DOI: 10.15825/1995-1191-2023-2-140-147

PROGNOSTIC SIGNIFICANCE OF GROWTH HORMONE IN PEDIATRIC LIVER TRANSPLANTATION

R.M. Kurabekova[1], O.V. Silina[1], O.M. Tsirulnikova[1, 2], I.E. Pashkova[1], O.E. Gichkun[1, 2], G.A. Olefirenko[1], S.Yu. Oleshkevich[1], A.R. Monakhov[1, 2], O.P. Shevchenko[1, 2]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Sechenov University, Moscow, Russian Federation

Growth hormone (GH) plays a leading role in the regulation of cell and tissue metabolism and growth. Its effects are mediated through the so-called somatomedins, among which the most important is the liver-produced insulin-like growth factor 1 (IGF-1). It has been reported that serum GH levels in liver recipients is related to the clinical transplant outcomes. **Objective:** to evaluate the prognostic significance of GH in pediatric liver transplantation (LT). **Materials and methods.** The study enrolled 148 children (61 boys) aged 2 to 60 months (median, 8) with end-stage liver disease resulting from biliary atresia (n = 86), biliary hypoplasia (n = 14), Byler disease (n = 15), Alagille syndrome (n = 12), Caroli syndrome (n = 5), and other liver diseases (n = 16, cryptogenic cirrhosis, fulminant and autoimmune hepatitis, Crigler–Najjar and Budd–Chiari syndromes, alpha-1 antitrypsin deficiency, glycogenosis and hepatoblastoma). All the patients were transplanted with the left lateral segment of the liver from a living related donor. GH concentrations were measured by enzyme immunoassay before, at one month and at one year after transplantation. **Results.** Median plasma GH levels in children with liver disease were 4.3 [1.6–7.2] ng/mL, significantly higher than in healthy children of the same age at 1.2 [0.3–2.4] ng/mL, p = 0.001, while mean height and body weight were lower than in healthy controls. GH levels decreased significantly after transplantation. At one month and one year later, the levels did not differ from those of healthy children (p = 0.74, p = 0.67, respectively). One month after transplantation, GH concentrations were lower in 1-year survivors than in non-survivors (p = 0.02); the diagnostically significant threshold GH level was 1.8 ng/mL. Prior to LT, plasma GH levels did not differ between 1-year survivors and non-survivors. Children with GH levels below 1.8 ng/mL post-LT were 9 times more likely to survive one year post-transplant than patients with levels above the threshold. **Conclusion.** GH concentrations in pediatric liver recipients is a positive prognostic indicator of pediatric LT outcomes.

Keywords: liver transplantation, growth hormone, pediatric transplantation, pediatric recipients.

INTRODUCTION

Pediatric liver transplantation (LT) for end-stage liver disease is currently the only radical method of treatment that can achieve not only high survival rates, but also full physical and social rehabilitation. According to international researchers, 1-year survival of living-donor pediatric liver recipients is 86–96% [1, 2]. The experience at the Shumakov National Medical Research Center of Transplantology and Artificial Organs shows that 1-year survival after transplantation exceeds 90% [3].

Currently, there are no accepted methods for predicting transplant outcomes in recipient children that are based on objective indicators. Validation of prediction methods is a promising approach to further improve LT outcomes in children [4, 5].

Important factors associated with longevity and quality of life are GH and IGF-1, which have received much attention in recent decades [6, 7].

GH and IGF-1 are significant links in humoral regulation of liver function: IGF-1 mediates anabolic and mitogenic effects of GH in peripheral tissues. Over 90% of IGF-1 circulating in systemic circulation is synthesized in the liver. IGF-1 production is regulated by GH, which stimulates its production by liver cells. In turn, IGF-1 regulates GH production in a negative feedback manner [8, 9].

The GH/IGF-1 axis controls cell and tissue growth, is closely connected with liver function and can affect survival of patients with liver diseases and transplant recipients [10, 11]. It is assumed that GH affects LT outcomes in children indirectly through regulation of growth and body weight, hepatocyte function and immune system activity [12–14]. In pediatric liver recipients, there is insufficient data on neurohumoral regulation of graft function.

