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Assessing Risk in Liver Transplantation

Special Reference to the Significance of a Positive Cytotoxic Crossmatch

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Objective
The authors determined the impact of a positive cytotoxic crossmatch on the outcome of liver transplantation.

Summary Background Data
Liver allografts rarely undergo hyperacute rejection, but transplants performed across a positive cytotoxic crossmatch tend to follow a different clinical course, with higher intraoperative blood use, postoperative graft dysfunction, and, in some cases, graft loss. How this affects overall graft survival has not been determined.

Methods
The authors provide a retrospective analysis of 1520 liver transplants performed between November 1989 and December 1993, with a minimum follow-up of 1 year. All cases had a cytotoxic crossmatch using serum pretreated with dithiothreitol.

Results
There were 1390 negative crossmatch and 130 positive crossmatch cases. There was no difference in overall graft survival, although early survival rates were lower in the positive crossmatch group, with the maximum difference at 6 months: 0.76 (95% confidence interval, 0.74–0.78) for a negative crossmatch *versus* 0.68 (95% confidence interval, 0.61–0.77) for a positive crossmatch. These differences become negligible by the 2-year mark. Using stepwise logistic regression, the authors identified seven variables independently associated with outcome: 1) donor age, 2) donor gender, 3) prior liver transplant, 4) medical urgency status, 5) ischemia time, 6) indication for transplantation, and 7) primary immunosuppressant.

Conclusions
The cytotoxic crossmatch is not statistically associated with overall graft survival after liver transplantation. However, early failure rates are higher in the positive crossmatch cases, a difference that disappears by the second year.

In the early days of kidney transplantation, it was established that going across a positive cytotoxic crossmatch carried a prohibitively high risk of hyperacute rejection,[1-3] making this a formal contraindication to the procedure. This has not been the case in liver transplantation. From the beginning, it was observed that the liver is unusually resistant to hyperacute rejection,[4-6] and an analysis of a large series from the cyclosporine era showed no difference in 2-year graft or patient survival when stratified according to crossmatch results,[7] casting doubt on the relevance of this test in clinical liver transplantation. However, this view began to change shortly thereafter.

A report published in 1987 suggested that patients with antibodies directed against donor class I human leukocyte antigens were more likely to have the vanishing bile duct syndrome develop,[8] although most of these patients did not have these antibodies present before the transplant. Of the four that did, only one went on to have this form of chronic rejection.[8] However, clear evidence of hyperacute rejection in recipients of liver allografts was reported over the next 2 years.[9,10]

Since then, a number of reports have appeared showing that a positive cytotoxic crossmatch adversely affects liver transplantation, including graft and patient survival,[11-14] although appropriate immunosuppressive therapy may abrogate this.[15] Some authors, however, have challenged these observations,[16] and we recently completed an analysis of risk factors in 419 patients undergoing 462 liver transplants, where we found no association between a positive cytotoxic crossmatch and graft failure.[17]

Although there appears to be enough evidence that liver transplants performed across a positive cytotoxic crossmatch behave differently than those done in the absence of preformed antibodies, it remains unclear whether their overall outcome also differs. To try to answer this important question, we carried out a multivariate analysis of risk factors on 1520 adult liver transplants, all of which had a cytotoxic crossmatch using pretransplant serum treated with dithiothreitol (DTT).[18-20]

MATERIAL AND METHODS

Patient Population

From November 5, 1989, to December 31, 1993, 1365 adult patients underwent 1556 liver transplants at Presbyterian University Hospital and the Veterans Administration Medical Center, Pittsburgh, PA; 1520 transplants had a cytotoxic crossmatch with DTT and form the basis for this report. Cases were excluded from analysis if the liver was received as part of a multivisceral transplant that included intestine. All grafts were flushed with University of Wisconsin solution. The start of the study period coincides with the introduction of DTT pretreatment in our routine clinical practice. The ending date of the study was chosen to allow at least 1 year of follow-up (patients were observed until January 18, 1995).

Variables Studied and Endpoints

Recipient variables were age, gender, indication for transplantation, UNOS status (United Network for Organ Sharing classification, see Definitions), whether the transplant was primary or a retransplantation, the results of the cytotoxic crossmatch (with DTT), primary immunosuppressive agent (*e.g.*, tacrolimus, cyclosporine, or tacrolimus rescue), and whether the graft was an ABO blood group mismatch.

Donor variables were age, gender, intensive care unit length of stay, harvest serum sodium, and total ischemia time.

