O. Zotova[1]
A. Lukianova[2]
M. Valchuk[1]
I. Vanko[1]
Y. Karol[3]
O. Shalay[1]
V. Loginsky[1]

[1]*SI «Institute of Blood Pathology and Transfusion Medicine, NAMS of Ukraine», Lviv*

[2]*Medical Biology Centre «Genom», Kyiv*

[3]*Communal Noncommercial Enterprise «Municipal Clinical Hospital № 5», Lviv, Ukraine*

Key Words: *acute myeloid leukemia, karyotype, cytogenetic abnormalities, monosomal karyotype, diagnosis, prognosis.*

PROGNOSTIC SIGNIFICANCE OF MONOSOMAL KARYOTYPE IN ADULT ACUTE MYELOID LEUKEMIA

Aim: *to detect the frequency, diagnostic and prognostic significance of monosomal karyotype (MK) in adult patients with acute myeloid leukemia (AML) and to determine the most common monosomies involved in MK*$^+$ *AML.* ***Materials and methods:*** *cytogenetic investigations of bone marrow and/or peripheral blood cells from 116 newly diagnosed adult patients with AML [range: 18–85 years, 70 (60%) males and 46 (40%) females] were performed. The methods of conventional cytogenetics (GTG) and fluorescence in situ hybridization (FISH) were used.* ***Results:*** *chromosomal abnormalities of various kinds were found in 68 (59%) patients. Taking into consideration the identified cytogenetic abnormalities, AML patients were classified into 3 risk groups: the group of patients with favorable cytogenetic markers t(8;21)(q22;q22), t(15;17)(q22;q11-21) and inv(16)(p13q22)/t(16;16)(p13;q22), the intermediate-risk group without significant prognostic markers and the group of patients with adverse prognostic factors [monosomies 5 and 7, deletions of 5q and 7q, rearrangements of 3q and 17p, t(9;22)(q34;q11), complex karyotype and MK]. MK was found in 7 (6%) patients. With respect to the distribution of monosomies in MK, 2 (29%) cases had one autosomal monosomy and 5 (71%) patients had ⩾ 3. One (14%) patient of the MK*$^+$ *AML cases had only monosomies, whereas 6 (86%) had also structural cytogenetic abnormalities, except the monosomies. The most common monosomies were: -5 (71%), -16 (57%), -7 (43%) and -17 (43%). The patients with MK*$^+$ *AML were classified into a new cytogenetic category of AML with a very poor prognosis. Median survival of these patients was 1 month and all patients died within 4 months.* ***Conclusions:*** *in our investigation chromosomal abnormalities of various kinds were found in 59% of adult patients with AML. Cytogenetic investigations are recommended for inclusion in the standard examination of patients with AML for diagnosis, prognosis of disease and selection the optimal treatment strategy.*

Acute myeloid leukemia (AML) is characterized by different clinical course and different sensitivity to therapy. Taking into consideration their significant prevalence, an intensive search for new prognostic criteria is conducted that may determine individual prognosis and define the most appropriate treatment approach for patients with AML. A key event in the development of AML is the restructuring of the progenitor cell genome, causing the disruption in molecular control of cell cycle, transcription and translation of major protein regulators. At the current level of knowledge the need for reassessment of standard prognostic criteria based mainly on clinical and routine hematological tests became apparent. Thus, the necessity to develop new prognostic systems became relevant. Simultaneously with conventional studies it would take into account the most specific signs of abnormal clones: genome of leukemic cells and its abnormality. Damage to the genome of leukemic cells is mainly represented by chromosomal rearrangements of proto-oncogenes or suppressor genes (translocations, inversions, deletions, loss of chromosomes, extra copies of chromosomes, etc.) and it can be detected using the conventional cytogenetic and molecular genetic methods. Using conventional cytogenetics, an abnormal karyotype can be detected in ~60% of adult AML patients. Based on the current World Health Organization (WHO) classification, more than two-thirds of AML can be categorized by their underlying cytogenetic or molecular genetic abnormalities. These genetic abnormalities are the most important factors in determining response to chemotherapy as well as outcome in AML. Taking into consideration the identified cytogenetic abnormalities, AML patients are currently classified into three risk groups, favorable, intermediate and adverse. The latter group includes AML with complex karyotype (CK) defined as 3 or more clonal abnormalities (excluding cytogenetic abnormalities listed under the WHO category «AML with recurrent genetic abnormalities»). Recently, a new cytogenetic category was introduced, namely, monosomal karyotype (MK) defined by the presence of two or more distinct autosomal monosomies (AM) or a single AM associated with one or more structural abnormalities, excluding core-binding factor AML and acute promyelocytic leukemia. In AML, MK has been shown to be prognostically worse than an otherwise CK [1–3].

