ORIGINAL PAPERS

Adv Clin Exp Med 2005, **14**, 6, 1151–1160
ISSN 1230-025X

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Clinical Relevance of Multidrug Resistance Proteins Expression in Patients with *de novo* Acute Myeloid Leukaemia*

Kliniczne znaczenie ekspresji białek oporności wielolekowej u chorych na ostre białaczki szpikowe *de novo*

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Abstract

Background. Early occurrence of the multidrug resistance (MDR) in *de novo* acute myeloid leukaemia (AML) may contribute to the treatment failure.
Objectives. Prospective investigatiom of the clinical significance of the MDR proteins overexpression and their functional augmenting, in the context of other AML prognostic factors, such as age, immunophenotype and cytogenetic profile.
Materials and Methods. The authors examined expression of MDR proteins (ABC transporters: MDR1, MRP1, MDR3, BCRP) and others (LRP and GSTπ) and performed MDR functional assay in peripheral blood blasts of 25 patients with *de novo* AML at diagnosis and after the first chemotherapy cycle consisting of a 3 + 7 combination of DNR/Ara-C. Multidrug resistance proteins presence and their functional activity (fluorescent dye efflux) were estimated by flow cytometry.
Results. Thirteen out of the 25 AML patients (52%) attained a complete remission (CR) with induction treatment, one had partial remission (PR) and eleven did not achieve remission. Out of eleven AML patients without CR, two died in aplasia and nine were classified as an early death due to disease progression. All AML patients who achieved remission were younger than 55 years. Among eleven AML patients without remission nine expressed CD34; 6 of them had intermediate and 5 unfavourable cytogenetic profile. In 10 out of 25 AML patients (40%) overexpression of MDR was shown at diagnosis, and was irreversible in 9 of them and their clinical outcome was poor (6 did not achieve CR, 1 had PR and one who obtained CR relapsed), whereas one patient who reversed achieved CR. The functional MDR1 assay showed the decreased Rh123 efflux, both at diagnosis and after the first chemotherapy cycle in 12 AML patients (48%) and it influenced patients outcome in a similar manner as MDR1 expression. At diagnosis other MDR proteins were also elevated in some AML patients: GSTπ in 19 (76%), LRP in 9 (36%), MRP in 4 (16%) and MDR3 in 2 (8%). In 7 AML patients (20%), both at diagnosis and after the first chemotherapy cycle the co-expression of MDR1 with MRP, LRP, GSTπ and Rh123 efflux impaired (3 patients), with LRP, GSTπ and Rh123 efflux impaired (1 patient) or with GSTπ and Rh123 efflux impaired (2 patients) resulted in 6/7 AML patients in chemotherapy resistance, and the remaining one who achieved remission was young and had favourable cytogenetic profile.
Conclusions. In view of the results obtained, the advanced age, unfavourable cytogenetic profile, overexpression of MDR1 at diagnosis and co-expression of other MDR proteins together with their functional activity contribute to the treatment failure in *de novo* AML (**Adv Clin Exp Med 2005, 14, 6, 1151–1160**).

Key words: acute myeloid leukaemia, multidrug resistance proteins, cytogenetic profile, chemotherapy efficacy.

* This work was supported by the State Committee for Scientific Research (KBN) No. 3/PO5B/18 422/2002 and PBZ-KBN-091/P05/2003.

Streszczenie

Wprowadzenie. Wczesne pojawienie się oporności wielolekowej (MDR) u chorych na ostrą białaczkę szpikową (o.b.s.) *de novo* może przyczyniać się do niepowodzenia leczenia.

Cel pracy. Prospektywna ocena klinicznego znaczenia wzrostu poziomu ekspresji białek MDR i ich zwiększonej aktywności w relacji do innych czynników prognostycznych w o.b.s., takich jak: wiek, immunofenotyp i profil cytogenetyczny.

Materiał i metody. Zbadano poziom ekspresji białek MDR (białka transportowe ABC: MDR1, MRP1, MDR3, BCRP) i innych (LRP i GSTπ) oraz wykonano czynnościowy test MDR u 25 chorych na o.b.s. *de novo* w czasie rozpoznania i po pierwszym cyklu chemioterapii (cykl 3 + 7 DNR/Ara-C). Oznaczanie obecności i funkcji białek MDR wykonano metodami cytometrii przepływowej.

