DOI: 10.15825/1995-1191-2021-2-13-20

CORRELATION BETWEEN INSULIN-LIKE GROWTH FACTOR 1 LEVELS AND TACROLIMUS DOSE IN PEDIATRIC LIVER RECIPIENTS

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Introduction. To prevent post-transplant complications associated with unbalanced immunosuppression, objective indicators reflecting the state of the immune system and associated with the immunosuppressant dose are required. In pediatric liver transplantation, an important indicator of hepatocellular function and restoration of anthropometric characteristics is insulin-like growth factor 1 (IGF-1), which exhibits both nonspecific and selective immunomodulator properties. **Objective:** to assess the correlation between growth hormone and IGF-1 levels and tacrolimus dose and blood concentrations in pediatric liver recipients and to determine the possibility of using the IGF-1 level in selecting the drug dose required to achieve its target concentration in the blood. **Materials and methods.** We examined 156 children aged from 2 to 105 (median – 8) months with liver cirrhosis of various etiology, who received liver from a living related donor. The concentration of growth hormone and IGF-1 was determined in blood plasma before, one month, and one year after transplantation using the enzyme-linked immunosorbent assay. Tacrolimus residual concentration was measured in the patient's whole blood by immunochemical method. **Results.** Growth hormone levels in the blood of pediatric liver recipients did not correlate with the dose or concentration of immunosuppressant tacrolimus one month or one year after transplantation, whereas the IGF-1 content was directly related to tacrolimus dose one year later ($r = 0.41$, $p = 0.001$), but not a month after surgery. The correlation coefficient was higher in uncomplicated post-transplant recipients ($r = 0.51$, $p = 0.002$) than in those with complications ($r = 0.26$, $p = 0.17$). The diagnostic efficiency of the IGF-1 level as an objective criterion for selecting the tacrolimus dose required to achieve its target blood concentration was 0.80 ± 0.11; 95% CI [0.58–1.00] ($p = 0.007$). In recipients with blood IGF-1 levels ≥115.7 ng/mL, the probability of prescribing a tacrolimus dose ≥0.25 mg/kg/day was 14 times higher than in children with lower blood IGF-1 levels. The estimated accuracy of the test was 83%, positive predictive value was 71%, and negative predictive value was 85%. **Conclusion.** The IGF-1 level was found to correlate with tacrolimus dose in liver transplant recipients one year after transplantation. The diagnostic efficiency of IGF-1 as a potential indicator for choosing the tacrolimus dose required to achieve its target blood concentration is 80%, which suggests further study of the test to assess the effectiveness of immunosuppression and selection of an individual immunosuppressant dose.

Keywords: living-donor liver transplantation, congenital biliary tract diseases, biomarker, effectiveness of immunosuppression.

INTRODUCTION

After organ transplantation, all patients are prescribed immunosuppressive drugs to prevent rejection, which in turn may have side effects [1, 2]. A 2–3-component immunosuppressive therapy in pediatric liver recipients includes tacrolimus (TAC), a calcineurin inhibitor. The difficulty in selecting the optimal dose of calcineurin inhibitors is associated with the narrow therapeutic index and significant variability of individual pharmacokinetics of drugs. If the drug dose is insufficient, graft rejection is possible, an excessive dose may lead to infectious complications.

The initial dose of immunosuppressants is prescribed taking into account clinical parameters like age, race, donor/recipient leukocyte antigen (HLA) compatibility, presence of HLA antibodies, etc. The effective TAC dose is selected based on the results of monitoring its residual concentration in the blood [3].

To reduce the incidence of complications, objective indicators reflecting the state of the immune system and associated with TAC dose or serum levels are needed [4, 5]. Methods currently being developed to assess the immunosuppression efficacy are based primarily on measurement of parameters characterizing the T cell respon-

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se: quantitative determination of T cell proliferation, T cell cytokine secretion, adenosine triphosphate (ATP) release in T cells, phenotyping of T cell determinants, etc. [6, 7]. Despite a significant number of studies, immunosuppression efficacy indicators have not found their application in clinical practice for a number of reasons, such as duration and complexity of analysis or its lack of accuracy and reproducibility [8, 9].

