When is steatosis too much for transplantation?

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1. Introduction

Steatosis is the most prevalent underlying condition in liver grafts available for transplantation. An incre-
ment in the use of steatotic grafts could significantly reduce the current disparity between organ demand and supply. Apart from the effect on the donor pool, non-alcoholic fatty liver disease (NAFLD) will additionally lead to more liver transplantsations, as it is projected to become the number one indication for OLT in North America by the year 2020 [1].

2. Definition and prevalence of liver steatosis

Steatosis is assessed according to the pattern and the amount of fatty infiltration in hepatic tissue sections. Traditionally, fatty accumulation has been classified morphologically as macrovesicular or microvesicular. Macrovesicular steatosis is characterised by a single, bulky fat vacuole in the hepatocyte that displaces the nucleus to the edge of the cell. Among a variety of disorders alcohol abuse, obesity, and diabetes mellitus are the main causes behind this form of lipid accumulation. The underlying pathogenesis is related to an excessive triglyceride accumulation in the liver, mainly due to an increased uptake of fatty acids released from adipose tissue and/or an augmented de novo synthesis. Defective hepatic export, secondary to reduced lipoprotein synthesis, or impaired β-oxidation of fatty acids further increase hepatic triglyceride content [2].

The less conspicuous microvesicular steatosis, usually related to toxins or metabolic disorders, is characterised by accumulation of tiny lipid vesicles in the cytoplasm of hepatocytes without nuclear dislocation. This manifestation is ascribed to reduced fatty-acid metabolism by hepatic mitochondria, caused either by impaired β-oxidation or disrupted activity of the mitochondrial electron-transport chain [3].

Current quantification and grading of liver steatosis originates from studies performed in the early 1990s, when steatosis was classified as mild, moderate, or severe [4] if, less than 30%, 30–60%, or more than 60% of hepatocytes, respectively, display fatty infiltrations [5].

The exact prevalence of steatosis in the general population has not been well defined. In autopsy studies of accidental deaths, hepatic steatosis is found in 6–11% of cases. In liver donors the prevalence is much higher with rates as high as 30% in cadaveric and 20% in potential living donors [6]. Since fatty liver disease is the hepatic component of the metabolic syndrome associated with obesity, which is a growing problem in Western countries [7], a further increase in the prevalence of steatosis in potential liver donors has to be expected in the future.

3. Assessment of donor hepatic fat

The assessment of a donor organ is one of the most difficult tasks for the transplant team. Since the success of OLT critically depends on the viability of the graft, it is crucial to establish the degree of steatosis that renders a donor organ unsuitable. The initial evaluation, based on visual inspection and palpation, is done by the donor surgeon. While macroscopic examination appears to be fairly reliable in determining the presence of severe grades of steatosis, it fails to detect moderate and mild degrees [5]. The positive predictive value of macroscopic appraisal at procurement was reported to be 71% for severe, 46% for moderate, but only 17% for mild steatosis [5]. When steatosis is suspected at inspection, 38% of liver transplant surgeons in the UK and 47% in the US proceed with the histological examination of the graft. Despite the low overall positive predictive value of macroscopic assessment, 50% of UK transplant surgeons never integrate histopathologic assessment into their decision-making process [8]. While the same survey indicated a much higher use of biopsies in the US, current OPTN data (as of April 14, 2006) reveals that only 28% of all 7593 cadaveric livers considered for OLT in 2005 were actually biopsied.

Although microscopic examination remains the “gold standard”, different tissue processing and staining techniques can affect detection and grading of steatosis. This was shown in 83 consecutive donor livers, where steatosis was diagnosed in 49% of Sudan III-stained sections, 47% of toluidine blue stained sections, 39% of frozen sections and 21% of deparaffinised hematoxylin/eosin (H&E)-stained sections [9]. In another study Oil Red O staining, very sensitive in the detection of fat, was shown to be highly technician-dependent and to yield a high false-positive staining rate [10]. Although H&E staining is of lower sensitivity compared to special lipid stains, it remains the most commonly used technique worldwide [8]. Due to time constraints frozen sections are preferred. Data on the clinical relevance of fat detected by more sensitive stains are still lacking.