Wyniki. U trzynastu z 25 chorych na o.b.s. (52%) nastąpiła całkowita remisja (CR) po leczeniu indukcyjnym, u 1 remisja częściowa (PR), a u 11 chorych nie stwierdzono remisji. Wszyscy chorzy na o.b.s., którzy uzyskali CR mieli mniej niż 55 lat, a CR była stabilna u 3 chorych w wieku poniżej 48 lat. Spośród 11 chorych na o.b.s., którzy nie osiągnęli CR 5 miało niekorzystny, a 6 – pośredni profil cytogenetyczny. U 10 z 25 chorych na o.b.s. (40%) zwiększona ekspresja MDR1 występowała w czasie rozpoznania, była nieodwracalna u 9 z nich i wpływała niekorzystnie na wynik leczenia (6 nie uzyskało CR, 1 miał PR, a u 1, który osiągnął CR, nastąpił nawrót choroby), podczas gdy chory, u którego nie stwierdzono ekspresji MDR1, po chemioterapii uzyskał CR. W teście czynnościowym MDR1 zwiększony wypływ Rh123 obserwowano zarówno w czasie rozpoznania, jak i po pierwszym cyklu chemioterapii u 12 chorych na o.b.s. (48%), co korelowało z wynikami terapii w sposób podobny jak obecność MDR1. W czasie rozpoznania u niektórych chorych stwierdzono również zwiększoną ekspresję innych białek MDR: GSTπ u 19 (76%), LRP u 9 (36%), MRP u 4 (16%) i MDR3 u 2 (8%). U 7 chorych na o.b.s. (28%) w czasie rozpoznania oraz po pierwszym cyklu chemioterapii występowała koekspresja MDR1 z MRP, LRP, GSTπ (3 chorych) i zwiększony wypływ Rh123; z LRP, GSTπ (1 chory) oraz z GSTπ (2 chorych), co skutkowało u 6/7 chorych opornością na chemioterapię, a jeden chory, który uzyskał remisję był młody i miał korzystny profil cytogenetyczny.

Wnioski. W świetle uzyskanych wyników starszy wiek, niekorzystny profil cytogenetyczny oraz zwiększona ekspresja MDR1, w czasie rozpoznania ostrej białaczki szpikowej *de novo* oraz koekspresja kilku (co najmniej dwu) białek oporności wielolekowej są czynnikami związanymi ze zmniejszonym prawdopodobieństwem CR. Znaczenie testu czynnościowego MDR1 dla przewidywania uzyskania całkowitej remisji w o.b.s. wydaje się porównywalne z oznaczaniem poziomu białka MDR1 (**Adv Clin Exp Med 2005, 14, 6, 1151–1160**).

Słowa kluczowe: ostra białaczka szpikowa, białka oporności wielolekowej, cytogenetyka, skuteczność chemioterapii.

Although treatment with cytosine arabinoside (Ara-C) and daunorubicin induces complete remission (CR) in about 65% of adults with *de novo* AML [1–3], many of those who respond to initial treatment relapse within 1–2 years after diagnosis [4]. The main mechanisms implicated in the pathogenesis of chemotherapy refractoriness are: resistance due to the changes in apoptotic pathways [5], and resistance due to the presence of ATP-dependent membrane efflux pumps (ABC transporter proteins) such as P – glycoprotein (MDR1) [6–9], multidrug resistance protein 1 (MRP1) or breast cancer resistance protein (BCRP) [10]. From among other proteins involved in nuclear-cytoplasmatic transport, which are not ABC transporters, the most important is lung resistance related protein (LRP) [11, 12]. However, individual AML patients may have combined overexpression of MDR1 (Pgp), MRP1 and/or LRP [11, 13 14].

The aim of this study was to investigate prospectively the prognostic value of the appearance of MDR transporter proteins: MDR1, MRP, MDR3, and BCRP, as well as LRP and the isoenzyme GST (GSTπ), together with their functional drug transporting activity, in blasts from patients with *de novo* AML and after the first course of chemotherapy. The results were evaluated considering the patients cytogenetic profiles, and other clinical and laboratory parameters in relation to the disease course, to determine the role of multidrug resistance in predicting clinical outcome.

Material and Methods

Twenty-five patients (14 females and 11 males, aged from 20 to 74, median age 47.4 years) with *de novo* acute myeloid leukaemia (AML) were analyzed. All patients were diagnosed and treated in the Department of Haematology Wroclaw Medical University (from 2002–March 2005). Morphological classification was performed on May-Grünwald-Giemsa stained bone marrow smears according to the French-American-British (FAB) criteria [15]. Among 25 AML patients there were following FAB subtypes: 2 MO (8%), 2 M1 (8%), 11 M2 (44%), 8 M4 (32%) and 2 M5a (8%). Chemotherapy consisted of daunorubicin (DNR 45 mg/m^2, *i.v.*, days 1–3) and cytosine arabinoside (Ara-C, 200 mg/m^2, *i.v.*, days 1–7). Complete remission status was defined according to the criteria of the CALGB [15]. The clinical characteristics of patients are listed in Tables 1 and 2.

