# Review on Liver Steatosis and its Impact on Liver Transplantation

**Abstract**

The lack of available organs for liver transplantation (LT) associated with the increased death rates among patients on the waiting lists for LT has triggered the use of so-called extended criteria donor (ECD) grafts. Among the wide range of these ECD livers, hepatic steatosis is one of the most frequent disorders, which is mostly related to an increasing prevalence of non-alcoholic fatty liver disease (NAFLD). NAFLD is increasingly significant in healthy individuals for its high worldwide prevalence, an association with the metabolic syndrome, such as insulin resistance, diabetes, dyslipidemia and obesity, and an association with liver-related morbidity and mortality. Using the ECD grafts especially those with NAFLD will markedly increase the donor pool for LT. Several approaches, including long-term and short-term measures, were identified for the management of NAFLD.

**Keywords:** Steatosis; Liver transplantation; Fatty liver; Ischemia; Reperfusion injury

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**Abbreviations:** ALT: Alanine Amino Transferases; AST: Aspartate Amino Transferases; AUROC: Area Under the Receiver Operating Characteristic Curve; BMI: Body Mass Index; CIT: Cold Ischemia Time; CT: Computed Tomography; D-MELD: Donor Age Multiplied with the Recipient Model for End-Stage Liver Disease; ECD: Extended Criteria Donor; EGD: Early Graft Dysfunction; ELF: Enhanced Liver Fibrosis; ESLD: End-Stage Liver Disease; FA: Fatty Acids; FIB-4: Fibrosis-4; FLI: Fatty Liver Index; FLIP: Fatty Liver Inhibition of Progression; GGT: Gamma-Glutamyl Transpeptidase; HCC: Hepatocellular Carcinoma; HCV: Hepatitis C Virus; IP: Ischemic Preconditioning; IR: Insulin Resistance; I/R: Ischemia Reperfusion; LDLT: Living Donor Liver Transplantation; LSM: Liver Stiffness Measurement; LT: Liver Transplantation; MaS: Macrosteatosis; MetS: Metabolic Syndrome; MiS: Microsteatosis; MELD: Model For End-Stage Liver Disease; MR: Magnetic Resonance; NAFL: Non-Alcoholic Fatty Liver; NAFLD: Non-Alcoholic Fatty Liver Disease; NAS: NAFLD Activity Score; NASH: Non-Alcoholic Steatohepatitis; NFS: NAFLD Fibrosis Score; NPV: Negative Predictive Value; PNF: Primary Non-Function; PPV: Positive Predictive Value; ROS: Reactive Oxygen Species; T2DM: Type 2 Diabetes Mellitus; Tnf-α: Tumor Necrosing Factor A; UNOS: United Network of Organ Sharing; WIT: Warm Ischemia Time

## Introduction

As organs shortage was anticipated as early as 1987, the development of what is called "marginal donors" was crucial [1]. A marginal graft could be defined as an organ with an increased risk for poor function or failure that may subject the recipient to higher risks of morbidity or mortality. However, there is no consensus about factors that define a graft as marginal and should be excluded from the use because of unacceptable risk to the recipient. Therefore, the decision to transplant a specific organ depends on the judgment of the transplant surgeon and consideration of the specific recipient [2]. Broadly there are two categories of marginal grafts. Firstly, there are grafts which carry a high risk of technical complications and/or impaired function, including the steatotic livers, non-heart beating donors, elderly donors, split livers, donors with high inotrope requirement or long ischaemia times. Secondly, grafts will be considered marginal if they carry a risk of transmission of infection and/or malignancy to the recipient [2]. Among the wide range of these ECD livers, hepatic steatosis is one of the most frequent disorders, which is mostly related to an increasing prevalence of fatty liver diseases [3].

### Definition

Fatty liver is defined as the fat accumulation of at least 5% of liver weight in the absence of other etiologies of liver disease or use of steatogenic medication [4]. Traditionally, fatty disorders of the liver have been classified into alcoholic and non-alcoholic. NAFLD includes a spectrum of diseases ranging from pure steatosis (non-alcoholic fatty liver [NAFL]) to NASH [5]. NAFL is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes. NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis [6]. NAFL is further subdivided into Macrosteatosis (MaS) and Microsteatosis (MiS). MaS are characterized by a single, bulky fat vacuole in hepatocytes, displacing the nucleus to the edge of the cell. In MiS, the cytoplasm of the hepatocytes contains tiny lipid vesicles without nuclear dislocation [7].

## Epidemiology

**Incidence and prevalence:** NAFLD is the most common chronic liver disease in the general population, posing an enormous burden on public healthcare costs. Its prevalence has increased as more patients develop a sedentary lifestyle, metabolic syndrome (MetS), and obesity. It is correlated with many factors, including body mass index (BMI), fat distribution, race, ethnicity, and sex [8]. Moreover, NAFLD occurrence increases with age and are generally higher in men than in pre-menopausal women. It also differs according to ethnicity, affecting 45% of Hispanic people, 33% of Caucasian and 24% of African-Americans in the USA, as well as increases in South Asians and United Kingdom [9]. In a recent meta-analysis, the global prevalence of NAFLD was estimated to be 25%, which is very similar to that of the MetS. Prevalence estimates averaged 32% in the Middle East, 30% in South America, 27% in Asia, 24% in Europe, 21% in North America and 13% in Africa [10].

**Natural history:** While pure steatosis is not associated with increased mortality in long-term follow-up studies, individuals with NASH have a reduced life expectancy. Recent evidence suggests that in addition to liver-related death (due to end-stage liver disease, portal hypertension and hepatocellular carcinoma (HCC), patients are exposed to increased frequency of cardiovascular diseases leading to excess mortality. Patients with either uncomplicated steatosis or NASH appear to be at risk of developing the full-blown MetS or its individual components, particularly type II diabetes mellitus (T2DM) and impaired fasting glucose. Progression to fibrosis has been demonstrated histologically in up to 30% of cases, but apparent regression may occur. Cirrhosis develops in up to 35% of those cases with a risk of liver failure or HCC [11]. Therefore, the increasing prevalence of NAFLD is expected to raise the number of liver transplantation (LT) candidates in the near future, and possibly become the most common indication for LT [3].

