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Original article

Development and evaluation of percentile curves of serum alanine aminotransferase in older adults: A multi-cohort study

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ABSTRACT

Introduction and Objectives: Age independently impacts alanine aminotransferase (ALT) levels. This study was conducted to develop age- and sex-specific ALT percentile curves among older adults and evaluate their diagnostic performance across two external cohorts.
Materials and Methods: We developed ALT percentile curves using data from a reference population aged 50–90 years (n = 20,039). We evaluated diagnostic performance of various ALT thresholds (40 U/L, American College of Gastroenterology [ACG]'s 33 U/L [men] and 25 U/L [women], and the new percentile curves) for infections of hepatitis B virus and hepatitis C virus, metabolic dysfunction associated steatotic liver disease, and excessive alcohol consumption in two external cohorts.
Results: ALT percentile curves declined with age. In men, the 95th percentile decreased from 31.4 U/L at 50 years to 21.7 U/L at 90 years; in women, from 26.1 U/L to 17.8 U/L. The 95th percentile curves achieved the highest Youden's index and area under the receiver operating characteristic (AUROC) across the three thresholds in two external validation cohorts, with the Youden's index and AUROC of 0.141 and 0.571 (95% CI: 0.555–0.586) in external cohort 1, and 0.435 and 0.717 (95% CI: 0.680–0.754) in external cohort 2, respectively.
Conclusions: The newly proposed ALT percentile curves may serve as a valuable reference for screening liver diseases in older adults.

1. Introduction

Serum alanine aminotransferase (ALT) levels are widely recognized as markers for hepatocellular injury and are routinely used in clinical practice to screen and monitor liver disorders [1]. Historically, the upper limit of normal (ULN) for ALT has been set at approximately 40 U/L, with minor variations across different healthcare institutions [2]. These ULN thresholds were established in the 1980s, originally aimed at screening blood donors for hepatitis A virus (HAV) and hepatitis B virus (HBV) infections. They were statistically derived to represent the 97.5th percentile of a population considered healthy [2,3]. However, these thresholds fail to account for factors such as alcohol consumption, metabolic dysfunction associated steatotic liver disease (MASLD), and hepatitis C virus (HCV) infection, potentially leading to an overestimation of the ULN values.

Several studies have indicated that the ULNs for serum ALT should be set lower than the commonly used standards, with sex-specific thresholds being necessary [2,4–6].

These studies recommend 29–33 U/L for men and 19–25 U/L for women. Lower ULN values have demonstrated improved sensitivity in identifying participants with liver diseases compared to the commonly used ULN of 40 U/L [2,5]. In agreement with those studies, the American College of Gastroenterology (ACG) recommends "new normal" levels for serum ALT of 33 U/L for men and 25 U/L for women, respectively [7]. Age has also been found to independently affect ALT levels [8,9]. Several studies have suggested the negative association between ALT level and age, although some have suggested an

Abbreviations: ALT, alanine aminotransferase; ACG, American College of Gastroenterology; HBV, hepatitis B virus; HCV, hepatitis C virus; MASLD, metabolic dysfunction associated steatotic liver disease; NAHNES, National Health and Nutrition Examination Survey; ULN, upper limit of normal

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inverted U-curve relationship [10]. Although many studies have established new ALT thresholds for pediatric populations, thresholds for older adults are still lacking. Furthermore, discrete ULNs do not fully capture the continuous changes that occur during biological development, potentially leading to inaccuracies in representing the extent and timing of age-related dynamics. Therefore, adopting a continuous approach using age- and sex-specific percentile curves provides a more suitable method for evaluating laboratory analytes. This methodology was consequently applied in our study [11].

Therefore, this study aimed to: 1) develop novel ALT percentile curves from a cohort of reference older adults; and 2) evaluate and compare the discrimination performance of new ALT percentile curves against other ULN thresholds in classifying four prevalent chronic liver diseases/conditions: HBV infection, HCV infection, MASLD, and excessive alcohol consumption, across two external validation cohorts.

2. Materials and Methods

2.1. Study overview

We used three different cohorts, including a derivation cohort to develop the age- and sex-specific percentile curves for ALT in older adults (50–90 years) and two external validation cohorts to assess the performance of the newly proposed percentile curves in classifying individuals with four common chronic liver diseases/conditions (Fig. 1).

