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# Proliferative Activity *in vitro* and Sample Preparation Evaluation in Conventional Cytogenetic Study in Patients with Hemoblastosis

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**Abstract** The success and effectiveness of conventional cytogenetic analysis (CCA) in patients with leukemia depends on the proliferative activity of leukemic cells in vitro and the related with it the number of metaphase plates on a microscopic preparation. The proliferative activity of leukemic cells in culture can vary significantly depending on the nosological form of leukemia, the individual characteristics of the course of the disease and the patient's condition. The aim of the study was to test a new assessment of the proliferative activity of cells and the number of metaphase plates in CCA. We have proposed a method for assessing cell growth based on counting metaphase plates on an area of a microscopic preparation equal to 287.5 $mm^2$ ($MFP/S_{287}$). The study revealed that this indicator is an objective criterion for the proliferative activity of cells cultured *in vitro* for CCA, and an effective tool for assessing the number of metaphase plates on a chromosomal preparation.

**Keywords** Conventional cytogenetic analysis, Proliferative activity of leukemic cells, Metaphase plates

## 1. Introduction

One of the mandatory stages of examination of patients with leukemia is the conventional cytogenetic study (CCS), since the analysis of metaphase chromosomes using light microscopy allows us to assess the state of the entire karyotype and identify not only marker and recurrent, but also variant and additional chromosomal changes. The data of standard karyotyping make it possible to verify the diagnosis, stratify patients into risk groups and predict the course of the disease [1,2].

The analysis of the karyotype of leukemic cells is carried out after performing a number of technical tasks, including the cell cultivation in a nutrient medium to obtain the maximum possible number of cells at the metaphase stage, multi-stage isolation of metaphase plates from biological material, preparation and staining of cytogenetical glass slide; scanning of cytogenetical preparations using light microscopy, search and photo-preservation of metaphase plates suitable for cytogenetic analysis. The cytogenetic analysis itself consists in identifying all chromosomes within each metaphase plate under study in accordance with the International System of Cytogenomic Nomenclature (ISCN) [3], assessing their quantitative integrity and structural immutability, detection of chromosomal anomalies (aneuploidies and derivatives) and identifying of rearrangements.

Nevertheless, in some cases, it is impossible to perform a CCS because of an insufficient number of metaphase plates. This fact may be due to both the unsatisfactory number of capable to division atypical mononuclears in the biomaterial and the extremely low proliferative activity of leukemia cells.

However, even the high proliferative activity of tumor cells does not guarantee obtaining a sufficient number of metaphase plates suitable for karyotype analysis. The presence of a cell in one or another stage of division depends on the duration of the cell cycle, which varies significantly among patients with hematological neoplasia. So, there is evidence that among patients with acute myeloid leukemia, the range of one cycle can be from 16 hours to 292 hours [4]. The duration of the cell cycle of individual tumor clones is unknown, therefore, cell division can be forcibly stopped using colchicine or its analogues not only when the chromosomes are in a state of maximum chromatin condensation and are located in the equatorial plane of the cell, but also in early metaphase. As a result, cytogenetic preparations, in addition to individual metaphase plates scattered among interphase nuclei, will also contain clusters of weakly spiralized and morphologically undifferentiated chromosomes.

The experience of karyotype studies in leukemia patients conducted by us with the use of CCS shows that despite the

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same conditions of cultivation in nutrient media and the standard method of preparation of chromosomal specimens, the number of metaphase plates has significant differences both in patients with different types of hemoblastosis and in patients with the same nosological form of the disease. The data of the scientific literature adduce various reasons for the variability of proliferative activity of cells *in vitro* [5,6,7], however, we have not found a convincing explanation for this fact in relation to leukemic cells. It is probably necessary to conduct a series of studies to understand the mechanisms of loss of growth activity of these cells during cultivation *in vitro*. At the same time, the technique of objective assessment of cell growth and the associated number of metaphase plates should become a necessary tool for such studies. In this regard, the aim of this study was to develop a method that allows to objectively assess the number of metaphase plates obtained from blood and bone marrow cells cultured *in vitro*.

