Definition of Healthy Ranges for Alanine Aminotransferase Levels: A 2021 Update

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The changing epidemiology of liver disease, and modifications in the recommended analytical methodology call for a re-evaluation of the upper reference limits (URLs) of alanine aminotransferase (ALT) levels. Using the same approach consolidated 20 years ago to define the healthy population, we defined the URL for the newly recommended International Federation of Clinical Chemistry (IFCC) standardized test. In a cross-sectional study, we examined 21,296 apparently healthy blood donors (age 18-65 years) and calculated the sex-specific URL by the 95th percentile in individuals without risk factors for liver disease. These were tested for the ability to predict liver damage in a subset of 745 participants with dysmetabolism, in an independent cohort of 977 unselected donors, and in 899 patients with chronic liver disease. ALT levels were measured by the IFCC test. Male sex, body mass index, glucose, lipids, ferritin, hypertension, and younger age were independent ALT predictors (*P* < 0.001). Updated URLs were identified at 42/30 U/L in males/females, approximately 30% lower than those currently recommended by the IFCC. Due to improved sensitivity, they conferred the ability to detect steatosis and significant fibrosis in individuals with dysmetabolism (odds ratio [OR] = 2.31, range 1.40-3.80, *P* = 0.001; and OR = 3.35, range 1.19-9.42, *P* = 0.021; respectively), although with a limited accuracy, and significant fibrosis in unselected donors (OR = 2.32, 1.02-5.31, *P* = 0.045). Updated URLs had a moderate to high accuracy to discriminate liver conditions (area under the receiver operating characteristic curve = 0.81, range 0.78-0.91). *Conclusion:* Updated URLs by the IFCC method were lower than those calculated in initial studies, but higher than those in use with the recommended old, nonstandardized method, and were able to better predict liver disease. The limited awareness that different techniques are still in use should be regarded as a possible source of medical errors. (*Hepatology Communications* 2021;0:1-9).

S erum alanine aminotransferase (ALT) is one of the most commonly requested biochemical tests in clinical practice and is an integral part of the diagnosis and management of patients with liver disease. Moreover, elevated ALT levels predict mortality independent of liver disease in the general population. The most likely interpretation of this association is that nonalcoholic fatty liver disease (NAFLD), which is most frequently related to metabolic dysfunction (MAFLD), is now the leading cause of liver damage.[1-4]

In 2002, our group showed that the upper reference limits (URLs) for ALT used at that time were based on reference populations, which included a substantial proportion of individuals with subclinical liver disease, thereby limiting the sensitivity of ALT URLs to detect

liver injury.[5] Thus, we proposed criteria for the definition of the reference population at low risk of liver disease by excluding carriers of viral hepatitis and individuals with metabolic syndrome and set the healthy thresholds for ALT at 30/19 U/L in males/females (M/F), respectively. The validity of this approach has been confirmed by studies focusing on the prediction of overall mortality[6] and liver-related events in the general population[7], as well in individuals with biopsy-proven normal liver.[8] Furthermore, the updated ALT thresholds have been widely adopted in clinical studies and guidelines.

To reduce variability of laboratory results, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) promoted a standardization of the analytical methods in clinical enzymology, including that for ALT determination.[9,10] The standardized method presents some technical differences when compared with those previously in use, the main one being a higher reaction temperature (37°C instead of 30°C), which increases enzyme activity and results in higher ALT values.[9] In addition, the upper thresholds recommended by this standardized method (59/41 U/L in M/F, respectively) were determined in a relatively small-size reference cohort, which consisted of individuals with risk factors for NAFLD.[11]

Thus, the modifications of the reference analytical methodology, and the changing epidemiology of the major causes of liver injury, with the rising role of NAFLD[1-3,12,13] call for a re-evaluation of the healthy ranges for ALT. The aim of this study was to examine the determinants and distribution of ALT by the IFCC method, to update the healthy ranges in a large cohort of apparently healthy individuals (ALT-URL-IFCC), and to validate their accuracy for detecting liver damage, in particular related to NAFLD.[14]

Patients and Methods

STUDY COHORTS

We considered 21,296 apparently healthy individuals, who presented for blood donation from May 2017 to April 2018 at the Transfusion Medicine and Hematology unit of the Fondazione Ca' Granda Hospital (Ca' Granda-2018 cohort). The enrollment criteria are reported in the Supporting Methods, and clinical features are presented in Table 1, upper panel. The study flow chart is presented in Supporting Fig. S1.

