ANNOTATION
to a dissertation work by Saliyeva Symbat Sarybaevna
on the topic: "Prognostic factors affecting the treatment effectiveness of children with malignant extracranial germ cell tumors", submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D110100 - "Medicine"

Relevance of the research topic.

Malignant tumors represent a significant medical and social problem. Studying tumors, especially in children, and even more so rare forms, is a relevant and important task.

Germ cell tumors (GCT) are a group of neoplasms, undifferentiated of benign and malignant origin, developing from germ cells. Despite the general theory of origin, GCTs are a heterogeneous group of neoplasms that occur in a wide age range and differ in localization, histology, and clinical behavior. The proportion of GCT accounts for 3% of all malignant neoplasms in children aged 0-14 years, 14% at the age of 15-19 years.

Currently, GCTs are among the most chemosensitive diseases and represent a model of a curable malignant process. The use of platinum-containing regimens of polychemotherapy made it possible to achieve a 5-year survival rate of about 85-90% in children with extracranial GCT [D'Angelo P., Depani S.]. The choice of treatment tactics today is based on histological verification of the diagnosis and integration of information obtained using visual diagnostic methods and classical tumor markers. However, some patients progress during or after standard therapy, which may be due to "insufficient treatment." The issue of preventing late complications after chemotherapy is also relevant due to "excessive treatment." Therefore, it is essential to investigate the prognostic factors that allow stratifying patients into risk groups and, deciding on further management tactics. Despite developed stratification protocols based on clinical prognostic factors, they do not always accurately predict treatment outcomes. Therefore, the search for new biological markers capable of improving the prognostic assessment of the disease and aiding in treatment individualization is a relevant direction.

MicroRNAs are promising biological markers that have demonstrated effectiveness in the diagnosis, prognosis, and monitoring of recurrences in various types of cancer. Recent studies have shown that some microRNAs, such as microRNA371-373 and microRNA302/367, are highly sensitive and specific markers for malignant GCTs (Roger D.P., Murray M.J.).

Thus, studying prognostic factors remains a relevant task aimed at improving treatment outcomes for patients with GCTs.

Scientific originality:

1. A clinical registry for children with extracranial GCT has been created for the first time in the Republic of Kazakhstan.

2. MicroRNA expression was studied in pediatric patients with gonadal and extragonadal localization of GCT for the first time.

The practical significance of the work:

1. The use of platinum-oriented chemotherapy according to the MAKEI protocol is highly effective in children with malignant extracranial GCT, which increased the overall five-year survival rate by 13%, from 68% to 81%.

2. The developed prognostic model based on clinical and morphological factors makes it possible to identify an unfavorable event at the early stages of development in patients with malignant GCT with an initially high level of AFP, impaired AFP kinetics and incomplete resection status.

3. MicroRNAs 371, 372, 373, 367,302d are particular and highly sensitive markers of malignant GCT compared to serum AFP. Monitoring these microRNAs will allow timely identification of the risk of an adverse event in patients with GCT.

Objective: to study prognostic factors affecting the effectiveness of treatment of malignant extracranial germ cell tumors to improve survival.

Research objectives:

1. To evaluate survival rates in children with extracranial germ cell tumors.

2. To carry out a one-factor analysis of clinical and morphological data associated with an unfavorable prognosis for extracranial germ cell tumors in children.

3. To conduct a multivariate analysis using the Cox regression model to identify independent predictors that significantly affect survival in children with extracranial germ cell tumors

4. Creation of a prognostic model of the probability of occurrence of an event based on clinical and morphological factors.

5. To study microRNA expression's diagnostic and prognostic value in malignant extracranial germ cell tumors in children.

Research Materials. The study utilized data from children under the age of 18 with extracranial GCT who received platinum-based chemotherapy according to the MAKEI2005 protocol in the oncology/hematology departments of the JSC "Scientific Center of Pediatrics and Pediatric Surgery" from February 2013 to May 2022.

To achieve the research objectives, a clinical registry for patients with extracranial GCT was established for the first time in the Republic of Kazakhstan. A total of 141 patients were registered during the analyzed period. According to the WHO histological classification, teratoma was morphologically verified in 45 patients (31.9%), and malignant types of GCT were identified in 96 patients (68.1%).

