features.

PATIENTS AND METHODS

Patient population. Major criteria for the diagnosis of AML-M0 adopted in this study are the following: (1) less than 3% blast cells stained by light microscopy MPO and Sudan black-B (SBB); (2) reactivity with CD33 and/or CD13 myeloid antigens; and (3) negativity for lymphoid antigens (the isolated expression of CD7 or terminal deoxynucleotidyld transferase (TdT) did not preclude the diagnosis of AML-M0). Two patients without expression of myeloid and lymphoid antigens were classified as AML-M0 based on the presence of 1% to 2% positive blast cells with MPO and SBB.8

Forty-one patients with a presumptive diagnosis of de novo AML-M0 were selected among approximately 700 newly diagnosed AMLs, seen at the University Institutes of Hematology in Brussels, Leuven, Mont-Godinne, Brugge, and in Ferrara since 1984. Fifteen patients were excluded from this analysis for the following reasons: (1) a diagnosis of AML-M1, of AML-M5, and of biphenotypic leukemia was thought to be more appropriate at review of cytology and immunophenotype; (2) patients with a cytologically similar subset of AML, in which at least 1% blasts with a lymphoid immunophenotype were present; (3) patients with at least 1% blasts with a nonlymphoid immunophenotype; (4) patients in whom a de novo diagnosis was not obtained; and (5) patients with a biphenotypic immunophenotype.

To compare cytogenetic findings in this cohort of patients and in a cytologically similar subset of AML, 42 patients with an AML-M1 FAB diagnosis, seen at our institutions during the study period, were selected for karyotype review. Differences in the distribution

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of clonal abnormalities among different groups were compared using the χ² test.

**Morphologic studies.** Review of bone marrow (BM) and peripheral blood smears stained by May-Grunwald-Giemsa and by cytochemical reactions including MPO, SBB, and alpha-naphthyl acetate esterase with and without fluoride inhibition, periodic-acid Schiff (PAS), and acid phosphatase (AcP) was performed, and the patients were classified according to the FAB criteria. Attention was devoted to the presence of dysplastic features of BM cells. When all three lineages were affected, AML with trilineage myelodysplasia (MDS) was diagnosed as previously reported.

**Immunologic studies.** Cytofluorimetric analysis of the phenotype of BM and/or peripheral blood cells was performed as previously described, gating primarily on the blast cell population. To minimize nonspecific Fc-receptor binding, all samples were preincubated with 2.5% human AB serum. Nonspecific isotypic mouse monoclonal antibodies (MoAbs) served as negative control for the primary agent.

The expression of following antigens was tested using commercially available reagents (Ortho Diagnostics, Raritan, NJ; Becton Dickinson, Mountain View, CA) (1) myeloid, erythroid, and platelet antigens: CD33, CD13, CD15, CD11b, CD14, glycophorine, CD41, and/or CD61; and (2) lymphoid antigens: CD7, CD2, CD3, CD5, CD22, CD10, and CD19. The expression of the CD34 stem cell marker was also tested: reactivity for the 17F11 MoAb, recognizing an epitope of the c-kit protein product (CD117), was assayed since 1993. A polyclonal antiserum was used for TdT assay (Gibco BRL, Gaithersburg, MD).

A sample was considered positive when 20% of blast cells showed fluorescence above control. However, in accord with previous reports, the 10% cutoff was thought to be more appropriate for the MoAb detecting the c-kit protein product.

**Cytogenetic and molecular genetic studies.** Cytogenetic analysis was performed at diagnosis in all patients by a synchronization technique with methotrexate and bromodeoxyuridine or thymidine. Metaphases were either R-banded or G-banded. Chromosome aberrations were described according to the International System for Human Cytogenetic Nomenclature. Complex karyotypes were defined by the presence of three or more events of translocation and nondisjunction in the same clone or by the presence of multiple unrelated clones. The configuration of the Ig and T-cell receptor (TCR) genes were analyzed at diagnosis in 15 patients for whom representative frozen samples were available.