The primary endpoint was graft failure (see Definitions), with cause of failure as a secondary endpoint.

Definitions

Definitions are as follows:

1. Graft failure. Patient death or retransplantation at any time during follow-up.
2. Medical urgency. UNOS 1: stable patient, waiting at home; UNOS 2: waiting at home, but requiring medical support; UNOS 3: unstable, in need of continuous hospitalization; and UNOS 4: requiring life-support systems. We should note that this classification was changed on April 1, 1995, but we will use the classification that was in effect during the study period throughout this article.
3. Total ischemia time. Time elapsed from aortic cross-clamping in the donor to portal or arterial revascularization, or both, in the recipient.
4. Indications. Refers to the primary diagnosis. In the case of a retransplantation, the diagnosis corresponds to the cause of graft failure for the preceding liver allograft (Table 1).

Cause of Graft Failure

Causes of graft failure are as follows:

1. Intraoperative. Cardiac arrest of any cause.

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Table 1. INDICATIONS FOR TRANSPLANTATION

Category	Diagnoses
Autoimmune	Autoimmune hepatitis
Cholestatic	PBC, PSC, cystic fibrosis, secondary biliary cirrhosis, and biliary atresia
Alcoholic	Ethanol-induced cirrhosis
Hepatitic	Hepatitis B, hepatitis C, etc.
Metabolic	α_1-Antitrypsin deficiency, Wilson's disease, hemochromatosis, etc.
Cryptogenic	All other etiologies of cirrhosis excluded
FHF	Fulminant hepatic failure
PNF-ischemia	Primary nonfunction and severe ischemic injury
HCC-cholangio	Hepatocellular carcinoma* and cholangiocarcinoma
Other malignancy	Secondary hepatic malignancies
Rejection, acute	Self-explanatory
Rejection, chronic	Self-explanatory
Technical	All technical complications
Other	Budd-Chiari syndrome, benign tumors, etc.

PBC = primary biliary cirrhosis; PSC = primary sclerosing cholangitis.
* Excluding incidental tumors.

2. Neurologic. Including, but not limited to, hemorrhagic or ischemic cerebrovascular accident, central pontine myelinolysis, anoxic encephalopathy, and brain herniation.
3. Cancer: *de novo.* Self-explanatory.
4. Cancer: *de novo,* PTLD. Post-transplantation lymphoproliferative disease.
5. Cancer: recurrent. Recurrent hepatobiliary or extrahepatic cancer.
6. Ischemic injury. Damage of the allograft, either before revascularization or after, that did not have a demonstrable immunologic etiology.
7. Primary non-function (PNF). A graft with such poor initial function that retransplantation or death occurred within 2 weeks. No technical or immunologic causes of failure can be identified.
8. Cardiac. Including, but not limited to, congestive heart failure, arrhythmias, and acute myocardial infarction.
9. MODS. Multiple organ dysfunction syndrome (MODS)[21] without concomitant documented sepsis, and where the liver dysfunction cannot be attributed to identifiable primary hepatic processes.
10. Sepsis. MODS from a documented infection (also known as secondary MODS[21]). Includes bacterial, viral, and fungal etiologies. If a patient dies in the early post-transplant period with a documented infection, the decision to assign it to sepsis, PNF, or ischemic injury is made based on whether there was poor function from the beginning (*i.e.,* a death from sepsis in a graft that never functioned well is coded as PNF or ischemic injury, as the case may be). An exception to this rule is the patient who goes into the transplant with an unrecognized infection (*e.g.,* positive blood cultures that are not reported back until after the surgery), in which case, it is assigned to sepsis regardless of the degree of initial dysfunction.
11. Hepatitis: *de novo.* Includes hepatitis B, hepatitis C, cytomegalovirus, and adenovirus.
12. Hepatitis: recurrent. Self-explanatory.
13. Technical. Including, but not limited to, hepatic artery thrombosis and severe hepatic artery stenosis, portal vein thrombosis, caval stenosis, ruptured pseudoaneurysms, hemorrhage after liver biopsy, bile strictures (whether single or multiple), bile leaks, and bile cast syndrome.
14. Rejection: acute. Including acute cellular and humoral rejections.[22]
15. Rejection: chronic. Occlusive arteriopathy or vanishing bile duct syndrome.[22]
16. Unknown. Lost to follow-up, unclassifiable.
17. Other. Self-explanatory.

When multiple processes were operating simultaneously, the assignment was made to the one considered to be most severe.

