Short- and Long-term Outcomes After Steatotic Liver Transplantation

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Objective: To determine if the use of steatotic grafts adversely affects outcomes in liver transplantation.

Design: A retrospective review of a prospectively maintained database.

Setting: A single center.

Patients: Four hundred ninety adults who underwent liver transplantation from January 1, 2002, to December 31, 2008, at a single center. Graft biopsies were available in 310 (63.3%) cases. Grafts were classified based on amount of macrovesicular steatosis: 5% or less (n = 222), more than 5% to less than 35% (n = 66), and 35% or more (n = 22).

Main Outcome Measures: Recipient demographics, Model for End-Stage Liver Disease (MELD) score, patient/graft survival, complications, transfusion rates, and liver function test results.

Results: One-, 3-, and 5-year patient and graft survivals, respectively, were similar (90.38%, 84.7%, and 74.4%, respectively, P = .3; and 88.7%, 82.5%, and 73.3%, respectively, P = .15). Median follow-up was 25 months. Recipient age, sex, body mass index, laboratory MELD score, and ischemia times were similar among all groups. Packed red blood cell (3 vs 8 U, P < .001), fresh frozen plasma (2 vs 4 U, P = .007), and cryoprecipitate transfusion rates were significantly increased in grafts with 35% or more steatosis. Intensive care unit (5 vs 11 days, P = .02) and hospital (11 vs 21 days, P < .001) stay was also increased in those with grafts with 35% or more steatosis compared with those with 5% or less steatosis. The grafts with 35% or more steatosis had higher transaminase peaks and longer times for bilirubin to normalize (P < .001).

Conclusions: Use of carefully selected steatotic grafts was not associated with higher rates of primary nonfunction or poorer outcomes. However, the use of steatotic grafts is associated with increased resource use in the perioperative period.

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Use of marginal grafts has increased by necessity, as there is still a discrepancy between the number of recipients on transplant waiting lists and the number of available donors. To reduce deaths on the waiting list, many centers use marginal donor grafts, which may be associated with increased risk of postoperative complications and potentially poorer outcomes. Liver steatosis is considered a risk factor for poorer outcomes after transplantation. At present, donor graft steatosis is encountered in many potential donors, and with the increasing incidence of obesity and diabetes in the population, this trend will likely continue in the future. Safe use of grafts with some steatosis has been demonstrated, but there is a high discard rate when grafts with more than 30% steatosis are encountered, for fear of primary nonfunction, delayed graft function, increased posttransplant complications, or poor long-term outcomes following transplantation. A survey of UK and US surgeons reported that no one would use a graft with severe steatosis (>60%) and those with more than 30% steatosis were used very cautiously. Inferior outcomes using steatotic grafts have also been suggested if the donor cold ischemia time is longer and if steatotic grafts from older donors are used.

Determining the degree of steatosis can be a challenge. The donor age and body mass index are sometimes indicative, and liver function tests are generally not helpful, and visualization by the procuring surgeon may be inaccurate. Biopsy with frozen section is the only clinically reliable method to estimate the fat content at the time of the procurement. Inferior out-
comes are more likely with increasing grades of steatosis. However, often the assessment of the frozen section is variable, with recognized artifacts due to freezing liver tissue and many biopsies being evaluated at night by inexperienced staff, resulting in variability both in assessment of potential grafts as well as reporting in the literature. In addition, without special stains, it can be difficult to distinguish steatosis from artifact caused by the frozen section process. Most centers only consider the degree of macrovesicular steatosis as relevant, but this is also controversial because some centers consider the percentage of microvesicular steatosis in the graft. The aim of this study was to evaluate the short- and long-term outcomes of moderate to severe steatotic grafts for liver transplantation.

