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Updated thresholds for serum alanine aminotransferase level in a large-scale population study composed of 34 346 subjects

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SUMMARY

Background
The sensitivity of current upper limit of normal (ULN) of serum alanine aminotransferase (ALT) levels for detecting chronic liver disease has been challenged recently.

Aim
To identify modulating factors for serum ALT levels and to refine its ULN threshold.

Methods
We enrolled 34 346 consecutive subjects who completed the health check-up at Taipei Veterans General Hospital from 2002 to 2009. ULN was set for healthy ALT level to the 95th percentile of the reference healthy population.

Results
A group of 21 282 subjects were used as a training set to define an ULN with the highest sensitivity; afterwards, this ULN was validated in another set of 13 064 subjects. A reference healthy population was selected from the training set after excluding subjects with any abnormalities in independent risk factors associated with elevated serum ALT level (>40 IU/L) by multivariate analysis like body mass index, waist circumference, glucose, cholesterol, high-density lipoprotein-cholesterol, triglyceride, hepatitis B virus surface antigen, anti-hepatitis C virus antibody and fatty liver. The new ULN of serum ALT level defined as the 95% percentile in the healthy population were 21 IU/L and 17 IU/L for men and women respectively. These cut-off values had the highest Youden's index and areas under the corresponding receiver operating curves among four widely applied thresholds in both the training and validation sets.

Conclusions
The suggested threshold of upper limit of normal provides better discrimination between healthy and unhealthy status. Viral hepatitis, metabolic syndrome and fatty liver are the major risk factors of elevated serum alanine aminotransferase levels.

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INTRODUCTION

Serum alanine aminotransferase (ALT) levels reflect the degree of liver cell damage and serum ALT assay is widely applied for screening and following-up of liver disorders in daily practice.[1] Its current upper limit of normal (ULN) is usually set at around 40 IU/L, although there may be slight variations among different laboratories.[2] These ULN thresholds were established in the 1980s to screen for hepatitis A virus and hepatitis B virus (HBV) infections among blood donors, and statistically set as the value at the 97.5th percentile of the 'healthy reference population'.[1] However, the effects of hepatitis C virus (HCV) infections and non-alcoholic fatty liver disease (NAFLD) had not been considered.[2–4]

Several studies demonstrate that substantial number of patients with chronic hepatitis B (CHB) or chronic hepatitis C (CHC) infections and normal serum ALT levels by current thresholds still have significant fibrosis and inflammation, and are at risk of liver disease progression, especially those with high-normal serum ALT (levels of 0.5–1 times ULN).[5–8] This may cause an underestimation of the prevalence of chronic liver diseases and the degree of liver damage in patients with CHB or CHC according to current ULN thresholds of ALT.

Consequently, the ULN thresholds of ALT have been challenged recently.[2, 5, 6, 9, 10] From a large-scale study in Italian blood donors, Prati and colleagues suggested an updated ULN of ALT of 30 IU/L for men and 19 IU/L for women to recognise HCV viraemia.[2] Another study from Korea conducted by Kang also revised the ULN of 'healthy' serum ALT level to 31 IU/L for men and 23 IU/L for women to better identify subjects with metabolic syndrome.[10] Thus, the ULN threshold of ALT needs to be revised to decrease the influences of HCV infection or NAFLD and to represent better, true healthy subjects. It is warranted to select a more optimal ULN threshold of ALT that takes account of sensitivity and specificity simultaneously for the discrimination of occult chronic liver diseases from healthy status. Consequently, this large-scale cohort study in Taiwan was conducted to identify modulating factors for serum ALT levels and to refine the ULN threshold of ALT.

