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P-glycoprotein expression and prognostic value in acute myeloid leukemia

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Abstract

Background and Objective. **Drug resistance has become a major cause of treatment failure in patients with acute leukemia. P-glycoprotein (Pgp), which is associated with the multidrug resistance (MDR) phenotype, has been reported to be an important predictor of treatment outcome. The aim of this study was to analyze the value of Pgp expression in bone marrow or peripheral blood as a predictor of the response to remission induction chemotherapy as well as the duration of remission in patients with *de novo* acute myeloid leukemia (AML).**

Design and Methods. **We examined the expression of Pgp in 82 patients with *de novo* AML using an immunocytochemical assay with the C219 monoclonal antibody.**

Results. **Twenty-seven of the 82 patients (33%) were C219-positive in from 1% to 100% of their cells. Thirteen cases (16%) showed a positive reaction in more than 50% of the leukemic cells. Only hyperleukocytosis was significantly associated with higher expression of Pgp. Although 8 of the 13 cases (62%) with more than 50% of cells having Pgp expression were CD34-positive, this association was not statistically significant. A univariate analysis of resistance to induction therapy showed a significantly higher resistance rate in patients with increased Pgp expression (P=0.01) as well as in those patients with decreased reactivity to myeloperoxidase. The multivariate analysis revealed the independent prognostic value of Pgp expression. C219 reactivity did not have an influence on remission duration.**

Interpretation and Conclusions. **Our data indicate that P-glycoprotein expression is a reliable marker of resistance to induction treatment in patients with *de novo* AML.**

Key words: *de novo* AML, induction failure, P-glycoprotein, multidrug resistance, MDR1

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Although complete remission (CR) rates of 50% to 80% are currently obtained in adults with *de novo* acute myeloid leukemia (AML), prognosis is still poor since only 20% of patients eventually reach long-term disease-free survival.[1] At present, advances in supportive care have translated into a dramatic decrease in early deaths from hemorrhagic and infectious complications that accounted for the vast majority of induction failures in the past. As a consequence of that, drug resistance has become a major cause of treatment failure in patients with acute leukemia. During the last two decades, a large number of studies have been designed to investigate the underlying mechanisms involved in the resistance of neoplastic cells to chemotherapy. The so-called pleiotropic or multidrug resistance (MDR) includes the resistance to many natural products used in the treatment of acute leukemias such as anthracyclines, vincristine, amsacrine, mitoxantrone and etoposide. This mechanism is mediated by an overexpression of a 170 kd transmembranous glycoprotein called P-glycoprotein (P-170), which acts as an ATP-dependent efflux pump ejecting drugs out of the cell.[2,3] Synthesis of P-glycoprotein is encoded for by the MDR1 gene. Several attempts have been made to relate the expression of the gene product to chemotherapy response in terms of remission rate and remission duration in patients with AML.[4-12] However, due to the great variability in the expression of both the P-glycoprotein and the mRNA of MDR1, the clinical relevance of the MDR phenotype has not been definitely established in AML.

In this study, we analyze the value of P-glycoprotein expression in predicting the response to remission induction chemotherapy and the duration of remission in a series of 82 patients with *de novo* AML.

Materials and Methods

Patients

We analyzed P-glycoprotein expression in a cohort of 82 patients with newly diagnosed acute non-lymphoblastic leukemia recruited from April 1990 to December 1994. The mean age of the patients was 49±22 years (range, 7-98). There were 47 men and 35 women. According to the French-American-British

(FAB) classification, 80 cases were diagnosed as AML and 2 as acute undifferentiated leukemia (AUL). Immunophenotyping was performed by direct immunofluorescence and immunocytochemistry techniques. The main clinical and biological characteristics of the patient population are listed in Table 1.

Treatment

All patients were given remission induction and consolidation chemotherapy with daunorubicin (45 mg/m^2/d, days 1 to 3), Ara-C (100 mg/m^2/12h, days 1 to 7), and etoposide (100 mg/m^2/d, days 1 to 3). Patients under 60 years of age received as intensification an additional course of Ara-C (1 g/m^2/12h, days 1-4) plus mitoxantrone (10-12 mg/m^2/d, days 5-7) followed by an autologous peripheral blood stem cell transplantation. Patients over 60 years of age received a single course of mitoxantrone (10 mg/m^2/d, days 1 to 3) plus Ara-C (150 mg/m^2/d, days 1 to 7).

