https://doi.org/10.23873/2074-0506-2021-13-1-33-40

Predictive value of serum cytokine level in the assessment of complications after liver transplantation

A.Yu. Maksimova✉[1], E.N. Bessonova[2], V.V. Bazarnyy[1]

[1]*Ural State Medical University,*

3 Repin St., Ekaterinburg 620028 Russia;

[2]*Sverdlovsk Regional Clinical Hospital № 1,*

185 Volgogradskaya St., Ekaterinburg 620102 Russia

✉Corresponding author: Arina Yu. Maksimova, Junior Researcher, of Central Research Laboratory, Department of General Pathology, Ural State Medical University, oreshek92@list.ru

Abstract

Introduction. *One of the urgent tasks in modern transplantology is the search of biomarkers for predicting and early diagnosis of graft dysfunction.*

Objective. *The study objective was to determine the biomarkers of graft dysfunction.*

Material and methods. *We have examined 19 recipients who underwent liver transplantation and 36 healthy blood donors. Levels of 7 serum cytokines were measured by multiparametric fluorescence analysis with magnetic microspheres (xMAP technology, Luminex 200, USA). Statistical analysis was carried out by methods of nonparametric statistics. To determine the predictive value of the test, a ROC-analysis was performed.*

Results and discussion. *We found that the interleukin-8 level was 3.6 times higher in recipients with graft dysfunction compared to those who had an uneventful postoperative course. The diagnostic sensitivity of the*

test was 75%, the specificity was 91%, and negative predictive value was 84.6.

Conclusion. *Serum interleukin-8 measurement provides a biomarker for early predicting a post-transplant graft dysfunction development.*

Keywords: liver transplantation, cytokines, graft dysfunction

Conflict of interests Authors declare no conflict of interest

Financing The study was performed without external funding

For citation: Maksimova AYu, Bessonova EN, Bazarnyy VV. Predictive value of serum cytokine level in the assessment of complications after liver transplantation. *Transplantologiya. The Russian Journal of Transplantation.* 2021;13(1):33–40. (In Russ.). https://doi. org/10.23873/2074-0506-2021-13-1-33-40

BAR, Balance of Risk

DRI, Donor Risk Index

DSens, diagnostic sensitivity

DSpec, diagnostic specificity

IL, interleukin

MELD, Model of the End Stage Liver Disease

PDGF-BB, Platelet-derived Growth Factor-BB

PIGF, Placental Growth Factor

ROC analysis, Receiver Operating Characteristic analysis

SA-PE, streptavidin-R-phycoerythrin

SOFT, Survival Outcomes Following Liver Transplantation

UCLA-FRS, University of California Los Angeles-Futility Risk Score

VEGF-A, vascular endothelial growth factor-A

Introduction

Liver transplantation is a commonly accepted radical treatment for patients with end-stage chronic liver disease and hepatocellular cancer. Thanks to the recent advances in surgery, immunosuppression, and conservative therapy, the achievement of good short-term results in recipient survival after transplantation has become possible [1, 2]. However, despite this, in technical terms, the operation remains a complex surgical intervention with a high risk of developing minimum a single complication. The causes of their occurrence are diverse. The most common are the biliary tract complications, such as bile leakage, bile duct strictures (5-30%), the infectious, bacterial, viral, fungal complications, and mixed infections (18-28%), arterial complications, such as thrombosis, hepatic artery stenosis (3.8%), and a graft dysfunction (8-24%) [3, 4]. Therefore, timely detection and prevention of complications in the postoperative period remains an urgent problem in transplantation.

One of the ways to solve this problem is primarily to predict the development of a graft dysfunction. Previously proposed predictive models UCLA-FRS (University of California Los Angeles-Futility Risk Score), SOFT (Survival Outcomes Following Liver Transplantation), DRI (Donor Risk Index), BAR (Balance of Risk) have not been widely used in liver transplantation, as being based on a large list of donor and recipient parameters thus creating technical difficulties [5, 6]. In view of this, the search for laboratory biomarkers for predicting postoperative complications and functional disorders of the transplanted organ continues. A. Perrakis et al. suggested using blood procalcitonin level as a marker of complications and a prognostic factor of mortality in the post-transplant setting, but its use was limited, since that parameter naturally predicted only the dysfunction that occurred due to bacterial and fungal infections [3, 7].

