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ORIGINAL ARTICLE

Changes in the concentration of freely circulating mutant DNA and wild-type DNA of the *H3F3A* (K27M) gene in the blood and cerebrospinal fluid of children with diffuse midline gliomas during a course of radiation therapy

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ABSTRACT

Purpose of the study. To study the possibility of detecting freely circulating DNA of the *H3F3A* (K27M) gene in blood plasma and cerebrospinal fluid in the lumbar spine in children with diffuse midline gliomas (DMG) during a course of radiation therapy (RT).
Materials and methods. Molecular genetic studies were carried out by digital PCR. 96 samples of lumbar cerebrospinal fluid and 288 samples of peripheral blood plasma from 96 pediatric patients were analyzed. The concentration of circulating tumor (ctDNA) mutant DNA and wild-type DNA of the *H3F3A* (K27M) gene was determined in the studied material against the background of a course of RT. Lumbar cerebrospinal fluid sampling was performed once at the beginning of therapy, blood sampling was performed three times: The 1st test before the start of RT, the 2nd against the background of a total dose 10–15 Gy, and the 3rd after the completion of the RT course. Patients are divided into the following groups: patients with stabilization of brain tumor growth during early magnetic resonance (MR) control 3 months after completion of the course of RT; patients with disease progression during the same follow-up period who underwent radiation or chemoradiotherapy.
Results. When the disease stabilized after a RT course during treatment, the concentration level of both the mutant variant of ctDNA and wild-type ctDNA significantly decreased in the third blood fraction. The absence of changes or an increase in the concentration of mutant ctDNA and wild-type ctDNA of the *H3F3A* (K27M) gene by the end of the course of radiation therapy was typical for patients with disease progression in the form of the appearance of metastatic foci in the central nervous system or continued tumor growth. At the same time, the concentration of wild-type DNA of the *H3F3A* (K27M) gene in the group of patients with progression was higher both in the lumbar cerebrospinal fluid and in the first fraction of blood plasma.
Connclusion. Determination of the concentration and dynamics of circulating tumor DNA of the mutant and wild type of the *H3F3A* (K27M) gene in blood plasma and lumbar cerebrospinal fluid in children with diffuse median gliomas of the brain during radiation therapy is promising from the point of view of predicting the effectiveness of therapy.

Keywords: glioma, diffuse median glioma, digital drip PCR, *H3F3A*, K27M, circulating tumor DNA (ctDNA)

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Compliance with ethical standards: this research has been carried out in compliance with the ethical principles set forth by the World Medical Association Declaration of Helsinki, 1964, ed. 2013. The study was discussed and approved at a meeting by the Scientific Council of the Russian Scientific Center of Roentgenoradiology (Scientific Protocol No. 3/2022, 12/12/2022, Protocol No. 7). Informed consent was received from all the participants of the study

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3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

Изменения концентрации свободно циркулирующей мутантной ДНК и ДНК дикого типа гена *H3F3A* (K27M) в крови и люмбальном ликворе у детей с диффузными срединными глиомами на фоне курса лучевой терапии

