Redefining the alanine aminotransferase upper limit of normal improves the prediction of metabolic syndrome risk

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Background Multiple studies have recently proposed the lowering of upper limit of normal (ULN) for alanine aminotransferase (ALT) to improve the diagnostic sensitivity for viral hepatitides and metabolic syndrome (MS). We have tried to validate some of the proposed ULNs in the diagnosis of MS.

Methods We used data from the HepaMeta Study conducted in 2011 in Slovakia, which explored the prevalence of MS in eastern Slovakia. Patients were tested for the criteria of MS and ALT. Different, previously published, ALT cutoffs were then used to calculate odds' ratios, sensitivity, specificity, and accuracy of MS and its components.

Results Manufacturers' recommended ULN used in our institution (0.8 μkat/l, 47 U/l for men and 0.6 μkat/l, 35 U/l for women) failed to predict any significant risk of MS. Lowered cutoff (72% of the original ULN) identified the patients with the highest age-adjusted probability of MS (odds ratio 3.194, 95% confidence interval 1.398–7.295). ALT was significantly associated with elevated levels of triacylglycerols, hyperglycemia, and obesity.

Conclusion In patients with MS, one must consider liver involvement if the patient has ALT levels in the upper third of the reference range. There is the need for discussion about the feasibility of lower ALT ULN in clinical practice. Eur J Gastroenterol Hepatol 27:405–411

Introduction

Alanine aminotransferase (ALT) is an enzyme found in the cytoplasm of hepatocytes, as well as in the muscle and kidney cells. As serum ALT levels rise as a consequence of hepatocellular injury, serum ALT levels can effectively identify an ongoing liver disease process [1]. ALT is usually considered to be the most liver-specific among typical liver tests [2]. However, serum ALT elevation can occur in several myopathic diseases such as dermatomyositis and polymyositis and muscular dystrophy [3]. The clinical usefulness of ALT is in its sensitivity and relative specificity for the detection of liver damage.

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The association of ALT and metabolic syndrome (MS) is well documented by a number of published papers. A meta-analysis published in 2013 concluded that 5 U/l ALT increment increases the risk of MS 1.13 times [95% confidence interval (CI) 1.11–1.16] [4]. Furthermore, non-alcoholic fatty liver disease (NAFLD) is the most common cause of elevated serum ALT levels in patients with negative viral hepatitis serology [5]. On the other hand, patients with ALT higher than the currently recommended ULN did not have a significantly higher risk of MS [odds ratio (OR) 0.73; 95% CI 0.4–1.32] [6].

Upper limit of normal (ULN) of ALT has been set at around 40 U/l since the 1950s, when serum ALT levels were used as a surrogate marker for non-A, non-B hepatitis among blood donors before identifying hepatitis C virus (HCV) [7]. Although at that time the patients were considered to be healthy, it is now assumed that reference populations for the determination of ALT ULN included many asymptomatic patients with NAFLD, and thus calculating ALT levels has not been performed in truly healthy populations [8].

Recently, multiple studies have proposed the lowering of ALT ULN in the setting of various diagnoses. A review published by Pacifico *et al.* [3] documented 13 attempts to introduce revised lower ULN for ALT. This has created a rather confusing situation in which every author lowered ALT ULN differently in both absolute and relative terms. The major source of heterogeneity is the detection method itself. Incubation of test serum with pyridoxal phosphate increases the detected ALT activity by roughly 30% [9].

In addition to that are differences of ULN recommended by suppliers of various ALT detection kits and differences between laboratories [10]. This heterogeneity on one hand improves the performance of ALT in detection of diseases in the particular study population, and on the other hand it complicates the comparison of new ULN among various populations. This leads to complications in the search for a universally valid ULN of the ALT and its eventual adoption into routine clinical praxis.

Therefore, we have tried to validate some of the proposed lower ULN of ALT in the diagnosis of MS on our own study population and express it in relative terms for better comparison among various studies.

A similar need for the update of ULN has arisen for aspartate aminotransferase (AST) as well; however, no association between AST and MS has been reported [11,12]. Furthermore, AST is less hepatocyte specific than ALT, and various isoenzymes of AST exist [3]; therefore, we have not included AST in this analysis.