## 2. Methods

The study was carried out on the basis of the Laboratory of Molecular Genetics and Cytogenetics of the Republican Specialized Scientific and Practical Medical Center of Hematology (RSSPMCH) of the Ministry of Health of the Republic of Uzbekistan in 2019-2021. The object of the study was metaphase plates obtained from leukemia cells of blood (BC) and bone marrow (BMC) of patients with chronic myeloproliferative neoplasia (CMPN) (n=150), as well as from mitogen-stimulated T-lymphocytes of blood of persons without hematological neoplasia (n=84).

*In vitro* cultivation of BMC and BC of patients with CMPN was carried out in a nutrient medium containing 5 ml of RPMI-1640 with glutamine, 1 ml of fetal bovine serum (FBS), 6 ml of 0.1% colchicine solution; cultivation was carried out for 21 hours at a temperature of 37.0℃. *In vitro* cultivation of BC of subjects without hematological neoplasia was carried out in a nutrient medium containing 5 ml of RPMI-1640 with glutamine, 1 ml of FBS, 35 ml of 0.1% solution of the phytohemagglutinin (PHA) mitogen. Cell division was stopped at the metaphase stage with 0.1% colchicine solution, which was added to the medium in an amount of 40 ml 30 minutes before the beginning of hypotension. Cultivation was carried out for 72 hours at a temperature of 37.0℃.

The cultured biomaterial was precipitated by centrifugation (1500 turn in minute, 7 min). A 0.56% solution of potassium chloride was added to the cellular sediment and incubated for 25 minutes at a temperature of 37.0℃. After the incubation was completed, the biomaterial was fixed three times using ethanol and glacial acetic acid in a ratio of 3:1 – to obtain a precipitate containing metaphase plates. Cytogenetic specimens were prepared by dripping drops of cooled suspended sediment onto a glass slide from a height of 50 cm; the specimens were dried at room temperature for 24 hours.

Differential staining (GTG-binding) of cytogenetic preparations was performed using trypsin (0.025 g / 10 ml of distilled water) and Giemsa dye diluted on a phosphate buffer (PhB) (0.5 ml dye: 10 ml PhB).

The proliferative activity of cultured *in vitro* cells was evaluated using a direct light microscope ZEISS Axio Scope. A1 with magnification of the microscope of ×200 by counting the number of metaphase plates (MFP) per unit surface area of the chromosomal glass slide (S) equal to 287.5 $mm^2$.

Statistical analysis of the data obtained was carried out using the calculation of statistical indicators of the standard deviation (σ), Student's t-test when comparing relative and average values. The differences were considered statistically significant at $p<0.05$.

## 3. Results

The evaluation of the proliferative activity of *in vitro* cultured leukemia cells of patients with chronic myeloproliferative neoplasia (CMPN), as well as mitogen-stimulated lymphocytes of conditionally healthy individuals was carried out by counting the number of metaphase plates (MFP) on the surface area of the chromosomal glass slide (S), which is equal to the visual field of ten transverse scans of a standard slide with a width of 25 mm when magnified by a microscope ×200.

The scanned surface area of the chromosomal preparation (S, $mm^2$) was calculated according to the formula developed by us (1):

$$S = W \bullet D_{FV} \bullet n \quad (1)$$

where W – is the width of the slide (standard width is 25 mm); $D_{FV}$ – is the diameter of the field of view of the microscope, which is 1.15 mm at magnification ×200; n – is the number of transverse scans of the chromosomal glass slide (n=10).

The diameter of the field of view of the microscope ($D_{FV}$, mm) was determined by the standard formula (2) [8]:

$$D_{FV} = FN / (M_o \bullet M_T) \quad (2)$$

where FN – is the diameter of the eyepiece, which is standard 23 mm; Mo – is the magnification of the lens (×20); Mt – is the magnification of the tube lens (×1).

Thus, the surface area of one transverse scan of a chromosomal glass slide was 28.75 $mm^2$, and the surface area of ten transverse scans (S) was 287.50 $mm^2$.

