International Journal of Applied Mathematics

Volume 35 No. 4 2022, 625-632
ISSN: 1311-1728 (printed version); ISSN: 1314-8060 (on-line version)
doi: http://dx.doi.org/10.12732/ijam.v35i4.10

DYNAMIC CHANGES IN BLOOD PARAMETERS OF LIVER TRANSPLANTATION RECIPIENTS PRE AND POST COVID-19 INFECTION

Nonka N. Yurukova

University Hospital "Lozenets"
Sofia - 1407, BULGARIA

Abstract: The effect of liver transplantation (LT) on the severity and mortality of coronavirus disease 2019 (COVID-19) remained controversial. There is still no consensus on whether liver transplantation (LT) recipients with COVID-19 are at greater risk of developing severe or fatal COVID-19. It is not completely clear what is the course of the disease and what laboratory changes occur. The present study was undertaken to identify the dynamic changes in blood parameters of LT recipients pre and post COVID-19 infection which may be used to diagnose the severity and thus assess the prognosis of such patients. Our collected data are from a Bulgarian liver transplantation program at a single center for adult recipients of LT who were followed up from May, 2020, through May, 2022 in the pandemic environment. The current study aims analyzing the statistically significant differences in over 50 biochemical blood parameters in the cohort of LT recipients pre and post SARS-CoV-2 infection.

AMS Subject Classification: 92C40, 62B15
Key Words: liver transplant recipients; SARS-CoV-2 infection; statistical methods and analysis; dynamic changes

1. Study design and target populations

This is a prospective case study launched after the out-break of COVID-19 in Bulgaria on May 2020. The study was conducted in regional referral hospital for

Received: June 30, 2022 © 2022 Academic Publications

LT patients Lozenetz Hospital in accordance with the Declaration of Helsinki. The protocol was approved by the ethical board of the promoting centre. All data generated or analyzed during this study are included in this published article. All consecutive adult LT recipients with concomitant confirmed SARS-CoV-2 infection (with any symptom profile or level of disease severity) were prospectively enrolled in this study at the time of diagnosis of COVID-19 and were followed-up until May 2022.

Five of the patients (38%) required hospital treatment with mean hospital stay of 11.6 (8-15) days. We closely tracked the dynamic changes in over 50 biochemical blood parameters, including of a severe COVID-19 patient which was hospitalized for 13 days and which had a fatal outcome.

Collected data regarding COVID-19 infection were in thirteen LT patients. Among patients with COVID-19 infection 10 (77%) were vaccinated and 3 (23%) were not vaccinated. Ten of the patients (77%) with COVID-19 infection were male and 3 (23%) were female. The mean age of patients with COVID-19 infection was 54 ± 13 years and the mean post-transplantation time was 8.5 years. Twelve of the patients (92%) with COVID-19 infection were on calcineurin inhibitors, 9 (69%) were on antimetabolites and 2 (15%) were on mTOR-inhibitors.

Among patients with COVID-19 infection with concomitant Diabetes mellitus were 7 (54%), Hypertension 10 (77%), Cardiovascular disease 3 (23%), Chronic kidney disease 3 (23%) and with NAFLD 5 (38%). The mean BMI in patients with COVID-19 infection was 28.4 ± 6.1.

2. Statistical methods

Continuous variables of clinical blood parameters are shown as mean and standard deviation. Categorical variables are shown as numbers and percent (%). Student's t-test for dependent samples (see [1]) or Tukey's Honestly Significant Difference test (see [2]) can be used to find means that are significantly different from each other. The paired sample t-test, sometimes called the dependent sample t-test, is a statistical procedure used to determine whether the mean difference between two sets of n observations is zero. In a paired sample t-test, each subject or entity is measured twice, resulting in pairs of observations $(x_i, y_i)\,(i = 1, 2, ..., n)$. Common applications of the paired sample t-test include case-control studies or repeated-measures designs. The unit of measure and the subject might be the same but there is a difference in time logs. In our case we are interesting of a patient's routine blood parameters measured before x_i and

after $y_i (i = 1, 2, ..., n)$ COVID-19 infection (n – number of the patients in the sample).