2.2. Participants and measurement

2.2.1. The derivation cohort

The derivation cohort consisted of population-based health checkup data collected between 2016 and 2022 in Deqing, China. Since 2009, the Chinese government has been annually providing Basic Public Health Services (BPHS) free of charge. These services assess the health status of civilian and non-institutionalized Chinese civilians in primary care settings [12]. The BPHS offers complimentary primary healthcare services, such as routine health check-ups and health counselling, to local residents. Health check-ups were conducted on-site at community health centers (CHCs) located in Deqing, a county in Zhejiang province, China, which comprises 47 communities and has a resident population of 0.5 million. The detailed variables and measurements used in this study are provided in Supplementary Table 1. In establishing a reference population of 'healthy' subjects, participants were carefully selected by excluding individuals meeting specific criteria [5,13,14]. These included outliers in ALT levels, identified by Tukey's method adjusted for sex and age groups annually [15]. Additionally, individuals with metabolic risk factors such as elevated fasting glucose (≥105 mg/dL), low HDL cholesterol (≤40 mg/dL for men, ≤50 mg/dL for women), high triglycerides (≥200 mg/dL), elevated total cholesterol (≥220 mg/dL), or a BMI ≥23 kg/m^2 as per WHO guidelines for the Asian population were excluded [16]. Those reporting alcohol intake exceeding 30 g/day for men or 20 g/day for women, as well as those positive for HCV antibody or hepatitis B surface antigen, were also excluded from the study cohort. Fig. 2 provides a detailed flowchart illustrating the selection process of study participants in the derivation cohort.

2.2.2. Two external validation cohorts

External validation cohort 1 initially included 15,560 participants from the National Health and Nutrition Examination Survey (NHANES) from 2017 to 2020. The NHANES comprises multiple waves of surveys conducted by the Centers for Disease Control and Prevention (CDC), targeting a nationally representative sample of the non-institutionalized civilian population in the United States [17]. The study design, sampling, and measurement of the NHANES data are previously described in detail [18,19]. The external validation cohort 1 was constructed by excluding participants: 1) aged <50 years

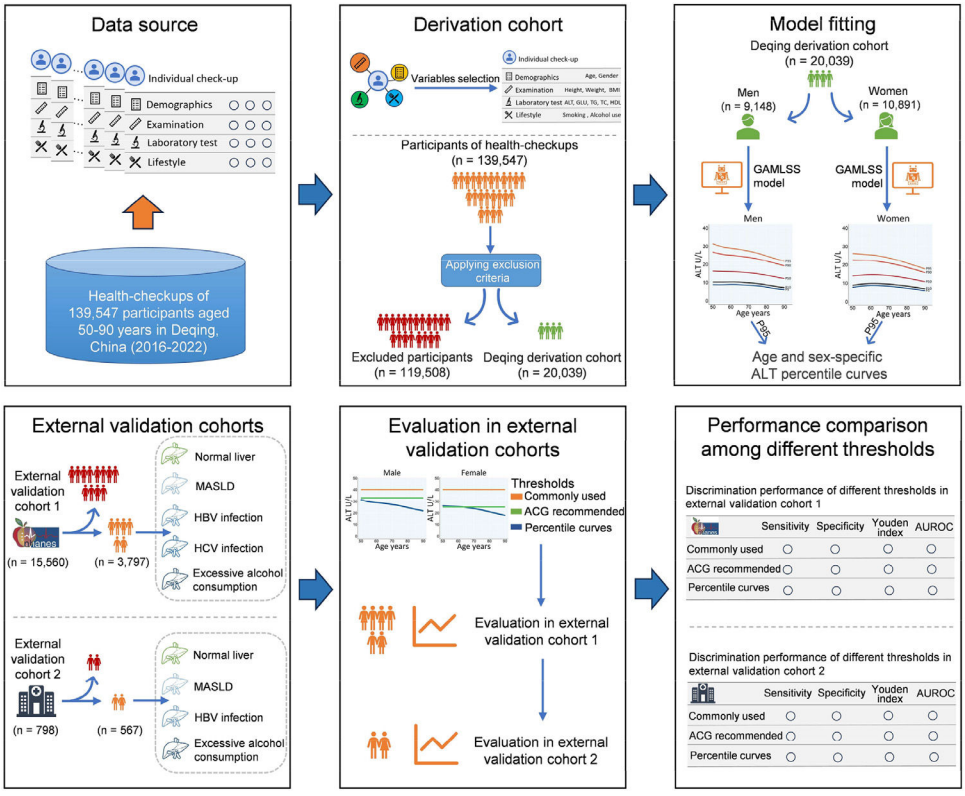

Fig. 1. Overview of the study design.ALT, alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; MASLD, metabolic dysfunction associated steatotic liver disease.

