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PREDICTORS OF HEPATIC STEATOSIS IN LIVING LIVER DONORS

M.G. Minina[1, 2], D.V. Voronov[1], A.A. Nevredimov[1], E.A. Tenchurina[1]

[1] Botkin City Clinical Hospital, Moscow, Russian Federation

[2] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

Fatty liver disease (steatosis) is considered a risk factor in donor liver transplantation (LT). Macrosteatosis (>50%) is associated with primary graft dysfunction and may reduce long-term recipient survival. **Objective:** to identify predictors of macrovesicular steatosis (>50%) by analyzing donor characteristics. **Materials and methods.** The retrospective study included 525 potential liver donors between January 1, 2019 and December 31, 2020. Clinical and morphological characteristics of donors were studied using logistic regression and receiver operating characteristic (ROC) analysis. Threshold values of parameters demonstrating statistical significance in multivariate analysis as predictors of >50% hepatic steatosis were obtained by ROC analysis based on calculation of the optimal cutoff point. **Results.** Diabetes mellitus (DM), cause of donor's death (traumatic brain injury), alanine transaminase (ALT) >90 units/L and aspartate transaminase (AST) >110 units/L were predictors of >50% steatosis, revealed by time-zero biopsy in the donor. Almost identical sensitivity and specificity indicators were determined in ROC analysis for liver enzymes – ALT and AST – which were 69.1 and 80.6; 72.2 and 81.1, respectively. Given the obtained values, we can say that with elevated levels of liver enzymes in the donor's blood, there is a high degree of probability of liver parenchymal damage, but low sensitivity indicates possible multifactoriality of liver damage, and fatty liver disease may be one of the factors, but there may also be no damage to the liver parenchyma. At the same time, the rather high specificity revealed in ROC analysis for liver enzymes is a reliable sign of the absence of fatty liver disease at enzyme values less than the threshold. **Conclusion.** The thresholds established for ALT and AST and their corresponding levels of sensitivity and specificity indicate that these parameters have a relatively low predictive level in the context of the presence of severe fatty liver disease in a donor. This allows, nevertheless, to use models built on their basis as screening models in the primary evaluation of liver donors.

Keywords: steatosis, extended criteria liver donors, metabolic associated fatty liver disease.

INTRODUCTION

Donor hepatic steatosis is an independent risk factor that has some significant impact on post-transplant complications, such as reperfusion injury, early graft dysfunction, and overall recipient survival. According to studies, ≥50% severe macrovesicular steatosis has the greatest negative impact on the effectiveness of LT and development of post-LT complications [1]. About 30–51% of donor liver transplants have some degree of steatosis [2, 3]. Prevalence of hepatic steatosis keeps on increasing to date due to the increasing number of donors with obesity and a history of non-alcoholic fatty liver disease (NAFLD) [4, 5].

The so-called time-zero liver biopsy performed during laparotomy remains the gold standard for diagnosing steatosis in donors [6, 7]. However, even at the stage of initial donor assessment, donor specialists should tentatively predict the level of possible steatosis, based on the donor's available clinical characteristics. Similar studies have been conducted by foreign authors, who established a positive correlation between hepatic steatosis and body mass index (BMI) [10, 11].

Rinella M.E. et al. performed a comparative analysis of the predictive value of BMI, liver chemistry tests, imaging studies in potential living liver donors, as possible indicators of grade of steatosis, confirmed morphologically. For example, the authors showed there was a significant correlation between BMI and overall grade of steatosis [12]. Another study demonstrated that skin folds on the body, ALT levels and serum lipid levels correlate with the severity of fatty hepatosis, although not significantly [13]. Jeong-Hoon Lee et al. developed the liver steatosis index: a multivariate analysis indicated that high serum ALT to serum AST ratio, high BMI, and DM were independent risk factors of NAFLD [14]. At the same time, hepatic steatosis was ruled out at liver steatosis <30, while values >60 reliably indicates the presence of hepatic steatosis.

The approximate level of steatosis in a donor can also be determined by the transplant surgeon during laparotomy by liver visualization and palpation. The advantage of this method lies in its apparent ease

Corresponding author: Elmira Tenchurina. Address: 5, Vtoroy Botkinsky proezd, Moscow, 125284, Russian Federation. Phone: (967) 113-87-64. E-mail: arimle@inbox.ru

of implementation. However, the accuracy of macroscopic assessment directly depends on the severity of steatosis and is 71% for severe, 46% for moderate and only 17% for mild steatosis [7–9]. According to some researchers, the positive predictive score on visual inspection was 65.6% for severe macrovesicular steatosis, while the rate of macrosteatosis overdiagnosis was 10.0% on visual inspection [7].

