Clinical Significance of Surface Antigen Expression in Children With Acute Myeloid Leukemia: Results of Study AML-BFM-87

By Ursula Creutzig, Jochen Harbott, Christian Sperling, Jörg Ritter, Martin Zimmermann, Helmut Löffler, Hansjörg Riehm, Günther Schellong, and Wolf-Dieter Ludwig

Immunophenotyping using a panel of 15 antibodies was performed in 267 (87%) and cytogenetic analysis in 196 (64%) of 307 children under 17 years of age enrolled in the AML-BFM-87 study. Treatment consisted of cytosine arabinoside, daunorubicin, etoposide induction and a 6-week seven-drug consolidation chemotherapy, followed by two blocks of high-dose cytosine arabinoside with or without cranial irradiation and maintenance therapy for 1 year. Five-year event-free survival for patients with immunophenotypic data was 0.43 ± 0.03 SE. The diagnostic value of the pan-myeloid reagents CD13, CD33, and CD65 for the recognition of childhood acute myeloid leukemia (AML) was high with a sensitivity of 98% (positivity of at least one of these antigens), whereas, with the exception of CD41 for French American British (FAB) subtype M7, the expression of single cell-surface antigens showed no correlation with morphologic or cytogenetic subgroups. On the other hand, characteristic subgroups of AML defined by morphologic features and karyotypes could be described by low or high rates of surface antigen expression compared with those of other patients. These immunophenotypic features most probably associated with specific entities include expression of CD34 or CD13 and absence of CD14 or CD4 in M2 with Auer rods/t(8;21); absence of HLA-DR, CD34, and CD14, but expression of CD33 in M3/t(15;17); positivity of either CD34 or CD13 and either CD14 or CD2 for M4E0/inv(16); and absence of either CD34 or CD13 and expression of either CD33 or CDw65 and either CD15 or CD4 for M5/t(9;11). In FAB M0, positivity of one or two of the three panmyeloid-associated markers (CD13/33/w65) was common; and cytogenetic results frequently showed random abnormalities. Expression of lymphoid-, progenitor- and most myeloid-associated antigens had no influence on the prognosis, whereas the outcome was significantly better for children with M2 with Auer rods, M3, or M4E0 or for those with the associated karyotypes t(8;21)/t(15;17) and inv(16) than for other patients. 

ACUTE MYELOID LEUKEMIAS (AMLs) comprise a heterogeneous group of diseases that differ in their etiology, pathogenesis, and natural history, as well as in their prognosis. In the past, AMLs were mainly classified according to morphology- and cytochemistry-based criteria proposed by the French American British (FAB) group.1,2

More recently, immunophenotyping and cytogenetics have been routinely applied to the characterization of leukemic cells in AML.3,4 Although the detection of chromosomal abnormalities has emerged as one of the most important pretreatment prognostic factors in AML,5-8 immunophenotyping using a large panel of monoclonal antibodies (MoAbs) to myeloid and lymphoid lineage as well as progenitor cell-associated antigens has provided valuable information for lineage assignment of myeloperoxidase-negative (MPO−) acute leukemias, often referred to as acute undifferentiated leukemia or minimally differentiated AML (AML-M0),9,12 and has enabled the identification of acute megakaryoblastic leukemia13 as well as AML coexpressing lymphoid-associated antigens.14-16

An integrated classification mainly based on specific chromosomal anomalies and distinctive morphologic (FAB) subtypes in AML has been proposed in an attempt to more precisely define biologically and clinically relevant entities as well as to provide new prognostic insights.17 More recently, the so-called morphologic, immunologic, and cytogenetic (MIC) working classification of AML has been substantiated and expanded by the detection of distinctive immunophenotypic features within cytogenetically and morphologically defined subgroups, such as coexpression of CD19 and CD56 in the M2/t(8;21) subtype of AML,18-21 and of CD2 in M3/t(15;17)20-23 and M4E0/inv(16)24 as well as a high incidence of CD34 expression in both secondary and de novo AML with aberrations of chromosome 5 and/or 7.25-27

The identification of more homogeneous subgroups of biologically similar cases could be of utmost importance for both an improved understanding of the leukemic process and the definition of prognostically relevant risk groups useful for individualization of treatment strategies. Although a large variety of clinical and biologic characteristics have been claimed to influence the prognosis of AML, very few of them—including age, previous hematologic disorder, certain cytogenetic aberrations, response to induction chemotherapy, and specific morphologic features within individual FAB subgroups such as Auer rods and eosinophils, especially in children—have been generally accepted as independent predictors of treatment outcome.4,6,8,28-31

In contrast with the findings in acute lymphocytic leukemia (ALL),32,33 the prognostic significance of surface antigen expression in AML is still a matter of controversy. Although some investigators, especially in childhood AML, could not show any correlation between the expression of individual progenitor-, myeloid- and lymphoid-associated antigens and
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From the University Children’s Hospital Münster, Münster, Germany; University Children’s Hospital Gießen, Gießen, Germany; the Department of Pediatric Hematology and Oncology, Medizinische Hochschule Hannover, Hannover, Germany; Robert-Rössle Clinic, Department of Medical Oncology and Applied Molecular Biology, Humbold-University of Berlin, Berlin, Germany; University Hospital Kiel, Kiel, Germany. Submitted November 15, 1994; accepted May 16, 1995. Supported by the Deutsche Krebshilfe.