A prospectively maintained clinical transplant database including recipient and donor demographics, clinical details, and recipient outcomes was used for this study. All adult transplantations performed from January 1, 2002, to December 31, 2008, were reviewed. The study was approved by the institutional review board at Washington University in St Louis. Data were collected from all the cadaveric liver grafts that had undergone biopsy, either at the time of the donor procurement or after reperfusion during the liver transplantation. Some grafts had biopsies taken at both instances and these were correlated to compare the consistency of the biopsy evaluations. Biopsies were taken as a wedge from the left lateral segment and/or with an 18G Monopry core biopsy needle (C. R. Bard Inc, Covington, Georgia). The procurement biopsies were analyzed after being frozen and sectioned either at the same facility as procurement or by the pathologists at our transplant center. All postreperfusion biopsies were analyzed at our institution with uniform criteria for determining the degree of steatosis, and the cohort was subdivided based on these biopsies. For both frozen and permanent sections, liver tissue was stained with hematoxylin-eosin. Macrovesicular steatosis was described based on the percentage of large droplet fat occupying the surface area of the parenchyma. At our institution we are less concerned with microvesicular steatosis, as is true in most other centers. Patients were divided into the following groups: group 1, 5% or less macrovesicular steatosis; group 2, more than 5% to less than 35% steatosis; and group 3, 35% or more steatosis. The preservation solution preferentially used was histidine-tryptophan-ketoglutarate solution, and the University of Wisconsin solution was used only if pancreas recovery was planned. Type of donation (standard brain death vs non–heart beating donors) and cold ischemic times were assessed. Recipient demographics, Model for End-Stage Liver Disease (MELD) score at the time of transplantation, postoperative complications, and reoperation rates were examined. To compare the groups' posttransplant liver function, peak transaminases (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]), bilirubin, and normalized ratio levels as well as the time for these to return to normal were recorded. Hospital and intensive care unit (ICU) length of stay was recorded, and patient and graft survival was calculated and compared for all groups.

At our center we use a standard 3-drug immunosuppression regimen, including tacrolimus, steroids (stopped by 4-6 months), and an antimetabolite (usually mycophenolate). Antibody induction therapy is not used at our center in liver transplantation, except in cases of multiorgan transplantation. Posttransplantation bolus steroid administration was used only for biopsy-proven severe rejection. The t test or Fisher exact test was used for analysis as appropriate. One-way analysis of variance was used to compare the groups. Kaplan-Meier curves and the log-rank test were used for survival studies.

### METHODS

#### RESULTS

From January 1, 2002, to December 31, 2008, 578 liver transplantations were performed, of which 490 were in adults. Graft biopsies were taken in 310 (63.3%) of these grafts. Eighty-seven of these biopsies were obtained at the time of procurement and 223 were postreperfusion biopsies of the graft after reimplantation at the time of liver transplantation. Biopsies were taken at both the donor procurement and postreperfusion in 55 grafts. The donor biopsy interpretation correlated with the postreperfusion biopsy in 54 of 55 (98%) biopsies. The 1 biopsy that did not correlate reported no steatosis in the donor frozen section, but the postreperfusion biopsy reported 30% macrovesicular steatosis. Median follow-up time was 25 months (range, 4-66 months). Grafts were classified based on the amount of macrovesicular steatosis: 5% or less (group 1, n=222); more than 5% to less than 35% (group 2, n=66); and 33%
or more (group 3, n=22). Of the 87 biopsies taken at procurement, 9 of 22 (41%) were in group 3, 22 of 66 (33%) in group 2, and 56 of 222 (22%) in group 1.

RECIPIENT FEATURES

Most transplantations were performed for hepatitis C cirrhosis and alcoholic cirrhosis (Table 1). Median recipient age was 53 years; body mass index (calculated as weight in kilograms divided by height in meters squared) was 28; true MELD score was 20 (Table 2); no significant differences were seen in these measures among the 3 groups. There was no significant difference between groups with regard to the presence of pretransplant ascites, previous abdominal surgery, or transplantation for hepatocellular carcinoma with exception points, suggesting no difference in surgical complexity between recipient groups.

DONOR FEATURES

Donor age was greater in group 2, and body mass index was greater in groups 2 and 3. No donor in group 3 was a non–heart beating donor or was hepatitis C positive. The number of donor deaths from cerebrovascular accident was similar in all groups. The cold ischemic time (time from cross-clamp to out of ice prior to implantation) in all groups was similar (group 1, 5.7 hours; group 2, 6.1 hours; and group 3, 5.5 hours; P=.17) (Table 2 and Table 3).