Research Methods: Clinical, laboratory-diagnostic, instrumental, statistical. The clinical research method involved collecting patient history and conducting physical examinations. Laboratory-diagnostic and instrumental research methods included determining serum levels of tumor markers (AFP, hCG, LDH), visual research methods (ultrasound, CT/MRI), histological and immunohistochemical examination of tumor tissues, quantitative polymerase chain reaction (PCR) with reverse transcription.

The study of microRNA expression consisted of 2 stages: retrospective - microRNA expression in tumor tissue, and prospective - microRNA expression in

both tumor tissue and patient blood serum at different stages of therapy. In the retrospective analysis, tissues from 84 children with GCT and 11 children with non-oncological pathology (control group) were studied. Ten microRNAs potentially associated with GCT (microRNA clusters 371-373, microRNA302/367, microRNA375, microRNA200) were used for analysis based on literature review. The prospective analysis examined the expression of 9 microRNAs in tumor tissue and blood serum from 20 children with GCT and 7 children with non-oncological pathology. MicroRNA200b was excluded from the analysis based on retrospective analysis.

Statistical analysis included determination of normality of the data distribution, survival estimation, regression analysis, and ROC analysis. To determine normality of the data distribution Shapiro-Wilk test, histograms were conducted, revealing that quantitative data were not normally distributed, warranting the use of non-parametric tests. Mann-Whitney test was applied for comparative analysis between two groups, while Kruskal-Wallis test was utilized for comparing three groups. Survival analysis was performed using Kaplan-Meier method and compared using log-rank test. Coefficients of correlation were used to assess the quality of the model's variable relationships. The overall effect of factors was evaluated using Cox proportional hazards regression model. The study also assessed the diagnostic test's informativeness, specifically determining its sensitivity and specificity. Based on sensitivity and specificity values, characteristic curves (ROC curves) were constructed using the biomedical software package "Medcalc," version 12.2.1.0.

The main provisions submitted for protection:

1. The overall survival rate for five years in children with extracranial GCT was 84%, and the event-free survival rate was 79%, comparable to the results of large centers worldwide.

2. Resection status, initial AFP level of more than 10,000 ng/ml and delayed AFP kinetics are statistically significant predictors of an unfavorable prognosis for response to therapy.

3. The prognostic model created on the basis of clinical and morphological factors makes it possible to identify a category of patients with a high risk of an event. In patients with resection status R0, the probability of occurrence of an event is 9.1 times lower than in patients with resection status R1 and 15.8 times lower than in patients with resection status R2. Patients with an initial AFP level of more than 10,000 ng/ml have a 3.9 times greater risk of an adverse outcome compared to the group of patients with an AFP level of less than 10,000 ng/ml. Patients with impaired AFP kinetics are 3.2 times more likely to have an event compared to the group with a normal decrease in AFP levels.

4. MicroRNAs 371-373, 367, and 302d are highly diagnostic and prognostic markers of malignant GCT and can potentially be used as new biomarkers that allow determining a personalized approach to treatment.

Conclusions:

1. The five-year survival rate of all patients included in this study was 84%, and event-free survival was 79%. In the group of malignant GCT, the total five-year survival rate was 81%, and the event-free survival rate was 73%.

2. Unfavorable prognostic factors in children with extracranial GCT are: extragonadal tumor location ($p<0,0001$), advanced stage of the disease ($p=0,0141$), initial AFP level of more than 10,000 ng/ml ($p=0,0216$), delayed AFP kinetics ($p=0,0002$), incomplete resection ($p<0,0001$) and incomplete response to therapy ($p=0,0079$).

3. Multivariate analysis revealed statistically significant predictors of the occurrence of an adverse event: incomplete tumor resection, baseline AFP level of more than 10,000 ng/ml and slowing of AFP kinetics ($p<0,0001$).

4. According to the developed prognostic model, in patients with complete removal of the tumor, the probability of occurrence of the event is 9,1 times lower than in patients with microscopic residual tumor and 15,8 times lower than in patients with macroscopic residual tumor. With an initial AFP level of more than 10,000 ng/ml, the risks increase by 3,9 times compared with AFP of less than 10,000 ng/ml, with a violation of the kinetics of AFP is 3,2 times higher compared with a normal decrease in AFP.

5. Overexpression of microRNA302/367 and microRNA371-373 is characteristic of malignant GCT and is not typical of teratomas.

6. Serum microRNAs302d, 367, 371, 372, 373 are potential markers of diagnosis, prediction, and monitoring of patients with malignant GCT, demonstrating high specificity and sensitivity. Monitoring microRNA levels in blood serum at the stages of therapy shows high informativeness in detecting events earlier than serum AFP.

