

Review

Alanine Aminotransferase-Old Biomarker and New Concept: A Review

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Abstract

Measurement of serum alanine aminotransferase (ALT) is a common, readily available, and inexpensive laboratory assay in clinical practice. ALT activity is not only measured to detect liver disease, but also to monitor overall health. ALT activity is influenced by various factors, including viral hepatitis, alcohol consumption, and medication. Recently, the impact of metabolic abnormalities on ALT variation has raised concern due to the worldwide obesity epidemic. The normal ranges for ALT have been updated and validated considering the metabolic covariates in the various ethnic districts. The interaction between metabolic and demographic factors on ALT variation has also been discussed in previous studies. In addition, an extremely low ALT value might reflect the process of aging, and frailty in older adults has been raised as another clinically significant feature of this enzyme, to be followed with additional epidemiologic investigation. Timely updated, comprehensive, and systematic introduction of ALT activity is necessary to aid clinicians make better use of this enzyme.

Key words: serum alanine aminotransferase, ALT, activity

1. Introduction

Serum alanine aminotransferase (ALT) is a readily available, inexpensive, and routine biochemical assay used in clinical practice [1]. Initially, many concerns were voiced regarding the clinical significance of ALT activity on viral and toxic hepatitis, muscular dystrophy, and other muscular diseases [2], which might cause a substantial increase in the ALT level [3] in a relatively low percentage of the overall population. However, more and more metabolic disorders, such as obesity, hyperlipidemia, and diabetes mellitus (DM) have been observed independently associated with mild-to-moderate ALT elevation [4]. The series of metabolic disorders were referred to metabolic syndrome (MetS) [5], and featured insulin resistance and obesity. The clinical implication of ALT elevation in representing MetS has caused worldwide

concern [4, 6, 7] in Western and Eastern countries with the rapidly increasing prevalence of obesity [8]. Currently, ALT measurement is not only widely used in detecting the incidence, development, and prognosis of liver disease with obvious clinical symptoms, but also provides reference on screening the overall health status during health check-ups [1, 9].

Some demographic factors, such as gender and age, might also interfere with the ALT level in the general population [10-13]. Even the laboratory method and the diurnal variation might interfere with the ALT level, which has been discussed in previous studies [14-16]. For a comprehensive association linked to many disease processes, co-morbidities, and concurrent diseases, the clinicians should carefully evaluate and explain the clinical implication under-

lying the abnormal ALT level when faced with an individual patient. The relative confounders should be adjusted. Thus, the overview provided in this paper was intended to summarize the clinical factors that influence the ALT level based on previous reports and provide better knowledge of this liver enzyme as a tool in clinical estimation.

2. Chemical Formation and Physiology of ALT

ALT is an enzyme that is mainly aggregated in the cytosol of the hepatocyte [17]. ALT consists of 496 amino acids, has a half-life of 47 ± 10 hours [18, 19], and is coded by the ALT gene, which is on the long arm of chromosome 8 [19]. Physically, the ALT enzyme catalyzes the transfer of amino groups from L-alanine to α -ketoglutarate, and the converted products are L-glutamate and pyruvate (Figure 1) in the liver, which is a critical process of the tricarboxylic acid (TCA) cycle. In this process, the coenzyme, pyridoxal phosphate, is required [17, 20].

ALT is mainly aggregated in the cytosol of the hepatocyte. ALT activity in hepatic cells is approximately 3000 times higher than serum ALT activity [17]. When liver injury occurs, ALT is released from injured liver cells and causes a significant elevation in serum ALT activity. ALT also exists in muscles, adipose tissues, intestines, colon, prostate, and brain [21, 22]; however, the concentration of ALT in these organs is much lower than the liver [1, 23, 24].