Expression of P-glycoprotein

For the study of P-glycoprotein, we used an immunocytochemistry method based on the amplified streptavidin-biotin system and the C219 monoclonal antibody (MoAb) (Centocor, Malvern, PA, USA), a mouse MoAb (IgG2) that recognizes an epitope localized in the inner cell membrane. Briefly, this technique was performed as follows: peripheral blood and bone marrow samples previously frozen at –20°C were defrosted at room temperature and combined with pure acetone for 10 minutes. Afterwards, samples were dried and incubated for 30 minutes with the primary MoAb (C219 10 µg/mL) in a dilution 1:10 in TRIS saline buffer (TSB). After washing with TSB, samples were incubated for 20 min. with a complex of biotinylated anti-mouse rabbit antibody diluted 1:5 in TSB. They were washed again in TSB, and reacted with the streptavidin-alkaline phosphatase complex and sodic acid at 0.1% for 20 minutes. After a new washing with TSB, the chromogenic substrate was added and samples were incubated for 20 minutes at room temperature. The preparation was finally washed with distilled water and counterstained with hematoxylin for 30 seconds. In the negative control the MoAb was replaced by regular mouse ascitic fluid. As the positive control, we used peripheral blood or bone marrow samples of patients who expressed C219-positivity in 100% of the analyzed cells. Samples with more than 1% positive blasts were considered as positive.

Statistical analysis

Correlations were calculated using the chi-square test. A P value of 0.05 or less was considered statistically significant. CR was defined according to the Cancer and Leukemia Group B (CALGB) criteria.[13] Remission duration was measured from the date of CR until relapse. Remission duration curves were calculated by the actuarial method of Kaplan and Meier and compared by the log-rank test. In order to determine the prognostic value of the reactivity to MoAb C219 in the induction response, we excluded patients who died before their response could be evaluated (failure types 3, 4 and 5 according to Preisler).[14] All calculations were performed using the DM, 4F, 1L and 2L programs from the Biomedical Data Package statistical library (BMDP Statistical Software, Los Angeles, CA, USA). Variables that were significant in the univariate analysis were also used in a multivariate Cox regression analysis in order to identify the most significant independent prognostic factors. The following covariates were analyzed: age, sex, clinical features (infection, hemorrhage, splenomegaly, hepatomegaly, lymph node enlargement, neurologic involvement, skin lesions, enlarged gums), peripheral blood parameters (hemoglobin, leukocyte count, absolute neutrophil count, platelets, blast cells), biochemical serum values, bone marrow characteristics (cellularity, percent of blasts, Auer rods, myeloperoxidase or Sudan black B, ANAE, NASDA, CAE, PAS), FAB classification and immunologic markers.

Table 1. Patient characteristics.

	N (%)		C219≤50%		C219>50%		p
Patients	82	(100)	69	(84)	13	(16)	
Age (years)							
<15	6	(7)	5	(7)	1	(8)	
15-60	45	(66)	39	(57)	6	(46)	NS
>60	31	(38)	25	(36)	6	(46)	
Sex							
Male	47	(57)	38	(55)	9	(69)	
Female	35	(43)	31	(45)	4	(31)	NS
WBC (x10^9/L)							
≤ 30	58	(71)	52	(75)	6	(46)	
> 30	24	(29)	17	(25)	7	(54)	0.03
FAB Subtype							
M0	3	(4)	3	(5)	0		
M1	22	(27)	19	(28)	3	(23)	
M2	19	(23)	16	(23)	3	(23)	
M3	9	(11)	7	(10)	2	(15)	NS
M4	13	(16)	12	(18)	1	(8)	
M5	5	(6)	5	q(5)	0		
M6	7	(9)	5	(7)	2	(15)	
M7	2	(2)	1	(2)	1	(8)	
AUL	2	(2)	1	(2)	1	(8)	
CD34 (%)							
≤ 20	31	(42)	26	(43)	5	(38)	
>20	42	(58)	34	(57)	8	(62)	NS

NS: not significant.