Methods have been detailed elsewhere. After DNA extraction by standard techniques, digestion with Bgl II, BamHI, EcoRI, HindIII, and Kpn I, respectively, was performed. DNA fragments were size-fractionated on 0.7% agarose gels and blotted onto Hybond N+ filters. Hybridization to probes labeled by primer extension was performed. Ig gene rearrangement was assessed using a heavy chain joining (JH) region probe (a 3.8-kb BamHI-HindIII fragment) and two constant region probes, Ccx and Cx (2.7- and 0.8-kb EcoRI fragments; from Dr R. Dalla Favaera, New York University Medical Center, New York, NY). TCR genes were analyzed for the δ, γ, and β chains, respectively. The TCR-δ rearrangement was studied with the Jδ S16 probe (a 1.5-kb Sac I fragment; from Dr T.H. Rabbitts, Laboratory of Molecular Biology, MRC, Cambridge, UK). The TCR-γ configuration was investigated with the Jy 1.3 probe (0.8-kb EcoRI-HindIII fragment; from Dr J. Bolhuis, Dr D. den Hoed Cancer Centre, Rotterdam, The Netherlands). The TCR-β gene was examined with a constant region CDNA probe (a 0.4-kb Bgl II fragment; from Dr T.W. Mak, Ontario Cancer Institute, Toronto, Canada).

**Clinical data.** Clinical records were reviewed with particular reference to the profession, hematologic data at presentation, outcome of induction therapy, presence of an MDS phase after achievement of CR, and cytologic features at relapse. CR was defined by the presence of less than 5% BM blast cells with more than 1.5 × 10⁹/L neutrophils and more than 100 × 10⁹/L platelets and hemoglobin greater than 10 g/dL.

**RESULTS**

**Morphology and cytochemistry.** All patients classified as AML-M0 showed little or no maturation along the granulocytic lineage. The overall morphologic picture was that of undifferentiated leukemia, with small- to medium-sized cells, round nuclei, and open chromatin. One or more nucleoli were evident, and scanty, moderately basophilic cytoplasm was observed in the majority of cases, whereas heterogeneity of cell size was observed in some cases. In 9 cases, 1% to 2% positive cells for the MPO and SBB stain were detected, whereas only occasional MPO+ cells were observed in the remaining 17 cases. Weak diffuse positivity for the nonspecific esterase stain that was not inhibited by sodium fluoride was observed in 15 cases; PAS block-labeled positivity or strong, localized positivity for the AcP was not observed in any patient, whereas small granular positivity for the PAS stain was detected in 10 cases. A moderate increase (2% to 5% of total cellularity) of morphologically normal eosinophils in late stage of differentiation was noted in 4 cases (nos. 13, 15, 16, and 26).

Because of overwhelming blast cell infiltrate of the BM, morphologic features of the residual nonblast cell population could not be assessed in 10 patients. Dysplastic features of the nonblast cell population fulfilling criteria for the definition of trilineage MDS were present in 7 cases (nos. 1, 4, 8, 10, 11, 14, and 21), whereas dysplastic features were confined to 1 or 2 cell lineages in 2 and 3 patients, respectively.

**Immunophenotype.** The immunologic profile of our patients is shown in Table 1. Positivity for two or more myeloid-associated antigens was detected in 22 cases, in the absence of coordinate expression of lymphoid-associated antigens; whereas, in 2 cases, only one myeloid marker (either CD13 or CD33) was found to be positive in more than 20% of blast cells (cases no. 11 and 15). Two patients with SBB and MPO positivity in 1% to 2% blast cells had a stem cell phenotype with CD34 and HLA-DR positivity with negative myeloid and lymphoid markers (patients no. 13 and 25). No patient expressed the CD14, glycophorine, and CD41 antigens normally detected in leukemias with monocytic, erythroid, or megakaryoblastic differentiation, respectively.

**Cytogenetic and molecular genetic findings.** Results of chromosome investigations are detailed in Table 2. Clonal abnormalities were detected at diagnosis in 21 patients.
complex karyotypes, including 2p and 12p rearrangements carrying -515q- and/or -717q- were detected in 13 pa-
tients; in 8 cases, these abnormalities were associated with
5q- and/or -717q- were consistently present in all abnor-
mal metaphases, possibly representing the primary chromo-
some change. Trisomy 21 was present as the sole anomaly
in 4 of 13 and 7 cases and 3 of 15 cases, respectively (see Table
2). In 2 cases (no. 9 and 18), both the IgH and the TCR
arrangement of the TCR-β gene in all cases examined.

Clinical features. Hematologic findings at presentation
in 4 of 15 cases and 3 of 15 cases, respectively (Table
2). In 2 cases (no. 9 and 18), both the IgH and the TCR
genes were in a rearranged configuration. There was no re-
arrangement of the TCR-β gene in all cases examined.

