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Research Article

On the operational definition of fatty liver

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We discuss the present operational definition of fatty liver and its shortcomings.

On the diagnosis of fatty liver

Fatty liver or liver steatosis is presently defined as an intrahepatic triglyceride content > 5%[1]. A strict diagnosis of fatty liver requires a liver biopsy, which is presently used to classify steatosis as light (> 5% to ≤ 33%), moderate (> 33% to ≤ 66%), or severe (> 66%)[2].

Although a liver biopsy is the reference method for the diagnosis of fatty liver, it is an imperfect gold standard because of sampling error. A needle biopsy is, in fact, approximately 1/20000th of the weight of the liver[2], and even if the most common intrahepatic fat pattern is the diffuse form, there are also heterogeneous, focal, multinodular, perilesional, perivascular, subcapsular, and lobar forms[3].

In addition, a liver biopsy is an invasive procedure and cannot be employed outside tertiary care centers, and even in these centers, it is nearly always performed on selected patients[4]. Importantly, a liver biopsy is also the reference method for the diagnosis of both intrahepatic inflammation and liver fibrosis, as we shall see below.

Proton magnetic resonance spectroscopy or quantitative fat/water selective magnetic resonance imaging (MRI) can detect an intrahepatic triglyceride content > 5% (corresponding to a proton density fat fraction > 5.6%) and could thus be used to diagnose fatty liver[1]. However, proton magnetic resonance spectroscopy is available only in a few centers and mostly for research purposes. In addition, there is not a linear association between 5% fatty hepatocytes and 5% of the whole liver weight fat content[5].

Liver ultrasonography is the imaging method most commonly employed to diagnose fatty liver in the general population and in clinical practice[1]. When compared with liver biopsy, liver ultrasonography has a true positive fraction of 85% (95% confidence interval 80 to 89%), a true negative fraction of 94% (88 to 97%), a positive diagnostic likelihood ratio of 13.3 (6.4 to 27.6), and a negative diagnostic likelihood ratio of 0.16 (0.12 to 0.22) for the detection of an intrahepatic triglyceride content > 33%[6]. Degrees of fatty liver < 33% may go undetected by liver ultrasonography, especially when < 10%[6].

A further option to study fatty liver in the general population is the use of surrogate markers[1]. One of such markers, the fatty liver index (FLI), which we developed against liver ultrasonography in the general population of the Dionysos Nutrition & Liver Study, is based on four common measurements, i.e. body mass index, waist circumference, gamma-glutamyl-transferase, and triglycerides[7]. FLI has gained increasing attention in the last decade because of its association with prevalent and incident cardio-metabolic disease. More importantly, for its ability to serve as a surrogate marker of fatty liver, FLI has been successfully cross-validated in external populations[8]. Although the cut-points of FLI proposed by us to rule in and rule out fatty liver in the general population of Campogalliano (Modena, Italy), are commonly employed in the literature[7], it is much better to recalibrate FLI in (a subsample of) the population of interest, as we advised in the original paper and as it was recently done by some researchers in the Shanxi Province of China[9][10].

Liver biopsy remains the only method able to distinguish intrahepatic inflammation from fibrosis, whereas liver elastometry and multiparametric magnetic resonance provide an indirect and combined measure of the two parameters, represented by two distinct vectors that are not yet distinguishable one from the other in both US and MRI-based imaging techniques[11][12][13][14].

On the dichotomization of fatty liver into alcoholic and non-alcoholic fatty liver