3. Physiologic Factors Associate with the Serum ALT Level

The impact of some physiological factors has been shown to be associated with the serum ALT level. Extreme physical exertion can induce a short-term, reversible elevation in ALT. In a study focused on Thai boxers, the ALT level was 2-2.25-fold higher than the baseline value after a fight in 20 male adolescent Thai boxers [25]. In addition, an elevation in ALT was also observed in 9 runners who took part in a 1600 km ultra-marathon [26]; specifically, a 4-fold increase in ALT activity was observed on the 4th day of running compared to the baseline value. Many authors attribute the elevation in ALT after physical exertion to muscle injury [27-29]. In contrast, modest physical

activity is helpful in normalizing ALT levels [30, 31]. In addition, the serum ALT values in those who exercise at customary levels were lower than those who do not exercise or exercise more strenuously than customary [3, 32]. ALT activity has a diurnal variation in a given population. Co'rdoba et al [14] observed a circadian variation in ALT activity, with the nadir value at 04:00h, and the peak value at 16:00h in 12 patients with chronic liver disease. All physiologic confounders should be taken into consideration and the outside environment should be standardized when comparison of ALT activity is performed.

4. Demographic Characteristics Influence the Serum ALT Level

Several demographic covariates may contribute to disparities in the ALT level. Specifically, age has been shown to be associated with the ALT level in prior cross-sectional and prospective studies [11-13]. Elinav et al [12] reported an inverted curve with peak ALT levels at 40-55 years in a group of Israeli participants. Further, Dong et al [11, 13] reported a significant decrease in a large sample of community participants through cross-sectional and prospective studies.

In addition, gender plays a crucial role in modulating discrepancies in ALT activity. In a study focused on blood donors without a diagnosis of hepatitis from London, the ALT level was much higher in males than females [33]. Many population-based health examinations have also shown significant discrepancies between genders under similar living conditions [4, 34-36]. Some scholars have attributed the gender-based differences in ALT levels to the hormonal differences between males and females [10].

Ethnicity difference in ALT levels has also been observed. In the Third National Health and Nutrition Examination Survey (NHANES III), Mexican-Americans had a higher prevalence of ALT elevation compared to other ethnicities [4, 37]. Indeed, the increased prevalence in ALT elevation might be correlated with the higher prevalence of metabolic syndrome (MetS) [37, 38], which is considered to be a major cause of ALT elevation in Mexican-Americans [6, 39, 40].

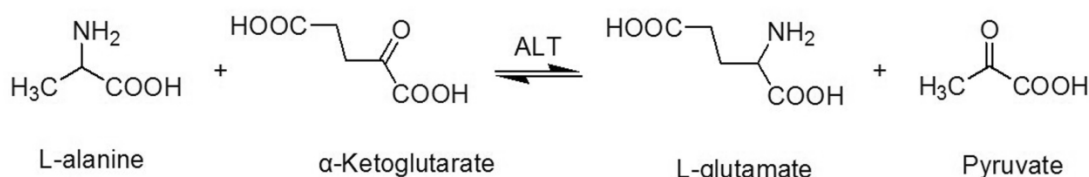


Figure 1. The transamination reaction catalyzed by alanine aminotransferase.

5. Clinical Factors Associated with Serum ALT level

5.1 Hepatic-related causes

5.1.1 Viral hepatitis (mainly hepatitis B virus [HBV] and hepatitis C virus [HCV] infections)

Viral hepatitis infection is the leading or secondary cause of ALT elevation in populations worldwide [4, 35, 36]. ALT activity is an indicator of liver injury in patients with acute and chronic viral hepatitis [41].

With respect to HBV infection, ALT elevation is often observed in the process of the cytolytic immune response (acute phase) and the following ineffective HBV clearance (chronic phase) [42]. Liaw et al [42] have described a fluctuation in ALT activity during the process of HBV infection. ALT activity is a crucial reference indicator in treatment selection and the evaluation of prognosis in patients infected with HBV [42-44]. Nevertheless, controversy exists and Lai et al [45] reported significant fibrosis and inflammation in 37% of patients infected with HBV and persistently normal ALT levels [45]. Unlike HBV infections, the ALT level is less meaningful for diagnosis and prognosis of HCV treatment. More patients infected with HCV progress to chronic hepatitis with persistent hepatocyte injury [41]. Greater than 6 in 10 of common HCV carriers have normal ALT levels or mildly elevated ALT levels (< 2 times the upper limit of normal [ULN]) with rare hepatic histologic lesions confirmed by liver biopsy [46]. Recently, Ruhl et al [47] suggested that lowering the ULN of the ALT level (29 IU/L for men and 22 IU/L for women) was the best cut-off value to identify HCV infectors in the US population with a high prevalence of HCV infection. Otherwise, the HCV RNA titer is closely linked to the ALT elevation. Cathy et al [48] reported that approximately 68% of patients with positive HCV-RNA levels have ALT elevations in asymptomatic blood donors who tested positive for antibodies to the HCV (anti-HCV).