4. Should routine biopsy be performed in candidates for living donations?

Liver biopsy, although relatively safe when performed by experienced physicians, leads to hospital admission in 2–3% of patients due to complications. Mortality rate following percutaneous liver biopsy amounts to 1 in 10,000–12,000. When evaluating possible candidates for living donation the risk has to be carefully weighed against the information gained from the biopsy. To keep invasive diagnostic procedures to a minimum, multiple surrogate markers and imaging techniques have been evaluated for their ability to detect hepatic fat. A study on the role of radiological modalities in patients with NAFLD comparing ultrasound, computerised tomography and magnetic resonance imaging to liver biopsy showed that the severity of hepatic steatosis can be accurately determined only when there is moderate or severe fatty infiltration [11]. Whether body mass index (BMI) can be used to predict steatosis and consequently spare living donors a liver biopsy is still controversial. While in one study none of the
subjects with normal BMI had steatotic livers [6], others reported only a weak correlation between BMI and biopsy with 9% of candidates displaying a normal BMI having 10% or greater steatosis [12]. Considering that accurate quantification of hepatic fat is neither provided by BMI nor by imaging studies, and that biopsy can disclose additional hepatic anomalies, some transplant surgeons make pre-operative liver biopsy mandatory as already discussed in the third forum on liver transplantation [13,14].

### 5. Impact of steatosis on the outcome of liver transplantation

A link between macrovesicular steatosis and PNF of liver grafts was suggested in the late 1980s [15]. Subsequent studies revealed a correlation between the extent of initial poor graft function as well as recipient survival and the quantity of fatty infiltration [4,5]. These findings, yielding a novel semi-quantitative classification for hepatic steatosis, stimulated transplant sur-
geons to analyse the impact of steatosis on the outcome of liver transplantation (Table 1). Although interpretation of these individual studies is hampered by differing staining methods and definitions of PDF and PNF, they offer important insights into the critical issue of using fatty liver for OLT. For example, liver steatosis has emerged as the single most important variable determining graft function after transplantation in a multivariate analysis [16]. Several studies showed that transplantation of livers with severe steatosis resulted in PNF in about 80% of the cases. Thus, exclusion of such fatty livers was promptly accompanied by a drop of PNF-rate from 8.5% to 1.4%, the difference closely mirroring the overall exclusion rate of 7% [10].

Based on these findings, most transplant surgeons would currently consider that grafts with severe steatosis should not be used for transplantation. The attitude among surgeons varies regarding the use of mildly or moderately steatotic grafts. Although increased postoperative aspartate aminotransferase (AST) levels have been reported after transplantation of mildly steatotic livers [5], in most series mild steatosis (<30%) did neither affect long-term graft function nor patient survival and therefore is not considered to be an obstacle to the use of such grafts. This data has provided the rationale for routine utilisation of mildly steatotic grafts in most centres.

The consensus to use mildly steatotic livers and to discard those with severe steatosis is reflected in the current practice of US transplant centres (Fig. 1). In contrast, assignment of moderately steatotic grafts (between 30% and 60%) remains controversial. While some investigators have reported increased rates of PNF (13% versus 3%) [17], others found identical 1-year graft and patient survival when transplanting livers with moderate steatosis compared with nonfatty livers [5,10,18,19]. Whereas graft and patient survival were not adversely affected in these studies, PDF was uniformly more prevalent in the steatotic groups. The impact of primary graft dysfunction on long-term graft outcome is still unclear.

Moderately steatotic grafts qualify as marginal grafts because these organs have been associated with poor clinical outcome, particularly when associated with additional risk factors, such as prolonged cold ischemia or advanced donor age. This view is supported by the finding that transplantation of such grafts under emergency conditions reduces patient survival [5]. As discussed in the two previous articles of this forum, it might be wise to match steatotic grafts to appropriate recipients. For example, results of a recent work on the assignment of steatotic livers using the model for end-stage liver disease (MELD) suggest that grafts with >30% fatty infiltration can be safely used in low-risk recipients, whereas these organs should be discarded in recipients with a high MELD score (e.g. >30) [20]. However, from the perspective of the entire population on the waiting list, such livers, despite bearing a high risk of PDF and PNF, may still serve the sickest patients, as already discussed in this forum by Burra et al.