Prior studies have shown that the number of MFPs calculated over the surface area of 28.75 $mm^2$ in different sections of each chromosomal glass slide did not have a significant standard deviation (σ). Nevertheless, in order to minimize the error for further research, we calculated the total number of MFPs on 10 cross-scan fields of each chromosomal glass slide. Thus, to assess the proliferative activity of cultured *in vitro* cells and the number of metaphase plates on a chromosomal glass slide for a conventional cytogenetic study, we used the MFP/S287

indicator.

Using the MFP/$S_{287}$ indicator, we evaluated the proliferative activity *in vitro* of venous blood cells (BC) in patients with CMPN and control subjects who do not have oncohematological disease. It should be noted that leukemia cells circulating in the blood of patients with CMPN are capable of proliferation due to their cancerous nature, whereas the ability to divide of blood cells (lymphocytes) of subjects without hemoblastosis was stimulated *in vitro* when exposed to the PHA mitogen.

The study showed that the average value of MFP/$S_{287}$ in the control group (n=84) was 286.86±32.50, whereas in the group of patients with CMPN (n=150) this indicator reached only 153.07±43.68 (t=2.46; p=0.0147). In addition, we found a significant variation in the values of MFP/$S_{287}$ in both groups, which proves the variability of the proliferative activity of cells during their cultivation *in vitro*, which was more pronounced in patients with hemoblastosis. Stratification of a group of patients with CMPN and a group of people without hemoblastosis into subgroups, considering the values of MFP/$S_{287}$, revealed a statistically significant difference between patients with CMPN and control subjects within each of the subgroups (Table 1).

Thus, the study conducted using the MFP/$S_{287}$ indicator allowed us to state the fact that the proliferative activity of cells and the number of metaphase plates obtained for CCS, under the same conditions of cultivation, fixation and preparation of chromosomal glass slide, can be different. It was shown that the values of the MFP/$S_{287}$ index can be variable both in blastosis caused by oncohematological pathology and in induction of cell growth by mitogen. At the same time, the proliferative activity of *in vitro* cultured leukemic cells of patients with CMPN was significantly lower than that of PHA-stimulated blood cells of subjects without hemoblastosis.

In the scientific literature, the question of the possibility and appropriateness of using venous blood of patients with CMPN for conventional cytogenetic examination remains controversial to date. At the time of initial diagnosis, during cytogenetic analysis, it is recommended to study bone marrow aspirate cells of patients with CMPN as a biomaterial, but in some cases (when blast cell circulation) it is also permissible to work with blood [4,9].

Our comparative study of the proliferative activity of leukemia cells, the source of which were venous blood and bone marrow of patients with CMPN, showed that the average value of MFP/$S_{287}$ obtained by culturing blood cells (BC) was 153.07±43.68, while the average value of MFP/$S_{287}$ obtained by culturing bone marrow cells (BMC) was 299.53±112.16 (t=1.22; p>0.2249) (Table 2). Similarly, we did not find a significant difference between the proliferative activity of BMC and BC in the subgroups "No MFP", "MFP 1-200" and "MFP 201-400".

The obtained result indicates the absence of differences in the proliferative activity *in vitro* of leukemic cells located in the bone marrow and venous blood of patients with CMPN. The only exception is the situation when the MFP/$S_{287}$ index exceeds 400 metaphases per unit area of the glass slide. In this case, the number of MFPs from the BMC significantly exceeded the number of MFPs from the BC (16.0% and 6.7%; t=2.58; p<0.05). However, in general, the number of cases when the MFP/$S_{287}$ index exceeded the value of 400 in our study was small (Table 2).

Thus, our study did not find statistically significant difference in the mean value of MFP/$S_{287}$ for BC and BMC cells in patients with CMPN. The ratio of patients with CMPN with different levels of MFP/$S_{287}$ on the glass slide was comparable for the BC and the BMC and also had no significant difference.