Results

Twenty-seven of the 82 patients with AML (33%) were C219-positive. In these cases, the percentage of positive cells ranged from 1% to 100%. When differ-

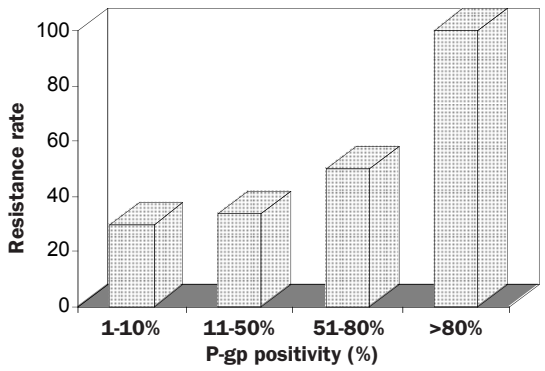

Figure 1. Resistance rate according to C219 positivity.

ent thresholds for C219-positive cells were considered we observed a direct relationship between the proportion of C219 positive blast cells and the resistance rate to the remission induction treatment (Figure 1). The threshold of 50% established a clear cut-off with prognostic relevance and we chose this value to study the impact of P-glycoprotein expression and other prognostic factors. In our study, only 13 cases (16%) showed a positive reaction to the MoAb C219 in more than 50% of the leukemic cells.

Relationship between P-glycoprotein expression and clinical and biological characteristics

Hyperleukocytosis was significantly associated to a higher expression of P-glycoprotein: a leukocyte count > 30×10^9/L was observed in 7 out of 13 patients (52%) who had more than 50% of C219-positive cells and only in 17 out of 69 patients (25%) with less than 50% C219-positive cells (p=0.03). We did not observe any significant correlation between P-glycoprotein expression and age, sex, general symptoms at diagnosis, enlargement of the liver, spleen or lymph nodes, or biochemical serum values.

None of the morphological parameters analyzed were significantly related to the C219-reactivity. The expression of CD34 was positive in 42 out of 73 patients (58%) in whom it was determined. Although 8 of the 13 cases (62%) with higher expression of P-glycoprotein were CD34 positive, the difference was not statistically significant.

Analysis of resistance to induction therapy

Of 82 patients in whom the C219 reactivity was determined, 16 did not receive induction treatment due to advanced age (12 cases over 70 years of age) and 10 patients died from complications of induction chemotherapy. Of the 56 remaining patients in which the response to induction was analyzed, 35 (53%) achieved CR and 21 (47%) had resistant disease (Table 2).

Table 2. Results of induction remission treatment.

	N	*(%)*
Patients treated	66	
Complete remission	35	(53)
Failure	31	(47)
Type		
1 (absolute resistance)	9	
2 (relative resistance)	12	
3	1	
4	5	
5	4	

Table 3. Results of induction treatment according to P-glycoprotein expression.

C219 expression (%)	*Resistance/total (%)*	*p*	*Median CR duration (months)*	*p*
≤ 10	12/40 (30)		16	
11-50	2/6 (33)	0.01	8	NS
>50	7/10 (70)		4	

NS: not significant.

A univariate analysis showed a significantly higher resistance rate in patients with spleen enlargement, as well as in those with absence of Auer rods or decreased reactivity to myeloperoxidase (MPO) or Sudan black B (SBB) stain. Increased reactivity to MoAb C219 was also significantly associated with a higher resistance rate (Table 3). Interestingly, none of the four patients with over 80% of positive cells reached CR (p=0.01). The multivariate analysis of resistance to induction treatment revealed the independent prognostic value of MPO/SBB and MoAb C219 reactivity. A lower proportion of both MPO/SBB positive cells and an increased number of cells positive to MoAb C219 were predictive of a poor prognosis.

Analysis of the remission duration

Of the 35 patients who achieved CR, 17 relapsed and one died while in remission. The actuarial probability of remaining in remission after 12 months was 50%. A leukocyte count greater than 30×10^9/L and lower MPO/SBB reactivity were significantly associated with a shorter remission duration. C219 reactivity had no influence on remission duration (Table 3).