Fatty liver is presently classified into non-alcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AFLD). The European Association for the Study of the Liver (EASL) presently suggests that, after (at least) hepatitis B infection, hepatitis C infection, and steatogenic drugs have been ruled out, NAFLD should be diagnosed when ethanol intake is ≤ 20 g/day in women and ≤ 30 g/day in men (**Figure 1**).
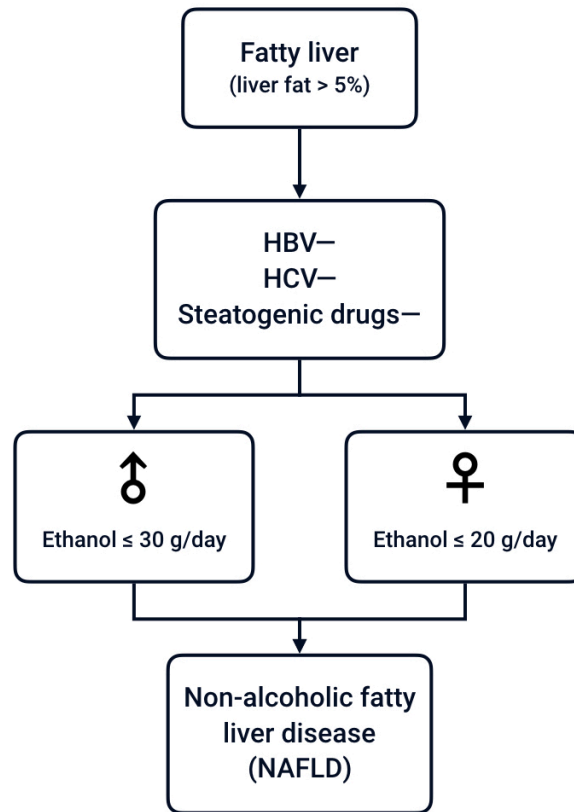
Figure 1 - The diagnosis of non-alcoholic fatty liver disease. Abbreviations: HBV = hepatitis B virus; HCV = hepatitis C virus; NAFLD = non-alcoholic fatty liver disease.

The NAFLD *vs.* AFLD dichotomization has several limitations.

Besides the loss of information inherent to any dichotomization[15], its most important criticism is that a simple cut-off of low daily alcohol intake cannot really distinguish alcohol- and non-alcohol-induced liver damage. The toxicity from low alcohol intake is in fact determined by genetic, alimentary, and lifestyle factors, and some individuals may well have alcohol-induced fatty liver while drinking alcohol below the proposed cut-point[16]. On the other hand, the gut microbiota may produce alcohol contributing to liver damage[17].

Another problem of the NAFLD *vs.* AFLD dichotomization is that it requires the use of an instrument accurate enough to detect small differences in ethanol intake[18]. Even the 7-day weighed food record that we employed in the Dionysos Nutrition & Liver study may not be accurate enough to detect small

differences in ethanol intake[19]. To gain some understanding of the problems caused by dichotomization and/or measurement error, **Figure 2** simulates the prevalence of NAFLD obtained by varying the cut-point of ethanol intake from 0 to 40 g/day by steps of 5 g/day in the general population investigated by the Dionysos Nutrition & Liver Study[19].
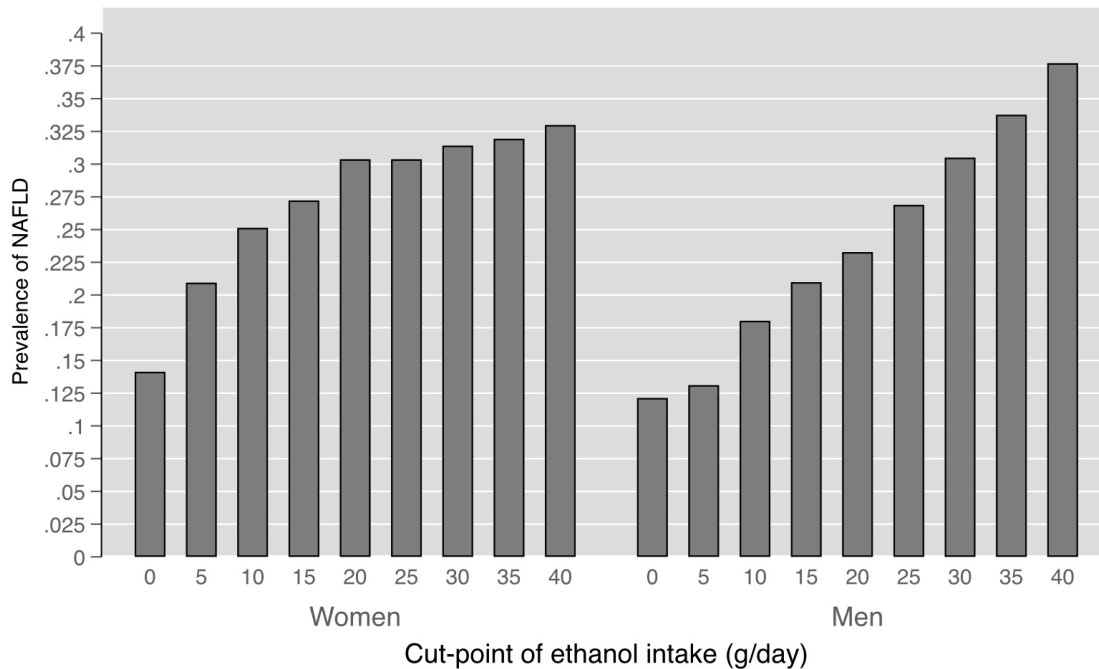
Recalculated from Bedogni G. Hepatology. 2005;42:44
Ethanol intake obtained from a 7-day weighted-food diary