MATERIALS AND METHODS

Study population

The study population consisted 34 346 consecutive subjects who completed the health check-up service at Taipei Veterans General Hospital from 2002 to 2009. There were 18 681 men (54.4%) and 15665 (45.6%) women, and all of them underwent complete clinical evaluation, laboratory examination and abdominal sonography. The ULN of waist circumference (WC) for Asian populations were 90 cm and were 80 cm for men and women respectively.[10] Body mass index (BMI) was calculated by dividing the body weight (in kilograms) by the square body height (in metres), and those with BMI > 24 kg/m^2 were defined as abnormal. Blood pressure (BP) was measured after the subjects had been seated for more than 5 min. Systolic and diastolic BP were recorded as the means of three consecutive readings, with a difference in systolic BP < 10 mmHg. The ULN of systolic and diastolic BP were 130 and 85 mmHg respectively. Fatty liver was diagnosed by ultrasonography according to the criteria of the American Gastroenterology Association.[11]

The study complies with the standards of the Declaration of Helsinki and has been approved by Institutional Review Board of Taipei Veterans General Hospital (2011-08-010IC).

Biochemical and serologic markers

Venous blood sample was collected after an overnight fast. Serum HBV surface antigen (HBsAg) was tested using radio-immunoassay (Abbott Laboratories, North Chicago, IL, USA) whereas antibody to hepatitis C virus (anti-HCV) was measured using second-generation enzyme immunoassay (Abbott Laboratories). Serum biochemical tests were measured using Roche/Hitachi Modular Analytics Systems (Roche Diagnostics GmbH, Mannheim, Germany). The reference limits of these tests were as follows: ALT level 40 IU/L; gamma-glutamyltransferase (GGT) level 51 IU/L; total cholesterol level, 200 mg/dL; high-density lipoprotein-cholesterol (HDL-C) level, 40 mg/dL for men and 50 mg/dL for women; low-density lipoprotein-cholesterol (LDL-C) level, 130 mg/dL; triglyceride (TG) level, 150 mg/dL; fasting glucose level, 100 mg/dL; and 2-h post-load plasma glucose level, 150 mg/dL.

Statistical analysis

The 21 282 persons who underwent physical check-up in the earlier 2/3 period of examination (October 2002–December 2006) were defined as the training set whereas the remaining 13 064 persons (January 2007–August 2009) were defined the validation set. (Figure 1) Initially, risk factors associated with abnormal serum ALT levels were assessed using the current threshold (>40 IU/L) in the training set. The reference healthy population was then selected by excluding subjects with any independent

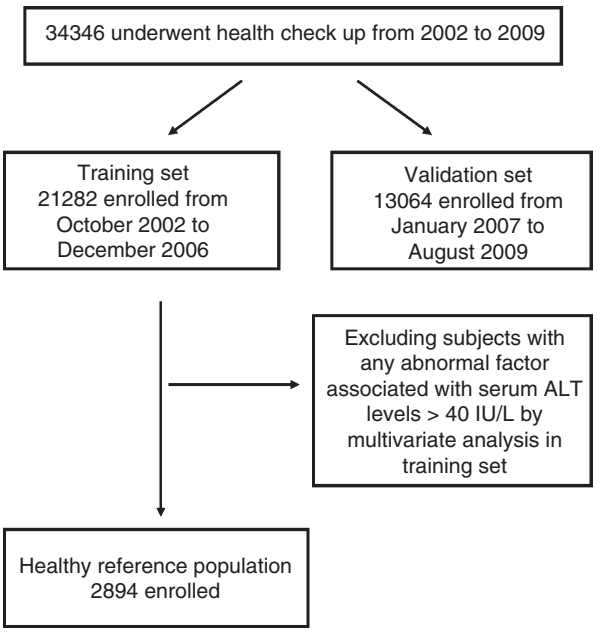

Figure 1 | The flow of subjects in the study.

risk factor correlating with abnormal serum ALT levels by multivariate analysis.

Subsequently, the mean, standard deviation and 95% CI of the whole population, subjects without viral hepatitis and the reference healthy population were estimated and stratified by gender and age. By this method, the ULN was set for healthy ALT level to the 95th percentile of the reference healthy population, which was commonly applied for distribution as a continuous variable.[2, 9, 10]

Pearson chi-squared analysis was used to compare categorical variables, whereas the Student *t*-test was performed to compare continuous variables. Variables with statistical significance ($P < 0.05$) or proximate to it ($P < 0.1$) by univariate analysis underwent multivariate analysis via the logistic regression model with the forward stepwise selection procedure.

