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SYSTEMIC INFLAMMATORY INDICES IN PATIENTS WITH MALIGNANT GLIOMAS AND EFFECTS OF PLATELET SECRETOME *IN VITRO*

Background. To date, no significant clinical progress has been achieved in the treatment of brain malignant gliomas (MG), and the active search for non-invasive circulating biomarkers continues. The prognostic significance of the ratio of the main peripheral blood cell populations of patients with MG is evaluated. Considerable attention is paid to the secretome of platelets (Pt) of peripheral blood. **Aim.** To evaluate the indicators of the peripheral blood cell population ratios in patients with brain MG and to study the influence of the secretome of Pt (SPt) of the peripheral blood of patients with brain MG in cell cultures *in vitro*. **Materials and Methods.** We studied samples of peripheral blood from patients with glioma CNS WHO grade G2 (n = 5), G3 (n = 12), and G4 (n = 20). The peripheral blood cell counts were analyzed in the preoperative period on an automatic hematology analyzer. The *in vitro* study of SPt was performed on the U251 human glioblastoma cell line cultured with SPt from MG patients or SPt pre-incubated with anti-TGF-β1 antibody. Cell cultures were observed for 72 h, and mitotic index (MI) was calculated. **Results.** In MG patients, the count of peripheral blood leukocytes and neutrophils increased ($p < 0.05$). The neutrophil-to-lymphocyte ratio (NLR) and systemic immune-inflammation index (SII) increased by 2—3 times compared to control. Nevertheless, correlation analysis did not reveal significant relationships between quantitative indicators of peripheral blood cells and the tumor malignancy degree in MG patients. The MI in U251 cells increased under the influence of SPt from patients with MG ($p < 0.021$), correlated with the tumor degree of malignancy ($r = 0.246$, $p = 0.014$). Pre-incubation of SPt with anti-TGF-β1 antibody tends to neutralize this promitotic effect. **Conclusion.** In MG patients, the integral indicators of NLR and SII increased but no significant relationship with the degree of tumor malignancy was found. In U251 cells, promitotic effects of SPt of MG patients partially decreased by anti-TGF-β1 antibody.

Keywords: malignant glioma, platelets, secretome, U251 cell line, mitotic index, TGF-β1, systemic inflammatory indexes.

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Despite large-scale studies of the causes and mechanisms of growth of primary brain tumors, first of all, glial ones, no significant clinical progress in their treatment has been achieved to date. The incidence of malignant primary brain tumors in Ukraine, according to the National Cancer Registry, is 5.0% per 100,000 population, and the mortality rate is 3.5% [1]. Malignant gliomas (MG) of the brain (WHO grade 3—4) predominate among primary central nervous system (CNS) tumors, most of which are glioblastomas (glioblastoma multiforme, GBM, WHO grade 4) [2, 3]. It is believed that MG arises from glial cells and multipotent progenitor cells, tumor stem cells (TSCs), which play an important role in MG multiresistance to adjuvant treatment and progressive course [4, 5]. The active search for and improvement of methods of diagnosis and treatment of MG with the involvement of the latest cellular and molecular technologies continues.

One of the tools for diagnosis and monitoring of cancer, which is gaining clinical application, is a liquid biopsy, a method that has an additional advantage, namely non-invasiveness [6—8]. Therefore, it can be carried out more often, which allows objectively monitoring the dynamics of the disease course, the effectiveness of chemotherapy, as well as a control of possible tumor recurrence.

The importance of molecular biomarkers for tumor diagnosis, prognosis, and prediction of response to therapy is taken into account in the updated edition of the CNS tumor classification approved by WHO experts (2021) [9]. Molecular biomarkers used for the histological verification of MG have limitations: 1) not all of them are valid in terms of the prognostic potential; 2) due to heterogeneity, tumor tissue samples from the same tumor site may differ in the morphology and structure; 3) biopsy or surgical resection of tumor tissue (i.e., invasive intervention) is required. Therefore, finding and validating non-invasive circulating biomarkers (NICB) is an urgent task [10—12]. The list of potential biological sources of NICB is limited and includes peripheral blood (serum and cellular elements), cerebrospinal fluid, cystic fluid, urine, and saliva. Accordingly, cells, their proteome, secretome, transcriptome, miRNA, and extracellular vesicles (exosomes, EXs) can be used as research objects [11].

