Transpl Int (2003) 16: 486–493
DOI 10.1007/s00147-003-0572-8

ORIGINAL ARTICLE

Marginal grafts: finding the correct treatment for fatty livers

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Received: 12 March 2002
Revised: 16 August 2002
Accepted: 28 January 2003
Published online: 28 March 2003

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Abstract The influence of steatosis on the outcome of orthotopic liver transplantation (OLT) was evaluated in 860 liver transplantations carried out in 784 patients from October 1990 to August 2001. Donor variables considered were: age, hepatic enzymes, bilirubin, total and warm ischemia times, macrovesicular and microvesicular steatosis. Recipient variables considered were: age, UNOS status, Child–Pugh score and indication for OLT. Patient and graft survival were the main outcome indicators. Macrovesicular steatosis affecting 15% or more of the hepatocytes was the only variable independently associated with shorter patient and graft survival ($P=0.0012$ and 0.0028). A significantly worse prognosis was to be expected if >15% macrovesicular steatosis was associated with a total ischemia time >10 h ($P=0.048$), or donor age >65 years ($P=0.016$) or with HCV-positive recipients ($P=0.0014$). From our study we can conclude that macrovesicular steatosis involving 15% or more of the hepatocytes identifies marginal livers. The risk of graft non-function or patient loss after OLT rises if macrovesicular steatosis >15% is associated with long ischemia time, high donor age, or HCV positivity in recipients.

Keywords Steatosis · Liver transplantation

Introduction

Steatosis is a common feature of marginal liver function. Donor livers with significant amounts of fat have, in the past, been reported to perform so poorly after grafting that they often had to be discarded. However, in the light of ongoing organ shortages, several centers have recently been forced to make use of steatotic liver implants, and they have done so with encouraging results. To optimize the use of steatotic livers, we evaluate in this study the influence of the type of steatosis—macrovesicular or microvesicular—and the influence of other donor and recipient characteristics on liver function after grafting.

Patients and methods

We evaluated 860 consecutive liver transplantations that were performed at our center in 784 patients between October 1990 and August 2001. Recipients were matched to donors on the basis of blood-group compatibility, age, dimension of the organ, and overall clinical conditions.

Liver biopsies were performed on heart-beating donors before the aorta was clamped. The biopsies were fixed in buffered formalin, embedded in paraffin, and stained with hematoxylin–eosin. If severe steatosis was suspected, the specimen was submitted to quick fixation by a rapid micro-wave-assisted method. Biopsy slides were examined retrospectively. They were assessed blind, by a single pathologist, for the following variables: type ofsteatosis (macrovesicular and microvesicular) and its extent, necrosis and inflammatory infiltrate, fibrosis (mild or severe), bile-duct lesions, and other relevant lesions. Macro-steatosis and

Table 1 Recipient variables: overall

Variable	OLTs ($n=860$)			
Recipients	784			
Gender	M: 555 (70.8%)	F: 229 (29.2%)		
Age (years)	Median: 51	Range: 1–68		
Child–Pugh status	A: 83 (11%)	B: 280 (36%)	C: 421 (53%)	
UNOS status	1: 56 (6.6%)	2A: 74 (8.6%)	2B: 206 (23.9%)	3: 524 (60.9%)

micro-steatosis were evaluated semi-quantitatively in ten consecutive fields (magnification ×25), independently from the lobular distribution.

The following donor variables were considered: age, hepatic enzymes (AST, ALT) and bilirubin in terminal phase, total ischemia time, warm ischemia time, macrovesicular and microvesicular steatosis, need of vasopressor, serum sodium levels, occurrence of cardiac arrest, and duration of ICU stay. The following recipient characteristics were analyzed: age, gender, UNOS status, Child–Pugh score, and indication for orthotopic liver transplantation (OLT).

Statistical analysis

Statistical analysis was performed with SPSS (SPSS, Chicago, Ill, USA). Continuous variables are expressed as median (minimum–maximum), and categorical variables as fractions. A (two-tailed) *t*-test or ANOVA test was used to test for differences between means. Pearson's non-parametric chi-square test was used to compare categorical variables. Univariate survival analysis was performed with the Kaplan–Meier estimator with log rank test to compare strata. Variables found to have univariate significance with a proportional risk tested with log minus log plot, were analyzed with the Cox multivariate regression model. The significance level for all tests was set at $P<0.05$.