The newly developed ULN for healthy ALT levels and three other thresholds (current threshold by manufacturers, Prati and Kang) were then validated to identify true healthy subjects in the training and validation sets.[2, 10] The accuracy of these four different ULN thresholds of ALT for predicting subjects with any abnormal finding was determined by testing the sensitivity, specificity and by calculating the area under the curve for the corresponding receiver operating curves (AUROC) and Youden's index. The AUROC was expressed as plots of the test sensitivity vs. 1 - specificity, whereas Youden's index was calculated by sensitivity + specificity − 1.[12] A two-tailed $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Factors associated with elevated serum ALT levels by current ULN threshold in the training set

The demographic data of subjects in the training set are shown in Table 1. Univariate analysis showed that subjects with serum ALT levels >40 IU/L were younger in age and had higher male-to-female ratio, higher BMI, larger WC, higher systolic and diastolic BP, higher fasting and 2-h post-load plasma glucose levels and higher total cholesterol, LDL-C, TG and GGT levels, but lower HDL-C levels and lower platelet counts than those with normal serum ALT levels by the current threshold. The incidence of HBsAg positivity, anti-HCV positivity and fatty liver were also higher in subjects with higher serum ALT levels.

By multivariate analysis, younger age, male sex, BMI > 24 kg/m^2, larger WC ($\geq$90 cm for men and $\geq$80 cm for women), glucose $\geq$ 100 mg/dL, cholesterol >200 mg/dL, lower HDL (<40mg/dL for men and <50mg/dL for women respectively), TG $\geq$ 150 mg/dL, presence of HBsAg, HCV positivity and fatty liver were the independent risk factors associated with abnormal serum ALT levels in the training set. (Table 2)

Distributions of serum ALT levels stratified by gender, age and study group in the training set

The distributions of serum ALT levels stratified by gender, age and the three study groups in the training set (i.e. whole study population, training set population excluding those with HBV or HCV infections and reference healthy population) were then evaluated. (Table 3) After excluding examinees with abnormal data in any independent risk factor associated with abnormal serum ALT levels, including BMI, WC, glucose, cholesterol, HDL, TG, HBsAg, anti-HCV and fatty liver, 2894 subjects were enrolled as the reference healthy population.

Serum ALT levels were higher in men than in women in all three populations. Regarding age, whole study population and subjects excluding viral hepatitis population both exhibited reciprocal association between ALT levels and age, with the highest levels occurring in 30–49 years for men and 50–69 years for women. For the

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Table 1 | Univariate analysis of factors associated with abnormal ALT in training set

Parameters	All (n = 21 282)	≤ 40 IU/L (n = 18 294)	>40 IU/L (n = 2988)	P value
Age, years*	52.4 ± 13.1	52.7 ± 13.3	50.7 ± 11.7	<0.0001
Male, n (%)	11 787 (55.4)	9606 (52.5)	2181 (73.0)	<0.0001
Body mass index, kg/m^2*	23.8 ± 3.5	23.4 ± 3.3	25.9 ± 3.7	<0.0001
Waist circumference, cm*	83.9 ± 10.1	83.0 ± 9.8	90.0 ± 10.2	<0.0001
Systolic BP, mmHg*	125.5 ± 18.6	125.0 ± 18.7	128.5 ± 17.9	<0.0001
Diastolic BP, mmHg*	78.2 ± 13.6	77.8 ± 13.1	80.9 ± 16.2	<0.0001
Fasting glucose, mg/dL*	97.0 ± 25.6	96.0 ± 24.6	103.6 ± 30.4	<0.0001
2-h post-load glucose, mg/dL*	121 ± 52.7	119.5 ± 50.9	133.6 ± 61.5	<0.0001
Cholesterol, mg/dL*	199.3 ± 37.0	198.4 ± 36.6	204.6 ± 38.8	<0.0001
HDL-C, mg/dL*	54.2 ± 14.6	55.1 ± 14.6	48.6 ± 12.8	<0.0001
LDL-C, mg/dL*	124.6 ± 32.7	123.8 ± 32.4	129.4 ± 34.0	<0.0001
Triglycerides, mg/dL*	127.2 ± 80.5	120.5 ± 73.7	168.1 ± 104.5	<0.0001
GGT, IU/L*	24.8 ± 36.3	20.1 ± 18.8	53.1 ± 79.2	<0.0001
HBsAg positivity, n (%)	2333 (11.0)	1803 (9.9)	530 (17.7)	<0.0001
Anti-HCV positivity, n (%)	583 (2.7)	387 (2.1)	196 (6.6)	<0.0001
Platelet, mm^3*	243.8 ± 60.8	244.4 ± 60.4	239.8 ± 63.2	0.0001
Fatty liver, n (%)	8878 (41.7)	6749 (36.9)	2129 (71.3)	<0.0001