Separate studies have already evaluated the prognostic significance of the ratios of the main peripheral blood cell populations in MG patients: neutrophil (Neu) to lymphocyte (Ly) ratio (NLR), platelet (Pt) to Ly ratio (PLR), and monocyte (Mo) to Ly ratio (MLR) [13—16]. Considerable attention is paid to Pt in peripheral blood [17, 18]. Under physiological conditions, non-activated Pt circulating in the peripheral blood represents a biconvex (lens-like) structure, while the surface of activated cells is covered with branches of the cell membrane [19]. Pts are anucleated fragments formed from bone marrow megakaryocytes and contain numerous granules (α-, δ- (dense granules), γ-, λ) with peptides and low molecular weight proteins (signaling molecules) [20, 21], which are excreted during Pt activation and participate in intracellular signaling and recruitment of immune cells and progenitor mesenchymal stem cells [22, 23]. Pts as "scanning soldiers" of the immune system [17, 24] regulate inflammation and are involved in the innate and acquired immune response [25, 26]. Molecular mediators secreted by Pt induce DNA synthesis and mitotic division, stimulate cell growth, chemotaxis, angiogenesis, cell proliferation and migration, and activate the synthesis of extracellular matrix components, thus playing an important role in the processes of maintaining homeostasis, physiological healing, and recovery [22], as well as in tumor progression [27], contributing to neoangiogenesis, proliferation, metastasis, and escape of tumor cells from the immune surveillance [28].

Thrombotic and thromboembolic events are frequent complications in patients with solid tu-

mors. Pts and TSCs share a common localization in GBM tissue [29]. When infiltrating tumor tissue [30], Pts, in addition to containing numerous granules with mediators of protumoral, angiogenic action [17, 27, 31], are also able to absorb, transport, and release EXs with tumor-associated RNA and, thus, be involved in various stages of tumor emergence and progression [32]. Tumor cells can directly and/or indirectly influence the RNA content in Pt [33]: in so-called "tumor-educated platelets", the mRNA repertoire changes [34, 35]. Tumor cells, through the release of EXs, transfer mutant RNA to Pts, which absorb and transport them [31, 35], promoting the spread of tumor RNA through the bloodstream, and Pts efficiently release protumor EXs, which provides a tumor survival "strategy", as suggested by some authors [36]. Thus, the study of the Pt properties in patients with brain MG is of particular importance.

The aim of the work was to evaluate the indicators of the peripheral blood cell population ratios in patients with brain MG and to study the influence of the secretome of Pt (SPt) of the peripheral blood of the patients *in vitro* using the U-251 cell line. This cell line, isolated in the 1970s from a GBM patient, possesses such characteristic features as a high level of protein expression (GFAP, S100B, Nestin) [37, 38], high proliferative activity, and the presence of a *TP53* gene mutation [39], which makes it a valid experimental model.

Materials and Methods

Patients. We have studied peripheral blood samples from patients undergoing examination and treatment at the State Institution "Romodanov Neurosurgery Institute, National Academy of Medical Sciences of Ukraine" (SI "INS NAMS"): neuro-oncology patients (n = 37) and persons without diagnosed neoplasms (comparison group, n = 5), after provision of informed consent. In both the MG patient group and comparison group, the study participants had an approximately equal distribution by gender (40% females and 60% males). The average age of the patients was 44.5 (28—65) years for females and 56 (28—69) for males.

The diagnosis of a primary tumor was verified by histological examination: 5 cases of glioma CNS WHO grade G2 (MG G2), 12 cases of glioma CNS WHO grade G3 (MG G3), and 20 cases of glioblastoma CNS WHO grade G4 (MG G4), according to the approved WHO updated edition of the CNS tumor classification (2021) [9]. The study was conducted within the scope of scientific research work (state registration No. 0122U000327 by the protocol agreed by the Bioethics Committee of the SI "INS NAMS" (protocol No. 2 dated 04.14.2021).

The criteria for including patients in the study were:

- presence of MG;
- instrumental and histological verification of the diagnosis;
- functional status ≥ 70% by the Karnovsky scale;
- age > 18 years;
- the patient's voluntary consent to participate in the study;
- primary case of glioma.

The criteria for excluding from the study:

- presence of gross somatic pathology (state of decompensation);
- lack of multispiral computed tomography and magnetic resonance imaging data of the brain;
- functional state < 70% by the Karnovsky scale;
- age < 18 years.

Peripheral blood leukocyte (PBL) count. Pt, Neu, Ly, and Mo in the peripheral blood of patients were determined at the examination stage (in the preoperative period) on an automatic hematology analyzer Swelab Alfa (Austria), and the ratio of the main cell populations (NLR (Neu/Ly), PLR (Pt /Ly), and MLR (Mo/Ly)) were

calculated. The systemic immune-inflammation index (SII) was calculated by the formula:

$$SII = \frac{Pt \times Neu}{Ly} \quad [16]$$

Study in vitro was performed on human GBM cell line U251 (obtained from the "Bank of Cell Lines from Human and Animal Tissues", R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv).

