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The quality of a liver graft

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Abstract Because transplantation success is influenced by the quality of the graft, the objective of this study was to find parameters to evaluate transplant livers in the recipient centre. In 64 liver grafts, the venous effluates of a portal back-table flush were investigated for various parameters. Amongst them, glutathione S-transferase (GST), glutamate dehydrogenase (GLDH) and the leucocyte count were found superior in predicting graft survival. Using the combination of these parameters, 100-day graft survival of between 95% (all parameters positive) and 0% (all parameters negative) was predicted. We concluded that good liver grafts are characterized by a low width of injury (cytosolic component: GST), a low depth of injury (mitochondrial component: GLDH), as well as by a potential to induce tolerance (passenger leucocytes). Perfusate analysis seems to be a valuable tool to recognize problematic grafts in advance and to quantify the "graft factor" in considerations concerning quality control.

Key words Liver graft quality · Effluates · Prediction of survival · Glutathione S-transferase · Glutamate dehydrogenase · Leucocyte count

Introduction

At present, most industrial processes are subject to stringent quality control. In transplantation, a highly interdisciplinary medical process, attempts are made to establish objective quality assurance mechanisms. For the procurement of graft-specific information in the recipient centre, tissue samples (by biopsy) or preservation media samples (by perfusion) can be used. The degree of fat content can be checked easily by a biopsy. However, the time needed for further analysis (e.g. immunohistochemical staining) is too long to influence an intraoperative decision. In contrast, perfusates can be obtained with little effort. The methods of clinical chemistry are established in the infrastructure and are rapid enough to provide reliable and fast information [1]. Some parameters from perfusates have been used in former studies to predict early liver graft viability [2] and to compare University of Wisconsin (UW) and HTK preservation [4]. The objective of this study was to find parameters for receiving centres to evaluate the quality of a liver graft using perfusate diagnostics.

Patients and methods

Perfusates of 64 consecutive liver grafts from June 1994 to June 1997 in 55 recipients (30 males, 25 females) were assayed. After transport to the recipient centre, the liver was checked for morphological abnormalities, the vessels were dissected and the graft was perfused with 500 ml of UW (Viaspan, DuPont Pharma, Bad Homburg, Germany). A standard infusion system was used to rinse the solution from the container to a 16-Ch Foley catheter in the portal vein. The first 60 ml of the effluent from the right liver vein was collected in three samples of 20 ml each. The samples were split and, except the sample for cell counting, centrifuged for 10 min at 2000 *g*. They were kept on ice until parameter estimation.

The parameters AST, ALT, glutamate dehydrogenase (GLDH), LDH, AP, lactate and CK-non-M were estimated by a standard routine laboratory analysis according to the recommen-

Table 1 Predictive value of different parameters for liver perfusates for 100-day graft survival

Parameter	Threshold value	Prediction rate [%]	Specificity [%]	Sensitivity [%]	AUC
Leucocytes[a]	1500/µl	63	65	59	5757
GST	6400 U/l	62	93	30	5713
GLDH	70 U/l	70	93	40	5620
AST	2300 U/l	58	88	18	5527
ALT	2300 U/l	58	83	29	5405
CK-non-M	120 U/l	61	44	76	5363
LDH	7200 U/l	55	80	27	5268
Lactate	10.4 mM	56	72	33	5088
AP	47 U/l	58	77	27	4071

[a] The correlation for leucocytes is vice versa. High leucocyte concentrations result in high function rates

dations of the German Society for Clinical Chemistry. Glutathione S-transferase (GST) activity was estimated by the method described by Habig [3], adapted to an automatic sampling system (Vitalab Eclipse, Merck, Darmstadt, Germany). The reacting buffer consisted of 0.1 *M* Na/K-phosphate, pH 6.5 (KH_2PO_4, Na_2HPO_4), 4 % (v/v) ethanol, 2.5 m*M* glutathione and 1 m*M* 1-Chloro-2,4-Dinitrobenzene. A sample volume of 5 µl was mixed with 800 µl of the reagent. The incubation time was programmed to 48 s, followed by a delay time of 10 s and a time for kinetic measuring of 20 s at $\lambda = 340$ nm. The formation of 1 µmol 1-Chloro-2,4-Dinitobenzene ($\varepsilon = 9.6\ mM^{-1} \cdot cm^{-1}$) conjugate per minute at 25 °C was defined as 1 U. The background activity of 0.009 min^{-1} (= 151 U/l in the sample) was automatically determined and subtracted. All chemicals were purchased from Sigma (St. Louis, USA).