Methods

We used data from the cross-sectional HepaMeta study conducted in 2011 in Slovakia. This project aimed to map the prevalence of viral hepatitis B/C and MS in the population living in Eastern Slovakia, including Roma settlements.

Population sample and procedure

Participants were selected from the patient databases of general practitioners operating in the catchment area. We contacted 26 general practitioners, and 17 agreed to take a part in our study (response rate 65%). Sample participants from the Roma population were recruited directly in the settlements by cooperation with the local Roma community workers. From these Roma settlements, a total of 452 participated after providing information about our study. A total of 710 individuals from the non-Roma population were randomly chosen from a list of patients. These individuals were contacted through phone and mail by trained research assistants, who provided the information about our study and invited them to participate. A total of 403 individuals participated in our study. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty at Safarik University in Kosice. Participation in the study was fully voluntary and anonymous. Inclusion criteria for the respondents were as follows: no preventive medical check-up in the past 2 years, no acute illness, appropriate age and being able to take time off from work during the week of data collection in the ambulance of their general practitioner. Exclusion criteria included antiviral or immunosuppressive therapy, known anti-HCV, HBsAg, or HIV antibody positivity. Excessive alcohol use was defined as intake of over 20 g of alcohol on average daily.

Detailed information about our study and its procedures was given to all the patients, and informed consent was signed before medical check-ups. Trained medical personnel collected the blood and urine samples and performed anthropometric measures in offices of cooperating general practitioners.

Laboratory testing

Clinical biochemistry tests [total serum cholesterol, HDL, LDL, triacylglycerol (TAG), γ-glutamyl transferase (GGT), ALT, and fasting plasma glucose] were performed using Advia 1650 and Advia 2400 autoanalyzers (Siemens, Erlangen, Germany). ALT was determined, according to IFCC recommendation, after the addition of a 0.1 mmol/l solution of pyridoxal phosphate. ALT requires the coenzyme pyridoxal-5′-phosphate for maximum activity; therefore, pyridoxine (vitamin B_6) deficiency could potentially be a major factor influencing the ALT results [3]. Pyridoxal-5′-phosphate levels could change ALT activity as much as 30% [9]. However, this deficiency is uncommon and typical for alcohol abusers. As we have excluded participants with significant alcohol intake, we did not test pyridoxal-5′-phosphate levels in the serum. Furthermore, anthropometric measures including weight, height, waist circumference, and blood pressure (average of three measurements) were also documented.

Definition of ALT cutoff points

We did not have ambition to define yet another new ULN in our population, which is, in our opinion, also contraproductive. On the other hand, we evaluated the accuracy and validity of already published values summarized in a review by Pacifico *et al.* [3]. Because of differences in the laboratory-specific reference ranges for ALT in all published studies, we used relative values expressed as percentage of the original ULN reported in selected studies. Percentage was then recalculated to absolute values using our current, laboratory-recommended ULN (ULN 100%) as baseline. This step was necessary because of variability of normal ranges of ALT in the published studies. None of the published studies reported the exact method for ALT determination, specifically the use of pyridoxal-5′-phosphate; therefore, we had no other option for dealing with presumed 30% variability caused by this factor.

After evaluation of published data, we ruled out studies with children or adolescents [13–15] or studies that selected the study cohort on the basis of age or BMI [16, 17]. The updated ULN in the remaining studies ranged from 42.7 to 132.5% of the original ULN. As the ULN reported by Park *et al.* [18] was the only one higher than the original and the authors had not clearly defined the low-risk population from which this ULN was derived, we did not include this ULN in our analysis. The remaining studies presented updated ULNs that were fairly similar; therefore, we have chosen the cutoffs proposed by Prati *et al.* [19], Kariv *et al.* [20], and Wu *et al.* [21] on the basis of the number of participants and definition of 'healthy' population.