Corresponding author: Rivada Kurabekova. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (499) 190-53-41. E-mail: kourabr@yandex.ru

The aim of this work is to evaluate the prognostic significance of GH in liver transplantation in young children.

MATERIALS AND METHODS

The study included 148 children, 61 boys and 87 girls aged 2 to 60 months (median, 8) with end-stage liver disease. The study protocol was approved by the local ethics committee at Shumakov National Medical Research Center of Transplantology and Artificial Organs. To participate in the study, patients' legal representatives signed a written informed consent, which is kept in their medical records.

In the children enrolled for the study, the causes of liver failure were biliary atresia (n = 86), biliary hypoplasia (n = 14), Byler disease (n = 15), Alagille syndrome (n = 12), Caroli syndrome (n = 5), and other conditions (n = 16), such as cryptogenic cirrhosis, fulminant hepatitis, autoimmune hepatitis, Crigler–Najjar syndrome, Budd–Chiari syndrome, alpha-1 antitrypsin deficiency, glycogenosis, and hepatoblastoma. All patients underwent left lateral LT from a living related donor. After transplantation, patients received double- or triple-drug immunosuppressive therapy, which included tacrolimus, mycophenolates, and corticosteroids.

The comparison group consisted of 16 healthy children, 9 boys and 7 girls, examined after treatment for intestinal dysbacteriosis. The median age in the comparison group was 12 (6–25) months. To compare the recipients' anthropometric indicators, represented by mean values and mean quartile deviation, we used the WHO reference data for healthy children of the same age [15].

We measured growth hormone content in plasma obtained from venous blood collected on an empty stomach between 8 and 10 o'clock in the morning. Blood was collected in disposable plastic tubes (BD Vacutainer, Becton Dickinson, USA) containing anticoagulants (ethylenediaminetetraacetic acid or sodium citrate). Blood plasma obtained by centrifugation at 1500 g for 10 minutes was stored at –500 °C until analysis. Plasma GH levels were measured by enzyme immunoassay using a reagent kit (DBC, Canada) according to the manufacturer's instructions. The results of GH level measurements are represented by median and interquartile range values, 25th to 75th percentile.

Statistical analysis was carried out using parametric and nonparametric statistics methods. Fisher's exact test was used to compare parametric samples. The Mann–Whitney U test was used to compare independent nonparametric variables; paired Wilcoxon test was used to compare dependent samples; correlation analysis was performed according to Spearman's correlation. Differences were considered statistically significant at $p < 0.05$.

A receiver operating characteristic (ROC) analysis was performed to assess the information content of the test. The area under the ROC curve (AUC) reflects the probability with which the test is able to separate one group of patients from another. As a null hypothesis, it was assumed that the area under the ROC curve does not differ from 0.5. The threshold GH level, separating patients from healthy ones, was determined by plotting the dependences of sensitivity and specificity on plasma GH levels.

The diagnostic sensitivity and specificity of the test, as well as the optimal GH threshold level was determined at the point of maximum sum of sensitivity and specificity. Test sensitivity was defined as the proportion of patients with a positive test among all patients. Test specificity was defined as the proportion of healthy people with a negative result among all healthy people.

Relative risk (RR) was calculated using a four-field contingency table for the marker concentration threshold and estimated 95% confidence interval (CI). The RR value was considered statistically significant ($p < 0.05$) if the lower CI limit was above 1.

We also calculated test accuracy (Ac), positive predictive value (PPV) and negative predictive value (NPV). Method accuracy was defined as the proportion of correct results, the ratio of the number of true positive and true negative results to the total number of tests. The PPV, reflecting the probability of becoming ill with a positive test, was determined as the proportion of true positives in the total number of all positive results. The NPV, i.e., the probability of not getting sick with a negative test was determined as the proportion of true negatives in the total number of negative results.

Calculations were made using computer statistical programs MS Office Excel (MS, USA), SPSS Statistics 20 (IBM, USA), and Statistica 7.0 (StatSoft, Inc., USA).

RESULTS

The indication for LT in children aged 2–60 months (median, 8) was end-stage liver disease, which was, in 90% of cases, caused by congenital and hereditary hepatobiliary diseases and in 10% of cases by rare metabolic disorders. The main characteristics of liver recipients included in the study are presented in Table 1.