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РЕЗЮМЕ

Цель исследования. Изучить выявляемость свободно циркулирующей ДНК гена *H3F3A* (K27M) в плазме крови и люмбальном ликворе у детей с диффузными срединными глиомами (ДСГ) на фоне курса лучевой терапии (ЛТ).
Материалы и методы. Молекулярно-генетические исследования проводились методом цифровой полимеразной цепной реакции (ПЦР). Проанализировано 96 образцов люмбального ликвора и 288 образцов плазмы периферической крови 96 пациентов детского возраста. В исследуемом материале определялась концентрация циркулирующей опухолевой (цоДНК) мутантной ДНК и ДНК дикого типа гена *H3F3A* (K27M) на фоне проводимого курса ЛТ. Забор люмбального ликвора проводился однократно в начале ЛТ, забор крови – трижды: 1-я проба – до начала ЛТ, 2-я проба – на фоне суммарной очаговой дозы (СОД) 10–15 Грей (Гр), и 3-я – после завершения курса ЛТ. Пациенты, которые получили лучевую или химиолучевую терапию, были разделены на следующие группы: 1-я группа включала в себя пациентов со стабилизацией роста опухоли головного мозга в сроки раннего магнитно-резонансного (МР) контроля, 2-я группа - пациентов с прогрессированием заболевания.
Результаты. При стабилизации заболевания после проведенного курса ЛТ на фоне лечения уровень концентрации как мутантного варианта цоДНК, так и цоДНК дикого типа достоверно снижался в анализе крови при третьем заборе. Отсутствие изменений или увеличение уровня концентрации мутантной цоДНК и цоДНК дикого типа гена *H3F3A* (K27M) к концу курса ЛТ было характерно для пациентов продолженным ростом опухоли с прогрессированием заболевания в виде появления метастатических очагов в центральной нервной системе. При этом концентрация ДНК дикого типа гена H3F3A (K27M) в группе пациентов с прогрессированием была более высокой как в люмбальном ликворе, так и в анализе крови при первом заборе.
Заключение. Определение концентрации и динамики циркулирующей опухолевой ДНК мутантного и дикого типа гена *H3F3A* (K27M) в плазме крови и люмбальном ликворе у детей с диффузными срединными глиомами головного мозга в процессе ЛТ является перспективным с точки зрения прогноза эффективности проводимой терапии.

Ключевые слова: глиома, диффузные срединная глиома, цифровая капельная ПЦР, ген *H3F3A*, мутация K27M, циркулирующая опухолевая ДНК (цоДНК)

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование обсуждено и одобрено на заседании Ученого совета ФГБУ «Российский научный центр рентгенорадиологии» Министерства здравоохранения Российской Федерации (научный протокол № 3/2022 от 12.12.2022 г., протокол № 7). Информированное согласие получено от всех участников исследования

Финансирование: финансирование данной работы не проводилось

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи

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INTRODUCTION

Over the past 10 years, there has been a fundamental paradigm shift in the field of diffuse midline gliomas (DMG) diagnosis, where among the most significant discoveries is the K27M mutation in the *H3F3A* or *HIST1H3B* genes, which encode histone variants H3, H3.3 and H3.1. The H3K27M mutation gives odds in gliomagenesis a head start due to persistent clonogenicity and aberrant differentiation and determines the associated changes in histone and DNA methylation [1]. The preservation of proliferative clonogenic states increases the likelihood of acquiring additional mutations in nascent neomorphic cells. In addition, aberrant differentiation can change the organization of tissues and create a microenvironment that promotes the development of tumors. Both of them are potential consequences of the H3K27M mutation, and may contribute to the occurrence of DMG [1]. To date, the detection of the K27M mutation in the *H3F3A* gene is recommended in a number of foreign countries to assess the prognosis of the disease and the choice of treatment tactics [2–4]. The detection of a mutation in DMG is associated with an extremely aggressive clinical course and an unfavorable prognosis, [5–8] regardless of histological examination data, therefore, when the K27M mutation is detected in the *H3F3A* gene, the tumor is classified as grade 4 malignancy [4, 6, 7].

When comparing adult and pediatric patients with central nervous system (CNS) tumors, it was shown that in adults, the K27M mutation occurs with the highest frequency in high-grade gliomas (HGGs) of the thalamus and spinal cord, and in children – with diffuse median gliomas of the brain, while the frequency of the K27M mutation in the *H3F3A* gene can reach 94 % [6, 9, 10]. An important difference is that in children suffering from DMG, the presence of the K27M mutation is an extremely unfavorable prognostic factor, and for supratentorial gliomas in adults, this gene change is not clinically significant. There were no significant differences in survival and clinical course of the disease for adult patients with and without the K27M mutation, H3K27M may be present both in histologically verified HGGs and in low-grade gliomas (LGGs) [9].

In children with HGG with the K27M mutation, they have a more aggressive clinical course in comparison with HGG of a different genetic nature. In this regard, the identification of mutant forms has prognostic value and most studies are focused specifically on the study of the mutant DNA of the *H3F3A* gene in DMGs, especially among children [2]. At the same time, there is practically no information on the prognostic role of determining changes in the concentration of wild-type DNA during treatment and the ratio of concentrations of mutant DNA and wild-type *H3F3A* gene [11] among diffuse midline gliomas, although for tumors of other localizations, an increase in the concentration of wild-type DNA is a poor prognostic factor [12, 13].