MATERIALS AND METHODS

The retrospective study included 525 potential liver donors between January 1, 2019, and December 31, 2020. During the study, the pool of potential donors was divided into three groups depending on the severity of morphologically confirmed hepatic steatosis. Clinical and morphological characteristics were assessed by logistic regression and ROC analysis. Borderline values of the indicators demonstrating statistical significance in multivariate analysis as predictors of >50% hepatic steatosis were obtained in ROC analysis based on calculation of the optimal cutoff threshold. In a four-field conjugacy table, sensitivity and specificity scores were calculated for characteristics with variable values.

RESULTS AND DISCUSSION

To analyze the clinical characteristics of donors, the entire pool of effective liver donors included in the study was divided into three groups depending on the degree of fatty liver disease as established by time-zero liver biopsy in the donor: group 1, ≤30% steatosis; group 2, 31–50% steatosis; group 3, >50% steatosis. Donors (58/525 people, 11.1%), who did not undergo morphological examination for one reason or another were excluded from this analysis.

It is noteworthy that in groups 2 and 3, more than half of the donors, 51.3% and 58.6%, respectively, had a BMI <30, while the number of donors in % with BMI ≥30 indicating obesity was almost identical in groups 2 and 3; in group 3, there was even some decrease in obese donors. A more detailed analysis of donors revealed that the difference in BMI in groups 2 and 3 was due to the fact that donors with subtotal and total liver steatosis, presumably of alcoholic genesis, had low BMI, which was a factor in distorting the significance of this index at the level of >50% liver steatosis. Similarly, traumatic brain injury (TBI) as a cause of death prevailed in donors with a history of alcoholic hepatitis, which is confirmed by available data – the proportion of donors with TBI in the group with >50% steatosis was 33.3%, whereas in the group with ≤30% steatosis, it was 17.9%. The mean value of the blood platelets in liver donors in group 3 with >50% steatosis was 183.6×10^9/L, which is lower than in groups 1 and 2. We believe that this fact may be related to reduced production of thrombopoietin (a glycoprotein hormone produced mainly by the liver) in livers with subtotal fatty hepatosis). Markus Peck-Radosavljevic et al. studied thrombocytopenia <50,000/μL in patients with chronic liver disease as a result of decreased production of thrombopoietin in it [16]. Thus, theoretically, thrombocytopenia may serve as a nonspecific indicator of reduced liver function, including against the background of steatosis. In our opinion, this fact requires further study.

ALT and AST, as the best-known markers of liver damage up to and including necrosis, had the highest mean values in group 3 with >50% steatosis, 88.6 units/L and 124.1 units/L, respectively. Incidence of DM in group 3 donors was more than twice as high relative to group 1, 7.6% vs 20.9% (Table 1). The mean value of total bilirubin (TBil) in donors in all donor groups did not exceed reference values; however, there was a slight increase in TBil level in the steatosis groups relative to group 1.

Next, logistic regression analysis was performed to identify reliable predictors of >50% steatosis. Donor characteristics that showed statistical significance of $p < 0.05$ in logistic regression were taken into account. The following factors demonstrated statistical significance in the context of predicting the presence of severe steatosis in liver donors: cause of donor death – TBI, BMI ≥30 kg/m^2, presence of DM in donors, with a rather high OR value of 2.91, increased liver enzymes, TBil, and reduced platelet count. Age is an important factor in the evaluation of donor livers for transplantation, but no proven relationship with the level of hepatic steatosis was found. Various publications have suggested that the ratio of age and sex is the main physiological predictor of developing hepatic steatosis [17], but whereas fatty hepatosis occurs more often in men at a young age [18], when reaching 50 years of age, it is equally common in men and women. The mean age of potential liver donors in group 3 with >50% steatosis was 49.0 years, which is comparable with the above data and confirms our results that there is no relationship between incidence of severe liver steatosis and donor's age and/or gender. Liver steatosis reduces insulin clearance, and has a negative effect on hepatic insulin resistance, leading to increased plasma glucose levels, compensatory hyperinsulinemia, and progression of type 2 DM [19]. In our study, blood glucose levels showed no correlation with severe hepatic steatosis; we tend to associate the elevated blood glucose levels detected in donors with donors' brain death conditions. We considered blood platelets, regardless of the fact that they demonstrated a significant relationship with 50% hepatic steatosis, as highly variable and nonspecific and were excluded from the final predictors (Table 2).