The updated ALT-URL-IFCC levels were estimated by the 95th percentile observed in individuals (n = 9,195) without viral hepatitis (hepatitis B surface

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TABLE 1. CLINICAL FEATURES OF THE STUDY COHORTS (UPPER PANEL: 21,296 APPARENTLY HEALTHY INDIVIDUALS FROM THE CA' GRANDA-2018 COHORT, WHO WERE INCLUDED IN THE STUDY; LOWER PANEL: 745 INDIVIDUALS FROM THE LIVER-BIBLE-2020 COHORT)

Ca' Granda-2018 Cohort, Whole Population	Males (n = 13,741, 64.5%)	Females (n = 7,555, 35.5%)	P Value
Age, years	41.3 (12.9)	37.8 (13.6)	<0.0001
BMI, kg/m^2	24.9 (3.2)	23.0 (3.6)	<0.0001
Obesity, yes	957 (7.0)	408 (5.4)	<0.0001
Glucose, mg/dL	86 (10)	83 (12)	<0.0001
Impaired fasting glucose, yes	323 (2.4)	186 (2.5)	0.60
Total cholesterol, mg/dL	184 (35)	182 (33)	<0.0001
Triglycerides, mg/dL	97 (57)	77 (38)	<0.0001
Arterial hypertension	4,229 (30.9)	1,007 (13.4)	<0.0001
ALT, U/L	26.2 (13.3)	18.9 (13.5)	<0.0001
HBsAg, positive	251 (1.8)	247 (3.3)	<0.0001
Anti-HCV Ab, positive	254 (1.9)	246 (3.3)	<0.0001
First-time donors	1,902 (13.8)	1,496 (19.8)	<0.0001

Ca' Granda-2018 Cohort, Population at Low Risk for Liver Disease	Males (n = 4,855, 64.5%)	Females (n = 4,340, 35.5%)	P Value
Age, years	37.1 (12.9)	35.8 (13.0)	<0.0001
BMI, kg/m^2	22.7 (2.1)	21.4 (2.0)	<0.0001
Obesity, yes	0	0	1
Glucose, mg/dL	83 (7)	81 (7)	<0.0001
Impaired fasting glucose, yes	0	0	1
Total cholesterol, mg/dL	172 (30)	175 (28)	<0.0001
Triglycerides, mg/dL	73 (26)	67 (24)	<0.0001
Arterial hypertension	957 (19.9)	366 (8.5)	<0.0001
ALT, U/L	23.7 (12.8)	18.0 (8.4)	<0.0001
HBsAg, positive	0	0	1
Anti-HCV Ab, positive	0	0	1
First-time donors	786 (16.4)	883 (20.3)	<0.0001

Liver Bible-2020 Cohort	Males (n = 632, 84.8%)	Females (n = 113, 15.2%)	
Age, years	53.8 (6.4)	53.7 (6.2)	0.88
BMI, kg/m^2	28.5 (2.9)	29.0 (3.5)	0.21
Obesity, yes	140 (22.2)	33 (29.2)	0.12
Glucose, mg/dL	96 (13)	94 (13)	0.15
Impaired fasting glucose, yes	34 (5.4)	14 (12.5)	0.010
Total cholesterol, mg/dL	200 (31)	211 (32)	0.001
Triglycerides, mg/dL	172 (94)	155 (67)	0.018
Arterial hypertension	440 (69.6)	82 (72.6)	0.58
ALT, U/L	31.5 (13.9)	24.0 (13.9)	<0.0001

Note: Data are shown as mean (SD) or frequency (%). P values were determined at univariate logistic regression models.