Figure 2 – Simulation of NAFLD prevalence obtained by varying the cut-point of ethanol intake from 0 to 40 g/day by steps of 5 g/day in the general population of the Dionysos Nutrition & Liver Study.

At the time of the Dionysos Nutrition & Liver Study, the cut-point of ethanol intake suggested by EASL to separate NAFLD from AFLD was 20 g/day for both men and women. If we adopt the current EASL cut-point of 30 g/day, the prevalence of NAFLD in the men of the Dionysos Nutrition & Liver Study would be substantially higher. However, the (lack of) association between fatty liver and continuous ethanol intake that we have reported in the same population would obviously not change[7]. This alerts to a clear problem with dichotomization[15].

On the diagnosis of non-alcoholic steatohepatitis

NAFLD is presently classified into uncomplicated non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH)[1] (**Figure 3**).
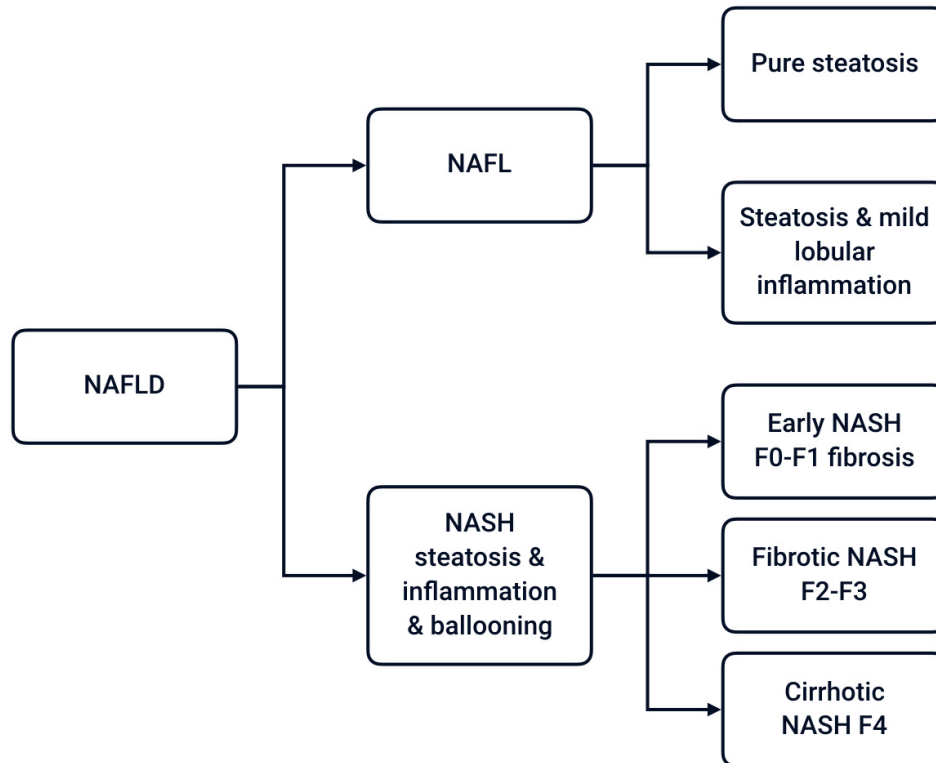

Figure 3 - Classification of non-alcoholic [fatty liver] disease into non-alcoholic fatty liver and non-alcoholic steatohepatitis. Abbreviations: NAFLD = non-alcoholic fatty liver disease; NAFL = non-alcoholic fatty liver; NASH = non-alcoholic steatohepatitis.