U251 cells were cultured in MEM nutrient medium with L-glutamine (Biowest, France), 1 mM sodium pyruvate, 10% fetal calf serum (FTS, Biowest, France) in culture plastic vials (TPP Techno Plastic Products AG, Switzerland) in a CO_2 incubator (Nuve, Turkey) under standard conditions (95% humidity, 37 °C, 5% CO_2).

The nutrient medium was changed every three days. Next, the cells (2 x 10^6) were transferred to 35 mm plastic Petri dishes (SPL, Korea) containing coverslips pre-coated with polyethyleneimine (Sigma-Aldrich, GmbH, Germany), then nutrient medium (2 ml) was added, and the cells were cultured until reaching a monolayer (~70 %).

The cell growth rate constant (μ) and cell doubling time (g) were calculated using the formulas [40]:

$$\mu = \frac{ln(N_2) - ln(N_1)}{t_2 - t_1},$$

where N_1 is the initial number of cells, N_2 is the final number of cells, t_1 is the start time, and t_2 is the final time.

$$g = \frac{ln2}{\mu},$$

where μ is the cell growth rate constant.

Preparation of peripheral blood Pt secretome (SPt). Pts were isolated from the peripheral blood (10.0 ml) of patients with gliomas using differential centrifugation by the protocol [41]. The concentration of cells was brought up to 1×10^6/ml, and three cycles of freezing at –80 °C and thawing to 37 °C were performed to activate and release Pt factors. Then the cell suspension was centrifuged, and the supernatant was passed through a microbiological filter PES (pore size 0.2 μm, MDI, India) to obtain a modified medium containing SPt. The total protein concentration was determined by Lowry and by photometric test according to the Bradford method using an automated bioanalyzer Respons 920 DiaSys (Diagnostic Systems International, Germany). SPt samples were standardized to a concentration of 1.0 mg/ml, aliquoted, and stored at –20 °C.

Study of SPt effects in vitro. To study the direct effects of SPt, the samples containing SPt from patients with gliomas (MG G2, MG G3, MG G4) (0.10 ml/1 ml of nutrient medium) were added to the cultured U251 cells. In parallel, the same SPt samples were pre-incubated for 30 min with monoclonal anti-transforming growth factor-β1 (anti-TGF-β1) antibody (clone 9016.2, Sigma, USA, 0.50 μg/ml) and then added to U251 cells. The cells were incubated for 72 h under standard conditions and dynamically observed with microphotographic registration on an inverted Nikon S-100 microscope (Japan). For microscopic studies, the cells were fixed in 10% neutral formalin (Bio-Optica, Italy) and stained with hematoxylin-eosin. Microscopic examination and photoregistration of cytological preparations of cultures were carried out on a NIKON Eclipse E200 light-optical photomicroscope (Japan). The changes in the mitotic activity were determined in the growth zone. The mitotic index (MI, %) was calculated as the proportion of cells with mitoses per 100 cells. The quantitative studies of control and experimental cultures were carried out in 10 representative fields of view with a standard measuring scale (object-micrometer). The analysis was performed by processing the digital images of cultures in 10 arbitrarily selected fields of view for each sample at the same magnification using ImageView software.

Statistical analysis of the data was carried out using the package of statistical programs "Statistica 8.0", the software StatSoft, Inc. (2007). Non-parametric methods of variational statistics were applied (Kruskel — Wallis ANOVA rank discriminant analysis for multiple comparisons of several independent groups, Mann — Whitney U-test for pairwise comparison of independent groups, Wilcoxon test for pairwise comparison of dependent groups (in observational dynamics). The normality of data distribution was determined by the Shapiro — Wilk test. Data are presented as (M ± SE), where M is the mean value and SE is the standard deviation from the mean value. Correlation analysis was performed using Spearman's rank correlation. Statistically significant differences were considered at $p < 0.05$.

Results

Peripheral blood cell ratios in MG patients. The counts of PBL ($p = 0.006$), Neu ($p = 0.008$), and the indices of NLR ($p = 0.047$) and SII ($p = 0.05$) were found to be significantly increased in MG patients compared to the control group.