The observed frequency of recurrent primary chromosome
changes of complex karyotypes and of unbalanced chromo-
some rearrangements in AML-M0 with respect to AML-M1
is shown in Table 3. Molecular genetic studies showed clonal
rearrangement of the IgH chain gene and of the TCR genes
in 4 of 15 cases and 3 of 15 cases, respectively (see Table
2). In 2 cases (no. 9 and 18), both the IgH and the TCR
genes were in a rearranged configuration. There was no re-
arrangement of the TCR-β gene in all cases examined.

Clinical features. Hematologic findings at presentation
and correlation of chromosome findings and clinicobiologic
features in patients with AML-M0 are summarized in Tables
4 and 5. The profession was known for 20 patients, 7 of
whom (no. 1, 2, 6, 7, 13, 14, and 15; 2 truck drivers, 2
factory painters, and 3 farmers) were considered exposed to
petroleum products, organic solvents, or pesticides. Mye-
locytopenia and of unbalanced chromosome rearrangements
in AML-M0 with respect to AML-M1 is shown in Table 3. Molecular genetic studies showed clonal

### Table 2. Karyotype and Clinical Outcome in 26 Cases of AML-M0

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Karyotype (No. of Cells)</th>
<th>Outcome (Duration of CR)</th>
<th>Survival*</th>
<th>IgH</th>
<th>TCRβ/γ/δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>45,XX, –7(1)/46,XX[47]</td>
<td>PR</td>
<td>3</td>
<td>G</td>
<td>G/G/G</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>45,XX,del(5)(q13q31), –7(10)</td>
<td>NR</td>
<td>3</td>
<td>G</td>
<td>G/G/G</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>46,XY,del(5)(q12q32)[3]/46,XY[13]</td>
<td>CR (21)</td>
<td>23</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>44,XX,del(2)(17q;7),del(5)(q12q32), –7, add(12)(p12), +mar(10)</td>
<td>NR</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>47,XY,del(7)(q12q31), +mar(10)</td>
<td>CR (2)</td>
<td>ABMT</td>
<td>8</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>45,XY, –7(1)/46,XX[18]</td>
<td>CR (2)</td>
<td>10</td>
<td>R</td>
<td>R/G/G</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>46,XY,del(5)(q12q32)[2],del(6)(q21), –7, der(12)(17q11p12), +mar(10)[3]/46,XY[12]</td>
<td>CF (2) ABMT</td>
<td>3</td>
<td>5</td>
<td>G/G/G</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>50,XY,del(2)(15q), –5add(7)(q22)+8add(21p11), +mar(1), +mar(2), +r, +r(10)</td>
<td>NR</td>
<td>1</td>
<td>G</td>
<td>G/G/G</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>45,XY,del(1)(q24q42),del(5)(q21q32), –8,der(12) (t8;12)<a href="25">q21p13</a></td>
<td>CR (9)</td>
<td>14</td>
<td>R</td>
<td>R/G/G</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>45,XY,del(5)[q14q32],del(7)(17q22), -17(4)<a href="q13q33">45, ideom del(9)</a>[-1]</td>
<td>CR (2)</td>
<td>BMT</td>
<td>8</td>
<td>G/G/G</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>45,XY, –7(1)/45, ideom, der(3)[q7][9]</td>
<td>NR</td>
<td>8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>47,XY, +13(6)/46,XY[9]</td>
<td>NR</td>
<td>NA</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>47,XY, +13(2)/49,XY, ideom, +9, +11(6)/46,XY[4]</td>
<td>PR</td>
<td>3</td>
<td>G</td>
<td>G/G/G</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>47,XY, +13(3)/47,XY, ideom, –7, +mar(7)</td>
<td>NR</td>
<td>5</td>
<td>G</td>
<td>G/G/G</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>45,XY, +13(3)[49,XY, +8, –10, +13(12)</td>
<td>CR (3) BMT</td>
<td>40+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>46,XX,(t6;9)[q12p23(14)/46,XX[14]</td>
<td>CR (5)</td>
<td>9</td>
<td>ND</td>
<td>G/G/G</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>45,XY,der(16)(16q10;7)[1]/44, ideom, –5, –14(6y)/46,XY[1]</td>
<td>CR (5)</td>
<td>10</td>
<td>R</td>
<td>R/G/G</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>59,45,XY,add(9)[p12], –12(4y)/46,XY, ideom, +8(3)/46,XY[7]</td>
<td>ED &lt;1</td>
<td>G</td>
<td>R</td>
<td>G/R/G</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>66,47,XY, +20(3)/46,XY[7]</td>
<td>CR (4)</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>45,XX, –7(1)/46,XX[6]</td>
<td>CR (2)</td>
<td>37</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>46,XX[16]</td>
<td>CR (3) BMT</td>
<td>15</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>49,46,XY[20]</td>
<td>CR (2)</td>
<td>9</td>
<td>G</td>
<td>G/G/G</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>46,XX[16]</td>
<td>CR (3) BMT</td>
<td>96+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>46,XX[16]</td>
<td>CR (duration NA)</td>
<td>NA</td>
<td>R</td>
<td>G/G/G</td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>46,XX[20]</td>
<td>NA</td>
<td>NA</td>
<td>R</td>
<td>G/G/G</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete remission; PR, partial remission; NR, no response; ED, early death; NA, not available; BMT, BM transplantation; ABMT, autologous BM transplantation; G, germline; R, rearranged; ND, not done.

