Tab. 3. *Multivariate logistic regression in search for independent predictors of T-cell mediated rejection in 184 patients undergoing liver transplantation from deceased donors*

Variable	Coefficient	Std. Error	Odds ratio	95% CI	P
Autoimmune hepatitis	1.559	0.634	4.76	1.37 to 16.46	0.014
Absence of ascites	1.146	0.533	3.15	1.11 to 8.95	0.032
Tacrolimus level 5th day post-LTx	-0.161	0.080	0.85	0.73 to 0.997	0.045
Significance level:	P = 0,0009	Cox & Snell: R^2	= 0,09786	Nagelkerke: R^2	= 0,1891
Variables entered: AIH, ascites, D5 tacrolimus level, age, alcohol, creatinine pre-LTx					
Variables not retained: age, alcohol, serum creatinine pre-LTx					

TCMR (table 2) displays the comparison between TCMR and no-TCMR groups. Average age in the group with TCMR vs. no-TCMR was 54.3 (46.3, 59.8) years and 42.3 (37.6, 57.7) years (p = 0.073). Etiology of liver disease was significantly different in AIH 33.3 % vs 6.7 % (p = 0.001); ALD (Alcohol-related Liver Disease) 23.8 % vs 50.9 % (p = 0.035); PSC (Primary Sclerosing Cholangitis) 19.0 % vs 6.7 % (p = 0.13); PBC (Primary Biliary Cholangitis) 14.3 % vs 8.0 % (p = NS); viral hepatitis (HBV, HCV) 4.8 % vs 12.9 % (p = NS); NAFLD (Non-Alcoholic Fatty Liver Disease) 4.8 % vs 11.0 % (p = NS); MW (Morbus Wilson) 4.8 % vs 1.8 % (p = NS) for both groups respectively. Median cold ischemia time (CIT) was 400.0 vs 377.0 min (p = NS) respectively. Representation of individual blood groups (BG) did not show any difference. Tacrolimus concentration at day 5 post LTx was 5.90 (4.00, 9.30) ng/ml vs 4.80 (2.60, 7.00) ng/ml (p = 0.097); tacrolimus concentration at discharge from the hospital was 9.00 (6.80, 11.3) ng/ml vs 8.9 (7.50, 10.6) ng/ml (p = NS) respectively. Length of hospital stay (LOS, group TCMR vs no-TCMR) was 35.0 vs 24.5 days (p = 0.001) respectively.

In univariate analysis, recipient age, alcoholic and autoimmune etiology, pre-transplant serum creatinine, ascites and 5th day tacrolimus concentration were identified as factors being associated with the TCMR. Multivariate logistic model displayed in table 3 reveals three independent risk factors of TCMR: autoimmune liver disease (OR = 4.76, 95% CI 1.37-16.46; p = 0.014); absence of clinically significant ascites prior to LTx (OR = 3.15; 95% CI 1.11-8.95, p = 0.024) and 5th day tacrolimus concentration (OR = 0.85, 95% CI 0.73-0.997, p = 0.045).

Analysis of overall survival post LTx and the survival outcome of TCMR are displayed in figures 1 and 2. Kaplan-Meier survival curves reveal no difference in overall survival between genders (fig. 1) and according to TCMR status (fig. 2).

Discussion

In our study, in a single low-volume liver transplant center over past 10 years, TCMR prevalence was 11.4 %. Three independent risk factors were identified: autoimmune liver disease, absence of clinically significant ascites prior to liver transplantation and lower serum tacrolimus level on the 5th post-transplant day.

The prevalence of TCMR following liver transplantation varies greatly among studies (3, 4) and is mostly dependent on the diagnostic strategy that has been used to define TCMR. Newer studies evaluating tacrolimus-based regimen tend to report lower incidence of TCMR compared with older studies using cyclosporin (22). Combination regimen with tacrolimus and mycophenolate has further decreased the risk. Studies with protocol biopsies have also reported a slightly higher prevalence of TCMR compared with studies when liver biopsies were performed only when clinical or laboratory evidence of graft dysfunction was suspected (23). Our study reports TCMR prevalence in the lower end of the reported intervals. The observation might be explained by lower prevalence of autoimmune liver disease and by our diagnostic strategy based on clinical and biochemical evidence. Per-protocol biopsies at pre-specified intervals after liver transplantation were not performed in our center (2, 3, 5, 23). Liver histology was used only for TCMR confirmation thereby underestimating the true prevalence of histological TCMR. However, possible under-diagnosis of TCMR did not appear to have any adverse impact on patients´ prognosis.

As for TCMR risk factors, patients with AIH had a 4.76-times higher likelihood of TCMR than other etiologies. We also tested the hypothesis, that both autoimmune hepatitis and primary sclerosing cholangitis carry a higher risk of TCMR. However, odds ratio for both diseases in the same multivariate model would be much lower (1.16) compared with odds ratio for solo autoimmune hepatitis (table 3). The absence of clinically significant ascites (stage 2 and 3) prior to LTx, increased the risk 3.15 times. Tacrolimus concentration in TCMR group was lower compared with non-TCMR group, but the difference did not reach statistical significance probably due to different numbers in both groups. However, in multivariate analysis after correction for age, alcohol etiology, autoimmune etiology, creatinine and ascites we found an independent association between lower tacrolimus concentration on day 5 and the higher risk of TCMR. This finding appears consistent with previous reports and also appears plausible. In the end, it is the serum concentration of this immunosupressive drug as the single modifiable risk factor of TCMR. We did not confirm significant association between TCMR and Child-Pugh, MELD scores, the blood type or the length of cold ischemia time. However, the presence of TCMR, with a need for its adequate treatment, led to a significantly prolonged hospital stay compared with patients without TCMR. In the literature, autoimmune liver disease has been frequently reported as ACR risk factor (4, 23, 24). The impact of liver function prior to LTx on the risk of TCMR has been evaluated in 133 patients by Gomez-Manero et al [25]. The study reports that patients with a Child-Pugh score of A and those without ascites had higher risk of TCMR following LTx. In addition, a multicenter study has also identified a trend for lower pre-transplant MELD score among patients developing port-transplant TCMR (26). In our study, MELD score did not predict TCMR, but it was lower than in the mentioned reports. Different selection criteria and liver allocation protocols between centers might also explain observed differences. More severe liver disease in patients with ascites likely leads to more