Brazilian Journal of TRANSPLANTATION

ORIGINAL ARTICLE

Time Between Start of Tacrolimus Use and Target Serum Level in Patients After Adult Liver Transplantation

Paola Hoff Alves[1,*], Vittoria Sampaio[1], Alexandre de Araújo[2], Caroline Tortato[1], Mário Reis Álvares da Silva[2]

1.Hospital de Clínicas de Porto Alegre – Seção de Farmácia Clínica – Serviço de Farmácia – Porto Alegre/RS – Brazil

2.Hospital de Clínicas de Porto Alegre – Serviço de Gastroenterologia e Hepatologia – Porto Alegre/RS – Brazil

https://doi.org/10.53855/bjt.v25i4.467_IN

Correspondence author:
phoffalves@hcpa.edu.br

Section Editor:
Ilka de Fátima Santana Ferreira Boin

Received:
May 18, 2022

Approved:
Sept. 14, 2022

Conflict of interest
Nothing to declare.

How to Cite:
Alves PH, Sampaio V, Araújo A, Tortato C. Time Between Start of Tacrolimus Use and Target Serum Level in Patients After Adult Liver Transplantation Hepático Adulto. BJT. 2022.25(04):e0322. https://doi.org/10.53855/bjt.v25i4.467_IN

eISSN
2764-1589

ABSTRACT

Introduction: Tacrolimus is one of the most used immunosuppressants in patients after liver transplantation and there is often a wide variation in its serum level. Assessing the mean time to reach the target serum level of tacrolimus after liver transplantation is critical, as very low levels increase with chances of graft rejection and very high levels are associated with toxicity. **Methodology:** Observational, retrospective cohort study conducted at Hospital de Clínicas de Porto Alegre. Liver transplant patients between December 2012 and December 2017 using oral tacrolimus were included. Patients who reached a serum level between 6–8 ng/mL during month 1 post-transplant were considered as target serum levels. **Results:** 78% (67/87) of patients reached the target serum level within 10 days after liver transplantation. Patients who experienced rejection over 1 year after transplantation took around 9 days to reach the target serum level during the index hospital stay. Patients who did not present rejection reached target serum level within 7 days after starting the immunosuppressant. **Conclusion:** Our work, considering therapeutic target values between 6–8 ng/mL in patients after immediate liver transplantation, demonstrated that this population performed a mean time that seems to determine a good prognosis, since in less than 2 weeks 78% of patients reached target serum level close to what was recommended.

Descriptors: Immunosuppression; Serum Level; Liver Transplant.

INTRODUCTION

According to the Brazilian Association of Organ Transplants, from January 1997 to March 2020, 27,670 liver transplants were performed in Brazil. Indicated, among others, in cases of cirrhosis due to autoimmune or nonautoimmune reasons, some metabolic disorders, acute liver failure, chronic liver failure, and hepatocellular carcinoma, liver transplantation is the last treatment option available and requires commitment from its candidates, since the number of organs offered for donation does not meet the demand 2020.[1-3] In Brazil, from January to March 2020, 796 candidates joined the waiting list, however 160 of these died while waiting for liver transplantation.[1]

To maintain the health of the graft and prevent associated serious complications, solid organ transplantation involves the almost lifelong use of immunosuppressive therapy and other drugs.[4] Immunosuppressive drugs act at different sites in the T-cell cascade and are intended to reduce or inhibit the recipient's immune response to alloantigens present in the donor's transplanted organ. They are classified into: calcineurin inhibitors (inhibit IL-2 synthesis), purine synthesis inhibitors (inhibit nucleic acid synthesis), mammalian target of rapamycin (mTOR) enzyme inhibitors

(inhibit the growth proliferation signal of smooth muscle cells and hematopoietic lineages), and corticosteroids (act at various levels of the cascade). [5] T cells are known to play a central role in the adaptive or acquired immune response, from which, after their activation, they trigger the production and release of soluble molecules that aim to fight the antigen, in the case, the transplanted organ (graft).[6]