The PBL counts exceeded the reference values and indicators of the comparison group in all cases: in MG G2 by 1.7 times ($p = 0.00008$), MG G3 and MG G4 twice (respectively $p = 0.006$, $p = 0.016$, Fig. 1, *a*). The changes in Pt, Ly, and Mo counts varied and did not reach a statistically significant level (Fig. 1, *b*, *c*, *d*). The Neu counts exceeded the reference values in all cases: in MG G2 (twice), MG G3 (by 2.4 times, $p = 0.008$), and MG G4 (by 2.7 times, $p = 0.0002$) (Fig. 1, *e*). According to the absolute content of peripheral blood cells, the indices of PLR and MLR ratios were variable and did not reach a statistically significant level (Fig. 1, *f*, *h*). Instead, the NLR and SII ratio indices increased by 2—3 times in MG patients, reaching statistically significant differences in patients with MG G4 compared to control ($p = 0.002$, $p = 0.0032$, respectively, Fig. 1, *g*, *i*). Nevertheless, the correlation analysis of the studied sampling data did not reveal significant relationships between quantitative indicators of peripheral blood cells and the tumor malignancy degree in MG patients.

The analysis of the studied indicators by gender revealed a tendency to some excess of PLR, NLR, and SII ratio indices in female patients compared to those in male patients, which did not reach a significant level, obviously, due to small samples.

Effect of SPt of MG patients in vitro. An assessment of the growth dynamics of U251 cells showed that the growth rate constant of the culture was 0.10595 $days^{-1}$ and the doubling time of cells in the culture was 6.974 days.

After seeding U251 cells on coverslips, within 24 h the formation of multi-layered, ordered, rounded cell conglomerates was observed, which had unclear outlines with signs of cell migration to the periphery of microaggregates without distinct signs of differentiation characterized by narrow cytoplasm and moderate nuclear polymorphism, as well as cells similar to astrocytes with developed appendages (Fig. 2, *a*).

After 72 h, an increased growth activity and stratification of cellular microaggregates with a predominance of large polymorphic astrocytic cells of unipolar, rhomboid, or polygonal shape with elongated numerous processes that formed intercellular connections with a specific reticular pattern were observed. In the growth zone of cultures, the cells at different stages of the mitotic division were determined (Fig. 2, *b*), and MI reached an average of (0.78 ± 0.06)%.

U251 cells cultivated in the medium containing SPt from MG patients were characterized by active growth with the formation of spherical cell conglomerates, from which tumor cells migrated radially. A monolayer of polygonal and elongated cells with long branched processes forming reticular structures was observed between the conglomerates (Fig. 2, *b*, *c*, *d*). The growth zone