The comparison group included 16 virtually healthy children, 9 boys and 7 girls, who were examined after treatment for intestinal dysbacteriosis. The median age in the comparison group was 12 (6–25) months.

The age and sex composition of the recipient children included in the study and children in the comparison group did not differ ($p = 0.78$ and $p = 0.84$, respectively).

The mean height of the patients included in the study was 71.2 ± 8.2 cm and was significantly lower than the mean reference value for healthy children of the same age (75 ± 6 cm according to WHO data, $p = 0.00$). The body weight of the recipients was 7.9 ± 2.3 kg and was lower than in healthy children, 9.5 ± 2 kg, $p = 0.00$.

Table 1

Main characteristics of liver recipients

Characteristics	Indicators
Number (n)	148
Age (months), median (range)	8 (2–60)
Sex (n, %):	
Boys	61 (87)
Girls	41 (59)
Liver disease (n, %):	
Biliary atresia	86 (58.1)
Biliary hypoplasia	14 (9.5)
Alagille syndrome	12 (8.1)
Byler disease	15 (10.1)
Caroli syndrome	5 (3.4)
Others:	16 (10.8)
Cryptogenic cirrhosis	
Fulminant hepatitis	
Autoimmune hepatitis	
Crigler–Najjar syndrome	
Budd–Chiari syndrome	
Alpha-1 antitrypsin deficiency	
Glycogenosis	
Hepatoblastoma	

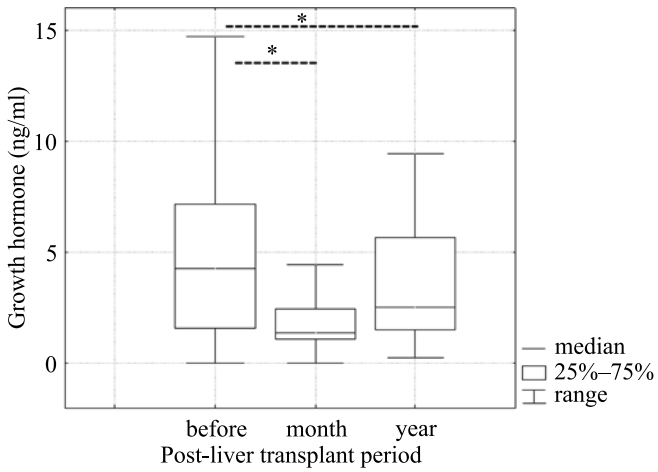

Fig. 1. GH levels in children before and at various times after LT. *, $p < 0.05$ compared to pre-transplant levels

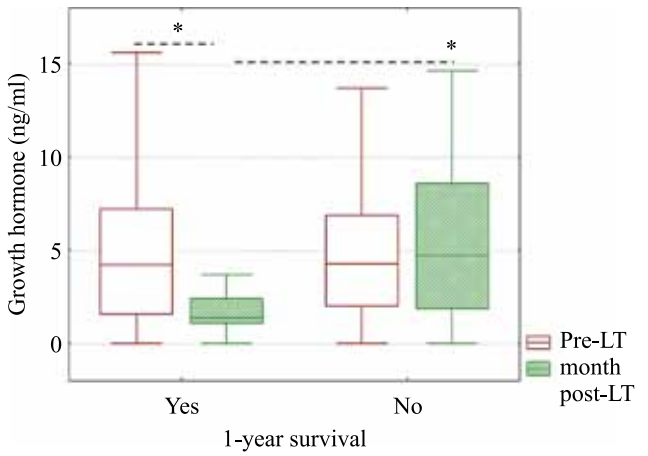

Fig. 2. GH levels before and one month after liver transplantation in 1-year survivors and non-survivors. *, $p < 0.05$

Recipients' height one year after transplantation averaged 82.1 ± 7.6 cm and remained significantly lower than the mean reference value for healthy patients of the same age (according to WHO data, 87 ± 7 cm, p = 0.00) [15]. Recipients' mean body weight of 11.5 ± 2.2 kg did not statistically differ from the reference values for healthy children of the same age, 12 ± 2 kg, p = 0.06.

