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Effluent Levels of Hyaluronic Acid Can Predict Ultimate Graft Outcome After Clinical Liver Transplantation: A Prospective Series

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PRIMARY nonfunction (PNF) of the liver is an inexact term and investigators continue to debate the site and origin of preservation injury. Some grafts with PNF fail in the operating room, while other grafts have to be replaced between 1 and 14 days postoperatively. The pathology of such grafts displays a spectrum of findings ranging from marked hepatocellular swelling and cholestasis to massive ischemic necrosis. Both recipient and donor etiological factors potentially contribute to PNF. Donor factors include the physiological condition of the heart-beating cadaver before organ procurement, the methods of preservation, period of cold ischemia, and various reperfusion phenomena. To date, there has been no proven method to discriminate in advance of transplantation between clinically acceptable and unacceptable liver allografts.

Because the critical target of preservation injury has been increasingly perceived to be the hepatocyte microvasculature (HMV),[1,2] we attempted in this study to correlate hyaluronic acid (HA), an HMV-specific marker,[3–5] with ultimate graft outcome.

Hyaluronic acid is a glycosoaminoglycan weighing 4 to 8 million kd. It is synthesized in the cell plasma membrane, deposited in the extracellular matrix, transported by the lymphatics, and uniquely catabolized by the liver HMV.[3–5] Serum HA has been used to monitor liver graft function[6] and as a prognostic marker after Tylenol poisoning.[7] Because HA is concentrated in HMV cells, the rationale for the present study was that preservation injury to these cells will result in the leakage of this substance into the organ effluent, where it can be collected and assayed on the backtable before liver transplantation.

In a preliminary retrospective study, the feasibility was demonstrated of correlating effluent HA levels with graft function and viability in an accurate and reproducible manner. Effluent HA values of ≤400 μg/L were associated with unequivocal early graft function, and grafts with HA values of >400 μg/L were at high risk of developing PNF.[8] Therefore, we undertook the following prospective study.

MATERIALS AND METHODS

Seventy consecutive primary, crossmatch–negative, adult orthotopic livers were transplanted into 70 adult recipients. Standard organ procurement techniques were used. All livers were preserved in University of Wisconsin (UW) solution at 4°C. After variable periods of cold storage, 150 mL of cold Ringers lactate was flushed through the portal vein. The suprahepatic cava was clamped and the effluent was collected through the infrahepatic cava. These livers were then reflushed with 500 mL of cold UW solution. These perfusions were performed on the backtable while the livers were being prepared for implantation. All patients received 1 g of methylprednisolone in the operating room and were then maintained on FK 506 and 20 mg/d of methylprednisolone. Recipients were monitored daily for liver function. Ultrasounds, biopsies, and other invasive procedures were performed as clinically indicated.

Table 1. Graft Survival

Group	Cases (n)	Unequivocal Graft Survival	Failure From PNF	Failure From Other Causes
HA ≤400.0 μg/L	58	55 (94.8%)	0	3
HA >400.00 μg/L	12	6 (50%)	6 (50%)	0

Overall 90-day graft survival = 87.1% (61 of 70); overall incidence of PNF = 8.6% (6 of 70).

Assay

The effluent was assayed for HA values using a radiometric assay marketed by Pharmacia Diagnostics.[8] All samples, including standards, were run in duplicate. Variation of results never exceeded 2.2%. The assays can be performed in <3 hours.

Statistical Analysis

The Mann-Whitney U test, a nonparametric equivalent to the standard two-sample *t* test, was used to compare HA and liver function values between groups.

Univariant logistic regression was used to predict PNF based on HA values.

RESULTS

Graft Survival

Table 1 shows an overall 90-day graft survival of 87.1% (61 of 70). PNF was only noted in livers with effluent HA

Table 2. Donor Age and Cold Ischemia Time

Group	Cases (n)	Donor Age (range)	CIT (range)
HA ≤ 400.0 μg/L	58	34.3 ± 14.4 y* (13–60)	14.7 ± 4.3 h* (5.5–23.5)
HA > 400.0 μg/L	12	40.6 ± 11.0 y* (17–55)	14.2 ± 3.8 h* (10.2–21.0)

*NS.

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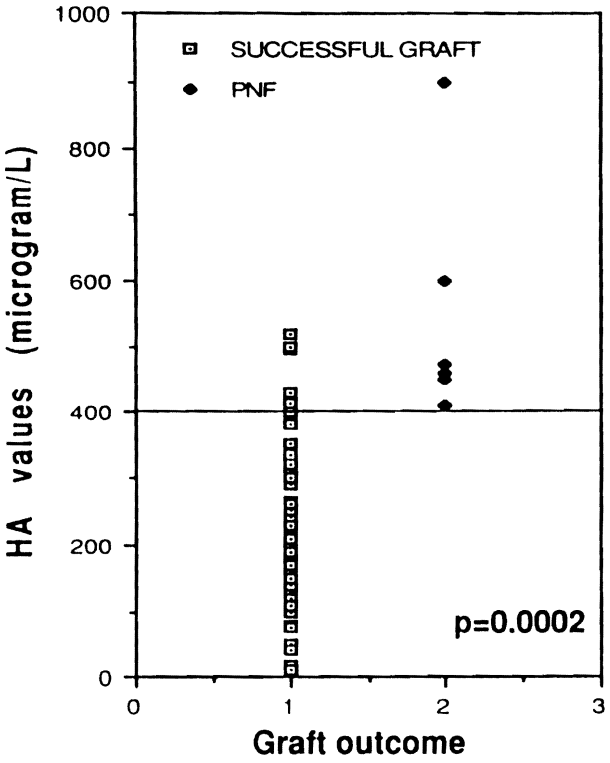