Presented in part at the 1993 annual meeting of The American Society of Hematology.

Address reprint requests to Ursula Creutzig, MD, PhD, Universitätss Kinderklinik, Albert-Schweitzer-Str. 33, D-48129 Münster, Germany.

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the treatment outcome, others suggested a significant influence of specific immunophenotypic features on the complete remission (CR) rate, the CR duration, and/or the survival. Among the antigens implicated in having an adverse prognostic effect are CD7, CD9, CD11b, CD13, CD14, CD41, CD45,44, HLA-DR, CD34, CD10, CD19, and TdT. On the other hand, the presence of CD15, CD42b, CD61, and TdT has been associated with a better treatment outcome. Other authors could not confirm these findings (eg, CD2, CD34, TdT) but the comparability of the results is hampered by methodologic differences in the detection of antigen expression as well as by differences in patient populations studied and treatment regimens administered.

The present AML-BFM-87 study involved prospective immunophenotyping examinations in addition to morphologic and cytogenetic analyses in a large number of children with newly diagnosed AML. Its objectives were (1) to determine the diagnostic sensitivity of immunophenotyping and its contribution to the subclassification of AML; (2) to examine the relationship between surface antigen expression, FAB subtypes, chromosomal abnormalities, and clinical characteristics; and (3) to evaluate the prognostic significance of different immunophenotypic features, and to compare it with that of other prognostic features, especially morphology and cytogenetic findings.

**PATIENTS AND METHODS**

**Patients**

A group of 307 previously untreated children with AML under 17 years of age were enrolled in the cooperative AML-BFM-87 study between December 1986 and October 1992. Informed consent was obtained from all patients.

**Diagnosis**

The diagnosis and FAB subtype of AML was determined irrespective of the immunophenotyping studies from Pappenheim-stained bone marrow (BM) and blood smears. Additional cytochemical reactions included periodic acid-Schiff (PAS), myeloperoxidase (MPO), α-naphthylacetate esterase, and acid phosphatase, which were routinely performed centrally at the University Children’s Hospital in Münster. All smears were also reviewed by at least one independent investigator (H. Löffler, Kiel), who also was not informed about the immunophenotyping results. The diagnosis of M0- and M7-subtypes always required the confirmation by immunologic methods.

**Treatment**

The treatment strategy of the AML-BFM-87 protocol is summarized in Fig 1. Therapy started with an 8-day induction course, comprised of cytosine arabinoside (Ara-C), daunorubicin, and etoposide, followed by a 6-week consolidation therapy with seven different drugs and two blocks of late intensification with high-dose Ara-C (3 g/m²) and etoposide. Maintenance therapy consisted of thioguanine daily, and Ara-C for 4 days every 4 weeks up to a total period of 18 months. During the first two and a half years of the study, cranial irradiation was given to 60 of 143 patients and, afterwards, to all patients in complete remission (CR). Allogeneic BM transplantation in first CR was recommended for children of the high-risk group only.

**Immunophenotyping**

Extensive immunologic marker analyses were performed in 267 of 307 children (87%). Immunophenotyping was performed at the central reference laboratory of the AML-BFM studies (Universitätsklinikum Steglitz, Berlin) for 251 of these patients and for 16 of them at other institutions. Heparinized fresh BM or peripheral blood samples were isolated by Ficoll-Hypaque density gradient centrifugation (Pharmacia, Bio-tech, Freiburg, Germany), and cell-surface antigens were detected by a standard indirect immunofluorescence (IF) assay as previously described. To avoid nonspecific Fc IgG-receptor-mediated binding of MoAbs, cells were treated with heat-inactivated 10% rabbit serum (GIBCO BRL, Egggenstein, Germany) before immunostaining as well as in subsequent incubation steps with primary or second reagents. Fluorescent labelings of surface membrane antigens were evaluated immediately by flow cytometry using a FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, CA). Data acquisition in flow cytometry was performed using FACScan Research or Lysis II software (Becton Dickinson). Isotype-matched nonreactive mouse MoAbs at the same protein concentrations were used as negative controls in all experiments. Although the percent viability of fresh leukemic cell suspensions usually exceeded 90%, dead cells were excluded from flow cytometric analysis with propidium iodide (Sigma Chemical Co, St Louis, MO) gating.

The MoAbs used, all available from commercial sources, were as follows: (1) panmyeloid antigens: CD13 (My7, Coulter Clone, Hialeah, FL), CD33 (My9, Coulter Clone), and CDw65 (VIM-2, Becton Dickinson) with epi-illumination and phase-contrast devices. Although not used routinely in the prospective study, CD3 (Leu-4, Becton Dickinson) was tested in most AML cases with coexpression of T-lymphoid markers to detect the possible contamination by residual CD3+ mature T cells.

Coexpression of lymphoid and myeloid antigens by single cells was confirmed by standard two-color flow-cytometric analysis using appropriate MoAbs directly conjugated to fluorescein isothiocyanate or phycoerythrin. Although not used routinely in the prospective study, CD3 (Leu-4, Becton Dickinson) was tested in most AML cases with coexpression of T-lymphoid markers to detect the possible contamination by residual CD3+ mature T cells.