Because the immediate cause of death in patients with liver failure is most commonly sepsis or MODS, the cause of graft failure was assigned to the liver pathology in those cases where the allograft dysfunction was the primary identifiable process (*e.g.,* chronic rejection rather than pneumonia).

If a patient died of sepsis in the face of ongoing acute rejection, the cause of failure was assigned to rejection, even if the liver function was not deemed to be severely impaired (we assumed a causal role for the increased immunosuppression required to treat the acute rejection). Exceptions to this rule were patients who first became septic and had the immunosuppression stopped and only then proceeded to reject.

Cytotoxic Crossmatch

Recipient sera were obtained immediately before starting the transplant operation, and an aliquot treated with DTT for 30 minutes to inactivate IgM antibodies.[18–20] A T-cell crossmatch then was performed, with T lymphocytes isolated from donor lymph nodes, using the modified Amos technique.[23] Crossmatches using untreated sera also were done, as a control, but we only consider the results of the crossmatch with DTT. The crossmatch results were scored in the following way:

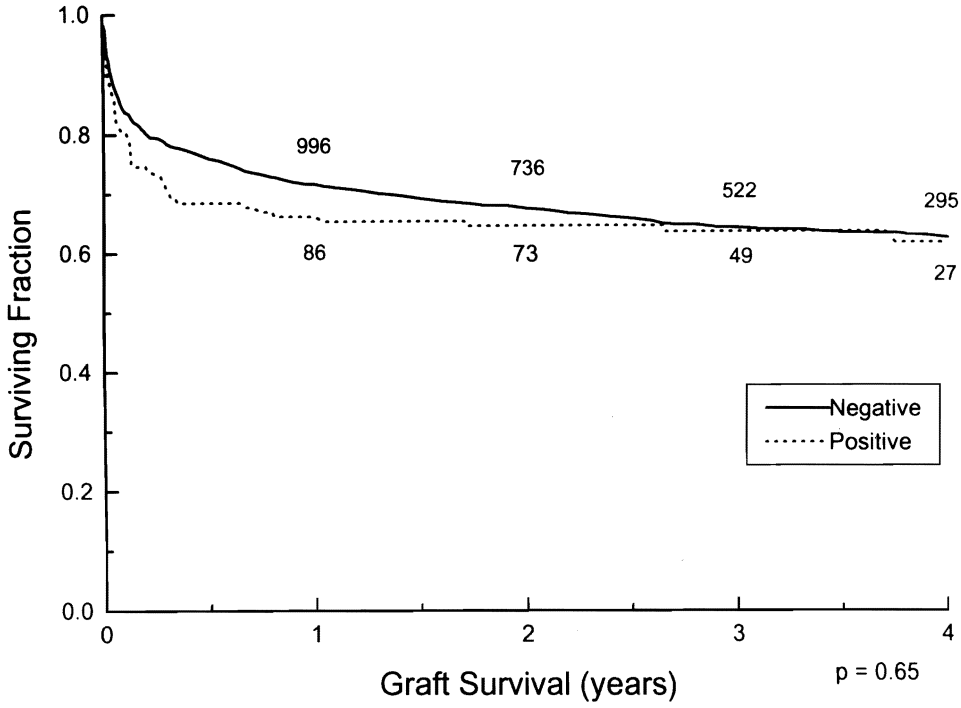

Figure 1. Kaplan-Meier graft survival curves of liver transplantations done in the absence (n = 1390) or presence (n = 130) of a positive cytotoxic crossmatch. There is no difference in the overall results, but the early graft failure rate is higher in the positive crossmatch group. The numbers above and below the curves represent the grafts at risk in the negative and positive crossmatch groups, respectively.

- 0: Uninterpretable
- 1: Negative (1%–10% kill)
- 2: Doubtful positive (11%–20% kill)
- 4: Weak positive (21%–50% kill)
- 6: Positive (51%–80% kill)
- 8: Strong positive (> 80% kill)

For the purposes of this study, we considered a crossmatch to be positive only if there was >50% cell kill. These results were not known prospectively.

Statistical Analysis

Continuous variables are presented as the mean ± standard deviation and categorical variables as proportions. A two-tailed t test was used to test for differences between means. Pearson's chi square was used to test for differences among categorical variables. Exact p values were calculated for unbalanced or sparse contingency tables. If an association was found in variables containing more than two categories, a Tukey–Kramer multiple comparisons procedure on k groups with Bernoulli responses[24] was used to identify the individual categories that were significantly different. The level of significance was set at 0.05.

