Clinical value of the quantitative expression of the three epitopes of CD34 in 300 cases of acute myeloid leukemia

MARC MAYNADIE,1 LUC GERLAND,1 SERGE AHO,2 FRANÇOIS GIRODON,1 MICHEL BERNIER,3 CORINNE BRUNET,4 LYDIA CAMPOS,3 SYLVIE DALIPHARD,4 VERONIQUE DENEYS,7 ANNE FALKENRODT,3 MARIE-CHRISTINE JACOB,9 EMILIENNE KÜHLEIN,10 GENEVIÈVE LECALVEZ,11 ANNIE FALKENRODT,8 MARIE-CHRISTINE JACOB,9 LYDIA CAMPOS,5 SYLVIE DALIPHARD,6 VÉRONIQUE DENEYS,7 FRANÇOIS GIRODON,1 MICHEL BERNIER,3 CORINNE BRUNET,4 MARC MAYNADIÉ,1 LUC GERLAND,1 SERGE AHO,2

Background and Objectives. The various epitopes of the CD34 molecule have been classified according to their different sensitivities to enzymatic cleavage by neuraminidase, chymopapain and a glycoprotease from Pasteurella haemolytica. Although monoclonal antibodies have been developed that specifically identify these epitopes, few studies have evaluated the distribution and quantitative expression of such epitopes on leukemic blasts.

Design and Methods. We report here a prospective multicenter study in which we examined and quantified the expression of the 3 classes of CD34 on fresh leukemic blast cells from 300 cases of acute myeloid leukemia (AML). The binding of monoclonal antibodies was studied by flow cytometry, allowing evaluation of blast cell positivity as well as their mean fluorescence intensity. These quantitative data were made comparable between centers by means of a calibration curve established with the same reagents in all laboratories.

Results. Quantitative expression of class I epitope was significantly higher than that of class II and class III epitopes (p<0.0001). The three classes were more frequently expressed in M0 and M1 and less in M3 and M5. The highest levels of CD34 expression were observed in M2, M0 and M1 and the lowest in M3, M5 and BAL for class II and III. CD34 expression was lower for all classes in cases with a normal karyotype, compared to in cases with structural or numerical abnormalities.

Interpretation and Conclusions. In cases with a t(9;22) the expression of class I was significantly higher than that of class II and III and the opposite was observed in AML with t(15;17). Moreover, as a whole, a high intensity of class III CD34 appeared to be a marker of good prognosis.

© 2002, Ferrata Storti Foundation

Key words: CD34, quantification, prognosis, acute myeloid leukemia

Acute Leukemias

research paper
haematologica 2002; 87:795-803

©2002, Ferrata Storti Foundation

Correspondence: Dr. Marc Maynadie, MD, Service d’Hématologie biologique, CHU de Dijon, 2 Boulevard Maréchal de Lattre de Tassigny, 21034 Dijon Cedex, France. Phone: international +33 380293314. Fax: international +33 380293660. E-mail: mmaynadie@chu-dijon.fr

The CD34 molecule is a transmembrane glycoprotein, expressed on hematopoietic progenitor cells as well as on vascular endothelium, high endothelial venules and some fibroblasts.1-4 The molecule has been characterized as a mucin-like sialoglycoprotein containing nine N-linked and a large number of O-linked glycosylation sites.5 Monoclonal antibodies (MoAbs) recognize different epitopes on the CD34 molecule and a classification has been proposed based on these epitopes' differential sensitivities to enzymatic cleavage.6,7 This classification was validated and adopted during the Leukocyte Typing V workshop.8 Class I epitopes are sensitive to cleavage by neuraminidase, chymopapain and a glycoprotease from Pasteurella haemolytica; class II epitopes are sensitive to enzymatic cleavage by chymopapain and the glycoprotease but resistant to neuraminidase; class III epitopes resist cleavage by all three enzymes.