Paired t-test is an appropriate statistical test that determines whether there is a statistically significant difference between the means of clinical parameters before and after COVID-19 infection. The null hypothesis for the dependent t-test is that the means from the two related groups are equal: $H_0 : \mu_{\text{before}} = \mu_{\text{after}}$. The alternative hypothesis is that the population means are not equal: $H_0 : \mu_{\text{before}} \neq \mu_{\text{after}}$. Values $p < 0.05$ of t-test statistic were adopted for statistically significant. At first the differences df and its mean value $\bar{d}$ are calculated:

$$d_i = x_i - y_i, (i = 1, 2, ..., n), \qquad \bar{d} = \frac{1}{n} \sum_{i=1}^{n} d_i.$$

The t-statistic for paired test is calculated using the following formula (see [1]): $t = \frac{\bar{d}\sqrt{n}}{S_d}$, where S_d is unbiased estimator of the variance of differences:

$$S_d = \sqrt{\frac{n \sum_{i=1}^{n} (d_i)^2 - \left(\sum_{i=1}^{n} d_i \right)^2}{n(n-1)}}.$$

Calculation depends on given degrees of the freedom $df = n-1$. If the calculated t value of t-test for dependent samples exceeds critical t at p=0.05, then the null hypothesis is rejected.

3. Results and discussion

Student's t-test for dependent samples (pre and post SARS-CoV-2 infection) was performed to identify the statistically significant differences in over 50 biochemical blood parameters (factors) for $n = 13$ LT patients. Statistical software Statistica 13.0, [3], was used to analyze the sample data. Using t-test statistics and p-level less than 0.05 for significance of differences we conclude that 12 of the factors are significant: Erythrocyte Sedimentation Rate (ESR), Hemoglobin (HGB), Hematocrit test (HCT), Creatinine (Creat), Glomerular Filtration Rate (GFR), Uric acid (Uric), Total Protein (TP), Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), Creatine kinase (CK), Total Iron Binding Capacity (TIBC), International Normalized Ratio (INR), and Fibrinogen (Fibrin). The results for estimated mean values of the significant parameters and its standard deviation (SD) for LT recipients pre and

post SARS-CoV-2 infection, p-value of the t-test for dependent samples are presented in Table 1.

Table 1: Characteristics of the estimated parameters with significant differences pre and post SARS-CoV-2 infection

Significant Parameter	Mean ± SD pre SARS-CoV-2 infection	Mean ± SD post SARS-CoV-2 infection	t-test p-level
HGB g/l	124.84±16.23	119.15±15.56	0.0343
ESR mm/h	23.62±17.26	34.15±24.83	0.0337
GFR	66.76±26.73	77.00±27.62	0.0027
HCT l/l	0.39±0.04	0.37±0.05	0.0243
Creat umol/l	152.69±179.35	138.15±175.24	0.0042
Uric umol/l	339.69±77.35	300.00±65.19	0.0259
TP g/l	73.15±5.81	66.62±6.95	0.0030
ASAT U/l	20.54±9.54	23.46±9.39	0.0173
ALAT U/l	26.31±16.10	36.08±21.66	0.0401
CK U/l	82.70±40.47	72.30±37.90	0.0391
TIBC umol/l	71.63±18.42	64.64±16.62	0.0268
Fibrin g/l	3.62±0.80	4.05±1.21	0.0490