In pediatric liver transplant (LT) recipients, the growth hormone/insulin-like growth factor 1 (IGF-1) system related to hepatocellular function, growth and body weight regulation are important indicators. elevated levels of growth hormone are combined with stunted growth and body weight due to reduced IGF-1 levels in the blood. Liver transplantation in children is accompanied by improved anthropometric parameters during restoration of growth hormone/IGF-1 system [10, 11].

IGF-1, synthesized mainly in the liver, mediates the peripheral effects of growth hormone, exerting a proliferative, regenerative, and antiapoptotic effect on various tissues [12]. The effect of IGF-1 on the immune system is associated with regulation of proliferation, differentiation, and metabolism of various cells. IGF-1 exhibits both nonspecific immunomodulator properties (it stimulates lymphopoiesis, immunoglobulin synthesis, T cell differentiation [13, 14], and demonstrates a selective inhibitory effect on IL-2-dependent lymphocyte growth [15].

Experimental studies have identified various mechanisms of interaction between IGF-1 and TAC. Particularly, it has been shown that both factors realize some of their effects through calcineurin-dependent cellular pathways [16–18]. In addition, it was found that intravenous and oral administration of TAC in rats increases the IGF-1 levels in the blood [19, 20]. The relationship between IGF-1 and TAC levels in the blood in paediatric liver recipients has not yet been studied.

The purpose of this study was to assess the correlation of growth hormone and IGF-1 levels with TAC dose and blood levels in pediatric liver recipients and to determine the possibility of using IGF-1 levels in selecting the drug dose required to achieve its target blood concentration.

MATERIALS AND METHODS

The study included 156 children with end-stage liver failure due to congenital and hereditary liver diseases, 65 boys and 91 girls aged from 2 to 105 (median 8) months. The etiology of liver failure included the following diseases: biliary atresia, Caroli syndrome, biliary hypoplasia, Alagille syndrome, Byler disease, and other rare liver diseases, including Crigler–Najjar syndrome, Von Gierke disease, alpha-1 antitrypsin deficiency, tyrosinemia, fulminant and autoimmune hepatitis, cryptogenic cirrhosis, and others (Table 1).

The patients included in the study underwent living-donor liver transplantation. The recipients received 2- or 3-component immunosuppressive therapy, which included tacrolimus, corticosteroids, and mycophenolates. The starting TAC dose at its first use was 0.1 mg/kg/day, in the early postoperative period – 0.2–0.3 mg/kg/day. The dose was further adjusted to achieve the target concentration of the drug in the blood – 4–8 ng/mL during the first month after transplantation and 3–6 ng/ml in the subsequent period.

Table 1

Patients included in the study

Number of patients		156
Age, months		8 (2–105)
Sex (M/F), number (%)		65 (42) / 91 (58)
Diseases, number of cases; %	Biliary atresia	86; 55%
	Caroli disease	15; 10%
	Biliary hypoplasia	14; 9%
	Alagille syndrome	12; 8%
	Byler's disease	7; 4%
	Others	22; 14%

TAC serum levels were measured in the patient's whole blood by immunochemical method on an ARCHITECT i2000 analyzer (Abbott, USA) using the ARCHITECT Tacrolimus Kit (Abbott, USA). Growth hormone levels were measured in blood plasma by ELISA using a specific reagent kit (DBC, Canada). IGF-1 levels were measured in blood plasma by sandwich enzyme-linked immunosorbent assay using the OCTEIA IGF-1 kit (IDS Ltd., UK). Optical density was measured using a Zenyth 340r automatic microplate reader (Biochrom Anthos, UK).

Data are presented as arithmetic mean (M) and standard deviation (SD) – M ± SD, upper and lower bounds of the 95% confidence interval (CI) with normal distribution of the indicator. Data from nonparametric samples are presented as median and interquartile range (2nd–3rd quartile or 25–75th percentile). Comparative statistical analysis was performed using nonparametric statistics methods: Mann–Whitney U-test, paired Wilcoxon test, Spearman correlation analysis. Differences were considered statistically significant when the probability of error was less than 0.05 ($p < 0.05$).

ROC (receiver operating characteristic) analysis was performed to evaluate the informativeness of the test. The area under the ROC curve reflects the likelihood that the test is able to separate one group of patients from another. The null hypothesis was that the area under the ROC curve does not differ from 0.5. Diagnostic sensitivity and specificity of the test, as well as optimal threshold value of the biomarker were determined at the point of maximum sum of sensitivity and specificity. Relative risk was determined using a four-field contingency table for the threshold IGF-1 concentration and estimated 95% CI. The RR value was considered statistically significant ($p < 0.05$) if the lower limit of CI was greater than 1. We

also calculated test accuracy (Ac), positive predictive value (PPV) and negative predictive value (NPV).