Plasma GH level in children with liver disease was 4.3 [1.6–7.2] ng/mL and was significantly higher than those in healthy children of the same age, 1.2 [0.3–2.4] ng/mL, p = 0.001. Data on GH levels are presented as median and interquartile range.

Fig. 1 shows a comparative analysis of plasma GH levels in children before, one month, and one year after LT.

A month after LT, plasma GH level in the recipients was 1.4 [1.1–2.4] ng/mL, significantly lower than before the operation (p = 0.001). A year after transplantation, GH level in recipients was 2.5 [1.5–5.7] ng/mL, significantly lower than before surgery (p = 0.049). One month and one year post-LT, plasma GH level in the recipients did not differ from the levels in healthy children (p = 0.74; p = 0.67, respectively).

To study the association of GH with clinical outcomes of pediatric LT, a comparative analysis of blood GH content in children who survived and did not survive one year after transplantation was performed. The pre-transplant GH level was not associated with transplant outcomes one year later (r = 0.03, p = 0.32). Data on plasma GH levels before and one month after transplantation in recipients who survived and did not survive one year are shown in Fig. 2.

GH levels one month after transplantation in survivors were 1.4 [1.1 to 2.4] ng/mL, were significantly lower than those before surgery at 4.2 [1.5 to 7.2] ng/mL, p = 0.00. In recipients who did not survive this period, the GH content after one month was 5.6 [1.9–8.6] ng/mL, almost no different from that before surgery, 4.5 [2.0–6.9] ng/ml, p = 0.68. That is, with a favorable LT outcome, there was a significant decrease in GH levels, and with an unfavorable outcome, GH levels did not change. Pre-transplant GH concentrations did not differ between survivors and non-survivors (p = 0.78); one month post-transplantation, plasma GH levels in survivors were significantly lower than in non-survivors.

To determine whether data on blood GH levels in recipients one month later can be used to predict outcomes one year later, we analyzed the relationship between test sensitivity and specificity at different GH levels (Fig. 3).

Analysis showed that the AUC was 0.74 ± 0.10 [0.54–0.95], statistically significantly different from 0.5 (p = 0.025).

To determine a diagnostically significant GH threshold level, we performed an analysis based on plots of the dependence of test sensitivity and specificity on GH levels in child recipients one month after LT (Fig. 4).

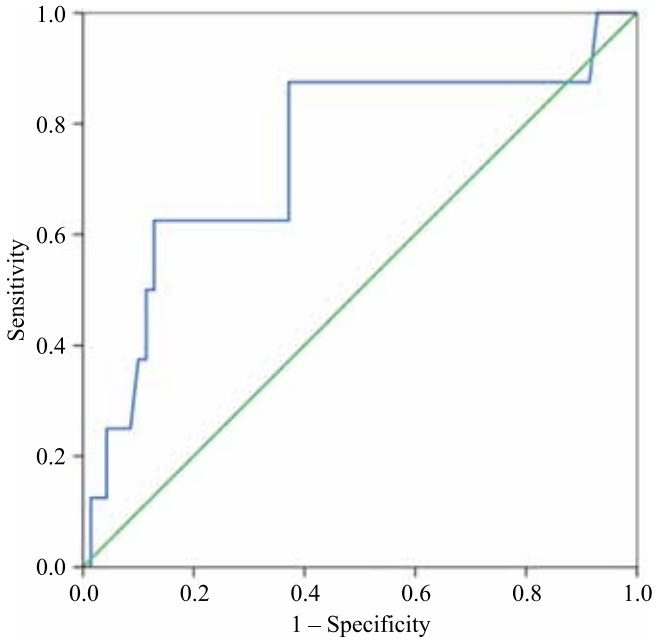

Fig. 3. ROC analysis of plasma GH levels in pediatric recipients one month after LT to assess 1-year survival, AUC = 0.74 ± 0.10; 95% CI 0.54–0.95, $p < 0.05$

Table 2

Characteristics of the test to assess 1-year recipient survival based on GH levels one month after LT

Characteristics	Values
AUROC, 95% CI	0.74 ± 0.10 [0.54–0.95]*
Sensitivity	0.875
Specificity	0.614
Growth hormone threshold	1.8 ng/mL
Relative risk, 95% CI	9.06 ± 1.04 [1.17–70.15]*
Test accuracy (Ac)	64%
Positive predictive value (PPV)	88%
Negative predictive value (NPV)	61%

*, $p < 0.05$.