LIVER ENZYMES

Peaks of AST and ALT were higher in both groups 2 and 3 (groups 1, 2, and 3, respectively, AST, 982 U/L vs 1405 U/L vs 2909 U/L, P<.001; and, ALT, 843 U/L vs 1107 U/L vs 2117 U/L [to convert to microkatals per liter, multiply by 0.01667], P<.001) (Figure 1A), but there was no difference in the time to peak levels (day 1, P=.5) or the time taken to return to normal (7-9 days, P=.9) (Figure 2). Bilirubin peak (Figure 1B) was not significantly different among groups, but time for bilirubin to normalize was longer (groups 1, 2, and 3, respectively, 2 vs 5 vs 10 days, P<.001) (Figure 2). Coagulation parameters were similar among groups; however, this was thought to be related to coagulation product use and not necessarily a true measure of posttransplant liver function (median normalized ratio peak, 2.2; range, 1.3-11.8).

LENGTH OF ICU AND HOSPITAL STAY

Intensive care unit (group 1 vs group 3, 5 vs 11 days; P=.02) and hospital stay (11 vs 21 days, P<.001) was significantly longer in patients who received grafts with 35% or more steatosis (Figure 3).

BLOOD PRODUCT TRANSFUSION RATES

Mean total (intraoperative + postoperative) packed red blood cell transfusion was significantly higher in the 35% or more steatotic group (group 3) compared with group 1 (8 vs 3 units, P=.002), suggesting that recipients of stea-
totically grafts require increased transfusion (Figure 4). Total fresh frozen plasma requirements were also increased in recipients receiving severely steatotic grafts as was intraoperative cryoprecipitate (group 1, 2 units; group 3, 6 units; \( P < .001 \)), but was rarely required postoperatively (Figure 4). Intraoperative transfusion of packed red blood cells (group 1, 3 units; group 3, 7 units; \( P = .01 \)) and fresh frozen plasma (group 1, 2.5 units; group 3, 5 units; \( P = .002 \)) was also increased in group 3.

**COMPLICATIONS AND REOPERATION RATES**

There was no difference between groups in the number of recipients reoperated on for bleeding after transplantation. Between 5% and 9% had bile leaks (\( P = .71 \)). Primary nonfunction occurred in 2 patients. In the first case, the donor liver biopsy was classified as “no steatosis” and the primary nonfunction was a result of technical difficulties during reimplantation, resulting in a warm ischemic time of 69 minutes. The patient was relisted and underwent transplantation the following day. In the second case, the donor liver biopsy demonstrated 10% steatosis, the transplant was straight forward with a warm ischemic time of 43 minutes and cold ischemic time of 8.5 hours; the explant pathology demonstrated massive reperfusion injury and necrosis. Both recipients did well after retransplantation. Other complications were not significantly different between groups (Table 4).

Multivariate analysis was performed for all factors that demonstrated significance on univariate analysis. Higher graft steatosis significantly increased the ICU length of stay, hospital length of stay, packed red blood cell transfusions, AST peak, ALT peak, and time for bilirubin normalization (Table 5).

**PATIENT AND GRAFT SURVIVAL**

The 1-year (groups 1, 2, and 3: 90.7%, 92.0% and 81.5%, respectively), 3-year (groups 1, 2, and 3: 86.1%, 86.4%, and 70.3%, respectively), and 5-year (groups 1, 2, and 3: 74.0%, 76.8%, and 70.3%, respectively; \( P = .3 \)) recipient survival was similar in all 3 groups (Figure 5A). The 1-year (groups 1, 2, and 3: 89.4%, 90.5%, and 77.0%, respectively), 3-year (groups 1, 2, and 3: 83.7%, 85.0%, and 65.6%, respectively), and 5-year (groups 1, 2, and 3: 73.7%, 75.5%, and 65.6%, respectively; \( P = .14 \)) graft survival was also similar (Figure 5B).

Six posttransplant biopsies, performed for a variety of reasons in recipients who received grafts with greater than 35% steatosis, showed complete dissipation of fat in the transplanted liver. The median transplantation to biopsy interval was 13 days (range, 9-75 days). Five of 6 (83%) biopsies in group 3, performed posttransplantation (median time to biopsy, 50 days; range, 10-80 days) also demonstrated no steatosis.