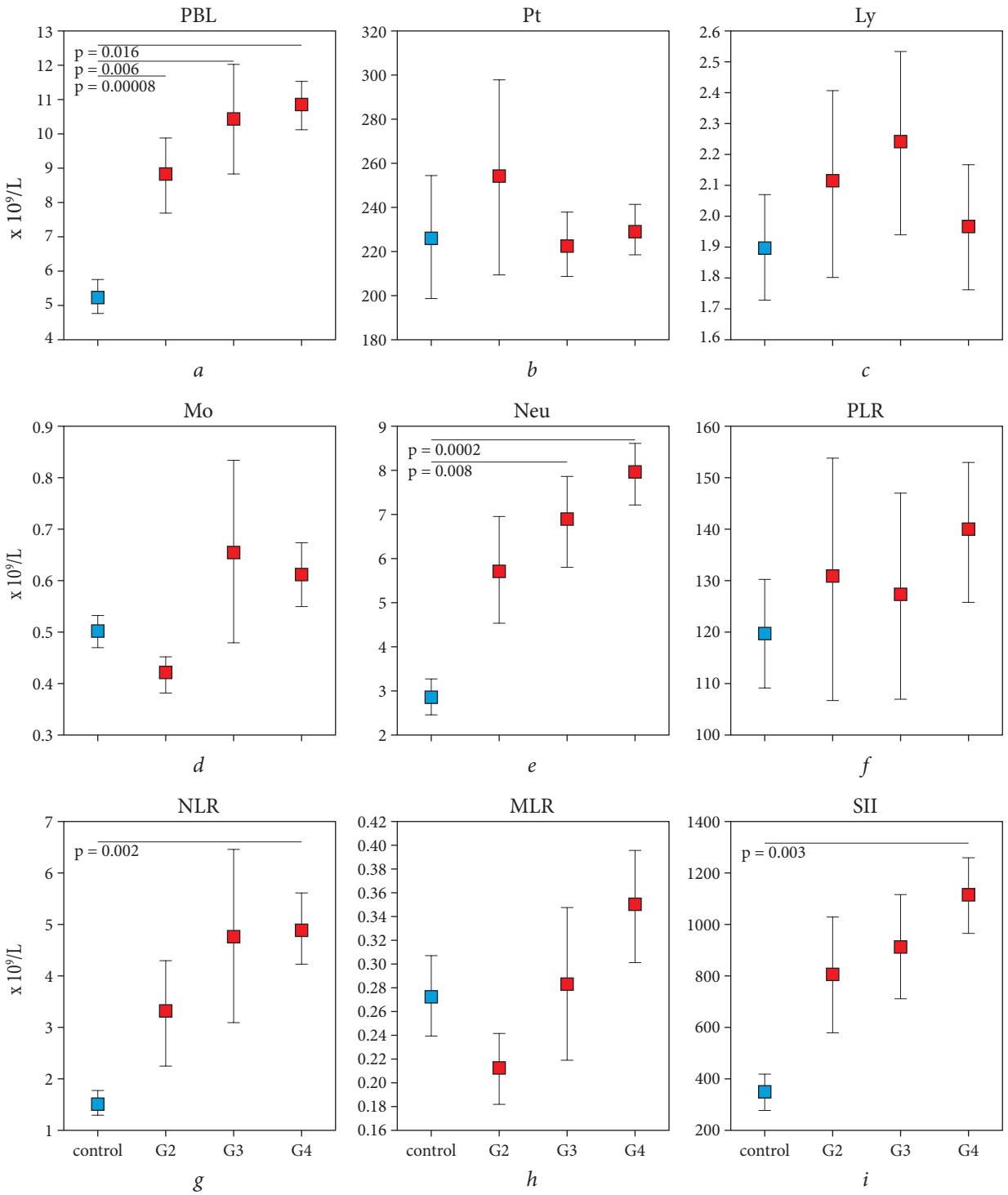

Fig. 1. Quantitative ratios of peripheral blood cells in patients with gliomas: *a* — absolute content of leukocytes (PBL), *b* — absolute content of platelets (Pt), *c* — absolute content of lymphocytes (Ly), *d* — absolute content of monocytes (Mo), *e* — absolute content of neutrophils (Neu), *f* — PLR ratio (Pt/Ly), *g* — NLR ratio (Neu/Ly), *h* — MLR ratio (Mo/Ly), *i* — systemic immune-inflammation index (SII). Control – comparison group of persons without diagnosed neoplasms (n = 5); G2 – cases of glioma CNS WHO grade G2 (n = 5); G3 — cases of glioma CNS WHO grade G3 (n = 12); G4 — cases of glioblastoma (CNS WHO grade G4) (n = 20). $p < 0.05$, Mann — Whitney test