antigen [HBsAg], hepatitis B virus DNA, and hepatitis C virus [HCV]-RNA–negative), and with normal body mass index (BMI; ≤25 kg/m^2), cholesterol (<240 mg/dL), triglycerides (<150 mg/dL), and glucose (<100 mg/dL) levels, and without regular alcohol intake nor regular or recent recreational drug use. In a sensitivity analysis, we replicated exactly the criteria for ALT-URL definition in 2002, which differed from the main analysis due to the definition of dyslipidemia based on glucose >105/98 mg/dL in M/F,

triglycerides >200 mg/dL, and/or total cholesterol >220 mg/dL, and absence of medications.[5]

The diagnostic accuracy of the updated ALT-URL-IFCC was validated in a subset of 745 individuals (a subset of the Ca' Granda-2018 cohort), consecutively enrolled from June 2019 to December 2020, who underwent noninvasive assessment of liver damage/fibrosis due to the presence of dysmetabolism (at least three criteria among overweight [BMI > 25 kg/m^2], arterial hypertension, hyperglycemia [>100 mg/dL], low high-density lipoprotein cholesterol [<45/55 in M/F], and increased triglycerides [>150 mg/dL]), and age at risk (40-65 years).[15] The clinical features of these individuals (Liver-Bible-2020 cohort) are reported in Table 1, lower panel. The presence of steatosis and of significant liver fibrosis were estimated by FibroScan with evaluation of continuous attenuation parameter (CAP > 255 dB/m) [16] and liver stiffness measurement (LSM > 7.9 kPa),[17] performed within 4 weeks of ALT measurement. The updated ALT-URL-IFCC was further tested for the ability to predict increased LSM and significant liver fibrosis in a previously described independent cohort of 977 healthy blood donors from Northern Italy (Lecco cohort).[18,19] Of these, 404 (41.7%) were females, and mean age was 47.2 ± 10 years and BMI was 24.3 ± 3.2 kg/m^2.

Additional validation cohorts in patients with liver diseases are reported in the Supporting Methods and Supporting Tables S1 and S2.[5,20]

ALT serum concentrations were measured at the Fondazione IRCCS Clinical laboratory, "Alessandro Manzoni" Hospital Lecco, and Royal Free Hospital according to the IFCC standardized method performed at 37°C with the addition of pyrodoxal-phosphate,[9,11] on Roche Cobas c702 automatic analyzers using Roche reagents (Roche Diagnostics, Monza, Italy). The study plan was approved by the review board of the Fondazione. Liver damage biomarkers evaluation was approved by the competent ethics committees, and subjects gave written informed consent to the procedures.

STATISTICAL ANALYSIS

The choice of defining an updated ALT-URL-IFCC by the 95th percentile in healthy individuals without risk factors for liver disease was chosen based on[21] (1) nonnormal, highly right-skewed distribution of ALT; (2) lack of association of low ALT levels with disease; (3) high prevalence of the most common liver disease (NAFLD) in unselected healthy individuals; (4) most frequent use of ALT-URL-IFCC to screen for NAFLD; and (5) ample validation of this approach.[5-8]

Serum ALT levels were fitted with univariate and multivariate general linear models. Models were adjusted for confounding factors, as specified. Not normally distributed variables, including ALT levels, were log-transformed before entering the models. Receiver operating characteristic curves, in which the sensitivity was plotted against the false-positive rate (1-specificity) were generated to evaluate new ALT thresholds.

Statistical analyses were carried out using the JMP professional 15.0 (SAS Institute, Cary, NC) and R statistical analysis software version 4.0.2 (http://www.R-project.org/). P values < 0.05 were considered statistically significant.

Results

Ca' GRANDA-2018 COHORT

The clinical features of the Ca' Granda-2018 cohort stratified by sex are presented in Table 1. Participants were predominantly young adults; two-thirds of them were males and regular blood donors. Most had normal glucose, lipid and ferritin levels, and a minority were obese or had impaired fasting glucose. Arterial hypertension was more common, especially in males, with almost one-third being affected by this condition. A small fraction of participants, who were primarily included among those presenting at first donation, were reactive for HBsAg or anti-HCV antibodies by screening assays, but infection was not necessarily confirmed by supplemental serology and nucleic acid testing. The sex-specific distribution of ALT levels in the whole Ca' Granda-2018 cohort is presented in Table 2, upper panel, and in Supporting Fig. S3A.