After the screening univariate analysis, those variables with a significance level of ≤0.3 were used in a stepwise logistic regression analysis[25] to identify the variables that are independent predictors of outcome. In the case of categorical variables, preliminary univariate logistic regression models were fit to determine what subcategories could be grouped together properly. Whenever possible, subcategories were considered individually only if they had ≥100 observations (to obtain more accurate estimates of the odds ratios). A similar preliminary analysis was carried out in the case of continuous variables to determine if they were represented more appropriately as categorical variables. Models were fit using forward inclusion and backward elimination with a likelihood ratio test. The presence of an interaction between variables was tested by introducing appropriate multiplicative terms. A significance level of 0.1 was used in the stepwise procedure.

Survival analysis was performed by means of the Kaplan-Meier method,[26] with the log-rank test to compare strata. All procedures were performed using SPSS (SPSS, Inc., Chicago, IL).

RESULTS

There were 1520 liver transplants performed in 1338 patients. Of these, there were 1479 isolated livers; 18 liver and kidney; 11 liver and pancreatic islets; 9 liver with bone marrow infusion; 1 liver and heart; 1 liver, kidney, and heart; and 1 liver and pancreatic islet combined with a bone marrow infusion. There were 1267 (83.4%) primary transplants and 253 (16.6%) retransplantations.

The crossmatch was negative in 1390 transplants and positive in 130. Eighteen cases in the positive crossmatch group were retransplantations, 6 of which had received

Table 2. CAUSE OF GRAFT FAILURE ACCORDING TO CROSSMATCH RESULTS

	Negative		Positive	
Cause of Failure	**Count**	**%**	**Count**	**%**
Acute rejection	9	1.8	4	8.3
Chronic rejection	14	2.8	2	4.2
PNF-ischemia	105	21.0	5	10.4
Sepsis-MODS	136	27.2	19	39.6
Technical	87	17.4	6	12.5
PTLD	7	1.4	1	2.1
Malignancy	40	8.0	1	2.1
Hepatitis, recurrent	28	5.6	2	4.2
Hepatitis, *de novo*	12	2.4	1	2.1
Cardiac	17	3.4	0	0
Neurologic	7	1.4	3	6.3
Intraoperative	10	2.0	2	4.2
Unknown	15	3.0	0	0
Other	13	2.6	2	4.2

PNF = primary nonfunction; MODS = multiple-organ dysfunction syndrome; PTLD = post-transplantation lymphoproliferative disease.
* No individual category reached statistical significance.

OKT3 with the previous graft (four had a strong positive crossmatch and two a negative crossmatch with the previous graft). Because OKT3 therapy can interfere with lymphocytotoxicity assays,[27] the analysis was conducted with and without these six cases, or excluding only the two cases with a previous negative crossmatch. The results essentially were identical, and all cases were included in the final analysis. Figure 1 shows the Kaplan-Meier graft survival curves, stratified according to the results of the crossmatch. There is no difference in overall graft survival; however, early survival is lower in the positive crossmatch group, with the difference being most pronounced at 6 months: 0.76 (95% confidence interval, 0.74–0.78) for a negative crossmatch *versus* 0.68 (95% confidence interval, 0.61–0.77) for a positive crossmatch. These differences become negligible by the 2-year mark.

There were 500 failed grafts with a negative crossmatch and 48 with a positive crossmatch; Table 2 summarizes the causes of failure. Although no individual failure category reached statistical significance, there are several trends worth noting. Failures due to rejection, both acute and chronic, were more common in the positive crossmatch group, and so were those due to sepsis-MODS. Interestingly, there were fewer failures due to PNF-ischemia than expected in the positive crossmatch group. The technical failures were analyzed separately; there was no difference among the causes of technical failure when stratifying according to crossmatch result. Specifically, failure rates due to hepatic artery thrombosis and biliary complications were identical (but the numbers are too small to make inferences).

To investigate what factors are associated independently with graft failure, we divided the transplants into two groups according to whether they were still functioning at the end of the follow-up period (group I, n = 972) or had failed (group II, n = 548). There were eight ABO mismatched grafts, five in group I and three in group II (p = 0.59). Recipient and donor characteristics are summarized in Tables 3 and 4, respectively.

There was no difference in terms of recipient age, gender, and the results of the cytotoxic crossmatch. However, both groups differed regarding the fraction that was UNOS status 4, had a history of a prior liver transplant, or had cyclosporine-based immunosuppression. The indications for transplantation also differed, with the failure group having proportionally fewer cases of cholestatic and autoimmune liver disease and a relative excess of PNF-ischemia (Table 3). Regarding donor characteristics, failed grafts were more likely to come from older or female donors (Table 4).