Few studies have been conducted in order to evaluate the distribution of these different epitopes on cell types expressing CD34. Class II and III, but not class I epitopes have been reported to be expressed on high endothelial venules.6,8 On normal hematopoietic progenitor cells, differences have been reported between immature and more mature progenitors. For some authors, expression of class II and class III epitopes appeared to be correlated.9-11 Conversely, others found class III epitope to be more broadly expressed than the two others.12,13 The expression of CD34 epitopes by leukemic cells from patients suffering from acute myeloid leukemia (AML), acute lymphoblastic leukemia or chronic myeloid leukemia, has been reported in 6 studies with probable overlap in the cases examined.10,12,14-17 The results showed some differences between expression of the three class-
es but none of these studies assessed the prognostic value of the various types of expression. Only very few studies have examined the quantitative expression of CD34, and then usually with only a single monoclonal antibody, on leukemic blasts.

Furthermore, these studies show discrepancies in the method used to evaluate CD34 expression, in terms of antibody binding capacity (ABC) or of molecules of equivalent soluble fluorochrome (MESF). From these rare studies, no definitive conclusion can be drawn on the interest of studying the 3 classes of CD34 epitopes on leukemic cells.

Here we report the results of a prospective study in which we examined and quantified the expression of the 3 classes of CD34 on fresh leukemic blast cells from 300 cases of AML diagnosed in laboratories of the Groupe d’Etude Immunologique des Leucémies (GEIL) network. The aim of this study was: i) to determine the interest of concomitantly testing the 3 classes of CD34 at diagnosis, (i.e. determine whether the use of only one reagent could lead to an erroneous immunophenotype indicating the absence of CD34); and ii) to evaluate the possible prognostic value of quantitative measurement of expression of CD34 epitopes.

Design and Methods

Patients

Between 1996 and 1999 AML cases diagnosed in 13 centers from the Groupe d’Etude Immunologique des Leucémies (GEIL) were studied. There were 300 cases, diagnosed by standard morphology and cytochemistry of peripheral blood and bone marrow smears according to the French-American-British (FAB) classification and immunophenotyped using a comprehensive panel of MoAbs against myeloid and lymphoid-associated antigens as proposed by the EGIL group (Table 1). Of these 300 patients, 286 were adults and 14 children. The median age of the adult male patients was 59±19 years [range: 16 to 96] and that of the adult female patients 57±20 years [range: 15 to 88]. The children were 10 boys and 4 girls, with a median age of, respectively, 7±6 and 14±3 years old. Fifty cases were secondary AML. Karyotype was performed in 145 cases and successful in 133. Information about the therapeutic protocol and outcome was available for 245 patients. The clinical data registered were the date of diagnosis, date of remission, remission duration and date of death if occurred. The follow-up was stopped on May 1st, 2001.

Immunophenotype

Bone marrow fresh blast cells were immunophenotyped according to the GEIL’s recommendations. Membrane and intracytoplasmic (c) labelings were performed in each center using direct or indirect immunofluorescence procedures. The panel of MoAbs used included CD19, CD10, CD20, CD21, CD22, CD23, CD24, cCD79, cytoplasmic and surface immunoglobulin for the B-lineage; CD2, cytoplasmic and surface CD3, CD4, CD5, CD7, CD8 for the T-lineage; and cytoplasmic and surface CD13, CD33, CD14, CD15, CD35, CD36, CD65, CD41, CD42, CD117, glycoporphin A for the myeloid lineage. The expression of HLA-DR, CD90, TdT, CD71, CD11a, CD11b, CD11c and CD38 was also often tested. Most of these antibodies were purchased by each laboratory from Beckman-Coulter (Hialeah, FL, USA), Becton Dickinson (Mountain View, CA, USA), Dako (Glostrup, Denmark) or An Der Grub (Vienna, Austria), but some were aliquoted and distributed within the group following global purchase. The presence of surface immunoglobulins was tested using fluorescein isothiocyanate (FITC)-conjugated goat F(ab’)_2 anti-human total Ig or conjugated antiserum directed to Ig light chains. Cytoplasmic µ-chains (cIg), cCD3, cCD79 and cCD13 were investigated either on fixed cytospin smears or by flow cytometric analysis after cell membrane permeabilization. A threshold of 20% or 10% fluorescent positive blasts was considered, depending on the type of marker used.