Diagnosis of AML was made on bone marrow (BM) and peripheral blood smears routinely obtained and evaluated according to the revised French-American-British (FAB) criteria [16], and the diagnosis of AML-M0 was made following the guidelines proposed by the same group [17]. CD34 and other myeloid surface markers (e.g. CD13, CD14, CD33, CD117, HLA-DR) were analyzed by flow cytometry. Cytogenetic analysis of the AML blasts was performed at diagnosis on aspirated BM cells cultured and harvested at standard conditions. At least 15 GTG-banded metaphases were analyzed from each patient following the recommendations of the International System for Human Cytogenetic Nomenclature (ISCN) [18] (tables 1 and 3). Risk groups were defined as follows: I – favourable, low risk: t(8;21) and inv(16); II – neutral, intermediate risk: patients with normal karyotype or without low or high risk-specific abnormalities; III – unfavourable, high risk: –5/del(5q), –7/del(7q), abn 12p, abn 11q, +11, +13, +21, +22, t(6;9), t(9;11), t(3;3), hypodiploid karyotypes or more than three independent aberrations [19].

In 25 AML patients examination was performed twice: at diagnosis and two days after the first course of chemotherapy (3 + 7 combination of DNR/Ara-C).

The expression of multidrug resistance proteins: MDR1, MDR3, MRP1, BCRP LRP and GSTπ was evaluated by flow cytometry in peripheral blood blasts, using specific fluorescent labeled monoclonal antibodies: anti-MDR1, anti-MDR3 (both Chemicon, CA, USA), anti-MRP1 (Sigma, MI, USA), anti-LRP, anti-BCRP (both Transduction Laboratories, CA, USA) and anti-GSTπ (Chemicon, CA, USA). Properly matched, according to the AML subtype, specific monoclonal antibodies; anti-CD13, anti-CD33 or anti-CD34 were used simultaneously, in the same test, to discriminate leukaemic and normal cells. The sample was classified as a positive when the mean geometric canal of fluorescence intensity (FI) was at least 1.5-fold higher (+) than that of the negative (isotypic antibody) control. When the mean channel of fluorescence was between 2–3-fold higher than the control, the sample was classified as (++) and when the fluorescence intensity was more than 3-fold higher than the control – the sample was classified as (+++).

MDR1 and MRP1 transporter ABC proteins function was examined by measuring the efflux of the fluorescent dye Rh 123 (0.5 μg/mL, rhodamine 123, Sigma) by flow cytometry analysis [20], with leukaemic blasts gated with AML cell-specific antibodies. The transporter protein activity was estimated as positive, when Rh 123 efflux resulted in 2-fold (+), 3-fold (++) or more than 3-fold decrease in the mean geometric number of channel of fluorescence-1 (FL-1).

Results

Thirteen out of the 25 AML patients (52%) attained a complete remission (CR) with induction treatment, 1 had partial remission (PR) and 11 did not achieve remission. Out of 11 patients without CR aged from 20 to 74 (median 55.9), 2 died in aplasia and 9 were classified as an early death due to disease progression. Their cytogenetic profile was intermediate in 6 and unfavourable in 5 patients. Time to remission (TTR) lasted from 4 to 11 weeks (mean 6.8 weeks) and disease free time varied from 4 to 40 months (mean 12.3 months).

All AML patients with CR were younger than 55 years and 8 of them, with stable CR were 48 years old or younger (range of age 25 to 48 years, median 38.4). It must be underlined that none of 5 patients older than 60 years reached CR, 3 of them with intermediate (No 4, No 8, No 11) and 2 (no 10, No 19) with unfavourable cytogenetic profile (Table 1). Eight out of 13 AML patients have stable CR, and five relapsed and died. The patients who achieved stable CR were M0 – 1, M1 – 1, M2 – 4, M4 – 2.

Out of the 25 AML patients, 3 (12%) had favourable chromosomal abnormalities, 15 (60%) cytogenetics of intermediate prognosis and 7 (28%) cytogenetics of unfavourable prognosis (tables 1 and 2). Two patients (No 6 and 18) had t(8;21), 1 patient (No 25) had inv (16). In the cytogenetic group of intermediate prognosis, 11 had normal karyotypes, 3 patients had trisomy 8. Two patients had chromosome monosomy 7, one patient – monosomy 5, and 6 – complex cytogenetic abnormalities.

In AML patients without CR, 9/12 expressed CD34 whereas in the group with CR 4/13 expressed CD34.

Results of MDR proteins expression and their functional activity evaluation are summarized in Table 4 and Figure 1. Overexpression of MDR1 was shown at diagnosis in 10 out of 25 AML patients (40%), and in 9 of them MDR1 remained overexpressed after first course of chemotherapy, whereas 1 patient reversed and achieved CR (No 3). Among 9 patients, in whom MDR1 remained overexpressed after first chemotherapy cycle: 6 patients did not achieve remission, and out of the remaining 3, two achieved CR, but one of them relapsed and died (No 17), the second received allo-BMT and had a stable CR (No 25) and the third had PR (No 12). Among 6 MDR1 positive

Table 1. Clinical characteristics, immunophenotype and cytogenetics of 25 patients with *de novo* AML

Tabela 1. Charakterystyka kliniczna, immunofenotyp i cytogenetyka 25 chorych z *de novo* o.b.s.