Data were calculated using statistical programs: MS Office Excel (MS, USA), SPSS Statistics 20 (IBM, USA), and Statistica 7.0 (StatSoft, Inc., USA).

RESULTS AND DISCUSSION

The median and interquartile range of plasma levels of growth hormone and IGF-1 in children with liver failure were 4.3 (1.6 to 7.2) and 8.7 (0 to 24.8) ng/mL, respectively. After liver transplantation, growth hormone levels significantly decreased to 1.4 (1.1 to 2.5) ng/mL one month later ($p = 0.003$) and 2.5 (1.6 to 5.6) ng/mL one year after surgery ($p = 0.015$). Blood IGF-1 levels increased to 74.2 (52.1 to 126.6) ng/mL one month later ($p = 0.0001$) and 79.6 (42.9 to 111.7) ng/mL one year after surgery ($p = 0.0002$). Data obtained confirm previous reports and show that liver transplantation in children with end-stage liver disease is accompanied by increased IGF-1 levels and decreased concentrations of growth hormone in the blood, which is obviously a consequence of graft functioning [10, 21].

Table 2

Correlation between growth hormone (GH) levels and TAC levels and its dose at various periods after liver transplantation (LT)

TAC administration time after LT	TAC	Coefficient of correlation with growth hormone levels		
		before LT	month after LT	year after LT
Month	Concentration, ng/mL	0.16	0.05	0.01
	Dose, mg/kg/day	0.00	–0.05	–0.03
Year	Concentration, ng/mL	0.31	–0.17	0.24
	Dose, mg/kg/day	–0.13	0.07	–0.07

Table 3

Correlation between IGF-1 blood levels and serum TAC levels and its dose at different periods after liver transplantation (LT)

TAC administration time after LT	TAC	Coefficient of correlation with IGF-1 levels		
		before LT	month after LT	year after LT
Month	Concentration, ng/mL	0.10	0.20	0.17
	Dose, mg/kg/day	–0.05	0.05	0.19
Year	Concentration, ng/mL	0.07	0.09	0.26
	Dose, mg/kg/day	0.24	0.19	0.41*

* $p = 0.001$.

After transplantation, recipients received TAC at an average dose of 0.22 ± 0.12 mg/kg/day one month and 0.16 ± 0.07 mg/kg/day one year after LT. Patients' TAC serum levels averaged 5.9 ± 3.4 ng/mL one month and 7.2 ± 3.0 ng/mL one year after transplantation.

Correlation analysis of growth hormone content before and after transplantation with TAC levels and dose administered one month and one year after transplantation showed no statistically significant relationships between growth hormone levels and concentration or dose of the immunosuppressant (Table 2).

Analysis of the association of IGF-1 levels with the dose and blood concentration of the drug showed a significant direct correlation between the moderate strength ($r = 0.41$, $p = 0.001$) and TAC dose one year after liver transplantation (Table 3). No statistically significant associations with either dose or concentration were found before and one month after surgery.

Thus, the results obtained show that the level of growth hormone, unlike IGF-1, does not correlate with TAC dose or levels, although the content of these hormones in pediatric liver recipients one year after transplantation, as well as in healthy children, is related [11]. The absence of such a correlation may particularly be due to significant changes in the level of growth hormone in blood as a result of circadian rhythms of its secretion.

IGF-1 levels one year after transplantation correlates with the administered dose, but not with serum TAC levels. Absence of such a relationship may be due to the significant variability of immunosuppressant concentrations in the blood and the low values of residual drug concentrations.

It should be noted that the revealed correlation does not explain the causal relationship between IGF-1 levels and the immunosuppressant dose required/sufficient to achieve the target plasma concentration of the drug. The relationship between IGF-1 levels and TAC dose and the mechanisms underlying this interaction in pediatric liver recipients has not been studied and may be the subject of a separate study.

Experimental studies have shown an increase in IGF-1 blood levels in rats in response to TAC administration [19, 20], which agrees with the direct correlation between IGF-1 levels and TAC dose (obtained in our study) one year after transplantation, but does not explain the absence of such correlation one month after surgery. At the same time, considering the effect of IGF-1 on lymphocyte proliferation [14, 22] and the inhibitory effect of TAC on T-cell division [23, 24], we can assume that higher IGF-1 levels in the body leads to more active lymphocyte proliferation, which requires a higher TAC dose to inhibit.