**Risk factors:** Given the tight association with MetS, the whole spectrum of NAFLD mostly occurs in patients with obesity (60-95%), T2DM (28-55%), and hyperlipidemia (27-92%). In a study of patients undergoing bariatric surgery, NAFLD was found in up to 95%, with fibrosis in 74%, and mild necroinflammation in 24% of cases. The pattern of fat distribution is more important than BMI, and visceral fat has been associated with severity of inflammation and fibrosis. Abnormal glucose regulation per se carries an increased risk for NAFLD, whose prevalence rises from 27% in subjects with normal basal glucose to 43% in impaired fasting glucose and 62% in T2DM [9].

## Pathogenesis

Although the exact pathogenesis of NAFLD remains poorly understood, there were different hypotheses involved in NAFLD development. Hepatic lipid content is regulated by a balance between hepatic lipid uptake, synthesis, oxidation, and export. Most of the triglycerides are re-esterified fatty acids (FA) that originate from adipose tissue lipolysis and come into the liver [12]. The adipose tissue lipolysis provides approximately 60% of the FAs used for hepatic triglyceride synthesis. The rest of the hepatic FAs come from de novo lipogenesis within the liver (25%) and dietary intake (15%) [13]. Steatosis reflects a net retention of fat within hepatocytes and results from an imbalance between uptake of fat and its oxidation and export. The most consistent pathogenic factor is insulin resistance (IR), leading to enhanced lipolysis which in turn increases circulating free FAs and their uptake by the liver. Fat accumulating in the liver has several effects:

a) Up regulation of apoptosis

b) Upregulation of tumor necrosing factor α (TNF-α) which is pro-steatotic and pro-inflammatory

c) Mitochondrial dysfunction presumably increasing reactive oxygen species (ROS) and provoking lipid peroxidation of cell membranes

d) Induction of cytochrome P450 which generates ROS

e) Induction of pro-inflammatory genes such as TNF-α and cyclo-oxygenase enzyme-2 which induce additional inflammatory mediators which are also pro-fibrotic.

The net effect of the above is apoptosis, necro-inflammation, and fibrosis [14]. Moreover, excess hepatic fat results in increased very low-density lipoprotein secretion and some of the serum lipid abnormalities noted in MetS and NAFLD, including hypertri-glyceridemia, decreased high-density lipoprotein, and higher low-density lipoprotein [15]. Hepatic fibrosis is promoted by steatosis even in the absence of liver cell injury. Adipokines, hormones secreted by adipocytes, appear to be important regulators of hepatic fibrosis. Leptin, which is increased in the MetS, promotes fibrosis and induces pro-inflammatory cytokines while adiponectin, which is decreased in MetS, inhibits stellate cell activation. The net effect of increased leptin and decreased adiponectin is pro-inflammatory and pro-fibrotic mediators [14]. Another prevailing hypothesis considered that several insults or "hits" are involved in causing progressive liver injury with the initial hit, MaS results. IR most likely plays a central role in the net retention of lipids, particularly triglycerides, within the hepatocytes. This is thought to result from decreased disposal of FAs due to impaired mitochondrial β-oxidation. The second hit is generally attributed to oxidative stress, which causes peroxidation of lipids in the hepatocyte membrane with cytokine production and is in large part responsible for the progression from simple steatosis to NASH to liver cirrhosis [16,17]. Additional theories, such as the multiple-hit hypothesis, suggest other factors including clinical conditions that may also involve in NAFLD [18]. Elevated circulating levels of pro-inflammatory cytokines and reduced levels of anti-inflammatory factors cause a chronic low-grade inflammatory state that is recognized as an important pathogenic mechanism of NAFLD. Among clinical associations, diabetes and MetS, advanced age (>45 years), Hispanic ethnicity, female sex, and obesity (BMI> 30) are all related to more aggressive liver histology but without a clear distinction of the cause and effect [14,19,20].

New insights have been recently provided regarding the fat composition in the steatotic livers, particularly the omega-3 and omega-6 FA ratio. In this context, omega-3 FAs down regulate the sterol regulatory element binding protein-1 which enhances hepatic triglyceride accumulation. Moreover, omega-3 FAs upregulate peroxisomal proliferator-activated receptor a, which stimulates lipid degradation. Conversely, these actions can be offset by excessive intake of omega-6 FAs [3].

## Diagnosis

**Clinical presentation:** Most patients with NAFLD (48-100%) are asymptomatic. The liver disease is often discovered incidentally during the routine examination. When symptoms occur, they are usually nonspecific as vague right upper quadrant abdominal pain, fatigue, and malaise. Rarely, pruritus, anorexia, and nausea may develop. Jaundice, abdominal distension (ascites), gastrointestinal bleeding, and confusion (encephalopathy) are all indicative of advanced liver disease (decompensated cirrhosis), occurring late in the course. There are no pathognomonic signs of NAFLD. Obesity is the most common abnormality on physical examination, occurring in 30-100% while hepatomegaly has been reported in up to 75% of patients [21].

### Non-invasive tests

**Laboratory investigations:** Mild to moderate elevation of serum alanine (ALT) and aspartate aminotransferases (AST) and/ or alkaline phosphatase and gamma-glutamyl transpeptidase (GGT) is the most common laboratory abnormality that could be found in patients with NAFLD [22]. Unlike those with alcohol-induced steatohepatitis, who typically manifest disproportionate increases in the AST level relative to the ALT level, patients with NAFLD usually have an AST/ALT ratio <1. The AST/ALT ratio tends to increase with the development of cirrhosis, thus losing its diagnostic accuracy. Elevated serum lipid profiles and glucose concentrations are also common in NAFLD patients, reported in 25 to 75% of cases. A small percentage of patients with NAFLD may have a low-titer (≤1:320) antinuclear antibody (ANA) positivity [21].

### Clinico-laboratory predictor models

In order to restrict the large number of individuals potentially susceptible to undergo a liver biopsy on the account of suspected NAFLD, clinicians have evaluated the utility of several indices predictors for diagnosis of steatosis, NASH and fibrosis in NAFLD patients [11,23], including:

**Fatty liver index (FLI)**: this algorithm was based on four markers; BMI, waist circumference (cm), triglyceride (mg/dL) and GGT (U/L), which is confirmed to accurately identify NAFLD. This index has been used in population studies and has achieved an area under the receiver operating characteristic curve ("AUROC", which measures the accuracy of the test, with the values close to 1 represent a perfect test) of 0.84 in detecting fatty liver. FLI provides a score out of 100, indicating that a score <30 can rule out and a score ≥60 to rule in hepatic steatosis [24].