Discussion

In our series, the frequency of P-glycoprotein expression was 33%. Other authors have found a higher incidence,[15-17] but results are difficult to compare due to differences in the characteristics analyzed and the sensitivity and specificity of the various methods used.

The cut-off of positivity at which a patient is considered to have a MDR phenotype is not fully established. According to previous reports, we considered patients to be positive when they had more than 5%,[11,16,17] 20%[4,10,15] or 30% of reactive cells.[7] However, these cut-off levels are arbitrary and did not contribute to meaningful prognostic information. In our series, a clinically significant positivity, that is, the proportion of positive cells related to the response to chemotherapy, was observed with a cut-off of 50%. Thus, this was chosen as the best cut-point in the analysis of the relationship between P-glycoprotein expression and the different prognostic factors.

In agreement with other authors, we did not find any correlation between C219 positivity and age or sex.[4,7,9,17,18] On the other hand, AML with hyperleukocytosis showed a higher expression of P-glycoprotein, a finding also noted by others.[10,16] The remaining hematologic and biochemical characteristics were not clearly associated with P-glycoprotein expression. Other authors[9,10,19,20] have demonstrated increased P-glycoprotein expression or mRNA in the MDR1 gene in the most undifferentiated leukemias (M0, M1, M5) as well as decreased expression in the FAB subtypes with better prognosis (M3, M4E).[4,21-22] In our series we could not demonstrate a relationship between FAB subtype and reactivity to MoAb C219. An unexpected finding was the absence of reactivity to MoAb C219 in all AML-M0 and AML-M5 studied. However, we observed decreased P-glycoprotein expression in those patients with AML-M4, where only 1 of 13 patients showed a C219 positivity in more than 50% of cells.

The correlation between the MDR phenotype and the expression of the CD34 antigen is controversial. Some authors have reported a significant association between the expression of the MDR1 gene and the presence of the CD34 antigen,[1,4,7,22-24] findings not confirmed by others.[9,15,17] In our series, P-glycoprotein expression and CD34 positivity were not significantly associated.

Concerning MDR expression being prognostic of the occurrence of resistance to chemotherapy, we found, as expected, a direct relationship between P-glycoprotein expression and the resistance rates. In the multivariate analysis, positivity to C219 and the MPO reaction had an independent prognostic value and the regression model predicted resistance accurately. In our study, as in that by Campos *et al.*,[4] none of the patients with more than 80% positivity of the blast cells reached CR. In other words, the predictive value of MDR expression has been demonstrated,[4,6,7,10,12,15-19,26] even though only a few authors make the independent prognostic value clear after a multivariate analysis.[4,10,23,24,28] However, despite the important prognostic role of the level of the P-glycoprotein expression in patients with AML, other mechanisms may overcome the resistance to chemotherapy allowing patients with significant positivity to MoAb C219 to attain remission.

In this, as in other series,[4] the increase of positivity to MoAb C219 was associated with a shorter remission duration. However, differences were not statistically significant, probably because of the small number of P-glycoprotein positive patients that reached CR.

In conclusion, detection of MDR mediated by the P-glycoprotein is a reliable marker of resistance to induction treatment in patients with *de novo* AML. Therefore, we consider that determination of P-glycoprotein should be included in the initial work-up of these patients. P-glycoprotein expression should be taken into account when designing therapeutic programs with MDR modulators or chemotherapeutic regimens with agents not affected by this mechanism in order to establish its effectiveness in future clinical trials.

Contributions and Acknowledgments

LS was responsible for the study design, interpretation and writing of the paper. IJ and GM reviewed the study and contributed to data analysis and paper writing, AS was responsible for the immunophenotyping, YG-G was involved in the immunocytochemical studies, FG and MPS were responsible for the morphologic diagnosis, JdlR contributed to paper writing, MAS was responsible for the final version of the paper and was the senior author.

The order in which the names of the authors appear is based on their contribution to the study.

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Disclosures

Conflict of interest: none.

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