CD34 expression

MoAbs directed against the three different classes of CD34 epitopes were purchased by the group,
Quantification of CD34 epitopes in AML

Aliquots were distributed to each center to be studied by flow cytometry. They were Immu133 (Beckman-Coulter) directed against the class I CD34 epitope, QBEND-10 (Beckman-Coulter) directed against class II and HPCA-2 (Becton Dickinson) directed against class III. As FITC-conjugated Immu133 was not available it was used as a purified MoAb and an FITC-conjugated anti-mouse Ig was used as second step reagent. This second-step reagent was also distributed to the group and used in similar conditions in all participating laboratories. Class II and class III MoAbs were both FITC-conjugates. In each case, the percentage of positive cells and the mean fluorescence of the positive peak were recorded.

Quantification

A mixture of levels II, III and IV calibrated fluorescent polystyrene beads (Immunobrite®, Beckman-Coulter) was distributed to each center. The dye included in the polystyrene emits between 525 and 700 nm when excited at 488 nm. These beads are calibrated against a solution of laser grade fluorescent dye and titrated as molecules of equivalent soluble fluorochrome (MESF) per microbead. Their titers are respectively 31,500, 115,000 and 460,000 MESF. A control bead (Standardbrite®, Beckman-Coulter) was also distributed to each laboratory. A specific flow cytometry protocol was established in each participating center, allowing the three peaks of fluorescence of the Immunobrite mixture to be visualized on the same histogram. For each patient tested, this protocol was used to run the mixture of beads, followed by the control bead and subsequently the blasts stained with each of the CD34 MoAbs. The mean fluorescence channel values of the 3 calibrated beads were plotted to establish a calibration curve (Figure 1). The mean

Figure 1. The mixture of levels II, III and IV of calibrated beads was run in the flow cytometer (A). The mean fluorescence channel of each bead was recorded and plotted to establish a calibration curve (B).
fluorescence channel values of the control bead and of the 3 classes of CD34 stained cells were used to calculate and compare absolute fluorescence intensity.

Statistical analysis

In order to compare expression of the three classes of CD34 epitopes, a Friedman two-way ANOVA was performed. The Kendall coefficient was calculated to assess agreement. The duration of disease-free survival (DFS) was defined as the time between achieving a complete remission and the date of first relapse, death or last follow-up (May 1st, 2001). Survival was defined as the time between the date of diagnosis and the date of death or last follow-up. Probabilities of survival were estimated by the Kaplan-Meier method. Univariate and multivariate analyses were performed using the Cox model. Expression of the different markers was first considered as a continuous variable and then the optimized cutoff point method was used to delineate groups of expression. Analyses were performed using STATA software (Stata corporation, TX, USA).

Results

Immunophenotype

Among the markers studied in the AML immunophenotype, CD13, CD33 and CD117 were the myeloid markers most often expressed (97%, 95% and 80%, respectively). CD35, CD36, CD15 and CD65 were positive in 30 to 40% of the cases. The megakaryocytic lineage-associated markers CD41 and CD42 were positive in M7 and in M6 cases.

Table 3. Expression of the three classes of CD34 in MESF and in percentage of fluorescent blast cells (mean; min-max) in each AML subtype.