**The HAIR (Hypertension, ALT, IR) model:** is an assessment of morbidly obese patients (BMI> 35), to develop NASH, by assigning a point each for the presence of hypertension, elevated ALT (>40 U/L) and IR Index >5. A HAIR score of ≥2 was 80% sensitive and 89% specific for NASH [25].

**The NASH clinical scoring system:** scores for DM, hypertension, elevated AST (≥27 IU/L), elevated ALT (≥27 IU/L), sleep apnea and race) is also used for morbidly obese patients, and had an AUROC of 0.8, sensitivity 22% and specificity 99%. This system has some limitations as the normal reference ranges were not defined and patients with NASH may have serum transaminases within the normal reference range [26].

**The NASH predictive index** incorporates age, BMI, homeostatic model assessment (HOMA)-IR, AST, ALT and sex (female), and produced an AUROC of 0.86, sensitivity 56%, and specificity 100% [27].

**The NASH test:** uses a combination of 13 clinical and biochemical variables including age, gender, and weight, height, and serum levels of cholesterol, triglycerides, α-2macroglobulin, apolipoprotein A1, haptoglobin, GGT, ALT, AST, and bilirubin. The AUROC for diagnosing NASH as determined by liver biopsy was 0.78 and by the test 0.79, with a sensitivity of 29%, specificity of 98%, positive predictive value (PPV) of 91%, and negative predictive value (NPV) of 71%. The NASH Test has been validated from different centers as a simple non-invasive test for diagnosis of NASH [28].

**The AAR (AST: ALT Ratio) model:** The AST-to-ALT ratio is the simplest predictive model for fibrosis. ALT is typically higher than AST in NAFLD; however, having an AAR >1 is suggestive of the presence of advanced fibrosis. AAR has a good negative predictive value (NPV) to rule out advanced fibrosis [29].

**The APRI (AST and Platelet Ratio index):** is calculated using the AST elevation (AST level divided by the upper limit of normal for the laboratory) and the platelet count per mm3 divided by 1000. APRI has been designed to detect significant fibrosis and cirrhosis and suggested cutoffs for significant fibrosis are >1 and for cirrhosis >1.5. In a recent study involving 358 patients with biopsy-proven NAFLD, the AUROC, sensitivity, and specificity of APRI with a cutoff >1 for significant fibrosis were, respectively, 0.67, 30, and 93 % and a cutoff >1.5 for cirrhosis were 0.94, 76, and 72 %, respectively [30].

**NAFLD fibrosis score (NFS)**: this score is a widely accepted tool in excluding advanced fibrosis evaluates six variables; age, hyperglycemia, BMI, platelet count, albumin, and AST/ALT ratio. It has AUROC 0.82, sensitivity 82%, specificity 77%, PPV 56%, and NPV 93%. This score is used to classify the probability of fibrosis as <-1.5 for low probability and >-1.5 to <0.67 for intermediate probability and >0.67 for high probability [31]. It has been acknowledged by the current NAFLD guidelines as a clinically useful tool for identifying advanced fibrosis in NAFLD. Another advantage of the NFS is its ability to provide prognostic information and identify patients with NAFLD who are at increased risk for liver-related complications (such as ascites and gastroesophageal varices) or death. A major limitation of this score is that a significant percentage (20-58%) of patients fall between the two proposed cutoff values and therefore, they have an indeterminate score [32].

**Fibrometer**: This tool uses age, weight, fasting glucose, AST, ALT, ferritin and platelet count to diagnose significant fibrosis [33]. The Fibrometer provides the probability of significant fibrosis and the percentage of hepatic fibrosis. Calès et al. [34] compared the Fibro Meter to NFS and found that the FibroMeter provides a more reliable diagnosis of significant fibrosis than the NFS. This tool can be used to confirm or disconfirm advanced fibrosis in NAFLD patients [34].

**The BARD (BMI, AST, ALT Ratio and Diabetes) score:** combines clinical features of BMI and the presence or absence of diabetes, with the AST/ALT ratio. It has been designed to detect advanced

fibrosis. Using a cutoff point >2, the sensitivity for the detection of advanced fibrosis was between 86.8 and 100 %, while the specificity ranged from 32.5 to 34.7 % in different studies. The main limitation of the BARD score is its low specificity, giving a high false positivity, based on the overestimation of BMI and presence of diabetes [35].

**The BAAT (BMI, Age, ALT, Triglyceride) score:** including age (>50 years), BMI (>28 kg/m$^2$), ALT (>2 × N), and serum triglycerides (>1.7 mmol/L). Each variable received a score of 0 or 1. Although a total score of 0 had sensitivity close to 100% and a specificity of 47%, a high total score of 4 gave a sensitivity of 14% and a specificity of 100% for detection of septal fibrosis to assess advanced liver fibrosis in overweight patients. For a cutoff BAAT score of 2, the sensitivity, specificity, PPV, and NPV were 71, 80, 61, and 86%, with an AUROC of 0.83 [36].

**Fibrosis-4 (FIB-4) index:** Although the FIB-4 index was originally developed to stage liver fibrosis in patients with hepatitis C infection, it has shown promising results in patients with NAFLD. This algorithm utilizes platelet count, ALT, AST, and age with dual cutoff values. For diagnosing advanced fibrosis, the cutoff is 1.45, giving an AUROC of 0.87 with a sensitivity of 74% and a specificity of 71%, resulting in a PPV of 22% and NPV of 73%. The upper cutoff is 3.25, giving an AUROC of 0.88 with a sensitivity of 26% and specificity of 98% and providing PPV of 75% and NPV of 85%. Utilizing the two cutoffs is clinically useful since the below the lower cutoff is good for excluding advanced fibrosis and patients above the upper cutoff are more likely to have advanced fibrosis [37].

**FibroTest:** is another panel that predicts the presence of fibrosis by using five biomarkers (haptoglobin, α2-macroglobulin, apolipoprotein A1, total bilirubin, and GGT). The diagnostic value of Fibro Test was assessed in a large cohort of NAFLD patients and demonstrated that it can reliably predict advanced fibrosis with an AUROC of 0.88 [38]. However, caution should be exercised when interpreting the results of this test in those with Gilbert's syndrome and cholestasis and also in acute inflammation as these conditions will increase bilirubin and haptoglobin level, respectively. FibroTest is available commercially in the USA as a part of the NASH-FibroSURE® (LabCorp, NC) [32].