Results

Tables 1 and 2 show the characteristics of recipient and donor populations. Recipient median age was 51 (1–68) years; a Child–Pugh score of C and a UNOS status of 3 defined the most represented groups of patients. Donor median age was 46 (0–85) years; median total ischemia time was 587 (47–1,309) min, and median warm ischemia time was 30 (11–125) min. Median macrovesicular steatosis was 5% (range 0–85), and median microvesicular steatosis was 10% (range 0–98).

Indications for OLT are summarized in Table 3. HCV-related cirrhosis represented the main indication in our center (43% of all transplant patients).

The overall 5-year patient survival rate was 79%, while HCV-positive recipients had a significantly different outcome (72%; Fig. 1). The overall 5-year graft survival rate was also different for HCV-positive recipients (70% vs 66%) (Fig. 2).

In our analysis, graft survival significantly decreases with rising macrovesicular steatosis values. This result can also be observed when macrovesicular steatosis was treated as a discrete variable and also when it was dichotomized at various points. As a value over 15% turned out to be relevant for graft survival, we decided to select this lowest significant value for successive analyses.

Table 2 Donor variables: overall

Variable	Median	Range
Age (years)	46	0–85
Macrovesicular steatosis (%)	5	0–85
Microvesicular steatosis (%)	10	0–98
Cold ischemia time (min)	558	28–1,284
Warm ischemia time (min)	30	11–125
Total ischemia time (min)	587	47–1,309
Bilirubin (mg/dl)	0.8	0.1–29
AST (U/l)	42	4–3,000
ALT (U/l)	32	5–4,018
Na^+ (mEq/l)	148	105–187
Days in ICU	3	1–132
Dopamine >10 μ/kg per min	Yes: 267 (31%)	No: 593 (69%)
Cardiac arrest	Yes: 57 (6.6%)	No: 803 (93.4%)

Table 3 Indications for first OLT

Indication	*n*	%
Viral cirrhosis	545	69
Viral cirrhosis + hepatocarcinoma	1,603	
HCV-related cirrhosis	37	43
Alcohol-induced cirrhosis	68	9
Cholestatic cirrhosis	67	8
Fulminant hepatitis	29	4
Metabolic disease	21	3
Autoimmune cirrhosis	8	1
Other	46	6

In 64 cases we transplanted livers with >15% macrovesicular steatosis; Tables 4 and 5 show the characteristics of recipients and donors of these grafts. No significant differences were found between this group and all the other OLTs, except for recipient gender. Patient and graft survival provided a significant difference from the other group ($P=0.0012$ and RR = 1.7; $P=0.0028$ and RR = 1.5, respectively; Figs. 3 and 4). We also analyzed microvesicular steatosis, but the results were not relevant for graft survival, neither taken at any cut off point, nor taken as a continuous variable ($P=0.67$, RR = 0.997, CI 95% = 0.98–1.01).

The outcome of patients receiving >15% macrosteatotic livers was significantly worsened by total ischemia times >10 h ($P=0.048$, RR = 1.56; Fig. 5). Tables 6 and 7 show the characteristics of recipients and