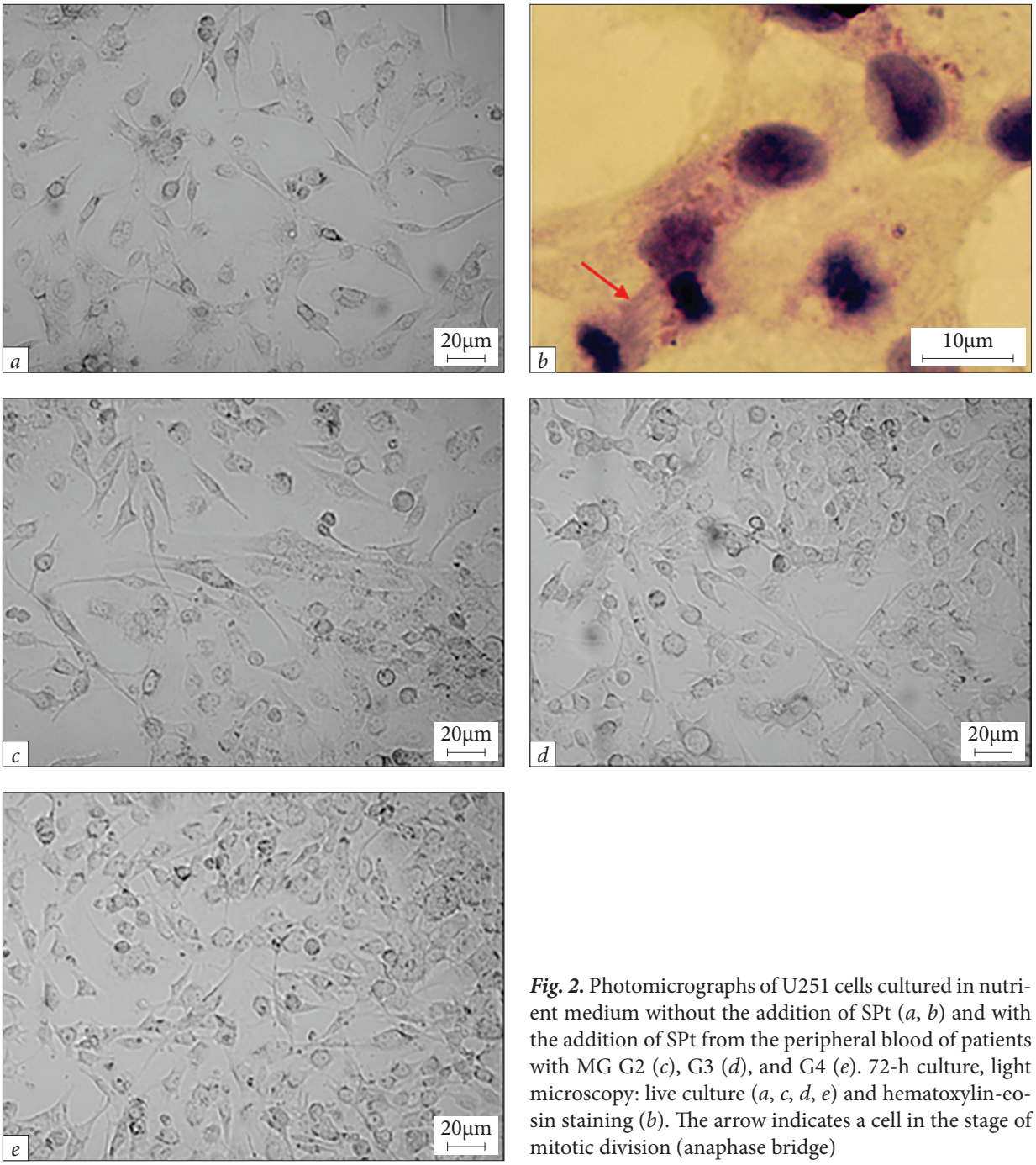

Fig. 2. Photomicrographs of U251 cells cultured in nutrient medium without the addition of SPt (*a*, *b*) and with the addition of SPt from the peripheral blood of patients with MG G2 (*c*), G3 (*d*), and G4 (*e*). 72-h culture, light microscopy: live culture (*a*, *c*, *d*, *e*) and hematoxylin-eosin staining (*b*). The arrow indicates a cell in the stage of mitotic division (anaphase bridge)

of cell cultures filled the entire area of the cultivation field.

The MI dynamics of U251 cells exposed to SPt of MG patients was characterized by a significant increase ($p = 0.00001$; Fig. 3, *a*). MI of cells cultured with SPt from patients with MG G2, MG G3, and MG G4 increased by 1.5, 1.6, and 1.8 times, respectively, compared to the control cells, which correlated directly with the degree of tumor malignancy ($r = 0.246$, $p = 0.014$).

