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Does Histopathology Predict the Outcome of Fatty Liver Resection and Transplantation?

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Abstract:

Hepatic steatosis is widely considered as substantial risk factor for postoperative complications after major hepatectomy and liver transplantation. Nonetheless, studies have been inconsistent regarding the extent of steatosis pertinent to aggravation of liver injury. Furthermore, a significant number of studies failed to show any link between hepatic steatosis and worse postoperative outcome. The confusion is further nourished by the conflicting observations on the impact of steatosis on survival rates following colorectal liver metastasectomy and also on the regenerative capacity of the fatty liver. We assume that these controversies are related to inconsistent evaluation of hepatic steatosis even among expert pathologists. In this mini-review, we will underline the limitations of the histo-pathological assessment of hepatic steatosis. The emerging role of chemical composition of hepatic lipids, particularly the balance between Ω-3 and Ω-6 fatty acids, in liver protection/injury will be highlighted. Finally, the conflicting studies on the impact of various histo-pathological grades and types of hepatic steatosis on the clinical outcome after liver resection and transplantation will be analyzed.

Keywords: Steatosis, microstaetosis, macrosteatosis, liver resection, transplantation

1 Introduction:

Patients with hepatic steatosis are commonly assumed to be more vulnerable to postoperative complications after liver resection and transplantation [1]. The current literature is abundant in correlating hepatic steatosis with postoperative complications and mortality after resectional and transplant surgery of the liver [2-7], however several studies failed to show a negative impact of steatosis on the clinical outcome [8, 9]. In the setting of liver regeneration, steatosis is thought to jeopardize restoration of liver volume after partial hepatectomy [1]. Recently, impairment of fatty liver regeneration in mice was shown to be related to inhibition of growth arrest and DAN damage-inducible protein (GADD34) and that overexpression of the same protein ameliorates liver regeneration [10]. Clinically, obese patients with liver steatosis show weak hepatic regenerative

response to major liver resection as reflected by reduced liver volumetric gain compared with matched non-obese controls [11]. In sharp contrast, liver regeneration is not significantly affected in high fat diet-induced simple steatosis in rats, compared with the lean animals [12]. Furthermore, a mild degree of diet-induced simple steatosis was demonstrated to enhance liver regeneration in mice [13]. This pro-proliverative influence was associated with increased expression of fatty acid transport protein and hepatocyte growth factor [13].

The ambiguous definition of liver steatosis in many studies and variability in the diagnostic methods appear, in our view, to be seriously affecting the validity of such studies. Steatosis of the liver is characterized quantitatively in relation to the percentage of hepatocytes containing lipid droplets into mild (<30%), moderate (30-60%) and severe (>60%) grades [14]. Qualitatively, hepatic steatosis is separated into two entities (micro- and macrosteatosis) according to the size of lipid droplets and the location of nucleus in the hepatocyte cytoplasm [14]. This approach continues to be applied despite several pitfalls during the histological workup and the inconsistency among pathologists.

2 Histopathology, Imaging and Chemistry of Hepatic Lipids: Which Is Critical for the outcome of Liver Resection and Transplantation?

Assessment of the grade and type and the distinction between simple steatosis and non-alcoholic steatohepatitis traditionally relies on microscopic evaluation by pathologists [14]. This subjective histo-pathological evaluation has gained clinical grounds since 1989, when pathologists from Pittsburgh described two cases of primary non-function due to high rate of macrosteatosis in many hepatocytes [15]. On this basis, several studies demonstrated that liver steatosis is a substantial risk factor for poor outcome after major hepatectomy [16, 17] and orthotopic liver transplantation (OLT) [18-21], while others failed to document negative effect [22-26]. The microscopic diagnosis of hepatic steatosis encompasses a number of pitfalls, as discussed below, which may significantly influence the interpretation. There is emerging evidence that modern imaging techniques may provide more precise quantification of hepatic fat content [27]. Moreover, the chemical composition of liver fat is evolving as key-player in liver ischemia/reperfusion injury [28].

2.1 The size number of biopsy samples

The importance of the biopsy size is frequently underestimated. An adequate biopsy sample should not be less than 1.6 cm length and 1.2-1.8 mm width and comprises about 10 portal tracts [29]. Of note, this “adequate” tissue sample will approximately be 1:50.000 of the total volume of the liver, which will never be enough to mirror the status of the parenchyma of the whole organ [29]. In a study reported by Vuppalanchi et al [30], three cores of liver biopsies were obtained percutaneously from each of 50 patients with suspected liver steatosis. The evaluation by blinded pathologist led to a diagnosis of steatosis that was significantly higher when three samples were assessed compared with the assessment of only two samples [30].