COVID-19 has been recognized as a disease that affects multiple organ systems, resulting in a wide range of symptoms. The severity of these symptoms varies from asymptomatic to mild or to a lifethreatening illness. The progress of COVID-19 disease and its symptoms can be divided in two phases. First, the virus enters the target organ cells during the viral phase by interaction of spike (S) protein of SARS-COV-2 with ACE-2-receptors. After the viral phase, some patients develop a secondary phase, called the hyperinflammatory phase, which characterizes with hypercoagulative and hyperinflammatory response. Studies investigating the impact of the COVID-19 pandemic on LT recipients are currently limited. In this prospective case study, we analyzed data regarding COVID-19 infection in our 13 LT patients was compared with 18 LT

patients without COVID-19 infection. The aim is to discuss the risk factors that make LT patients more vulnerable for severe with COVID-19 or mortality and the impact of immunosuppressive therapy. Across the studies, gender, post-transplantation time or comorbidities such as hypertension, diabetes mellitus, cardiovascular diseases, chronic kidney disease and NAFLD were variably identified as independent risk factors for mortality or severe disease. However, overall, no comorbidity was generally reported as a major risk factor (see [4]). The role of comorbidities was strongly influenced by the important effect of age, as comorbidities increase with older age of the recipients. Age was commonly documented as a risk factor for mortality and composite outcomes. The role of advanced age in COVID-19 confirms what has been extensively observed in the general population (see [5]). As a low number of studies divided recipients in subgroups regarding interval after transplantation, there might be statistical power issues analyzing this effect of post-transplantation time. The largest part of the studies could not find an independent association between type of baseline immunosuppression and mortality or severe disease (see [6]). However, the short follow-up time in most of the studies might confound this, clarifying that long follow-up studies are needed to evaluate the modifications on graft function. Concerning the potential hypercoagulative response after binding of SARS-COV-2 to vascular ACE-2-receptors, more studies are necessary to address the role of prophylactic or therapeutic anticoagulation and RAAS-I use in LT recipients with severe disease. Despite an initial delay in IgG response, LT recipients show similar humoral and cellular immune responses after COVID-19 infection. In contrast, LT recipients showed a low immune response after vaccination. The influential role played by more sustained immunosuppression and by the use of antimetabolites on humoral response was confirmed by other studies (see [7]). More research is needed to address the direct effect of COVID-19 on the graft in lung transplant recipients, as well as the factors ameliorating the immune response after vaccination in SOT recipients. Liver transplantation recipients face a substantial threat of COVID-19 infection due to the chronic use of immunosuppression agents and increased comorbidities risks. Cytokine release syndrome is considered a significant cause of severe COVID-19 infection, including multiorgan dysfunction [4]. The chronically suppressed immune system in LT recipients may blunt the effect of inflammatory cascades. Studies have been reported that immunosuppression in LT patients can effectively reduce the hyperinflammatory in the clinical course of COVID-19 and potentially serve as a protecting factor to prevent cytokine release syndrome [5]. Innate and adaptive immunity may be altered in LT recipients taking combinations of immunosuppressive drugs for an extended period [6]. In the present study,

serially decreasing HGB and HCT were observed among the LT recipients pre and post COVID-19 infection and these decreases were statistically significant. In following LT recipients pre and post COVID-19 infection, the levels of creatinine, uric acid, and creatine kinase exhibited decreases. Accordingly, The GFR levels exhibited a statistically significant increase (p=0.0027). Significant increases were observed in ESR (p=0.0337). In addition, statistically significant increases were observed in fibrinogen. ESR and fibrinogen can be used along with CRP levels as inflammatory markers to assess the outcomes of patients with COVID 19 infection [7]. ESR levels have been found to exhibit a significant negative correlation with albumin, total protein, and total iron binding capacity levels, and positive correlation with fibrinogen. Hence, in COVID 19-infected patients, particularly those with LT, the ESR tends to increase temporary, [8]. Patients with severe COVID 19 infection, especially LT recipients, also exhibit a high incidence of liver involvement during their clinical course. COVID 19 may affect multiple organs due to disseminated intravascular coagulation (DIC), and hypoxia with hypoperfusion, [9]. Elevated levels of transaminases along with decreased total protein and total iron binding capacity levels have also been found in patients with COVID 19 with suspected liver inflammation or injury. In addition, certain drugs used in the treatment of COVID 19 have been found to aggravate liver damage [10]. In the present study, the levels of ASAT exhibited steady increases (p=0.0173). The ALAT levels also exhibited statistically significant increases (p=0.0401).