An ANOVA test was used to assess the statistical significance of linear regressions. Sensitivity was defined as the rate of true positive predictions in all positive cases. Specificity was the rate of true negative predictions in all negative cases. The receiver operative characteristic curve (ROC) was done to compare the prognostic potential of different parameters. As the target parameter of liver quality, the function of the graft over the time plotted according to the Kaplan-Meier method was used. Significance in this plot was estimated by the log-rank test. The results were expressed as mean ± SD.

Results

GST activity, GLDH activity and leucocyte number in the perfusates did not show any correlation to each other (Betrag (Spearman's Rho) < 0.1). Comparing the AUC of a sensitivity versus specificity plot (ROC) to predict 100-day graft loss, GST, GLDH and number of leucocytes were found superior compared to other parameters such as transaminases or LDH (Table 1). The specificity ranged from 65 % to 93 % and the sensitivity, from 30 % to 59 %. The cut-off levels, separating grafts with a good prognosis from those with a bad one, were less than 6400 U/l for GST, less than 70 U/l for GLDH and a leucocyte concentration larger than 1500/µl. The three parameters were independently predictive for the function rate.

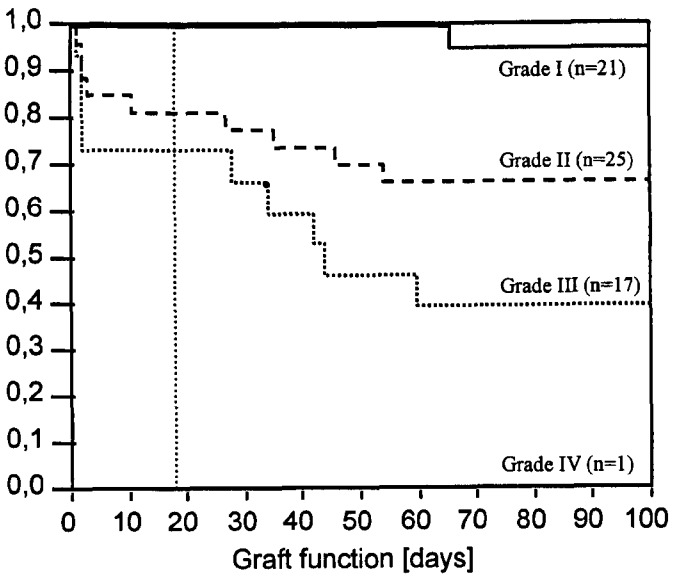

Fig. 1 Kaplan-Meier plot for graft function time: grouped by the grades of liver quality calculated from glutamate dehydrogenase (GLDH), glutathione S-transferase (GST) and leucocyte concentrations in the graft perfusate

The combination of these parameters resulted in an increase in prediction accuracy. After 100 days, 95 % of the grafts judged well by all parameters (grade I, $n = 21$) were still functioning. In the case of two parameters judged well, the function rate fell to 64 % (grade II, $n = 25$) and with only one positive parameter, it fell to 47 % (grade III, $n = 17$). The graft with a bad function prognosis indicated by all parameters (grade IV) failed after 18 days (Fig. 1).

Conclusions

From these results, we concluded that a good liver graft is characterized by a low width of injury (cytosolic component: GST), as well as by a low depth of injury (mitochondrial component: GLDH). Beside these parameters, grafts of high quality exhibited a potential to induce tolerance by the passenger leucocytes. We were even able to identify perioperatively liver grafts of high quality, with a risk of early graft loss of 5 % independent of recipient diagnosis. Unfortunately, the results did not provide arguments to reject grafts, because a 100-day graft survival of 47 % is often preferable to no transplantation. However, these results gave a valuable tool to recognize problematic grafts in advance and to quantify the "graft factor" in general quality considerations.

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