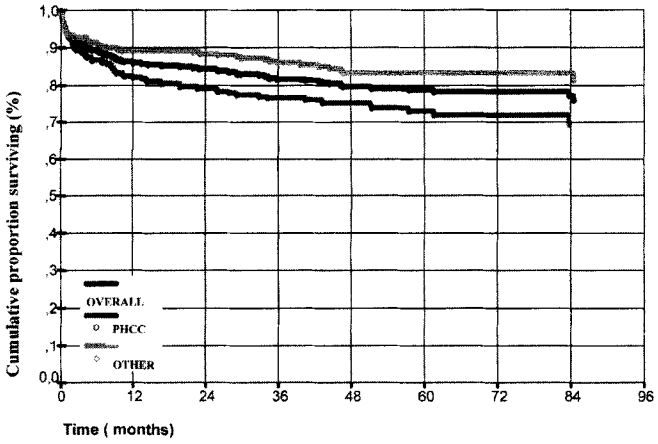

Fig. 1 Cumulative patient survival

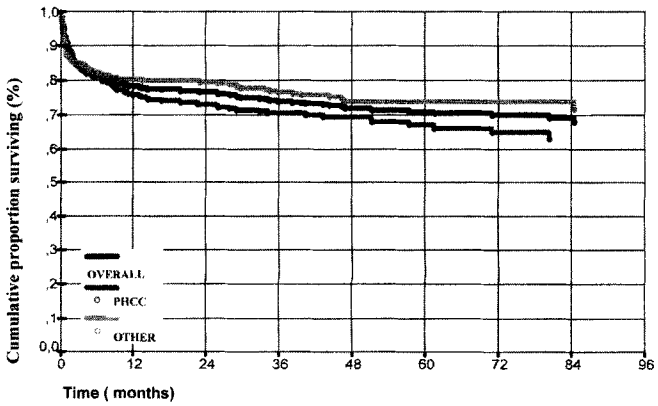

Fig. 2 Cumulative graft survival

donors with >10 h total ischemia time. No significant differences were found between this group and the other OLTs. We evaluated total ischemia time as a continuous co-variate in the group of >15% macro-steatotic livers, and we can confirm a significant influence on patient survival ($P=0.02$, RR = 1.5) (Table 8). Each additional hour of total ischemia time increased the risk of patient loss by 15%.

In the group with > 15% macrovesicular steatosis, a donor age >65 years influenced outcome with a significantly worsening rate of patient and graft survival ($P=0.0016$ and RR = 2.1; $P=0.001$ and RR = 2.5, respectively; Figs. 6 and 7). Tables 9 and 10 show the characteristics of recipients and donors for donor ages above 65 years. No significant differences were found between this group and the other OLTs, except for a less frequent use of vasopressors, for older recipients age and for shorter total, cold and warm ischemia time; this last point shows the effort in our center to reduce ischemia times when an older donor is available. Moreover, in our center we are inclined to match elderly donors with elderly recipients; however, in this cohort, recipient age did not determine the graft and patient survival rates. No significant differences were found dividing at median recipient age (median age: 55 years; graft: $P=0.4$; patient: $P=0.21$).

Figures 8 and 9 show a significantly worse prognosis for patient and graft if >15% macrovesicular steatosis was associated with HCV-positive recipients ($P=0.0014$ and $P=0.006$, respectively). Tables 11 and 12 show the characteristics of recipients and donors when recipients were HCV positive. Significant differences were found between this group and all the other OLTs in terms of

Table 4 Recipient variables: graft with macrovesicular steatosis > 15% (*NS* not significant)

Variable	OLTs ($n=64$)				*P* vs others
Gender	M: 87%	F: 13%			< 0.05
Age (years)	Median: 52	Range: 18–68			NS
Child–Pugh status	A: 18%	B: 28%	C: 54%		NS
UNOS status	1: 4.4%	2A: 4.4%	2B: 11.1%	3: 75.6%	NS

Table 5 Donor variables: graft with macrovesicular steatosis > 15% (*NS* not significant)

Variable	Median	Range	*P* vs others
Age (years)	54	14–72	NS
Macrovesicular steatosis (%)	22	15%–85	< 0.05
Microvesicular steatosis (%)	20	0–75	< 0.05
Cold ischemia time (min)	540	225–1,284	NS
Warm ischemia time (min)	27	15–98	NS
Total ischemia time (min)	574	250–1,309	NS
Bilirubin (mg/dl)	0.96	0.3–12	NS
AST (U/l)	41	8–592	NS
ALT (U/l)	32	10–363	NS
Na^+(mEq/l)	147	127–172	NS
Days in ICU	2	1–26	NS
Dopamine >10 μ/kg per min	Yes: 22 (34.4%)	No: 42 (65.6%)	NS
Cardiac arrest	Yes: 4 (6.25%)	No: 60 (93.75%)	NS