2.2 Tissue fixatives and staining methods

Different fixatives can alter the diagnosis of hepatic steatosis via induction of fusions or disintegration of LDs. Cold methanol removes most cellular phospholipids and enhances fusion of LDs. Acetone fixation extracts total cellular lipids with subsequent collapse of LDs [35]. Visualization of LDs is noticeably prejudiced by the staining method. Fukumoto et al [32] noticed that LDs labeled by Nile red display different shape compared with those stained by Sudan III, and oil red O. The authors found that ethanol and isopropanol used for Sudan III and oil red O staining, respectively, and glycerol used for mounting, cause fusion of adjacent LDs [32].

Immunofluorescence labeling for adipose differentiation-related protein (ADRP), a LD marker, was dislocated to the rim of large LDs which have formed as a result of fusion [32].

2.3 The inter-observer agreement:

High inter-observer disagreement was documents even among expert pathologists regarding both the quantitative and qualitative assessment of steatosis as well as steatohepatitis [33]. The assessment of 46 high-resolution images of H&E stained liver sections by 4 renowned pathologists disclosed poor concordance regarding the degree of total, macro- and microsteatosis. Lack of agreement was observed also with the semiquantitative evaluation; for instance, the diagnosis of marked steatosis (≥30%) varied from 22% to 46%. Pathologists were asked to interpret 4 features of steatohepatitis (lobular and portal inflammation, hepatocyte ballooning and Mallory's hyaline) as absent or present and to provide an overall diagnosis of steatohepatitis. A disagreement among pathologists was evident with regard to the assessment of all parameters as well as the final diagnosis of steatohepatitis [33]. These results possibly explain the inconsistency among published studies on the relevance of liver steatosis to liver surgery and transplantation.

2.4 Imaging techniques for assessment of liver steatosis:

Ultrasound examination of the liver is the most commonly used modality for assessment of steatosis [34]. Despite the ability of conventional cross-sectional imaging such as computed tomography (CT) and magnetic resonance imaging (MRI) to provide more reliable diagnosis, both techniques failed to show significant agreement with the conventional histopathological evaluation [35]. In a study on 161 live liver donors who underwent liver steatosis assessment by ultrasonography, computed tomography, proton magnetic resonance spectroscopy and dual gradient echo MRI, the later outperformed all other modalities [27]. However, this study provided no data on the relation between liver steatosis quantified by each method and the clinical outcome after live donor hepatectomy [27]. To overcome the uncertainty of histopathologic assessment, chemical lipid assay was applied as more reliable reference for the amount of intrahepatic fat in murine liver steatosis models [36, 37]. Significant agreement was observed between MRI estimation of the magnitude of hepatic fatty infiltration compared with chemical lipid assay [36, 37]. Moreover, two different studies showed that patients with liver content ≥ 5% [38] and > 10% (36) assessed by MRI exhibited higher grades of surgical complications, including organ failure and sepsis, compared with those who have <5% and <10% , respectively [36, 38].

2.5 The Role of chemical composition of hepatic lipids in hepatocellular protection/injury:

The histological evaluation of hepatic steatosis as a predictor of the clinical outcome ignores the chemical lipid composition [28]. Dietary models of hepatic steatosis in rats with similar total triglycerides, but different composition of fatty acids (FAs), show that increased saturated FAs is associated with enhanced liver injury and markers of endoplasmic reticulum stress such as X-box binding protein-1 mRNA splicing and glucose-regulated protein 78 expression [39]. Noteworthy, the rise of saturated FAs was associated with reduced liver regeneration in response to partial hepatectomy and increased lipopolysaccharide-induced liver damage [39].

In a rodent model of hepatic macrosteatosis, more pronounced hepatocellular injury after ischemic stress was shown in comparison with lean animals. The mechanism of injury was mostly related to reduced sinusoidal perfusion which has been successfully managed by preconditioning with dietary Ω-3 FAs [40]. In humans, three live liver donors with moderate steatosis were

treated with oral Ω-3 FAs capsules prior to right hemi-hepatectomy. This approach resulted in remarkable reduction of steatosis grade and the extent of macrosteatosis within one month [41].

Supplementation of ob/ob mice with Ω-3 FAs decreased hepatic levels of arachidonic acid before ischemia, diminished hepatic TXA2 production after reperfusion. As a result, significant amelioration of sinusoidal perfusion and protection against hepatocellular damage were conferred. Similar protection was observed by the use of selective TXA2 receptor blockage without alteration of the histological pattern of intrahepatic lipids (28). In humans, circulating levels of TXB2, a metabolite of TXA2, were remarkably increased during hepatic resection. Intravenous administration of TXA2 synthase inhibitor intraoperatively reduced plasma TXB2 and concomitantly blunted serum transaminase levels [42].