5.1.2 Alcohol intake

Excessive alcohol intake is another cause of ALT elevation in the general population. In an Italian population, 45.6% of altered liver tests have been attributed to excessive alcohol intake (≥ 28 g/day) [35], while in a US national population survey, excessive alcohol (>1 time/day) is a crucial cause of ALT elevation, second only to HCV infection [4], however, alcohol intake might be a time-and dose-dependent covariate that influences ALT activity. Short-term and light alcohol consumption was not shown to induce significant ALT elevation in adults [49, 50], however,

ethnicity differences exist regarding the biological consequences of alcohol abuse [51, 52]. In a UK study based on alcohol abusers from different areas worldwide, adults from South Asia were shown to be more susceptible to alcohol-related liver damage and the ALT levels were higher than in European alcohol abusers [51]. Indeed, the effect of mild alcohol intake on ALT activity can be distinguished from binge drinking. Moderate alcohol intake does not contribute to significant ALT elevation, especially in a normal weight population, due to its potential effect on improvement of insulin sensitivity [53-55].

5.1.3 Some medications

Pratt et al [9] listed the medications that might cause ALT elevations. A randomized controlled trial (RCT) indicated that the estimated odds ratios (ORs) of ALT elevation in active treatment groups (including acetaminophen, hydromorphone+acetaminophen, morphine+acetaminophen, and oxycodone+acetaminophen) were 2.57-3.08 compared to the placebo group involving 343 healthy participants, even at the recommended dose [56]. Another commonly used medication, statins, also causes mild ALT elevation [57, 58]. The mechanism underlying statin-associated ALT elevation is still unclear. Some scholars have suggested that the ALT elevation in statin users is attributed to cholesterol reduction in hepatocytes and co-morbid conditions, rather than liver damage or dysfunction [59, 60]. Therefore, the long-term medications should be carefully considered when faced with an unexplained ALT elevation. Pratt et al [9] also indicated that cessation of drug treatment is the best way to confirm the relationship between a drug and ALT elevation.

5.1.4 Coffee consumption

Of note, coffee intake might be a protective factor against ALT elevation. In NHANES III, there was a 50% and 70% decrease in ALT elevation amongst participants who consumed >2 cups of coffee/day or ≥ 373 mg of caffeine, respectively, compared to participants who did not consume coffee [61]. Lee et al [62] attributed the protective effects of caffeine to antioxidant activity.

5.1.5 Non-alcoholic fatty liver disease (NAFLD)

NAFLD is a spectrum of clinical and pathologic changes, from fatty liver alone to steatohepatitis [63]. NAFLD is common in asymptomatic patients, and the prevalence ranges from 10 to 24% worldwide [64]. Considered as a manifestation of the MetS in liver [65, 66], NAFLD has been strongly associated with ALT activity in previous studies [4, 35, 36, 67]. NAFLD is the common cause of unexplained mild ALT elevation [3, 68]. NAFLD in asymptomatic patients is often

serendipitously detected by liver biochemistry testing during routine health check-ups [69, 70]. Similar to the increasing prevalence of obesity [8], NAFLD is increasing and becoming a major health burden [71]. In spite of the non-linear correlation between the degree of ALT elevation and the histologic severity of NAFLD [72, 73], a mild ALT elevation is largely attributed to NAFLD. When faced with an unexplained ALT elevation (without viral hepatitis or a history of excessive alcohol intake), NAFLD should be considered in the differential diagnosis.

5.1.6 Autoimmune hepatitis

Autoimmune hepatitis is a less common liver disease than NAFLD [74], and the mechanism underlying autoimmune hepatitis is still unknown [75]. ALT elevation is an available auxiliary measurement in the diagnosis of autoimmune hepatitis [76].

Moreover, ALT activity is a crucial indicator in detecting the effect of immunosuppressive treatment, prognosis, and long-term survival in patients with autoimmune hepatitis. In a study based on 84 Japanese autoimmune hepatitis patients, a persistently low ALT level (≤ 40 U/l) was the threshold value that was associated with improved prognosis [77]. Another study also showed an association between persistent ALT elevation and poor survival in 69 autoimmune hepatitis patients [78]. ALT is considered to be a crucial non-invasive marker of inflammation in patients with autoimmune hepatitis [79].

