ORIGINAL ARTICLE

UDC 616-006.4:616.037+616.155

KI-67 AS A PROGNOSTIC FACTOR OF MYELODYSPLASTIC SYNDROME

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Patients with MDS RAEB II were examined. The decrease in dynamics of intracellular Ki-67 protein expression was determined in patients with MDS RAEB II with positive response to chemotherapy, and the increase in proliferative activity of haematopoietic cells of peripheral blood (PB) and bone marrow (BM) was determined in patients with MDS RAEB II in transformation and acute myeloid leukaemia (AML) after MDS.

Key words: *MDS, Ki-67, chemotherapy, peripheral blood, bone marrow.*

Modern haematology and oncology are searching for criteria that allow for determining the level of biological activity of cell with maximum objectivity.

With regard to new approaches, proliferative potential of different cells, including tumour cells, can be characterized on the basis of analysis of intracellular Ki-67 protein expression, which occurs during late G_1, S, G_2 and M phases of the cell cycle. Studies show that the rate of proliferative cell activity, on the one hand, reflects the degree of malignancy of the process, and on the other hand, it allows for prediction of the response to therapy based on proliferative potential of the cells [1]. Ki-67 is one of the well-known proliferation markers, which is expressed by the dividing cells that are absent in G_0 phase of the cell cycle. The appearance of Ki-67 protein coincides with the beginning of the mitotic cycle, and its content varies depending on the stage of mitosis. Studies of Ki-67 expression are used for prediction of clinical course of many malignancies, such as lymphomas, tumours of breast, pancreas, lungs, pituitary gland, stomach, colon, nervous system, and endometrium [1,2]. A significant 2-fold decrease in 5-year survival was found in the group of patients with uterine endometrial stromal sarcoma, the cells of which expressed Ki-67, as compared to the patients with Ki-67 negative cells [3].

In haematology, the rate of proliferative activity of lymphoid cells in lymphoproliferative diseases is an important parameter in diagnostics of the disease modification and determination of the degree of malignancy [4]. Studies show that the research of Ki-67 protein expression has the prognostic value, which allows for revealing the beginning of B-CLL progression 1–3 months prior to clinical and laboratory confirmation [5].

The increase in proliferative activity and appearance of resistance to apoptosis was registered in patients with CML in transformation of chronic phase into accelerated phase and blast crisis phase [6, 7, 8].

However, only the few studies highlight some aspects of proliferative activity of haematopoietic cells in myelodysplastic syndrome. No reliable data regarding Ki-67 protein expression in patients with different responses to treatment are available, and the role of this parameter as a prognostic criterion of MDS has not been established.

The objective of this work was to determine proliferative activity of haematopoietic cells of peripheral blood and bone marrow according to Ki-67 expression in patients with myelodysplastic syndrome — refractory anaemia with excess blasts II (MDS RAEB II) with different responses to CT (chemotherapy).

Materials and methods.

42 patients with MDS RAEB II were examined, 23 of them females and 19 males, aged 43 to 78 (median 67.5) years. All patients were registered and treated at the a polyclinic and department of diseases of the blood State Institution "Institute of Haematology and Transfusion of NAMS of Ukraine". Reference group is patients with MDS RAEB II and the group of partly healthy subjects (Table 1).

The diagnosis was established on the basis of data of general clinical, laboratory and instrumental methods of examination. Special haematological methods included: cytological, cytochemical, cytogenetical and histological studies of bone marrow (BM) and peripheral blood (PB) cells.

Proliferative activity of haematopoietic cells of PB and BM was studied according to the presence of Ki-67 marker by laser flow cytometry, which is deemed as DNA analysis alternative [9,10]. Intranuclear Ki-67 proliferation marker in haematopoietic cells was determined using monoclonal antibodies from PE Mouse Anti-Human Ki-67 Set (BD Pharmingen, USA) and FITC-conjugated Armenian Hamster IgG Monoclonal Isotype Control reagents.