3 Liver Steatosis and the Clinical Outcome of Liver Resection:

Many studies on the impact of steatosis on the outcome of liver surgery have been published over the last two decades. In the light of the aforementioned pitfalls of the microscopic evaluation of steatosis, clinical data on the impact of steatosis on the clinical outcome after liver resection are conflicting and often difficult to be convincingly interpreted.

In a retrospective analysis of a large series of liver resections involving 325 steatotic versus 997 non-steatotic patients, uni- and multivariate analyses showed no negative effect of steatosis on postoperative complications or patient survival. This result might be influenced by inclusion of minor liver resections [19]. Kooby et al [43] compared 325 patients with fatty liver with 160 lean controls regarding the clinical outcome after hepatic resection for liver neoplasms. The authors reported that marked steatosis was independent predictor of postoperative complications but not for 60-dat mortality [43]. Two studies demonstrated that marked steatosis (≥ 30%) is an independent risk factor for post-hepatectomy complications. However, data on the postoperative mortality were inconsistent [16, 17]. In a case-matched control study, steatosis of any grade significantly predicted postoperative complications. Cholestasis was significant risk factor for mortality after resection of the steatotic patients [6]. A retrospective study on 194 steatotic patients who underwent liver resection for colorectal liver metastasis (CRLM), morbidity, including infective complications, and admissions to intensive care unit correlated with increasing grades of steatosis [7]. A meta-analysis of 1000 patients enrolled in 4 observational studies showed that steatotic patients had twofold increase in postoperative morbidity and that the severe form carries nearly threefold higher risk of post-hepatectomy death [4]. In a study on the clinical outcome after major hepatectomy with portal vein resection, hepatic steatosis was associated with remarkably higher rate of postoperative mortality [3]. However, in 2715 patients, among them 927 were steatotics, Hamady et al [59] documented that patients with liver steatosis have substantially higher risk of postoperative liver failure and local recurrence [44]. Contrarily, no statistically significant increase in postoperative complications could be found following major hepatectomy in obese-steatotic patients compared with matched non obese- non steatotic controls, despite documented impairment in postoperative volumetric liver gain in the former group [11]. These data are further supported by a report on liver metastasectomy for CRLM in 513 non steatotic versus 421 patients with different degrees of steatosis. No significant difference in terms of postoperative morbidity, mortality or tumor recurrence could be documented between steatotic and non steatotic patients even after case-control matching [45]. In the same line, steatohepatitis but not simple steatosis was reported to increase overall and hepatectomy-related surgical complications[8, 9]. Analysis of data from LiverMetSurvey database showed

that liver steatosis that develops after neoadjuvant chemotherapy exerted no negative influence of peri-operative mortality, 5- year overall and cancer specific survival after first liver resection for CRLM [46]. Steatotic patients who were registered in the LiverMetSurvey database and underwent first liver resection of CRLM without prior chemotherapy similar 90-day perioperative mortality to those with normal liver parenchyma. In comparison with normal parenchymal background, steatosis was surprisingly associated with significantly improved overall and cancer specific survival [47]. The disagreement extends to studies on live donors for living donor liver transplantation (LDLT). For instance, postoperative transaminase and bilirubin levels as well as morbidity rates where not significantly different between donors with mild (5-30%) versus no macrosteatosis [25]. Nevertheless, in another study on LDLT, mild macrosteatosis (up to 20%) was documented as independent risk factor for postoperative hyperbilirubinemia [48]. These conflicting results could be related to inconsistent evaluation of steatosis in liver sections.

4 Graft Steatosis and Survival after Liver Transplantation:

The current shortage of high quality grafts has led to the increasing use of so-called “extended criteria” grafts, among which steatotic livers are the most common. Increasing degrees of steatosis, particularly the macrovesicular form, is thought to have detrimental effect on graft and patient survival after orthotopic liver transplantation (OLT) [49]. The vast majority of European transplant centers reject implantation of grafts with severe steatosis for any patient [5]. However, the results from studies reporting on the effect of hepatic steatosis on graft and recipient survival are not consistent.