In DMGs, due to the peculiarities of the anatomical location of tumors, it is difficult to obtain histological material using surgical intervention. Unfortunately, the use of targeted stereotactic biopsy does not always allow to obtain an adequate amount of material for histological and molecular analysis [14, 15]. When a diffuse tumor is biopsied, several samples are taken from different points, which sometimes does not allow to identify intra-tumor heterogeneity for an accurate diagnosis [16]. The use of a liquid biopsy method aimed at identifying biological markers by analyzing circulating tumor DNA (ctDNA) in blood plasma and lumbar liquor samples makes it possible to determine the molecular profile of a tumor without using traumatic invasive techniques. Modern approaches to monitoring the course of the disease that meet international standards use radiographic imaging – magnetic resonance imaging (MRI) to determine how the tumor reacts to treatment. It is worth noting that performing an MRI examination is an expensive procedure, and often, in the case of pediatric patients, requires the use of an anesthetic aid, which is difficult to access in the regions. In the case of DMG H3K27M, diffuse tumor growth and radiation-induced edema complicate the interpretation of images under dynamic observation. Studies have shown that the levels of tumor biomarkers in biological media, such as blood or cerebrospinal fluid, correlate with the course of the disease. Thus, consistent quantification of these biomarkers can help identify disease progression in advance. The diagnostic potential of using liquid biopsy in children with DMG has not been fully disclosed, although research in this direction is actively underway [7, 17]. In addition, the use of liquid biopsy in pediatric neuro-oncology lags behind similar methods in adults, however, these studies show that the technology has significant potential [7, 17–19].

The most accessible material for the liquid biopsy method is blood plasma, and for many solid tumors, the determination of ctDNA in plasma is an important diagnostic method [6, 7]. However, in DMG, the blood-brain barrier (BBB) significantly restricts the flow of ctDNA into the blood [20], therefore, an alternative source of ctDNA is the lumbar cerebrospinal fluid [7, 21]. In CNS tumors in children, molecular examination of the lumbar cerebrospinal fluid can also be a significant alternative to morphological verification at high risks or inability to obtain biopsy material [19].

To date, the most sensitive method for evaluating ctDNA, which allows obtaining adequate results with a small amount of material, is the digital drip PCR method [7]. In recent years, the number of studies conducted by this method has increased many times, including in CNS tumors [7, 11].

An integrated approach in liquid biopsy studies, especially in DMG, should include a combination of the choice of research material and molecular analysis methods [6, 7]. In recent years, the gold standard has become the study of ctDNA in both plasma and cerebrospinal fluid, which allows us to obtain the most accurate molecular data necessary for the diagnosis and prognosis of the course of the disease.

In this work, we examined the ctDNA of the *H3F3A* (K27M) gene both in blood plasma and in lumbar cerebrospinal fluid in children with diffuse midline gliomas during radiation therapy. Special attention was paid not only to the detectability of mutant ctDNA, but also to the ratio of the amount of mutant ctDNA to wild-type ctDNA – variant allele fraction (VAF), the change in H3.3K27M VAF over time ("delta VAF"), as well as its correlation with various clinical parameters [22]. In our opinion, the study of patients without the H3.3K27M mutation is important, but poorly studied. To date, we have not found information in the available literature on changes in the concentration of wild-type DNA of the *H3F3A* gene against the background of radiation therapy in children, which confirms the relevance of our work and the need for further development of molecular diagnostics and personalized therapy of DMG.

MATERIALS AND METHODS

The study included 96 children with diffuse midline gliomas of the brain who underwent radiation and chemoradiotherapy at the Department of Russian Scientific Center of Roentgenoradiology in the period from 2022 to 2024. The study cohort consisted of 53 (55 %) boys and 43 (45 %) girls aged 18 months to 18 years, the average age at the time of diagnosis was 8 years. Clinical indicators included gender, age, and the nature of disease progression – the appearance of metastatic dropouts in the central nervous system or continued tumor growth, but they had no significant differences in the study groups and associations with DNA concentrations in blood plasma and lumbar liquor ($p > 0.05$). When conducting an instrumental examination of patients based on the results of MRI of the brain natively and with contrast enhancement before the start of therapy, it was found that in all patients the tumors had diffuse growth and median location. Histological examination of tumors in 18 cases showed that HGG prevailed mainly, 6 of them had a K27M mutation in the *H3F3A* gene. The assessment of groups with continued growth and stabilization of the disease was carried out on the basis of MRI data of the brain without and with contrast enhancement (CE), performed within 3–4 months after completion of the course of RT.

