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COMPARISON OF BLOOD PARAMETERS IN LIVER TRANSPLANT PATIENTS WITH AND WITHOUT COVID-19 INFECTION

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Abstract: The available data on COVID-19 infection in liver transplant (LT) recipients are not conclusive and are limited to several case series. Transplant recipients are considered as "clinically extremely vulnerable" sub-population for COVID-19 because of lifelong immunosuppressive therapy and their higher rate of underlying comorbidities. We collected data from a Bulgarian liver transplant program at a single center for adult recipients of LT who were followed up from May, 2020, through May, 2022 despite the pandemic environment. The current study aims analyzing the statistically significant differences in over 50 biochemical blood parameters between cohorts of liver transplant patients with SARS-CoV-2 infection and LT patients without symptoms of infection.

AMS Subject Classification: 92C40
Key Words: liver transplant recipients; SARS-CoV-2 infection

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 LT patients Lozenetz Hospital in accordance with the Declaration of Helsinki. The protocol was approved by the ethical board of the promoting centre. All

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data generated or analyzed during this study are included in this published article. All consecutive adult LT patients 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. Collected data regarding COVID-19 infection in our thirteen LT patients was compared with eighteen LT patients without COVID-19 infection. Among patients with COVID-19 infection 10 (77%) were vaccinated and 3 (23%) were not vaccinated, compared to patients without COVID-19 infection 9 (50%) were vaccinated and 9 (50%) were not vaccinated. Ten of the patients (77%) with COVID-19 infection were male and 3 (23%) were female, vs. 14 (78%) of patients without COVID-19 infection were male and 4 (22%) were female. The mean age of patients with COVID-19 infection was 54 years, vs. the mean age of patients without COVID-19 infection was 45 years. The mean post-transplantation time in patients with COVID-19 infection was 8.5 years, vs. the mean post-transplantation time in patients without COVID-19 infection was 6.5 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, vs. 12 (67%) of patients without COVID-19 infection were on calcineurin inhibitors, 8 (44%) were on antimetabolites and 1 (6%) 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%), vs. 6 (33%), 10 (56%), 2 (11%) and 2 (11%) respectively among patients without COVID-19 infection. The mean BMI in patients with COVID-19 infection was 28.4, vs. 23 in patients without COVID-19 infection.

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 independent samples (see [1]) or Tukey's Honestly Significant Difference test (see [8]) can be used to find means that are significantly different from each other. We used Student's t-test, which is an appropriate statistical test that determines whether there is a statistically significant difference between the means of clinical parameters in two unrelated groups. The null hypothesis for the independent t-test is that the population means from the two unrelated groups are equal:

$$H_0 : \mu_1 = \mu_2.$$

We are looking to see if we can show that we can reject the null hypothesis and accept the alternative hypothesis, which is that the population means are not equal:

$$H_A : \mu_1 \neq \mu_2.$$

Values $p < 0.05$ of t-test statistic were adopted for statistically significant. The two sample t-statistic is calculated using the following formula:

$$t = \frac{\bar{x} - \bar{y}}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}},$$

where n_1 and n_2 are sizes of the considered two independent samples, $\bar{x}$, $\bar{y}$ are mean values of the data in first and second sample (estimators of populations mean μ_1 and μ_2) and S is calculated using unbiased estimators of populations variances s_x^2, s_y^2:

$$S = \sqrt{\frac{(n_1 = 1)s_x^2 + (n_2 - 1)s_y^2}{n_1 + n_2 - 2}}.$$

The calculation depends on the given degrees of the freedom $df = n_1 + n_2 - 2$. If the value of two samples t-test for independent samples exceeds critical t at p=0.05, then the null hypothesis is rejected. The independent t-test requires that the dependent variable is approximately normally distributed within each group. We use Lilliefors test (see [2]) as a test for normality. It is an improvement on the Kolmogorov-Smirnov (K-S) test - correcting the K-S for small values at the tails of probability distributions. The independent t-test assumes the variances of the two groups are equal in the population. If the variances are unequal, this can affect the Type I error rate. The checking that there was homogeneity of variance for both groups is assessed by Fisher's test for equality of variances (see [1]).

3. Results and discussion

Student's t-test for independent samples was performed to identify the statistically significant differences in over 50 biochemical blood parameters (factors) between the cohorts of liver transplanted patients with SARS-CoV-2 infection and LT patients without this infection. 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: Hemoglobin (HGB), Mean Corpuscular Volume (MCV), Glomerular Filtration Rate (GFR), Total Bilirubin (TBL), Direct Bilirubin (DBL), Albumin

(Alb), Sodium (Na), Iron, Calcium (Ca), Prothrombin Time (PT), International Normalized Ratio (INR) and Immunoglobulin M (IgM). The results for estimated mean values of the significant parameters and its standard deviation (SD) for both LT cohorts, p-value of the t-test are presented in Table 1.

Table 1: Characteristics of the parameters estimated as a significant

Significant Parameter	Mean ± SD without SARS-CoV-2 infection	Mean ± SD with SARS-CoV-2 infection	t-test p-level
HGB g/l	137.28±15.82	124.84±16.23	0.0413
MCV fl	94.05±6.23	84.84±0.57	0.0049
GFR	89.27±30.01	66.76±26.73	0.0395
TBL umol/l	14.03±4.30	19.33±9.12	0.0388
DBL umol/l	5.36±1.79	8.06±4.20	0.0209
Alb g/l	44.72±4.48	40.38±4.50	0.0127
Na mmol/l	138.67±5.45	142.76±2.74	0.0189
Ca mmol/l	2.44±0.11	2.33±0.15	0.0337
Iron umol/l	16.55±5.07	11.69±4.19	0.0084
PT %	95.44±14.23	78.00±28.73	0.0333
INR	1.01±0.02	1.43±0.76	0.0249
IgM	0.91±0.42	1.39±0.79	0.0358

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 mel-

litus, 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.

4. Conclusion

In summary, here we report the differences in clinical characteristics of a small cohort of LT patients with and without COVID-19 infection consecutively admitted to University Hospital “Lozenets” in Bulgaria. Our findings provide information about the characteristics of COVID-19, and may help us identify patients with a risk of complications and need of hospital admission. Early diagnosis and risk stratification are essential for the prompt initiation of different treatment options, modification of immunosuppression and prevention of complications and development of severe forms of COVID-19 in liver transplant recipients, especially in patients with comorbidities. During the course of treatment there may be a dose reduction of the immunosuppressive therapy but not

discontinuation, especially of the calcineurin inhibitor in mono- or dual-therapy regimens. More research is needed to address the direct effect of COVID-19 on the graft in liver transplant recipients, as well as the factors ameliorating the immune response after vaccination in LT recipients. The next step of our investigation is to analyzed the data using the simplest model for the spread of infection so called SIR model (see [9, 10]).

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