Aim of this study was to detect the frequency, diagnostic and prognostic significance of MK in adult patients with AML and to determine the most common monosomies involved in MK$^+$ AML.

MATERIALS AND METHODS

Cytogenetic investigations of bone marrow and/or peripheral blood cells from 116 newly diagnosed adult patients with AML [range: 18–85 years, 70 (60%) males and 46 (40%) females] were performed. Diagnosing of AML was fulfilled according to the WHO definition of > 20% of blasts in the bone marrow or peripheral blood and based on French-American-British classification (FAB). The methods of conventional cytogenetics (GTG) and fluorescence *in situ* hybridization (FISH) were used. Cytogenetic methods were carried out using standard techniques [4–7] and karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) [8]. Only clonal abnormalities were considered as positive results. Abnormalities were considered clonal if ⩾ 2 metaphases had the same aberration in the case of a structural abnormality or an extra chromosome, or if ⩾ 3 metaphases shared the same aberration in the case of a monosomy. CK was defined as 3 or more clonal abnormalities. MK was defined as the presence of two or more AM or one AM with at least one additional structural chromosomal abnormality.

RESULTS AND DISCUSSION

Cytogenetic investigations were performed in 116 newly diagnosed patients with AML. Chromosomal aberrations of various kinds were found in 68 (59%) cases. Among them presence of one karyotype abnormality was established in 37 (54%) cases, two abnormalities — in 13 (19%) cases and multiple structural and/or numerical changes (⩾ 3) — in 18 (27%) cases. The most common abnormalities were: monosomies Y, 5 and 7, trisomy 8, deletions of 5q and 7q, rearrangements of 3q, 12p, 17p and 11q23, translocations t(8;21)(q22;q22), t(9;22)(q34;q11), t(15;17)(q22;q11-21) and t(16;16)(p13;q22) or inv(16)(p13q22), marker and ring chromosomes and acentric structures. Taking into consideration the identified cytogenetic abnormalities patients were classified into risk groups according to the European LeukemiaNet (ELN) criteria [9]: a group of patients with favorable cytogenetic markers, an intermediate-risk group without significant prognostic markers and a group of patients with adverse prognostic factors. The first group includes AML with favorable clinical prognosis, namely with balanced chromosomal abnormalities — t(8;21)(q22;q22), t(15;17)(q22;q11-21) and inv(16)(p13q22)/t(16;16)(p13;q22). The second group consists of AML with normal karyotype or with other rare or atypical chromosomal aberrations that do not have prognostic significance. The third group comprises AML with a poor clinical prognosis, namely with chromosomal abnormalities such as loss or extra copies of chromosomes, deletions, translocations and multiple karyotype changes (⩾ 3) [monosomies 5 and 7, deletions of 5q and 7q, rearrangements of 3q and 17p, translocation t(9;22)(q34;q11), CK and MK].

MK was found in 7 (6%) cases of the 116 patients with AML. With respect to the distribution of monosomies in MK, 2 (29%) cases had 1 AM and 5 (71%) had 3 or more AM. The most common monosomies involved in MK$^+$ AML were: -5 (71%), -16 (57%), -7 (43%) and -17 (43%). Three patients (43%), besides abnormal ones, had 12–55% normal metaphases in their karyotypes. One (14%) of 7 patients with MK$^+$ AML had only monosomies, whereas other 6 (86%) had also structural cytogenetic abnormalities, besides monosomies. Spectrum of additional structural chromosomal aberrations associated with MK was as follows: del(6)(q13), del(13)(q11q22), add(12)(p13), add(16)(q12-13), der(10)t(10;18)(p12;q12), der(14)t(7;14)(q11;q21), der(15)t(15;17)(p11;q12), der(12)t(12;18)(p12;q12), add(2)(q36), marker and ring chromosomes and acentric structures (Table, Figure).