We selected quantitative donor factors for ROC analysis – BMI, ALT, AST, and TBil. Quantitative ROC analysis is characterized by AUC (area under the curve). The higher the AUC, the higher the quality of the classifier (factor), while the value of AUC ≤0.5 demonstrates the low predictive ability of a particular factor. Figure shows ROC curves and AUC values for BMI, ALT, AST and TBil: 0.567, 0.774, 0.750, 0.648, respectively. The greatest prognostic value with respect to >50% macrosteatosis are donor ALT and AST values, whose AUC is 0.774 and 0.750, respectively. Donor BMI and TBil were excluded as predictors of >50% steatosis because of low AUC values. So, we do not consider high TBil and increased body weight as screening predictors of steatosis in the donor (Fig.).

In ROC analysis, optimal cutoff thresholds were obtained by calculations so that the studied models for predicting severe donor steatosis could be used in practice. For ALT and AST values, the cutoff thresholds were 90 U/L and 110 U/L, respectively.

Taking into account the established thresholds, the sensitivity and specificity values for ALT and AST were obtained in the form of a contingency table. See Tables 3–4.

Almost identical indicators of sensitivity and specificity were revealed for liver enzymes ALT and AST – 69.1%, 80.6% and 72.2%, 81.1%, respectively.

Table 1

Comparative analysis of effective liver donor groups depending on steatosis grade

Factors		Group 1	Group 2	Group 3	P
		Steatosis 0–30%, n = 341	Steatosis 31–50%, n = 39	Steatosis >50%, n = 87	
Age, years, avr. (min–max)		48.8 (19–68)	51.2 (29–63)	49.0 (28–67)	0.46
Male / female, n (%)		233 (68.3) / 108 (31.7)	26 (66.7) / 13 (33.3)	64 (73.6) / 23 (26.4)	0.60
Stroke / traumatic brain injury, n (%)		280 (82.1) / 61 (17.9)	33 (84.6) / 6 (15.4)	58 (66.7) / 29 (33.3)	**0.004**
BMI, kg/m^2, n (%)	<30	251 (74.0)	20 (51.3)	51 (58.6)	**0.001**
	≥30	88 (26.0)	19 (48.7)	36 (41.4)	
DM, n (%)		26 (7.6)	4 (10.3)	18 (20.9)	**0.001**
Hypertension, n (%)		166 (48.7)	21 (58.3)	40 (46.0)	0.744
Platelets, ×10^9/L, avr. (min–max)		232.8 (14–567)	200.9 (66–330)	183.6 (18–469)	**<0.0001**
Arterial lactate, mmol/L, avr. (min–max)		2.9 (0.4–17.0)	3.0 (0.7–8.9)	3.4 (0.90–15.0)	0.308
ALT, U/L, avr. (min–max)		41.6 (1.8–783)	58.7 (15.6–459.0)	88.6 (6.60–319.0)	**<0.0001**
AST, U/L, avr. (min–max)		45.5 (1.5–947)	70.2 (7.1–729)	124.1 (16.9–729.6)	**<0.0001**
Total bilirubin, μmol/L, avr. (min–max)		11.8 (2.2–72)	17.2 (3.7–88.5)	16.5 (3.4–58.0)	**<0.0001**
Liver explanted / not explanted, n (%)		284 / 57 (83.3 / 16.7)	17 / 22 (43.6 / 56.4)	15 / 72 (17.2 / 82.8)	**<0.0001**

Note. avr., average value.

Table 2

Logistic regression of donor characteristics and >50% liver steatosis detected by time-zero biopsy in donors

Factors	OR	95% CI, min–max	P
Age, years	1.003	0.984–1.022	0.737
Gender, m/f	1.250	0.786–1.989	0.346
Cause of death, Stroke / TBI	1.903	1.172–3.088	**0.009**
BMI, kg/m^2	1.045	1.008–1.084	0.017
Hypertension	0.972	0.638–1.479	0.893
DM	2.908	1.576–5.364	**0.001**
ALT, U/L	1.009	1.005–1.013	**<0.0001**
AST, U/L	1.011	1.007–1.014	**<0.0001**
TBil, μmol/L,	1.033	1.015–1.052	**<0.0001**
Platelets, ×10^9/L	0.993	0.991–0.996	**<0.0001**
Glucose, mmol/L	1.042	0.991–1.094	0.106