**Table 1.** The Proportion of Patients with CMPN and Persons without Hemoblastosis (Control) with Different Levels of MFP/$S_{287}$

| MFP /$S_{287}$ | Number of persons without hemoblastosis (n=84) | | Number of patients with CRF (n=150) | | Statistical analysis |
|---|---|---|---|---|---|
| | n | %, M±m | n | %, M±m | |
| No MFP | 2 | 2,38±1,66 | 12 | 8,00±2,22 | t=2,03; p<0,05 |
| 1-200 | 31 | 36,90±5,27 | 103 | 68,67±3,79 | t=4,90; p<0,05 |
| 201-400 | 33 | 39,28±5,33 | 25 | 16,67±3,04 | t=3,69; p<0,05 |
| >400 | 18 | 21,43±4,48 | 10 | 6,67±2,04 | t=3.0; p<0,05 |
| M±m | 286,86±32,50 | | 153,07±43,68 | | t=2,46; p=0.0147 |

**Table 2.** The Ratio of Patients with CMPN with Different Levels of MFP/$S_{287}$, the Source of which was the BC and BMC

| MFP /$S_{287}$ | Patients with CMPN | | | | Statistical analysis |
|---|---|---|---|---|---|
| | Source of metaphases: BC (n=150) | | Source of metaphases: BMC (n=150) | | |
| | n | % | n | % | |
| No MFP | 12 | 8,00±2,22 | 12 | 8,00±2,22 | t=0,00; p>0,05 |
| 1-200 | 103 | 68,67±3,79 | 96 | 64,00±3,92 | t=0,86; p>0,05 |
| 201-400 | 25 | 16,67±3,04 | 18 | 12,00±2,65 | t=1,16; p>0,05 |
| >400 | 10 | 6,67±2,04 | 24 | 16,00±2,99 | t=2,58; p<0,05 |
| M±m | 153,07±43,68 | | 299,53±112,16 | | t=1,22; p>0,2249 |

To resolve the issue of the appropriateness of using venous blood as a biological material for obtaining metaphase plates for karyotyping, we stratified the group of patients with CMPN into 2 subgroups: the first subgroup (A) included patients with a predominant amount of MFP/$S_{287}$ on BMC preparations, and the second subgroup (B) included patients with a predominant amount MFP/$S_{287}$ on BC preparations. The study showed that the ratio of patients with CMPN (n=138), in whom the average value of MFP/S287 prevailed for chromosomal glass slide from the BC (B), was significantly greater than the ratio of patients in whom the average value of MFP/$S_{287}$ prevailed for chromosomal glass slide from the BMC (A) (57.2% and 42.7%; t=2.43; p<0.05). The results obtained may indicate that in the sample of patients with CMPN studied by us, the proliferative activity *in vitro* of the BC was higher than the proliferative activity of the BMC.

A comparative analysis of the value of MFP/$S_{287}$ for preparations from BMC and BC showed that in subgroup A, the average value of MFP/$S_{287}$ for BMC was significantly higher than for BC (654.2 MFP and 143.2 MFP; t=2.29, p<0.05). In subgroup B, the mean value of MFP/$S_{287}$ for BC cells was significantly higher than for BMC (181.1 MFP and 63.9 MFP; t=3.44, p<0.05) (Table 3). It should be noted that both in subgroup A and in subgroup B statistically significant differences in the mean value of MFP/S287 for chromosomal glass slide from the BMC and BC were identified among patients with CMPN with a range of this indicator in the span of «1-200». This result confirms the data we obtained earlier that the largest ratio of patients with CMPN had values of the MFP/$S_{287}$ index in the span of «1-200» (Table 1).

Thus, venous blood can be used as a source of leukemia cells to obtain cytological preparations for the study of the karyotype of patients with CMPN using the conventional method to the same extent as bone marrow.

In general, the results obtained indicate that the MFP/$S_{287}$ indicator makes it possible to objectively assess the number of metaphase plates on chromosomal glass slide obtained for karyotype analysis, and also allows us to conclude about the level of proliferative activity of cells cultured *in vitro*. This indicator can be used to count metaphase plates obtained from both blood cells and bone marrow cells, both when assessing the growth activity of tumor cells, and when working with normal cells stimulated by mitogens.