The threshold GH level, which corresponds to the maximum specificity (0.614) and sensitivity (0.875) of the test, was 1.8 ng/mL. The result means that patients with post-transplant GH levels below the threshold have a 61.4% chance of surviving one year post-transplant, while those with GH levels above the threshold have an 87.5% chance of not surviving this period.

Calculation of RR showed that at GH levels above the threshold, the RR of not surviving one year was 9.06 ± 1.04 [CI 1.17 to 70.15], $p < 0.05$. Thus, recipients with a blood GH level >1.8 ng/mL one month after transplantation had a 9-fold higher risk of not surviving one year than recipients with lower levels of the hormone.

Table 2 presents the calculated informative characteristics of the test.

As can be seen from the table, test accuracy was 64%, which corresponds to the overall proportion of correct results. The PPV reflecting the probability of not surviving a year with a GH level above 1.8 ng/mL was 88%, and the NPV reflecting the probability of surviving a year with a hormone value below the threshold was 61%.

DISCUSSION

Prediction of pediatric LT outcomes is important because it provides an opportunity for a more personalized approach to patient management in the early stages after surgery. Current widely used methods for predicting survival in patients with liver failure based on complex indicators or the results of biochemical studies reflecting liver function, such as albumin levels, are not effective enough. This can be partly down to intensive replacement therapy in the early post-transplant period in liver recipients [16–18].

The height and body weight of children with end-stage liver disease are significantly lower than those of healthy children of the same age. The present study found that after LT, children recover their body weight to the level of healthy children of the same age and there is

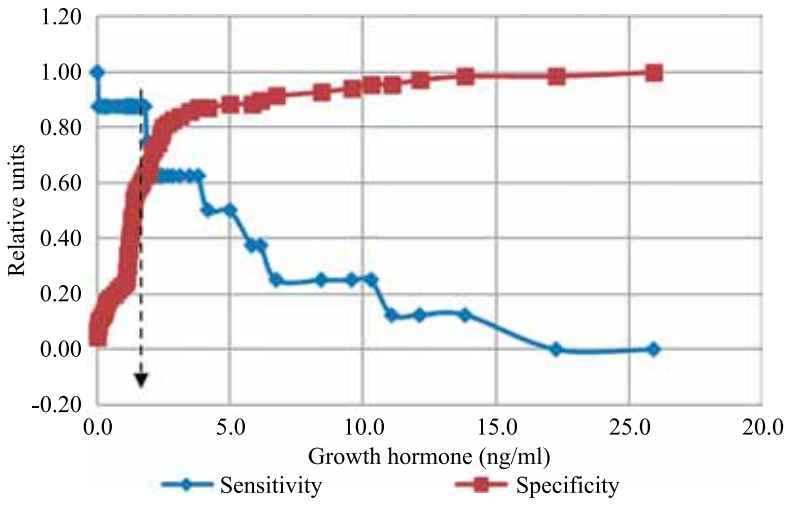

Fig. 4. Dependence of test sensitivity and specificity on GH concentrations in pediatric recipients one month post-LT

a tendency for increased average height of recipients. Earlier in our studies, it was shown that in children with hepatobiliary diseases, the GH level is elevated in combination with impaired IGF-1 synthesis in the liver and reduced IGF-1 levels in the blood; LT in children is accompanied by improved anthropometric parameters due to restoration of IGF-1 synthesis by donor liver cells and normalization of relations in the GH/IGF-1 axis [19, 20].

In this work, a reliable decrease in post-LT plasma GH levels was established. The decrease can be due to IGF-1 production by the graft; absence of significant GH dynamics is indirect evidence of insufficient IGF-1 production by a graft, i.e. graft functional failure. The findings confirm that liver graft plays a role in GH production.

The results of the present work showed that GH levels after one month negatively correlates with one-year transplant outcomes. This suggests that plasma GH tests can be used to predict LT outcomes in young children.