In the recent years, experts have suggested the potential role of interleukins (IL) and growth factors in the development of post-transplant complications [8-10]. An attempt was made to predict the development of a liver graft dysfunction based on the measurements of certain cytokine levels (IL-2, IL-4, IL-6, IL-8, IL-10, IL-12) [11-13]. However, such predictive methods are still under development.

Thus, the search for predictors of liver graft dysfunction remains relevant. This determined the objective of our study.

Material and methods

A prospective case-control study included 19 patients who underwent orthotopic liver transplantation at Sverdlovsk Regional Clinical Hospital № 1, Yekaterinburg. Criteria for inclusion in the study were the following: age over 18 years, primary liver transplantation, MELD (Model of the End Stage Liver Disease) score under 30. Depending on the outcome, all recipients were allocated in two groups: those with an uneventful postoperative course (group I; n=11), and those with complications that led to a graft dysfunction (group II; n=8). The liver graft dysfunction was assessed according to the criteria previously proposed by K. Laidler et al.: the blood bilirubin level of no lower than 10 mg/mL, the international normalized ratio of at least 1.6, blood transaminase levels (alanine aminotransferase, aspartate aminotransferase) of no lower than 1500 IU/L [14]. The control group consisted of 36 conditionally healthy blood donors, comparable in gender and age to the study group patients.

At the second scheduled follow-up visit at 3 months after orthotopic liver transplantation, blood samples were collected from the ulnar vein of recipients to study the levels of serum cytokines. The levels of IL-6, IL-15, receptor antagonist IL-1RA, IL-8, placental growth factor

(PIGF), platelet-derived growth factor (PDGF-BB), and vascular endothelial growth factor (VEGF-A) were measured by multiplex fluorescence-based assay with magnetic microsphere beads (xMAP technology, Luminex 200, USA) using a detection and quantification systems Procarta Plex Human Cytokine /Chemokine/ and Procarta Plex Simplex Human Growth Factor (Invitrogen, USA) according to the manufacturer's specification. The biological sample was combined and incubated in a 96-well plate with a mixture of specially prepared magnetic microsphere beads internally dyed with precise proportions of red and infrared fluorophores. Varying color and intensity of fluorescence in the beads creates hundreds of different fluorescent profiles that can be individually examined and classified in a single sample. After adding a mixture of detecting antibodies specific to the cytokines under study and streptavidin-R-phycoerythrin (SA-PE), the resulting suspension was analyzed using a Luminex 200 flow chamber. To detect magnetic particles, the Luminex instrument contains two lasers: red to detect the spectral signature, and green to quantify the amount of SA-PE fluorescence, which is proportional to the amount of protein present in the sample. The concentration of each cytokine was calculated based on the mean fluorescence intensity of the particles. To construct the calibration relationship curve, seven consecutive 4-fold dilutions of the calibrator were prepared. The results were processed using the xPONENT software package.

For statistical analysis, the studied samples were tested for the normality of the distribution across three parameters: indirect, graphical, and calculated. The sample was found not keeping to the normal distribution, so the parameters of quantitative variables which distribution was different from normal are presented by the medians (Me) with 25% and 75% quartiles. The nonparametric two-way Mann-Whitney test was

used to identify the differences in quantitative characteristics between groups. The relationship between variables was assessed using Spearman's rank correlation coefficient. A Receiver Operator Characteristic analysis (ROC-analysis) was also performed with the calculation of diagnostic sensitivity (DSens), specificity (DSpec), and the Area Under the Curve (AUC) (0.9–1.0 being excellent, 0.8–0.9 very good, 0.7–0.8 good, 0.6–0.7 satisfactory). An additional criterion, a negative predictive value was used. The results were processed using Statistica 10.0 software (Dell (Stat Soft), USA).