**COMMENT**

Several studies\(^2,9\) show that severely steatotic (>60%) donor grafts have a high risk of failure following liver transplantation. It was the aim of the current study to examine the effect of lesser-degree, but still significant, donor graft steatosis on postoperative liver function as well as patient outcomes. Having a reliable donor assessment is essential when choosing a steatotic graft for transplantation. Currently, the most accurate way to re-
producibly assess the degree of steatosis pretransplantation is a frozen-section biopsy assessment, as surgeon visualization without biopsy may be very inaccurate. In fact, 1 study demonstrated predictive values of only 71%, 46%, and 17% for severe, moderate, and mild steatosis, respectively,\textsuperscript{10,11} when visualization only was used to grade the degree. However, even frozen sections of the donor liver can be associated with inaccuracies if not performed carefully owing to small spaces created artifactualy by the act of freezing liver tissue. Wedge biopsy or needle cores are taken and most commonly stained with hematoxylin-eosin, in which the approximate percentage area of nonstaining vacuolization is described as fatty change.\textsuperscript{10} In our study, the donor graft biopsies correlated with the postreperfusion biopsies 98% of the time, providing confidence in our pretransplant frozen section assessment. Macrovesicular steatosis is the most important factor predicting post–orthotopic liver transplantation allograft function. There is little evidence suggesting that microvesicular steatosis affects allograft function, and thus we did not incorporate this parameter into our assessment. In the current study we only included outcome data when a graft biopsy was performed, since the precise degree of steatosis estimated by the donor surgeon without biopsy confirmation is unreliable.

The current study demonstrates that donor liver macrovesicular graft steatosis, even that greater than 35%, does not preclude safe use in liver transplantation. However, there are significant clinical problems that can be associated with the use of these organs. First, postoperative transaminase peaks were higher in all steatotic grafts, with AST normalizing in 7 to 9 days and ALT by 24 days, suggesting that the reperfusion injury is temporary even in severely steatotic grafts. Severely steatotic grafts remain cholestatic for longer than mild or moderate steatosis, suggesting a greater level of short-term dysfunction in this setting. Coagulation parameters were not different among groups. However, the recipients of steatotic grafts received greater numbers of red blood cell, fresh frozen plasma, and cryoprecipitate transfusions, possibly masking a potentially higher normalized ratio in these recipients. Recipients of severely steatotic grafts required increased resource use, including increased length of stay, both ICU and overall. Two cases of primary nonfunction were identi-

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<th>Table 4. Postoperative Complications of Steatotic Liver Transplantation</th>
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<td>Complication</td>
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<td>Hemoperitoneum\textsuperscript{a}</td>
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<td>Ischemic cholangiopathy</td>
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<td>PNF</td>
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<td>Hepatic artery thrombosis</td>
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<td>Retransplantation</td>
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<td>5%-34% Steatosis (n=66)</td>
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<td>35% Steatosis (n=22)</td>
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Abbreviation: PNF, primary nonfunction.
\textsuperscript{a}Patients requiring additional laparotomy for bleeding.