**The enhanced liver fibrosis (ELF) test:** ELF test is an algorithm that includes hyaluronic acid, amino-terminal propeptide of type III procollagen, and tissue inhibitor of metalloproteinase 1. It had a good performance in distinguishing advanced fibrosis with a cutoff 10.3 with an AUROC of 0.90, but lower AUROC of 0.82 for significant fibrosis with a cutoff 9.3 and 0.76 for the absence of fibrosis with a cutoff <8.5. Interestingly, the ELF test was shown to be a good predictor of liver-related morbidity and mortality in a group of patients with a chronic liver disease, including 44 patients with NAFLD, suggesting that it might be a promising prognostic tool [39].

**Palekar et al. [40] Model:** a clinical model to distinguish NASH from simple steatosis by combining 5 different variables including age (≥50 years), female gender, AST (≥45 U/L), body mass index (BMI ≥30 kg/m2), and AST/ALT ratio (≥0.8), and serum hyaluronic acid (≥55 mg/L). The AUC for this model was 0.76. The presence of 3 or more of these factors had a sensitivity, specificity, PPV, and NPV for NASH diagnosis of 74%, 66%, 68%, and 71%, respectively [40].

## Imaging study

Several noninvasive imaging techniques can provide semi-quantitative data in NAFLD with variable accuracy measures. These techniques include:

**Ultrasonography (US):** it can identify hepatic steatosis and have been advocated as a diagnostic test for NAFLD. US have an overall sensitivity of 89% with 93% specificity [41], but markedly decreased in those with the mild disease when the amount of fat in the liver is less than 30% or in the presence of fibrosis [42]. The ultrasonic feature in NAFLD is the "bright" liver with increased parenchymal echotexture compared with the kidneys and associated with vascular blurring. Areas of focal sparring can give the appearance of metastases. The PPV and NPV of ultrasound in patients with abnormal liver chemistries and other causes of liver disease ruled out are 96% and 19% respectively [14]. On the basis of increasing echogenicity of the liver parenchyma compared with that of the right kidney and decreased visualization of the diaphragm and intrahepatic vessel borders, steatosis in each patient was graded as none (normal US liver structure), mild (slight increase of echogenicity, normal visualization), moderate (diffuse increase of echogenicity, slight impaired visualization), or severe (marked increase of echogenicity, poor or no visualization) [43]. A review conducted by Festi et al. [44] concluded that ultrasonography should be used as a first-line diagnostic tool because of its evaluation of liver steatosis and other abdominal organs [44].

**Computed tomography (CT):** Fatty infiltration of the liver produces a low-density hepatic parenchyma on CT scanning. In a direct comparison of CT with the US, US were found to be more sensitive in detecting fatty change. However, when fatty change is patchy or focal or when a semi-quantitative assessment is required, CT scan and MRI are superior to US [21].

**Magnetic resonance (MRI) imaging:** T1-weighted dual-echo MR imaging and MR spectroscopy are superior to US and CT scans and strongly correlate with histopathologic steatosis assessment. In contrast to US and CT, T1-weighted dual-echo MR imaging and MR spectroscopy are better able to depict differences across steatosis grades. These modalities showed the best diagnostic accuracies [45].

**FibroScan (Transient elastography):** it is another noninvasive method to assess liver fibrosis. This method measures liver stiffness, which was originally designed for the hepatitis C population, but is now being used in the NAFLD population. The FibroScan sends a pulse through the skin, which is circulated through the liver. The velocity of the wave, which is correlated with liver stiffness, is measured by ultrasound. The stiffer the liver the greater the degree of fibrosis. The liver stiffness measurement is used to assess the current stage of liver fibrosis. The cutoffs are 4.85, 7.38, 9.28, 13.33 and 25.34 kPa which represent stages, 0 (no steatosis), 1 (perivenular and/or perisinusoidal fibrosis), 2 (combined pericellular portal fibrosis), 3 (septal fibrosis) and 4

(cirrhosis) [23]. Transient elastography has been validated as a screening tool for advanced fibrosis in NAFLD, with a high NPV of 97% however, it cannot be used to rule out inflammation of the liver [46]. Overall, these noninvasive measurements, including the clinic-laboratory predictor models and imaging techniques to assess NAFLD patients should be used prior to a liver biopsy as they pose minimal risk to the patient. However, liver biopsy should be considered in patients when these noninvasive tests are inconclusive, or the patients have risk factors associated with advanced fibrosis, such as age >50 years, the presence of diabetes, morbid obesity or metabolic syndrome [23].

**Invasive test (Liver biopsy):** Despite the utility of several imaging modalities in the diagnosis of diffuse fatty disorders of the liver, none is sufficiently sensitive to detect fibrosis or cirrhosis. Therefore, liver biopsy is considered as the gold standard for diagnosis in such conditions [21]. More recently, the NASH clinical research network (NASH-CRN) stated that NASH was ''established by the presence of a characteristic pattern of steatosis, inflammation, and hepatocellular ballooning on liver biopsies in the absence of significant alcohol consumption,'' thereby eliminating sinusoidal fibrosis from the definition. A distinction between no NASH, borderline, and definite NASH has also been proposed, underlining current difficulty in establishing a clear distinction between what constitutes NASH and what does not [47]. Despite the availability of different lipid stains such as Sudan III, toluidine blue, and Oil Red O, frozen-section H&E staining remains the most commonly used technique worldwide due to time constraints [7]. Steatosis is typically characterized quantitatively and qualitatively. The quantitative evaluation is based on the percentage of hepatocytes containing cytoplasmic fat inclusions. There are many quantitative systems that grade liver steatosis. Ploeg et al. [48] classified steatosis into mild, moderate and severe if ≥5% and <30%, ≥ 30% and ≤ 60%, or ≥ 60% of hepatocytes contain fat vacuoles within the cytoplasm, respectively [48]. Brunt et al. [49] described the mild, moderate and severe degrees of steatosis if between 5% and 33%, between 33% and 66%, or more than 66%, respectively [49]. In the clinical setting of LT, Ploeg et al. [48] description is sounder when studying graft steatosis. As described before, fatty infiltration is separated qualitatively into two categories, MaS and MiS. MaS are characterized by a single, bulky fat vacuole in hepatocytes, displacing the nucleus to the edge of the cell. In MiS, the cytoplasm of the hepatocytes contains tiny lipid vesicles without nuclear dislocation. MiS are usually encountered in mitochondrial disruption following acute viral, toxin- or drug-induced injury, sepsis, and in some metabolic disorders. However, the quantitative methods provide information only on the total amount of fat, omitting any data on the chemical composition of hepatic lipids, novel objective tools such as measurement of the omega-6 and omega-3 FA levels in liver biopsy samples, may help prediction of the magnitude of reperfusion injury [3]. Importantly, other histopathological features should be carefully assessed in the presence of steatosis including inflammation, fibrosis, and ballooning degeneration. Different systems were developed for discrimination between simple steatosis and NASH, including:

**The NAFLD Activity Score (NAS):** Kleiner et al. [50] proposed the NAS which specifically includes only features of active injury that are potentially reversible in the short term. The score is defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2); thus, ranging from 0 to 8. Fibrosis, which is both less reversible and generally thought to be a result of disease activity, is not included as a component of the activity score. The separation of fibrosis from other features of activity is an accepted paradigm for staging and grading for both NASH and chronic hepatitis. Cases with NAS of 0 to 2 were largely considered not diagnostic of steatohepatitis; on the other hand, most cases with scores of ≥5 were diagnosed as steatohepatitis. Cases with activity scores of 3 and 4 were divided almost evenly between the 3 diagnostic categories [50].

**The histopathological Fatty Liver Inhibition of Progression (FLIP) algorithm:** Bedossa et al. [47] defined NAFLD by the presence of steatosis in ≥5% of hepatocytes, and NASH by the presence, in addition, of hepatocellular ballooning of any degree and lobular inflammatory infiltrates of any amount. Therefore, steatosis was used as the criteria for entry into the algorithm weighted by hepatocellular ballooning and lobular inflammation. In the algorithm tree, all cases with at least grade 1 steatosis were diagnosed NAFLD independently of other criteria, whereas when each of the three features (steatosis, ballooning, lobular inflammation) was classified as at least grade 1, then the lesion was categorized as NASH [47].

**The SAF (steatosis, activity and fibrosis) score:** this score is calculated by the steatosis score (S) that assess the quantities of large or medium-sized lipid droplets, but not foamy microvesicles, from 0 to 3 (S0: <5%; S1: 5%-33%, mild; S2: 34%-66%, moderate; S3: >67%, marked). Activity grade (A, from 0-4) was the unweighted addition of hepatocyte ballooning (0-2) and lobular inflammation (0-2) as described previously. Cases with $A_0$ (A = 0) had no activity, $A_1$ (A = 1), mild activity, $A_2$ (A = 2), moderate activity, $A_3$ (A ≥ 3) severe activity. Stage of fibrosis (F) was assessed using the score described by NASH-CRN as follows; stage 0 ($F_0$) none; stage 1 ($F_1$): 1a or 1b perisinusoidal zone 3 or 1c portal fibrosis, stage 2 ($F_2$): perisinusoidal and periportal fibrosis without bridging, stage 3 ($F_3$): bridging fibrosis and stage 4 ($F_4$): cirrhosis [47].

## Complications

NAFLD, which recently evidenced as a systemic disease, is associated with a significantly higher overall mortality and morbidity compared to the general population, particularly from vascular and liver-related complications. It could be stratified as a major risk factor in developing hypertension, coronary artery diseases, left ventricular diastolic dysfunction and hypertrophy, excessive fat accumulation in the pericardial area, and aortic valve sclerosis, as well as increased carotid artery intimal and medial thickness and prevalence of carotid atherosclerotic plaques [51,52]. NAFLD, and its associated metabolic factors, also have a negative impact on other liver diseases. With hepatitis C, obesity, IR, and steatosis are predictors of progression to advanced liver fibrosis and are also associated with a lower sustained viral response rates to interferon-based therapies. A proportion of cases of HCC have also been attributed to NAFLD but the overall contribution of metabolic risk factors to HCC is an unresolved issue until now. There is also evidence that the outcome of the alcoholic liver disease is worse in the obese and those with MetS. Fatty liver disease attributable to metabolic

risk factors is increasingly noted in those infected with hepatitis B virus (HBV), but whether this has any implications for disease outcomes remains unclear [22]. In addition, NAFLD could be strongly associated with obstructive sleep apnea syndrome [53] and chronic kidney diseases [20]. NAFLD is also associated with colorectal cancer, metabolic bone disease (vitamin D deficiency and osteoporosis) and rare metabolic diseases (lipodystrophies and glycogen storage diseases) [54]. Despite its high prevalence and clinical significance, knowledge and awareness of NAFLD are poor, not only among the general population but also among general practitioners [52].

## Impact of steatosis on LT

Nowadays, NAFLD has a great concern in regards to LT; from becoming the second most common cause of transplant candidates due to its high worldwide prevalence and from the other hand, the urgent need for increasing the donor pool by using marginal donors with graft steatosis, especially in living donor LT (LDLT) [55]. The assessment of the donor liver fat is a difficult task for the transplant team. An initial evaluation, based on visual inspection and palpation, is first done during graft procurement. However, criteria such as color and texture of the graft depend only on the experience of the explanting surgeon and thus remain subjective. Despite this general agreement, a European survey showed that liver biopsy at the time of procurement for LT is rarely performed. However, several transplant programs consider a liver biopsy as mandatory before discarding a potential liver graft [3]. It was well recognized that the implantation of a donor's liver with severe fatty infiltration (>60%) is frequently associated with an increased incidence of postoperative complications. In the past, grafts with ≥30% fatty infiltration are not acceptable for transplantation [56]. Furthermore, even the use of donor livers with less fatty infiltration (<30%) has been reported to result in a bad outcome after LT. Conversely, some authors reported excellent results after transplantation using severely steatotic liver grafts [57-60]. Noteworthy, all recipients disclosed a low MELD score and short ischemia times [3]. However, the critical shortage of organ donors, especially in countries such as middle- and far-eastern countries, where cadaveric donors are almost less available, often mandates the use of such grafts with different degree of steatosis [61].