Practical recommendations:

1. Platinum-containing chemotherapy regimens are adequate, with a high probability of survival (81%) in children with malignant extracranial GCT.

2. MicroRNAs 371,372,373,367, and 302d are potential predictors of events in patients with malignant GCT, even more highly sensitive than AFP.

3. For early diagnosis of events during the first 24 months after the end of treatment, careful monitoring of serum biochemical markers (AFP and HCG) is recommended since most relapses occur during this period.

4. The developed scheme of stratification of patients with malignant GCT, taking into account the expression of serum microRNAs, will allow doctors of practical healthcare to timely identify the contingent of the risk group for the occurrence of adverse events with a further choice of management tactics.

Personal contribution of a doctoral student. The author directly participated in all stages of the study: from setting goals and objectives, developing the design of the study, conducting a thorough literature review, engaging in data collection, developing a clinical register, analysis of the data obtained, statistical processing, and interpretation of the results. The author has prepared a project to study the expression of microRNAs in blood serum. This fragment of the dissertation work was carried out within the framework of a target program funded by the Ministry of Health of the Republic of Kazakhstan: BR11065390

"Development and development of innovative technologies for early diagnosis and treatment of malignant diseases taking into account modern genomics approaches."

Approbation of the work.

The results of the research conducted on the topic of the dissertation were reported and discussed at conferences at the Republican and international levels:

- International scientific and practical conference "Young researcher: challenges and prospects for developing modern pediatrics and pediatric surgery," 01.03.2019, Almaty. Report on the topic: "Clinical and morphological features of extracranial germinogenic cell tumors in children."
- VII Congress of Oncologists and Radiologists of Kazakhstan. Session of young scientists. October 18-19 , 2019Nur-Sultan. Report on the topic: "Prognostic factors in the treatment of extracranial germinogenic cell tumors in children."
- Scientific and practical conference "Integration of pediatric science, education, and practice," within the framework of the University Days 2019 Program "Pediatrics of the XXI century. Modern challenges and trends", in Almaty, December 6, 2019. Report on "Assessment of prognostic factors in extracranial germinogenic cell tumors in children."
- International scientific and practical conference "Young researcher: challenges and prospects for the development of modern pediatrics and pediatric surgery" in celebration of the 90th anniversary of KazNMU named after S.D.Asfendiyarov, March 3, 2020. Report on the topic: "The role of prognostic factors in the treatment of extracranial germinogenic cell tumors in children". The information was awarded the diploma of 1st place in the nominations for "Best oral report."
- V Congress of Oncologists of the Republic of Moldova, October 8-9, 2020. Report on the topic: "Results of treatment of children and adolescents with extracranial germinogenic cell tumors."
- International scientific and practical conference "Young researcher: challenges and prospects for the development of modern pediatrics and pediatric surgery," dedicated to the memory of N.N.Akhparov, April 22, 2020. Report on the topic: "Analysis of microRNA expression in germinogenic cell tumors."
- V International Scientific and Educational Forum "Ana men Bala," May 19-20, 2022. Report: "Oncological alertness in teratomas in newborns."
- International scientific and practical conference "Modern therapeutic and diagnostic technologies in pediatric oncology and hematology," dedicated to the 30th anniversary of the onomatology service in the Republic of Kazakhstan, May 11-12, 2023. Report on the topic: "Serum microRNAs in the diagnosis and monitoring of germinogenic cell tumors."
- SIOP ASIA XV Congress, Yerevan, Armenia. 18-21 May 2023. Poster presentation "Circulating microRNAs in diagnosing children with germ cell tumors."

Publications on the topic of the dissertation.

Based on the materials of the dissertation, 14 printed works were published, including:

- Scientific publication in a journal indexed in the Scopus database with at least 25 percentile – 2, less than 25 percentile – 2.
- In journals recommended by the Committee for Quality Assurance in the Field of Education and Science of the Republic of Kazakhstan – 3.
- In the materials of international conferences – 7.

Received 4 authors' certificates

A methodological recommendation has been developed and published: "Early diagnosis and treatment of children with extracranial germinogenic cell tumors".

The structure and scope of the dissertation work.

The dissertation work is presented on 126 pages of computer text. It consists of references, a literature review, a description of materials and research methods, own research results, discussion, conclusion, a list of references from sources, and appendices. The work is illustrated with 51 pictures and 28 tables. The bibliographic list contains 189 references.