As most of the previously published, updated ULNs are in the range of 50–70% of the original ULN, we also empirically defined cutoff points at 80 and 60% of our own ULN (100%). The cutoff points that were used are summarized in Table 1. The GGT cutoffs that were used were set at 100% ULN (men 1.0 μkat/l, women 0.65 μkat/l).

Table 1. Different ALT upper limits of normal

	Relative to current ULN (%)	Men [μkat/l (U/l)]	Women [μkat/l (U/l)]
Our current ULN	ULN (100)	0.8 (47)	0.6 (35)
Arbitrary 80% of ULN	ULN (80)	0.64 (38)	0.48 (28)
Prati *et al.* [19]	ULN (75) men ULN (63) women	0.6 (35)	0.38 (22)
Kariv *et al.* [20]	ULN (72)	0.58 (34)	0.43 (25)
Arbitrary 60% of ULN	ULN (60)	0.48 (28)	0.36 (21)
Wu *et al.* [21]	ULN (53) men ULN (43) women	0.42 (25)	0.26 (15)

ALT, alanine aminotransferase; ULN, upper limit of normal.

Criteria of MS

Standard International Diabetes Federation criteria were used. Patients were considered to have MS when central obesity was present (waist circumference > 94 cm for men and > 80 cm for women or BMI > 30 kg/m^2) and when any two of the following factors were present.

(1) Raised triglycerides of 150 mg/dl or more (1.7 mmol/l) or specific treatment for this lipid abnormality.
(2) Reduced HDL-c less than 40 mg/dl (1.03 mmol/l) in men and less than 50 mg/dl (1.29 mmol/l) in women, or specific treatment for this lipid abnormality.
(3) Raised blood pressure – systolic blood pressure of 130 or more or diastolic blood pressure of 85 mmHg or more – or treatment of previously diagnosed hypertension.
(4) Raised fasting plasma glucose of 100 mg/dl or more (5.6 mmol/l), or previously diagnosed type 2 diabetes. If the blood glucose is above 5.6 mmol/l or 100 mg/dl, oral glucose tolerance test is strongly recommended but is not necessary [22].

Statistical analysis

Categorical data are presented in absolute count and percentages, and interval data are presented as median ± interquartile range because of nonparametric distribution. Measurement of statistical significance of difference between interval data with non-normal distribution was done by Mann–Whitney test. The performance of ALT in diagnosis of MS in raw data (without adjustment) was assessed by receiver operating characteristic (ROC) analysis. For risk based on categorical data, unadjusted ORs with 95% CIs were calculated from the contingency table. Age-adjusted ORs for the risk of MS were calculated by multivariate logistic regression. Sensitivity, specificity, and positive and negative predictive values for MS were calculated for each cutoff.

Results

Characterization of the study cohort

Out of 855 participants, 344 were men and 511 were women. A total of 168 patients were excluded on the basis of the following exclusion criteria: hepatitis B/C positive, 57; significant alcohol intake, 68; significant alcohol use with viral hepatitis B positivity, 10; and missing data, 33 patients. Altogether, 687 participants were available for analysis. Further patients were excluded per analysis because of missing ALT (four patients) or GGT (three patients) data. Descriptions of study parameter distributions are summarized in Table 2.

Median age was 34.7 years; women were slightly older than men. Probably because of the relatively low median age in the study cohort, women had lower median values of all the parameters positively associated with MS risk. Patients with MS had elevated levels of both ALT (0.17 ± 0.1 vs. 0.21 ± 0.15 μkat/l) and GGT (0.26 ± 0.16 vs. 0.39 ± 00.38 μkat/l); *P* values for both equals 0.0001.

Among patients with MS, only 16 (2.3%) participants were taking hypolipidemic medication, and 11 (1.6%) participants diagnosed with diabetes mellitus were on peroral antidiabetics. No patient was treated with insulin. In all, 61 patients (7.1%) were taking antihypertensive medication.

Association of elevated ALT and MS

In the ROC analysis of ALT association with MS, the area under ROC was 0.6, with a 95% CI of 0.549–0.651. We then grouped the patients by the presence of elevated ALT according to each of the proposed ULNs (Table 3). The difference in the proportion of patients with MS was seen to be increasing with the decrease of ULN.