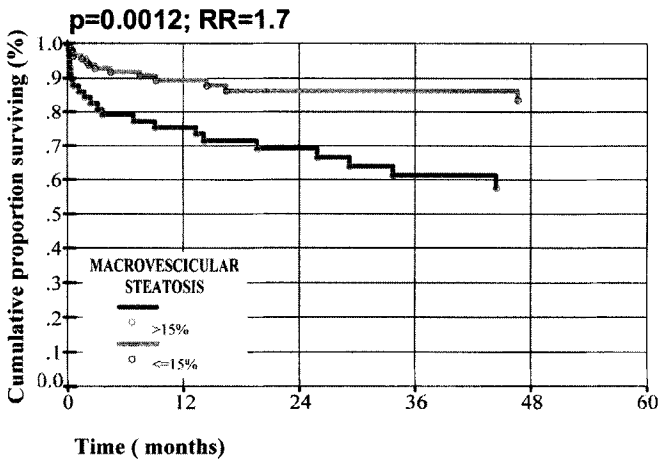

Fig. 3 Cumulative patient survival: macro-steatosis >15% vs macro-steatosis ≤15%

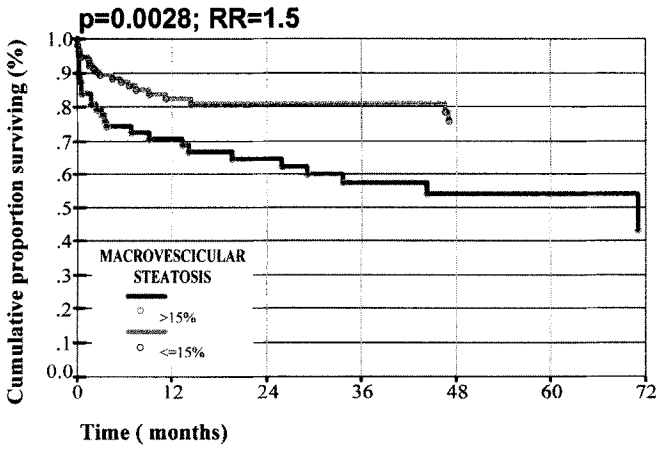

Fig. 4 Cumulative graft survival: macro-steatosis >15% vs macro-steatosis ≤15%

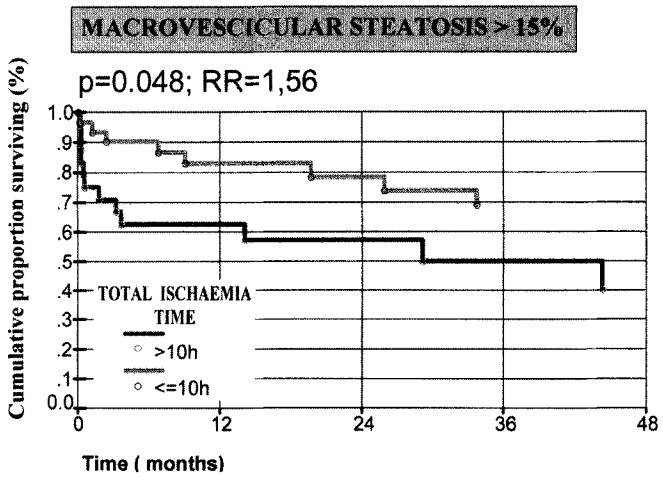

Fig. 5 Cumulative patient survival with macro-steatosis >15%; total ischemia time >10 h vs total ischemia time <10 h

gender, older recipient and donor age, more frequent UNOS-3 recipients, longer total and cold ischemia times and more cardiac arrests in donors. None of the other variables taken into account significantly influenced graft or patient survival.

Discussion

Several studies have shown that severe steatosis is a significant risk factor of PRNF or ENF, whereas mild to moderate steatosis does not preclude transplantation, as the results are similar to those obtained with normal livers [4, 10, 11, 12, 14, 15, 16, 17, 18]. However, recent studies have reported that severe steatosis is also compatible with adequate function if it is of the microvesicular type [2, 3, 6]. Donor livers with as many as 60% to 100% of the hepatocytes containing fat in microvesicles have been safely used without an increased risk of PRNF or of poor early function of the graft.