For nuclear staining of terminal deoxynucleotidyl transferase (TdT), cytoplasmic preparations of leukemic blasts were fixed in methanol, subsequently assayed by indirect IF using heterologous rabbit anti-TdT antiserum (Supertechs, Bethesda, MD), and evaluated for IF using Zeiss microscopes (Zeiss, Oberkochen, Germany) equipped with epi-illumination and phase-contrast devices. A leukemic cell sample was considered positive for surface anti-
Table 1. Initial Patient Data in Study AML-BFM-87

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>307</th>
<th>267</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>7.9</td>
<td>7.8</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>CNS involvement*</td>
<td>36/301 (12%)</td>
<td>31/260 (12%)</td>
</tr>
<tr>
<td>Extramedullary organ involvement</td>
<td>113/295 (38%)</td>
<td>95/257 (37%)</td>
</tr>
<tr>
<td>Liver &amp;&gt;=5 cm BCM</td>
<td>63/304 (21%)</td>
<td>55/264 (21%)</td>
</tr>
<tr>
<td>Spleen &amp;&gt;=5 cm BCM</td>
<td>52/303 (17%)</td>
<td>44/263 (17%)</td>
</tr>
<tr>
<td>Median WBC/mm³ (range)</td>
<td>27 × 10⁹</td>
<td>28 × 10⁹</td>
</tr>
<tr>
<td>(0.460-0.528 × 10⁹)</td>
<td>(0.800-0.528 × 10⁹)</td>
<td></td>
</tr>
<tr>
<td>Median hemoglobin (g/dL)</td>
<td>8.2</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Abbreviations: CNS, central nervous system; BCM, below costal margin.

* CNS involvement = >10/mm³ leukemic blasts in the cerebrospinal fluid.

gen expression if more than 20% of the cells expressed fluorescence intensity greater than 98% of the normal control cells. Positivity for TdT was arbitrarily defined as greater than 5% of cells exhibiting nuclear fluorescence as compared with negative control samples.

Cytogenetics

BM samples were centrally karyotyped in Giessen by J. Harbott and F. Lampert. They were incubated in RPMI 1640 plus 20% fetal calf serum (10⁶ cells/mL) for a 24- and 48-hour culture. The cell suspension was brought to hypotonic solution (KCl, 15 minutes) and fixed in methanol-acetic acid (3:1). After being washed several times, the cells were dropped on a cold wet slide to spread the metaphases. G-banding was performed after trypsin pretreatment (10 to 15 seconds) after air drying, 3 to 5 days later. Karyotyping followed the criteria of the Third International Workshop on Leukemia 198056 and the International Standard for Human Cytogenetic Nomenclature (ISCN) 1985 and 1991.57,58

Routinely, a total of 20 banded metaphases were karyotyped. Cultures with less than five cells assessable for karyotyping were excluded. Karyotyping was successful in 75% (147/196) of the samples obtained.

Statistical Analysis

Relationships of marker reactivity to cytogenetic results, other patient characteristics, and response to treatment (response rate) were determined by Fisher’s exact test or the chi-square test.

Variables for assessing outcome were the response rate (rate of patients achieving CR), the event-free survival (EFS) and the event-free interval (EFI). EFS was calculated from the time of diagnosis to the first event (relapse or death of any cause) or censoring (date of last follow-up), whereas EFI was defined as the time from CR until the first event for those patients achieving CR. Survival curves, standard errors, and tests for differences in EFS between subgroups were calculated by standard methods.59,60 Cox regression was used for multivariate analysis. All tests were descriptive and explorative. Calculations were performed with Statistical Analysis System (SAS), version 6.03 (SAS Institute, Cary, NC).

Follow-up data were updated on August 1, 1994.

Definitions

The sensitivity, specificity, and predictive value61 of the presence or absence of the most characteristic morphologic subtype-associated antigen combinations or abnormal karyotypes were determined to define the potential role of immunophenotyping and karyotyping in facilitating morphologic subtype analysis. For example, if an AML patient is or is not found to have a combination of antigens known to be associated with a particular morphologic subtype, FAB Mx, the informational value of the extent of contribution to the subtype diagnosis Mx is given by the following ratios:

Sensitivity is the likelihood of correct association (definitely positive) to a particular morphologic subtype: (number of FAB Mx patients with the MoAb combination [specific karyotype])/(all FAB Mx patients tested for MoAb combination with aberrations)].

Specificity is the likelihood of precise elimination (definitely negative) of other morphologic subgroups: (number of non-FAB Mx patients without the MoAb combination [specific karyotype])/(all non-FAB Mx patients tested for MoAb combination with aberrations)].

Predictive value is the probability of finding an FAB-type-associated MoAb combination (or chromosome aberration): (number of FAB Mx patients with the MoAb combination [specific karyotype])/(all AML patients with the MoAb combination [specific karyotype]).

Patient Characteristics

Table 1 gives an overview on the initial clinical data and distribution of FAB subtypes of the 307 patients in the AML-BFM-87 study and of the 267 patients with immunophenotypic data.

RESULTS

Overall Results

Table 2 presents results of the AML-BFM-87 study for the total group and for patients with immunologic data. CR was achieved by 230 of 307 (75%) and 199 of 267 (75%) patients respectively. The Kaplan-Meier estimation of the EFS and EFI was in the same range for both patients with immunologic data and the total group.