INDEPENDENT PREDICTORS OF ALT LEVELS IN HEALTHY INDIVIDUALS

The independent predictors of ALT levels in the entire Ca' Granda-2018 cohort and in the subjects stratified by sex are given in Table 3. As expected, the

TABLE 2. DISTRIBUTION OF ALT SERUM ACTIVITY (U/L) STRATIFIED BY SEX IN THE WHOLE CA' GRANDA-2018 COHORT AND IN INDIVIDUALS WITHOUT RISK FACTORS FOR LIVER DISEASE

ALT Percentile

Whole Cohort	Males (n = 13,741)	Females (n = 7,555)
5	13	10
25	18	14
50	23	17
75	30	21
95	48	33
Without Risk Factors for Liver Disease	Males (n = 4,855)	Females (n = 4,330)
5	13	10
25	17	13
50	21	16
75	27	20
95 (URL)	42	30

Note: The distribution of ALT percentiles was obtained in participants stratified by sex.

main predictor of ALT levels was male sex. Higher ALT values were very robustly associated with adiposity, as estimated by the BMI, but also with total cholesterol and ferritin levels. In the overall cohort and in males, there was also an independent association with glucose, triglycerides, and the presence of arterial hypertension. Younger age was also associated with ALT.

In the overall cohort and in females, there was a link between regular physical activity and a statistically significant, but quantitatively limited, increase in ALT. In the overall cohort and in males there was a protective association between a history of recreational cannabis use (tolerated in terms of suitability to donation when occurred more than 1 month apart from the presentation) and ALT. There was no significant association between regular intake of alcoholic beverages or positive viral markers (in individuals presenting for the first evaluation for blood donation) and ALT.

DEFINITION OF ALT-URL-IFCC

In the Ca' Granda-2018 cohort, 4,855 males (35.3%) and 4,340 females (57.4%) had no risk factors for liver disease (Table 1, middle panel). The distribution of ALT levels in these individuals without

TABLE 3. INDEPENDENT DETERMINANTS OF SERUM ALT LEVELS (LOG U/L) IN THE WHOLE CA' GRANDA-2018 COHORT OF 21,296 HEALTHY INDIVIDUALS, AND IN MALES/FEMALES SEPARATELY

	Estimate	SEM	P Value
Overall Cohort, n = 21,296			
Sex, F	−0.103	0.003	$6*10^{-238}$
Age, years	−0.001	0.002	$2*10^{-10}$
BMI, kg/m^2	+0.022	0.001	$2*10^{-159}$
Glucose, 10 mg/dL	+0.010	0.006	0.003
Triglycerides, 10 mg/dL	+0.004	0.003	$5*10^{-16}$
Total cholesterol, 10mg/dL	+0.010	0.001	$2*10^{-46}$
Ferritin, log ng/mL	+0.083	0.003	$2*10^{-117}$
Arterial hypertension, yes	+0.012	0.003	0.0002
Physical activity, moderate/severe	+0.007	0.002	0.013
HBsAg or anti-HCV Ab, positive	+0.004	0.008	0.59
Recreational drug use, yes	−0.011	0.004	0.019
Regular alcohol drinking, yes	−0.006	0.004	0.16
Males, n = 13,741			
	Estimate	SEM	P Value
Age, years	−0.004	0.000	$4*10^{-34}$
BMI, kg/m^2	+0.029	0.001	$1*10^{-153}$
Glucose, mg/dL	+0.001	0.000	0.0001
Triglycerides, mg/dL	+0.0005	0.0004	$1*10^{-16}$
Total cholesterol, mg/dL	+0.001	0.000	$3*10^{-46}$
Ferritin, log ng/mL	+0.078	0.004	$3*10^{-70}$
Arterial hypertension, yes	+0.012	0.003	0.001
Physical activity, moderate/severe	+0.001	0.003	0.79
HBsAg or anti-HCV Ab, positive	+0.018	0.011	0.13
Recreational drug use, yes	−0.015	0.005	0.005
Regular alcohol drinking, yes	+0.004	0.005	0.50
Females, n = 7,555			
	Estimate	SEM	P Value
Age, years	−0.001	0.003	$3*10^{-7}$
BMI, kg/m^2	+0.014	0.001	$2*10^{-28}$
Glucose, 10 mg/dL	+0.000	0.000	0.65
Triglycerides, 10 mg/dL	+0.000	0.000	0.97
Total cholesterol, 10 mg/dL	+0.010	0.002	$3*10^{-7}$
Ferritin, log ng/ml	+0.070	0.006	$2*10^{-29}$
Arterial hypertension, yes	+0.010	0.006	0.097
Physical activity, moderate/severe	+0.018	0.005	$9*10^{-5}$
HBsAg or anti-HCV Ab, positive	+0.004	0.008	0.75
Recreational drug use, yes	−0.006	0.008	0.48
Regular alcohol drinking, yes	−0.009	0.005	0.098