To date, in the recommendations for the diagnosis and treatment of chronic myeloid leukemia (CML) [10] for the identification of a cytogenetic marker of pathogenetic translocation t(9;22)(q34;q11.2) – Ph-chromosome, as well as additional chromosomal abnormalities, it is proposed to use only the bone marrow as biological material. At the same time, a number of authors believe that in some cases (for example, during the circulation of blast cells) it is also permissible to work with venous blood [4,9]. The results obtained by us indicate that venous blood can be used as a source of leukemic cells to obtain chromosomal preparations for CCS in patients with CMPN to the same extent as bone marrow. Obtaining a bone marrow punctate is an invasive and painful procedure. At the same time, in some cases, it is not possible to obtain chromosomal preparations from bone marrow cells, whereas parallel cultivation of BC makes it possible to isolate the number of metaphase plates necessary for karyotyping. Since conducting a CCS is a mandatory study in patients with CMPN, it is necessary to use any methodological possibilities to obtain a result.

Thus, the MFP/$S_{287}$ indicators are an objective criterion and an effective tool for assessing the number of metaphase plates on a chromosomal preparation. The data obtained by us show the possibility of using MFP/$S_{287}$ as a criterion for the proliferative activity of cells cultured during CCS.

It should also be noted that the MFP/$S_{287}$ indicator, reflecting the proliferative activity of cells, can be used to determine the relative number of leukemia stem cells (LSCs) representing cell populations with proliferative potential and capable of generating leukemia clones. However, until now, little is known about the phenotype, function, and expression profiles of these leukemic stem cells [11,12,13]. The practical interest in determining the amount of LSCs in CML is due to their resistance to therapy, including tyrosine kinase inhibitors [14,15]. In this regard, the determination of the number of LSCs based on the MFP/$S_{287}$ indicator can become a prognostic factor of treatment failure and relapse of the disease. However, this hypothesis requires further research.

**Table 3.** The Average Value of MFP/$S_{287}$ for Chromosomal Glass Slide from the BMC and BC of Patients with CMPN

| MFP /$S_{287}$ | (A) BMC > BC | | | | Statistical analysis | (B) BC > BMC | | | | Statistical analysis |
|---|---|---|---|---|---|---|---|---|---|---|
| | BMC | | BC | | | BC | | BMC | | |
| | n | (M±m) | | (M±m) | | n | (M±m) | n | (M±m) | |
| No MFP | 0 | - | 3 | 0 | - | 1 | 0 | 6 | 0 | - |
| 1-200 | 27 | 99,96±9,15 | 48 | 58,91±6,32 | t=3,69; p=0,0004 | 51 | 73,35±6,16 | 66 | 44,77±5,15 | t=3,56; p=0,0005 |
| 201-400 | 10 | 310,30±19,61 | 6 | 258,67±19,84 | t=1,85; p=0,0870 | 19 | 280,05±8,15 | 6 | 275,83±13,52 | t=0,27; p=0,7917 |
| >400 | 22 | 1490,86±374,94 | 2 | 2034,50±1001,87 | t=0,51 p=0,6166 | 8 | 655,62±116,01 | 1 | 547±0,0 | t=0,94; p=0,3852 |
| All patients (n=138) | 59 | 654,25±207,48 | 59 | 143,24±80,96 | t=2,29; p=0,0236 | 79 | 181,10±31,63 | 79 | 63,91±12,61 | t=3,44; p=0,0007 |

## 4. Discussion

The determination of karyotype abnormalities is an integral part of modern hematology: cytogenetic analysis data are among the mandatory differential diagnostic criteria [10,16]. Thus, the diagnosis of chronic myeloid leukemia (CML) is established on the basis of clinical and laboratory studies with the mandatory detection of t(9;22)(q34;q11.2), the cytogenetic sign of which are derivatives of chromosomes 9 and 22 (Ph-chromosome) (Figure 1) [17,18,19,20].

To confirm the classical or variant t(9;22)(q34;q11), as well as to identify additional clonal chromosomal abnormalities in Ph-positive and Ph-negative cells during diagnosis, monitoring of the course and therapy of CMPN a conventional cytogenetic research of the bone marrow with analysis at least 20 metaphase [9,21]. Conventional cytogenetic research using GTG-banding technology makes it possible to assess the state of the entire karyotype and individual chromosomes, to identify marker, recurrent and unique chromosomal anomalies, including quantitative mutations, as well as interchromosomal and intrachromosomal rearrangements. Being able to cover a wide range of potential karyotype changes occurring in hemoblastoses, conventional cytogenetic research, however, has a number of limitations, the most significant of which is the need to obtain a sufficient number of metaphase plates with chromosomes identified in accordance with ISCN.