Results

Statistical analysis of differences in the blood levels of 7 cytokines between healthy volunteers and liver recipients was performed (Table). In the group of patients with an uneventful post-transplant course, the levels of IL-1RA, PDGF-BB, PIGF, VEGF-A were lower than in the control group by 7.6, 2, 216.1, 9.7 times, respectively, those differences being statistically significant. The levels of IL-15 and IL-6 were 0.4 and 10.4 times higher, respectively, versus the control group. With the graft dysfunction development, the levels of cytokines IL-1RA, PDGF-BB, PIGF, and VEGF were also significantly lower: by 7.6, 1.9, 3.6, 4.8 times; and a statistically significant increase in the levels was observed not only for IL-15, IL-6, but also for IL-8 by 0.4, 10.4, 3.6 times versus controls.

Table. Blood serum cytokine concentrations (pg/mL) in liver recipients

Parameters	Control group Me (25%;75%)	Group I Me (25%;75%)	Group II Me (25%;75%)	p1	p2	p3
PDGF-BB	552.0 (457.0;1753.6)	270 (242.0;317.4)	280 (196.6;346.1)	p<0.05	p<0.05	p>0.05
PIGF	363.2 (290.9;481.5)	1.68 (1.62;249.8)	98.7 (1.6;273)	p<0.05	p<0.05	p>0.05
VEGF-A	990.5 (807.4;1505.8)	101.9 (54.5;295.8)	205.9 (158.2;251.4)	p<0.05	p<0.05	p>0.05
IL-6	0 (0;0)	10.47 (5.2;10.47)	10.47 (7.86;10.47)	p<0.05	p<0.05	p>0.05
IL-15	1.5 (0;5.3)	3.54 (3.54;3.54)	3.5 (2.6;3.5)	p>0.05	p>0.05	p>0.05
IL-1RA	326.1 (0;502.2)	42.7 (0.05;42.7)	42.7 (32.0;73.8)	p<0.01	p>0.05	p>0.05
IL-8	0 (0;3.6)	0 (0;0.09)	3.6 (1.3;19.3)	p>0.05	p<0.05	p<0.01

Notes:

p1 - statistical significance of differences between the control group and the group of patients with an uneventful postoperative course

p2 - statistical significance of differences between the control group and the group of recipients with graft dysfunction

p3 - statistical significance of differences between the group of recipients with graft dysfunction and the group of patients with an uneventful postoperative course

Since the study conduct was ultimately aimed at preventing complications, our attention was focused on their potential predictors. Based on the study results presented in the Table, IL-8 can claim such a role. This hypothesis is based on the fact that this parameter was 3.6 times higher in recipients with graft dysfunction than in patients with an uneventful postoperative course and in the controls (Mann-Whitney; $p<0.01$, statistically significant).

The diagnostic significance of IL-8 levels in the blood of recipients after transplantation was assessed using ROC-analysis, which resulted in calculation of the test sensitivity as equal 75%, the test specificity as equal 91%. Meantime, the area under the ROC-curve was 0.87, which, in

accordance with the expert scale for AUC values, indicates a very good quality of the model. Probably, if the test result is negative, there will be no signs of graft dysfunction.