Performance of different ALT categories was assessed by computing univariate ORs from the contingency table. The highest proportion of patients with MS in the group with elevated ALT was present when ULN proposed by Kariv *et al.* [20] was used. Elevated GGT with a cutoff at 100% conferred the highest probability of MS, followed by ALT cutoff proposed by Kariv and colleagues set at 72% of original ULN. Furthermore, classification of patients according to the ALT cutoff based on the original ULN did not identify subjects with increased probability of MS. Classification of these patients according to any of the lowered ULNs identified subjects with increased odds of MS (Table 4).

Sensitivity and specificity of ALT in MS prediction

Sensitivity, specificity, and positive and negative predictive values were calculated for each cutoff. Table 5 shows that ALT ULN proposed by Kariv and colleagues at 72% of the

Table 2. Summary statistics of the study cohort

	Total (*n* = 687)	Men (*n* = 251)	Women (*n* = 436)	*P*
Age	34.7 (13.83)	34.06 (13.08)	34.87 (13.59)	0.026
BMI (kg/m^2)	25.0 (7.62)	25.9 (6.85)	24.41 (7.55)	0.002
Waist (cm)	87 (20.0)	91 (19.0)	83 (20.00)	< 0.0001
sTK (mmHg)	119 (19.0)	123 (17.0)	117 (18.00)	< 0.0001
dTK (mmHg)	74 (13.0)	75 (13.0)	73 (12.33)	0.011
Glucose (mmol/l)	4.74 (0.66)	4.83 (0.69)	4.67 (0.66)	< 0.0001
Total cholesterol (mmol/l)	4.90 (1.26)	4.88 (1.38)	4.92 (1.23)	0.5
HDL-c (mmol/l)	1.15 (0.41)	1.06 (0.38)	1.21 (0.45)	< 0.0001
LDL-c (mmol/l)	2.56 (0.92)	2.62 (0.99)	2.52 (0.83)	0.142
TG (mmol/l)	1.06 (0.77)	1.13 (0.79)	0.980 (0.71)	0.001
MS prevalence (%)	24.7	23.6	25.5	0.33

Data presented as median (interquartile range) for continuous variables.
dTK, diastolic blood pressure; MS, metabolic syndrome; sTK, systolic blood pressure.

Table 3. Proportion of patients with MS in groups based on different upper limits of normal for ALT (unadjusted data)

	ALT normal [*N* (%)]		ALT elevated [*N* (%)]			
	MS	No MS	MS	No MS	OR (for MS)	95% CI
ALT – ULN 100%	164 (24.7)	500 (75.3)	6 (37.5)	10 (62.5)	1.82	0.65–5.11
ALT – ULN 80%	159 (24.2)	498 (75.8)	11 (47.8)	12 (52.2)	2.87	1.24–6.62
ALT – ULN 75%[a]; 63%[b] [19]	155 (24.0)	492 (76)	15 (45.5)	18 (54.5)	2.64	1.30–5.36
ALT – ULN 72% [20]	155 (23.8)	496 (76.2)	15 (51.7)	14 (48.3)	3.42	1.61–7.25
ALT – ULN 60%	150 (23.7)	483 (76.3)	20 (43.5)	27 (57.4)	2.38	1.30–4.37
ALT – ULN 53%[a]; 43%[b] [21]	139 (23.2)	460 (76.8)	31 (38.8)	50 (61.7)	2.05	1.26–3.34
GGT – 100% ULN	147 (23)	493 (88)	23 (57.5)	17 (42.5)	4.53	2.36–8.70

ALT, alanine aminotransferase; CI, confidence interval; GGT, γ-glutamyl transferase; MS, metabolic syndrome; OR, odds ratio; ULN, upper limit of normal.
[a]men.
[b]women.