The scheme of radiation therapy

Radiation therapy was performed using Varian Clinac 2100 linear accelerators, True Beam, and the Varian Eclipse dosimetric calculation system. During therapy, the traditional version of fractionation of the dose of 1.8–2 Gy was used, with a total focal dose of up to 54 Gy. In the presence of pronounced perifocal edema and symptoms of developing intracranial hypertension, treatment began in the mode of multifractionation in single doses of 1.0–1.1 Gy 2 times a day with an interval between fractions of 4–6 hours with a gradual transition to the usual fractionation mode as the condition stabilizes, but with a correction of the total dose over the period of multifractionation in the direction of its increase equivalent to 54 Gy. In patients with a histologically confirmed diagnosis of HGG, a course of RT was performed with parallel radio modification with temozolomide, 75 mg/m^2, daily against the background of the entire course of RT.

Obtaining research material

During the study, we received samples of peripheral blood plasma and lumbar cerebrospinal fluid from 97 patients. Samples of lumbar cerebrospinal

fluid were taken once against the background of radiation therapy. Blood plasma was taken at three stages: before the start of therapy, during radiation therapy and after completion of the course of radiation therapy.

Isolation of circulating DNA from lumbar cerebrospinal fluid

To isolate ctDNA from the lumbar liquor, we used Sileks kits, which are based on the use of SileksMagNA-Direct particles (particles for selective binding of nucleic acids). The extraction procedure was carried out according to the protocol provided by the manufacturer. The collection of cerebrospinal fluid and the beginning of the procedure for isolation of circulating tumor DNA did not exceed 30 minutes. The lumbar liquor was centrifuged at 1,500 revolutions per minute for 5 minutes, and a superabsorbent fraction with a volume of 0.7 to 2 ml was used to isolate ctDNA. In our work, mutant ctDNA of the *H3F3A* gene was isolated from 96 cerebrospinal fluid samples in 33, and wild-type ctDNA of the *H3F3A* gene was isolated in all 96 cerebrospinal fluid samples (Fig. 1).

Isolation of circulating DNA from blood plasma

Plasma preparation. Plasma was separated immediately after receiving a blood sample. Sileks kits based on SileksMagNA-Direct particles were used to isolate circulating DNA from blood plasma. The isolation procedure was carried out according to the manufacturer's protocol. Of the 288 peripheral blood plasma samples obtained, mutant ctDNA of the *H3F3A* gene was isolated in 29, wild-type ctDNA of the *H3F3A* gene was isolated in all studied samples.

Determination of the K27M mutation in the *H3F3A* gene by digital droplet PCR (ddPCR)

Highly sensitive screening of the *H3F3A* (K27M) mutation using Digital Droplet PCR (ddPCR) technology using the *H3F3A* (K28M) Screening Kit (Bio-Rad, USA) and the QX100 Droplet Digital PCR System (Bio-Rad, USA) was used.

For ddPCR formulation, BioRad reagents were used according to the research protocol. The DNA probes used to detect the amplification products of the studied and normalizing genes were labeled FAM and HEX. The PCR mixture was placed in a droplet generator, where a water-oil emulsion was created from 20 µl of the sample in which the amount of DNA under study was to be determined, and up to 20,000 drops of 1 nl were formed in each tube. In this case, the genetic material is randomly distributed into droplets: both target DNA and background DNA fall into them. The process of distributing the target DNA by droplets is purely random and obeys the law of distribution of small Poisson numbers. Before dividing the sample into drops, it is not nec-