4. Conclusion

In summary, here we report the dynamic changes in routine blood indicators of a small cohort of LT recipients with COVID-19 infection consecutively admitted to University Hospital "Lozenetz" in Bulgaria. Our findings provide information about the blood characteristics of COVID-19, and may help identify patients with a risk of complications and need of hospital admission. COVID-19-related systemic inflammation has been shown to causes multiorgan damages. Hyperinflammation leads to the progression of disease to severe forms. The assessment of inflammatory markers, especially in LT recipients, may guide clinicians in making decisions regarding the severity of the disease. The next step of our investigation is to use mathematical model for prediction of Covid-19 dynamics (see [11]).

References

[1] G. Snedecor, W. Cochran, *Statistical Methods*, Iowa State University Press, Iowa (1989).

[2] E. Hincal, B. Kaymakamzade, N. Gokbulut, Humidity level on Covid-19 with control strategies, *International Journal of Applied Mathematics*, **34**, No 4 (2021), 795–802; doi:10.12732/ijam.v34i4.14.

[3] StatSoft, Inc., STATISTICA Manual (Data Analysis Software System), Version 13.0 (2019).

[4] C. Ronco, T. Reis, Kidney involvement in COVID-19 and rationale for extracorporeal therapies, *Nat. Rev. Nephrol*, **16**, No 6 (2020), 308–310; doi: 10.1038/s41581-020-0284-7.

[5] A. Romanelli, S. Mascolo, Immunosuppression drug-related and clinical manifestation of coronavirus disease 2019: a therapeutical hypothesis, *Amer. J. Transplan.*, **20**, No 7 (2020), 1947–1948; doi: 10.1111/ajt.15905.

[6] V. Terzieva, Y. Uzunova, R. Gornev, L. Spassov, Regulatory T cells in the mosaic of liver transplantation tolerance, In: V. Mihaylov (Ed.), *Organ Donation and Transplantation*, London (2020); https://www.intechopen.com/chapters/73970; doi: 10.5772/intechopen.94362.

[7] S. Thompson, M. K. Bohn, N. Mancini, et al., Horvath and the IFCC taskforce on COVID 19. IFCC interim guidelines on biochemical/hematological monitoring of COVID 19 patients, *Clin. Chem. Lab. Med.*, **58**, No 12 (2020), 2009–2016; doi: 10.1515/cclm-2020-1414.

[8] L. Zhang, Y. Peng, Q. Zheng, L. Jiang, S. Tang, P. Chen, Retrospective analysis of clinical characteristics and laboratory results of COVID 19 patients, *European J. of Inflammation*, **19** (2021), 1–7; doi:10.1177/20587392211011919.

[9] S. Ghahramani, R. Tabrizi, K.B. Lankarani, S. M. Kashani, S. Rezaei, N. Zeidi, M. Akbari, S.T. Heydari, H. Akbari, P. Nowrouzi-Sohrabi, F. Ahmadizar, Laboratory features of severe vs. Non-severe COVID 19 patients in Asian populations: A systematic review and meta analysis, *Eur. J. Med. Res.*, **25**, Art. No 30 (2020), 1-10; https://doi.org/10.1186/s40001-020-00432-3.

[10] C. Danwang, F.T. Endomba, J.R. Nkeck, D.L.A. Wouna, A. Robert, J.J. Noubiap, A meta-analysis of potential biomarkers associated with severity of coronavirus disease 2019 (COVID 19), *Biomark. Res.* **8**, Art. No 37 (2020), 1–13; https://doi.org/10.1186/s40364-020-00217-0.

[11] L. Lazarova, N. Stoikovikj, A. Stojanova, M. Mileva, M. Ljubenovska, Mathematical model for prediction of Covid-19 dynamics, *International Journal of Applied Mathematics*, **35**, No 1 (2022), 119–133; doi:10.12732/ijam.v35i1.9.