For the purpose of the study, PB or BM samples of 50 мL were placed in control and study tubes. Erythrocytes in the samples were destroyed using FACS Lysing Solution (BD, USA). After washing, the cells obtained were fixed by 4 %

formaldehyde solution during 10 minutes. The cells were treated with 0.1 % saponin solution at room temperature during 5 minutes. After washing, anti-Ki-67 MCA was added into the test tube, isotypical control was added into the control tube, and both tubes were incubated in the dark at room temperature during 20 minutes. Cytofluorometric studies were conducted using a flow cytofluorometer (FACScan, Becton Dickinson, USA) with argon laser at the wavelength of 488 nm operated by LYSYS-II ver. 1.1. (Becton Dickinson, USA); WinMDi 2.8 (Joseph Trotter, Scripps Institute, La Jolla, USA) and Microsoft Excel 2000.

Prior to initiation of the treatment with cytostatics, all complications in patients were compensated: haemorrhagic manifestations — by transfusions of platelet concentrate and administration of haemostatics, infectious and inflammatory processes — by prescription of adequate antibacterial or antiviral therapy.

Control bone marrow study for the assessment of cytostatic therapy results was conducted after completion of the chemotherapy course. Peripheral blood analysis was conducted every 10 days.

All patients with MDS included in the study were assigned into respective groups:

Group 0 — patients with the diagnosis of MDS RAEB I progression, treated with CT (6-MP 50–100 mL/day or thioguanine 40–120 mL/day) without any positive effect;

Group I — patients with the diagnosis of MDS RAEB II from Group I and subjects newly diagnosed with MDS RAEB II;

Group II — patients with MDS RAEB II, which have responded to CT positively;

Group III — patients from Group II and subjects newly diagnosed with MDS RAEB II, which have not responded to treatment;

Group IV included patients with AML transformed from MDS RAEB II.

To determine the authenticity of the studied parameters and justification of the validity of the results used standard statistical methods. Average values in the tables and text matching Poisson distributing presented as M ± m. Parametric indices were compared using t-test (Stu'yudenta) a bidirectional version. The degree of association between categorical indicators expressed as relative risk (RR).

Statements about the presence of significant differences assumed by the probability of error less than 0.05.

Digital data was analyzed using the software package Statistica 10,0 (StatSoft, USA) and the program "Excel" from the package "Microsoft office 2010".

The results of studies

42 patients with MDS RAEB II with the positive response to treatment in transformation and AML after MDS were examined. Reference group — patients with MDS RAEB I progression and apparently healthy subjects (Table 1).

The state of 71 % of the examined patients was assessed as moderately severe. Anaemia syndrome manifestations — weakness, performance impairment,

Table 1

Compared results of Ki-67 expression by haematopoietic PB and BM cells, and haematological parameters in patients with MDS RAEB II.

Parameter	Group 0 (n=13)	Group I (n=42)	Group II (n=15)	Group III (n=27)	Group IV (n=9)	Control (n=30)
	MDS RAEB I progression	MDS RAEB II	MDS RAEB II with positive response to treatment	MDS RAEB II in transformation	AML after MDS	apparently healthy subjects
Erythrocytes • 10^{12}/L	1.7 ± 0.2	2.1 ± 0.7	2.6 ± 0.5	1.8 ± 0.5	1.9 ± 0.5	4.5 ± 1.3
Haemoglobin g/L	65.5 ± 1.2	64.5 ± 2.3	82.5 ± 1.6	63.5 ± 0.3	67.5 ± 1.9	148.3 ± 2.6
Leucocytes •10^9/L	2.5 ± 0.5	2.6 ± 0.8	3.4 ± 0.5	15.9 ± 2.9	19.4 ± 3.5	6.9 ± 1.2
Platelets •10^9/L	59.5 ± 12.5	54.7 ± 9.5	78.3 ± 1.8	58.7 ± 4.3	53.5 ± 6.7	248.5 ± 16.4
Blasts of blood %	1.7 ± 0.05	2.2 ± 0.05	0.9 ± 0.05	7.8 ± 0.5	8.4 ± 1.5	0
Blasts of bone marrow %	10.5 ± 2.5	16.7 ± 2.3	8.9 ± 1.4	21.5 ± 1.6	36.4 ± 4.9	2.1 ± 0.5
Ki-67 of PB %	8.4 ± 0.5	12.3 ± 0.5	2.7 ± 0.5	18.3 ± 0.6	23.8 ± 2.5	0.7 ± 0.01
Ki-67 of BM %	19.4 ± 2.6	18.9 ± 0.5	12.6 ± 0.5	31.7 ± 1.5	47.5 ± 5.3	4.3 ± 0.01