Among the total pool of leukemia cells, only a small part of them has proliferative potential, resulting from a number of genetic and epigenetic changes, as well as diversification. Being in the niches of the bone marrow, with the participation of adhesion mechanisms, LSCs are regulated by the microenvironment, surface antigens, cytokines, chemokines, level of reactive oxygen species and other intracellular signals [11,12,13,22]. At the same time, the resting LSCs may be transcriptionally inactive (silent) in CML and expression of *BCR-ABL1* will not be detected [23]. From a clinical side, LSCs in CML are of fundamental interest, since they are resistant to most modern therapies, including tyrosine kinase inhibitors, and are the main cause of treatment failure and disease recurrence [14,15].

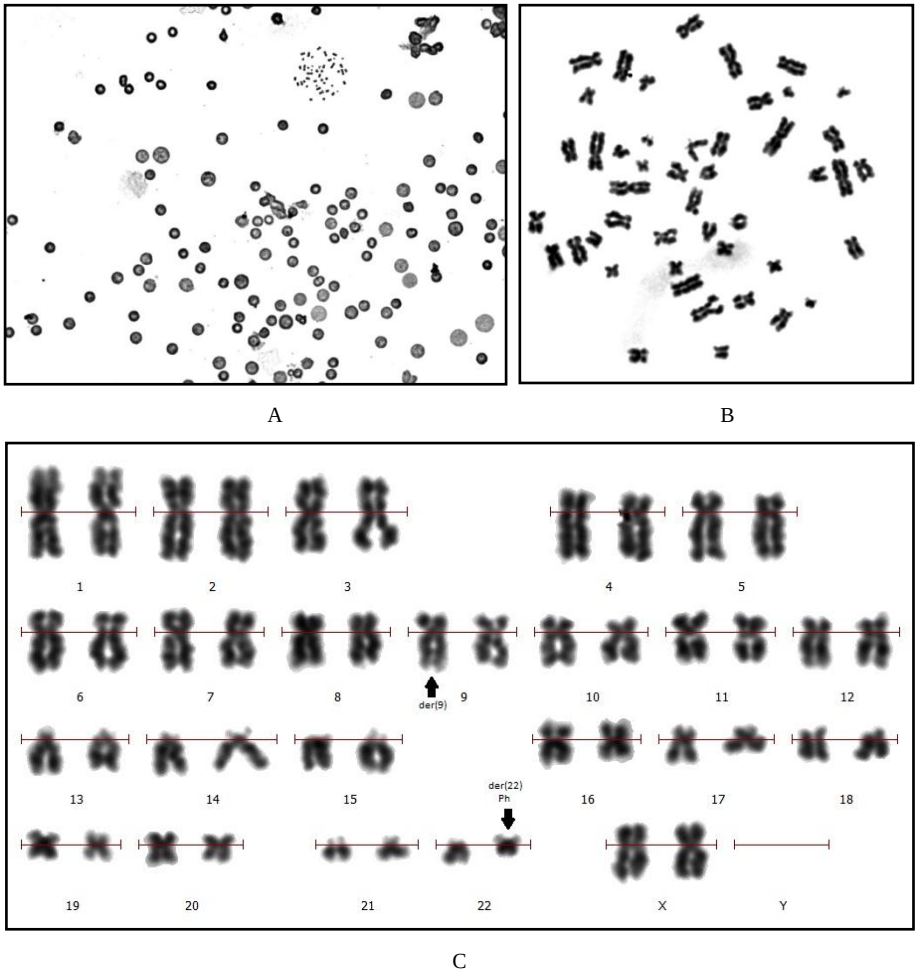


**Figure 1.** The karyotype analysis in CML diagnosis. A. Cytogenetical glass slide of a patient with CML: in the field of view are the non-dividing cell nuclei and MFP (microscope magnification ×200, camera: PROGRES® MF$^{cool}$). B. The same MFP in microscope magnification ×1000 (Oil Immersion Lens, 100×). C. A karyogram of the CML patient with chromosomal abnormalities (program for automated karyotyping: Video Test-Kario 3.1). Derivatives of chromosomes 9 and 22 (Ph-chromosome) are indicated by arrows; karyotype: 46,XX,t(9;22)(q34;q11.2)