Table 3. RECIPIENT CHARACTERISTICS

	Group I (n = 972)	Group II (n = 548)	Significance
Age (y)	49.1 ± 12.2	49.5 ± 12.3	p = 0.49
Sex (M/F)	590/382	347/201	p = 0.31
UNOS 4 (%)	46.6	58.4	p = 0.00001
Prior transplant (%)	11.7	25.4	p < 0.00005
Positive crossmatch (%)	8.4	8.8	p = 0.83
Primary immunosuppressant (%)			
Tacrolimus	88.7	88.2	
Cyclosporine	2.2	4.9	
Tacrolimus rescue	9.1	6.9	p = 0.005
Indication (%)			
Hepatitic	23.8	23.9	
Cholestatic	18.4	9.5	
Alcoholic	20.7	15.3	
Cryptogenic	7.7	9.5	
Autoimmune	4.3	1.5	
HCC-cholangio	5.3	8.9	
Other malignancy	0.4	1.5	
PNF-ischemia	4.5	10.4	
Metabolic	3.0	3.1	
FHF	1.9	2.0	
Chronic rejection	2.1	3.6	
Acute rejection	0.3	0.4	
Technical	3.9	5.8	
Other	3.7	4.6	p < 0.00005

UNOS = United Network for Organ Sharing; HCC-cholangio = hepatocellular carcinoma and cholangiocarcinoma; PNF = primary nonfunction; FHF = fulminant hepatic failure.
Group I = grafts still functioning at the end of the follow-up period; Group II = failed grafts.

Table 4. DONOR CHARACTERISTICS

	Group I (n = 972)	Group II (n = 548)	Significance
Age (% >45 y)	24.6	35.6	p = 0.00001
Sex (% females)	30.6	40.3	p = 0.0001
ICU length of stay (days)	4.1 ± 8.0	3.9 ± 3.9	p = 0.65
Harvest serum sodium (%)			
<136	8.1	9.3	
136–145	31.0	29.4	
146–160	45.3	44.2	
>160	15.6	17.1	p = 0.71
Ischemia time (hr)	13.8 ± 3.7	14.1 ± 3.9	p = 0.22

ICU = intensive care unit.
Group I = grafts still functioning at the end of the follow-up period; Group II = failed grafts.

The results of the multivariate analysis are summarized in Table 5. As in our previous work, studying a smaller sample,[17] we found that donor age and gender were associated independently with outcome. There was no evidence of an interaction between these two factors (*i.e.*, their effects were only additive). The odds of failure were more than twice as high for patients with a prior liver transplant and almost three times as high for those receiving cyclosporine-based immunosuppression (compared with those receiving tacrolimus-based immunosuppression). Transplants performed on patients UNOS status 4 also carry a modest increase in the risk of failure (odds ratio 1.3), and for each additional 6 hours of ischemia, the odds ratio is 1.27.

Table 5 also lists the failure risk associated with different indications for transplantation, using cholestatic as the reference category. The cholestatic group had the lowest risk of failure, followed by alcoholic cirrhosis, whereas at the other end of the spectrum, we find those patients transplanted for primary hepatobiliary malignancies (HCC-cholangio). Transplants performed on patients UNOS status 4 or with a history of a previous liver transplant were more likely to fail because of sepsis-MODS than from other causes ($p < 0.05$). No association was found between other risk factors and graft failure categories.

Because the early failure rate is higher in the positive crossmatch group, we then investigated whether the factors that are independently associated with early failure differ from those associated with overall failure. The transplants were divided into two groups: according to whether they functioned for more that 6 months (group Ie, n = 1146) or failed within this interval (group IIe, n = 374). There were seven ABO-mismatched grafts in group Ie and one in group IIe (p = 0.37). Recipient and donor characteristics are summarized in Tables 6 and 7, respectively.

There was no difference in recipient age and gender. Early failures were more likely to be associated with a history of prior liver transplant, UNOS status 4, and cyclosporine-based immunosuppression. There also were proportionally more cases of PNF-ischemia among the early failure group. In contrast to the overall failures, however, early failures had a borderline association with the cytotoxic crossmatch (more likely to fail early with a positive crossmatch, p = 0.055; refer to Table 6). There were no differences between early and overall failures regarding donor characteristics, with both age and gender being associated with early failure (Table 7).