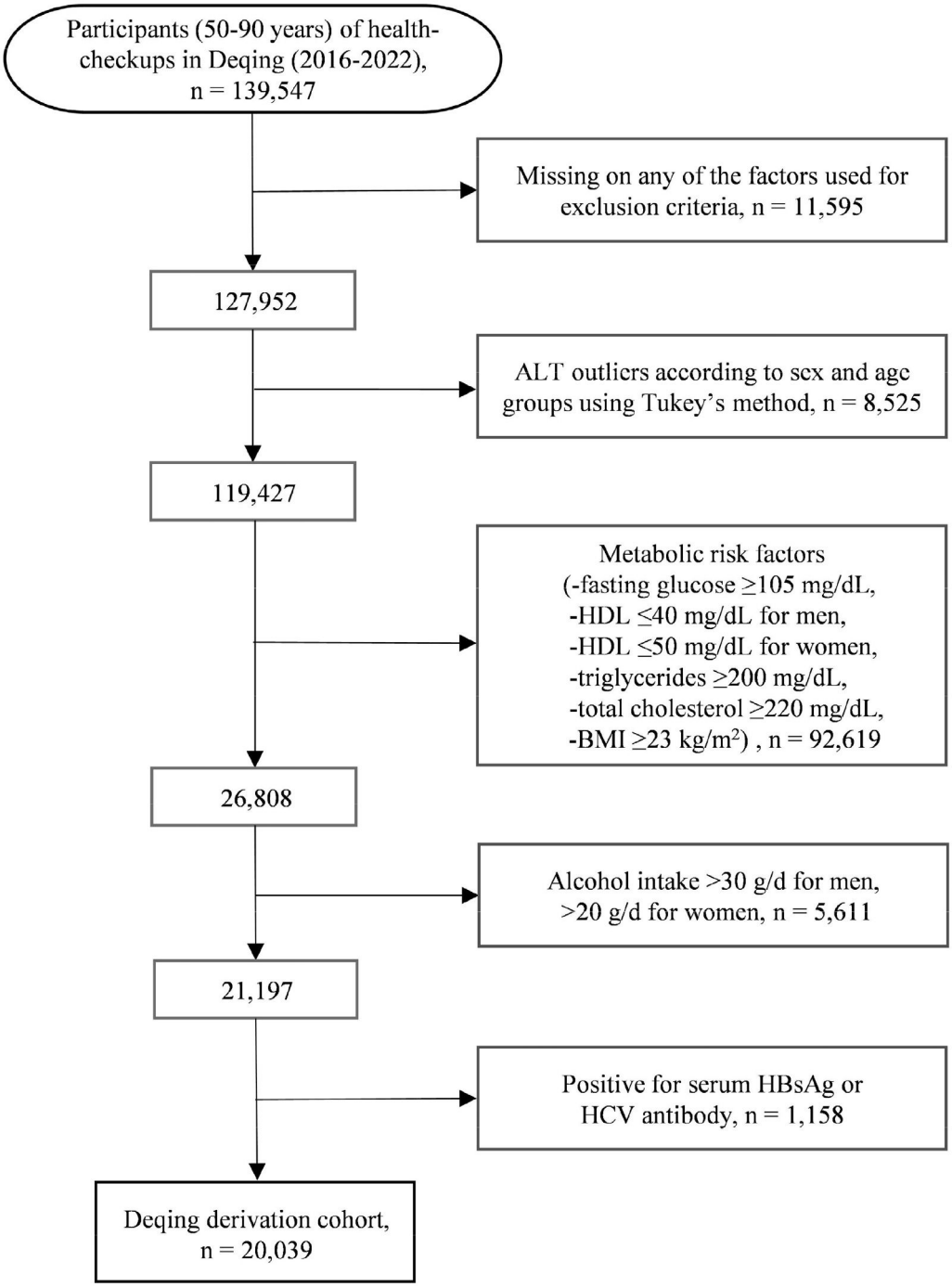

Fig. 2. Flowchart of study participants in the derivation cohort.ALT, alanine aminotransferase; HDL, high-density lipoprotein; BMI, body mass index; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

(n = 10,573), 2) having missing data on ALT measurement (n = 739), 3) having missing information on alcohol use (n = 248), HBV or HCV infection (n = 28) or information related to MASLD's definition (components for Fatty Liver Index [FLI] calculation including serum levels of γ-GTP and triglycerides, BMI, and waist circumference) (n = 175). Finally, external validation cohort 1 comprised 3,797 participants. The detailed flowchart of participant selection is shown in Supplementary Fig. 1A. As an external validation cohort for the newly proposed ALT percentile curves, the participants in this cohort were classified as having four common chronic liver diseases/conditions (HBV infection, HCV infection, MASLD, and excessive alcohol consumption). HBV and HCV infections were defined by the presence of hepatitis B surface antigen and hepatitis C RNA positivity, respectively. Excessive alcohol consumption was defined as a daily alcohol intake of more than 30 g for men and 20 g for women [20]. MASLD was defined as having a Fatty Liver Index score ≥60 [21], after excluding alternative causes. The following equation was used to calculate the FLI:

$$FLI = \frac{(e^{(0.953 * ln(triglycerides) + 0.139 * BMI + 0.718 * ln(GGT) + 0.053 * waist\ circumference - 15.745)})}{(1 + e^{(0.953 * ln(triglycerides) + 0.139 * BMI + 0.718 * ln(GGT) + 0.053 * waist\ circumference - 15.745)})} * 100$$

The characteristics of participants in external cohort 1 are presented in Supplementary Table 3.