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<th>Table 5. Multivariate Analysis Performed on All Factors That Demonstrated Significance on Univariate Analysis</th>
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<td>Output Variable Tested</td>
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<td>Intensive care unit stay</td>
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<td>Hospital stay</td>
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<td>Packed red blood cell transfusion</td>
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<td>Fresh frozen plasma transfusion</td>
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<tr>
<td>Aspartate aminotransferase peak</td>
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<td>Alanine aminotransferase peak</td>
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<td>Time for bilirubin to return to normal</td>
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ALT by 24 days, suggesting that the reperfusion injury is temporary even in severely steatotic grafts. Severely steatotic grafts remain cholestatic for longer than mild or moderate steatosis, suggesting a greater level of short-term dysfunction in this setting. Coagulation parameters were not different among groups. However, the recipients of steatotic grafts received greater numbers of red blood cell, fresh frozen plasma, and cryoprecipitate transfusions, possibly masking a potentially higher normalized ratio in these recipients. Recipients of severely steatotic grafts required increased resource use, including increased length of stay, both ICU and overall. Two cases of primary nonfunction were identi-
fied in our population, but neither was thought to be related to steatosis of the donor graft. Outcome reports in the literature assessing graft steatosis have been conflicting. In some, steatosis has been shown to be an independent risk factor for poorer outcomes.12,13 Verran et al10 reported on the use of 120 steatotic donor grafts based on macrovesicular steatosis and found increased graft loss in recipients who received moderately or severely steatotic grafts. A review from the United Network for Organ Sharing database from 1987 to 2001 demonstrated no difference in outcomes based on donor body mass index,14 a possible surrogate for hepatic steatosis. Angele et al1 categorized 225 liver transplants in 208 recipients and divided these into categories with less than 30% or 30% or more steatosis in the graft. With a median follow-up of 34 months, they found no difference in 5-year survival (68% vs 58%, respectively), retransplantation rates, or perioperative complication rates, but they did not report resource use. McCormack et al15 also reported comparable 3-year survival in 20 recipients receiving grafts with severe steatosis. However, they incorporated microvesicular steatosis in this definition, and actually only 7 of the 20 cases had greater than 30% macrovesicular steatosis in the donor graft. Interestingly, animal and human studies suggest that fat disappears from liver grafts within 14 days of the transplant procedure.15 In 1 report assessing 55 living donor liver transplantation recipients, biopsies were performed on a protocol basis on posttransplant day 10. Seven donor grafts had 15% to 30% macrovesicular steatosis at the time of transplantation, but the day 10 biopsy demonstrated a mean decrease from 21.4% to 4.4% steatosis. In our series, 100% of posttransplant biopsies in the greater than 35% steatosis group and 85% of the moderately steatotic grafts demonstrated complete disappearance of steatosis when biopsies were performed more than 10 days after liver transplantation.

A recent study demonstrated increased resource use when marginal donors were used in liver transplantation,16 but these authors did not specifically focus on steatotic grafts. Axelrod et al16 demonstrated a length of stay 10 days longer for recipients of marginal donor livers (donor risk index > 2.5) compared with ideal donor livers (donor risk index < 1.0). When marginal donor livers were used in patients with high MELD scores (> 35), the length of stay increased from 23 to 41 days. Interestingly, the donor risk index does not include assessment of graft steatosis, perhaps because this variable is not reported on a routine basis and is subject to bias, based on the experience level of the local pathologist. In our cohort, there was no difference in MELD scores among the groups, and we tend to avoid use of severely steatotic grafts in very sick patients.

Our center has avoided factors that may magnify the effect of severe steatosis, such as cold ischemic time longer than 10 hours, non–heart beating donors, and elderly donors,17 as confounding variables. By overlapping the donor and recipient operations, we are able to keep our cold ischemia time short, especially in the severely steatotic groups. The longest cold time in this group was 9 hours and the median was 5.5 hours. To avoid any extra time that may be required to assess the degree of graft steatosis at the time of procurement, our policy is to take the biopsy immediately on visualization of the liver and have a pathologist on standby to read the biopsy if we have a high suspicion for steatosis in the donor (ie, high body mass index, diabetes, etc) if a predonation biopsy is unable to be obtained. A second concern in steatotic grafts is donor age. Age older than 65 years has been shown to be a risk factor in using these grafts.17 In group 3 of our cohort of recipients, no donor older than 69 years was included.

In conclusion, use of severely steatotic grafts appears to have similar long-term outcomes to nonsteatotic grafts, but their use is associated with increased blood product use, longer ICU and overall length of stay, and delayed normalization of liver test results compared with nonsteatotic grafts. In light of ongoing donor shortages, steatotic grafts should be used; however, this is associated with increased resource use in recipients of these grafts. On this basis, centers should implement strategies to minimize cold ischemia time and avoid severely steatotic elderly donors. The risks associated with steatotic liver grafts appear to be short-term perioperative risks, and long-term steatotic grafts provide excellent function once recipients are beyond the initial transplant period.
fewer livers overall will be transplanted. Insurance companies will naturally favor the programs that offer the lowest cost transplants, yet by steering the beneficiaries to these programs, they may not be offering them the best overall chances.

I have several questions for the authors:

1. I presume, since a large portion of the biopsies were done at donor procurement, that these were not interpreted by a single pathologist. While the authors noted a strong correlation in fat content between the frozen section and the permanent section when both were available, my question is: Have they examined the correlation between different pathologists or between the interpretation by pathologists vs the in 1 interpretation by the surgeons? In our experience, there can be great variability in this area, and this could affect the results of their analysis.