Table 8 lists the results of the multivariate analysis. Except for the indication for transplantation, variables found to be associated independently with overall failure also were associated with early failure. This includes donor gender, which in our previous study,[17] in a smaller patient population, failed to reach significance in the early failure group. The cytotoxic crossmatch also was associated with early failure (odds ratio for a positive crossmatch: 1.5).

DISCUSSION

In contrast to the kidney,[1–3] a positive cytotoxic crossmatch is not a contraindication to liver transplantation. This policy arose out of necessity, because of the short

Table 5. VARIABLES INDEPENDENTLY ASSOCIATED WITH GRAFT FAILURE

	Odds Ratio	95% CI
Donor age (>45 y)	1.7	1.3–2.1
Female donor sex	1.4	1.1–1.7
Prior liver transplant	2.3	1.5–3.5
UNOS 4	1.3	1.1–1.7
Ischemia time	1.27*	1.1–1.5*
Indication		
Cholestatic	Reference	
Hepatitic	1.9	1.3–2.8
Alcoholic	1.4	0.94–2.1
Cryptogenic	2.2	1.3–3.6
PNF-ischemia	2.0	1.0–3.8
HCC-cholangio	3.6	2.2–6.0
Other	1.5	0.98–2.4
Primary immunosuppression		
Tacrolimus	Reference	
Cyclosporine	2.8	1.5–5.1
Tacrolimus rescue	0.80	0.5–1.2

CI = confidence interval; UNOS = United Network for organ sharing; PNF = primary nonfunction; HCC-cholangio = hepatocellular carcinoma and cholangiocarcinoma.
* For each 6-hour increment in ischemia time.

Table 6. RECIPIENT CHARACTERISTICS: ANALYSIS OF EARLY GRAFT FAILURES

	Group Ie (n = 1146)	Group IIe (n = 374)	Significance
Age (yr)	49.0 ± 12.2	50.1 ± 12.5	p = 0.13
Sex (M/F)	713/433	224/150	p = 0.42
UNOS 4 (%)	47.2	62.0	p < 0.00005
Prior transplant (%)	12.6	29.1	p < 0.00005
Positive crossmatch (%)	7.8	11.0	p = 0.055
Primary immunosuppressant (%)			
Tacrolimus	88.5	88.8	
Cyclosporine	2.2	6.1	
Tacrolimus rescue	9.3	5.1	p = 0.00004
Indication (%)			
Hepatitic	24.4	21.9	
Cholestatic	16.5	11.2	
Alcoholic	20.2	14.4	
Cryptogenic	7.9	9.6	
Autoimmune	3.8	1.9	
HCC-cholangio	6.9	5.9	
Other malignancy	0.9	0.5	
PNF-ischemia	4.6	12.8	
Metabolic	2.6	4.3	
FHF	1.7	2.7	
Chronic rejection	2.6	2.7	
Acute rejection	0.3	0.3	
Technical	3.9	6.7	
Other	3.7	5.1	p < 0.00005

UNOS = United Network for Organ Sharing; HCC-cholangio = hepatocellular carcinoma and cholangiocarcinoma; PNF = primary nonfunction; FHF = fulminant hepatic failure.
Group Ie = grafts still functioning at the 6-month cut-off; Group IIe = grafts that failed before the 6-month cut-off.

preservation times initially allowed by the liver, and was bolstered by the observation that this organ is uniquely resistant to antibody-mediated rejection.[4–6] In fact, in an analysis of the largest series to come out of the cyclosporine era, transplants done across a positive cytotoxic crossmatch were found to have a slightly better (albeit not statistically significant) survival than those done in the presence of a negative crossmatch.[7] This led the authors to speculate whether preformed antibodies might actually have a protective or tolerogenic effect in liver transplantation.

We now know this is not the case. Although highly unusual, the liver can experience hyperacute rejection.[9,10,28] Transplants carried out in the presence of preformed antibodies tend to follow a different clinical course, with higher intraoperative blood use, postoperative graft dysfunction, and, in some cases, graft loss. This seems to be more so for isoagglutinins[29–31] than lymphocytotoxic antibodies.[11,12,32] Biopsies of crossmatch-positive cases show a higher incidence of "preservation injury" and acute cellular rejection than those of matched controls,[12] and graft failure rates have been reported to be higher in presensitized patients.[11–14]