The absence of correlation between IGF-1 levels and TAC dose one month after transplantation may be due to the fact that the optimal dose of the immunosuppressant in the majority of recipients during this period has not

yet been selected. The presence of correlation one year after transplantation may be due to the fact that by this period, the optimal TAC dose can be chosen in a part of recipients, at least with a favorable postoperative period. This is confirmed by the results of analysis of correlation between IGF-1 levels and TAC dose depending on complications: the correlation coefficient in recipients with uneventful post-transplantation period (r = 0.51, p = 002) is higher than in recipients with complications (r = 0.26, p = 0.17).

Moreover, the direct correlation between IGF levels and TAC dose revealed in our work is consistent with the experimental data on the common mechanisms of realization of some effects of IGF and TAC through calcineurin cellular pathways [16, 17, 20], which probably suggests competition for common binding sites and, consequently, direct correlation between the content of these factors in the blood mediated through them.

However, despite the lack of study of the mechanisms underlying the identified relationship, the obtained result allows us to consider the IGF-1 blood levels as a potential indicator for selection of a drug dose in pediatric liver recipients.

Based on regression analysis between IGF-1 levels and TAC dose in recipients without complications one year after transplantation (Fig. 1), we obtained a linear dependence equation: $y = 0.091 + 0.0007 \times x$, which allows the calculation of TAC dose (y) by IGF-1 levels (x). The coefficient of determination of linear regression (r^2) is 0.34, r = 0.58, p = 0.0002.

To assess the informative characteristics of IGF-1 levels as an objective criterion for TAC dose selection, the recipients were divided into 2 groups: those receiving low (0.03–0.24 mg/kg/day) and higher TAC dose (0.25–0.36 mg/kg/day). IGF-1 levels in these groups were 7.1 (0.1 to 25.5) and 13.2 (11.0 to 24.4) ng/mL before transplantation, respectively. One month later, they were 86.3 (56.5–161.3) and 115.0 (82.4–168.4) ng/ml, respectively. There were no significant differences in IGF-1 levels in children who received high and low doses of TAC both before (p = 0.212) and one month after transplantation (p = 0.302). One year after surgery, however, recipients who received a higher TAC dose had significantly higher IGF-1 levels: 138.6 (110.2 to 189.7) ng/mL than those who received a lower dose: 64.3 (43.5 to 100.0) ng/mL (p = 0.005) (Fig. 2).

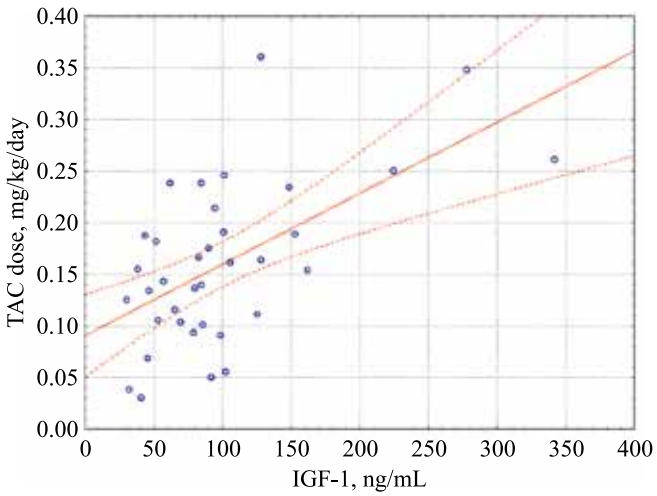

Fig. 1. Linear regression analysis of IGF-1 blood levels with TAC dose in pediatric recipients without complications one year after liver transplantation ($y = 0.091 + 0.0007 \times x$), $r^2 = 0.34$, r = 0.58, p = 0.0002