BP, blood pressure; GGT, gamma-glutamyltransferase; HBsAg, hepatitis B surface antigen; HDL-C, high-density lipoprotein-cholesterol; HCV, hepatitis C virus; LDL-C, low-density lipoprotein-cholesterol.

* Expressed as mean ± s.d.

healthy reference population, the distributions of ALT levels were relatively stable across different age groups both in men and women. The 95th percentile of serum ALT levels were 21.03 IU/L for men and 17.29 IU/L for women. Consequently, healthy serum ALT values were defined as those below 21 IU/L for men and 17 IU/L for women.

Validation of different ULN thresholds of ALT for predicting unhealthy status

The discriminative ability of predicting unhealthy status of the newly suggested ULN of ALT and three other thresholds was then compared. In the training set, 18 388 (86.4%) subjects with an abnormal data in the nine independent risk factors associated with elevated serum ALT levels were classified as having 'unhealthy' status, whereas the remaining 2894 (13.6%) were defined as having 'healthy' status. Regarding the validation set, 11 174 (85.5%) subjects were 'unhealthy' whereas the remaining 1890 (14.5%) were 'healthy'.

In the training set, among the 7838 women 'unhealthy' subjects, 7057 (90.04%) had normal serum ALT levels by the current threshold of the manufacturers (<40 IU/L). (Table 4) With regard to the validation set, 4403 of the 4947 female 'unhealthy' subjects (89.00%) had serum ALT level less than 40 IU/L. In contrast, the proportions were only 42.18% in the training set and were 33.05% in the validation set by the suggested threshold of this study. Similarly, the proportions of subjects with normal serum ALT levels by the current threshold (40 IU/L) among the male subjects with unhealthy status were 79.74% and 74.92% in the training and validation sets respectively. On the contrary, the proportions were only 34.89% in the training set and were 25.47% in the validation set by the suggested threshold of this study. The proportions of unhealthy status in subjects with serum ALT levels within the ULN were lowest by the newly suggested threshold and highest by the current threshold of the manufacturers both in the training and validation sets regardless of gender. (Table 4) This suggested that majority of subjects with serum ALT levels less than the ULN by current thresholds were not truly healthy.

Of the four thresholds of ULN of ALT, the newly suggested threshold posited in this study had the highest Youden's index and AUROC in the training and validation sets in both male and female subjects. (Table 5) Thus, the threshold of this study had the best discriminative ability for predicting unhealthy status.