* Months; +, indicates the patient is alive.
relapse were consistent with a diagnosis of AML-MO in 5 cases; whereas, the features of AML-M1 were observed in 4 cases, and those of AML-M2, in 1 case. Three transplanted patients (no. 16, 22, and 24) are alive and free of leukemia at 15, 40, and 96 months; whereas the remaining patients died at less than 1 to 37 months, with a median overall survival of 8 months.

**DISCUSSION**

**Diagnosis of AML-M0.** Although the formulation of a generally accepted system of classification of acute leukemias by the FAB group (5,12) has provided a framework of reference for the identification of various subtypes of AML, the unequivocal recognition of AML-M0 may still pose some problems, with particular reference to the possibility of confusing early lymphoblastic leukemia with inappropriate expression of myeloid antigens.

The diagnosis of AML-M0 in our patients fulfilled the FAB criteria and was supported by immunophenotyping and by cytochemical features of the blast cell population. Interestingly, SBB appeared to be more sensitive than MPO in this study, and 7 patients who would have otherwise been classified as AML-M0 because of the presence of less than 3% positivity for MPO were included among AML-M1 at cytologic review, showing 3% to 12% SBB-positive cells.

While a pro-B–lymphoid nature of leukemic cells could be ruled out by negativity for early markers of B-lymphoid differentiation, such as CD19 and CD10, the differential diagnosis with early T-cell acute lymphoblastic leukemia was not unequivocal for those patients with CD7 positivity. This problem may be particularly important, especially for those patients, such as case no. 9, showing a clonally rearranged pattern for the TCR gene.

In the absence of consensus on the diagnostic importance that should be attributed to lineage-associated antigens, we felt that a diagnosis of AML-M0 was appropriate in these patients, given the absence of coordinate expression of T-lymphoid features (ie, negativity for the AcP stain, for the TdT as well as for the CD5, CD2, and CD3 molecules). It is noteworthy that, although the finding of inappropriate rearrangement of the IgH chain and TCRγ or γ gene is not surprising in AML, especially in those patients with blast cell immaturity and TdT positivity, the incidence of such genetic events in our series is not dissimilar as compared with previous studies of unselected AML cases.

Clinical and cytologic follow-up in this series yields the following observations confirming the "nonlymphoid nature" of these leukemias: (1) the presence of trilineage MDS in 7 of 16 evaluable cases; (2) an MDS phase preceding overt relapse in 2 cases achieving CR; and (3) a more differentiated myeloid phenotype at relapse in 5 of 10 cases, in the absence of lineage switch. These features would be unusual in lymphoblastic leukemias because trilineage MDS has been described in approximately 15% de novo AML and, more frequently, in erythroleukemia and megakaryoblastic leukemias.

Immunologic findings in AML-M0 document the consistent expression of the CD34 stem cell marker in association with CD13 and/or CD33 and with other myeloid associated antigens, such as CD11b and CD15. However, no patient was found with isolated expression of CD15 or CD11b, thus confirming the notion that CD13 and CD33 are to be considered the most sensitive and reliable markers for the immunologic diagnosis of AML with minimal myeloid differentiation.

Finally, attention should be drawn to the fact that the anti-CD117 MoAb 17F11, recognizing an epitope of c-kit protein product, functioning as a receptor for the stem cell factor (c-kit ligand), was found to be reactive with more than 10% leukemic cells in 50% of our cases (3 of 6). The distribution of the CD117 antigen in different FAB subtypes of AML is still controversial, some investigators having found 100% positive cases in AML-M0 and AML-M1 and others having reported on a 14.9% positivity in children AML-M1. Obviously, more cases need to be studied to clarify the clinicobiologic significance of CD117 positivity in AML.