In order to keep immunosuppressive drugs within the appropriate therapeutic range, monitoring the blood levels of the drugs is vital to the management of patients after transplantation.[7] In post-liver transplant patients, the collection of blood tests, especially the serum level (SL) of the immunosuppressant tacrolimus (TAC) is standard routine, and a variation in these levels, sometimes abruptly, is often observed. Very low levels of tacrolimus increase the chances of graft rejection, and very high levels are associated with toxicity, nephrotoxicity being one of the most important adverse effects.[8]

Some factors impact on the variability of tacrolimus blood levels, such as pharmacogenetic factors, gastrointestinal factors (interfering with absorption), hypoalbuminemia, and others. Therefore, knowing the mean time to reach the immunosuppressive therapeutic target can guide strategies to optimize therapy, preventing early graft dysfunction and avoiding adverse reactions. The objective of this work was to determine the mean time to reach the SL target of tacrolimus in adult post-liver transplant patients.

METHODS

This is an observational, retrospective cohort study that was conducted through chart analysis at the Hospital de Clínicas de Porto Alegre, with adult liver transplant patients between December 2012 and December 2017.

The study population consisted of patients who met the following inclusion criteria: patients over 18 years of age, liver transplant recipients, and those taking oral tacrolimus as immunosuppressant. Patients who had other organ transplant, patients on sublingual and intravenous tacrolimus, and/or death at index hospitalization or retransplantation were excluded from the study.

The following variables were analyzed: gender, age at the time of transplant, underlying disease (indication for transplant), date of transplant, glomerular filtration rate, creatinine, tacrolimus SL values, and index hospitalization time. In addition, the presence of the following comorbidities was evaluated: diabetes, renal failure, neoplasms, bone fracture, tuberculosis, dyslipidemia, biliary tract complications, hepatitis C, hepatitis B, cytomegalovirus, hypothyroidism, hepatitis from other causes, metabolic syndrome, osteoporosis, steatosis, nonalcoholic fatty liver disease, and acute rejection.

DEFINITIONS

Target blood level of tacrolimus

Patients who achieved SL between 6–8 ng/mL during the first month after transplant according to institutional protocol were considered on target.

The mean time to SL target of tacrolimus was evaluated in days, which was also followed during hospitalization and up to one month after transplantation, with the delta range calculated from the date of drug initiation to the date of the first value between 6–8 ng/mL. A spot analysis of SL at D30 post-transplant was performed.

Acute rejection

All patients who had biopsy-confirmed rejection within one year after transplantation were considered.

The continuous variable "mean time to SL target of tacrolimus" was described as mean ± standard deviation and/or median (25–75%), as distribution. Categorical variables, such as underlying disease, were described in absolute (n) and relative (%) frequency. The collected data were stored in an electronic database (Microsoft Excel) and analyzed using the statistical program Statistical Package For Social Sciences (SPSS) version 18.0. The collection of data from the medical records was performed after approval by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre and received approval under opinion No. 44616421600005327.

RESULTS

In the period from January 2012 to December 2017, 125 patients underwent liver transplantation at the hospital in question. Of these, 38 patients met exclusion criteria, and considering this, 87 liver transplant patients were included in the study and followed up for 1 month after transplantation.

The average age was 55.7 years, and 64.4% of the patients were male. Regarding the reason for transplantation, most patients underwent transplantation for the following diseases: 56.3% (49/87) cirrhosis due to hepatitis C virus (HCV), 10.3% (9/87) cirrhosis due to HCV + alcohol, 4.6% (4/87) cirrhosis due to hepatitis B virus (HBV), 3.4% (3/87) due to alcoholic cirrhosis, and the remaining 25.4% (22/87) had other underlying diseases.

From the 87 patients included in the study, a total of 763 tacrolimus SL samples were obtained, resulting in an average of 8 samples per patient. The average length of stay for these patients was 14 days.

There were 78% of patients who reached the target SL within a mean of 10 (± 3.2) days after liver transplantation. Patients who experienced rejection over 1 year after transplantation took around 9 days to reach the target SL during index hospitalization. The patients who did not show rejection, on the other hand, reached SL target within 7 days of starting the immunosuppressant. Figure 1 shows the general distribution of tacrolimus blood levels over 30 days after transplantation.