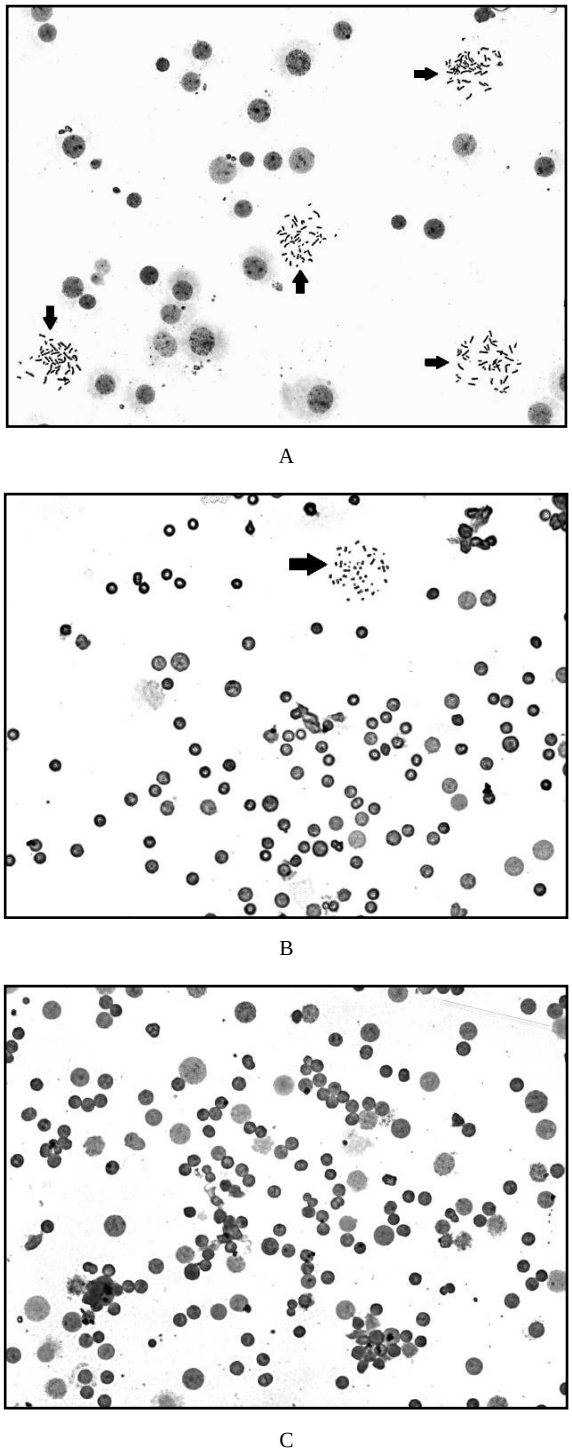

**Figure 2.** Cytogenetical preparations with a sediment. In the field of view of the microscope are the non-dividing cell nuclei and MFPs (MFPs are indicated by arrows, microscope magnification ×200, camera: PROGRES® MF$^{cool}$). A. The sediment of a patient without hemoblastosis with PHA-stimulated lymphocyte division. B, C. The sediment of a patients with CML

The loss of connection with the cells of the bone marrow microenvironment contributes to the transition of LSCs into the active growth phase of the cell cycle. The extramedullary pool of LSCs circulating in blood retains the ability to proliferate. However, despite on reduced need for external signals and high proliferative potential, the growth activity of LSCs is significantly inhibited when they cultivated in growth medium. At the same time, a decrease in the ability to divide is observed both for leukemic cells isolated from the bone marrow and for leukemic cells isolated from the blood, where they were in a free-circulating state. As practice shows, the degree of proliferative activity of leukemia cells can have significant differences, and even individual clones of leukemia cells have different proliferative potential. The reasons for the loss of proliferative activity of LSCs when they are extracted from the body and placed in cultural conditions and the detailed mechanisms of proliferation regulation in the absence of support for endogenous motivators of cell division currently remain unclear.