Table 2. Results of Study AML-BFM-87

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Total</th>
<th>Immunologic Data Available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early deaths*</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>Partial/nonresponse</td>
<td>49</td>
<td>41</td>
</tr>
<tr>
<td>CR achieved</td>
<td>230 (75%)</td>
<td>199 (75%)</td>
</tr>
<tr>
<td>Allogeneic/autologous BM transplantation in first CR</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Relapses</td>
<td>92</td>
<td>76</td>
</tr>
<tr>
<td>Median follow-up (mos)</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>EFS of 5 years</td>
<td>.43 ± .03</td>
<td>.43 ± .03</td>
</tr>
<tr>
<td>EFI of 5 years</td>
<td>.54 ± .05</td>
<td>.57 ± .04</td>
</tr>
</tbody>
</table>

* Includes 4 deaths before therapy.
Table 3. Correlation of Surface Antigen Expression With FAB Subtypes

<table>
<thead>
<tr>
<th>Antigen</th>
<th>FAB Subtype (N)</th>
<th>Progenitor-Associated</th>
<th>Panmyeloid</th>
<th>Monocyte/Granulocyte-Associated</th>
<th>Lineage-Specific</th>
<th>Lymphoid-Associated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. HLA-DR*</td>
<td>No. CD34*</td>
<td>No. CD13*</td>
<td>No. CD33*</td>
<td>No. CDw65*</td>
<td>No. CD14*</td>
</tr>
<tr>
<td>M0 (15)</td>
<td>7 (54%*)</td>
<td>7 (64%)</td>
<td>6 (46%)</td>
<td>8 (62%*)</td>
<td>7 (58%)</td>
<td>0 (0%*)</td>
</tr>
<tr>
<td>M1 (10)</td>
<td>5 (63%)</td>
<td>6 (75%)</td>
<td>6 (60%)</td>
<td>9 (90%)</td>
<td>6 (60%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>M1 Auer (14)</td>
<td>12 (100%)</td>
<td>10 (81%*)</td>
<td>10 (83%)</td>
<td>9 (76%)</td>
<td>6 (62%)</td>
<td>6 (62%)</td>
</tr>
<tr>
<td>M2 (21)</td>
<td>8 (38%*)</td>
<td>5 (31%)</td>
<td>14 (67%)</td>
<td>20 (95%)</td>
<td>17 (89%)</td>
<td>6 (32%)</td>
</tr>
<tr>
<td>M2 Auer (51)</td>
<td>48 (96%*)</td>
<td>30 (64%*)</td>
<td>47 (94%)</td>
<td>40 (82%)</td>
<td>26 (53%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>M3 (14)</td>
<td>0 (0%*)</td>
<td>1 (8%*)</td>
<td>11 (79%)</td>
<td>10 (77%)</td>
<td>0 (0%*)</td>
<td>3 (23%*)</td>
</tr>
<tr>
<td>M4 (26)</td>
<td>22 (92%)</td>
<td>7 (32%)</td>
<td>18 (75%)</td>
<td>22 (92%)</td>
<td>19 (88%)</td>
<td>6 (27%)</td>
</tr>
<tr>
<td>M4Eo (35)</td>
<td>31 (89%)</td>
<td>21 (68%*)</td>
<td>32 (94%*)</td>
<td>31 (91%)</td>
<td>24 (71%*)</td>
<td>20 (59%)</td>
</tr>
<tr>
<td>M5 (58)</td>
<td>54 (90%*)</td>
<td>10 (21%*)</td>
<td>13 (25%*)</td>
<td>49 (91%)</td>
<td>50 (99%*)</td>
<td>22 (42%*)</td>
</tr>
<tr>
<td>M6 (7)</td>
<td>5 (83%)</td>
<td>1 (20%)</td>
<td>4 (60%)</td>
<td>4 (67%)</td>
<td>4 (67%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>M7 (19)</td>
<td>9 (60%)</td>
<td>5 (32%)</td>
<td>4 (31%*)</td>
<td>8 (62%*)</td>
<td>3 (23%*)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total Positive</td>
<td>201 (80%)</td>
<td>103 (45%)</td>
<td>159 (64%)</td>
<td>222 (88%)</td>
<td>196 (81%)</td>
<td>63 (27%)</td>
</tr>
<tr>
<td>Total No. of Patients Tested</td>
<td>251</td>
<td>229</td>
<td>248</td>
<td>252</td>
<td>242</td>
<td>234</td>
</tr>
</tbody>
</table>

Results of immunophenotyping are given as number and percentage of positive cases. > or < indicates percent of positive samples higher or lower than in other patients.
* Fisher's test, P = .05.
† One patient with basophilic leukemia excluded.
Immunophenotype

The expression of 13 cell surface antigens and TdT for the group of 267 assessable children is presented in Table 3. The total number of patients varied for each marker tested, mainly because of insufficient cell numbers.

Leukemic blasts from 233 of 237 (98%) of the patient samples available expressed one or more of the panmyeloid markers tested (ie, CD13, CD33, CDw65). Expression of at least two of these antigens on AML blasts was found in 210 of 237 patients (89%), whereas 27 patients (11%) disclosed only one (N = 23) or none (N = 4) of these markers with 14 of them being classified as FAB M0 or M7.

The other myeloid-associated antigens (ie, CD14, CD15, CD41, glycophorin A) were detected less frequently and in varying proportions depending on the maturational stage and FAB-subtype of myeloid leukemic cells.