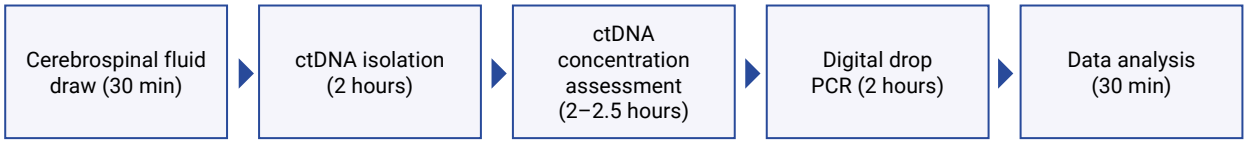

Fig. 1. Isolation of circulating tumor DNA by drip PCR from lumbar cerebrospinal fluid

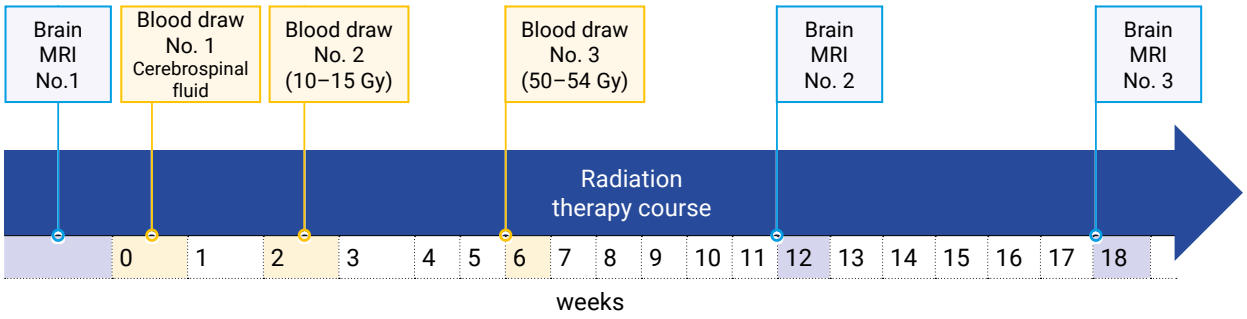

Fig. 2. Study design

essary to dilute it to a concentration so that each drop contains either 0 or 1 copy of the target DNA: when analyzing the results, situations are taken into account when there is more than one copy of the target in one drop. According to the Poisson distribution, either one matrix chain gets into the drop, or none gets into it. Samples were transferred from the droplet generator to the applicator. The amplification was carried out in "real time" mode. After amplification, the tablet was placed in a "BIO-RAD QX 100TM DROPLET READER" device, where the signal from the fluorescent labels was read. In a drop with a matrix, amplification is many times more efficient than with other types of PCR, which is due to the presence of all components of the PCR mixture in the nanoscale. During amplification, enzymatic cleavage of TaqMan probes occurs, as a result of which the fluorescence efficiency of the droplet increases many times. The product accumulated during the amplification is detected in each drop separately, at a rate of 1500 drops/s. Based on the ratio of the total number of microdrops and the number of microdrops in which the fluorescence level exceeds the background, the reader calculates the absolute amount of DNA in one microliter of the sample. The results were recorded in the "Quanta Self 16" program.

The database of clinical cases was formed using electronic databases of Microsoft Excel tables. Statistical processing was carried out using the SPSS software for Windows, version 26.0 (SPSS, Chicago, Illinois, USA) and Statistica, version 13.

The normality of the sample distribution was checked using the Kolmogorov-Smirnov criterion. The reliability of the differences was determined using the Mann-Whitney criterion. The exact one-sided Fisher criterion was used to evaluate qualitative features. The results of comparing quantitative data were considered statistically significant at $p < 0.05$.

STUDY RESULTS AND DISCUSSION

1. Mutation analysis

In recent years, scientists have focused on H3.3K27M mutant DMG [19], since the *H3F3A* (K27M) mutation is more common than G34V/R mutations in children with highly malignant diffuse astrocytomas [23–25]. This mutation is considered as a potential diagnostic marker for the identification of these tumors, similar to the use of IDH1/2 mutations for the diagnosis of diffuse gliomas in adults.

All H3K27M mutations described in DMG in most cases have the same epigenomic consequences for the PRC2 complex (PRC2 – Polycomb repressive complex 2 – conservative protein complex) as a whole [26, 27], despite the different functions and genomic distribution of many variants. It is important to note that the life expectancy of patients largely depends on the type of histone where the K27M mutation is present. Back in 2014, Wu et al. It was found that patients with a mutation in histone H3.1 respond better to radiation therapy, have a less aggressive course and are less likely to have metastases [28]. Therefore, the assessment of the type of histone mutation can be used as a predictive stratification factor in future prospective studies [4].