In order to evaluate the clinical performance of the newly developed ALT percentile curves, we conducted a retrospective study at the Department of Gastroenterology, First Affiliated Hospital of Zhejiang University, between January 1 and April 1, 2023. The initial cohort comprised 795 consecutive hospitalized patients with various gastroenterological diseases such as gastrointestinal polyps, Crohn's disease, ulcerative colitis, and gastrointestinal bleeding. Eligible patients were required to possess complete test results for HBV and HCV infections and undergo ultrasound (US) or computed tomography (CT) liver examination. We excluded patients with diseases that might influence ALT levels, which could bias the discrimination of the proposed ALT percentile curves [4]. Finally, external validation cohort 2 consisted of 567 participants. The detailed flowchart of participant selection is shown in Supplementary Fig. 1B. Two independent physicians retrospectively examined the electronic medical records (EMRs) to classify patients into normal liver, HBV, HCV, MASLD, and excessive alcohol consumption, resolving any discrepancies by consultation with a senior physician. HBV and HCV infections were defined by hepatitis B surface antigen and hepatitis C RNA positivity, respectively. Excessive alcohol consumption was defined as a daily alcohol intake of more than 30 g for men and 20 g for women. MASLD was defined as the presence of fatty liver diagnosed via US or CT, with subsequent exclusion of alternative causes [22]. Fatty liver was diagnosed using widely used imaging modalities, including US and CT. Ultrasound suggests hepatic steatosis through signs like increased brightness, liver-to-kidney contrast, beam attenuation, and clear vessel and gallbladder walls [23]. The liver-to-spleen signal intensity ratio on unenhanced CT scans has been the most commonly used parameter to discriminate hepatic steatosis, with reported specificity and sensitivity of 100% and 82%, respectively [24]. The patient characteristics in external cohort 2 are reported in Supplementary Table 4.

2.3. Statistical analysis

ALT levels vary by sex and age, necessitating a method that accounts for these differences as continuous functions over age. Recent studies advocate for this approach to accurately capture the physiological changes in laboratory analytes [9,25,26]. To achieve this, percentile curves for ALT were developed using the generalized additive model for location, scale, and shape (GAMLSS) implemented in the R package gamlss. This modeling technique predicts percentile curves (5th, 10th, 50th, 90th, and 95th) based on four parameters: location (μ), scale (σ), skewness (ν), and kurtosis (τ). The GAMLSS was fitted to the data using penalized maximum likelihood estimation, and the model fit was evaluated using the generalized Akaike Information Criterion (GAIC). Comprehensive details of the statistical models employed can be found in the Supplementary Methods.

The newly proposed 95th percentile curves were evaluated in external validation cohorts 1 and 2 to classify individuals with normal livers and those with chronic liver diseases/conditions, respectively. Evaluation parameters, including sensitivity, specificity, Youden's index (sensitivity + specificity - 1), and AUROC, were calculated and compared with the commonly used reference of 40 U/L and the ACG's recommended thresholds of 33 U/L for men and 25 U/L for women. All statistical analyses were performed using R software (version 4.3.0).

2.4. Ethical considerations

The study protocol was reviewed and approved by the Institutional Review Board of the First Affiliated Hospital of Zhejiang University and was conducted in accordance with the Declaration of Helsinki. The derivation cohort utilized data from health checkups conducted as part of the BPHS. External validation cohort 1 used publicly available NHANES data. External validation cohort 2 was retrospectively analyzed. Therefore, the requirement for written informed consent was exempted.

3. Results

3.1. Participant characteristics

After applying exclusion criteria, we ultimately included 20,039 participants who were considered to be healthy used as derivation cohort, consisting of 9,148 males and 10,891 females (Table 1). For the healthy subgroups, the mean ages for men (68.9 ±9.5 years) were older than that of women (65.6 ±10.0 years). The mean ALT was also higher in men (17.2 ±11.0 U/L) than that in women (15.7 ±10.4 U/L). The healthy men have lower mean of serum total cholesterol (172.1 ±24.8 v.s.186.1 ±21.2, mg/dL), serum triglyceride (85.9 ±30.4 vs. 92.3 ±30.7, mg/dL), and HDL (54.1 ±9.7 vs. 60.4 ±8.1 mg/dL) than that of females. Men were more likely to report tobacco and alcohol use than females. Compared to those healthy people, the unhealthy

Table 1
Clinical and behavior characteristics of total population, healthy subgroup and unhealthy subgroup in the derivation cohort.