Evidence for coexpression of at least one of the T- or B-lymphoid-associated antigens was found in 42% (111 of 267) of the patients and confirmed by two-color IF studies or marked numerical overlap of percentage of cells expressing the respective antigens. Coexpression of T-lymphoid features (CD2, CD4, CD7) occurred much more frequently than coexpression of the B-lymphoid-associated antigen CD19 (2%) and CD10 (1%, data not shown).

Among the non-lineage-restricted markers, HLA-DR was the most commonly expressed antigen (80%), whereas CD34 and TdT were expressed with intermediate or low frequency (45% and 21%, respectively).

Correlation of Surface Antigen Expression With FAB Subtypes and Cytogenetics

The frequency of surface-marker and TdT expression in relationship to FAB classification is also shown in Table 3.

We did not find the expression of any individual myeloid- or lymphoid-associated surface antigen to clearly correlate with a particular FAB subtype, except for CD41 with FAB M7 (predictive value, 71%).

However, the expression of several of these antigens was not distributed equally, and characteristic associations could be identified between surface antigen profiles and morphologic subtypes (Table 3) as well as chromosomal abnormalities (Table 4).

In FAB M0, negativity of one or two of the three panmyeloid antigens tested, especially CD13 or CDw65, was frequently observed (9 of 11 patients), and expression of CD34, TdT as well as T-lymphoid features was not uncommon. Similar immunophenotypic features were found in patients with random abnormalities, eg, significantly more frequent negativity of at least two of the three panmyeloid antigens than in other karyotypic subgroups (11/23 v 3/71, P = .0001).

FAB M1 (with or without Auer rods) followed a similar pattern of antigen expression as M0 with a relatively high incidence of CD7 coexpression as well as CD34 and TdT positivity. In FAB M2 with Auer rods and in t(8;21) the progenitor-associated antigens HLA-DR and CD34 were expressed by blast cells in most patients, and TdT activity was found in about one third of them.

Consistent with previous findings, FAB M3 was characterized by the absence of HLA-DR (specificity, 85%), only occasional expression of CD15, usually positivity of the panmyeloid markers, and a relatively high frequency of CD2 coexpression. FAB M4E0 and inv(16) resembled M2 with Auer rods in its antigenic profile, but showed a stronger expression of CD13 and monocyte-associated features such as CD14 and CD4. Coexpression of CD2 was observed in 23% and 40% of patients with M4E0 and inv(16), respectively. In contrast with FAB M0, M1 and M4 subtypes, surface expression of CD13 and CD34 was detected in only 21% to 25% of patients with FAB M5 and in less than 14% with t(9;11), whereas expression of HLA-DR, CD33, and CDw65 was found in the vast majority of these patients.

CD15 and CD4 were also commonly expressed in FAB M5, whereas 42% of all M5 patients and only 29% with t(9;11) were positive for CD14. In FAB M7, CD41 was found to be expressed in five patients, and CD42b or CD61 in 10 others.

Table 5 shows the predictive value as well as the sensitivity and specificity of the most characteristic immunophenotypic features within different morphologic subtypes.

Correlation of Other Features With FAB Subtypes

MPO positivity (>3%) was associated with the FAB types M1, M2, M3, and M4 and was only rarely found in other subtypes (eg, 26% in FAB M5). Blasts with the karyotypes t(8;21), t(15;17), and inv(16) were always MPO+, whereas the rate of positive samples was significantly lower in patients with t(9;11) (29% positive, P = .03) and in those with random abnormalities (57% positive, P = .001) than in other patients.

The information value of a specific aberration for a morphologic subtype diagnosis is presented in Table 5. The predictive value of t(15;17) for FAB M3 is 1.00, meaning that all children with this aberration were classified as M3. A high predictive value (>75%) was also exhibited by the aberrations t(8;21) for M2 with Auer rods, inv(16) for M4E0, and t(9;11) for M5. The aberration t(8;21) was only exceptionally seen in M2 patients without Auer rods (1/10 patients).

Relationship Between Immunophenotype and Treatment Outcome

The prognostic significance of the expression of surface membrane antigens and TdT, as to the CR rate and EFS, is summarized in Table 6.

Neither the surface antigens tested nor TdT reactivity was associated with a higher or lower CR rate except for CDw65+ AMLs. The analysis of EFS curves likewise failed to show any prognostic significance for the different myeloid-, progenitor cell-, and lymphoid-associated antigens investigated. Patients with leukemic blasts disclosing expression of only one or none of the three panmyeloid antigens analyzed had a somewhat lower CR rate (17/27 patients or 63% v 164/210 or 78%, P = .08), but did not differ significantly in EFS from patients whose leukemic cells expressed more than two of the panmyeloid markers (EFS .37 ± .09 SE v .44 ± .04 SE, P log rank = .23).
Table 4. Correlation of Surface Antigen Expression With Cytogenetic Findings