Table

Results of cytogenetic investigations of leukemic cells from patients with MK+ AML

№	Age	Sex	FAB classification	Karyotype	Overall survival, months
1	46	Female	AML M5	43~46, XX, -5, -8, -10, +11, -16, -17, -17, +1~4 mar[cp20]	0
2	48	Male	AML M5	36~43, X, -Y, -13, -14, -15, -16, -17, -18, -19, -20, -21, -22 [cp10]/46, XY[12]	1
3	85	Male	AML ?	45, XY, -5, del(6)(q13), add(12)(p13), del(13)(q12;q22), -16, +mar [13]/46, XY[12]	No data
4	52	Male	AML M6	44~45, XY, -4, -5, -5, -6, -7, -9, -9, -10, -15, -22, +1~8 mar [cp27]	4
5	76	Male	AML M1	40~45, X, -Y, -3, der (3), -4, -5, -7, der(15)t(15;17)(p11;q12), add(16)(q12-13), -16, -17, -20, +mar, +r [cp22]/46, XY[3]	No data
6	55	Male	AML ?, post MDS	43~59, XY, +Y, +1, +2, -3, -5, +6, +7, +8, -11, -12, +13, +16, +18, +18, +19, +20, +22, +1~4 mar,+1~2 dmin [cp20]	1
7	56	Male	AML M2	45, XY, (2)(q36), der(10)t(10;18)(p12;q12), -18 [13]/45, XY, -7, der(12)t(12;18)(p12;q12), der(14)t(7;14)(q11;q21)[7]	1

Using conventional cytogenetics, an abnormal karyotype can be detected in ~60% of adult AML patients [2]. In our investigation frequency of chromosomal abnormalities of various kinds was 59%, which is comparable to the data reported in the literature. According to the karyotype analysis AML patients were classified into risk groups taking into consideration the ELN criteria [9]: the group of patients with favorable cytogenetic markers [t(8;21)(q22;q22), t(15;17)(q22;q11-21) and inv(16)(p13q22)/t(16;16)(p13;q22)], the intermediate risk group without significant prognostic markers and the group with adverse prognostic factors [monosomies 5 and 7, deletions of 5q and 7q, rearrangements of 3q and 17p, translocation t(9;22)(q34;q11) and CK]. The latter group also included AML cases with MK defined by the presence of two or more distinct AM or a single AM associated with one or more structural abnormalities. Distribution of AML patients into risk groups according to the identified prognostic markers allows us to choose the most appropriate treatment approach for them, namely the intensity of therapy, the necessity of allogeneic stem cell transplantation in the first remission, the necessity of the prescription of tyrosine kinase inhibitors for AML pa-