It has been postulated that steatotic livers are more sensitive and less tolerant to ischemia-reperfusion (I/R) injury, leading to worse clinical outcomes. If severe, I/R injury can lead to liver failure and death [62]. The exact etiology for the increased susceptibility of steatotic livers to I/R injury is not well-known. Several different hypotheses have been proposed to explain the increased susceptibility, including impaired hepatic microcirculation and mitochondrial dysfunction [62]. MaS is associated with accumulation of intracellular lipid, increasing hepatocyte volume leading to obstruction of the adjacent sinusoid space, and increasing the vascular resistance in the hepatic microcirculation [63,64]. This may potentially impair oxygen and nutrient delivery following reperfusion to an already susceptible organ. The increased lipid levels in steatotic livers may also lead to mitochondrial dysfunction through the formation of ROS [65]. Mitochondrial energy supply is fundamental to cellular viability, and the interruption of key mitochondrial processes disrupts normal cellular bioenergetics, impairs cellular function,

and subsequently leads to cell death by either necrosis or apoptosis [66]. However, MiS is frequently regarded as a mild and reversible condition and is usually disregarded as a risk factor for graft dysfunction, it has been established that graft MiS is an independent factor influencing the early graft function (EGD) [61]. Cieslak et al. [67] observed that a 12% increase in MiS was equivalent to a 50% increased risk for graft dysfunction [67]. MiS may develop because of severe and prolonged inhibition of mitochondrial β-oxidation of FAs during preservation-reperfusion injury [68]. MiS increases progressively and evolves in the liver during cold storage and worsens significantly before reperfusion in the recipient, whereas the degree of MaS remains constant [69]. On the contrary and despite the graft dysfunction risk, the use of livers exhibiting MiS does not decrease graft or patient survival rates [61].

On the other hand, the composition of hepatic lipids and particularly thromboxane $A_2$ levels are considered the principal modulators of I/R injury in the steatotic livers, while the size and morphology of lipid droplets are insignificant [70]. Other potential mechanisms that have been proposed include Kupffer cell dysfunction [71], more lipid peroxidation [72,73], more exuberant pro-inflammatory responses, including increased release of pro-inflammatory mediators such as TNF-α [74], and impaired leukocyte adhesion [75]. Therefore, it is likely that the increased vulnerability of the steatotic livers to more I/R injury are multifactorial, and further experimental studies are required to elucidate the underlying mechanisms [62]. Various types of I/R injury are implemented during both the donors and recipients' hepatic surgery, including warm ischemia in hepatic resection when hepatic inflow is temporarily occluded and cold and re-warming I/R injury when a donor's liver is re-perfused during LT [76].

## Warm ischemia time (WIT)

Whereas sinusoidal endothelial cells are the cells in the liver most susceptible to cold ischemia, hepatocytes are injured more readily by warm ischemia [69]. Experimentally, a WIT of 30 minutes plus 4-5 hours of cold preservation causes primary non-function (PNF) and the longer the WIT, the greater the cumulative release of ALT, AST, and lactate dehydrogenase in the perfusion solution. On the other hand, a shorter WIT increases the graft tolerance to cold storage with better outcomes [77]. The longer the WIT results in Hepatocyte vacuolation, sinusoidal congestion, and the appearance and aggravation of focal hepatocyte drop-out [78]. Simultaneously, the recipient will suffer from acute hemodynamic changes and an eliminated hepatic metabolism, as well as the cytokine, metabolite, and other toxin accumulation in the splanchnic system [79].

## Cold ischemia time (CIT)

This is defined as the time from cardiac arrest or cross-clamping and the initiation of in-situ cold flush in the donor to the removal of the organ from cold storage for implantation into the recipient [80]. Cold ischemia or cold storage is another factor that interferes with the incidence of graft dysfunction. The cutoff for CIT is not uniform; some studies reported that a CIT >10 hours is associated with poor outcomes after LT [81,82], and others have reported that CIT can be longer than 18 hours [48,83]. Decreasing temperatures

can induce cell injury through the release of proteases, leading to alterations in calcium homeostasis, modifications of the cytoskeleton, and local tissue destruction [84]. Cold preservation also induces several changes in the hepatocytes, such as cell swelling, intracellular acidosis, loss of mitochondrial function, and decreased ATP [76]. An analysis of donor and transplant-related factors using united network of organ sharing (UNOS)/ organ procurement and transplantation network registry data has shown that for every hour of CIT above 8 hours, the adjusted risk of graft failure increases by 2%. This has been confirmed in the continental European liver transplant experience; every 15 minute increase in CIT increases 1-year graft failure risk by 0.9%. In addition, the risk of developing non-anastomotic biliary strictures significantly increased with every hour increase in CIT, as biliary epithelium may be particularly sensitive to ischemia-induced oxidative stress after reperfusion [80]. In a recent systematic review on experimental studies, increased ischemia times, as well as increased severity of steatosis, was shown to correlate with a decrease in survival. Histologically, ≥30% MaS was associated with increased intraparenchymal hemorrhage, sinusoidal congestion, and necrosis. ALT, AST, prothrombin time, and bilirubin were increased in subjects with ≥30% MaS or mixed hepatic steatosis. Similar degrees of histological injury and levels of transaminases in MiS were noticed [62].

Moreover, almost all LT recipients are maintained on calcineurin inhibitors, which are associated with increased generation of ROS, more mitochondrial dysfunction, and lipid peroxidation. This may have important implications for the natural history of recurrence of NAFLD following LT. Whether calcineurin inhibitor dosing should be minimized in patients with recurrence of NASH is not known, but a theoretical possibility for such an approach to recurrence of NASH following LT exists [85]. The incidence of PNF after LT is 1.8% in normal grafts and 5.1% in those exhibiting greater than 30% steatosis. Therefore, steatosis ≥ 30% in a donor's liver is considered unacceptable. However, other studies demonstrated that moderate to severe steatosis is acceptable when other risk factors are excluded [76]. A large study using UNOS data showed that the presence of ≥30 % of MaS was an independent risk factor for impaired 1-year graft survival [86]. Interestingly, two Italian groups demonstrated that transplanting livers with moderate to severe MaS is an independent risk factor for the development of biliary complications after LT [87,88]. Perhaps, an impaired microcirculation at the sinusoidal level could be responsible for bile duct ischemic damage, resulting in a higher risk of biliary strictures [3].