No (Nr)	Age (Wiek)	Gender (Płeć)	FAB	Cytogenetics (Cytogenetyka)	Immunophenotype (Immunofenotyp)	TTR – weeks (tygodnie)	DFT – months (miesiące)	Therapy outcome (Wynik leczenia)
1	35	F (K)	M5a	46, XX intermediate	CD2, CD14, CD33	8	5	CR, relapse, death
2	20	F (K)	M0	45, XX, t(1;3)(p36;q27), t(1;13)(p32;q14), -7 unfavourable	CD2, CD7, CD33, DR	NR	No	death
3	38	M	M1	46, XY intermediate	CD3, CD7, CD13, DR, CD117	4	21	CR
4	62	M	M4	46, XY intermediate	CD13, CD33, CD34, DR, CD117, cMPO	NR	No	death
5	53	F (K)	M2	46, XX intermediate	CD3, CD13, CD33, CD34, DR, CD117	NR	No	Death
6	48	M	M2	45, X, -Y, t(8;21) favourable	CD33, CD34, CD117, DR	4	4	CR, relapse, death
7	38	F (K)	M4	46, XX intermediate	CD13, CD15, CD33, DR	4	16	CR
8	74	F (K)	M4	46, XX intermediate	CD13, CD15, CD33, CD34, CD117, DR	NR	No	death
9	45	F (K)	M2	46, XX intermediate	CD33, CD34, CD117, DR	4	10	CR
10	69	M	M4	48, XY, +13, i(17q), +19 unfavourable	CD13, CD15, CD33, DR	NR	No	death
11	70	M	M4	47, XY, +8 [2]/46, XY[13] intermediate	CD13, CD15, CD33, CD34, DR	NR	No	death
12	32	M	M2	47, XY, +8 intermediate	CD33, CD34, CD117, DR	8	8	PR
13	42	F (K)	M2	46, XX intermediate	CD13, CD33, CD117, DR	10	14	CR
14	52	M	M5a	46, XY, del(6) (q21q23) [7]/46, XY, idem, del(3)(q21)[1] /46, XY, idem, inv(8)[1]/46, XY[6] unfavourable	CD15, CD33, DR	6	2	CR, relapse, death
15	56	M	M2	46, XY intermediate	CD13, CD34, CD117, DR	NR	No	death
16	44	M	M1	92, XY[10]/46, XY[5] unfavourable	CD34, CD117, DR	NR	No	death
17	51	F (K)	M2	46, XX intermediate	CD13, CD34, CD117, DR	11	5	CR, relapse, death
18	25	F (K)	M2	45, X, -X, t(8;21) [10]/46, XX [10] favourable	CD13, CD33, CD34, CD117, DR	5	40	CR
19	69	F (K)	M4	46, XX, -7, +8, inv(12)(q15q24.1)[72]/46, XX[3] unfavourable	CD13, CD33, CD34, DR	NR	No	death
20	59	M	M2	46, XY intermediate	CD13, CD33, CD34, CD117, DR	NR	No	death
21	48	F (K)	M2	46, XX, del(9)(q22q32)[3]/46, XX [12] intermediate	CD13, CD33, CD34, CD117, DR	7	8	CR
22	46	F (K)	M0	44-45, XX, der(9), t(10;11)(p13;q23), der(16), t(22;9;16), (q11;p11;p11), -16(p11pter), -22 (q11pter) [cp20] unfavourable	CD2, CD7, CD33, DR	7	9	CR
23	39	F (K)	M2	39-45, XX, der(4), -5, del(7)(q22), der(11), -15, -15, der(16;12;?), i(17q), der(18), -19, -20, -22 [cp20] unfavourable	CD13, CD33, CD34, CD117, DR	NR	No	death
24	44	M	M4	46, XY intermediate	CD13, CD33, CD34, CD117, DR	10	6	CR, relapse, death
25	25	F (K)	M4	46, XX, inv(16)(p13q22) favourable	CD13, CD14, CD34, CD117, DR	7	24	CR

TTR – time to remission (weeks), DFT – disease free time (months).

TTR – czas do remisji (tygodnie), DFT – czas wolny od choroby (miesiące).

Table 2. Clinical characteristics of 25 *de novo* AML patients

Tabela 2. Charakterystyka kliniczna 25 chorych z *de novo* o.b.s.