5.2 Non-hepatic cause

5.2.1 Metabolic covariates

Except for apparent causes, such as viral hepatitis, alcohol intake, and some medications, the so-called unexplained causes of ALT elevation in some previous studies have mainly been attributed to MetS [4, 68]. Similar to the pandemic of obesity [8], MetS presents as a series of metabolic disorders, including glucose intolerance, central obesity, dyslipidaemia, and hypertension, has caused worldwide concern in the most recent decades.

Compared to the obvious cause of ALT elevation referred above, ALT elevation caused by MetS is mild and neglected. In a cross-sectional study, the ALT level in MetS patients, as defined by the National Cholesterol Education Programme Adult Treatment Panel III (NCEP-ATP-III) criteria, was approximately 30% higher than participants without MetS in a male population from south China [7]. This impact of MetS on ALT elevation, however, is progressive and cumulative with a linear trend [6, 34, 39]. Even within ULN values, which did not consider the impact of MetS and are higher than the updated values, the increasing prevalence of MetS is still correlated with the

increasing ALT level in the general population. In a community-based Korean population, the ORs for MetS in the highest quintiles of ALT were 7.1-fold higher than the reference quintile in men and 2.1-fold higher in women [80]. All of the enrolled participants were selected within the ULN values (the ULN value is 30 U/l for males and 19 U/l for females) [80]. Another Korean national health survey also showed a significantly increased prevalence of MetS components, as defined by NCEP-ATP-III criteria, in the subgroup with high-normal ALT levels [81]. With respect to the lipoproteins, ALT was shown to be stably and significantly associated with intermediate-density lipoprotein (IDL) and apolipoprotein B (ApoB) after adjusting various covariates in different models [82]. These associations were commonly attributed to the stable and independent effects of insulin resistance and fatty liver disease in subjects with ALT elevations [6, 49].

In addition, the impact of MetS components on ALT activities varies to some extent. These distinguishing effects emerged after logistic regression using all of the MetS components as covariates. The impact of the individual MetS components on ALT elevation was disproportionate [7]. The body mass index (BMI) and waist circumference (WC), representing the central obesity component of MetS, were more closely linked to the ALT elevation [4, 7, 36, 67, 83, 84], although, the underlying mechanism has not been elucidated. The possible explanation is that obesity, especially abdominal fat, is potentially involved in the visceral adipose deposition that causes hepatotoxic fatty acids [85]. In a US national population-based study [86], BMI lost significance when evaluating the association between obesity and ALT abnormalities after adjusting for leptin, insulin, and triglyceride concentrations, rather than the WC, which was representative of the visceral adipose deposition, indicating that BMI might be an intrinsic association between obesity and ALT abnormalities. Another viewpoint was that the obesity-ALT elevation association was modulated by insulin resistance (IR). In a national health survey from a Korean adolescent population, the prevalence of IR status was positively correlated with the degree of obesity [87]. The OR of obesity-induced ALT elevation was significantly decreased after adjusting the homeostasis model (HOMA-IR) as an index of IR status [88]. Despite the ill-defined intrinsic mechanism, central obesity is the crucial MetS component that most influences the ALT level in general population.

5.2.2 Celiac disease and muscle injury

Chronic ALT elevation is also found in several non-hepatic disorders, such as celiac disease and

muscle injury [27, 89, 90]. The intrinsic mechanism between celiac disease is not known. Approximately 40-57% of patients with celiac disease have abnormal liver tests [91-95]. Celiac disease patients with elevated ALT levels should be treated with gluten-free diet; doing so will restore ALT levels to normal in 75-95% of patients within 6 months [92, 94, 95]. It is well known that ALT elevation is often observed in patients with muscle necrosis [96] and the ALT elevation without evidence of liver disease should be considered due to muscle injury [27]. Otherwise, the increased creatine kinase and lactate dehydrogenase activities that occurred following muscle injury should be assayed to identify the cause of ALT elevation [9, 27].