The results of our multivariate analysis, on the largest clinical series where the effect of the crossmatch has been studied, indicate that a positive cytotoxic crossmatch has no discernible effect on eventual graft survival. Although this may come as a surprise considering the growing literature stating otherwise, a careful look at the data begins to show the complexity of this issue. Using stepwise logistic regression, we found seven variables associated independently with outcome: 1) donor age, 2) donor gender, 3) history of a prior liver transplant, 4) UNOS status 4, 5) ischemia time, 6) indication for transplantation, and 7) primary immunosuppressant (Table 5). There was no association between donor serum sodium at the time of procurement and graft outcome, in contrast to recent reports,[33,34] and the same can be said about the donor's length of stay in the intensive care unit. We reported recently on the effects of donor age and gender, with grafts procured from women or donors older than 45 years having a higher incidence of failure.[17] The current study, conducted on a much larger sample, confirms these results.

The adverse effect of increasing donor age had been surmised from the start of clinical liver transplantation, when a ceiling of 45 years was suggested for donor candidacy.[35] Over the ensuing years, a number of reports appeared, suggesting that outcome was not affected by increasing donor age,[36–38] presumably because of the liver's resistance to senescence.[39] However, we found recently that livers from donors older than 60 years have only a 43% 2-year survival *versus* 71% for the younger donors.[40] A detailed analysis showed that the risk of failure, as a function of donor age, remains constant until age 45 and increases sharply after that.[17]

Table 7. DONOR CHARACTERISTICS: ANALYSIS OF EARLY GRAFT FAILURES

	Group Ie (n = 1146)	Group IIe (n = 374)	Significance
Age (% >45 yr)	25.0	39.6	p < 0.00005
Sex (% females)	31.2	43.0	p = 0.00003
ICU length of stay (days)	4.05 ± 7.7	3.87 ± 3.5	p = 0.67
Harvest serum sodium (%)			
<136	8.3	9.4	
136–145	31.5	27.0	
146–160	44.8	45.3	
>160	15.4	18.3	p = 0.37
Ischemia time (hr)	13.8 ± 3.7	14.1 ± 4.0	p = 0.3

ICU = intensive care unit.
Group Ie = grafts still functioning at the 6-month cut-off; Group IIe = grafts that failed before the 6-month cut-off.

Table 8. VARIABLES INDEPENDENTLY ASSOCIATED WITH EARLY GRAFT FAILURE

	Odds Ratio	95% CI
Donor age (>45 yr)	1.9	1.4–2.5
Female donor sex	1.4	1.1–1.8
Prior liver transplant	2.8	1.9–3.7
UNOS 4 status	1.4	1.1–1.9
Ischemia time	1.28*	1.05–1.6*
Positive crossmatch	1.5	0.96–2.2
Primary immunosuppression		
Tacrolimus	Reference	
Cyclosporine	3.5	1.9–6.4
Tacrolimus rescue	0.5	0.3–0.9

CI = confidence interval; UNOS = United Network for Organ Sharing.
* For each 6-hour increment in ischemia time.

Grafts obtained from female donors show a modest degradation in survival (refer to Table 5), a finding we are at a loss to explain. Livers from female donors do slightly worse if given to male recipients than if given to female recipients (data not shown), but the difference in the risk of failure of a female-to-female *versus* a female-to-male combination is small, and it is unclear whether the gender of the recipient is a significant factor. Because of this, and our desire to simplify the model, we only consider the gender of the donor as a risk factor. We found no association between donor–recipient gender combination and specific causes of failure.

Risk factors such as a history of prior liver transplant, UNOS status 4, and ischemia time are well known.[22,41–45] Similarly, that the main indications for transplantation have different prognoses was recognized from the very beginning,[22] although this study marks the first time that their risks are calculated while controlling for the effects of confounding variables. Not surprisingly, the choice of primary immunosuppressant also was found to be associated with graft outcome, with grafts treated exclusively with cyclosporine-based immunosuppression being more likely to fail (odds ratio 2.8) than those treated exclusively with tacrolimus-based immunosuppression. This observation suffers from all the limitations inherent in retrospective analyses, but it is supported by a wealth of evidence obtained by our group[46–48] and others[49–51] that point toward the superiority of tacrolimus in liver transplantation.