Characteristics	Men			Women		
	Total population (n = 6,6321)	Healthy subgroup (n = 9148)	Unhealthy subgroup (n = 57,173)	Total population (n = 81,169)	Healthy subgroup (n = 10,891)	Unhealthy subgroup (n = 70,278)
Mean (SD)						
Age, years	66.1 (±9.0)	68.9 (±9.5)	65.7 (±8.8)	65.4 (±9.4)	65.6 (±10.0)	65.2 (±9.2)
BMI, kg/m^2	23.1 (±3.5)	20.5 (±1.7)	24.4 (±3.1)	23.8 (±3.7)	20.4 (±1.9)	24.3 (±3.2)
Total cholesterol, mg/dL	179.1 (±33.8)	172.1 (±24.8)	183.0 (±36.8)	195.1 (±36.1)	186.1 (±21.2)	196.7 (±38.1)
Triglyceride, mg/dL	112.0 (±58.3)	85.9 (±30.4)	130.9 (±75.7)	137.9 (±72.3)	92.3 (±30.7)	144.6 (±72.5)
Glucose, mg/dL	98.0 (±23.5)	87.6 (±8.8)	101.1 (±27.4)	98.6 (±24.2)	88.6 (±8.1)	100.3 (±25.8)
HDL, mg/dL	51.7 (±11.6)	54.1 (±9.7)	49.7 (±13.6)	52.2 (±11.3)	60.4 (±8.1)	50.6 (±11.4)
ALT, U/L	20.9 (±15.2)	17.2 (±11.0)	22.8 (±16.9)	19.6 (±14.6)	15.7 (±10.4)	20.1 (±15.2)
Number (%)						
Alcohol consumption						
Non-drinker	33,452 (61.2)	8,311 (90.9)	25,141 (55.3)	67,279 (97.6)	10,840 (99.5)	56,439 (96.2)
≤Alcohol upper limit*	3103 (5.7)	837 (9.1)	2,266 (5.0)	935 (1.4)	51 (0.5)	884 (1.5)
>Alcohol upper limit*	18,069 (33.1)	0 (0)	18,069 (39.7)	733 (1.1)	0 (0)	733 (1.3)
Cigarettes use						
None-smoker	23,662 (42.0)	4003 (44.3)	19,659 (41.6)	68,832 (99.3)	10,740 (99.4)	58,092 (99.3)
Current-smoker	25,683 (45.6)	4145 (45.5)	21,538 (45.6)	403 (0.6)	61 (0.5)	342 (0.6)
Ex-smoker	6944 (12.3)	926 (10.2)	6,018 (12.8)	58 (0.1)	8 (0.1)	50 (0.1)

BMI, body mass index; HDL, high-density lipoprotein; ALT, alanine aminotransferase.
* Alcohol upper limit refers to 30g per day for men and 20g per day for women. Data are shown as n (% values) and mean ± SD.

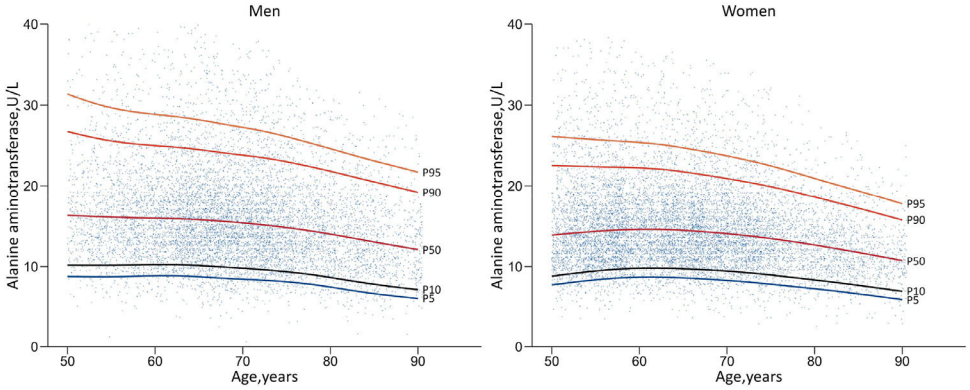

Fig. 3. Age- and sex-specific percentile curves of alanine aminotransferase (ALT) in men and women.

subgroup was more likely to have higher BMI, serum total cholesterol, serum triglyceride, fasting glucose, and ALT in both genders. However, HDL was generally lower in unhealthy individuals than in healthy individuals. The unhealthy subgroup was more likely to report alcohol use than the healthy subgroup; however, tobacco use was comparable in the two subgroups. Density plots revealed a clear distinction between the ALT distributions in the healthy and unhealthy subgroups. Specifically, the ALT distribution for the healthy subgroup was shifted to the left compared to the unhealthy subgroup. This leftward shift indicates that the ALT levels in the healthy subgroup were generally lower, suggesting that the individuals in this group were indeed healthy (Supplementary Fig. 2). The detailed information on ALT levels by age group and sex in derivation and validation cohorts is shown in Supplementary Table 2.

3.2. Age- and sex-specific percentile curves of ALT

Fig. 3 presents the 5th, 10th, 50th, 90th, and 95th percentile curves of ALT levels in the derivation cohort of male and female older adults. The ALT percentile curves exhibited similar patterns in men and women, characterized by a gradual decline with age. In men, the 95th percentile (P95) and 90th percentile (P90) demonstrated a sustained decrease over the entire age span, with a reduction in the rate of decline between ages 55–70 years. The 5th percentile (P5), 10th percentile (P10), 50th percentile (P50) in men appeared to plateau from the ages of 50–70, after which they gradually began to decline. In contrast to men, P90 and P95 remained stable initially between the ages 50–60 years, followed by a gradual drop, accelerating at the age of 70 in women. The median ALT serum concentration ranged from 12.1 to 16.4 U/L in men and from 10.7 to 13.9 U/L in women. In men, the 95th percentile decreased from 31.4 U/L at 50 years to 21.7 U/L at 90 years, whereas in women, it decreased from 26.1 U/L at 50 years to 17.8 U/L at 90 years. Detailed reference values for ALT, including P5, P10, P50, P90, and P95, along with the coefficient of variation (sigma), skewness (nu), and kurtosis (tau), are provided for age groups and are disaggregated by sex in Supplementary Table 5 and Supplementary Table 6.