Fig 1. Individual HA values and graft outcome.

levels >400 μg/L. None of 58 livers with low effluent HA levels developed PNF, while 6 of 12 livers with high effluent HA levels developed PNF ($P < .005$). Graft survival beyond 90 days was achieved in 55 of 58 livers with low effluent HA values compared with 6 of 12 livers with high effluent HA values ($P < .001$). Three livers with low effluent HA levels failed between the 7th and 10th days postoperatively. These livers were lost to hepatic artery thrombosis in two cases and death from pneumonia in the third.

Study Parameters

Donor Age and Cold Ischemia Time. No significant differences are noted in either variable between the two groups (Table 2).

Peak Asparate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Levels After Orthotopic Liver Transplantation. Asparate aminotransferase and ALT values in the first 7 postoperative days were both significantly elevated in the group with high effluent HA levels (>400 μg/L) vs the group with low effluent HA levels (≤400 μg/L) ($P < .006$ and $P < .0005$) (Table 3).

Table 3. AST and ALT Levels After OLT

Group	AST (IU/L)	ALT (IU/L)
HA ≤ 400.0 μg/L	1287 ± 1326 (n = 57)	983 ± 1041 (n = 57)
HA > 400.0 μg/L	4687 ± 3439 (n = 12)	3774 ± 2438 (n = 12)
P*	<.0006	<.0005

*Wilcoxon sign rank test.

Table 4. Analysis of False-Positive Livers

Group	Clinical Group	AST (IU/L)	ALT (IU/L)
1	≤400.0 (n = 58)	1287 ± 1326	983 ± 1041
2	PNF (n = 6)	5137 ± 4110	4046 ± 3545
3	False-positive (n = 6)	4237 ± 2940	3502 ± 2230

AST: group 1 vs group 3, $P < .003$; group 1 vs group 2, $P < .05$; group 2 vs group 3, P = NS.
ALT: group 1 vs group 3, $P < .002$; group 1 vs group 2, $P < .05$; group 2 vs group 3, P = NS.

Individual Effluent HA Levels and Graft Outcome. Mean HA values of successful grafts were 218.6 ± 16.2 (mean ± SE) μg/L vs mean values for PNF grafts of 549.3 ± 74.9 μg/L (Fig 1). All six grafts lost to PNF had significantly higher HA levels when compared with successful grafts ($P = .0002$). All PNF grafts had effluent HA values >400 μg/L.

Analysis of False-Positive Livers

False positives were defined as livers that were successfully transplanted despite effluent HA levels >400 μg/L. However, these false-positive livers had a pattern of enzyme release similar to the six livers that experienced PNF (Table 4).

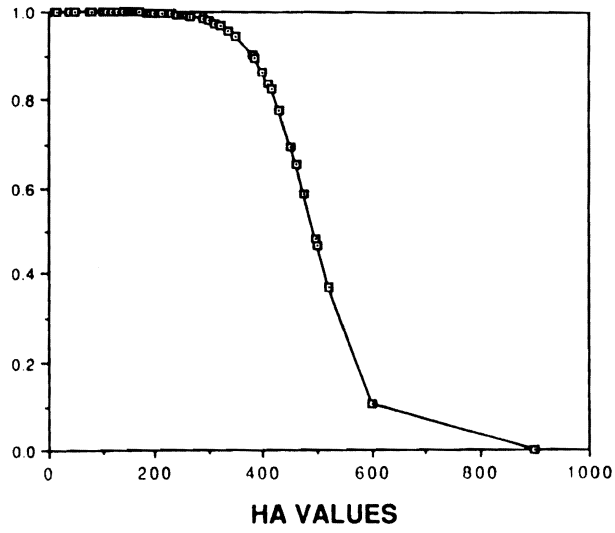

Fig 2. HA values analyzed as predictors of graft outcome using stepwise logistic regression. Analysis showed that an increase of effluent HA values is significantly associated with an increase in the probability of PNF (improved chi-square of 22.135; $P = .0001$).

Degree of Discrimination

Logistic regression was used to test whether graft success and PNF was associated with effluent HA levels (Fig 2). Analysis showed that HA values were significantly associated with both events (improved chi-square of 22.135, $P = .0001$). This implies that an increase in effluent HA levels is associated with an increase in the probability of PNF.

DISCUSSION AND CONCLUSIONS

Six percent to 8% of transplanted livers never adequately function, with consequent death or retransplantation.[9] PNF is the leading cause of early graft loss and is responsible for enormous morbidity, mortality, and expense. We believe that systematic measurements of HA effluent levels will allow detection of livers that already have been seriously damaged and are at high risk of developing PNF. In this study, half the 12 livers failed from PNF in the high-risk group (HA >400 μg/L). Conversely, livers with low HA values were uniformly suitable for transplantation, with no examples of PNF among 58 livers in the good risk group.

Even though six livers with HA values >400 μg/L ultimately survived and were classified as false positives, these livers exhibited a severe injury pattern just short of catastrophic. The position of these livers on a risk scale can be seen with logistic regression analysis in Fig 2. If the HA value in future cases were known in advance, the use of these organs would be unwarranted.

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