Note: P values were determined at multivariate generalized linear models, adjusted for the confounders specified in the table.

risk factors stratified by sex is presented in Table 2 (lower panel) and Supporting Fig. S3B. The updated ALT-URL-IFCC (95th percentile) were identified

at 42 U/L (95% confidence interval [CI] = 40-43) in males, and 30 U/L (95% CI = 29-32) in females. Although a relation between age and ALT levels was found, the extent of the variation in terms of U/L per year was limited (Table 3), and the introduction of age-specific cutoffs was not justified. Sensitivity analyses are presented in the Supporting Results and Supporting Table S3.

ALT predictors in participants without risk factors for liver disease were similar to those observed in the overall cohort and are reported in the Supporting Results and Supporting Tables S4 and S5.

A detailed characterization of participants with persistently increased ALT levels, and of the underlying causes of liver disease, is reported in the Supporting Results and in Supporting Table S6.

IMPACT OF UPDATED ALT-URL-IFCC ON THE PREDICTION OF FATTY LIVER DISEASE

The clinical features of the Liver-Bible-2020 cohort are presented in Table 1, lower panel. Steatosis was detected in 510 (68.6%), and significant fibrosis in 16 (2.2%). The diagnostic accuracy of the new *ALT-URL-IFCC* versus old ALT-URL-IFCC for discriminating liver damage is presented in Table 4. Although the previously adopted ALT-URL-IFCC was not helpful in the detection of both steatosis or fibrosis, the new ALT-URL-IFCC was associated with both steatosis (P = 0.001) and with significant fibrosis (P = 0.021), conferring the possibility to detect significant fibrosis, despite with low accuracy (area under the receiver operating characteristic curve [AUROC] = 0.61). The advantage of the updated ALT-URL-IFCC was conferred by the higher sensitivity to detect liver damage.

In the Liver-Bible-2020 cohort, ALT levels were proportional to hepatic fact content, as estimated by CAP (estimate = 18.6 ± 3.6, P = $2{*}10^{-7}$), and to liver stiffness (estimate = 0.88 ± 0.11, P = $6{*}10^{-15}$). The best ALT cutoffs for steatosis detection were at 35 U/L in males (sensitivity = 0.36, specificity = 0.80) and 22 U/L in females (sensitivity = 0.59, specificity = 0.69). Significant fibrosis was not detected in females. The best cutoffs for fibrosis detection were at 27 U/L in males (sensitivity = 0.94, specificity = 0.45). However, 24.6% of individuals in the Ca' Granda-2018 cohort (31.4% of those with and 15.8% of those without risk factors, P < 0.0001) had ALT > 27 U/L.