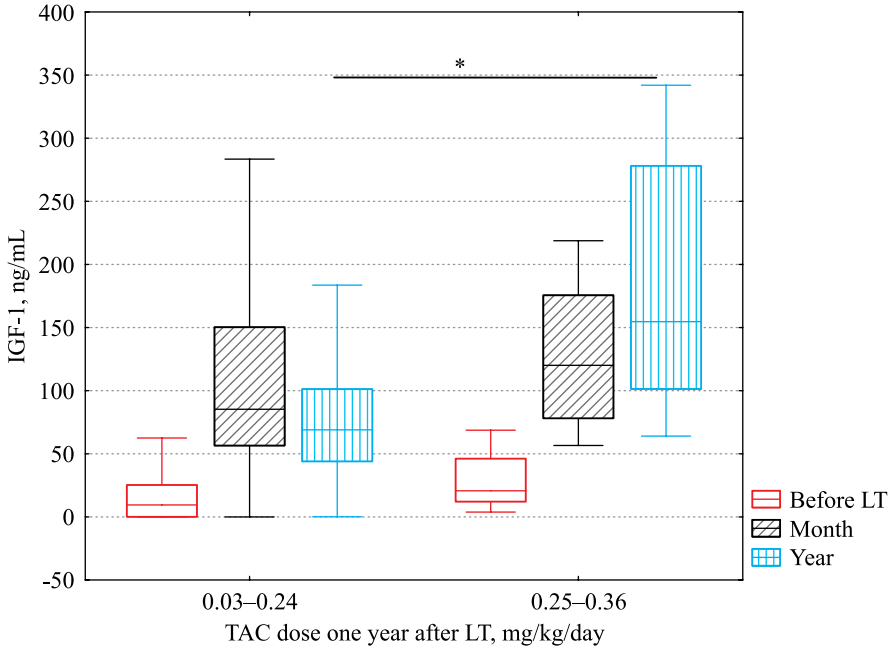

Fig. 2. Dynamics of IGF-1 blood levels in pediatric recipients who received different TAC doses one year after liver transplantation (a/LT), * p = 0.001

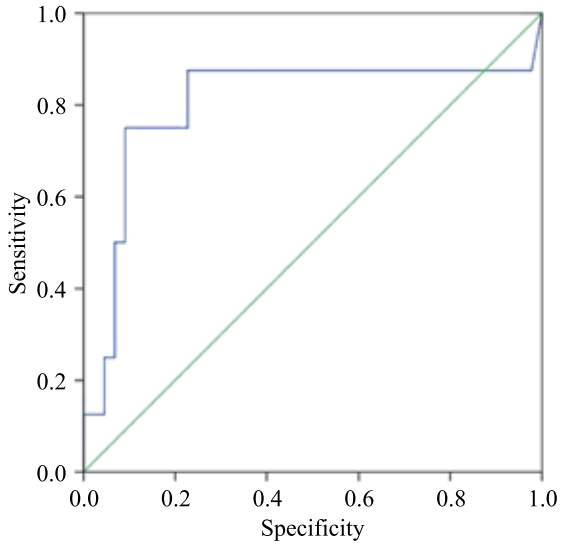

Fig. 3. ROC analysis of IGF-1 blood levels in pediatric recipients one year after liver transplantation (LT), as an indicator for choosing a TAC dose one year after LT: AUC = 0.80 ± 0.11; 95% CI 0.58–1.00, p = 0.007

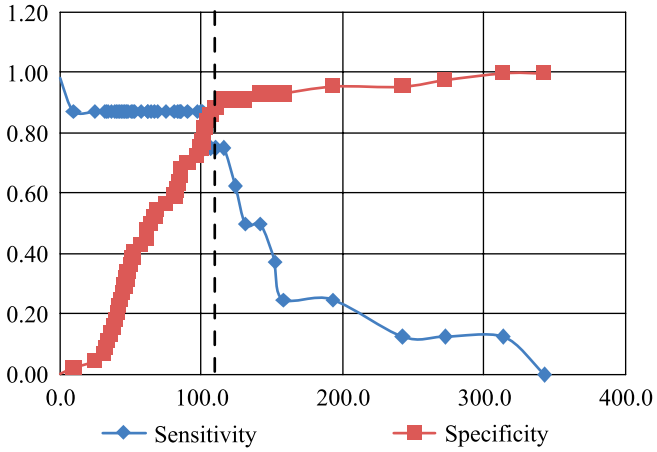

Fig. 4. Sensitivity and specificity of IGF-1 blood levels in pediatric recipients one year after liver transplantation (LT) as an indicator for TAC dose adjustment a year after LT

Table 4

Characteristics of a TAC dose requirement test based on IGF-1 levels one year after liver transplantation

Characteristics	Values
TAC dose requirement test	≥0.25 mg/kg/day
AUROC, 95% CI	0.80 ± 0.11; [0.58–1.00]
Sensitivity	0.75
Specificity	0.91
IGF-1 threshold	115.7 ng/mL
Relative risk, 95% CI	14.3 ± 1.0; [2.0–106.1]*
Test accuracy (Ac)	83%
Positive predictive value (PPV)	71%
Negative predictive value (NPV)	85%

* p < 0.05.