2. I noticed that the cold ischemia times are very short, in all groups, both steatotic and nonsteatotic, averaging 6 hours or less. Despite overlapping donor and recipient operations, our mean cold time is around 8 hours. As the authors acknowledge in their manuscript, this may explain why they have such excellent results with steatotic livers, which are known to be particularly prone to ischemia injury. My second question is, to what extent do the authors believe that the short cold time can be attributed in part to the fact that two-thirds of the livers that are recovered by the St Louis OPO [Organ Procurement Organization], the Mid America Transplant Services, are recovered at a specialized organ recovery facility at the OPO, rather than at a donor hospital? I know a number of OPOs, including our own, are considering the development of their own non-hospital organ recovery facilities.

3. Finally, I am interested in the group that had greater than 35% steatosis. What was the upper limit of this group? Fifty percent? Sixty percent? Are there any livers that are too fatty to transplant?

Dr Chapman: As Dr Punch points out, this has been a difficult and thorny problem in particular in the liver transplant arena, where for a long time it has been known that increasing steatosis in the donor liver was associated with an increased ischemia-reperfusion injury that can be associated with graft failure. As we push the frontier to accept more and more marginal donors, defining the limits for acceptable degrees of steatosis has been a challenge. Today the risk of significant donor liver steatosis is increasing, occurring in 10% of donors or more. In our series, 7% of the donors that were utilized would be considered severe steatosis. To further compound this problem, we have had difficulty in assessing these results across centers. Dr Punch is at the University of Michigan, where the SRTR [Scientific Registry for Transplant Recipients] maintains an excellent national database. This national database can be used for assessing results on various donor and recipient factors, including short- and long-term transplant outcomes. Unfortunately graft steatosis is not a variable that is reliably collected on a national basis so we can’t assess this variable on a large-scale basis. While DRI [donor risk index] predictive formulas exist, we can’t use steatosis currently to predict outcome.

In response to Dr Punch’s specific questions, what do you do in the middle of the night to assess the degree of steatosis, who is assessing the liver biopsy, and the overall suitability of the liver itself for use as a transplant organ? In our experience, we try whenever possible to have one of our own surgeons present at the donor recovery. Surgeons become as good as an average pathologist at liver biopsy interpretation, so we are looking and trying to help make that determination ourselves. All of our biopsies are brought back and reviewed by one of our experienced liver pathologists. This has helped in the reliability of biopsy interpretation and perhaps allowed for the high
degree of agreement between our donor biopsy performed in the field compared with our postreperfusion biopsy interpreted by our own liver pathologist.

Dr Punch raised a question about our short cold ischemia times. We work as carefully as we can to maintain a short cold ischemia time especially in our steatotic grafts. We do this in a couple of ways, first by overlapping the donor and recipient procedure. Second, he pointed out a unique feature in our own OPO. The OPO maintains operating rooms in the OPO office, so for standard deceased donors once they have been declared brain dead, the donors are moved to the OPO and this includes flying the donor into St Louis if they are at an outlying area. This was started in 2000. It has been a very effective technique at both cost and travel time reduction and it is something that I think we are going to see more and more of around the country. Currently 70% of our donors are actually recovered at our local OPO outside of the hospital setting. It is located just 5 minutes from our own medical center, so at the time of the donor recovery if there is a question about the degree of steatosis, the transplanting surgeon can drive 5 minutes, look at the liver, and involve our own pathologist if needed. This is an important factor to keep our cold ischemia times short.

One final point. In this series, we have used steatotic grafts with up to 60% macrosteatosis. We have not gone beyond that point. I think for grafts that have higher degrees of steatosis, perhaps 40% or more, we have shied away from using grafts that also have concomitant inflammation. If there is any degree of steatohepatitis, if there is any degree of fibrosis, we decline those grafts. We do not use severely steatotic grafts from donors who are also hepatitis C positive or steatotic grafts from DCD [donation after cardiac arrest] donors. Nevertheless, steatotic grafts are not likely to function well early postoperatively, mainly manifested by increased coagulopathy. The patients bleed more and they have a higher likelihood of needing reexploration. In summary, carefully selected liver grafts that have moderate to severe steatosis from standard brain dead donors can be utilized for liver transplantation with good results. However, resource utilization is generally increased in this patient group.

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