This conclusion is not shared by two studies, which found an increased rate of ENF after implantation of micro-steatotic livers compared with macro-steatotic livers [7, 20]. However, these studies did not determine the percentage of macrovesicular steatosis and evaluated the performance of steatotic livers at re-transplantation only; in this setting, additional risk factors for liver malfunction are involved, and it becomes difficult to extrapolate the risk posed by steatosis.

There is a need for the morphological type of fatty infiltration to be distinguished [19, 21]. Only histological examination is of help in ascertaining type and extent of liver steatosis; the analysis of the other clinical and biochemical characteristics of our donors did not reveal any feature indicative of the type of fatty infiltration.

Our data confirm that donor livers with microvesicular steatosis can safely be used to expand the donor pool; microvesicular steatosis, even when severe, did not influence the outcome of patient or graft. As confirmation of good liver function in the patients who received micro-steatotic livers, lipids were rapidly mobilized after transplantation, with a 30% to 50% decrease in the number of hepatocytes that displayed fatty infiltration in biopsies performed 1 month after surgery. In contrast, macrovesicular steatosis involving 15% or more of the hepatocytes in the donor liver was significantly associated with shorter patient and graft survival after transplantation.

In a previous series of 311 consecutive liver transplantations we learned that macrovesicular steatosis over 25% is a risk factor for patient and graft survival [21]. Although moderate steatosis is usually defined as a fat content of 30–60%, in our study a value of 15% turned out to be relevant for graft survival. We therefore cannot easily utilize grafts with macrovesicular steatosis

Table 6 Recipient variables: ischemia time >10 h (*NS* not significant)

Variable	OLTs ($n=406$)				*P* vs others
Gender	M: 69.2%	F: 30.8%			NS
Age (years)	Median: 50.3	Range: 1–64			NS
Child–Pugh status	A: 8.4%	B: 38%	C: 53.6%		NS
UNOS status	1: 3.4%	2A: 6.1%	2B: 11.2%	3: 79.3%	NS

Table 7 Donor variables: ischemia time >10 h (*NS* not significant)

Variable	Median	Range	*P* (vs others)
Age (years)	45	4–85	NS
Macrovesicular steatosis (%)	10	0–70	NS
Microvesicular steatosis (%)	10	0–98	NS
Cold ischemia time (min)	670	475–1,284	<0.05
Warm ischemia time (min)	30	11–125	NS
Total ischemia time (min)	701	587–1,309	<0.05
Bilirubin (mg/dl)	0.8	0.15–21	NS
AST (U/l)	30.5	4–3,000	NS
ALT (U/l)	31	6–1,883	NS
Na+ (mEq/l)	148	105–187	NS
Days in ICU	3	1–33	NS
Dopamine >10 μ/kg per min	Yes: 123 (30.3%)	No: 283 (69.7%)	NS
Cardiac arrest	Yes: 27 (6.65%)	No: 379 (93.35%)	NS

Table 8 Total ischemia time (h) as continuous co-variate

Factor	Patients		Graft	
	Overall	Macrovesicular steatosis >15%	Overall	Macrovesicular steatosis >15%
P (significant)	0.06	0.02	0.1	0.03
RR	1.05	1.15	1.03	1.13

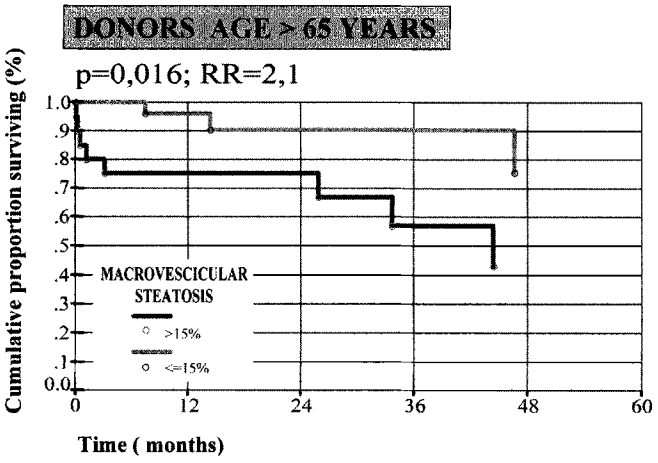

Fig. 6 Cumulative patient survival with donor age >65 years; macro-steatosis >15% vs macro-steatosis ≤15%