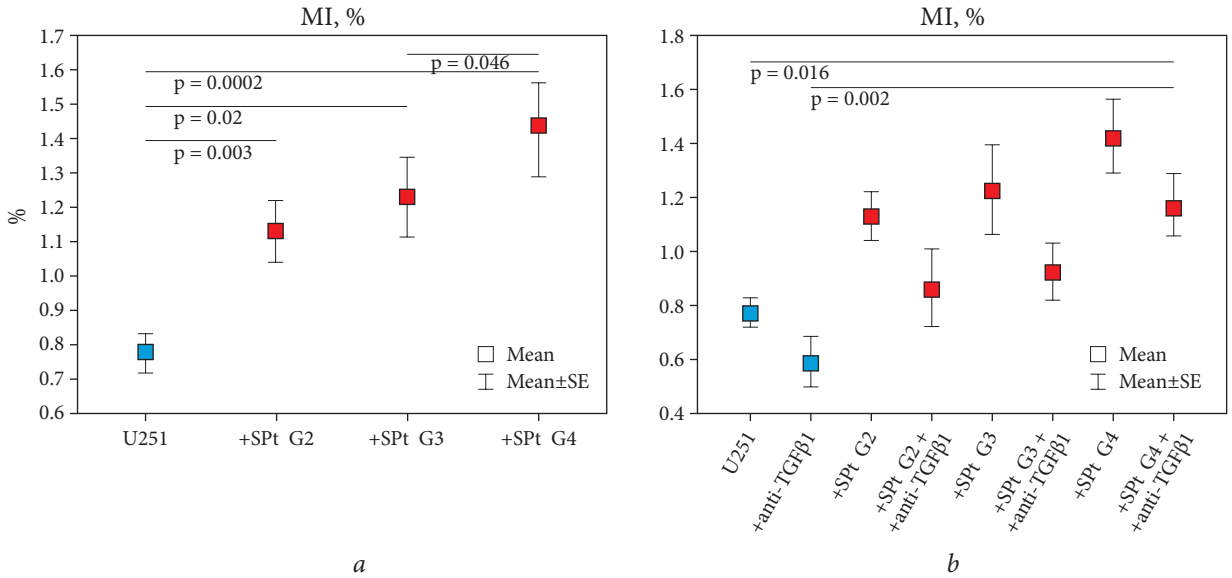

Fig. 3. Indicators of mitotic activity (mitotic index, MI, %) in U251 cells under the influence of SPt from patients with MG of various malignancy (*a*) and SPt pre-incubated with anti-TGF-β1 (*b*). SPt G2 — SPt of patients with glioma CNS WHO grade G2 (n = 5); SPt G3 — SPt of patients with glioma CNS WHO grade G3 (n = 12); SPt G4 — SPt of patients with glioblastoma (CNS WHO grade G4) (n = 20)

At the same time, the MI of U251 cells cultured with SPt from patients with MG pre-incubated for 30 min with anti-TGF-β1 antibody (0.50 μg/ml) decreased in all experimental variants by 1.3 times ($p > 0.05$) compared to the corresponding values of SPt-treated cells (Fig. 3, *b*), however, not reaching the value of the control cells. Moreover, the MI decreased also in the U251 cell line treated with anti-TGF-β1 antibody only.

Discussion

The prognostic significance of the ratio of the main cell populations of peripheral blood in MG patients has been extensively studied in recent years [13—16]. Patients included in our study showed significant signs of systemic inflammation such as increased counts of PBL and Neu, NLR, and SII. However, correlation analysis showed no significant relationships between the quantitative parameters of peripheral blood cells and the tumor malignancy degree.

Nevertheless, the integral quantitative indicators such as NLR and SII, which increased in MG patients, may be the subject of future attention, because other researchers also denoted an increase in NLR and SII in patients with gliomas, as well as the probable prognostic significance of the NLR ratio [15, 16].

According to some authors, a high PLR value in the preoperative period predicts a poor prognosis for patients with glioma [13], and a decrease in Pt count correlates with the overall survival [14, 17]. At the same time, the increased number of Pt correlates positively with the proliferation of TSCs and negatively with the overall survival of patients with GBM [29]. In our study, no unidirectional significant changes in Pt counts in the peripheral blood of MG patients and, accordingly, in the PLR index were found.

The Pt-released mediators are the basis of their protumoral activity representing a fundamental component of the tumor microenvironment (TME) and an important aspect of tumor biology participating in tumor initiation and

progression. TME also modulates the Pt function, directly inducing Pt aggregation with tumor cells and triggering the release of granules. At the same time, Pts absorb and transport tumor EXs with mutant RNA, and also actively and effectively release protumor EXs, contributing to tumor spread [31, 35, 36]. The so-called "tumor-educated" Pts have an altered mRNA profile [34, 35] and an increased content of angiogenesis regulatory proteins (VEGF, PDGF, bFGF) [42]. They enhance the dissemination of tumor cells, activating the function of endotheliocytes and recruiting immunocytes to primary and metastatic tumors [43]. It should be noted that changes in the blood-brain barrier in MG deserve special study, but indirectly the content of biologically active substances depends on the degree of changes in its structure in case of the occurrence and progression of tumors.

Taking into account the above data on Pt mediators and their effects, we studied the influence of SPt of the peripheral blood of patients with MG *in vitro* on U251 cells. SPt from MG patients caused a significant increase in their mitotic activity in direct correlation with the degree of tumor malignancy. These results are generally consistent with the data [29], which showed a 3-fold increase in the proliferation of TSC cell lines isolated from MG patients.

The obtained data are consistent with known data on the ability of Pt to secrete growth factors from α-granules, such as PDGF, TGF-β, IGF, VEGF, EGF, and bFGF, which regulate migration and cell proliferation and exert a mitogenic effect [20—22, 27, 28]. Among the listed growth factors produced by Pt, potentially included in the SPt of MG patients, our attention was drawn to the pleiotropic cytokine TGF-β known as an important agent of the malignant phenotype of human brain glioma, as its expression is significantly increased in high-grade gliomas. It potentially acts as a promoter of cell motility, invasion, metastasis, and maintenance of TSCs. As a result of mutations in MG, the TGF-β Smad-dependent canonical signaling pathway is switched to a Smad-independent one [44]. It is also known that Pt, due to the release of TGF-β, can provoke the epithelial-mesenchymal transition and reduce tumor cell apoptosis [24, 45].