Data (Dane)	Median (Średnia) ± SD	Range (Zakres)
Age – years (Wiek – lata)	47.4 ± 14.6	20–74
Haemoglobin (Hemoglobina) g/dl	9.08 ± 1.39	6.7–13.5
Erythrocytes (Erytrocyty) T/l	2.88 ± 0.54	2.04–4.03
Leucocytes (Leukocyty) G/l	50.46 ± 55.63	1.2–185.0
Peripheral blasts (Odsetek blastów we krwi obwodowej) %	61.4 ± 28.1	10–98
Peripheral blasts (Liczba blastów we krwi obwodowej) G/l	41.03 ± 53.06	1.3–173.9
Platelets (Płytki krwi) G/l	71.2 ± 80.07	4.0–352.0
CD34 expression (Ekspresja CD34)	16/25	
FAB classification (Klasyfikacja FAB)	n	%
M0	2	8
M1	2	8
M2	11	44
M4	8	32
M5a	2	8
Karyotype (Kariotyp)		
Favourable (Korzystny)	3	
Intermediate (Pośredni)	15	
Unfavourable (Niekorzystny)	7	

Table 3. Cytogenetic analysis of 25 patients with *de novo* AML

Tabela 3. Analiza cytogenetyczna 25 chorych z *de novo* o.b.s.

Cytogenetic group (Grupa cytogenetyczna)	Karyotype classification criteria* (Kryteria klasyfikacji kariotypu)*	n	%
Favourable (Korzystny)	t(8;21), inv(16)	3	12
Intermediate (Pośredni)	normal karyotype or with < 3 aberrations other than unfavourable or favourable ones	15	60
Unfavourable (Niekorzystny)	5/del(5q), -7/del (7q), t(6;9), polyploid complex karyotype (with ≥ 3 independent aberrations)	7	28

* According to (ICSN) but taking into account only aberrations present in analyzed patients.

* Zgodnie z (ICSN), lecz biorąc pod uwagę jedynie aberracje obecne u omawianych chorych.

patients without remission, 3 had intermediate (No 4, No 8, No 15) and 3 (No 2, No 19, No 23) unfavourable cytogenetic profile. Patients with intermediate cytogenetic profiles were older (56, 62 and 74 years, respectively) than those with unfavourable profile (20, 39 and 69 years, respectively). Out of three AML patients who were MDR1 negative at diagnosis and became positive after the first chemotherapy cycle one had no remission (No 5) and the remaining two (No 14 and 24) achieved CR, but then relapsed and died.

Twelve out of 25 AML patients were MDR1 negative both at diagnosis and after first cycle of chemotherapy. Eight of them achieved CR, which was stable in 5 cases (No 7, 9, 13, 21 and 22). Four out of 5 MDR1 negative patients with stable CR had intermediate, and only one (No 22) had an unfavourable cytogenetic profile.

MRP was elevated at diagnosis in four AML patients (16%), in one (No 12) it reversed after induction chemotherapy and among the remaining 3 patients who were MRP positive after first chemotherapy cycle, 2 did not obtain remission (No 8, No 23), but 1 got CR (No 25). Their cytogenetic profiles were as follows: intermediate (No 8 and 12), unfavourable (No 23) and favourable (No 25).

The combined overexpression of MDR1 and MRP1 was found in 6 patients (24%); two of them had CR (No 3 and No 25), one had PR (No 12) and three had no remission (No 2, No 8 and No 23).

LRP expression was found at diagnosis in 9 out of 25 AML patients (36%). After first chemotherapy cycle it remained overexpressed in 8 cases and in 1 case reversed (No 3). Out of 8 LRP patients positive both at diagnosis and after first cycle of chemotherapy, 4 had no remission, 1 had a partial remission (No 12) and 3 achieved CR, which remained stable in 2 of them (No 9 and No 25) but one patient (No 14) relapsed after two months.

MDR3 was present at diagnosis, and remained overexpressed after first chemotherapy cycle, in

Table 4. MDR proteins before (A) and after (B) 1st cycle of chemotherapy in 25 AML patients

Tabela 4. Białka MDR przed (A) i po (B) 1. cyklu chemioterapii u 25 chorych na o.b.s.