DISCUSSION

This study assessed risk factors associated with elevated serum ALT levels and refined the new threshold for

Table 2 | Factors associated with abnormal ALT in the training set, by multivariate analysis

	B	SE	OR	95% CI	*P* value
Age (per years)	−0.496	0.030	0.6089	0.5746–0.6452	<0.0001
Gender (male/female)	0.684	0.039	1.9815	1.8362–2.1383	<0.0001
Body mass index (>24 kg/m^2/ ≤ 24 kg/m^2)	0.465	0.043	1.5923	1.4623–1.7339	<0.0001
WC (male: ≥ 90 cm/<90 cm; female: ≥ 80 cm/80 cm)	0.395	0.041	1.4841	1.3691–1.6089	<0.0001
Glucose (≥ 100 mg/dL/<100 mg/dL)	0.337	0.038	1.4003	1.3007–1.5077	<0.0001
Cholesterol (>200 mg/dL/ ≤ 200 mg/dL)	0.271	0.034	1.3115	1.2259–1.4031	<0.0001
HDL-C (male:<40 mg/dL/ ≥ 40 mg/dL; female: <50 mg/dL/ ≥ 50 mg/dL)	0.252	0.038	1.2869	1.1953–1.3855	<0.0001
Triglycerides (≥ 150 mg/dL/<150 mg/dL)	0.387	0.038	1.4721	1.3677–1.5844	<0.0001
HBsAg (±)	0.848	0.047	2.3347	2.1301–2.5590	<0.0001
Anti-HCV (±)	1.679	0.081	5.3602	4.5752–6.2799	<0.0001
Fatty liver (yes/no)	0.978	0.040	2.6604	2.4618–2.8751	<0.0001

B, β regression coefficient; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HDL-C, high-density lipoprotein-cholesterol; OR, odds ratio; S.E., standard error; WC, waist circumference.

Table 3 | Serum ALT levels stratified by gender, age and study population in the training set

	Number		ALT levels					
			Male			Female		
Age (years)	Male	Female	Mean	s.d.	95% CI	Mean	s.d.	95% CI
Whole study population (*n* = 21 282)								
All	11 787	9495	31.63	29.60	(31.09–32.16)	23.17	20.89	(22.75–23.59)
<30	431	460	31.20	39.40	(27.47–34.93)	17.41	15.75	(15.97–18.85)
30–39	1411	1190	36.56	35.89	(34.69–38.43)	19.45	20.42	(18.29–20.61)
40–49	2721	2245	35.21	36.18	(33.85–36.57)	20.35	20.00	(19.52–21.17)
50–59	3776	3284	31.56	24.28	(30.79–32.34)	25.73	20.69	(25.02–26.44)
60–69	1898	1545	29.76	27.32	(28.53–30.99)	26.43	23.80	(25.24–27.62)
70–79	1138	640	24.27	17.77	(23.23–25.30)	23.32	18.87	(21.85–24.78)
≥ 80	412	131	21.02	15.58	(19.51–22.53)	22.18	17.38	(19.18–25.19)
Excluding viral hepatitis population (*n* = 18 411)								
All	10119	8292	29.50	21.61	(29.08–29.92)	21.90	17.90	(21.51–22.28)
<30	370	412	27.86	24.50	(25.36–30.37)	16.75	15.12	(15.28–18.21)
30–39	1182	1036	34.17	29.15	(32.51–35.84)	18.02	16.60	(17.00–19.03)
40–49	2242	1944	32.87	21.90	(31.96–33.78)	19.07	15.17	(18.39–19.74)
50–59	3258	2865	29.77	18.80	(29.12–30.41)	24.57	19.03	(23.87–25.26)
60–69	1642	1339	27.52	22.59	(26.42–28.61)	25.08	20.98	(23.95–26.20)
70–79	1043	576	23.08	14.30	(22.21–23.95)	21.69	13.20	(20.61–22.77)
≥ 80	382	120	20.72	15.42	(19.17–22.27)	20.85	15.21	(18.10–23.60)
Reference healthy population (*n* = 2894)								
All	1237	1657	20.40	11.36	(19.76–21.03)	16.80	10.15	(16.31–17.29)
<30	141	233	18.09	8.55	(16.67–19.52)	15.32	15.35	(13.34–17.30)
30–39	220	438	20.22	9.21	(18.99–21.44)	16.10	8.29	(15.32–16.88)
40–49	245	538	21.91	12.71	(20.31–23.51)	16.45	9.61	(15.63–17.26)
50–59	298	356	21.58	13.73	(20.01–23.14)	18.81	8.98	(17.87–19.74)
60–69	145	69	20.86	12.56	(18.79–22.92)	17.88	7.76	(16.02–19.75)
70–79	129	19	18.64	7.69	(17.30–19.98)	19.32	7.67	(15.62–23.01)
≥ 80	59	4	17.03	6.21	(15.42–18.65)	18.75	3.30	(13.49–24.01)

s.d., standard deviation.