Table 4. Age-adjusted OR for metabolic syndrome based on ALT categories with different upper limits of normal

	OR	95% CI	*P*
ALT – ULN 100%	1.795	0.583–5.525	0.307
ALT – ULN 80%	2.551	1.007–6.494	0.048
ALT – ULN 75%[a]; 63%[b] [19]	2.309	1.051–5.073	0.037
ALT – ULN 72% [20]	3.194	1.398–7.295	0.006
ALT – ULN 60%	2.597	1.321–5.128	0.006
ALT – ULN 53%[a]; 43%[b] [21]	2.079	1.223–3.535	0.007
GGT – 100% ULN	3.076	1.517–6.237	0.002

ALT, alanine aminotransferase; CI, confidence interval; GGT, γ-glutamyl transferase; MS, metabolic syndrome; OR, odds ratio; ULN, upper limit of normal.
[a]men.
[b]women.

original ULN had the highest accuracy in diagnosis of MS. All of the new cutoffs, despite being lower than the original, had low sensitivity. The ULN proposed by Wu and colleagues had the highest sensitivity (18.2%), but in absolute terms it is unsatisfactory and with significant trade-off of specificity.

Different ALT cutoffs for the prediction of meeting individual MS criteria

We also evaluated the performance of ALT in the prediction of individual MS criteria. The highest area under receiver operating characteristic (auROC) was for the prediction of hypertriglyceridemia (auROC 0.626; 95% CI 0.603–0.706], followed by hyperglycemia (auROC 0.626; 95% CI 0.550–0.702), obesity (auROC 0.608; 95% CI 0.565–0.650), and arterial hypertension (auROC 0.585; 95% CI 0.537–0.633). On the other hand, ALT was not better than chance in the prediction of decreased HDL-c (auROC 0.499; 95% CI 0.455–0.542).

Analysis of the association between various upper limits of normal of ALT and MS criteria yielded the following results. The original ULN of ALT was not significantly associated with any MS component. After lowering the ULN of ALT to various degrees, there was a statistically significant association with elevated levels of TAGs, hyperglycemia, and obesity. Any ALT ULN was not associated with decreased HDL or arterial hypertension (Table 6).

Discussion

The definition of ALT ULN is very important for routine clinical practice, as this allows the physician to quickly select the patients with probable liver damage. In recent years, however, there has been accumulating evidence that even patients with ALT in the 'normal range' have increased risk of death from liver-related causes. A study published by Kim and colleagues in 2004 provided prospective information on 94 533 men and 47 522 women with health insurance from Korea Medical Insurance Corporation. These individuals were followed up for 8 years. Authors reported that individuals with ALT in the upper half of the normal range had a significantly higher risk of liver-related mortality. Compared with the ALT activity less than 20 U/l, risk ratios of death for men with ALT in the 20–29 and 30–39 U/l were 2.5 (95% CI 2.0–3.0) and 9.5 (95% CI 7.9–11.5), respectively. In women, these relative risks were 3.8 (1.9–7.7) and 6.6 (1.5–25.6), respectively [23]. These data indicate that some people with ALT in the normal range possibly have liver disease.

Table 5. Sensitivity and specificity of ALT in metabolic syndrome prediction in whole cohort

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
ALT – ULN 100%	3.5	98.0	37.5	75.2	74.3
ALT – ULN 80%	6.5	97.6	47.8	75.8	74.8
ALT – ULN 75%[a]; 63%[b] [19]	8.8	96.5	45.5	76.0	74.5
ALT – ULN 72% [20]	8.8	97.3	51.7	76.2	75.1
ALT – ULN 60%	11.8	94.9	43.5	76.3	74.1
ALT – ULN 53%[a]	18.2	90.4	38.8	76.8	72.3
ALT – ULN 43%[b] [21]					
GGT – 100% ULN	13.5	96.7	57.5	77.0	75.8

Accuracy = (true positives + true negatives)/all patients.
ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; NPV, negative predictive value; PPV, positive predictive value; ULN, upper limit of normal.
[a]men.
[b]women.