**Cytogenetic profile of AML-M0: Comparison with unselected AML cases and with AML-M1.** A preliminary methodological problem in the definition of the cytogenetic profile of AML-M0 was represented by the selection of an appropriate control group serving as reference for comparative analysis. Data for comparison of AML-M0 with unse-
lected AML cases were derived from the report of the Sixth International Workshop on Chromosomes in Leukemia (SIWCL). In addition, we elected to include into this study original cytogenetic data from a cohort of patients affected with de novo AML without maturation, corresponding to the M1 type of the FAB classification, to be able to compare chromosome findings in these cytologically similar forms of leukemia, the distinction of which may not be immediate on cytologic grounds and may appear somewhat artificial from a biologic point of view.

According to our data, de novo AML-M0 stands out as a disease entity with a higher percentage of abnormal karyotypes (80.7%) as compared with unselected cases of AML reported at the SIWCL, where up to 46% cytogenetically normal cases were found. In addition, the type of chromosome changes in AML-M0 differs significantly with respect to the general cytogenetic profile of AML, a conclusion reinforced by a literature review of previously reported cases with AML-M0 diagnosed according to the FAB proposals (see Table 6).

None of the chromosome translocations associated with well-defined cytologic subsets of AML was encountered in this series, nor was any previously unrecognized translocation found as a recurrent chromosome change in our patients with AML-M0. Notably, 11q23 rearrangements, possibly associated with stem cell involvement in acute leukemia, were not observed in this series. Chromosome changes, such as 5q and 7q abnormalities, normally found in many FAB subtypes of AML were detected in 13 of 21 (61.9%) AML-M0 cases with abnormal karyotype, a figure significantly higher than the 21% derived from 357 de novo AML cases with abnormal karyotype reported at the SIWCL.

Interestingly, a 66% and 72.4% frequency of 5q and/or 7q aberrations was found in two studies of 39 and 58 therapy-related AML (t-AML) cases with abnormal karyotype. These findings show that some cytogenetic features in our patients with AML-M0 recall those typically found in a subset of t-AML and may suggest that similar leukemogenic mechanism may frequently be operative in these forms of leukemia.

The cytogenetic profile of AML-M0 shows similar differences even when compared with the cytologically closest form of leukemia, namely AML-M1. In addition, analysis of our data shows that trisomy 13 and aberrations of 2p are more frequently observed in AML-M0, that 3q21/2q26 rearrangements may be confined to AML-M1, and that the observed frequency of primary chromosome changes in AML-M0 differs significantly with respect to AML-M1. Besides abnormalities of 5q and 7q, complex karyotypes and unbalanced chromosome changes leading to gain or loss of genetic material, normally regarded as characteristic chromosomal changes in t-AML, appeared more frequently in AML-M0 than in AML-M1. In this respect, it is interesting to note that a view is emerging that balanced reciprocal translocations and unbalanced chromosome abnormalities may contribute differently to malignant transformation, the former type possibly resulting from exposure to agents targeting DNA topoisomerase II and the latter type being characteristically associated with genetic damage following "in vivo" and "in vitro" exposure to mutagens, such as alkylating agents. Finally, it is noteworthy that aberrations of 5q and 7q and deletions or translocations of 12p are also very common in erythroleukemia, which is usually regarded as a stem cell disorder with multiple cell-lineage involvement. In the absence of reliable markers of early erythroid differentiation, the theoretical possibility should be considered that some leukemias fulfilling the FAB criteria for AML-M0 may in fact represent proliferations of immature erythroid precursors.

Correlation of chromosome findings with clinicobiologic features. Further insights into the significance of chromosome findings in AML-M0 may be derived from the observation that, in this study, 11 cases had −5/5q− and/or −7/7q− as a possible primary chromosome change, 5 cases had +13, 5 patients had different nonrecurring primary chromosome aberrations, and 5 patients had a normal karyotype. As shown in Table 5, analysis of clinicobiologic findings in these cytogenetic groups of AML-M0, yields important