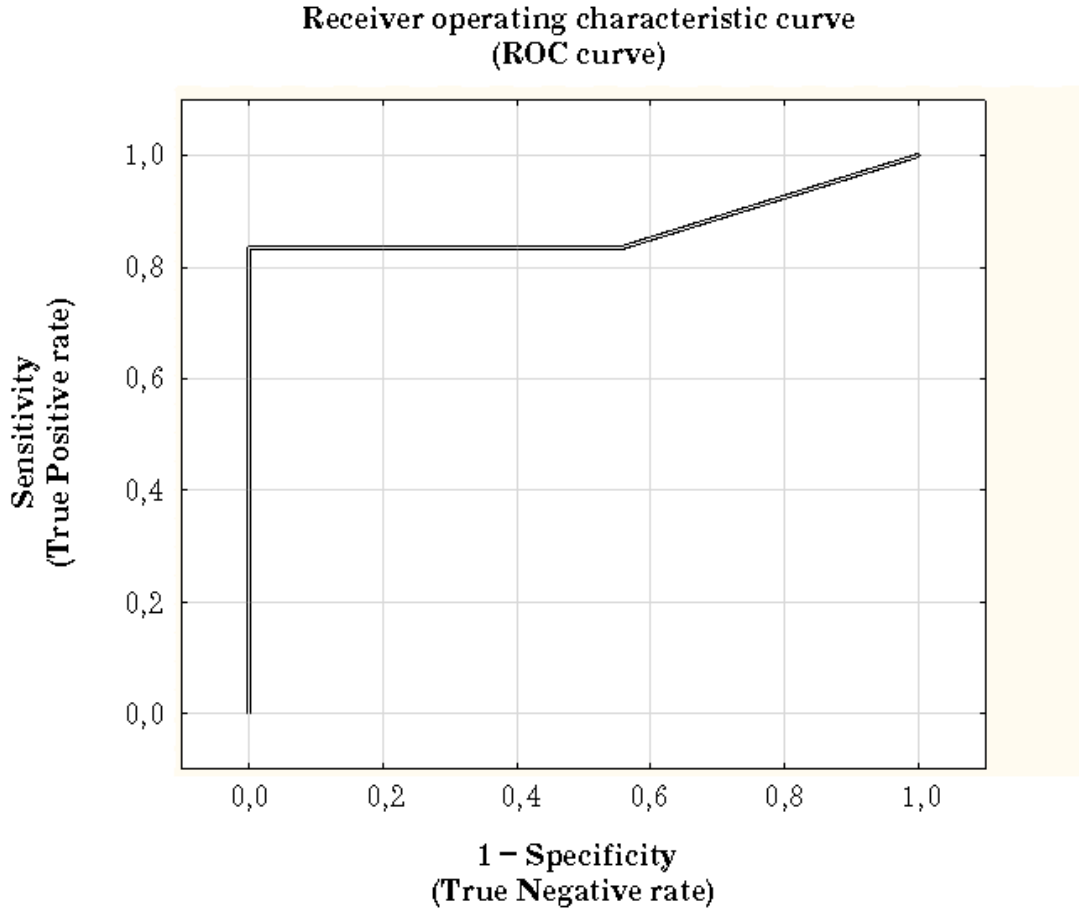

Figure. Diagnostic characteristics of the model for predicting liver graft dysfunction

Discussion

The results obtained indicate that all patients after liver transplantation have an imbalance of pro- and anti-inflammatory cytokines, which is partly consistent with the data of a number of investigators [15-18]. Meanwhile, statistically significant differences between the groups of patients with an uneventful postoperative course versus those with the graft dysfunction were found only in the IL-8 level.

It is known that IL-8 is one of the main proinflammatory chemokines, which is synthesized mainly by monocytes and macrophages [19]. However, it has recently been established that hepatocytes and Kupffer cells are the main sources of IL-8 in chronic liver diseases [20, 21]. So, in the group of patients with the graft dysfunction, the intrahepatic IL-8 synthesis provokes migration of activated neutrophils, leukocytes, and macrophages into the liver [20]. As a result, local inflammation occurs, which initiates liver fibrogenesis. IL-8 is also involved in the formation of a profibrogenic microenvironment due to the activation of collagen-producing liver stellate cells [20]. Moreover, this cytokine triggers trans-differentiation of mature hepatocytes into cholangiocytes by activating the Notch signaling pathway, thereby reducing the number of functioning hepatocytes in the liver [21].

Given the proinflammatory function of this cytokine, in 2017 and 2018 a number of multicenter randomized trials of the drug Reparixin (a non-competitive allosteric blocker of CXCL8 receptor) were conducted, which confirmed this drug efficacy in preventing the development of early graft dysfunction by reducing the IL-8 activity, and as a result, blocking a number of activities related to the recruitment of leukocytes [22].

All the above suggests the interrelation of pathophysiological processes in graft dysfunction development with pleiotropic effects of IL-8. Using this parameter as a tool for predicting/early diagnosing this complication will allow stratifying recipients with regard to their post-transplant course, timely correcting patient's condition, and administering an appropriate treatment.

This study has a number of limitations associated with a small sample size, the need to measure the level of serum cytokines in the period before orthotopic transplantation and before the clinical

manifestations arise. However, the results we have obtained can become the basis for further studies, indicating the potential of using the blood IL-8 level measurement in clinical practice as a predictor of graft dysfunction.

Conclusions

1. The imbalance of cytokines in the post-transplantation period has been revealed. The most significant was the increase in the blood level of interleukin-8, which was 3.6 times higher in the group with graft dysfunction than in the group with a favorable outcome.