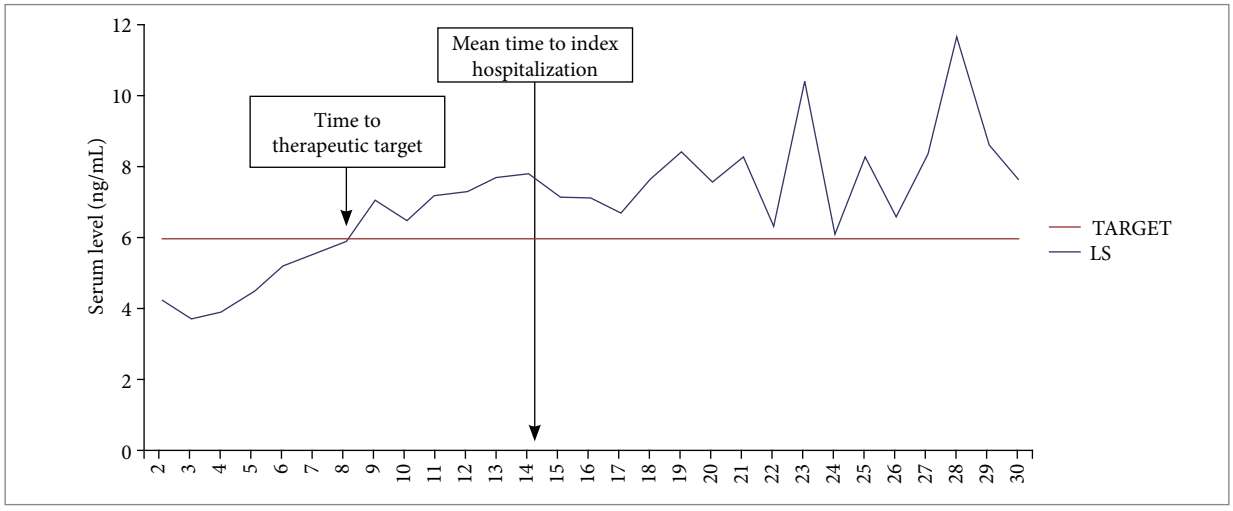

Figure 1. Distribution of mean tacrolimus blood levels of the study population in relation to post-transplant days.

In the longitudinal evaluation, SL were determined at time D30 after initiation of tacrolimus use, being the target value 27% of the time.

DISCUSSION

A total of 87 patients were included in this study, and the average age of the patients was 55.7 years, and most of them were male (64.4%). The characteristics of the study population in terms of age and sex are similar to previous studies with liver transplant patients. In the study by Su et al.,[9] whose objective was to describe trends in age among liver transplant registrants and recipients in the United States between 2002 and 2014, the mean age increased from 51.2 years in 2002 to 55.7 years in 2014. Another study,[10] which evaluated the rate of variability of tacrolimus SL in New York between 2007 and 2010, showed that of the 150 patients included in the study, 47% (70 patients) had their transplant between 51 and 60 years of age. The increase in the age of transplantation may reflect the increase in life expectancy of the population and the advances in recent years in therapies related to the underlying diseases, such as new drugs for treating HCV that have fewer adverse effects and greater efficacy than interferon, reflecting the control of the disease and prolonging the need for transplantation.

Advanced age alone is not a cause of graft rejection; however, several studies have shown that the risk of immunological and nonimmunological complications is higher in liver transplant recipients with advanced age. Elderly transplant recipients are considered weak immune responders, graft and patient survival in this age group can be significantly reduced in a single episode of acute rejection.[11–28]

Regarding gender, a study by Bhat et al.,[12] whose objective was to identify patients at higher risk of neoplasms after liver transplantation in a large multicenter database, reported that 64.6% of transplant patients were male, which is similar to that found in this study.