In this study, variants of allelic fractions (VAF) H3.3K27M were evaluated – the ratio of the concentration of freely circulating mutant DNA to the wild-type DNA of the *H3F3A* (K27M) gene in samples of lumbar liquor, as well as in blood plasma. At the same time, blood plasma samples were taken before the start of radiation therapy (sample 1), during radiation therapy (sample 2) and after the end of radiation therapy (sample 3) (Fig. 2). A total of 8 parameters were evaluated. Thus, we determined the concentration and assessed the dynamics of changes, against the background of radiation therapy, not only mutant DNA, but also wild-type DNA of the *H3F3A* (K27M) gene. It is necessary to understand the molecular features of the development of DMG from various angles in order to make it possible to improve and create new therapeutic strategies.

2. Correlation status of the *H3F3A* (K27M) gene and clinical and pathological characteristics

Previous studies have shown that K27M-mutant DSGs are associated with significantly shorter-term survival [28]. Moreover, in a multivariate analysis that also took into account the effect of treatment, the type of histone H3 mutation was a more accurate predictor of survival duration than the assessment of the clinical and radiological risk of DMG [11, 30].

When analyzing the data we obtained, we established the presence of a significant correlation between progression and mutant ctDNA of the *H3F3A* (K27M) gene obtained from the lumbar liquor fraction (Table 1).

In a recent preclinical study, Grasso et al., investigating the efficacy of panobinostat in DMG, established its effectiveness against cells containing both mutant DNA and wild-type DNA for the *H3F3A* (K27M) gene *in vitro*, although cells with the H3K27M mutation developed resistance to panobinostat within a few weeks after exposure to low doses of the drug. It is worth noting that panobinostat treatment significantly prolongs the survival of mice with tumors without mutation in the *H3F3A* gene [31]. These results led to the initiation of NCT02717455 (clinaltrials.gov), a clinical trial of panobinostat (Phase I–LBH589) conducted by the Pediatric Brain Tumor Consortium (PBTC) for the treatment of children with recurrent or progressive HGG.

In this regard, we paid special attention to the change in the concentration of wild-type DNA for the *H3F3A* (K27M) gene, suggesting that this phenomenon may become one of the effective prognostic markers of tumor progression and the effectiveness of therapy. The analysis of the mutual correlations of the concentrations of wild and mutant DNA of this gene in different fractions of blood plasma showed a number of interesting dependencies, for example, the concentration of mutant DNA K27M in the lumbar liquor had highly reliable correlations with the concentration of the same mutant DNA in the first and second fractions of blood plasma (Table 2).

There was also a high correlation of the concentration of mutant DNA of the *H3F3A* (K27M) gene in the second fraction of blood with cerebrospinal fluid, with the first fraction and the third, as well as with the concentration level of the wild-type gene in the third blood sample (Table 3).

Evaluation of the results of the study of the mutation status in blood plasma and lumbar cerebrospinal fluid of patients using ddPCR showed high

Table 1. Correlation between mutant DNA of the *H3F3A* gene (K27M) and disease progression

Value	Correlations are significant when $p < 0.05$
	Progression
K27Mmut in cerebrospinal fluid	0.271440

Table 2. Correlation between the mutant DNA of the *H3F3A* gene (K27M) in cerebrospinal fluid and the first two blood samples on the background of RT

Value	Correlations are significant when $p < 0.05$
	mut(K27M cerebrospinal fluid)
mut(K27M draw 1)	0.555019
mut(K27M draw 2)	0.384082

Table 3. Correlation between the mutant DNA of the *H3F3A* (K27M) gene in the second blood sample and cerebrospinal fluid draws, the first and third blood samples against the background of RT mutant variant of the gene, as well as the wild-type gene in the third sample