NASH is presently defined as the presence of steatosis, inflammation, and ballooning at liver biopsy in a patient with NAFLD[1]. Liver biopsy cannot distinguish alcohol- from non-alcohol-induced steatohepatitis, and the current separation of NASH and alcoholic steatohepatitis is based on the dichotomization of alcohol intake with the limitations described above. We refer the reader to a recent review for details and controversies on the histopathology of NASH[2].

Here, we are interested only in the methodological consequences of the fact that liver biopsy is central to the diagnosis of steatohepatitis, independently of its etiology. This requirement implies that

NASH cannot be diagnosed in the general population. Even if accurate indirect markers of NASH were available, which they are not[1], they would have been developed in tertiary care centers where liver biopsy can be performed, most often on selected subsets of patients[4]. Accordingly, their diagnostic performance is likely to be completely different in the general population because of the different case-mix of individuals[20]. Thus, the available estimates of the prevalence of NASH in the general population should be taken with caution, especially when they are based on indirect and demonstrably unreliable markers of NASH such as altered liver enzymes in the presence of fatty liver after the exclusion of some of the known risk factors for this latter[21][22].

On the diagnosis of liver fibrosis

The clinical relevance of NASH stems from its potential association with liver fibrosis[1]. **Figure 4** shows the time to the progression of one stage of fibrosis for NAFL *vs.* NASH according to a recent metanalysis of longitudinal studies performed in tertiary care centers[23].

|	N	Studies	Fibrosis progression (years to progress 1 stage, mean [95%CI])
NAFLD	366	11	7.7 [5.5 to 14.8]
NAFL	133	6	14.3 [9.1 to 50.0]
NASH	116	7	7.1 [4.8 to 14.3]

Figure 4 - Time to the progression of one stage of fibrosis in non-alcoholic fatty liver disease.

Abbreviations: NAFLD = non-alcoholic fatty liver disease; NAFL = non-alcoholic fatty liver; NASH = non-alcoholic steatohepatitis.

As stated above, only liver biopsy can distinguish inflammation from fibrosis and provide an accurate grading and staging of these two histologic features. Many surrogate markers and an increasing number of elastographic techniques are nonetheless available to provide a non-invasive measure of the combined vectors of inflammation and fibrosis without being able to discriminate among them[13][24][25]. As for NASH, these methods were calibrated against liver biopsy in tertiary care centers and

most often on selected sets of patients so that their diagnostic performance is likely to be completely different in the general population because of the different case-mix of individuals[20]. Thus, the available estimates of the prevalence of NASH-associated liver fibrosis in the general population should be taken with caution[21][22].