Regarding the reason for transplantation, most patients (56.3%, 49/87) had HCV cirrhosis, 10.3% (9/87) had HCV + alcohol cirrhosis, 4.6% (4/87) had HBV cirrhosis, 3.4% (3/87) had alcoholic cirrhosis, and the remaining 25.4% (22/87) had other underlying diseases. In older studies, such as that of Ciesek and Wedemeyer,[13] it is reported that HCV infection was the leading cause of liver transplantation worldwide, which compares with the study by Supelana et al.,[10] who evaluated the rate of variability

of tacrolimus LS in New York between 2007 and 2010, and showed that of the 150 patients included in the study, 77 (51%) underwent transplantation for the underlying disease HCV cirrhosis.

During the last decade, the composition of the waiting list and indications for liver transplantation have changed significantly in Europe and worldwide due to the development of new antiviral drugs, changes in lifestyle and nutritional behavior.[14]

Since the introduction of direct-acting antiviral drugs,[15,16] the proportion of liver transplantation due to HCV infection has decreased significantly from 21% in 2014 to 11% in 2017 according to the European Liver Transplant Registry. On the other hand, cancer (mainly hepatocellular carcinoma) was the indication with the largest increase from 12% in 1997 to 24% between 2007 and 2016.[14] Similar observations were also reported by the Nordic Transplant Registry,[17] in which hepatocellular carcinoma as a primary indication for liver transplantation increased from 2.5% in 1994 to 20% in 2015. Another study, which aimed to analyze the epidemiological profile of patients on the waiting list for liver transplantation in Espírito Santo between January 2015 and January 2018, concluded that of 244 patients included in the study, 56 (22.95%) had primary liver cancer as their underlying disease, followed by alcoholic cirrhosis in 53 (21.72%) patients.[18] The period in which the population of this study was analyzed may have been the reason why the main cause of transplantation was still HCV hepatitis.

Evaluating the collections for SL determination, a total of 763 samples were obtained, resulting in an average of 8 samples per patient. The average length of stay for these patients was 14 days. According to Neuberger et al.,[19] it is recommended to collect 1 sample per patient every 2 or 3 days for the first 15 days after transplantation, so within a 14-day period, 7 samples were collected, which reflects adequacy of our process, in which the data were similar. The protocol of collecting every 3 days is based on the average time of stability of the drug, which has an average half-life in whole blood of approximately 43 h; therefore, daily collection is not necessary, since the pharmacokinetics of the drug will not reflect reliable values of SL, and may act as a confounder in adjusting the dose.

Controversial issues are discussed with regard to the target SL. In our population, we consider the recommendation of Neuberger et al.,[19] who advocate therapeutic target at levels between 6–8 ng/mL in the first month post liver transplant. Our finding demonstrated that most patients achieved SL target within an average of 10 days after initiation of use, and in the subgroup analysis patients who had acute rejection achieved target in 9 days versus 7 of those without rejection.

Rodríguez-Perálvarez et al.,[20] who evaluated tacrolimus exposure in the first 15 days after liver transplantation, found that patients with levels greater than 7 ng/mL had less moderate/severe rejection compared to patients with levels less than 7 ng/mL. Jia et al.,[21] whose objective was to investigate the effect of the strategy of 'minimizing tacrolimus exposure on long-term survival of patients after liver transplantation, using lower therapeutic targets as a target and consequently lower doses, demonstrated that patients with a mean tacrolimus blood concentration of 5–10 ng/mL did not impact long-term survival, even considering lower therapeutic target.

Another study, which aimed to investigate the association of variation in tacrolimus levels with clinical outcomes in a sample of 127 patients from 2006 to 2013, found that there was variation greater than or equal to two standard deviations in 41% of patients, characterizing high variability of the drug. Of these patients, 8 had rejection (15%), but the association of rejection with variation was not significant. However, there was a significant association between a rate of change of SL greater than or equal to two standard deviations with mortality and survival, concluding that worse outcomes are associated with increased variation in tacrolimus blood levels. Speculation is that the nonsignificant statistical association with the rejection variable may be justified by the sample size.[22]