Based on the assessment of diagnostic efficiency of GH levels using common C-statistic methods: construction of ROC curve, determination of threshold values and calculation of informative characteristics of test, we show that plasma GH levels in liver recipient children one month after transplantation can be used to predict one-year survival with a 74 ± 10% probability. A test probability level of about 75% or more, is generally considered an indicator of a good test. A threshold GH level of 1.8 ng/mL separates high and low risk recipients from those at risk of not surviving 12 months after transplantation. Recipients with GH levels below the threshold (1.8 ng/mL) were 9 times more likely to survive the year than those with levels above the threshold. The overall accuracy of this test was 64%. The PPV and NPV were 88% and 61%, respectively. At GH levels below the threshold (negative test), the probability of one-year survival was 61%. At GH levels above the threshold (positive test), the probability of not surviving one year after transplantation was 88%. Thus, the test results have a high probability of predicting LT outcomes.

The data we obtained on the relationship between transplant outcomes and plasma GH levels in liver recipients are consistent with the data obtained by other authors, who showed the importance of body weight, which directly depends on GH levels, in survival. In adult liver recipients, a positive correlation of lower blood GH concentrations after transplantation with 3-month and 3-year survival was also established [10, 21].

The present work is an observational and retrospective hypothesis-driven study. To use GH as an objective laboratory criterion in predicting LT outcomes in children, a prospective clinical study of its diagnostic efficacy is needed.

CONCLUSION

Our work shows that GH levels in children one month after LT can serve as a positive prognostic indicator of transplant outcome; patients with GH levels below the threshold (1.8 ng/mL) may be 9 times more likely to survive one year after transplantation than those with hormone levels above the threshold. The results obtained may be useful for personalization of patient management, as well as for understanding the links between the neuroendocrine system and factors affecting liver graft function.

The authors declare no conflict of interest.

REFERENCES

1. *Moosburner S, Wiering L, Gül-Klein S, Ritschl P, Dziodzio T, Raschzok N et al*. Over 30 Years of Pediatric Liver Transplantation at the Charité-Universitätsmedizin Berlin. *J Clin Med.* 2022; 11 (4): 900. doi: 10.3390/jcm11040900.
2. *Yoon KC, Song S, Lee S, Kim OK, Hong SK, Yi NJ et al*. Outcomes of Split Liver Transplantation vs Living Donor Liver Transplantation in Pediatric Patients: A 5-Year Follow-Up Study in Korea. *Ann Transplant.* 2022; 3 (27): 935682. doi: 10.12659/AOT.
3. *Gautier SV, Khomyakov SM*. Organ donation and transplantation in the Russian Federation in 2021. 14th Report from the Registry of the Russian Transplant Society. *Russian Journal of Transplantology and Artificial Organs*. 2022; 24 (3): 8–31. https://doi.org/10.15825/1995-1191-2022-3-8-31.
4. *Hong SK, Yi NJ, Hong K, Han ES, Lee JM, Choi Y et al*. Risk Factors Affecting Outcomes in Pediatric Liver Transplantation: A Real-World Single-Center Experience. *Ann Transplant.* 2021; 28 (26): 929145. doi: 10.12659/AOT.
5. *Minich A, Arisar FAQ, Shaikh NS, Herman L, Azhie A, Orchanian-Cheff A et al*. Predictors of patient survival following liver transplant in non-alcoholic steatohepatitis: A systematic review and meta-analysis. *EClinicalMedicine.* 2022; 50: 101534. doi: 10.1016/j.eclinm.2022.101534.
6. *Berryman DE, Christiansen JS, Johannsson G, Thorner MO, Kopchick JJ*. Role of the GH/IGF-1 axis in lifespan and healthspan: lessons from animal models. *Growth Horm IGF Res.* 2008; 18 (6): 455–471. doi: 10.1016/j.ghir.2008.05.005.
7. *Junnila RK, List EO, Berryman DE, Murrey JW, Kopchick JJ*. The GH/IGF-1 axis in ageing and longevity. *Nat Rev Endocrinol.* 2013; 9 (6): 366–376. doi: 10.1038/nrendo.2013.67.
8. *Maes M, Sokal E, Otte JB*. Growth factors in children with end-stage liver disease before and after liver transplantation: a review. *Pediatr Transplant.* 1997; 1 (2): 171–175.
9. *Takahashi Y*. The Role of Growth Hormone and Insulin-Like Growth Factor-I in the Liver. *Int J Mol Sci.* 2017; 18 (7): 1447. doi: 10.3390/ijms18071447.