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Progenitor-Associated</th>
<th>Pannmyeloid</th>
<th>Monocyte/Granulocyte-Associated</th>
<th>Lineage-Specific</th>
<th>Lymphoid-Associated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. HLA-DR*</td>
<td>No. CD34*</td>
<td>No. CD13*</td>
<td>No. CD33*</td>
<td>No. CDw65*</td>
</tr>
<tr>
<td>ND (137)</td>
<td>105 (81%)</td>
<td>44 (40%)</td>
<td>82 (65%)</td>
<td>115 (91%)</td>
<td>97 (82%)</td>
</tr>
<tr>
<td>Normal (29)</td>
<td>22 (79%)</td>
<td>6 (26%)</td>
<td>20 (74%)</td>
<td>26 (93%)</td>
<td>25 (93%)</td>
</tr>
<tr>
<td>Random (47)</td>
<td>28 (12%)</td>
<td>27 (68%)</td>
<td>24 (63%)</td>
<td>30 (75%)</td>
<td>24 (62%)</td>
</tr>
<tr>
<td>+81 (19)</td>
<td>11 (79%)</td>
<td>4 (31%)</td>
<td>11 (85%)</td>
<td>12 (86%)</td>
<td>10 (77%)</td>
</tr>
<tr>
<td>1;8;21;i (25)</td>
<td>18 (89%)</td>
<td>15 (68%)</td>
<td>16 (80%)</td>
<td>18 (80%)</td>
<td>19 (60%)</td>
</tr>
<tr>
<td>t(15;17)i (15)</td>
<td>0 (0%)</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>inv(16)i (12)</td>
<td>11 (92%)</td>
<td>11 (92%)</td>
<td>11 (100%)</td>
<td>10 (93%)</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>t(9;11)i (17)</td>
<td>14 (93%)</td>
<td>1 (8%)</td>
<td>2 (14%)</td>
<td>12 (87%)</td>
<td>12 (92%)</td>
</tr>
<tr>
<td>11q23;i (8)</td>
<td>4 (57%)</td>
<td>2 (29%)</td>
<td>3 (43%)</td>
<td>6 (88%)</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>−7/−q31 (6)</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>3 (100%)</td>
<td>2 (67%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Total positive</td>
<td>201 (80%)</td>
<td>104 (48%)</td>
<td>160 (65%)</td>
<td>223 (88%)</td>
<td>197 (81%)</td>
</tr>
</tbody>
</table>

The total number of patients for each marker tested varies, mainly because of insufficient cell numbers. In addition, immunophenotypic results were not available for all patients with adequate cytogenetic findings. Results of immunophenotyping are given as number and percentage of positive cases. > or < indicates percent of positive samples higher or lower than in other patients.

Abbreviations: ND, no cytogenetic data available.
* Fisher's test, P < .05.
† Indicates a single abnormality as given or associated with other abnormalities.
Coexpression of any of the T- or B-lymphoid-associated antigens did not predict a difference in either the CR rate or the EFS, irrespective of whether the presence of a single marker was evaluated (Table 6), or whether the patients with leukemic blasts expressing at least one of the lymphoid-associated antigens were compared with those disclosing no reactivity with lymphoid markers (Fig 2).

An examination of the possible prognostic relevance of the combination of two or three antigens for the CR rate yielded no further information, but the small patient numbers in most of these subgroups have to be taken into consideration.

Influence of Karyotypes

The estimated probability of a 5-year EFS was significantly different in children with the specific karyotypes t(8;21), t(15;17), and inv(16) as compared with other patients with a known karyotype (EFS = 0.57 ± 0.08 SE v.32 ± 0.05 SE, P log rank = .01) (Fig 3). This was mainly due to differences in initial response rates as the corresponding values after achieving remission were similar (EFS = 0.60 ± 0.08 SE v.47 ± 0.06 SE, P log rank = .34).

Multivariate Analysis

A separate Cox multivariate analysis of EFS was performed for each single surface antigen tested. As additional covariables, the following patient characteristics found to be predictive for an unfavorable outcome in the AML-BFM-83 and AML-BFM-87 studies were included: white blood cell count greater than 100,000/mm³, age less than 2 years, FAB subtypes other than M1/M2 with Auer rods, M3, M4Eo, and, in addition, the therapy response on day 15 with greater than 5% residual blasts in the BM. Except for the absence of CD14, showing a trend towards a shorter EFS (P = .06), no additional influence of surface markers on EFS was detected.

**DISCUSSION**

In the present study including a large number of uniformly treated children with AML, we prospectively investigated the diagnostic sensitivity of a comprehensive panel of MoAbs reactive with myeloid- and lymphoid-associated, as well as non-lineage-restricted antigens and examined potential correlations between the morphologic features, the immunophenotype, the karyotype, and the response to standardized chemotherapy. Although none of the anti-myeloid MoAbs applied in this study recognized the blast cells of all AML patients, it was possible to confirm the diagnostic value of the pannmyeloid reagents CD13, CD33, and CDw65 for the recognition of the vast majority of childhood AML. Even though individual immunophenotypic features with exclusive distribution in FAB subtypes could not be identified except for CD41 and CD61 expression in acute megakaryoblastic leukemia, we were able to describe characteristic as-
associations between certain FAB subtypes, surface antigen expression, and chromosomal abnormalities, mainly those associated with a favorable prognosis, thus underlining the clinical importance of a detailed pretherapeutic characterization of myeloid leukemic cells. In contrast with recent studies in adult AML, no prognostic significance could be attached to the expression of any of the myeloid- or lymphoid-associated or non-lineage-restricted antigens.

**Contribution of Immunophenotype to Diagnosis**

The diagnostic value of the panmyeloid reagents CD13, CD33, and CDw65 for the recognition of childhood AML was high with a sensitivity of 98% (positivity of at least one of these antigens). As in the Pediatric Oncology Group (POG) study, the MoAbs CD13 and CD33 detected most (94%) of the children with AML (POG study, 88%), whereas the sensitivity of CD33 and CDw65 was even higher (97%).