ROC analysis showed that the area under the curve (AUC) was 0.80 ± 0.11; 95% CI [0.58–1.00], p = 0.007 (Fig. 3). Based on an analysis of the dependence of test sensitivity and specificity on IGF levels, the threshold level of the biomarker was determined (Fig. 4) and relative risk values were calculated. The test accuracy, the positive predictive value and negative predictive value were also measured (Table 4).

According to results obtained, the IGF-1 levels in recipients makes it possible to determine the TAC dose with an 80% probability. Recipients with IGF-1 levels ≥115.7 ng/mL had a 14-fold higher risk of TAC dosing ≥0.25 mg/kg/day than children with lower IGF-1 levels. The estimated test accuracy was 83%, and the probabilities of positive and negative predictions were 71% and 85%, respectively, which are considered "good test" indicators.

Current methods for assessing the efficacy of immunosuppression based on the T cell response are complex and time-consuming [8, 9]. Determination of IGF-1 levels by immunoassay takes about 6 hours and is an acceptable method in terms of complexity and duration for clinical transplantology. Besides, IGF-1 has important properties that allow it to be considered as a potential biomarker for TAC dose selection, at least in liver recipients, because more than 90% of IGF-1 circulating in the systemic bloodstream is synthesized in the liver [25, 26]. IGF-1 levels are significantly higher than that of other peptide hormones, and IGF-1 half-life is 2–4 hours, which is 6–10 times longer than that of other similar hormones. IGF-1 levels in the blood plasma of healthy children, unlike growth hormone, whose secretion varies throughout the day, is quite stable, it gradually increases with age and reaches its maximum values at puberty.

The present study is observational, retrospective and hypothesis-driven, which requires a prospective study to confirm or refute. In addition, the study did not examine the possible influence of other factors on serum IGF levels, such as the recipients' age, concentrations of various hormones, doses of corticosteroids and mycophenolates administered during immunosuppressive therapy, etc., since the authors assumed that these factors do not differ significantly in patients of the same age group.

The authors declare no conflict of interest.

REFERENCES

1. *Miloh T, Barton A, Wheeler J, Pham Y, Hewitt W, Keegan T et al.* Immunosuppression in pediatric liver transplant recipients: Unique aspects. *Liver Transpl.* 2017; 23 (2): 244–256.
2. *Catic-Dordevic A, Cvetkovic T, Stefanovic N, Velickovic-Radovanovic R.* Current Biochemical Monitoring and Risk Management of Immunosuppressive Therapy after Transplantation. *J Med Biochem.* 2017; 36 (1): 1–7.