10. *Nicolini D, Mocchegiani F, Palmonella G, Coletta M, Brugia M, Montalti R et al*: Postoperative Insulin-Like Growth Factor 1 Levels Reflect the Graft's Function and Predict Survival after Liver Transplantation. *PLoS One.* 2015; 10 (7): e0133153. doi: 10.1371/journal.pone.0133153.
11. *Wu D, Zhang L, Ma S, Zhao Y, Chen R, Zhang F et al*. Low Growth Hormone Levels Predict Poor Outcome of Hepatitis B Virus-Related Acute-on-Chronic Liver Failure. *Front Med.* 2021; 8: 655863. doi: 10.3389/fmed.2021.655863.
12. *Dichtel LE, Cordoba-Chacon J, Kineman RD*. Growth hormone and insulin-like growth factor I regulation of nonalcoholic fatty liver disease. *J Clin Endocrinol Metab.* 2022; 107 (7): 1812–1824. doi: 1210/clinem/dgac088.
13. *Abdel-Wahab R, Hassan MM, George B, Carmagnani Pestana R, Xiao L, Lacin S et al*. Impact of Integrating Insulin-Like Growth Factor 1 Levels into Model for End-Stage Liver Disease Score for Survival Prediction in Hepatocellular Carcinoma Patients. *Oncology.* 2020; 98 (12): 836–846. doi: 10.1159/000502482.
14. *Liu Z, Cordoba-Chacon J, Kineman RD, Cronstein BN, Muzumdar R, Gong Z et al*. Growth Hormone Control of Hepatic Lipid Metabolism. *Diabetes.* 2016; 65 (12): 3598–3609. doi: 10.2337/db16-0649.
15. WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatr Suppl.* 2006; 450: 76–85. doi: 10.1111/j.1651-2227.2006.tb02378.x.
16. *Wong CS, Lee WC, Jenq CC, Tian YC, Chang MY, Lin CY et al*. Scoring short-term mortality after liver transplantation. *Liver Transpl.* 2010; 16 (2): 138–146. doi: 10.1002/lt.21969.
17. *Hayashi PH, Forman L, Steinberg T, Bak T, Wachs M, Kugelmas M et al*. Model for End-Stage Liver Disease score does not predict patient or graft survival in living donor liver transplant recipients. *Liver Transpl.* 2003; 9 (7): 737–740. doi: 10.1053/jlts.2003.50122.
18. *Pan HC, Jenq CC, Lee WC, Tsai MH, Fan PC, Chang CH et al*. Scoring systems for predicting mortality after liver transplantation. *PLoS One.* 2014; 9 (9): e107138. doi: 10.1371/journal.pone.0107138.
19. *Shevchenko OP, Tsirulnikova OM, Tsirulnikova IE, Kurabekova RM, Olefirenko GA, Stepanova OI, Gautier SV*. Dynamics of insulin-like growth factor-1 (igf-1) in children after ab0-incompatible liver transplantation. *Russian Journal of Transplantology and Artificial Organs*. 2014; 16 (2): 46–51. (In Russ.) https://doi.org/10.15825/1995-1191-2014-2-46-51.
20. *Kurabekova RM, Tsiroulnikova OM, Pashkova IE, Makarova LV, Mozheiko NP, Monakhov AR, Shevchenko OP*. Association between Growth Hormone and Insulin-like Growth Factor-1 (IGF-1) Levels, Liver Function and Short-Term Survival of Paediatric Liver Recipients. *Russian Journal of Gastroenterology, Hepatology, Coloproctology*. 2020; 30 (4): 44–51. (In Russ.) https://doi.org/10.22416/1382-4376-2020-30-4-44-51.
21. *Salso A, Tisone G, Tariciotti L, Lenci I, Manzia TM, Baiocchi L*. Relationship between GH/IGF-1 axis, graft recovery, and early survival in patients undergoing liver transplantation. *Biomed Res Int.* 2014; 2014: 240873. doi: 10.1155/2014/240873.

The article was submitted to the journal on 14.04.2023