TABLE 4. COMPARISON OF THE DIAGNOSTIC ACCURACY OF NEWLY ESTABLISHED VERSUS PREVIOUS ALT-URL-IFCC FOR THE PRESENCE OF HEPATIC STEATOSIS AND SIGNIFICANT FIBROSIS IN THE LIVER-BIBLE-2020 COHORT (N = 745)

	Steatosis		Significant Fibrosis	
	Old ALT-URL-IFCC (59/41 in M/F)	New ALT-URL-IFCC (42/30 in M/F)	Old ALT-URL-IFCC (59/41 in M/F)	New ALT-URL-IFCC (42/30 in M/F)
OR (95% CI)	1.80 (0.77-4.19)	2.31 (1.40-3.80)	3.09 (0.67-14.19)	3.35 (1.19-9.42)
P value	0.17	0.001	0.15	0.021
AUROC	0.51	0.55	0.54	0.61
Prevalence	0.69 (0.65-0.72)	0.69 (0.65-0.72)	0.02 (0.01-0.03)	0.02 (0.01-0.03)
Sensitivity	0.05 (0.04-0.08)	0.19 (0.15-0.22)	0.12 (0.03-0.36)	0.38 (0.18-0.61)
Specificity	0.97 (0.94-0.98)	0.91 (0.87-0.94)	0.96 (0.94-0.97)	0.85 (0.82-0.87)
PPV	0.79 (0.63-0.90)	0.82 (0.74-0.88)	0.06 (0.02-0.19)	0.05 (0.02-0.11)
NPV	0.32 (0.28-0.35)	0.34 (0.30-0.38)	0.98 (0.97-0.99)	0.98 (0.97-0.99)
Positive LR	1.76 (0.78-3.98)	2.06 (1.32-3.22)	2.83 (0.74-10.81)	2.47 (1.28-4.76)
Negative LR	0.98 (0.95-1.01)	0.89 (0.84-0.95)	0.91 (0.76-1.10)	0.74 (0.50-1.08)

Note: In AUROC analysis, ALT was treated as a binary variable, as a measure of the accuracy of different cutoffs. *P* values were determined at univariate logistic regression models.
Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

In the Lecco cohort, which was not selected for dysmetabolism, the updated ALT-URL-IFCC predicted abnormal LSM (odds ratio [OR] = 2.14, 1.16-3.95; P = 0.014; sensitivity = 0.24, specificity = 0.87; positive likelihood ratio (LR+) = 1.87, negative likelihood ratio (LR-) = 0.87), and significant liver fibrosis (OR = 2.32, 1.02-5.31; P = 0.045; sensitivity = 0.26, specificity = 0.87; LR+ = 1.98, LR- = 0.85), although with limited accuracy. The thresholds for individuals without dysmetabolism and risk factors for liver disease in the Lecco cohort were almost coincident to the updated ALT-URL-IFCC (i.e., 43 U/L [95% CI 40-55] in males [n = 176] and 32 U/L [95% CI 30-35] in females [n = 193].

The accuracy of the updated ALT-URL-IFCC for detecting liver disease in clinical cohorts was moderate to high (AUROC = 0.78-0.93) and is reported in the Supporting Results and Supporting Tables S7 and S8.

Discussion

In this study, we updated the healthy ranges for ALT with the new standardized IFCC methodology and the changing epidemiology of liver disease. NAFLD is now the leading cause of liver disease, and it affects at least one-fourth of the subjects enrolled as healthy volunteers in clinical trials, which leads to some concern on the safety of participants and validity of study findings.[22] There is currently low awareness of the differences between the methodologies available to measure ALT levels, and new uncertainties concerning the thresholds to be adopted in general practice to screen for liver disease. Although more accurate methods to discriminate chronic liver disease and fibrosis are available,[15,17] ALT still represent the most used liver damage biomarker worldwide.[23] Furthermore, noninvasive scores of fibrosis still rely on the evaluation of ALT levels, and by introducing a source of heterogeneity, the concomitant use of two different measurement methods and different definitions of the reference populations may negatively affect the accuracy of such approaches.[24] The new IFCC methodology of ALT measurement was developed to improve the standardization among and within the different labs and clinical settings, but it is not yet widely adopted worldwide. Our main aim was to pragmatically set thresholds able to identify apparently healthy individuals at higher risk of liver disease, and in particular NAFLD, in the

general population. We therefore repeated with the proposed analytic method and in the current epidemiological situation the same approach successfully used 20 years ago.[5] We used the 95th percentile, as we were specifically interested in the upper bound of reference range, as a potential indicator of liver injury.[5]