3. *Venkataramanan R, Shaw LM, Sarkozi L, Mullins R, Pirsch J, MacFarlane G et al*. Clinical utility of monitoring tacrolimus blood concentrations in liver transplant patients. *J Clin Pharmacol.* 2001; 41 (5): 542–551.
4. *Sood S, Haifer C, Yu L, Pavlovic J, Churilov L, Gow PJ et al*. A novel immune function biomarker identifies patients at risk of clinical events early following liver transplantation. *Liver Transpl.* 2017; 23 (4): 487–497.
5. *Sharon E, Shi H, Kharbanda S, Koh W, Martin LR, Khush KK et al*. Quantification of transplant-derived circulating cell-free DNA in absence of a donor genotype. *PLoS Comput Biol.* 2017; 13 (8).
6. *Shipkova M, Wieland E*. Editorial: Immune monitoring in solid organ transplantation. *Clin Biochem.* 2016; 49 (4–5): 317–319.
7. *Crespo E, Bestard O*. Biomarkers to assess donor-reactive T-cell responses in kidney transplant patients. *Clin Biochem.* 2016; 49 (4–5): 329–337.
8. *Schlickeiser S, Boes D, Streitz M, Sawitzki B*. The use of novel diagnostics to individualize immunosuppression following transplantation. *Transpl Int.* 2015; 28 (8): 911–920.
9. *Mannon RB, Morris RE, Abecassis M, Axelrod D, Bala S, Friedman GS et al*. Use of biomarkers to improve immunosuppressive drug development and outcomes in renal organ transplantation: A meeting report. *Am J Transplant.* 2020; 20 (6): 1495–1502.
10. *Shevchenko OP, Tsirulnikova OM, Tsirulnikova IE, Kurabekova RM, Olefirenko GA, Stepanova OI et al*. Dynamics of insulin-like growth factor-1 (IGF-1) in children after AB0-incompatible liver transplantation. *Vestnik transplantologii i iskusstvennykh organov*. 2014; XVI (2): 46–51.
11. *Kurabekova RM, Tsiroulnikova OM, Pashkova IE, Makarova LV, Mozheyko 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. *Gastroenterology, Hepatology, Coloproctology*. 2020; 30 (4): 44–51.
12. *Weigent DA*. Lymphocyte GH-axis hormones in immunity. *Cell Immunol.* 2013; 285 (1–2): 118–132.
13. *Van Buul-Offers SC, Kooijman R*. The role of growth hormone and insulin-like growth factors in the immune system. *Cell Mol Life Sci.* 1998; 54 (10): 1083–1094.
14. *Robbins K, McCabe S, Scheiner T, Strasser J, Clark R, Jardieu P*. Immunological effects of insulin-like growth factor-I – enhancement of immunoglobulin synthesis. *Clin Exp Immunol.* 1994; 95 (2): 337–342.
15. *Walsh PT, Smith LM, O'Connor R*. Insulin-like growth factor-1 activates Akt and Jun N-terminal kinases (JNKs) in promoting the survival of T lymphocytes. *Immunology.* 2002; 107 (4): 461–471.
16. *Li SY, Fang CX, Aberle NS, 2nd, Ren BH, Ceylan-Isik AF, Ren J*. Inhibition of PI-3 kinase/Akt/mTOR, but not calcineurin signaling, reverses insulin-like growth factor I-induced protection against glucose toxicity in cardiomyocyte contractile function. *J Endocrinol.* 2005; 186 (3): 491–503.
17. *González-Juanatey JR, Piñeiro R, Iglesias MJ, Gualillo O, Kelly PA, Diéguez C et al*. GH prevents apoptosis in cardiomyocytes cultured in vitro through a calcineurin-dependent mechanism. *J Endocrinol.* 2004; 180 (2): 325–335.
18. *Maestri M, Dionigi P, Pettenazza P, Visconti F, Rademacher J, Gaspari A et al*. Treatment of the nephrotoxicity of immunosuppressive drugs with insulin-like growth factor-I. *Minerva Chir.* 1998; 53 (5): 391–396.
19. *Mabuchi M, Kawamura I, Takeshita S, Fushimi M, Takakura S, Hirosumi J et al*. Insulin-like growth factor-I enhances choleretic action of FK506 in rats. *Transplant Proc.* 2003; 35 (4): 1596–1598.
20. *Kawamura I, Takeshita S, Fushimi M, Mabuchi M, Seki J, Goto T*. Induction of choleresis by immunosuppressant FK506 through stimulation of insulin-like growth factor-I production in the liver of rats. *Eur J Pharmacol.* 2001; 419 (1): 99–105.
21. *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.
22. *Clark R, Strasser J, McCabe S, Robbins K, Jardieu P*. Insulin-like growth factor-1 stimulation of lymphopoiesis. *J Clin Invest.* 1993; 92 (2): 540–548.
23. *Naesens M, Kuypers DR, Sarwal M*. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol.* 2009; 4 (2): 481–508.
24. *Kershner RP, Fitzsimmons WE*. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation.* 1996; 62 (7): 920–926.
25. *De Palo EF, Bassanello M, Lancerin F, Spinella P, Gatti R, D'Amico D et al*. GH/IGF system, cirrhosis and liver transplantation. *Clin Chim Acta.* 2001; 310 (1): 31–37.
26. *Leung KC, Ho KK*. Measurement of growth hormone, insulin-like growth factor I and their binding proteins: the clinical aspects. *Clin Chim Acta.* 2001; 313 (1–2): 119–123.

The article was submitted to the journal on 1.03.2021