No (Nr)	Age (Wiek)	Gender (Płeć)	FAB	A/B	ABC transporters (Białka transportowe ABC)				Functional assay** (Test czynnościowy)	MDR proteins (Białka MDR)	
					MDR1*	MRP*	MDR3*	BCRP*		LRP*	GSTπ*
1	35	F (K)	M5	A B	– –	– +	– +	– –	– –	– ++	++ +++
2	20	F (K)	M0	A B	+ +	+ –	– –	+ +	+ +	+ +	+ +
3	38	M	M1	A B	+ –	+ –	++ +	+ –	+ –	++ –	++ –
4	62	M	M4	A B	+ ++	– –	– –	– +	+ +	+ +	+ +
5	53	F (K)	M2	A B	– ++	– ++	– +	– –	– +/–	– ++	– +++
6	48	M	M2	A B	– –	– –	– –	– –	– –	– –	– –
7	38	F (K)	M4	A B	– –	– –	– –	– –	– –	– –	++ +
8	74	F (K)	M4	A B	+ +	+ +	– –	– –	++ ++	+ ++	++ ++
9	45	F (K)	M2	A B	– –	– –	– –	– +	+ +	+ +	+ +
10	69	M	M4	A B	– –	– –	– –	– –	– –	– –	+ –
11	70	M	M4	A B	– –	– –	– –	– –	– –	– +	+ +
12	32	M	M2	A B	+ +	+ –	– –	– –	+ +	+ +	+ +
13	42	F (K)	M2	A B	– –	– –	– –	– –	– –	– –	++ +
14	52	M	M5a	A B	– ++	– –	– –	– –	++ ++	+ +	++ +
15	56	M	M2	A B	+ +	– –	– –	– –	+ +	– +	+ +
16	44	M	M1	A B	– –	– –	– –	– –	– –	– –	+ –
17	51	F (K)	M2	A B	+ +	– –	– –	– –	– –	–/+ –/+	+ +
18	25	F (K)	M2	A B	– –	– –	– –	– –	– –	– –	– +
19	69	F (K)	M4	A B	+ +	– –	– +	– –	+++ ++	– +	+ +
20	59	M	M2	A B	– –	– –	– –	– –	– –	– –	– –
21	48	F (K)	M2	A B	– –	– –	– –	– –	+ +	– –	– –
22	46	F (K)	M0	A B	– –	– –	– –	– +	– –	– –	+ +
23	39	F (K)	M2	A B	+ +	++ ++	– –	– –	+ +	+ +	++ +++
24	44	M	M4	A B	– +	– –	– –	– –	– –	– –	– –
25	25	F (K)	M4	A B	++ ++	+ +	+ +	– –	+++ +	++ ++	+ +

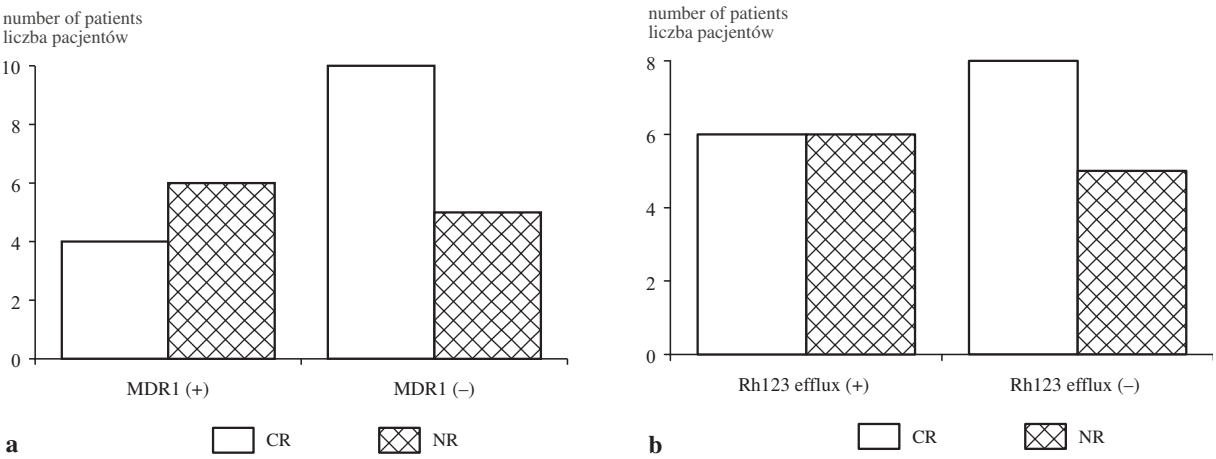

Fig. 1. Remission rate in AML patients whose blasts: **a** – expressed and not expressed MDR1 protein, estimated with anti-MDR1 monoclonal antibody; **b** – demonstrating the functional activity (Rh123 fluorescent dye efflux) of multidrug resistance transporter proteins (MDR1 and MRP), both measured by flow cytometry. CR – complete remission; NR – remission not achieved

Ryc. 1. Częstość remisji u chorych na o.b.s., których blasty: **a** – wykazywały ekspresję białka MDR1 oznaczanego za pomocą monoklonalnego przeciwciała anty-MDR1, oraz **b** – wykazywały aktywność białek transportowych (MDR1 i MRP1) mierzoną testem czynnościowym z zastosowaniem fluorescencyjnego barwnika Rh123. Obydwa oznaczenia wykonano metodami cytometrii przepływowej. CR – całkowita remisja; NR – brak remisji

2 patients (No 3, No 25), but it seemed not to influence their chemotherapy outcome, as their both achieved CR. After the first cycle of chemotherapy MDR3 was found in 3 patients (No 1, No 5 and No 19): 2 of them had no remission and 1 (No 1) relapsed.

Elevated BCPR was found at diagnosis in 2 patients; after chemotherapy it was reversed in case No 3 (CR) and remained overexpressed in the second one – No 2 (NR).