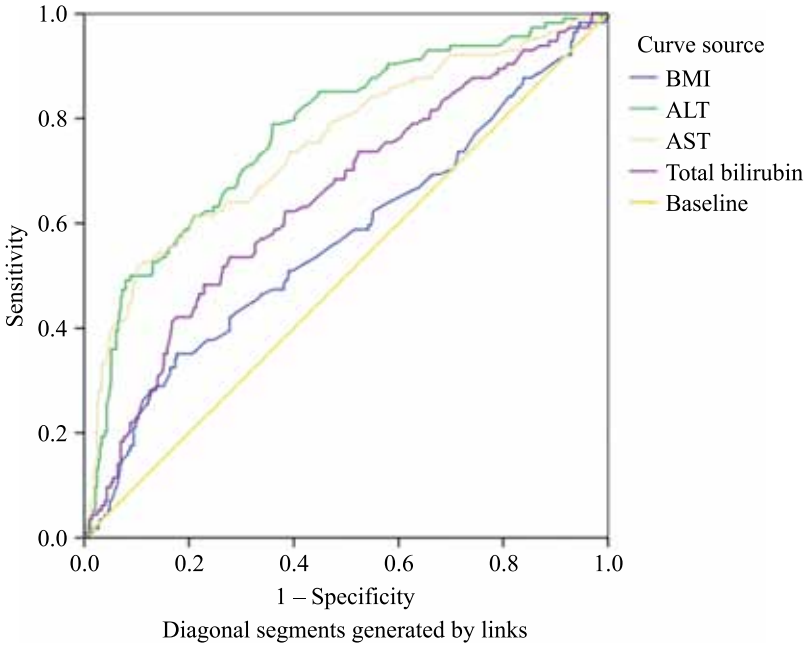
Note. OR, odds ratio; CI, confidence interval.

Given the values obtained, we can say that if the level of liver enzymes reaches and passes the threshold values we have identified, liver parenchyma damage is highly probable, but low sensitivity indicates a possible multifactorial nature of such damage; steatosis may be one of the factors.

CONCLUSION

Predictors of morphologically confirmed >50% steatosis in liver donors were identified. Donor age, sex, and blood glucose levels are not reliable predictors of hepatic >50% steatosis. BMI and TBil have low AUC values (0.56 and 0.645, respectively) in ROC analysis. Therefore, we believe they cannot be used as screening predictors of this pathology. The factor such as thrombocytopenia, although demonstrating a significant correlation with >50% hepatic steatosis, is nonetheless too

variable and nonspecific, and may be associated with various causes that were not considered in this study. TBI, as the cause of donor death, correlates with total and subtotal liver steatosis of alcoholic origin. Among the considered possible predictors of >50% hepatic steatosis, transaminases (ALT, AST) with relatively low sensitivity (69.1% and 72.2%, respectively) showed acceptable specificity (80.6% and 81.1%, respectively), which means that if these parameters are increased (ALT >90 U/L, AST >110 U/L) in a potential liver donor, significant fatty hepatosis can be predicted with a certain degree of probability. A significant association with >50% steatosis



Area under the curve

Validation result variables	Area	Standard error	Asymptotic value	Asymptotic 95% CI	
				Lower bound	Upper bound
BMI	0.567	0.033	0.034	0.503	0.631
ALT	0.774	0.026	0.000	0.723	0.825
AST	0.750	0.028	0.000	0.695	0.805
TBil	0.648	0.030	0.000	0.589	0.708

Fig. ROC curves and AUC for BMI, ALT, AST and TBil

Table 3

Sensitivity and specificity for ALT

	ALT <90	ALT >90	Total:
Steatosis <50	324	17	341
Steatosis >50	78	38	116
Total:	402	55	457
Specificity	324 : (324 + 78) × 100%		**80.6**
Sensitivity		38 : (38 + 17) × 100%	**69.1**

Table 4

Sensitivity and specificity for AST

	ACT <110	ACT >110	Total:
Steatosis <50	327	15	342
Steatosis >50	76	39	115
Total:	403	54	457
Specificity	327 : (327 + 76) × 100%		**81.1**
Sensitivity		39 : (39 + 15) × 100%	**72.2**

was demonstrated by DM in liver donors. Type 2 DM and ALT and AST values above the cutoff point thresholds should raise the concern of specialists during the initial donor evaluation for severe steatosis.

The authors declare no conflict of interest.

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