<table>
<thead>
<tr>
<th>AML subtype</th>
<th>MESF</th>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>29,126</td>
<td>21,903</td>
<td>22,601</td>
<td>&lt; 10^-4</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>%</td>
<td>41±37</td>
<td>40±37</td>
<td>39±37</td>
<td></td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>M0</td>
<td>39,764</td>
<td>16,387</td>
<td>17,528</td>
<td>&lt;10^-4</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>%</td>
<td>71±30</td>
<td>72±30</td>
<td>71±29</td>
<td></td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>M1</td>
<td>25,029</td>
<td>18,787</td>
<td>17,528</td>
<td>&lt;10^-4</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>%</td>
<td>47±36</td>
<td>56±37</td>
<td>57±38</td>
<td></td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>M2</td>
<td>31,990</td>
<td>19,518</td>
<td>21,744</td>
<td>&lt;10^-4</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>%</td>
<td>41±24</td>
<td>45±25</td>
<td>45±26</td>
<td></td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>M3</td>
<td>11,607</td>
<td>18,787</td>
<td>17,528</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>48±29</td>
<td>52±29</td>
<td>56±29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>25,495</td>
<td>15,970</td>
<td>17,408</td>
<td>&lt;10^-4</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>%</td>
<td>43±29</td>
<td>41±24</td>
<td>43±31</td>
<td></td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>M5</td>
<td>14,551</td>
<td>9,399</td>
<td>8,462</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>10±29</td>
<td>9±27</td>
<td>9±29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>26,259</td>
<td>10,910</td>
<td>12,259</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>27±27</td>
<td>26±26</td>
<td>25±25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B A L</td>
<td>27,219</td>
<td>9,010</td>
<td>11,208</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>42±34</td>
<td>56±27</td>
<td>47±21</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Others</td>
<td>39,651</td>
<td>41,569</td>
<td>39,689</td>
<td>&lt;10^-4</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>33±38</td>
<td>27±25</td>
<td>21±22</td>
<td></td>
<td>&lt;10^-4</td>
</tr>
</tbody>
</table>
Among T-lineage markers, CD2, CD7 and CD4 were the most often observed (21%, 26% and 28%, respectively). Among B-lineage markers, CD19 and CD24 were expressed in 10% and 7% of the cases, respectively, while cCD79 expression was noted in the 8 BAL and in 2 AML of unknown subtype. The BAL cases were characterized by co-expression of myeloid and B-lineage markers in 8 cases, and none by that of myeloid and T-lineage markers according to the EGIL's score i.e. a score of more than two points in more than one lineage.

CD34 expression

The class I, class II and class III epitopes of CD34 were studied in, respectively, 269 cases (90%), 285 cases (95%) and 296 cases (99%). The mean percentages of fluorescent cells were, respectively: 41±37%, 40±37% and 39±37% (p<10−4) (Figure 2). The Kendall factor was 0.96. When a 20% positive cut-off was used, class I was positive in 153 cases (57%), class II in 164 cases (57.5%) and class III in 165 cases (56%). The 3 epitopes were co-expressed in 130 cases and all absent in 104 cases. Varying patterns were observed in 26 cases (Table 2). According to FAB subtypes, the three classes of CD34 were more frequently expressed in M0, and M1 subtypes and less in M3 and M5 subtypes (Table 3).

Quantification

The mean quantification value obtained with the control bead was 113,475±7,190 MESF which yields an interlaboratory CV of 6.3% (Figure 3). Overall, expression of the class I epitope was statistically significantly higher (29,126±23,409 MESF) than that of class II (21,903±19,168 MESF) and class III epitopes (22,601±17,433 MESF) (p<10−4) (Figure 4). No significant difference was noted between class II and class III expression. The Kendall correlation factor was 0.87 between the 3 classes in terms of MESF. No significant difference was observed between primary and secondary cases regarding class I and II epitopes expression, but the expression of class III CD34 was significantly higher in secondary cases (24,630±19,720 MESF) than in primary cases (18,747±15,850 MESF) (p=0.002). Analysis by type of AML (Table 3) showed that the mean MESF was higher for the class I epitope for all types of AML and for the BAL but not for the M3 subtype in which the class III epitope was more strongly expressed. The highest CD34 expressions were found in M2, M0 and M1 subtypes for class II and class III epitopes while for class I they were observed in M2 and M0 subtypes and BAL. The lowest expressions were observed in M3, M5 and BAL for class II and III and in M3, M5 and M1 subtypes for class I. In cases with a normal karyotype, CD34 expression was lower for the three classes of epitopes than in cases with structural or numerical abnormalities (Table 4). Class I epitope expression was the highest in all types of abnormalities. It was the highest in cases with a t(8;21) but the difference did not reach statistical significance while in cases with a t(9;22), expression of the class I epitope was significantly higher than that of class II and III epitopes (p=0.04). The opposite was observed in AML with t(15;17) (p=0.02) in which the class I epitope was expressed at a significantly lower level. Structural abnormalities