Table 6. Odds ratios of individual ALT upper limits of normal for predicting the risk of metabolic syndrome components

	TAG	HDL	Hyperglycemia	Hypertension	Obesity
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
ALT – ULN 100%	2.364 (0.844–6.62)	1.899 (0.653–5.525)	0.7 (0.091–5.395)	1.324 (0.475–3.691)	2.664 (0.85–8.345)
ALT – ULN 80%	2.571 (1.089–6.07)	1.336 (0.571–3.131)	2.316 (0.761–7.048)	1.426 (0.607–3.35)	4.304 (1.448–12.791)
ALT – ULN 75%[a]; 63%[b] [19]	3.034 (1.481–6.216)	1.519 (0.735–3.138)	3.096 (1.282–7.475)	1.658 (0.815–3.374)	3.403 (1.456–7.953)
ALT – ULN 72% [20]	3.339 (1.566–7.117)	1.65 (0.756–3.604)	2.315 (0.849–6.311)	1.579 (0.740–3.369)	4.4 (1.658–11.675)
ALT – ULN 60%	2.971 (1.599–5.518)	1.35 (0.732–2.491)	2.419 (1.071–5.461)	1.503 (0.809–2.795)	2.329 (1.203–4.509)
ALT – ULN 53%[a]; 43%[b] [21]	2.659 (1.617–4.373)	1.173 (0.731–1.879)	2.349 (1.207–4.572)	1.398 (0.859–2.275)	2.67 (1.581–4.512)
GGT – 100% ULN	4.826 (2.516–9.259)	2.357 (1.158–4.8)	4.048 (1.869–8.765)	2.607 (1.369–4.962)	4.291 (1.866–9.866)

ALT, alanine aminotransferase; CI, confidence interval; GGT, γ-glutamyl transferase; OR, odds ratio; TAG, triacylglycerol; ULN, upper limit of normal.
[a]men.
[b]women.

This is also supported by a number of studies that reported significantly lower ALT ULN compared with currently recommended ULN in a truly healthy population. A large population-based study from Israel included all participants, aged 15–90 years, who underwent ALT testing in the beginning of 2002. From the total of 346 530 people, the authors excluded all patients with a high risk of liver disease on the basis of other abnormal test results and/or diagnoses. Afterwards, they analyzed the distribution of ALT activity in the sera of 17 496 participants with a low risk of liver disease. The 95th percentile, which is often used as ULN, was found to be only 72% of the original, manufacturer-recommended ULN (decrease from 52 to 37.5 U/l) [20]. Similar data were presented in a recent paper by Pacifico and colleagues who reviewed 13 studies that aimed to update ULN for ALT. The relative decrease ranged from 12.5 to 47.8% in men and from 30 to 57.5% in women.

The situation is even more complicated by interlaboratory variability, which is caused by different analytical methods, as well as different cutoffs recommend by the test kits' manufacturers. An interesting survey was published in 2009 by Dutta and colleagues. The authors investigated the variability of ALT ULN in the state of Indiana, USA. They have confirmed that a wide variability exists among the recommended ULNs (31–72 U/l). Although there was a statistically significant analyzer-to-analyzer variability, these differences were not clinically significant. The authors conclude that the widely variable ALT ULN could be owing to variable reference intervals established by different manufacturers, and studies should be undertaken to identify outcome-based reference intervals for ALT [24]. This variability could be probably caused in part by differences in the analytical methods, particularly the addition of pyridoxal phosphate. However, in one older study, the authors conclude that the magnitude of ALT stimulation by pyridoxal phosphate depends on complex factors and cannot be attributed only to the analytical methods [9]. Unfortunately, none of the studies evaluated in this study reported the method of ALT determination in detail, particularly regarding the incubation with pyridoxal phosphate. It is probable that the determinations were performed according to IFCC recommendation, which includes the addition of pyridoxal phosphate [25]. However, as it was not explicitly confirmed in individual studies, we were unable to take this factor into account.

Another possible source of heterogeneity is the relationship of ALT activity and in-vivo concentration of pyridoxal phosphate. It has been proven that the in-vivo activity of ALT correlates with the pyridoxal phosphate levels; however, this correlation is only partially clinically significant. In one study, which involved geriatric patients, only 36.7% with pyridoxal phosphate deficiency had ALT activity below reference value [26]. Pyridoxal phosphate deficiency is also more common with advanced age or alcohol abuse [27]. In our study, we included young patients (median age 34.7 years) and excluded patients with significant alcohol use; therefore, the potential bias caused by pyridoxal phosphate deficiency is minimal.