GSTπ was elevated at diagnosis in 19/25 AML patients and persisted in 16/18 after first chemotherapy cycle. Out of 3 patients in whom GSTπ reversed after induction therapy, 1 achieved CR (No 3) and out of 16 in whom it remained elevated, 7 had had no remission, 1 had PR and 10 had CR, but 3 of them relapsed early (No 1, 14 and 17).

Increased Rh123 efflux both at diagnosis and after the first chemotherapy cycle, was observed in 12 AML patients (48%); 6 of them had no remission, 1 had PR (No 11). After induction therapy, in 4/5 of patients with CR, the Rh123 efflux remained elevated; three of them had stable CR (No 9, No 21 and No 25) and one relapsed early (No 14). One patient, in whose blasts Rh123 efflux was reversed after the first chemotherapy cycle, achieved a stable CR (No 3).

Out of 7 AML patients with the co-expression of: MDR1 with MRP, LRP, GSTπ and high Rh123 efflux (No 2, No 8, No 23); LRP, GSTπ and Rh123 high efflux (No 4); GSTπ and Rh123 high efflux (No 15, No 19), at diagnosis and after the first chemotherapy cycle, 6 patients did not achieve CR; 3 of them had unfavourable and 3 intermediate cytogenetic profile.

The exception was a patient (No 25, with AML M4, 25-year-old woman) with favourable cytogenetic profile, who, in spite of the co-expression of MDR1, MRP, LRP, MDR3, GSTπ and increased Rh123 efflux at diagnosis and after the first chemotherapy cycle, obtained stable CR. Another patient (No 3 with AML M1, 38-year-old man), with intermediate cytogenetic profile, also had co-expression of MDR1 with MRP, LRP, MDR3, BCRP, GSTπ and increased Rh123 efflux at diagnosis, but reversed after the first cycle of chemotherapy, and he also achieved stable CR.

* Protein expression was assessed by flow cytometry as +, ++, or +++ when increase in mean channel of fluorescence intensity in tested sample as compared to control, was 1.5–2 times, 2–3 times, or higher than 3 times, respectively.
** Rh 123 efflux was assessed as +, ++, or +++ when decrease in mean channel of fluorescence intensity in tested group as compared to control, was 2 times, 3 times, or higher than 3 times, respectively.

* Ekspresję białek przyjmowano jako +, ++, lub +++, gdy średni kanał intensywności fluorescencji w badanej próbce w porównaniu do grupy kontrolnej był wyższy, odpowiednio: 1,5–2 razy, 2–3 razy lub ponad 3 razy.
** Wypływ Rh123 oceniano jako +, ++, lub +++, gdy średni kanał intensywności fluorescencji w badanej próbce w porównaniu do grupy kontrolnej był niższy, odpowiednio: 1,5–2 razy, 2–3 razy lub ponad 3 razy.

Discussion

In this prospective study of patients with *de novo* AML the prognostic value of expression and function of the MDR-transporter proteins: MDR1 (Pgp), MRP1, MDR3, BCRP, LRP and GSTπ, was assessed in the context of clinical and laboratory features.

Thirteen out of the 25 AML patients (52%) attained a complete remission (CR) with induction treatment, one had partial remission (PR) and eleven did not achieved remission. Out of eleven AML patients without CR two died in aplasia and nine were classified as an early death due to the disease progression.

All (13) AML patients with CR were younger than 55 years and 8 of them, in whom CR was stable, were 48 years old or younger. Described results are in agreement with those obtained by many other groups. Schoch et al. [21] analyzed 1225 AML patients, and found that up to 49 years, the age has no major impact on prognosis, but in patients 50 years old or older, the influence of age on outcome increases.

Older age of patients with unfavourable cytogenetic profile limits achievement of CR, as none of described 5 patients older than 60 years, with intermediate (3 patients) or unfavourable (2 patients) cytogenetic profile, achieved CR. Three patients with favourable cytogenetic profile achieved CR, and out of 7 AML patients with unfavourable cytogenetic data, 4 had no remission and out of 3 with CR, 1 had an early relapse, and the remaining 2 patients were relatively young (25 and 46 years old, respectively) and did not express MDR1.

Presented results confirm that overexpression of MDR1 in AML patients at diagnosis (10/25; 40%) and its persistence after induction therapy (9/25) indicate for bad prognosis (6 patients did not achieved remission, 1 had PR and 3 had CR, but one of them had an early relapse). The cytogenetic profile in MDR1 positive AML without remission was intermediate in 3 and unfavourable in another 3 patients. Filipits et al. [22] described similar results, evaluating an association of MDR1 expression with a poor response to chemotherapy. In 111 patients with *de novo* AML, with low, intermediate and high MDR1 expression, the CR rates were 77%, 68% and 38%, respectively. Pirker et al. [23] observed that increased levels of MDR1 mRNA in blast cells at diagnosis correlated with unfavourable treatment outcome in 63 AML patients. Presented observations are in agreement with results of many other studies, showing that MDR1 overexpression predicts lower CR rates (22, 24, 25, 26) shortening of overall survival (OS) [3] or short disease-free survival (DFS) [27]. A SWOG trial showed correlation of Pgp expression with poor treatment outcome, but not with OS or relapse-free survival (RFS) [28].