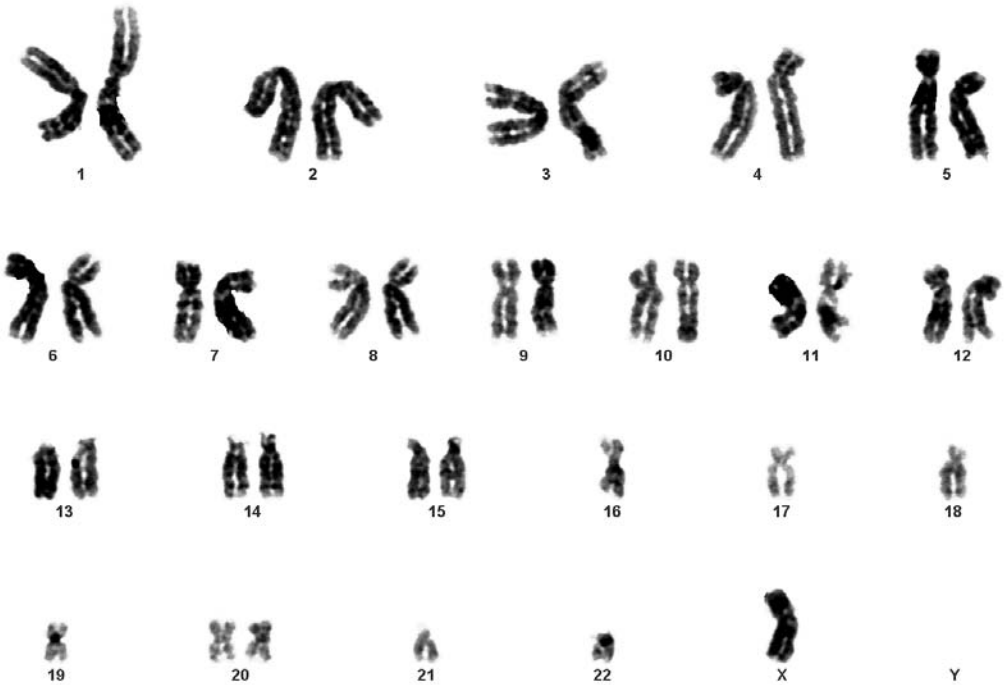

Figure. Karyotype of leukemic cell from MK$^+$ AML patient (Table, № 2) — 39, X, -Y, -16, -17, -18, -19, -21, -22

tients with t(9;22)(q34;q11) or differentiating agent — all-trans retinoic acid (ATRA) for acute promyelocytic leukemia patients with t(15;17)(q22;q11-21).

Using conventional cytogenetics, MK can be detected in ~10% of patients with AML at the age < 60 years and ~13–5% of patients at the age > 60 years [2]. In our investigation the frequency of MK was 6%, which is not much lower in comparison with the data reported in the literature. 14% of patients of the MK$^+$ AML cases had only monosomies, whereas 86% of patients had also structural cytogenetic abnormalities of various kinds, besides monosomies. It is reported that the most common monosomies involved in MK$^+$ AML are -5 (21–55%), -7 (23–45%), -16 (7–16%), -17 (11–22%), -18 (10–19%), -20 (8–19%) [2]. In our investigation, the most common monosomies were: -5 (71%), -16 (57%), -7 (43%) and -17 (43%).

All patients with MK$^+$ AML are classified into a new cytogenetic category of AML with a very poor prognosis, even after allogeneic stem cell transplantation [2]. Our data largely confirm this. 2 patients of 7 cases with MK$^+$ AML refused treatment, 5 patients started intensive induction therapy. The response to induction therapy was as follows: complete remission was not achieved in any of the patients (0 of 5); refractory disease and death was observed in 20% (1 of 5); early death in 80% (4 of 5) of them. Median survival of those patients was 1 month and all patients died within 4 months.

In our investigation, 4 (57%) patients of 7 cases with MK$^+$ AML had only abnormal metaphases in their karyotypes, whereas other 3 (43%) had also 12–55% normal metaphases, besides abnormal ones. It is reported that the presence of ⩾ 10 of cells with normal metaphases in MK$^+$ AML can be a prognostic factor and has been associated with longer survival. But how residual normal metaphases translate to longer survival is unclear [10].

The mechanisms responsible for MK$^+$ AML are still unclear, but it may be associated with deletions or mutations in *TP53* gene and multiple drug resistance. In fact, recent evidence indicated that *TP53* alterations occur in 70% of MK$^+$ AML patients and these abnormalities are more frequent in patients with CK$^+$/MK$^+$ AML that in those with CK$^+$/MK$^-$ AML. They potentially lead to a chromosome instability pattern that is usually a result of a single catastrophic event knows as chromothripsis. Thus, *TP53* alterations appear to be one molecular basis for this MK$^+$ AML subset and, in particular, *TP53* alterations suggest an important role for p53 in leukemogenesis [2].

CONCLUSIONS

1. Chromosomal abnormalities in leukemia cells were found in 59% of 116 newly diagnosed adult patients with AML by conventional cytogenetic methods.

2. Taking into consideration the identified cytogenetic abnormalities AML patients were classified into risk groups: the group of patients with favorable cytogenetic markers [t(8;21)(q22;q22), t(15;17)(q22;q11-21) and inv(16)(p13q22)/t(16;16)(p13;q22)], the intermediate risk group without significant prognostic markers and the group with adverse prognostic factors [monosomies 5 and 7, deletions of 5q and 7q, rearrangements of 3q and 17p, translocation t(9;22)(q34;q11), CK and MK].