What is the significance, then, of a positive cytotoxic crossmatch? An answer is suggested in Figure 1, which shows that graft survival is lower in the early post-transplant period in positive crossmatch cases, but the difference vanishes by the second year. Multivariate analysis also shows that crossmatch is associated with early outcome (*i.e.*, within 6 months, refer to Table 8), but not with overall outcome. Although it did not reach statistical significance, positive crossmatch grafts were more likely to fail because of rejection or sepsis-MODS (Table 2). Taken together, these observations support the idea that a positive cytotoxic crossmatch adversely impacts liver transplant outcome, but the degree of background noise is still so high that its overall importance is masked. Therefore, with a positive crossmatch, we pay up front. This agrees with the findings of Takaya et al.,[11] who showed lower 1-year graft survival in positive crossmatch cases compared with a matched control group. Similar findings were reported by Katz et al.[13] in a small series. The durability of grafts that make it through the high risk early period may be explained by the change in the complement components of the recipient to predominantly donor phenotype. In xenograft models, this alteration in host environment has been shown to interdict hyperacute (humoral) rejection.[52]

Our results do stand in sharp contrast to those of Nikaein et al.,[14] who recently reported overall lower patient and graft survivals when going across a positive cytotoxic crossmatch (448 negative and 34 positive). Besides our larger sample size, their study differs in several ways. Whereas we use a modified Amos technique,[23] the Baylor group reports using both standard NIH[53] and antihuman globulin (AHG)[54] techniques. Unfortunately the authors do not state in their report how many patients were crossmatched with each technique, so it is not clear if this could explain our different findings. In the AHG technique, an antihuman Ig antibody is added to increase the cytotoxic potential of lymphocyte-bound antibody, making it not only a more sensitive method to detect low concentrations of complement fixing antibodies, but also one that is capable of detecting antibodies that do not fix complement.[54] It has been suggested in the kidney transplant literature that AHG-dependent antibodies will not cause immediate graft loss, but rather failures within the first few months; this effect seems to be primarily limited to retransplantations.[55,56] Whether a similar mechanism could be operating in liver transplantation remains to be determined.

Another difference is that in the Baylor study, a positive crossmatch was defined as >10% kill, and their exclusion criteria included retransplants, patients treated with tacrolimus, and those dying within 1 week.[14] However, when we repeated our survival analysis excluding cases with a previous transplant, and using >10% cell kill as the definition of a positive crossmatch, we still found no difference in overall survival (data not shown). Because almost 90% of our patients were treated with tacrolimus, we could not exclude them from the analysis, which brings up the question of whether our results are related to the different immunosuppressive regimens.

Knowing the results of the crossmatch certainly is use-

ful, because it helps identify a high-risk group that requires special attention and, most likely, more aggressive immunosuppression. However, we disagree totally with the position that transplanting across a positive cytotoxic crossmatch is "inadvisable,"[57] because the overall results, at least in terms of graft failure rates, are indistinguishable. There is much interest, both on the part of society and that of practitioners, to be able to understand what factors determine the outcome of liver transplantation and to use that information to maximize the benefit that can be derived from our chronically insufficient supply of organs. We have yet to reach that point, but a picture is starting to emerge, one that hints at the great complexity of this problem. We do know enough already to state that, other than a few well-defined formal contraindications, there probably is no single risk factor so influential that it could be used to preclude someone from transplantation. This decision will have to be made based on a consideration of how multiple risk factors act together. Determining how best to model these factors and their interactions will require a great deal of work. An example of this is the age of the donor, which in this study, we treated as a categorical variable, mostly for convenience of exposition. However, we have already shown that the risk associated with donor age does not go up abruptly and to a constant level (as a step function would) after 45 years.[17] Rather, the risk increases every year (after age 45), and this information should probably be included in the model, for example through a local regression procedure or a regression spline.[17] We should be alert to the misuse of certain statistical models, especially Cox proportional hazards.[58] As part of an ongoing study, we formally have tested the proportionality of hazards for variables that are associated with outcome and found that several of them (*e.g.*, UNOS status, prior transplant, indications for transplantation) violate this crucial assumption (unpublished observations, 1995). If we are to use Cox regression, we must use an extension of the model designed to deal with nonproportional hazards.[59,60]

In summary, we have studied the effect of a positive cytotoxic crossmatch on the outcome of liver transplantation. Although the graft failure rate is higher in the early post-transplant period when going across a positive crossmatch, the difference disappears by the second year. Using multivariate analysis, we identified seven variables that are associated independently with outcome: 1) donor age, 2) donor gender, 3) history of a prior liver transplant, 4) UNOS status 4, 5) ischemia time, 6) indication for transplantation, and 7) primary immunosuppressant. The results of the crossmatch should not be used to decide against transplantation, but they do identify a high-risk group of patients that requires special attention and, probably, more aggressive immunosuppression.

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