With the above in mind, we conducted additional studies of U251 cells under the influence of SPt from patients with MG pre-incubated with anti-TGF-β1 antibody. Such a pretreatment caused a decrease in MI of the cultured U251 cells and evidenced the neutralizing effect of anti-TGF-β1 on SPt of MG patients, indicating that its promitotic effect is partially caused by the presence of TGF-β1 in the composition of SPt.

Thus, SPt in MG cases can be considered an important component of the microenvironment and a fundamental aspect of tumor biology due to its influence on the processes of tumor initiation and progression and can serve as a potential NICB of molecular diagnosis and response monitoring at different stages of cancer treatment.

The obtained results indicate the important role of Pt in the morphogenesis of MG and justify the expediency of further in-depth research and clinical and morphological comparison.

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ДОСЛІДЖЕННЯ *IN VITRO* ЕФЕКТІВ СЕКРЕТОМУ ТРОМБОЦИТІВ ПЕРИФЕРИЧНОЇ КРОВІ ПАЦІЄНТІВ ІЗ ЗЛОЯКІСНИМИ ГЛІОМАМИ

Стан питання. На сьогодні не досягнуто значного клінічного прогресу в лікуванні злоякісних гліом (ЗГ) головного мозку; триває активний пошук неінвазивних циркулюючих біомаркерів. Оцінюється прогностична значущість кількісних співвідношень основних клітинних популяцій периферичної крові хворих зі ЗГ. Значну увагу приділяють тромбоцитам (Тр) периферичної крові через вміст гранул із сигнальними молекулами, які секретуються назовні та беруть участь у внутрішньоклітинній передачі сигналів, прогресії пухлин, неоангіогенезі, проліферації, метастазуванні та уникненні імунного нагляду. **Мета.** Вивчити *in vitro* дію секретому Тр (СТр) периферичної крові пацієнтів з гліомою головного мозку в культурах клітин гліобластоми людини лінії U251 та оцінити її зв'язок з кількісними показниками співвідношень клітинних популяцій периферичної крові. **Матеріали та методи.** Дослідження проведено в культурах клітин U251 з використанням СТр периферичної крові пацієнтів з гліомами 2-го (G2, n = 5), 3-го (G3, n = 12) та 4-го (G4, n = 20) ступенів злоякісності (відповідно до затвердженої ВООЗ оновленої редакції класифікації пухлин ЦНС). Формували групи культур клітин U251: 1) без додавання СТр (контроль); 2) з додаванням СТр пацієнтів зі ЗГ; 3) з додаванням СТр, попередньо проінкубованих протягом 30 хв з антитілами до TGF-β1; 4) з додаванням антитіл до TGF-β1. За культурами клітин спостерігали протягом 72 год і визначали мітотичний індекс (MI, %). Дослідження вмісту клітинних популяцій периферичної крові пацієнтів проводили в доопераційному періоді на автоматичному гематологічному аналізаторі. **Результати.** У пацієнтів зі ЗГ підвищувався вміст лейкоцитів периферичної крові та нейтрофілів ($p < 0,05$). Співвідношення нейтрофілів до лімфоцитів (СНЛ) та індекс системного запалення (ІСЗ) зростали у 2—3 рази в порівнянні з контролем, однак зв'язку цих показників зі ступенем злоякісності пухлин виявлено не було. За впливу СТр пацієнтів у культурах клітин U251 підвищувався MI ($p < 0,021$), корелюючи зі ступенем злоякісності пухлини ($r = 0,246$, $p = 0,014$), натомість у разі СТр, попередньо проінкубованих з антитілами до TGF-β1, він зменшувався ($p > 0,05$). **Висновки.** У пацієнтів зі ЗГ підвищуються інтегральні показники СНЛ та ІСЗ. У культурах клітин гліобластоми людини лінії U251 встановлено промітотичні ефекти після впливу СТр пацієнтів зі ЗГ, які корелюють зі ступенем злоякісності пухлини. Попередня інкубація СТр пацієнтів зі ЗГ з антитілами до TGF-β1 частково нейтралізує їхні промітотичні ефекти.

Ключові слова: злоякісна гліома, тромбоцити, секретом, U251, мітотичний індекс, TGF-β1.