3.3. Discrimination performance of different thresholds of ALT in external validation cohort 1

Table 2 presents the evaluation metrics of the proposed percentile curves and comparisons with other thresholds in external validation cohort 1. The commonly used threshold of 40 U/L exhibited a low sensitivity of 9.4% (95% CI: 8.4 %–10.3%) and high specificity of 97.4% (95% CI:97.0% %–98.0%). The ACG's recommended ALT thresholds showed an increased sensitivity of 20.1% (95% CI: 18.8%–21.3%), but with a decreased specificity of 91.6% (95% CI: 90.9%–92.6%). The percentile curves exhibited the highest sensitivity among the thresholds considered at 27.4% (95% CI: 26.0%–28.8%). However, the specificity of the proposed percentile curves was low at 86.6% (95% CI: 85.7% –87.8%). When it comes to overall evaluation metrics, the newly proposed percentile curves appear to yield the best performance with the Youden's index of 0.141 and AUROC of 0.571(95% CI: 0.555 –0.586), followed by the ACG thresholds with the Youden's index of 0.117 and AUROC of 0.559 (95% CI: 0.543–0.575), and commonly

Table 2
Discrimination performance of different thresholds of ALT for predicting chronic liver diseases/conditions in external validation cohort 1.

		Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	Youden's index	AUROC (95%CI)
All causes	Commonly used	9.4 (8.4 - 10.3)	97.4 (97.0 - 98.0)	0.068	0.534 (0.518 - 0.550)
	ACG recommended	20.1 (18.8 - 21.3)	91.6 (90.9 - 92.6)	0.117	0.559 (0.543 - 0.575)
	Percentile curves	27.4 (26.0 - 28.8)	86.6 (85.7 - 87.8)	0.141	0.571 (0.555 - 0.586)
MASLD	Commonly used	9.0 (8.0 - 9.9)	94.6 (93.8 - 95.3)	0.035	0.518 (0.502 - 0.533)
	ACG recommended	19.2 (17.9 - 20.4)	86.5 (85.4 - 87.6)	0.056	0.528 (0.512 - 0.544)
	Percentile curves	27.1 (25.6 - 28.5)	80.9 (79.7 - 82.2)	0.080	0.540 (0.524 - 0.556)
HBV infection	Commonly used	18.8 (17.5 - 20.0)	93.3 (92.5 - 94.1)	0.120	0.560 (0.544 - 0.576)
	ACG recommended	31.2 (29.8 - 32.7)	84.4 (83.3 - 85.6)	0.157	0.578 (0.563 - 0.594)
	Percentile curves	50.0 (48.4 - 51.6)	78.0 (76.7 - 79.3)	0.280	0.640 (0.625 - 0.655)
HCV infection	Commonly used	48.3 (46.7 - 49.9)	93.9 (93.1 - 94.6)	0.422	0.711 (0.696 - 0.725)
	ACG recommended	63.8 (62.3 - 65.3)	85.1 (84.0 - 86.2)	0.489	0.744 (0.731 - 0.758)
	Percentile curves	70.7 (69.2 - 72.1)	78.7 (77.4 - 80.0)	0.493	0.747 (0.733 - 0.761)
Excessive alcohol consumption	Commonly used	9.9 (8.9 - 10.8)	94.2 (93.5 - 94.9)	0.041	0.520 (0.504 - 0.536)
	ACG recommended	21.3 (20.0 - 22.6)	86.1 (85.0 - 87.2)	0.074	0.537 (0.521 - 0.553)
	Percentile curves	27.6 (26.2 - 29.1)	79.6 (78.3 - 80.9)	0.073	0.536 (0.520 - 0.552)

HBV, hepatitis B virus; HCV, hepatitis C virus; MASLD, metabolic dysfunction associated steatotic liver disease; ACG, American College of Gastroenterology. Commonly used refers to 40 U/L, regardless of sex; ACG recommended refers to 33 U/L for men, 25 U/L for women; Percentile curves refer to newly age and sex-specific thresholds.

used threshold with the Youden's index of 0.068 and AUROC of 0.534 (95% CI: 0.518−0.550). Regarding the discrimination of specific liver diseases, all three thresholds achieved the best performance in discriminating patients with HCV infection, followed by those with HBV infection, MASLD, and excessive alcohol consumption. The evaluation results for the men and women in this cohort are presented in Supplementary Table 7 and Supplementary Table 8, respectively. The sex-stratified results are consistent with the overall analysis.