Table 2. Number of cases in which the expression of the three classes of CD34 was concordant or discordant. A 20% cutoff of fluorescent cells was used.

<table>
<thead>
<tr>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>130</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>7</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>104</td>
</tr>
</tbody>
</table>

Table 4. MESF values of the three classes of CD34 (mean, min-max) in cases of AML according to the karyotype status.

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>n</th>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>50</td>
<td>15,245</td>
<td>8,928</td>
<td>9,903</td>
<td>&lt;10−4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,117-58,545</td>
<td>115-76,998</td>
<td>735-50,723</td>
<td></td>
</tr>
<tr>
<td>Structural abnormalities</td>
<td>45</td>
<td>62,456</td>
<td>19,994</td>
<td>20,621</td>
<td>&lt;10−4</td>
</tr>
<tr>
<td>t(9;21)</td>
<td>4</td>
<td>21,942</td>
<td>19,228</td>
<td>19,540</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,541-65,015</td>
<td>3,226-63,335</td>
<td>1,122-63,684</td>
<td>0.04</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>6</td>
<td>78,705</td>
<td>38,483</td>
<td>36,128</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18,669-84,422</td>
<td>6,982-78,797</td>
<td>13,508-58,294</td>
<td></td>
</tr>
<tr>
<td>t(15;17)</td>
<td>8</td>
<td>14,717</td>
<td>16,399</td>
<td>17,601</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5,695-32,714</td>
<td>2,563-46,424</td>
<td>3,658-54,930</td>
<td>0.02</td>
</tr>
<tr>
<td>del 5q</td>
<td>6</td>
<td>40,167</td>
<td>25,599</td>
<td>19,984</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,718-150,652</td>
<td>816-26,886</td>
<td>7,338-45,674</td>
<td>0.05</td>
</tr>
<tr>
<td>Hyperdiploidy</td>
<td>18</td>
<td>27,432</td>
<td>14,166</td>
<td>13,887</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,894-13,411</td>
<td>1,894-60,185</td>
<td>1,894-35,318</td>
<td></td>
</tr>
<tr>
<td>Hypodiploidy</td>
<td>15</td>
<td>72,538</td>
<td>10,340</td>
<td>12,770</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>832-7,6530</td>
<td>852-47,775</td>
<td>39,45,068</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

Among T-lineage markers, CD2, CD7 and CD4 were the most often observed (21%, 26% and 28%, respectively). Among B-lineage markers, CD19 and CD24 were expressed in 10% and 7% of the cases, respectively, while cCD79 expression was noted in the 8 BAL and in 2 AML of unknown subtype. The BAL cases were characterized by co-expression of myeloid and B-lineage markers in 8 cases, and none by that of myeloid and T-lineage markers according to the EGIL’s score i.e. a score of more than two points in more than one lineage.
were pooled because of the low number of each of them and CD34 expression of the three epitopes was higher in cases with structural rather than numerical abnormalities (p<10⁻⁴).

Prognostic factors

The median follow-up was 12 months (range 0.5 to 92 months). The median disease-free survival (DFS), calculated on 136 treated cases, was 7 months and the 3-year DFS was 8.7±4.1%. In univariate analysis, significant correlations were noted between the age of the patient (p<10⁻⁴), class III CD34 expression intensity (p=0.05) and DFS. By multivariate analysis, only age (p<10⁻⁴) remained correlated. Overall survival (OS) was calculated on 162 cases. The median OS was 13 months and the 3-year OS was 13±3.1%. In univariate and multivariate analyses, significant positive correlations were noted between age (p<10⁻⁴), class III CD34 expression intensity (p=0.04) and OS (Figure 5). Such correlations remained significant (p=0.02) when OS was calculated on 143 treated patients with a median OS of 21 months.