ULN of ALT from a large population composed of subjects who underwent physical check-up. The major finding is that most subjects with normal serum ALT levels by the current threshold still have unhealthy status, including viral hepatitis, metabolic syndrome and fatty liver. The study also demonstrates that the optimal

threshold of ULN for ALT is 21 IU/L for men and 17 IU/L for women for better discrimination between healthy and unhealthy status.

For patients with chronic hepatitis, the current threshold for ULN of serum ALT levels (40 IU/L) has been challenged in several points of view. First, Lai showed that 37% of CHB patients with serum ALT levels <40 IU/L still had significant liver inflammation and fibrosis, especially for those with serum ALT levels between 26 and 40 IU/L.[13] For CHC patients, Boccato demonstrated that among 33 patients with normal serum ALT levels, 13 (39.4%) had progression of fibrosis on repeated liver biopsy, with a mean interval of 7.8 years.[6] These studies suggest that substantial patients with normal serum ALT levels by current thresholds still have ongoing hepatic necro-inflammation and fibrosis, and are not truly healthy.

Second, one large population-based prospective study in Korea observed that subjects with serum ALT levels >20 IU/L had significantly higher rates of mortality from liver diseases than their counterparts.[8] Another study conducted by Yuen also demonstrated that CHB patients high-normal serum ALT (levels 0.5–1-times ULN) had higher incidences of developing complications of cirrhosis than those with serum ALT levels less than 0.5-times ULN.[7] Regarding hepatocellular carcinoma (HCC), Kumada demonstrated that serum ALT level >20 IU/L was an independent risk factor associated with the development of HCC in patients with CHC.[14] Similarly, for HBV carriers, the REVEAL study from Taiwan also showed that serum ALT level >15 IU/L predicted HCC occurrence and was an important predictor of the nomogram for estimating HCC risk in their model.[15] This implies that subjects with serum ALT levels more than 0.5-time ULN may not only have significant liver inflammation and fibrosis but also carry a risk of developing cirrhosis and HCC.

Third, for patients who received adequate anti-viral therapy, serum ALT levels often decreased to levels below 30 U/L when there was sustained virological response.[10, 16] Fourth, in the current study, majority of subjects who had serum ALT levels within the ULN by current threshold were still unhealthy. Taken together, the ULN of serum ALT levels by the current threshold could not reflect the true healthy status and needed to be revised.

Table 4 | The numbers and proportions of subjects with normal and elevated serum ALT levels defined by the four thresholds of the ULN stratified by healthy status