2. Based on the ROC-analysis, fairly high values of diagnostic sensitivity of 75%, diagnostic specificity of 91%, and negative predictive value of this parameter were established. This indicates the potential of its use in clinical practice as a predictor of graft dysfunction.

References

1. Pereira CS, Carvalho AT, Bosco AD, Forgiarini Júnior LA. The Perme scale score as a predictor of functional status and complications after discharge from the intensive care unit in patients undergoing liver transplantation. Escala Perme como preditor de funcionalidade e complicações após a alta da unidade de terapia intensiva em pacientes submetidos a transplante hepático. *Rev Bras Ter Intensiva.* 2019;31(1):57–62. PMID: 30970092

2. Jadlowiec CC, Taner T. Liver transplantation: Current status and challenges. *World J Gastroenterology.* 2016;22(18):4438–4445. PMID: 27182155 https://doi.org/10.3748/wjg.v22.i18.4438

3. Zazula R, Prucha M, Tyll T, Kieslichova E. Induction of procalcitonin in liver transplant patients treated with anti-thymocyte

globulin. *Crit Care.* 2007;11(6):R131. PMID: 18088403 https://doi.org/10.1186/cc6202

4. Khubutiya MSh, Zhao AV, Shadrin KB. Postoperative complications in liver transplant recipients: modern considerations of pathogenesis and main areas of prophylaxis and treatment. *Russian Journal of Transplantology and Artificial Organs.* 2009;11(2):60–66. (In Russ.).

5. Martínez JA, Pacheco S, Bachler JP, Jarufe N, Briceno E, Guerra J, et al. Accuracy of the BAR score in the prediction of survival after liver transplantation. *Ann Hepatol.* 2019;18(2):386–392. https://doi.org/10.1016/j.aohep.2019.01.002

6. Schlegel A, Linecker M, Kron P, Györi G, Oliveira ML, Müllhaupt B, et al. Risk assessment in high- and low-MELD liver transplantation. *Am J Transplant.* 2017;17(4):1050–1063. PMID: 27676319 https://doi.org/10.1111/ajt.14065

7. Perrakis A, Stirkat F, Croner RS, Vassos N, Raptis D, Yedibela S, et al. Prognostic and diagnostic value of procalcitonin in the post-transplant setting after liver transplantation. *Arch Med Sci.* 2016;12(2):372–379. PMID: 27186183 https://doi.org/10.5114/aoms.2016.59264

8. Hwang W, Lee J. Pathophysiologic implications of cytokines secretion during liver transplantation surgery. *Int J Med Sci.* 2018;15(14):1737–1745. PMID: 30588198 https://doi.org/10.7150/ijms.28382

9. Hassan L, Bueno P, Ferrón-Celma I, Ramia JM, Garrote D, Muffak K, et al. Early postoperative response of cytokines in liver transplant recipients. *Transplant Proc.* 2006;38(8):2488–2491. https://doi.org/10.1016/j.transproceed.2006.08.054

10. Klune JR, Bartels C, Luo J, Yokota S, Du Q, Geller DA. IL-23 mediates murine liver transplantation ischemia-reperfusion injury via IFN-γ/IRF-1 pathway. *Am J Physiol Gastrointest Liver Physiol.* 2018;315(6):991–1002. https://doi.org/10.1152/ajpgi.00231.2018

11. Woo YS, Lee KH, Lee KT, Lee JK, Kim JM, Kwon CHD, et al. Postoperative changes of liver enzymes can distinguish between biliary stricture and graft rejection after living donor liver transplantation: A longitudinal study. *Medicine (Baltimore).* 2017;96(40):e6892. https://doi.org/10.1097/MD.0000000000006892

12. Kim JM, Kim JH, Lee S-Y, Park JB, Kwon CHD, Kim SJ, et al. Prediction of biliary complications after living-donor liver transplantation based on serum cytokine profile. *Transplant Proc.* 2014;46(3):861–864. https://doi.org/10.1016/j.transproceed.2013.11.038