Discussion

This study reports the frequency and intensity of expression of the three classes of CD34 on blast cells from AML patients. Significant correlations were observed between these parameters and the FAB type of the cells as well as several karyotypic anomalies. Moreover, class III epitope expression should be considered as a prognostic marker in terms of antigen density, a better prognosis appearing to be associated with high expression.

CD34 has so far been the most commonly used marker for the identification of hematopoietic stem and progenitor cells.²,⁶,²⁶ The expression of this sialomucin is high on the earliest hematopoietic cells and decreases to undetectable levels by the stage when maturing progenitor cells lose the capacity to form colonies in vitro.²⁷ CD34 has also been reported to be expressed on blast cells of roughly 40% of cases of AML, with large differences in published series (30% to 60% of the cases).²⁸,²⁹

These differences could be due to the fact that MoAbs to different epitopes of CD34 were used to define expression in the various series of patients. Surprisingly, few studies have been published on comparison of the expression of these 3 epitopes in AML.

In the series of 300 cases reported here, the expression of the three classes of CD34 epitopes, in percentage of labeled blasts, was similar, being close to 40% of fluorescent cells as a mean. There was a good correlation between the three epitopes,
yet nearly 10% of the cases showed highly discrepant expressions, with blast cells positive for only 1 or 2 of the epitopes. In our series the three classes of CD34 were more frequently expressed on AML with an immature immunophenotype than on more mature subtypes. In a previous study of 20 AML cases, Steen et al. reported that class I and II epitopes were less often expressed than class III on more mature AML (M 3/M 4-M 5), 12 In 19 AML cases, Egeland and Gaudernak reported that class II was less frequently expressed than classes I and III. 15 We do not confirm these results as we did not observe significant differences between the three classes of epitopes between AML subtypes in terms of expression frequency.

Another way to analyze the expression of a molecule is to quantify it. Among different available systems, we chose to use the Immunobrite calibration standard beads, which yield highly reproducible quantimetric assessments at a multicenter scale as demonstrated in a previous study conducted within the GEl group, and allow calibration of fluorescence intensities between flow cytometers. 30 For both studies the coefficient of variation of the control bead was lower than 7%. Furthermore, this reagent is very stable over time which is very important for a large and long multicenter study (13 centers from 1996 to 1999), and does not require any particular manipulation. As the beads have been calibrated against a solution of laser grade fluorescent dye, they were titrated in MESF units and allowed a precise quantification of antigen expression with these arbitrary units.

As for the proportions of labeled cells, we observed a good correlation between the quantitative expression of the three classes of CD34. A bias could have been introduced by the fact that our class I MoAb was not available as a conjugate, although care was taken to work in excess conditions. However, class I was not always the CD34 epitope expressed with the highest fluorescence (i.e. AML-M 3) confirming that data in AML were comparable between the three epitopes. In two different studies published previously, quantification of unconjugated MoAb against the three classes of CD34 was performed using the QIFIkit system and class I levels were found to be lower than class II and class III levels. 11, 31 These studies were performed on 13 normal bone marrow samples 31 and on peripheral blood or cytapheresis products 31 indicating that on normal cells class I was less expressed than class II and III epitopes. Our results suggest that the situation appears to be different in AML. Moreover, Lanza et al., 31 using a different quantimetry technique, reported even higher levels of CD34 class I expression on AML than those we observed here. Serke et al. 31 have compared quantification systems of CD34 expression. Similar levels were reported for class II and class III antibodies using either indirect immunofluorescence quantified by the QIFIkit system or direct immunofluorescence quantified by the Quantibrite system. Our results using the Immunobrite system are also close to those reported by these authors.