Following the Metavir system, liver fibrosis is currently classified in 5 stages: F0, F1, F2, F3, and F4, where F0 means absence of fibrosis and F4 is synonymous with liver cirrhosis[2]. Instead of the common practice of dichotomizing fibrosis, much can be gained by treating it as an ordinal outcome and by developing predictive models giving cumulative probabilities[26]. As an example of this application, **Figure 5** gives the cumulative probability of the F0-F1 *vs.* F2-F3 *vs.* F4 stages of fibrosis as measured by Fibroscan among a series of Italian HCV patients[27]. According to the employed ordinal generalized logistic model, a patient with a liver stiffness of 12 kPa would have a 2% (95%CI 0 to 5%) probability of F0-F1, an 85% (72 to 99%) probability of F2-F3, and a 13% (1 to 25%) probability of F4 fibrosis.
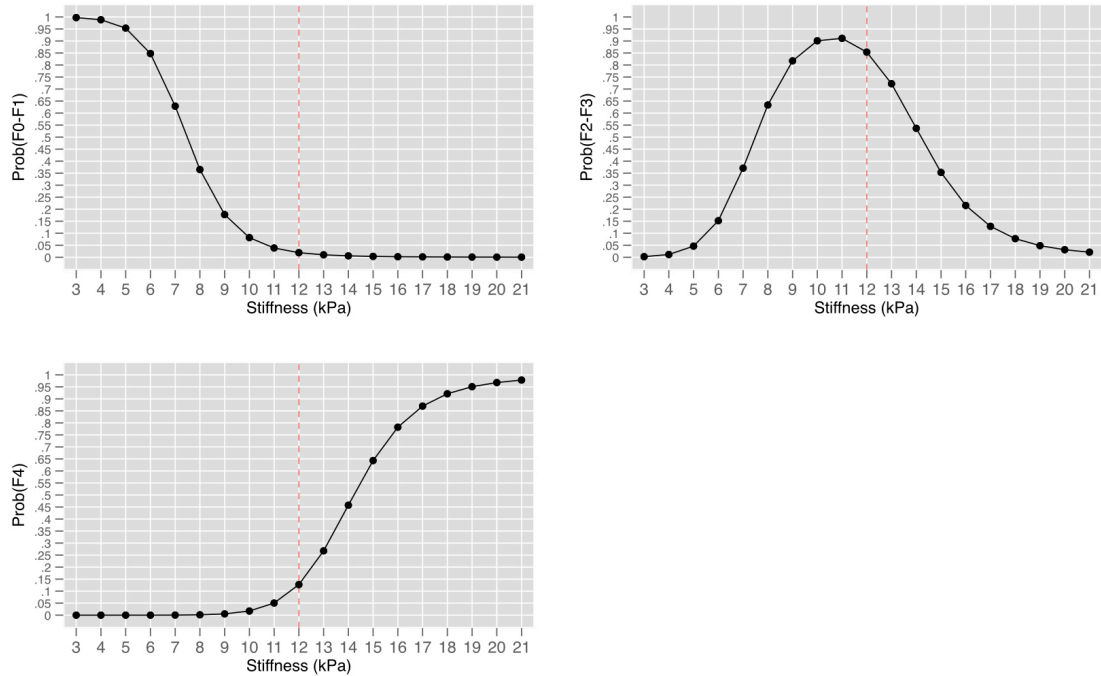
Recalculated from Arena et al. Hepatology 2013;58:65

Figure 5 - Cumulative probability of the F0-F1 *vs.* F2-F3 and F4 stages of liver fibrosis as predicted by an ordinal generalized logistic regression model (see reference [27] for details). According to this model, a patient with a liver stiffness of 12 kPa (red line) would have a 2% (95%CI 0 to 5%) probability of F0-F1, an 85% (72 to 99%) probability of F2-F3, and a 13% probability (1 to 25%) of F4 fibrosis. Abbreviations: Prob. = probability.

Conclusion

We have described some limitations of the current operational definition of <u>fatty liver</u> and <u>NAFLD</u>. They can be summarized as follows:

1. <u>Fatty liver</u> is presently defined as an intrahepatic triglyceride content > 5%, but the most commonly used diagnostic technique, i.e., liver ultrasonography, cannot reliably detect values of <u>fatty liver</u> < 10% and is much better at detecting values > 33%. This is likely to cause an underestimation of fatty liver;

2. Instead of dichotomizing <u>fatty liver</u> into <u>NALFD</u> and AFLD, it would be more useful if epidemiological studies could evaluate the association between <u>fatty liver</u> and continuous or

ordinal ethanol intake with the possibility of studying its independent contribution and interaction with other factors, e.g., body mass index;

3. The present estimates of the burden of <u>NASH</u> and its complications (fibrosis, cirrhosis, hepatocarcinoma) in the general population are based on indirect assumptions and may not be reliable. The real burden of NASH and NASH-associated fibrosis in the general population is likely to remain unknown.

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