Since most transplant centres have focused their attention on macrovesicular steatosis, the clinical impact of microsteatosis remains unclear. Experimental data point to a negative impact of both micro- and macrosteatosis on ischemic injury [21], yet, clinical studies report contradictory results.

Livers revealing moderate to severe microvesicular steatosis (>30% of hepatocytes containing fat) have been safely used for transplantation without increasing the risk of PNF in some studies [18,22]. In contrast, moderately microsteatotic grafts (30–60%) have been associated with PNF in 60% of the cases [23]. As this latter study included only patients undergoing retransplantation, data interpretation is severely hampered. Therefore, until further clinical data become available, liver grafts with microvesicular fat inclusions should be routinely used to expand the donor pool.

In analogy to deceased donors, excessive steatosis is commonly regarded as a contraindication to living donor liver transplantation (LDLT), although only scant data regarding the maximal acceptable degree of steatosis is currently available. While some investigators found no functional impairment using organs containing less than 30% steatosis [24], most groups prefer to use only living donor grafts with less than 20% macrosteatosis [25]. After transplantation, regression of mild steatosis was recently observed after 7 days in 7 patients, suggesting that mild steatosis is rapidly reversible after LDLT [24,26].

On the other side, these potential donors should still be advised to lose weight by simple measures such as low-calorie diet, total abstinence from alcohol, and exercise. This approach can significantly reduce the total amount of hepatic steatosis as shown in one study (from 49% to 20% after weight reduction) [27].
6. Protective strategies in steatotic grafts

During liver transplantation blood supply is interrupted exposing liver parenchyma to cold and normothermic ischemia. Fatty livers are particularly susceptible to ischemic injury [21]. Thus, protective strategies have been the focus of numerous experimental studies, as extensively reviewed elsewhere [28]. While many experimental interventions were found to protect the ischemic liver, none has been successfully translated into clinical practice. A good example is ischemic preconditioning (IP). After the initial description in 1996, the protective effect of IP could be proven in patients undergoing major liver resection, yielding even a superior benefit in those with steatotic livers (20–50% steatosis) [29]. Recent clinical studies, however, failed to show clear protective effects in the transplant setting [30].

Besides weight reduction, dietary modulation of omega-3 polyunsaturated fatty acids (PUFA) could emerge as an easily applicable protective strategy in potential living donors. Experimental omega-3 PUFA supplementation has been shown to reduce hepatic triglyceride content and convert macro- to microvesicular steatosis, resulting in significant improvement of hepatic blood flow [31,32]. Recent data from our laboratory suggest that the increased ischemic injury of macrosteatotic livers in ob/ob mice is related to the reduced availability of hepatic omega-3 PUFA content. Intravital fluorescent microscopy displayed impaired microcirculation in macrosteatotic livers, particularly after ischemic insult. Dietary omega-3 PUFA supplementation dramatically improved microcirculation resulting in a significant reduction of hepatocellular injury (Fig. 2). Therefore, omega-3 PUFA pretreatment of potential living donors with confirmed macrovesicular steatosis might render their livers acceptable for transplantation.

In conclusion, steatotic livers with less than 60% fatty infiltration should be accepted for OLT. While mildly (<30%) steatotic grafts should be used routinely, possibly irrespective of other factors, caution should be applied using organs with moderate (30–60%) steatosis. For example such livers may not be used in very sick patients, e.g. with a MELD score >30, particularly when associated with other risk factors. There is a consensus that severely steatotic grafts should not be used for transplantation until novel protective strategies are available. Of note, this consensus is based on very few convincing studies.

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References

Can non-heart-beating donors replace cadaveric heart-beating liver donors?

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1. Introduction

Recently non-heart-beating donation (or also called donation after cardiac death) has re-emerged as a major potential way of increasing the supply of organs for transplantation. The success of renal transplantation from non-heart-beating donors (NHBD) [1] has led to...