The proportion of children with AML expressing one or more of the lymphoid-associated (predominantly T-cell-associated) antigens was high (42%), and in the same range as recently reported in other pediatric studies.

In view of previous data clearly showing the expression of CD4 on pluripotent hematopoietic stem cells as well as on cells of the monocytic lineage and CD7 during early stages of normal myeloid differentiation, these antigens cannot be regarded as T-lineage-specific markers. Recent results from our group and others showing T-cell receptor and/or gene rearrangements in only a small subset of AML patients with coexpression of T-cell-associated antigens are in line with this statement. In accordance with other studies, CD4 expression was found predominantly in FAB subtypes with monocytic differentiation (M4 or M5), but was also detected in other FAB subtypes; CD7 expression, often combined with TdT positivity, was significantly associated with the immature subtypes M0 and M1.

CD2 AML, suggested in previous studies to represent a distinct biologic and clinical entity, was observed in 11% of our AML cases with a higher incidence in M3 and M4E0 subtypes, whereas expression of CD10 or the pan-B-lineage-associated antigen CD19 was rarely found.

**Immunophenotypic Features in Corresponding Cytogenetic and Morphologic Subgroups**

Mitelman and Heim have calculated diagnostic values for chromosomal aberrations among FAB subtypes in more than 3,000 AML patients. The highest predictive values (>80%) were found for M3 if t(15;17), for M2 if t(8;21), for M4 if inv(16), and for M5 if t(9;11) was present.

By including morphologic features such as Auer rods in the M2 subtype and abnormal eosinophils in the M4 subtype, surface antigen expression, and cytogenetic findings, we were able to calculate the sensitivity, specificity, and predictive value of a given chromosomal abnormality or immunophenotype for the diagnosis of a morphologic subtype. These analyses indicated that the predictive value of immunophenotypic features for morphologic subtypes was low as compared with specific chromosomal aberrations. On the other hand, our data indicate that some immunophenotyping profiles, as discussed below, may be helpful for the recognition of clinically relevant entities such as M2(t(8;21), M3(t(15;17), M4E0/inv(16), and M5(t(11q), especially in cases with equivocal morphology and missing cytogenetic/molecular genetic results.

In accordance with the results of Hurwitz et al., we also found a high frequency of CD34 expression in 23 children with t(8;21)-associated AML, whereas T-cell-associated antigens, especially CD7, but also CD2 and CD4, were rarely positive. In contrast with their results and recent studies in adult patients suggesting frequent coexpression of CD19 in AML with cytogenetically proven t(8;21), we observed a clearcut CD19 positivity in only 1 patient. This discrepancy may be caused by the usually weak and variable expression
of CD19 on AML cells requiring special gating strategies to separate blasts from whole mononuclear cell fractions in flow-cytometric analysis and by the use of different CD19 MoAbs (e.g., Leu-12 and/or B4 in the studies by Hurwitz et al18 and Kita et al19 versus HD37 in our analysis). However, other technical factors (see review by Drexler et al26) including the application of different staining techniques and the use of cryopreserved rather than fresh leukemic cells have to be taken into consideration.18,34,50

The previously described characteristic immunophenotypic features of acute promyelocytic leukemia (APL)23,31,70,71 such as low expression or absence of HLA-DR, CD14, CD15 (as detected with MoAbs VIM-D5 or VIM-C6), and CD34 could be confirmed by our results in 14 children with APL defined by the t(15;17) aberration and/or cytomorphology. Interestingly, our results as well as a recent study including 35 patients with cytogenetically and/or molecularly proven t(15;17)71 disclosed only a marginal correlation between CD2 positivity and APL and, therefore, contrast with other reports suggesting a significant association between coexpression of CD2 and M3 subtype.21-23 In accordance with the data reported by Paietta et al,27 leukemic blasts of children with AML in our study were characterized by a high CD33 expression and absent or weak CD7 reactivity, thus expanding the immunophenotypic features predictive for the diagnosis of APL.

The diagnostic value of multiparameter flow-cytometric analysis for the detection of small subpopulations with aberrant immunophenotypic features (e.g., CD2 coexpression) has recently been shown in AML.44 M4Eo with inv(16) has recently been shown in AML M4Eo with inv(16).24 Our results disclosed coexpression of CD2 in 4 of 10 children with M4Eo and inv(16) and in 7 of 31 with M4Eo as defined by cytomorphology. Routine immunologic marker analyses in most multicentre trials,35-40,42-44,50,72 including the AML-BFM-87 study, were performed by applying indirect IF assays and using cut-off levels of 20% to 30% positive blast cells to define antigen positivity of a given leukemic cell population. It is obvious that this procedure may have underestimated the incidence of aberrant phenotypic features in AML when only a small subpopulation of leukemic blasts express the respective surface antigens such as CD2 in M4Eo with inv(16) or CD19 in M2 with t(8;21).18,19,21,24 Other characteristic immunologic features of M4Eo, including consistent expression of CD13, frequent positivity of CD34, and absence of CD7, were also observed in this series of childhood AML patients.

Previous studies in patients with acute monocytic leukemia and t(9;11) failed to show a characteristic immunophenotypic pattern on leukemic blasts.14,73 Therefore, it is noteworthy that most of the 16 children with t(9;11) in this study disclosed similar immunophenotypic features with strong expression of HLA-DR, CD33, CDw65, and CD4, whereas CD13, CD14, and CD34 were positive on less than 30% of cases.