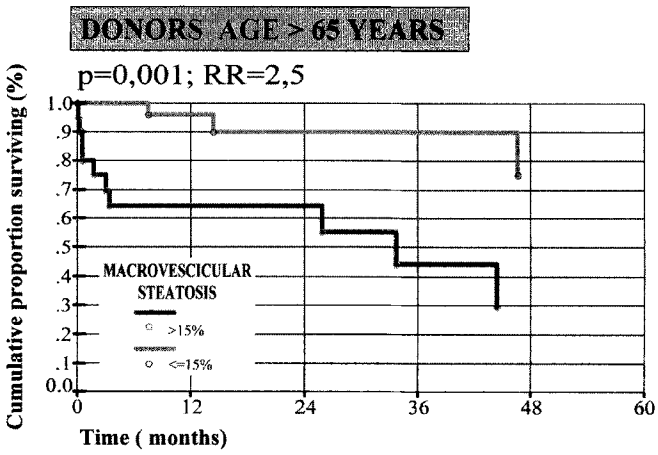

Fig. 7 Cumulative graft survival with donor age >65 years; macro-steatosis >15% vs macro-steatosis ≤15%

of over 15%, especially when the donor presents further risk factors.

Fukumory et al. [8] reported that steatotic livers are more susceptible to cold ischemia injury. In our experience, total ischemia time longer than 10 h significantly influenced the negative outcome of livers with >15% macrovesicular steatosis: every additional hour significantly increased the relative risk (graft loss and patient loss).

At present, the procurement index of our interregional area AIRT (Interregional Transplant Association), calculated by division of the number of utilized organs by the maximum number of organs procurable from all utilized donors [9, 13], is 97%; the high rate is

Table 9 Recipient variables: donor age >65 years (*NS* not significant)

Variable	OLTs ($n=132$)				*P* vs others
Gender	M: 75.8%	F: 24.2%			NS
Age (years)	Median: 54.3	Range: 17.8–64.9			<0.05
Child–Pugh status	A: 9.4%	B: 32.3%	C: 58.3%		NS
UNOS status	1: 5.3%	2A: 6.4%	2B: 14.9%	3: 73.4%	NS

Table 10 Donor variables: donor age >65 years (*NS* not significant)

Variable	Median	Range	*P* vs others
Age (years)	71	66–85	<0.05
Macrovesicular steatosis (%)	1	0–70	NS
Microvesicular steatosis (%)	1	0–75	NS
Cold ischemia time (min)	531	177–796	<0.05
Warm ischemia time (min)	25	15–98	<0.05
Total ischemia time (min)	559	196–819	<0.05
Bilirubin (mg/dl)	0.8	0.2–12	NS
AST (U/l)	38	4–674	NS
ALT (U/l)	25	6–862	NS
Na^+ (mEq/l)	147	125–179	NS
Days in ICU	3	1–17	NS
Dopamine >10 μ/kg per min	Yes: 23 (17.4%)	No: 109 (82.6%)	<0.05
Cardiac arrest	Yes: 14 (10.6%)	No: 118 (89.4%)	NS

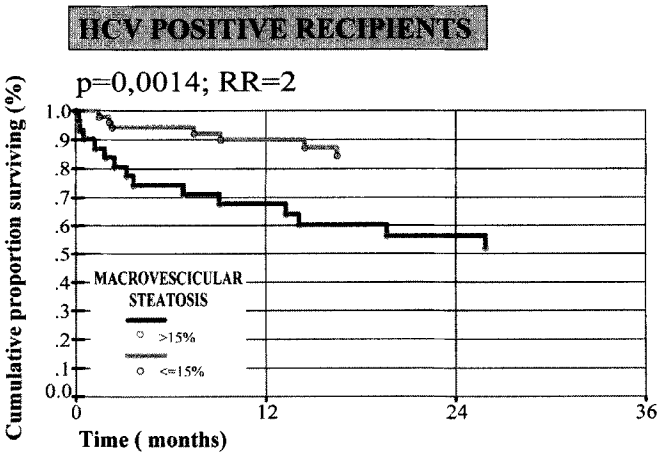

Fig. 8 Cumulative patient survival with HCV-positive recipients; macro-steatosis >15% vs macro-steatosis ≤15%