Out of 3 AML patients who were MDR1 negative at diagnosis and became positive after the first cycle of induction chemotherapy, the appearance of MDR1 expression seems to have some prognostic value, as neither of them achieved stable CR.

In presented study the MRP1 overexpression at diagnosis *de novo* AML was present in 16% cases, similarly to those (10%) observed in other studies [29, 30]. An evaluation at the mRNA level done by Schaich et al. [31] indicated that MRP1 predicts for OS or DFS only in patients with intermediate cytogenetic parameters. In the case of presented MRP1 positive patients, 2 of them out of 3 who did not obtained CR, had intermediate cytogenetic profile. However, in the study of Tsimberidou et al. [32] and also other groups, there was no correlation found between MRP1 overexpression and CR rates [25, 28, 29, 33], OS or DFS [25, 28, 33]. Although MRP1-mediated mechanisms may have independent prognostic value in *de novo* AML, its low overexpression frequency makes its importance questionable.

The co-expression of MDR1 and MRP1 was found in 6/25 AML patients (24%) and only two of them achieved CR. This finding is in agreement with the results of Legrand et al. [29], as they have shown that the combined activity of MRP1 and MDR1 is a more significant prognostic factor for poor outcome, measured by CR, RFS and OS duration, as compared to the MDR1 overexpression alone. Van der Kolk et al. [34] demonstrated that although MRP1 activity is not an independent prognostic factor for CR achievement, patients with both high MDR1 and MRP1 activity showed lower CR rate than patients with low MDR1 and MRP1 activities.

In our study LRP was elevated at diagnosis in 9 out of 25 AML patients (36%), and 3 of them obtained CR. In other studies LRP expression was observed in 35–50% of AML patients and has been reported by many groups to be an adverse prognostic factor [12, 13, 22, 25]. But other authors failed to show any correlations between LRP overexpression and CR rate or OS [31, 32, 35].

Presented data are not sufficient to estimate the clinical significance of MDR3 and BCRP overexpression. MDR3 was elevated in 2 cases only, and did not seem to influence the treatment outcome as both these patients achieved CR. BCRP was also increased in 2 patients, one of them obtained CR and the second did not. The importance of BCRP expression in AML is still controversial. Relatively high expression of BCRP was observed in approximately 30% of high-risk group AML patients, and did not correlate with MDR1

expression, suggesting that BCRP may prompt resistance to therapeutic agents at the absence of MDR1 [36]. Benderra et al. [37] analyzed the expression of BCRP and MDR1 in 149 AML patients, and found that BCRP was a prognostic factor for CR achievement (43% in BCRP positive patients *versus* 69% in BCRP negative patients), the 4-year disease-free survival (12% *vs.* 33%) and 4-year overall survival (19% *vs.* 38%). It revealed that AML patients, expressing both BCRP and MDR1, had the poorest prognosis.

The multidrug transporter proteins functional assay seems to have a prognostic value for CR achievement. Increased efflux of Rh123 was present in 11/25 AML (44%) and among those patients CR achieved only one (who reversed after induction chemotherapy); 6 patients had no remission at all.

GSTπ was elevated at diagnosis in 18/25 AML patients and remained overexpressed in 16/18 after first chemotherapy cycle; 7 of them were chemotherapy resistant and remaining 3 had CR, but followed by an early relapse.

Six out of 7 AML patients with the co-expression of functionally active MDR1 with other multidrug resistance proteins at the diagnosis and after the first chemotherapy cycle, had no remission; three of them had unfavourable and three intermediate cytogenetic profile. Exceptions were two patients, who obtained stable CR, but both of them were young (25 and 38 years old) and one of them had intermediate cytogenetic profile and the favourable with inv(16). Similarly to results obtained by Zwaan [38], 2 our AML patients with t (8;21) (No 6 and 18) but without overexpression of MDR proteins, both achieved CR.

It can be concluded that older age (no one over sixty achieved CR), cytogenetic profile (out of 7 patients with unfavourable cytogenetic abnormalities, 5 did not achieve CR), and MDR1 overexpression, or co-expression of several (at least two) multidrug resistance proteins at diagnosis, are the factors associated with an adverse prognosis and lower CR probability in AML patients.

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Received: 06.06.2005
Revised: 18.07.2005
Accepted: 18.07.2005

Praca wpłynęła do Redakcji: 06.06.20055 r.
Po recenzji: 18.07.2005 r.
Zaakceptowano do druku: 18.07.2005 r.