Revised ALT-URL-IFCC were 42/30 in M/F, higher than the healthy limits determined by the same approach at the same center using an old, non-standardized method (30/19),[5] but lower than the currently accepted reference range for the IFCC standardized method (59/41).[11] The main advantage of the proposed approach is that by the careful characterization of the risk factors in a large cohort, we were able to exclude from the reference population those at higher risk of liver disease and NAFLD, thereby providing "healthy limits" with improved ability to discriminate these conditions. The choice of the revised ALT-URL-IFCC was corroborated by the results of several sensitivity analyses. We also showed that the choice of the revised ALT-URL-IFCC was consistent with data derived from an independent cohort from the same geographical area.

The major ALT predictors in healthy individuals were male sex and increased adiposity. We also found a strong association with dyslipidemia, including high level of cholesterol and triglycerides, and ferritin levels, which reflect both insulin resistance and hepatic iron stores.[25-27] Hypertension and high glucose levels were also independent predictors. Overall, these data suggest that metabolic risk factors influencing hepatic fat accumulation represent the major determinant of ALT levels in healthy individuals. In keeping with this notion, we recently showed that inherited genetic variants influencing hepatic fat accumulation are the main determinant of serum ALT levels in the general population.[28] The determinants of ALT levels within the normal range, including recent physical activity and age, are discussed in detail in the Supporting Discussion.[5,8,29-33]

Due to the higher sensitivity compared with the thresholds previously proposed for the IFCC method,[11] the revised ALT-URL-IFCC rendered it possible to discriminate the presence of NAFLD and of significant liver fibrosis in individuals with dysmetabolism. The ability to predict significant fibrosis was confirmed in an independent cohort of unselected blood donors. However, in keeping with previous literature data,[34-37] 82% of individuals with steatosis and 54% of those with significant fibrosis had normal

ALT levels, even when the definition was based on the updated thresholds, which are lower than those currently advised. In the setting of dysmetabolism, an even lower threshold at 27 U/L was more accurate to discriminate the presence of significant fibrosis. However, this lower threshold with higher sensitivity to detect subtle liver damage would not be appropriate for the overall population because of the very low specificity,[38] as they would trigger an unacceptable amount of unnecessary follow-up tests and investigations.

This study has some limitations. These include the lack of direct assessment of the presence of fatty liver and fibrosis by FibroScan in the whole cohort.[17] However, we could evaluate a comprehensive panel or risk factors and viral markers. Although the concomitant assessment in diagnostic algorithms of other noninvasive predictors may improve the rate of liver disease diagnosis and classification,[39] the correct definition of the most appropriate ALT threshold to trigger further investigations remains of paramount importance. In addition, we could not compare ALT levels, determined by both the old and the new methodology, in the same samples. Furthermore, the updated ALT-URL-IFCC cannot be generalized to the developmental age group,[40] the elderly, populations of different ethnicity, or special populations.

In conclusion, we used a validated approach to update the healthy ranges of ALT determination by the new IFCC standardized methodology. In both sexes, the revised ALT-URL-IFCC (42/30 U/L in M/F) were about 30% lower than those calculated in initial studies,[11] but higher than those recommended for the old, nonstandardized method.[5] These revised ALT-URL-IFCC were able to identify individuals with liver injury related to dysmetabolism (e.g., NAFLD/MAFLD), which supports their use in general clinical practice. However, among patients with clinical NAFLD, ALT alone had limited accuracy to stratify the risk of progressive disease,[34-37] and additional testing is required.[39,41] Remarkably, the IFCC standardized methodology is not yet universally adopted, as the old, nonstandardized method is still in use in several laboratories. The existence of two methodologies producing substantially different ALT results is of concern, as it may lead to wrong diagnosis and clinical errors. While waiting for a harmonization of laboratory techniques, clinicians should be aware of which method has been used in each patient and apply the appropriate threshold.

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