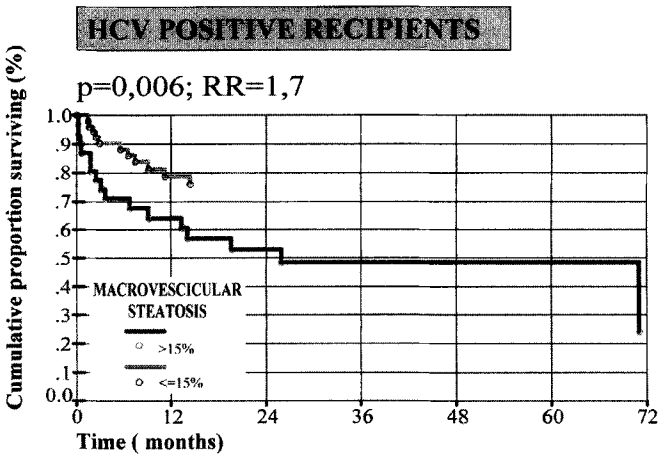

Fig. 9 Cumulative graft survival with HCV-positive recipients; macro-steatosis >15% vs macro-steatosis ≤15%

justified by our policy of maintaining ischemia times for marginal livers below 6 h. In emergency situations, we transplanted a small number of livers with >25% macrovesicular steatosis maintaining ischemia times under 6 h, the shortest possible time necessary to organize harvesting and transplantation. Obviously, it is not possible to obtain statistically significant results, but we must stress the good outcome of patients and grafts. Consequently, it is difficult for us to accept steatotic livers evaluated and refused by other centers, as in these cases ischemia times are always very much longer.

De Carlis et al. [5] and Briceno et al. [1] considered the negative association between steatosis and donor age. In our study, macrovesicular steatosis >15% associated with a donor age of over 65 years resulted in shorter patient and graft survival. Steatotic livers from younger donors can be used with better outcome.

A significantly worse prognosis was documented if macrovesicular steatosis >15% was associated with HCV-positive recipients. If the outcome of HCV-positive patients is to be optimized, this combination should always be avoided. This, however, presents an important problem to centers with 50% or more HCV-positive patients on the waiting list. How can these patients, for whom steatotic livers have to be avoided, be treated? A probable solution lies in living related-donor liver transplantation (LRLT).

Table 11 Recipient variables: HCV-positive recipients (*NS* not significant)

Variable	OLTs ($n=369$)				P vs others
Gender	M: 74.2%	F: 25.8%			0.05
Age (years)	Median: 54.2	Range: 1–67.5			<0.05
Child–Pugh status	A: 8.3%	B: 36.3%	C: 55.3%		NS
UNOS status	1: 2.3%	2A: 7.8%	2B: 20.5%	3: 69.4%	<0.05

Table 12 Donor variables: HCV-positive recipients (*NS* not significant)

Variable	Median	Range	P (vs others)
Age (years)	50	8–85	<0.05
Macrovesicular steatosis (%)	5	0–70	NS
Microvesicular steatosis (%)	10	0–90	NS
Cold ischemia time (min)	580	144–1,284	<0.05
Warm ischemia time (min)	29	11–105	NS
Total ischemia time (min)	612	163–1,309	<0.05
Bilirubin (mg/dl)	0,9	0.01–12	NS
AST (U/l)	42	5.2–1,635	NS
ALT (U/l)	32	6–1,273	NS
Na^+(mEq/l)	149	123–187	NS
Days in ICU	3	1–26	NS
Dopamine > 10 μ/kg per min	Yes: 122 (33%)	No: 247 (67%)	NS
Cardiac arrest	Yes: 30 (8.1%)	No: 339 (91.9%)	<0.05

In conclusion, macrovesicular steatosis involving 15% or more of the hepatocytes identifies marginal livers. Such livers should be used, but transplantation teams must be aware of the major risk when ischemia time >10 h, or donor age >65 years, or HCV-positive recipients, are associated. These combinations should be avoided whenever possible. If ENF develops, early re-transplantation must be performed.

Only this policy justifies the use of livers with high fat contents.

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