The absence of proliferatively active clones of leukemia cells in the cultured biomaterial is the main problem when performing CCS in patients with leukemia, and primarily in patients with myeloid hemoblastosis, since it makes it impossible to obtain important information about the state of the karyotype and the presence of chromosomal abnormalities of diagnostic and prognostic significance. If the growth of leukemia cells of lymphocytic origin can be stimulated by mitogens (phytohemaglutinin, lipopolysaccharide, pookweed), then for leukemia cells transformed from myeloid precursors, as well as undifferentiated cells, practical growth stimulators *in vitro* are not used. The significant importance has the unresolved issue that the use of cell growth stimulators in culture does not always lead to the desired effect, which is obtaining a sufficient number of dividing cells that can be stabilized at the metaphase stage. From a practical side, an important point is to find the reasons for the decrease in the growth activity of leukemia cells *in vitro* and ways to activate it in cultural conditions. All this requires a reliable and convenient method for assessing proliferative activity and the associated number of metaphases.

Most of the known methods for assessing cell proliferation cannot be used in routine practice when performing conventional cytogenetic analysis. Thus, the mitotic index (MI, ‰), which is the ratio of the number of dividing cells to the total number of cells in the observed cell population [24,25], and its variation – the metaphase index (the ratio of the number of metaphase plates to the total number of cell nuclei), cannot be used, because of the bias of the obtained indicators due to the randomness of the dispersion of metaphase plates suitable for chromosomal analysis and the significant variability in the number of nuclei of non-dividing cells. So, in the case of cytogenetic research to detect congenital chromosomal pathology when scanning cytological preparations obtained from PHA-stimulated blood cells, a small number of non-dividing cell nuclei and several metaphase plates are usually located in the field of view of the microscope (×200). With CML, the number of non-dividing cell nuclei (1000) required for the calculation of MI on a cytogenetical preparation may range from one to several dozen fields of view of a microscope (×200), whereas metaphase plates

suitable for chromosomal analysis may be unevenly scattered over a larger area of the preparation (Figure 2). A different picture is observed in myelodysplastic syndrome and in some cases of acute leukemia, when the interphase cell nuclei are single, and the search for the sufficient number of metaphase plates often requires scanning a significant area of the cytogenetical preparation.

In all these situations, the assessment of the proliferative activity of cultured *in vitro* cells, carried out by counting the number of metaphase plates (MFPs) on the area of the chromosomal preparation (S), which is equal to the visual field of ten transverse scans of a standard slide (287.50 $mm^2$), is the most objective method. The $MFP/S_{287}$ indicator makes it possible to objectively assess the number of metaphase plates on preparations obtained for karyotype analysis, and also allows us to conclude about the level of proliferative activity of cells cultured *in vitro*. This method can be used to count metaphase plates obtained from both blood cells and bone marrow cells, both when assessing the growth activity of tumor cells, and when working with normal cells stimulated by mitogens. At the same time, the $MFP/S_{287}$ index, equal to 286.86, corresponding to the average value of the number of metaphase plates of conditionally healthy subjects, can approximately serve as the value of optimal proliferative activity of cells *in vitro* during CCS.

It should be noted that the $MFP/S_{287}$ indicator allows not only to assess the level of proliferative activity of cells in culture, but also to conduct a comparative analysis of the growth activity of various types of blood and bone marrow cells in various oncohematological and lymphoproliferative diseases, as well as to evaluate the growth properties of culture media.

## 5. Conclusions

Despite the fact that the ratio of high-tech molecular genetic methods in the study of pathogenetic mechanisms of neoplastic diseases of the hematopoiesis system is huge, the cytogenetic method based on differential chromosome staining has not lost its importance. Classical karyotyping, based on cellular technologies, allows obtaining valuable information about the state of the karyotype, and serves as a conditional link between the molecular and organismic levels of human organization. Having certain advantages, the conventional cytogenetic method has a number of limitations, overcoming which can significantly increase its effectiveness. The search for opportunities to control the proliferative activity of leukemia cells in culture conditions in order to obtain metaphase plates necessary for karyotype analysis is one of the ways to optimize this technology.

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