The increasing number of studies that propose updated ULNs of ALT combined with the interlaboratory variability of the original ULN of ALT makes it very difficult to directly compare performance of each proposed ULN or potentially adopt the new ULN into clinical praxis. We tried to evaluate the proposed ULNs in our cohort of patients with MS. As the ULN recommended by our laboratory is different from the ULN in most of the studies (47 U/l for men and 35 U/l for women), we have decided to express different updated ULNs as a percentage relative to the original ULN. We understand that the change of the ALT ULN is not always proportional, but we still believe that the expression of the updated ULN in relative percentages is more accurate and compensates for the interlaboratory variability of the original ULN.

Over the past decade, more authors have published data about significantly increased liver-related morbidity of patients with ALT in the normal range in various diseases. In a recent meta-analysis of nine studies with a total of 830 patients with hepatitis B, Chao *et al.* [28] reports that approximately one-fifth of chronic hepatitis B patients with ALT of 40 U/l or less may have significant hepatic fibrosis. Similar data are available for hepatitis C. In a landmark paper from 2002 by Prati and colleagues, the authors first derived a new lower ULN for ALT by using an apparently healthy population, and then applied this new ULN in the detection of liver damage caused by chronic hepatitis C. The new ULN showed superior sensitivity in identifying participants with HCV viremia [sensitivity 76.3% (95% CI 69.1–83.6%] vs. 55% (95% CI 46.4–63.5%)] with acceptable trade-off in specificity [19].

MS and insulin resistance are tightly associated with the liver. In fact, nonalcoholic steatosis is considered to be a hepatic manifestation of the MS [29]. Already published

papers have confirmed that patients with MS have higher levels of serum ALT. In the paper published by Saely *et al.* [30], patients with MS had a mean level of ALT of 34 ± 21 U/l compared with patients without MS (29 ± 20 U/l). Similar results were published by Bedogni *et al.* [6], but the difference in the ALT levels was even greater. ALT levels, when considered as a continuous variable, were even associated with components of MS after adjustment for age and insulin resistance [31]. The strongest evidence about the association of ALT and MS comes from the meta-analysis by Liu *et al.* [4]. The authors reported that the pooled RR of MS was 1.13 (95% CI 1.11–1.16) in a dose–response analysis with 5 U/l ALT increment. There was also a significant difference between sexes. It is therefore surprising that after the classification of patients into normal and elevated ALT groups, patients with elevated ALT do not have increased risk of MS [OR 0.73 (95% CI 0.4–1.32)] [6]. This was confirmed in the study from the USA published by Fraser *et al.* [32] in which ALT over 40 U/l was not significantly associated with triglycerides, HDL-c, or glucose levels. On the other hand, Hsu and colleagues reported that patients with increased ALT (original ULN of 40 U/l) have a higher risk of MS (OR 2.1, 95% CI 1.68–2.57). Albeit this risk is lower than the risk conferred by liver steatosis on ultrasound (OR 3.8, 95% CI 3.15–4.68), it is still significant [12]. A study by Park *et al.* [33] in an adolescent population found similar results [OR for elevated ALT in MS patients was 6.2 (2.3, 16.8)]. This controversy is very interesting, and it could be caused by different study populations or differences among laboratories and essay methods.

Our data show that if we classify patients into normal and elevated ALT categories on the basis of the original ULN (in our case it is 0.8 μkat/l or 47 U/l for men and 0.6 μkat/l or 35 U/l for women), there is no difference in the proportion of patients with MS. This result confirms the findings by Bedogni *et al.* [6]. If, however, we decrease the ULN progressively, as can be seen in Table 3, the difference in the MS prevalence between groups increases. The highest difference in the prevalence of MS is when we set the ULN to 60–70% of the original ULN. When we take age-adjusted data into account (Table 4), the highest risk of MS for patients with elevated ULN is when the cutoff proposed by Kariv *et al.* [20] is used (72% of the original ULN). Furthermore, sensitivity/specificity analysis of individual ULN demonstrates that this ULN has the highest overall accuracy and positive predictive value of all tested ALT ULNs (Table 5). Similar data have been published by Kang and colleagues. In his study, authors decreased the ALT ULN to 31 U/l for men and 23 U/l for women. Prevalence of MS in patients with elevated ALT was 27.6% compared with 11.9% in patients without elevated ALT. These values are lower than in our study, but overall prevalence of MS in the study by Kang *et al.* [34] was not given and we presume that it is lower than in our study, because of the different criteria used (NCEP ATP III vs. IDF).