Note:
* $p < 0.05$ — comparison of Ki-67 expression data of Group 0 with data of Group I.
** $p < 0.05$ — comparison of Ki-67 expression data of Group 0 with data of Group II.
*** $p < 0.05$ — comparison of Ki-67 expression data of Group I with data of Group III.
**** $p < 0.05$ — comparison of Ki-67 expression data of Group III with data of Group IV.

dizziness — were observed in 95 % of patients. 11 (26 %) patients had severe anaemia and haemorrhagic syndrome, which were compensated by transfusions of erythrocytes and platelet concentrate. Patients suffered from weakness — 12 (28.6 %), increased body temperature — 15 (35.7 %), ossalgia / arthralgia — 9 (21.4 %) subjects, irrespective of the age and gender. Weight loss was registered in 19 (45.2 %) patients.

Leucocytes count in Groups I and 0 was identical ($p < 0.05$). Platelets count did not differ in Groups 0 and I ($p < 0.05$). The percentage of blast cells in leucograms of patients of Groups 0 and I was practically identical. However, the percentage of blast cells in BM of patients of Group I with the diagnosis of MDS RAEB II was 1.6 times higher than in patients of Group 0 with MDS RAEB I progression. The count of haematopoietic cells expressing intranuclear Ki-67 protein in peripheral blood was 1.5 times higher in patients with MDS RAEB II. The percentage of Ki-67 positive cells in BM was identical in patients of Groups 0 and I, but 4.5 and 4.4 times higher, respectively, than that in apparently healthy subjects (4.3 ± 0.01) (Table 1).

As a result of chemotherapy conducted, the positive response was obtained in 15 (35.7 %) patients with MDS RAEB II. In this group of patients, the general medical state improved, the manifestations of anaemia syndrome decreased; haemoglobin level in PB increased to 82.5 % that is 1.3 times higher than in patients of Group I; leucocytes count increased 1.3 times and platelets count increased 1.5 times as compared to their count in patients with MDS RAEB II (Group I) (Table 1). The percentage of blast cells in PB was less than 1 %; the percentage of blast cells in BM decreased 1.9 times after treatment, as compared to that (16.7 %) in Group I (MDS RAEB II) before treatment. The count of cells expressing intranuclear Ki-67 protein decreased 4.5 times in PB and 1.5 times in BM in the group with the positive response to treatment; however, the count of Ki-67 positive cells in PB was 3.8 times higher, and in BM 2.3 times higher than that in apparently healthy subjects.

Studying the further dynamics of the pathological process, i.e. MDS RAEB II in transformation and AML after MDS, the absence of increase in erythrocytes count was determined in Group III ($1.8 \pm 0.5 \cdot 10^{12}$/L) and in Group IV ($1.9 \pm 0{,}5 \cdot 10^{12}$/L) as compared to the data of Group I — MDS RAEB II. Haemoglobin concentration in all three groups was unchanged. Platelets count in patients of Group III — MDS RAEB II in transformation and Group IV — AML after MDS remained stably low and varied from $58.7 \pm 4.3 \cdot 10^9$/L to $53.5 \pm 6.7 \cdot 10^9$/L that is 4.2–4.6 times lower, respectively, than in the control group — apparently healthy subjects.