	Healthy status with normal ALT (%)	Healthy status with elevated ALT (%)	Unhealthy status with normal ALT (%)	Unhealthy status with elevated ALT (%)
Men				
Training set (n = 11 787, healthy: 1237, unhealthy: 10 550)				
Current	1193 (96.44%)	44 (3.56%)	8413 (79.74%)	2137 (20.26%)
Kang	1126 (91.03%)	111 (8.97%)	6877 (65.18%)	3673 (34.82%)
Prati	1106 (89.41%)	131 (10.59%)	6657 (63.10%)	3893 (36.90%)
Wu	838 (67.74%)	399 (32.26%)	3681 (34.89%)	6869 (65.11%)
Validation set (n = 6894, healthy: 667, unhealthy: 6227)				
Current	646 (96.85%)	21 (3.15%)	4665 (74.92%)	1562 (25.08%)
Kang	602 (90.25%)	65 (9.75%)	3633 (58.34%)	2594 (41.66%)
Prati	595 (89.21%)	72 (10.79%)	3475 (55.81%)	2572 (44.19%)
Wu	424 (63.57%)	243 (36.43%)	1586 (25.47%)	4641 (74.53%)
Women				
Training set (n = 9495, healthy: 1657, unhealthy: 7838)				
Current	1631 (98.43%)	26 (1.57%)	7057 (90.04%)	781 (9.96%)
Kang	1445 (87.21%)	212 (12.79%)	5276 (67.31%)	2562 (32.69%)
Prati	1259 (75.98%)	398 (24.02%)	4068 (51.90%)	3770 (48.10%)
Wu	1110 (66.99%)	547 (33.01%)	3306 (42.18%)	4532 (57.82%)
Validation set (n = 6170, healthy: 1223, unhealthy: 4947)				
Current	1201 (98.20%)	22 (1.80%)	4403 (89.00%)	544 (11.00%)
Kang	1067 (87.24%)	156 (12.76%)	3054 (61.73%)	1893 (38.27%)
Prati	907 (74.16%)	316 (25.84%)	2202 (44.51%)	2745 (55.49%)
Wu	777 (63.53%)	446 (36.47%)	1635 (33.05%)	3312 (66.95%)

ULN threshold of ALT, current 40 IU/L; Kang, 31 IU/L for men and 23 IU/L for women; Prati, 30 IU/L for men and 19 IU/L for women; Wu (this study), 21 IU/L for men and 17 IU/L for women.

Table 5 | Diagnostic accuracies of four thresholds of the ULN of ALT for predicting unhealthy status

	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Youden's index	AUROC (95% CI)
Men				
Training set				
Current	20.3(19.5–21.0)	96.4(95.3–97.4)	0.167	0.583(0.575–0.592)
Kang	34.8(33.9–35.7)	91.0(89.3–92.6)	0.258	0.629(0.620–0.638)
Prati	36.9(36.0–37.8)	89.4(87.6–91.1)	0.263	0.632(0.623–0.640)
Wu	65.1(64.2–66.0)	67.7(65.1–70.3)	0.328	0.664(0.656–0.673)
Validation set				
Current	25.1(24.0–26.2)	96.9(95.2–98.0)	0.220	0.610(0.598–0.621)
Kang	41.7(40.4–42.9)	90.3(87.7–92.4)	0.320	0.660(0.648–0.671)
Prati	44.2(43.0–45.5)	89.2(86.6–91.5)	0.334	0.667(0.656–0.678)
Wu	74.5(73.4–75.6)	63.6(59.8–67.2)	0.381	0.690(0.679–0.701)
Women				
Training set				
Current	10.0(9.3–10.6)	98.4(97.7–99.0)	0.084	0.542(0.532–0.552)
Kang	32.7(31.6–33.7)	87.2(85.5–88.8)	0.199	0.599(0.590–0.609)
Prati	48.1(47.0–49.2)	76.0(73.8–78.0)	0.241	0.620(0.611–0.630)
Wu	57.8(56.7–58.9)	67.0(64.7–69.3)	0.248	0.624(0.611–0.630)
Validation set				
Current	11.0(10.1–11.9)	98.2(97.3–98.9)	0.092	0.546(0.533–0.558)
Kang	38.3(36.9–39.6)	87.2(85.2–89.1)	0.255	0.628(0.615–0.640)
Prati	55.5(54.1–56.9)	74.2(71.6–76.6)	0.297	0.648(0.636–0.660)
Wu	67.0(65.6–68.3)	63.5(60.8–66.2)	0.305	0.652(0.640–0.664)

ULN threshold of ALT, current 40 IU/L; Kang, 31 IU/L for men and 23 IU/L for women; Prati, 30 IU/L for men and 19 IU/L for women; Wu (this study), 21 IU/L for men and 17 IU/L for women.