Analysis of the outcome of OLT in patients with variable grades of steatosis showed significant correlation between enhanced hepatocellular injury and increased degrees of steatosis. Severe renal failure requiring hemofiltration /hemodialysis occurred more frequently in recipients of severely steatotic grafts. Severe steatosis was associated with significant increase of early (90 days) mortality [50]. In a case-control matched study, morbidity and mortality after OLT were studied in 57 recipients of donor livers with up to 30% steatosis. The control group included 59 patients who received grafts without fatty infiltration [13]. Median transaminase value at the second postoperative day was significantly higher in the fatty liver group. Surprisingly, mild steatosis was associated with significant decline in the 4-month graft survival and in 2-year patient survival. In multivariate analysis, steatosis was independent risk factor for graft loss [18]. The clinical outcome of OLT in 115 patients who were categorized according to the grade of steatosis into four groups was prospectively analyzed [51]. There was no significant differences among all groups with regard to the demographic data, donor age, weight and body mass index, etiology of liver disease, indications of OLT and MELD (Model for End Stage Liver Disease) score [51]. Graft survival at 1-year was significantly lower with severe compared with absent and even mild and moderate steatosis groups [51]. In the same line, severe graft steatosis exerted a significant influence on one year patient survival compared with non-and mildly steatotic organs [58]. No negative effect on graft or patient survival could be documented in hepatitis C virus positive recipients; however, the study may be limited by the small number of patients in each subgroup [51]. Another study showed that graft survival is significantly lowered with increasing grades of steatosis at one month but not at 3-months or one year [16]. Of note, graft loss in presence of steatosis was significantly influenced by increased rates of recurrent hepatitis C and the older age [21].

Contrarily, liver grafts with more than 50% steatosis exhibited adequate initial graft function and no compromise to early (30-day) patient survival [36]. It should be noted that

prolonged ischemia was avoided in the fatty organs and non-among recipients of fatty liver suffered from other risk factors such as emergency or re-transplantation [52]. In another study, the outcome of the use of severely steatotic grafts for OLT was analyzed [5]. Despite the higher rate of primary graft dysfunction, renal failure, prolonged intensive care unit and hospital stay with severe steatosis, 60-day mortality and 3-year patient survival were not negatively affected [5]. In line with these data, a study on the cumulative graft survival in patients who received grafts with mild compared with moderate and severe steatosis showed no significant difference despite impaired graft function postoperatively [26]. In 2 groups of mild and moderate fatty graft recipients who were matched for age, gender, MELD score and cold ischemic time, moderate steatosis was not associated with any negative impact on patient survival at 1 year [53]. Nineteen recipients of moderately macrosteatotic grafts were compared with 195 matched controls. Despite increased postoperative complication rates steatotic graft recipients, short and long-term survival of the grafts and patients were similar [54]. Recently, Wong et al investigated the results of implantation of deceased donor grafts with severe forms of macrosteatosis > 60% versus ≤ 60 in 19 and 354 patients, respectively. Graft and patient survival rates were almost similar in both groups [55]. A Japanese group demonstrated that implantation of organs with mild and moderate steatosis results in comparable graft and patient survival with normal grafts while severe steatosis led to worse outcome in LDLT. Noteworthy, histological assessment and computed tomography were used for evaluation of steatosis [20]. In another series of LDLT (20), steatosis grades of the grafts biopsy ranged from mild (1- 20%) to moderate (21-50%). Steatotic donors had significantly higher body mass index. Although the peak transaminase levels were significantly higher with steatosis, both groups showed comparable 1-year graft survival [24].

In the setting of OLT for hepatitis C virus positive recipients, patient survival at 5 years was significantly decreased with increasing degree of steatosis. Graft survival at 3 years showed also significant inverse relation with the increasing degree of steatosis. Aside from gender, analysis of marginal donor variables showed no significant difference among steatotic and non steatotic donors [56]. In contrast, another group observed no negative influence when steatotic grafts were transplanted to patient's positive for hepatitis C virus [57]. In patients requiring re-transplantation, severe microsteatosis significantly lowered the 1-year graft survival [58]. Likewise, at post-transplant day 7, initial poor graft function was significantly related to presence of microvesicular steatosis [59]. High grade (>30%) macrosteatosis was also reported to induce remarkable shortening of both graft and patient survival [19]. Furthermore, in 311 consecutive OLTs, 5 among 8 patients with graft macrosteatosis of ≥ 25%, died within the first year. However, the effect of macrosteatosis on graft survival was not significant [60].

5 Conclusions and Perspectives:

The influence of intrahepatic lipids on the clinical outcome after fatty liver resection and transplantation is currently viewed from the side of shape and size of LDs and lipid quantity as assessed by imaging studies. However, the recent evidence on potential role for lipid metabolites, which are derived from the hepatic lipid content, warrants further consideration in future studies.

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