Studies have sought to determine factors that may identify predisposition to poor compliance and variability in tacrolimus SL in an attempt to act early with strategies to improve immunosuppression for these patients. In our work, we observed high variability of tacrolimus SL from day 17 post-transplant. Since the average length of stay was 14 days the high variability found may be related to poor medication adherence after discharge. One method suggested to try to identify early the patient at risk, is the medication level variation index (MLVI). Such an index represents the degree of fluctuation between blood drug levels on an individual basis, which can be calculated in liver transplant recipients by the standard deviation of tacrolimus SL. It is suggested that higher MLVI values represent erratic immunosuppression, considering low drug adherence as the most likely factor. In the study by Supelana et al.,[23] data were obtained from the medical records of 150 randomly selected adult liver transplant recipients. The MLVI was significantly higher in patients who had biopsy-confirmed rejection (mean MLVI = 3.8) compared to the rest of the cohort (mean MLVI = 2.3).

In contrast, Alves et al.[24] evaluated tacrolimus SL variation index scores in an adult post-liver transplant population and were unable to associate MLVI values with worse outcomes, in the paper the authors found similar rejection rates using an MLVI cutoff point of 2.5.

In the pediatric population, scores such as MLVI are widely used to determine prediction of poor adherence by associating high variability with poorer outcomes. In the study by Shemesh et al.,[25] 400 pediatric liver transplant recipients (1–17 years) were

followed for 2 years; 53% of adolescents with MLVI > 2 achieved acute late rejection by the end of the second year compared to 6% of those with MLVI ≤ 2.

In addition to poor adherence, other factors may be related to variability in tacrolimus SL and should be considered in strategies to obtain therapeutic targeting. Gastrointestinal factors such as diarrhea and vomiting can alter tacrolimus concentrations.[26] Hypoalbuminemia and anemia can modify tacrolimus distribution, increasing its circulating free fraction, resulting in expressive variability and increased exposure.[27]

Our work has some limitations. The first one is related to the type of study: cohort studies are subject to loss to follow-up, which can impact the sample size and consequent statistical power for association with the outcome, besides not being able to control for all confounding factors. We also considered limiting factors that we did not use direct patient intervention tools to assess adherence, that the study was conducted at a single center, and that the dosing regimen that correlates with variation in tacrolimus SL was not evaluated.

FINAL CONSIDERATIONS

The individualization of therapeutic targets to determine the most effective treatments is increasingly being discussed. Target blood levels for immunosuppression can vary according to the transplanted organ, and even within the same transplant population, reflecting the need for an integrated follow-up by the multiprofessional team in determining and following the therapeutic plan for these patients.

Our work, by considering therapeutic target values between 6–8 ng/mL in immediate post-liver transplant patients, demonstrated in our population that the average time to therapeutic target obtained seems to determine good prognosis based on previous work, since in less than 2 weeks 78% of patients reached SL target. It was also possible to observe that patients who took longer to target therapy had a higher rejection rate, thus suggesting the impact of subtherapeutic values on clinical outcomes.

The behavior of the postdischarge variation curve is striking and may be associated with poor drug adherence. More direct intervention studies with patients that relate adherence to clinical variability and outcomes are needed for better conclusions.

AUTHORS' CONTRIBUTION

Scientific and intellectual relevant contribution to the study: Alves HP, Araújo A, Silva MRA; **Conception and design:** Sampaio V, Alves HP, Araújo A; **Data analysis and interpretation:** Sampaio V, Alves HP; **Writing the manuscript:** Sampaio V; **Critical revision:** Alves HP, Araújo A, Tortato C, Silva MRA; **Approval of the final version:** Alves HP.

AVAILABILITY OF RESEARCH DATA

Data will be available upon request.

FUNDING

Not applicable.

ACKNOWLEDGEMENTS

Not applicable.