The biologic significance of the observed associations between cytogenetic abnormalities and immunophenotypic features is still unclear. It has been postulated that transcriptional mechanisms acting in certain AML subgroups may control the expression of correlated surface markers.21

**Prognostic Implications**

Results have been controversial concerning the prognostic implications of surface antigen expression in AML (see introduction). Several recent studies, especially in childhood ALL,27,23 have shown that the prognostic impact not only of immunophenotypic subgroups but also of chromosomal abnormalities is diminished by improved efficacy of chemotherapy, thus indicating that prognostic factors must be evaluated in the context of therapy delivered.

Our results, like those reported by the Children’s Cancer Study Group24 and the Pediatric Oncology Group,25 do not indicate that the expression of myeloid-, lymphoid-, and progenitor-cell-associated antigens can be applied for risk stratification in childhood AML at diagnosis.

The significantly better CR rate for CDw65+ patients, observed in this study as well as in adult AML patients with expression of CDw65,27,47 did not translate into longer remission duration or EFS. None of the other myeloid-lineage-associated antigens investigated in this study had a significant influence on either the CR rate or the EFS in univariate and multivariate analysis.

Several studies reported on a poor response to induction chemotherapy in adult patients with CD34+ AML.25,26,43,45,46 Noteworthy, however, is the fact that these studies often included patients with AML after exposure to radiation and/or cytotoxic therapy and AML evolving from myelodysplastic syndrome,25,26 both of which are associated with a poor prognosis under the usual therapy. Moreover, a higher incidence of CD34 expression was associated with aberrations of chromosome 5 and/or 7 in adult AML,25,36,45 thus casting doubt on the independent prognostic value of CD34 expression.

As in other recent studies dealing exclusively with pediatric patients,34,35 we were unable to show any significant influence of CD34 expression on treatment outcome in childhood AML. This is in line with other recent reports questioning the adverse prognostic impact of CD34.27,36,39,52,74
our study, CD34 expression was associated with FAB M1/M2 with Auer rods and M4Eo, whereas lower expression rates were found in FAB M3 and M5, suggesting that CD34+ AML comprises a heterogeneous group that is associated not only with poor but also with good prognostic factors.

The biologic and clinical impact of lymphoid coexpression on AML cells has been the subject of considerable interest and controversy in recent years. Expression of CD7, often associated with immature morphologic subtypes, has been regarded as an adverse prognostic factor in several recent studies. However, no correlation between the expression of CD7 or other lymphoid-associated antigens investigated (CD2, CD4, CD19) and the prognosis could be shown in our patient population.

In both the previous AML-BFM-87 and the present BFM-87 trial, we could define a standard- and a high-risk group based mainly on morphologic criteria (Fig 4). The criteria for standard risk—M1/M2 with Auer rods, M3, M4Eo—are closely related to specific prognostically favorable karyotype abnormalities (Table 5) as could be confirmed in our study (Fig 3). Differences in prognosis between the morphologically or cytogentically defined standard and high-risk groups were mainly caused by induction failure and less by relapse rate. However, in patients achieving CR, mode and intensity of therapy (eg, cranial irradiation) became important.

Future studies based on an integrated classification considering information from morphologic, immunophenotypic, cytogenetic, and molecular characterization of leukemic blasts will probably contribute to a better recognition of biologically and clinically relevant entities as well as to individualization of treatment strategies in AML.

ACKNOWLEDGMENT

We thank Barbara Komischke and Mathilde Martin for excellent technical assistance and Christa Lausch for assistance in the preparation of the manuscript.

Immunophenotyping of 16 patients was performed in the German laboratories of St. Burdach Universitäts-Kinderklinik, Düsseldorf; H. Kabisch, Universitäts-Kinderklinik, Hamburg; St. Thierfelder, Universitäts-Klinik, München; D. Stachel, v. Haunersches Kinderspital, München; W. Kaboth/C. Nerl, Technische Universität, München-Schwanberg.

The central laboratory for morphology was provided by P. Stappert, E. Kurzkaebe, Universitäts-Kinderklinik, Münster, Germany.

Principal investigators of study AML-BFM-87 in Germany: R. Mertens, Kinderklinik RWTH, Aachen; A. Gnekow, I. Kinderklinik Ludwigshafen; H. Kabisch, Kinderklinik der Freien Universität, Berlin; U. Bode, Universitäts-Kinderklinik, Bonn; J. Kühn, Universitäts-Kinderklinik, Universität, Miinster; H. Kabisch, Universitäts-Kinderklinik, Miinchen; W. Kaboth/C. Nerl, Technische Universität, München-Schwanberg.

The coordinators of study AML-BFM-87 were G. Schellong, J. Ritter, and U. Creutzig, Universitäts-Kinderklinik, Münster.

REFERENCES


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SURFACE ANTIGEN EXPRESSION IN CHILDHOOD AML


CD34$^+$ and CD14$^-$/DR$^-$ phenotypes with shorter survival. Leukemia 6:393, 1992


Clinical significance of surface antigen expression in children with acute myeloid leukemia: results of study AML-BFM-87

U Creutzig, J Harbott, C Sperling, J Ritter, M Zimmermann, H Loffler, H Riehm, G Schellong and WD Ludwig