3.4. Discrimination performance of different thresholds of ALT in external validation cohort 2

Table 3 presents the evaluation metrics of the proposed percentile curves and comparisons with other thresholds in external validation cohort 2. Similar findings were observed as in the validation cohort 1. For example, a comparison of the three thresholds suggested that the newly proposed percentile curves had the highest sensitivity, lowest specificity, and highest Youden's index and AUROC. The ACG thresholds had lower sensitivity, higher specificity, and lower Youden's index and AUROC. The commonly used threshold has the lowest sensitivity, highest specificity, and lowest Youden's index and AUROC. Notably, there were different findings in this cohort. The overall discrimination performance was much higher for all three thresholds in this cohort than in validation cohort 1. For instance, the percentile curves showed a sensitivity of 49.7% (95% CI: 45.6%−53.8%), specificity of 93.7% (95% CI: 91.7%−95.7%), the Youden's index of 0.435, and AUROC of 0.717 (95% CI: 0.680−0.754). All three thresholds achieved the best performance in discriminating patients with MASLD, followed by those with HBV and excessive alcohol consumption. The evaluation results for the men and women in this cohort are presented in Supplementary Table 9 and Supplementary Table 10, respectively. The sex-stratified results are consistent with the overall analysis.

4. Discussion

Serum liver chemistry tests offer a valuable and economical assessment of liver function, with ALT being the most common and relevant parameter indicating potential liver disease. However, the reference values for ALT are age- and sex-dependent, and few studies have accounted for the dynamic association of ALT with age. Our study, from one derivation cohort of 20,039 apparently 'healthy' older adults, presents the reference percentile curves of ALT for individuals aged 50 to 90 years. The derived percentile curves were validated using two external validation cohorts, and the evaluation parameters suggested that they achieved increased sensitivity, decreased specificity, and increased Youden's index and AUROC compared to the commonly used ALT threshold and the ACG's newly suggested threshold.

To our knowledge, this is the first study to propose reference percentile curves for ALT specifically in male and female older adults. ALT level, a key indicator of liver function, has a well-documented relationship with age and sex. Although age-specific thresholds for ALT have been established, research has predominantly focused on pediatric populations [9,13,27], in which physiological development significantly affects laboratory test results. Despite the extensive documentation of ALT levels, there is a notable lack of age-specific reference intervals for older adults. This gap exists despite the recognition that older adults undergo significant physiological changes that affect ALT levels. For example, both cross-sectional and longitudinal studies have indicated that ALT levels decline with age in both sexes [8,28], which underscores the necessity for age-specific reference values. Our study presents the percentile curves for ALT derived from healthy older adults, and is one of the largest cohort studies on this topic. By providing these reference percentile curves, our study supplements existing knowledge and offers a valuable tool for a more accurate assessment of liver health in older adults.

Recent studies have challenged the commonly reported reference of 40 U/L for normal ALT levels, highlighting differences based on sex [2,5]. For instance, a large-scale study of Italian blood donors conducted by Prati et al. recommended updated ULNs for ALT levels: 30 U/L for men and 19 U/L for women. Similarly, Lee et al. proposed new serum ALT ULNs of 33 U/L for men and 25 U/L for women from healthy Asian populations with normal liver histology, a recommendation adopted by the ACG's clinical guidelines [29]. Notably, using data from older adult populations as reference, we derived the downward percentile curves of ALT with age for both males and females. These curves revealed a decrease in ULN from 31.4 U/L at 50 years old to 21.7 U/L at 90 years old, and from 26.1 U/L at 50 years old to 17.8 U/L at 90 years old in females. Our newly proposed percentile curves were notably lower than those of previously reported ULNs, and this trend was even more pronounced in older individuals.

The relationship between ALT levels and age has been widely studied [8]. Several studies have suggested the negative association between ALT levels and age, indicating that ALT levels tend to decrease with age. However, some studies suggest an inverted U-shaped relationship, with ALT levels initially increasing and then decreasing in individuals aged ≥50 years. The changing epidemiology of viral hepatitis or metabolic risk factors with age is considered a potential explanation for the association between age and ALT levels [8]. However, previous longitudinal studies adjusted for these possible confounders and the downward association remained independent of the aforementioned risk factors [28]. Animal studies suggest that older livers demonstrate slower and compromised regenerative capacity, decreased organ weight, diminished inflammatory response

Table 3
Discrimination performance of different thresholds of ALT for predicting chronic liver diseases/conditions in external validation cohort 2.

		Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	Youden's index	AUROC (95%CI)
All causes	Commonly used	15.2 (12.2 - 18.1)	97.4 (96.1 - 98.7)	0.126	0.563 (0.522 - 0.603)
	ACG recommended	35.6 (31.7 - 39.5)	96.6 (95.1 - 98.1)	0.322	0.661 (0.622 - 0.700)
	Percentile curves	49.7 (45.6 - 53.8)	93.7 (91.7 - 95.7)	0.435	0.717 (0.680 - 0.754)
MASLD	Commonly used	17.9 (14.8 - 21.1)	96.6 (95.1 - 98.1)	0.145	0.572 (0.532 - 0.613)
	ACG recommended	43.3 (39.2 - 47.3)	94.8 (92.9 - 96.6)	0.38	0.690 (0.652 - 0.728)
	Percentile curves	59.0 (54.9 - 63.0)	90.9 (88.5 - 93.2)	0.498	0.749 (0.714 - 0.785)
HBV infection	Commonly used	3.0 (1.6 - 4.4)	93.0 (90.9 - 95.1)	-0.04	0.480 (0.439 - 0.521)
	ACG recommended	18.2 (15.0 - 21.3)	86.1 (83.3 - 88.9)	0.043	0.521 (0.481 - 0.562)
	Percentile curves	36.4 (32.4 - 40.3)	80.2 (76.9 - 83.4)	0.165	0.583 (0.542 - 0.623)
Excessive alcohol consumption	Commonly used	11.4 (8.8 - 14.0)	93.5 (91.5 - 95.5)	0.049	0.525 (0.484 - 0.566)
	ACG recommended	20.0 (16.7 - 23.3)	86.2 (83.4 - 89.1)	0.062	0.531 (0.490 - 0.572)
	Percentile curves	25.7 (22.1 - 29.3)	79.6 (76.3 - 82.9)	0.053	0.526 (0.485 - 0.567)

HBV, hepatitis B virus; HCV, hepatitis C virus; MASLD, metabolic dysfunction associated steatotic liver diseases, ACG, American College of Gastroenterology. Commonly used refers to 40 U/L, regardless of sex; ACG recommended refers to 33 U/L for men, 25 U/L for women; Percentile curves refer to newly age and sex-specific thresholds.

rates, and heightened fibrosis in comparison to younger livers [30]. Similarly, in humans, the aging liver undergoes progressive decreases in hemodynamics and size, changes that are likely associated with the accumulation of oxidative stress [31]. Although the exact mechanism remains unclear, the consistently reported inverse association between ALT levels and age, especially among older adults, underscores the necessity to develop reference percentile curves for older adults.

Overall, the newly proposed percentile curves for ALT showed significantly improved discrimination (reflected as increased Youden's index and AUROC) of the four specific liver diseases/conditions in validation cohorts 1 and 2 compared to the commonly used ALT ULN or the ACG's recommended sex-specific ULNs. Consistent with previous studies [4,14], lowering ALT ULNs resulted in increased sensitivity, but at the expense of decreased specificity. Our newly derived percentile curves achieved the highest sensitivity among the three different thresholds assessed, demonstrating a more than two-fold increase compared to the commonly used ALT ULN. Although this approach led to a decrease in specificity, the reduction was generally acceptable, with decreases of 11% and 3.7% observed in the external validation cohorts. As ALT is the most common indicator for chronic liver disease screening, higher sensitivity is crucial. This results in fewer false-negative outcomes, ensuring that fewer disease cases are missed. Therefore, the newly developed percentile curves may be valuable tools for chronic liver disease screening in older adults.

Our study is the first to derive a dynamic percentile curve of ALT in older adults that accounts for the dynamic association between ALT and age. This study stands out for its substantial sample size, meticulous exclusion criteria, and the robust consistency of results across various cohorts. Together, these elements significantly enhance the credibility and reliability of our findings. However, it is crucial to acknowledge several limitations before interpreting these findings. First, only the four most common chronic liver diseases/conditions that cause elevated ALT levels were assessed. There are some infrequent cases of elevated ALT levels as a result of medications, autoimmune hepatitis, and genetic liver disease, which were not considered in this study. Second, although most variables were collected objectively, alcohol intake, an important factor in elevated ALT levels and an important diagnostic factor for MASLD, was self-reported in this study. This type of data collection may have caused information or selection bias (participants were excluded because of missing data). Third, a single ALT result was used in this study; therefore, the longitudinal association of elevated ALT and the incidence of chronic liver diseases could not be concluded, which may warrant further cohort studies with repeated measurements of ALT. Fourth, strict screening criteria were applied in validation cohort 2 to minimize potential confounding effects on ALT levels. However, this contributed to a more selective cohort, which may limit the generalizability of the findings. Future studies with larger cohorts are warranted to confirm these observations.

5. Conclusions

The newly proposed ALT percentile curves outperform commonly used and ACG's thresholds for identifying chronic liver diseases. These percentile curves may serve as a valuable reference for screening liver diseases in older adults.

Author contributions

Yu Zhang: Data curation, formal analysis, writing – original draft; Shuwen Li: Methodology, formal analysis, writing – original draft; Kang Fu: Formal analysis, writing – original draft; Kailu Fang: Formal analysis, writing – original draft; Luyan Zheng: Formal analysis, writing – review; Yushi Lin: Formal analysis, writing – review; Yang Zheng: Visualization, methodology; Jie Wu: Conceptualization, supervision, writing – review & editing.

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Data availability statement

The datasets from both the derivation cohort and validation cohort 2 are not publicly accessible due to stringent security protocols and privacy regulations. However, researchers interested in these data for further study can obtain limited de-identified datasets by contacting the corresponding author, subject to reasonable requests. The validation cohort 1 dataset is publicly available by the National Center for Health Statistics via htt ps://w ww.cdc.gov/nchs/nhanes/index.htm.

Declaration of interests

None.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aohep.2025.101918.

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