13. Kurabekova RM, Tsiroulnikova OM, Pashkova IE, Olefirenko AG, Gichkun OE, Makarova LV, et al. Diagnostic effectiveness of transforming growth factor beta 1 in assessing the risk of developing graft dysfunction in liver recipient children. *Russian Journal of Gastroenterology, Hepatology, Coloproctology.* 2019;29(3):58–65. (In Russ.). https://doi.org/10.22416/1382-4376-2019-29-3-58-65

14. Bolondi G, Mocchegiani F, Montalti R, Nicolini D, Vivarelli M, De Pietri L. Predictive factors of short term outcome after liver transplantation: A review. *World J Gastroenterol.* 2016;22(26):5936–49. PMID: 27468188 https://doi.org/10.3748/wjg.v22.i26.5936

15. Meier RH, Meyer J, Montanari E, Stéphanie L, Balaphas A, Muller Y, et al. Interleukin-1 receptor antagonist modulates liver inflammation and fibrosis in mice in a model-dependent manner. *Int J Mol Sci.* 2019;20(6):1295. https://doi.org/10.3390/ijms20061295

16. Joshi M, Oltean M, Patil PB, Hallberg D, Kleman M, Holgersson J, et al. Chemokine-mediated robustaugmentation of liver

engraftment: a novel approach. *Stem Cells Transl Med.* 2015;4(1):21–30. PMID: 25473087 https://doi.org/10.5966/sctm.2014-0053

17. Chae MS, Kim JW, Chung HS, Park CS, Lee J, Choi JH, et al. The impact of serum cytokines in the development of early allograft dysfunction in living donor liver transplantation. *Medicine (Baltimore).* 2018;97(16):e0400. PMID: 29668595 https://doi.org/10.1097/MD.0000000000010400

18. Gras J, Wieërs G, Vaerman JL, Truong DQ, Sokal E, Otte JB, et al. Early immunological monitoring after pediatric liver transplantation: cytokine immune deviation and graft acceptance in 40 recipients. *Liver Transpl.* 2007;13(3):426–433. PMID: 17318867 https://doi.org/10.1002/lt.21084

19. Hull J, Ackerman H, Isles K, Hull J, Ackerman H, Pinder M, et al. Unusual haplotypic structure of IL-8, a susceptibility locus for a common respiratory virus. *Am J Hum Genet.* 2001;69(2):413–419. https://doi.org/10.1086/321291

20. Zimmermann HW, Seidler S, Gassler N, Nattermann J, Luedde T, Trautwein C, et al. Interleukin-8 is activated in patients with chronic liver diseases and associated with hepatic macrophage accumulation in human liver fibrosis. *PLoS One.* 2011;6(6):e21381. PMID: 21731723 https://doi.org/10.1371/journal.pone.0021381

21. Sasaki T, Suzuki Y, Kakisaka K, Wang T, Ishida K, Suzuki A, et al. IL-8 induces transdifferentiation of mature hepatocytes toward the cholangiocyte phenotype. *FEBS Open Bio.* 2019;9(12):2105–2116. PMID: 31651102 https://doi.org/10.1002/2211-5463.12750

22. ClinicalTrials.gov Identifier: NCT03031470; ID Number: TPL-RPX-01-Pilot study of reparixin for early allograft dysfunction prevention in liver transplantation. Available at:

https://clinicaltrials.gov/ct2/show/NCT03031470 [Accessed December 24, 2020]

Information about the authors

Arina Yu. Maksimova, Junior Researcher of Central Research Laboratory, Department of General Pathology, Ural State Medical University, https://orcid.org/0000-0001-8412-4315

50%, the development of the study concept and design, collecting material, statistical data processing, analysis of the data obtained, text preparation, editing

Elena N. Bessonova, Dr. Sci. (Med.), Head of Gastroenterology Department, Sverdlovsk Regional Clinical Hospital № 1, https://orcid.org/0000-0002-4223-3473

25%, analyzing the data obtained, text preparation, editing

Vladimir V. Bazarnyy, Dr. Sci. (Med.), Professor of the Department of Clinical Laboratory Diagnostics and Bacteriology, Ural State Medical University, http://orcid.org/0000-0003-0966-9571

25%, analyzing the data obtained, text preparation, editing

The article was received on October 31, 2020;
Approved after reviewing November 24, 2020;
Accepted for publication December 21, 2020