REFERENCES

1. Associação Brasileira de Transplantes de Órgãos. Registro Brasileiro de Transplantes. [acesso em: 05 out. 2020]. São Paulo: ABTO. Disponível em: www.abto.org.br/abtov03/Upload/file/RBT/2020/RBT-2020-1trim-leitura.pdf
2. Jadlowiec CC, Taner T. Liver transplantation: Current status and challenges. World J Gastroenterol. 2016;22(18):4438-45. https://doi.org/10.3748/wjg.v22.i18.4438
3. Azzam AZ. Liver transplantation as a management of hepatocellular carcinoma. World J Hepatol. 2015;7(10):1347-54. https://doi.org/10.4254/wjh.v7.i10.1347

4. Eaton CK, Gutierrez-Colina AM, Quast LF, Liverman R, Lee JL, Mee LL, et al. Multimethod assessment of medication nonadherence and barriers in adolescents and young adults with solid organ transplants. J Pediatr Psychol. 2018;43(7):789-99. https://doi.org/10.1093/jpepsy/jsy016

5. Ministério da Saúde. Secretaria de Ciência, Tecnologia, Inovação e Insumos Estratégicos em Saúde. Departamento de Gestão e Incorporação de Tecnologias e Inovação em Saúde. Protocolo Clínico e Diretrizes Terapêuticas para imunossupressão no transplante hepático em pediatria. Brasília: Ministério da Saúde, 2020. [acesso em: 07 out. 2020]. Disponível em: https://www.gov.br/conitec/pt-br/midias/protocolos/publicacoes_ms/pcdt_imunossupresso-no-transplante-heptico-em-pediatria_-isbn.pdf

6. Cruvinel WM, Mesquita Júnior D, Araújo JAP, Tieko T, Catelan TTT, Souza AWS, Silva NP, et al. Sistema imunitário – Parte I: Fundamentos da imunidade inata com ênfase nos mecanismos moleculares e celulares da resposta inflamatória. Rev Bras Reumatol. 2010;50(4):434-61. https://doi.org/10.1590/S0482-50042010000400008

7. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet. 1995;29(6):404-30. https://doi.org/10.2165/00003088-199529060-00003

8. Russell CL, Cetingok M, Hamburger KQ, Owens S, Thompson D, Hathaway D, et al. Medication adherence in older renal transplant recipients. Clin Nurs Res. 2010 May;19(2):95-112. https://doi.org/10.1177/1054773810362039

9. Su F, Yu L, Berry K, Liou IW, Landis CS, Rayhill SC, et al. Aging of liver transplant registrants and recipients: Trends and impact on waitlist outcomes, post-transplantation outcomes, and transplant-related survival benefit. Gastroenterology. 2016 Feb;150(2):441-53.e6; quiz e16. https://doi.org/10.1053/j.gastro.2015.10.043

10. Supelana C, Annunziato RA, Schiano TD, Anand R, Vaidya S, Chuang K, et al. Medication level variability index predicts rejection, possibly due to nonadherence, in adult liver transplant recipients. Liver Transpl. 2014;20(10):1168-77. https://doi.org/10.1002/lt.23930

11. Sonny A, Kelly D, Hammel JP, Albeldawi M, Zein N, Cywinski JB. Predictors of poor outcome among older liver transplant recipients. Clin Transplant. 2015;29(3):197-203. https://doi.org/10.1111/ctr.12500

12. Bhat M, Mara K, Dierkhising R, Watt KD. Gender, race and disease etiology predict de novo malignancy risk after liver transplantation: Insights for future individualized cancer screening guidance. Transplantation. 2019;103(1):91-100. https://doi.org/10.1097/tp.0000000000002113

13. Ciesek S, Wedemeyer H. Immunosuppression, liver injury and post-transplant HCV recurrence. J Viral Hepat. 2012;19(1):1-8. https://doi.org/10.1111/j.1365-2893.2011.01548.x

14. Adam R, Karam V, Cailliez V, O Grady JG, Mirza D, Cherqui D, et al. 2018 Annual Report of the European Liver Transplant Registry (ELTR) – 50-year evolution of liver transplantation. Transpl Int. 2018 Dec;31(12):1293-317. https://doi.org/10.1111/tri.13358

15. Schinazi R, Halfon P, Marcellin P, Asselah T. HCV direct-acting antiviral agents: The best interferon-free combinations. Liver Int. 2014;34(Suppl 1):69-78. https://doi.org/10.1111/liv.12423

16. Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. Gastroenterology. 2017;153(4):996-1005. https://doi.org/10.1053/j.gastro.2017.06.012

17. Espen Melum. The Nordic Liver Transplant Registry (NLTR): Annual report 2020. [acesso em: 01 dez 2021]. Disponível em: http://www.scandiatransplant.org/members/nltr/TheNordicLiverTransplantRegistryANNUALREPORT2020.pdf

18. Lemos LD, Silva M, Bertollo LA, Bertollo CA, Matos LA, Venturi AB, et al. Análise do perfil epidemiológico dos pacientes em lista de espera para transplante de fígado no Espírito Santo / Analysis of the epidemiological profile of patients on waiting list for liver transplantation in Espírito Santo. Arq Med Hosp Fac Cienc Med Santa Casa São Paulo. 2020;65:e16. https://doi.org/10.26432/1809-3019.2020.65.016

19. Neuberger JM, Bechstein WO, Kuypers DRJ, Burra P, Citterio F, De Geest S, et al. Practical Recommendations for long-term management of modifiable risks in kidney and liver transplant recipients: A guidance report and clinical checklist by the Consensus on Managing Modifiable Risk in Transplantation (COMMIT) Group. Transplantation. 2017;101(4):S1-56. https://doi.org/10.1097/TP.0000000000001651

20. Rodríguez-Perálvarez M, Germani G, Papastergiou V, Tsochatzis E, Thalassinos E, Luong TV, et al. Early tacrolimus exposure after liver transplantation: Relationship with moderate/severe acute rejection and long-term outcome. J Hepatol. 2013;58(2):262-70. https://doi.org/10.1016/j.jhep.2012.09.019

21. Jia JJ, Lin BY, He JJ, Geng L, Kadel D, Wang L, et al. "Minimizing tacrolimus" strategy and long-term survival after liver transplantation. World J Gastroenterol. 2014;20(32):11363-9. https://doi.org/10.3748/wjg.v20.i32.11363

22. Maciel NB, Schwambach KH, Blatt CR. Transplante hepático: Variação dos níveis sanguíneos de tacrolimo e desfechos de sobrevida, rejeição e óbito. Arq. Gastroenterol. 2021;58(3):370-6. https://doi.org/10.1590/S0004-2803.202100000-62

23. Supelana C, Annunziato RA, Schiano TD, Anand R, Vaidya S, Chuang K, et al. Medication level variability index predicts rejection, possibly due to nonadherence, in adult liver transplant recipients. Liver Transpl. 2014;20(10):1168-77. https://doi.org/10.1002/lt.23930

24. Alves PH, Adriao BY, Álvares da Silva M, et al. Applicability of the medication level variability index (MLVI) in Adult hepatic transplantation and association with graft rejection rates. J Bras Transpl. 2021;24(2):1-80. https:doi.org\10.53855/bjt.v24i2.009

25. Shemesh E, Bucuvalas JC, Anand R, Mazariegos GV, Alonso EM, Venick RS, et al. The Medication Level Variability Index (MLVI) predicts poor liver transplant outcomes: A prospective multi-site study. Am J Transplant. 2017;17(10):2668-78. https://doi.org/10.1111/ajt.14276

26. Hochleitner BW, Bösmüller C, Nehoda H, Frühwirt M, Simma B, Ellemunter H, et al. Increased tacrolimus levels during diarrhea. Transpl Int. 2001;14(4):230-3. https://doi.org/10.1007/s001470100331

27. Chen D, Guo F, Shi J, Zhang C, Wang Z, Fan J, et al. Association of hemoglobin levels, CYP3A5, and NR1I3 gene polymorphisms with tacrolimus pharmacokinetics in liver transplant patients. Drug Metab Pharmacokinet. 2014;29(3):249-53. https://doi.org/10.2133/dmpk.dmpk-13-rg-095

28. Fijter JW. The impact of age on rejection in kidney transplantation. Drugs Aging. 2005;22(5):433-49. https://doi.org/10.2165/00002512-200522050-00007