In the present study, BMI, WC, glucose, total cholesterol, HDL, TG and fatty liver are all independent risk factors correlated with elevated serum ALT levels, suggesting a close relationship among metabolic syndrome, central obesity, fatty liver and liver injury.[17–19] Metabolic syndrome and NAFLD have become the most common aetiologies of abnormal liver biochemistry tests both in high (such as Taiwan) and low endemic countries (like the USA) for viral hepatitis.[20–22] It can be expected that NAFLD and non-alcoholic steatohepatitis will be important risk factors of developing liver-related complications like cirrhosis and HCC worldwide in the near future.[4]

Moreover, recent studies further demonstrate that NAFLD is associated with cardiovascular disease and malignancies, including colon, pancreas, breast and kidney cancers.[15, 23–25] A community-based study conducted in the US also correlated NAFLD with an increased risk of overall death.[24] Consequently, NAFLD has become a growing global public health threat that should be diagnosed and managed early using adequate screening tools.

The proportions of subjects with serum ALT levels within the ULN in the unhealthy population are lowest by the new threshold suggested by the present study. However, although this reflected the lowest value, it also compromised specificity. To select the more suitable cut-off for differentiating healthy and unhealthy status, which taking both the sensitivity and specificity into consideration, the Youden's index and AUROC were compared among the suggested threshold and three other widely applied thresholds. The newly suggested cut-off value has the highest values by these two methods in both the training and validation sets, suggesting that this revised threshold has the best discriminative ability for detecting unhealthy status. Screening for viral hepatitis, metabolic syndrome and fatty liver, which all carry risks of developing liver-related complications and mortality, can be recommended for daily practice.

The major strengths of this study are the large sample size, detailed biochemistry data and consistency of the results between the training and validation sets strengthen the power of the results. However, there are also several limitations. First, we lack the detailed information on alcohol status from the retrospective analysis. Alcohol is an important risk factor correlated with elevated serum ALT levels in the Western countries.[26] However, a community study enrolling 3260

subjects in Taiwan has demonstrated that HBV infection, HCV infection and NAFLD are the major aetiologies for elevated serum ALT levels, and only 0.8% of subjects with elevated ALT were due to alcohol consumption.[20] Another population-based cross-sectional survey conducted in China also showed that alcohol drinking was not associated with serum ALT levels both in NAFLD and non-NAFLD subjects.[27] In our current study, the majority of subjects who underwent self-paid physical check-up were not alcoholism, and the mean serum GGT level was as low as 20.1 IU/L (ULN 51 IU/L) for those with serum ALT level 40 IU/L or less. (Table 1) This suggests that alcohol drinking may only have a minor impact on increasing serum ALT levels in Asian populations, including our current study. More prospective studies are warranted to elucidate the effect of alcoholism on elevated serum ALT levels in Asia.

Second, although autoimmune hepatitis and genetic liver diseases had extremely lower incidences than viral hepatitis and NAFLD in Taiwan, they may still influence the serum ALT levels in our cohort. Nevertheless, we could not evaluate their impact on the threshold of ULN of serum ALT levels. Third, this study is its cross-sectional design and its reliance on a single ALT testing. The long-term outcomes and liver-related complications between subjects with serum ALT levels within and beyond the newly suggested threshold cannot be compared.

Fourth, these newly suggested thresholds of ULN of serum ALT levels were generated from the Taiwanese population. Whether or not they could be extrapolated to the Western populations still need more studies to be validated. Moreover, although our newly suggested thresholds of ULN of serum ALT levels might help to identify more unhealthy subjects, it could simultaneously cause over diagnosis some healthy subjects as unhealthy. It would increase unnecessary medical costs and cause the anxiety about liver diseases among these subjects with non-existent liver diseases.

In conclusion, the thresholds of ULN of serum ALT levels should be lowered to 21 IU/L for men and 17 IU/L for women to detect more unhealthy subjects and for optimal discrimination between healthy and unhealthy status. Moreover, viral hepatitis, metabolic syndrome and fatty liver are the major independent risk factors associated with elevated serum ALT levels by current ULN thresholds.

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