The analysis of leucocytes count in PB shows the increase in leucocytes count in Groups III and IV, as compared to their count in Group I (MDS RAEB II). Leucocytes count increased 6.1 times in the group of patients with MDS RAEB II in transformation and 7.5 times in Group IV (AML after MDS), as compared to leucocytes count in Group I (MDS RAEB II). The percentage of blast cells in PB increased 3.5 times in Group III and 3.8 times in Group IV, as compared to the data of Group I. The percentage of blast cells in BM in patients with MDS RAEB II in transformation increased 1.2 times in Group III and 2.2 times in Group IV (AML after MDS).

In the course of assessment of intranuclear Ki-67 protein expression by haematopoietic cells of PB and BM in patients of the studied groups, the increase in their count was registered. Ki-67 cells count in PB of patients of Group III (MDS RAEB II in transformation) was 1.5 times higher and in Group IV (AML with MDS) 1.9 times higher than in the reference group II (MDS RAEB II), whereas Ki-67 positive cells count in patients of Group IV was 1.3 times higher, as compared to their count in patients of Group III (MDS RAEB II in transformation).

Therefore, the increase in the count of haematopoietic cells expressing intranuclear Ki-67 protein was observed in bone marrow of patients of those reference groups.

Thus, the percentage of Ki-67 positive cells in Group III (MDS RAEB II in transformation) is 1.7 times higher than in Group I, and the percentage of cells expressing Ki-67 protein in Group IV (AML with MDS) is 2.5 times higher than in the reference group I (MDS RAEB II), whereas the level of Ki-67 protein expression by BM cells in patients of Group IV is 1.5 times higher than in the group of patients with MDS RAEB II in transformation.

Conclusions:

The involvement of intranuclear Ki-67 protein in the mechanism of pathological process development and in the forming of response to treatment in patients with MDS RAEB II was determined.

1. The decrease in the count of cells expressing intranuclear Ki-67 protein of PB and BM was determined in patients with MDS RAEB II with the positive response to treatment, as compared to that in the group of MDS RAEB II in transformation and in the group of patients with AML after MDS, that may indicate the decrease in proliferative activity of haematopoietic BM cells.

2. The increase of Ki-67 protein expression by haematopoietic cells of PB and BM was determined in the groups of patients with MDS RAEB II in transformation and AML after MDS, as compared to that in the group of patients with MDS RAEB II before treatment.

The difference in the expression of intranuclear Ki-67 protein by haematopoietic cells of PB and BM in patients with MDS RAEB II with the positive response to CT and groups of patients, in which the pathological process had adverse development (MDS RAEB II in transformation and AML after MDS), indicates the involvement of Ki-67 protein in the pathogenesis of the disease, that may be used for assessment of the treatment efficacy and the degree of the tumour process severity.

Кофлікту інтересів немає.

Це дослідження не отримало ніякої фінансової підтримки від державної, громадської чи комерційної організації.

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KI-67 ЯК ФАКТОР ПРОГНОЗУ ПЕРЕБІГУ МІЄЛОДИСПЛАСТИЧНОГО СИНДРОМУ

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Було обстежено хворих на МДС РАНБ II. Визначено зменшення динаміки експресії внутрішньоклітинного білка Кі-67 у хворих на МДС РАНБ II при позитивній відповіді на хіміотерапію, та зростання проліферативної активності гемопоетичних клітин ПК та КМ у хворих на МДС РАНБ II в трансформації та ГМЛ після МДС.

Ключові слова: МДС, Кі-67,хіміотерапія ,периферична кров,кістковий мозок.

KI-67 КАК ФАКТОР ПРОГНОЗА ТЕЧЕНИЯ МИЕЛОДИСПЛАСТИЧЕСКОГО СИНДРОМА

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Было обследовано больныйх с МДС РАИБ II. Определено изменения динамики экспресии внутриядерного белка Кі-67 у больных МДС РАИБ II при положительном ответе на химиотерапию и увеличение пролиферативной активности гемопоэтических клеток ПК и КМ у больных МДС РАИБ II в трансформации и ОМЛ после МДС.

Ключевые слова: МДС, Кі-67, химиотерапия, периферическая кровь, костный мозг