## Treatment

In spite of the growing understanding of the global epidemic of NAFLD, there is no definite pharmacotherapy available. Only proposed benefits according to available evidence as lifestyle modification with regular exercise and dietary changes or bariatric surgery in morbidly obese patients are available. Other suggested means of pharmacological therapy as anti-inflammatory, insulin sensitizers or antioxidant agents have shown promising results, there is some concern for adverse effects and long-term safety [89].

**Long-term strategies:** These strategies are applicable for the treatment of hepatic steatosis in general population aiming for decreasing the impact of NAFLD on the general health. In addition, sticking to the appropriate strategies can increase the donor pool through providing healthier persons with healthier liver grafts. They are of greater concerns in LDLT programs.

**Non-pharmacologic treatment (lifestyle modification):** These interventions, aimed at correcting unhealthy lifestyles, simultaneously treat all the clinical manifestations of the MetS and are an effective treatment option in NAFLD. Lifestyle changes include weight loss, dietary changes, reduction of sedentarily and physical exercise. They are best accomplished through cognitive-behavioral therapy, which should always be implemented as a first-line therapeutic option, regardless of the severity NAFLD/ NASH [11]. European association for the study of the liver (EASL), diabetes (EASD), and obesity (EASO) clinical practice guidelines for the management of NAFLD proposed pragmatic approaches for lifestyle modification [54], including:

Energy restriction, in the form of 500-1000 kcal energy defect, to induce a weight loss of 500-1000g/week with 7-10% total weight loss target in obese patients. In addition, Long-term maintenance approach, combining physical activity according to the principles of cognitive-behavioral treatment should be achieved.

Dietary modification with adherence to the Mediterranean diet has been reported to reduce liver fat, including low-to-moderate fat and moderate-to-high carbohydrate intake, low-carbohydrate ketogenic diet or high-protein diets, and avoid fructose-containing beverages and foods [90,91]. Saturated fatty acids should be limited while increased consumption of food rich in mono- and polyunsaturated fatty acids (Omega-3) is advised [92].

Exercise/physical activity; 150-200 min/week of moderate-intensity aerobic physical activities in 3-5 sessions are generally preferred (brisk walking or stationary cycling). Resistance training is also effective and promotes musculoskeletal fitness, with effects on metabolic risk factors. Avoiding of a high rate of inactivity-promoting fatigue and daytime sleepiness as they reduce compliance with exercises.

**Pharmacologic treatment:** Drug therapy should be indicated for progressive NASH (bridging fibrosis and cirrhosis) but also for early-stage NASH with increased risk of fibrosis progression (age >50 years, diabetes, MetS, increased ALT) or active NASH with high necro-inflammatory activity. Whereas no drug has currently been tested in phase III trials and is approved for NASH by regulatory agencies, no specific therapy can be firmly recommended. Safety and tolerability are essential prerequisites for drug treatment, because of NASH-associated co-morbidities and polypharmacy, a potential source of drug-drug interactions [54].

**Insulin sensitizers:** Regarding metformin, there are scarce evidences for its histological efficacy in NASH. Thiazolidinediones have insulin-sensitizing effects. The pioglitazone vs. vitamin E vs. placebo for non-alcoholic steatohepatitis (PIVENS) trial for 2 years in patients without overt diabetes demonstrated that Pioglitazone improved all histological features (except for fibrosis) and achieved resolution of NASH more often than placebo [93]. These histological benefits occurred together with ALT improvement and partial correction of IR. Despite the safety and tolerability

profile, pioglitazone can be used for selected patients with NASH, particularly in T2DM where the drug has a registered use and caution should be taken in regard to its cardiovascular risks [54].

**Anti-obesity and lipid lowering agents:** Drug therapies of obesity (orlistat and sibutramine) have not shown a direct beneficial effect on the liver independent of the beneficial effect of the weight loss. However, these drugs may enhance the beneficial effects of behavioral therapy [11]. Lipid lowering agents (statins, fibrates, and omega-3 FAs) use in NAFLD are safe, with no increased risk of hepatotoxicity, and may even reduce aminotransferases with a possible role in delaying progression from NAFL to NASH [54].

**Antioxidant and cytoprotective agents:** in the PIVENS trial, vitamin E (800 IU/day) improved steatosis, inflammation and ballooning and induced resolution of NASH in 36% of patients vs. 21% in the placebo arm [93]. In addition, vitamin E may have a greater benefit when combined with other agents as ursodeoxycholic acid. Contrarily, concerns about vitamin E long-term safety exist as it may be associated with increased risk for cardiovascular events and prostate cancer in older patients > 50 years [89]. Obeticholic acid, which is a synthetic farnesoid X receptor agonist, decreased IR in T2DM and improved all NASH lesions and fibrosis in non-cirrhotic NASH patients after 72-weeks of treatment [94].

**Other Trials:** Promising novel agents with anti-inflammatory (Pentoxifylline) and antifibrotic agents that may improve liver enzymes and histology are also being tested in late-phase RCTs in NASH [54]. Coffee is rich in sources of bioactive phytochemicals including methylxanthines (caffeine), amino acids, phenolic acids, and polyphenols which may protect against liver disease [95]. In a recent meta-analysis of 16 studies comprising 3153 cases of hepatocellular carcinoma, the risk of HCC was found to be reduced by 40% with coffee intake compared to no coffee [96]. Emerging evidence, in animal studies, has shown that inhibiting renin angiotensin aldosterone system pathway decreases hepatic stellate cell activity, which in turn prevents fibrosis. Therefore, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers may be useful in treating NAFL/NASH as they may lead to decreased fibrosis. However, not many studies have been done in humans with NAFLD to assess the effectiveness of ACE inhibitors or angiotensin receptor blockers [89].

## Surgical treatment

**Venesection:** Hepatic iron accumulation is associated with IR, and iron depletion improves IR. In NAFLD, high ferritin levels are common. In these patients, a phlebotomy program to reduce iron stores to near iron deficiency improved the NAS, without worsening the fibrosis, but more data are needed [54].

**Bariatric surgery:** Although bariatric surgery is not specifically indicated in NAFLD, it may be useful in morbidly obese patients. A systematic review with meta-analysis has reported an improved liver histology following gastric bypass and laparoscopic banding [97]. Moreover, a recent cohort study confirmed that bariatric surgery-associated weight loss cleared NASH in 85% of patients and improved fibrosis in 34%, although the possible benefits should be balanced against perioperative complications [98].