Another updated ULN for ALT was derived from the population at low risk of liver disease by Zheng and colleagues. The authors set the ULN at around 50% of the original ULN for both sexes. The sensitivity of this ULN for diagnosis of NAFLD was 61.1% for men and 53.8% for women, and the specificity was 94.8 and 94.6%, respectively. These results, however, cannot be directly compared with the sensitivity and specificity for MS, as the diagnosis of NAFLD required steatosis on liver ultrasound and patients needed not to fulfill MS criteria [35]. Wu and colleagues compared the performance of updated lower ULN of ALT (52.5% of the original for men and 42.5% for women) in the diagnosis of composite outcome, which required positivity of at least one of following parameters: BMI more than 24 kg/m^2, waist more than 90 cm (men), 80 cm (women), glucose more than 100 mg/dl, cholesterol more than 200 mg/dl, HDL-c less than 40 mg/dl (men), less than 50 mg/dl (women), TAG more than 150 mg/dl, HBsAg, or anti-HCV positivity or ultrasonography-documented steatosis. Their updated ULN had higher Youden's index for diagnosis of this composite end point than original ULN (0.381 vs. 0.220 for men and 0.305 vs. 0.092 for women), although this radical decrease of ULN resulted in a significant decrease of specificity (63.6 vs. 96.9% for men and 63.5 vs. 98.2% for women) [21].

Conclusion

ALT is an important marker of liver damage. It is closely associated with the activity of chronic viral hepatitis A NAFLD. However, the sensitivity of its current ULN is very poor, especially in the diagnosis of the MS-associated liver damage. There have been multiple attempts to redefine the ALT ULN, but there are several drawbacks. All the papers published so far came up with a different ULN, and the results are not easily comparable because of (i) different definitions of healthy population that were used as a sample for new ULN, (ii) different 'original' (manufacturer recommended) ULNs of the ALT tests used in these studies, (iii) different approach to ALT sex variability in some studies, and (iv) different diseases used to assess the performance of the updated ALT ULN. We have therefore tried to adapt some of the proposed ALT ULNs for clinical use in our population. We have expressed updated ULNs relative to our original (manufacturer recommended) ALT ULN to compensate for the different essays used to test for ALT. We have found that patients with elevated ALT have the highest age-adjusted risk of MS when ALT ULN is set at around 70% of the original ULN, close to the ULN proposed by Kariv *et al.* [20]. In general, however, ALT is not, on its own, suitable for prediction of MS. Area under the curve was only 0.603 in our study, which corresponds to the published data [34].

Recent updates of normal ranges of various other biochemical parameters, such as triglycerides or LDL, demonstrate that the reference ranges of biochemical tests are not constant and they need to adapt to their current application. Our results, as well as the results published in respectable journals so far, suggest that in patients with MS one must consider liver involvement even if the patient has ALT levels in the upper third of the reference range, and there is the need for the discussion about the feasibility of lower ALT ULN in the clinical praxis.

The question remains as to how to approach a patient with ALT activity in the upper third of the currently used normal range. We see two distinct clinical scenarios that require specific approach. A population-based study from the USA showed that almost 40% of the population would have elevated ALT based on Prati criteria [3]. That makes

the lower ALT ULN not feasible for general liver disease screening. However, in patients with either documented liver disease (hepatitis B, C) or at risk of liver disease (MS), this lower ULN could be used to identify the patients with significant fibrosis [28], viremia [19], or NAFLD.

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Conflicts of interest

There are no conflicts of interest.

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