In a study of 66 cases of AML, those with class I epitope bright expression had 265,000 mean MESF and cases with dim expression had 49,000 mean MESF. 19 In another series reported by the same authors, class I epitope mean MESF was 115,000, 17 which is much higher than in our series. The difference could be explained by the fact that different calibration systems were used. No results were given in the study by Lanza et al. 37 about the subtype of the AML studied and more comparisons are not possible. Expression of the class II epitope, quantified in two studies using the Qbend-10 antibody, was 347,000 mean MESF in the first one 37 and the highest value observed in the second one was 400,000 ABC. 18 However these studies were performed using phycoerythrin (PE)-conjugated antibodies in both studies, impairing any comparison. Expression of class III epitope in terms of MESF does not appear to have been reported in the literature. Serke et al. reported a higher expression of the 8G12-PE class III antibody, calculated in fluorescence ratio, than of class I and II antibodies. 34 According to the karyotype status, we confirm that CD34 intensity was higher in AML cases with t(8;21) than in cases with other abnormalities as already mentioned by Porwit-MacDonald et al. using the class II Qbend10 antibody. 18 This was true for the three classes in our series. We also observed significant differences in the quantitative expression of CD34 epitopes according to FAB subtypes. The highest intensities were, surprisingly, found in the M 2 subtype for the three classes. M 0 and M 1 subtypes, which are more immature AML, yielded low intensities of CD34 expression, especially for class I. The lowest intensities were observed for AML at more mature stages of differentiation such as M 3 or M 5 subtypes. These data are in accordance with the previously described frequency of CD34 expression. 32, 33 The prognostic value of CD34 expression has been studied in a large number of series, and found to be either of good or bad prognosis. 32, 33 Such differences could be due to the fact that CD34 expression was appreciated in terms of expression fre-
quency and that in most of these studies only one monoclonal antibody was used.24 Despite the heterogeneity of our series, which included treated (n=213) and untreated (n=32) cases, the quantified expression of CD34 appears to provide different information as we found that high class III expression was a good prognostic factor as reflected by DFS and OS. This result remains valid when one considers only the homogeneous series of treated patients.

This study demonstrates an overall good correlation of the expression of CD34 three epitopes in about 90% of AML cases. Quantimetric analysis indicated that class I epitope is usually but not always expressed in greater amounts in AML. However, the quantimetric expression of CD34 class III epitope appears to be a new prognostic factor in this disease, and suggests that this class of CD34 should be preferentially used when only one is tested.

Contributions and Acknowledgments

MM carried out overall analyses and wrote the paper. LG recorded all data and made some descriptive analyses of the data. SA was responsible for the statistical analysis. GC and MCB provided advice about the manuscript and gave final approval. All others did the immunological flow cytometry study of the cases.

We thank physicians, cytologists, cytogenetic medical teams and technicians of each center participating in this study.

Funding

This work was supported by ARC (grant n° 6636) and by the GOELAM S group.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

References

21. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA,
Quantification of CD34 epitopes in AML


Hill C. Should comparison be made between survival of responding vs. non-responding patients? Bull Cancer 1993; 80:294-8.


Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Alberto Orfao, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Orfao and the Editors. Manuscript received December 27, 2001; accepted June 5, 2002.

What is already known on this topic

This study shows an overall good correlation between the expression of the three CD34 epitopes in AML blast cells. Despite this, quantitative differences in the levels of the CD34 expression were noted for the class I versus class II and III antibodies among t(9;22)+ and t(15;17) AML cases.

What this study adds

High intensity expression of class III CD34 appears to be a marker of good prognosis in acute myeloid leukaemia.

Potential implications for clinical practice

In clinical practice, careful selection of the anti-CD34 monoclonal antibody reagents for the phenotypic analysis of AML blasts has to be made, class III antibodies being preferred if only one reagent is to be used.

Alberto Orfao, Associate Editor