**Short-term strategies:** These strategies are reserved to the short perioperative period aiming for improving the outcomes after LT of a steatotic liver graft. They could be also useful in deceased donors. These strategies include ischemic preconditioning (IP) and liver defatting approaches.

**Ischemic preconditioning:** Several techniques have been developed as IP to improve the detrimental effects of I/R injury on marginal donor grafts, in particular, steatotic ones. Clinical studies have shown decreasing hepatocyte swelling and enzyme release after LT as well as less autophagy and possibly less rejection in steatotic livers exposed to IP [99,100]. A recent systematic review of experimental IP in steatotic livers [101] concluded that animals with preconditioned livers had improved outcomes, including increased survival, less histologic injury and improved liver function [102]. Several approaches for the IP were examined experimentally, including:

**Pharmacological preconditioning:** such as with anaesthetics, alpha- and beta-adrenergic blockers, antioxidants, and/or steroids have been suggested as potential hepatoprotective strategies for steatotic livers, but few have been reported in humans. Experimentally, some of these agents have been added to the cold storage preservation solution and found to reduce I/R injury-related markers in perfused steatotic livers. Beck-Schimmer et al. [103] hypothesized; in an RCT that sevoflurane anaesthesia would reduce postoperative liver injury and promote beneficial effects from an inducible nitric oxide pathway. In addition, it can significantly reduce the postoperative aminotransferase levels, in particularly in the steatotic livers [103]. Carvedilol, a beta- and alpha-adrenergic blocker to treat ischemic heart, was found also to reduce hepatic death markers, vascular resistance and ROS, as well as increased bile production and hepatic ATP levels post-reperfusion [104]. Moreover, intravenously administered the antioxidant glutathione or its precursor N-acetylcysteine shortly prior to reperfusion recovered the normal intracellular glutathione level and significantly reduced lipid peroxidation, I/R injury markers and hepatic death [73,105]. It is worth noting that none of these studies investigated the possibility of combining several pharmacological agents at once; therefore, it is possible that doing so would lead to a more dramatic decrease in sensitivity to I/R injury [102,106].

**Surgical preconditioning:** in which, the major blood vessels to the liver are clamped momentarily (Pringle manoeuvre). This has been shown to reduce lipid peroxidation, hepatic microcirculation failure, and neutrophil accumulation after subsequent I/R injury when applied to steatotic livers. IP has been shown to protect human livers against a subsequent period of ischemia in patients undergoing hemi hepatectomy as in live donors. The analysis of a subgroup of patients with mild-to-moderate steatosis presented reduced serum levels of liver damage markers (aminotransferases) when preconditioned [107].

**Heat shock preconditioning:** Another experimental approach to alleviate I/R injury is heat shock preconditioning, in which fatty liver donor rats were exposed to brief whole-body hyperthermia (10 minutes at 42.5 degrees Celsius) and allowed to recover [108]. This approach has been shown to preserve microcirculatory parameters (sinusoidal perfusion rate, sinusoidal diameter,

minimal leukocyte-endothelial adhesion) and prevent microvascular perfusion failure after surgically induced I/R in obese rats with steatotic livers [109]. Interestingly, there was a time window of efficacy ranging from 6h to 24 h after the heat shock. The protective mechanisms of heat shock preconditioning are not fully understood, but the utility of this approach warrants further studies that would enable a rational design of heat shock preconditioning regimens to improve effectiveness and practicality [106].

**Liver defatting:** Multiple studies described what is called "liver defatting", which have been performed in attempts to optimize the steatotic liver grafts prior to LT [106,110].

**Combined measures (Dietary, exercise and drug therapy):** A short-term intensive treatment with protein-rich diet (1000 kcal/day), exercise (600 kcal/day), and bezafibrate (400 mg/day) for 2-8 weeks in a small trial of 11 donors led to threefold decrease in MaS and reduced body weight and BMI. Alternatively, the consumption of omega-3 FAs for 1 month was used to decrease MaS in three candidates for LDLT who had biopsy-proven hepatic MaS >30% prior to donation [110]. In addition, administration of omega-3 FAs enhances the hepatic regeneration and functional recovery in the living donors [111].

**Machine perfusion:** The use of machine-based liver perfusion systems may also offer benefits and perhaps a way to test the function of the organ prior to implantation. The new preservation concepts include in situ warm oxygenated perfusion before the organ harvest (normothermic concept) [112] or hypothermic machine perfusion after organ procurement and transport to the transplantation centre (hypothermic concept) [113]. While the perfusion system may enable to determine the potential viability of the graft, wide application of perfusion system in marginal graft such as severe steatotic livers will need long-term data after LT [3].

**Optimizing the perfusion solution:** ultimately, the goal of liver defatting is to rapidly decrease the proportion of MaS hepatocytes, while maintaining high viability and functionality. Steatosis is the result of an imbalance between triglyceride synthesis and breakdown processes in hepatocytes. Therefore, to achieve significant defatting, the protocol of choice should shift this balance towards more efficient breakdown (lipolysis) and excretion of related by-products, as well as minimizing the synthesis [114,115]. The pathways responsible for lipid metabolism are well known; however, there is much work to be done on how best to modulate this metabolism using cocktails of agents in order to achieve rapid defatting without adversely affecting viability and other critical liver functions. As illustrated in figure 13, targeting multiple routes simultaneously may provide the most effective approach, but the rational design of defatting protocols will require a better understanding of the potential interactions among the relevant metabolic pathways in steatotic hepatocytes. This would be greatly facilitated if a suitable cell culture model of hepatic steatosis were available to perform both screening and mechanistic studies in an efficient manner, prior to testing in actual livers [106]. While many challenges need to be overcome, liver defatting is a potentially promising approach to reduce the sensitivity of steatotic livers to I/R injury, and a new modality that may enable the successful recovery of a large number of livers that would otherwise be discarded [106].

## Conclusion

Using the marginal donors especially those with NAFLD will markedly increase the donor pool for LT. Consequently, it will shorten the recipient deaths on the lengthy waiting list for LT. Several approaches, including long-term and short-term measures were identified for the management of NAFLD.

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## Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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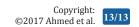
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