Value	Correlations are significant when $p < 0.05$
	mut(K27M draw 2)
mut(K27M cerebrospinal fluid)	0.384082
mut(K27M draw 1)	0.211165
mut(K27M draw 3)	0.360417
wt(K27M draw 3)	0.390472

informativeness of both blood plasma and lumbar cerebrospinal fluid, which is confirmed in studies where ctDNA are found in the blood and lumbar cerebrospinal fluid in the blood and lumbar cerebrospinal fluid, which are more sensitive and can reflect various types of mutations in glioma cells [11, 32, 33]. At the same time, it was previously stated that the presence of BBB means that the cerebrospinal fluid can provide a more detailed characteristic of the tumor than blood plasma, and contains certain biomarkers that are unlikely to be detected in plasma [34]. However, in our study, we found highly reliable correlations of the studied DNA in blood plasma and lumbar cerebrospinal fluid. At the same time, the concentration of mutant DNA of the *H3F3A* (K27M) gene in the lumbar cerebrospinal fluid also had highly significant correlations with the concentration of wild-type DNA of the *H3F3A* (K27M) gene in the first fraction of blood plasma. Thus, the assessment of VAF in blood plasma has a high prognostic significance in assessing the effectiveness of therapy.

3. Analysis of the dynamics of changes in the concentration of freely circulating DNA of the *H3F3A* (K27M) gene: wild and mutant type in the group with and without progression on the background of radiation therapy

Radiation therapy, as a standard strategy for the treatment of DMG, improves the quality of life of patients, after which 70–80 % of patients experience temporary relief of symptoms, as well as increased survival [24, 35, 36]. However, within 4–9 months, the disease progresses again. Ionizing radiation (AI) used in RT can inhibit tumor growth by inducing DNA damage directly or through reactive oxygen species (ROS) [37]. So far, there are no predictors for assessing the early effect of RT during treatment for children with DMG, only MR control brain monitoring after 1.5–3 months will allow to exclude or confirm the progression of the disease. Accordingly, it is impossible to personalize anti-relapse therapy in time.

During the ongoing study, the concentration level and VAF of mutant and wild ctDNA of the *H3F3A* (K27M) gene were studied for groups with stabiliza-

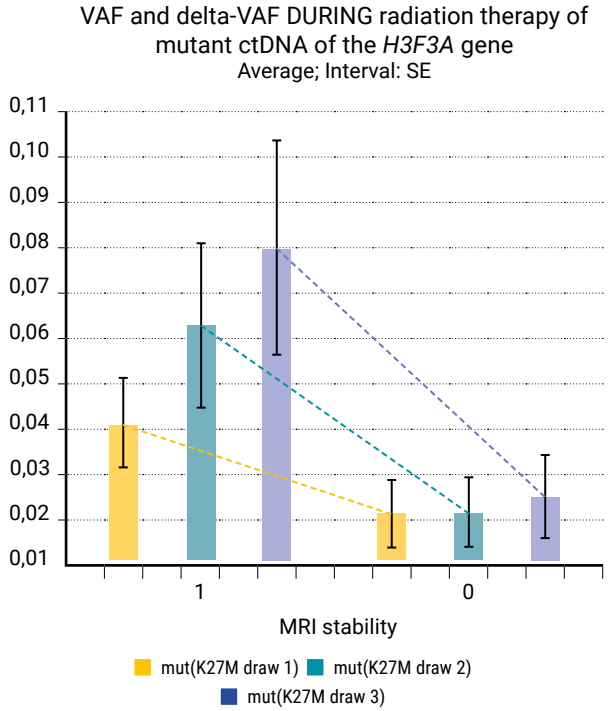

Fig. 3. VAF and delta-VAF against the background of radiation therapy of mutant ctDNA of the *H3F3A* (K27M) gene in blood plasma in a group of patients with continued growth (group 1) and stabilization (group 0), depending on the data of an MR brain study 3 months after completion of treatment