### Table 6. Cytogenetic and Clinicobiologic Findings in AML-M0: Data From the Literature

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>No. of Cases</th>
<th>Median Age (Range)*</th>
<th>Cr (Yes/Total)*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>12</td>
<td>50 (32-59)</td>
<td>4/7</td>
<td>1, 4, 8, 44, 48</td>
</tr>
<tr>
<td>−5/5q− and/or −7/7q−</td>
<td>10</td>
<td>62.0 (29-70)</td>
<td>0/6</td>
<td>1, 4, 8, 46</td>
</tr>
<tr>
<td>+8</td>
<td>7</td>
<td>62 (54-68)</td>
<td>2/3</td>
<td>1, 5, 8, 46</td>
</tr>
<tr>
<td>+19</td>
<td>4</td>
<td>45 (56-66)</td>
<td>1/1</td>
<td>0, 5, 8, 43, 45</td>
</tr>
<tr>
<td>+13</td>
<td>3</td>
<td>62 (61-87)</td>
<td>2/3</td>
<td>0, 8, 39, 40, 43</td>
</tr>
<tr>
<td>del(3)</td>
<td>2</td>
<td>61-70</td>
<td>0/2</td>
<td>8</td>
</tr>
<tr>
<td>t(12;13)(p13;q14)</td>
<td>2</td>
<td>42-51</td>
<td>NR</td>
<td>47</td>
</tr>
</tbody>
</table>

* Only those cases with clonal aberrations observed in at least 2 patients are shown. del(3p) was associated in both cases with −5 or −7; +19 was associated with +13 in 1 case. Abbreviation: NR, not reported.

* Data not available for all cases.
observations, some of which deserve particular attention. Those patients carrying aberrations of the long arms of chromosome 5 and/or 7 share with t-AML the primary chromosome change, the frequent presence of a complex karyotype, and, possibly, some additional aberrations such as rearrangements involving 12p.38 In addition, they may frequently show abnormalities of the short arm of chromosome 2, the presence of which was only detected sporadically in other forms of de novo or t-AML.39 Interestingly, a possible exposure to myelotoxic agents in the workplace could be documented in 4 cases.

Other hematologic features in these patients, such as the presence of trilineage MDS in frequent association with a relatively low white blood cell count and low percentage of BM blasts also recall the clinicobiologic picture commonly observed in t-AML.39 Most importantly, although patients in this cytogenetic subset of AML-M0 were generally young, they infrequently achieved CR under conventional myeloablative chemotherapy.

Trisomy 13 may identify a group of AML-M0 preferentially affecting elderly males with frequent professional exposure to myelotoxic agents. A moderate increase of BM eosinophils, an unusual finding in other patients with AML-M0, was observed in 3 of 5 cases, whereas the presence of trilineage MDS could only be assessed in 2 patients because of almost complete BM replacement by blast cells in the remaining 3 cases. All cases expressed either TdT or CD7 (3 cases and 2 cases, respectively).

Although +13 has been reported in a wide spectrum of myeloid neoplasias,31 our data seem to support the existence of a strong association between this numerical aberration and AML-M0, because +13 has only been found in 10 patients with other FAB subtypes of AML seen at our institutions during the study period. An association of +13 with cell immaturity in acute leukemia was previously described by Sreekantaiah et al,42 whereas a more heterogeneous cytologic picture was described by Dohner et al43 where a more heterogeneous cytologic picture was observed in 3 of 36 AML-M0 collected in a literature review (see Table 6) that trisomy 13 as the primary change.

Little is known about the prognostic implication of +13 in AML, although a relatively low CR rate was noted at review of 21 published cases.44 However, this finding must be weighed against the advanced age of most patients with +13. Not unexpectedly, 2 patients with +13 achieving CR in this series were less than 60.

Finally, unlike most karyotypically abnormal AML-M0, those patients with normal karyotype frequently showed positivity for "myeloid" cytochemistry in 1% to 2% cells and achieved CR under conventional chemotherapy. As shown in Table 3, a normal karyotype is more frequently encountered in AML-M1, thus suggesting that those cases of AML-M0 with few MPO+ blast cells may not be dissimilar, on cytogenetic grounds, from AML-M1, the distinction of these FAB subtypes in such cases being only represented by the arbitrary 3% cutoff for SBB/MPO positivity. In conclusion, these findings confirm that the recognition of AML-M0 is essential for a complete cytologic classification of AML. These findings also seem to indicate that the identification of AML-M0 as a distinct entity may be justified on cytogenetic and clinicobiologic grounds and indicate that chromosome changes may partially account for the unfavorable outcome usually associated with this leukemia.

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Cytogenetic profile of minimally differentiated (FAB M0) acute myeloid leukemia: correlation with clinicobiologic findings [see comments]

A Cuneo, A Ferrant, JL Michaux, M Boogaerts, H Demuynck, A Van Orshoven, A Criel, M Stul, P Dal Cin and J Hernandez