3. MK were found in 6% of patients with AML. The most common monosomies were: -5 (71%), -16 (57%), -7 (43%) and -17 (43%). 14% of patients of MK$^+$ AML cases had only monosomies, whereas 86% had also structural cytogenetic abnormalities, besides monosomies.

4. Patients with MK$^+$ AML were classified into a new cytogenetic category of AML with a very poor progno-

sis. Median survival of MK^+ AML patients was 1 month and all patients died within 4 months.

5. Cytogenetic investigations should be included in the standard examination of patients with AML for diagnosis, prognosis and selection the optimal treatment strategy.

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ПРОГНОСТИЧНЕ ЗНАЧЕННЯ МОНОСОМНОГО КАРІОТИПУ ПРИ ГОСТРІЙ МІЄЛОЇДНІЙ ЛЕЙКЕМІЇ У ДОРОСЛИХ

О.В. Зотова[1], А.С. Лук'янова[2], М.О. Вальчук[1], І.С. Ванько[1], Ю.С. Кароль[3], О.О. Шалай[1], В.Є. Логінський[1]

[1]ДУ «Інститут патології крові та трансфузійної медицини НАМН України», Львів

[2]Медико-біологічний центр «Геном», Київ

[3]Комунальне некомерційне підприємство «5-та Міська клінічна лікарня», Львів, Україна

Резюме. ***Мета:*** *оцінка частоти, діагностичного та прогностичного значення моносомного каріотипу (МК) у дорослих, хворих на гостру мієлоїдну лейкемію (ГМЛ), та визначення найбільш поширених моносомій, задіяних у МК.* ***Об'єкт і методи:*** *цитогенетичні дослідження клітин кісткового мозку та/або периферичної крові проведено у 116 нелікованих дорослих пацієнтів із ГМЛ (віком від 18 до 85 років, серед них 70 (60%) чоловіків і 46 (40%) жінок). Використовували метод класичної цитогенетики (GTG) та флуоресцентну in situ гібридизацію (FISH).* ***Результати:*** *хромосомні аномалії різного характеру виявлено у 68 (59%) хворих. З урахуванням цитогенетичних аномалій хворих на ГМЛ класифіковано на 3 групи ризику: зі сприятливими цитогенетичними маркерами [t(8;21)(q22;q22), t(15;17)(q22;q11-21) та inv(16)(p13q22)/t(16;16)(p13;q22)]; проміжного ризику без прогностично значущих маркерів; із несприятливими факторами прогнозу [моносомії 5 і 7, делеції 5q і 7q, перебудови 3q і 17p, t(9;22)(q34;q11), комплексний каріотип та МК]. МК виявлено у 7 (6%) пацієнтів. У 2 (29%) випадках спостерігали одну аутосомну моносомію, у 5 (71%) — ⩾ 3. Серед хворих на ГМЛ з МК в 1 (14%) пацієнта виявлено лише моносомії, а у 6 (86%) хворих одночасно з моносоміями відмічали також структурні перебудови. Найпоширенішими моносоміями були: -5 (71%), -16 (57%), -7 (43%) та -17 (43%). Пацієнтів з МК віднесено до нової цитогенетичної категорії ГМЛ — з дуже поганим прогнозом. Усі ці хворі прожили не більше 4 міс (медіана виживаності становила 1 міс).* ***Висновки:*** *цитогенетичні аномалії різного характеру виявлено у 59% дорослих пацієнтів із ГМЛ. Цитогенетичні методи рекомендовано включити у стандарти обстеження хворих на ГМЛ для діагностики, прогнозування перебігу хвороби та підбору оптимальної тактики лікування.*

Ключові слова: гостра мієлоїдна лейкемія, каріотип, цитогенетичні аномалії, моносомальний каріотип, діагноз, прогноз.

Correspondence:
Zotova O.
General Chuprynka str., 45, Lviv 79044
SI «Institute of Blood Pathology and Transfusion Medicine, NAMS of Ukraine»
E-mail: lnkzotova@gmail.com

Submitted: 14.05.2019