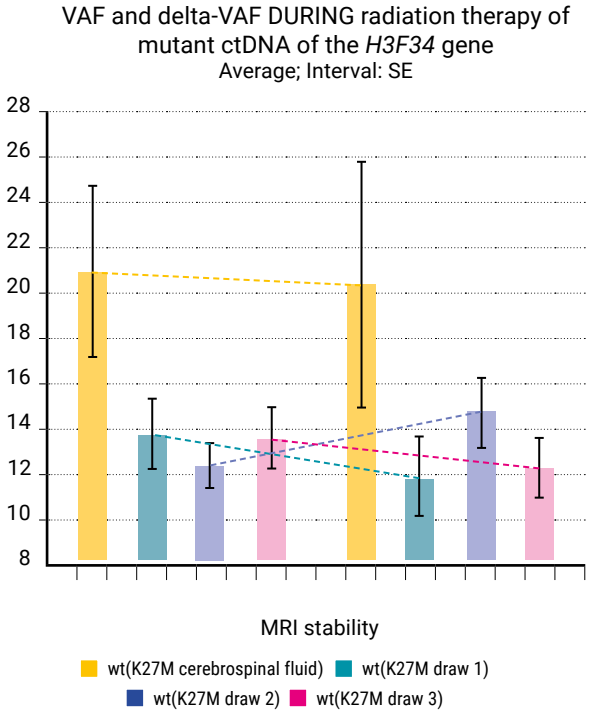

Fig. 4. VAF and delta VAF against the background of radiation therapy of wild-type ctDNA of the *H3F3A* (K27M) gene in cerebrospinal fluid and blood plasma in a group of patients with continued growth (group 1) and stabilization (group 0), depending on the data of an MRI study of the brain 3 months after completion of treatment

tion and progression against the background of RT. In the group of patients with stabilization, the concentration of mutant DNA of the *H3F3A* (K27M) gene in the blood was lower in three blood plasma samples compared with the concentration of mutant ctDNA in the group with early progression. VAF did not tend to significantly increase against the background of RT with stabilization of the disease. Whereas in children with early progression, the concentration of VAF in three plasma samples was 2–3 times higher compared to the group with a favorable prognosis, and the delta of VAF increased 2 times with each subsequent measurement against the background of RT (Fig. 3).

When analyzing the VAF and delta VAF wild-type ctDNA of the *H3F3A* (K27M) gene in cerebrospinal fluid and blood plasma, the following patterns were revealed in the group of patients with progression (group 1) and without progression (group 0), depending on the data of the MR brain examination 3 months after completion. The concentration level of wild-type ctDNA of the *H3F3A* (K27M) gene in the cerebrospinal fluid at the beginning of the course of RT was identical in both groups of patients. At the same time, in the group of patients with early progression (Fig. 4), the wild-type VAF ctDNA had the following pattern: a decrease in the RT process in the second plasma sample and an increase in the third sample against the background of completion of the RT course. Whereas in the group of patients with stabilization of the disease, the VAF of the wild-type ctDNA of the *H3F3A* (K27M) gene differed, namely: an increase in concentration in the second plasma sample and a significant decrease in it at the end of the course of RT. The delta of VAF in blood plasma, first of all, indicates the presence of a significant dependence between these indicators and the course of the disease.

The data obtained prove the diagnostic value of the wild-type ctDNA of the *H3F3A* (K27M) gene, allowing us to significantly expand the possibilities of molecular diagnostics and monitoring the effectiveness of treatment of DMG. In addition, based on the analysis of delta VAF during treatment, it is possible to predict early tumor recurrence after radiation therapy and timely initiation of personalized therapy.

CONCLUSION

1. Studies of VAF in blood plasma and lumbar cerebrospinal fluid of children with tumors diffuse midline gliomas before the start of radiation therapy are comparable and have equal diagnostic value.

2. High plasma concentrations of wild-type DNA of the *H3F3A* (K27M) gene correlate with early progression, which also affects survival rates.

3. Dynamic control of the DNA concentration of both mutant and wild-type *H3F3A* (K27M) gene in blood plasma and lumbar cerebrospinal fluid in children with diffuse midline gliomas during radiation therapy can be used to predict the effectiveness of RT. In addition, based on the analysis of the dynamics of the concentration levels of the wild-type DNA of the *H3F3A* (K27M) gene during treatment, it is possible to predict early tumor recurrence after radiation therapy.

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Kudinova E. A. – development of the research design, analysis of the data obtained, writing the text of the manuscript, interpretation of the results;
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Dzhikiya E. L. – development of the research design, analysis of the data obtained, writing the text of the manuscript, interpretation of the results;
Kaminskiy V. V. – review of publications on the topic of the article, a set of clinical material, interpretation of the results;
Antonenko F. F. – review of publications on the topic of the article;
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