5.2.3 Hemochromatosis

As an ethnic specific disease mainly occurring in individuals of Nordic descent, hemochromatosis is a less common cause of ALT elevation. *HFE* gene mutations are the major cause of hereditary hemochromatosis, and iron overload is the main cause of hepatic injury. Measurements of serum ferritin, total iron-binding capacity, and the *HEF* mutation test can help diagnose hemochromatosis [97]. Liver biopsy might be necessary if the aforementioned tests are negative in patients highly suspected to have hemochromatosis to evaluate the status of liver injury from iron overload.

6. Interaction on ALT Level between Distinguished Covariates

Except for the independent univariate impact, ALT fluctuation has also been shown to be influenced by multivariate interaction in several studies [54, 55, 84]. Among 13,580 US participants, after excluding the patient with hepatitis B or C infection, or iron overload, obesity significantly increased the risk of alcohol-related abnormal aminotransferase activity. The prevalence of abnormal aminotransferase activity was increased from <5% in the normal weight group to near 30% in the obesity subgroup [54]. Similarly, these effects were also observed in the Finnish population [55]. Piton et al [84] also described the interaction of ALT activity by neural network and recommended the distinguishing ULN of the ALT level to be classified by BMI and gender. In addition, the age and gender interaction was also observed and expressed in mathematical formulas [12, 98]. In contrast, our prior cross-sectional study did not show a synergistic effect between MetS and HBV infection, which were also considered as critical covariates associated with ALT elevation [7].

7. Several Concerns that Clinicians should Raise

7.1 Age as a critical covariate that should be emphasized

As a covariate influencing ALT activity, the association between age and ALT activity has been described in previous studies [11-13]. The impact of age on ALT fluctuation is not only present based on quantitative discrimination, but also on the diagnostic value in predicting all-cause and disease-specific mortality. A previous authoritative review [1] indicated that elevated ALT might predict higher mortality in a general population; however, this opinion might be questionable, especially in old adults. Inverse relationships between ALT activity and mortality were observed in several studies focused on older population [57, 99, 100], and this opinion was confirmed by subsequent meta-analysis [101]. Dong et al [11, 13] raised the concept that ALT activity might be influenced by accelerated aging and frailty in older adults independent of its traditional role in screening liver function. Although age lost significance in many multi-covariate analyses [7, 35, 36], clinicians should carefully explain the extremely low ALT level, especially in older population owing to its potential implication on increasing mortality.

7.2 ULN of ALT as a hot scientific topic

Earlier ULNs for the ALT level were defined in blood donors with non-B, non-C hepatitis, and ranged from 40 to 50 U/l [102-105]. These definitions did not consider that metabolic covariates caused liver injury with slight-to-moderate ALT elevation [3, 106]. Because of the increasing prevalence of patients with metabolic disorders, the clinical significance of ALT values have been recognized. Metabolic covariates have been enrolled when evaluating the ULNs of ALT activity by many scholars in recent decades. Prati et al [107] first raised the concept that subjects with metabolic abnormalities should be excluded in evaluating the ULN of ALT level for the potential risk to the general health. In agreement with Prati et al [107], many scholars have re-evaluated the ULN of ALT values in specific populations, including adults and adolescents [10, 47, 108-123].

The detailed ULNs of ALT levels are distinct for the intrinsic differences in ethnicity, gender, and age distribution. In addition, the difference in definition of so-called "healthy subjects" and statistical methods might also contribute to the variations in the ULNs. Pacifico et al [15] had summarized the reported ULNs of serum ALT levels in published studies [10, 84, 107-110, 113-118, 121]. This review is incomplete due to omitting some additional references [47, 111, 112,

119, 120, 122, 123] and indirect comparisons. The etiology of ALT elevation can be attributed to viral hepatitis, excessive alcohol consumption, and metabolic disorders, including fatty liver disease [4, 35, 36, 83], in population-based studies. Subjects with the above-mentioned problems should be excluded when evaluating the ULNs of ALT values in a given population. Therefore, we re-summarized the reported ULNs of ALT values by extracting the key exclusion information in various districts as a supplement and presented the information by category (Table 1, Fig-

ure 2). As shown in Figure 2, the ULNs of ALT levels in studies excluding the subjects with metabolic abnormalities were greater than those without (41.0 ± 10.8 vs. 27.1 ± 7.0 U/l). About one third decrease on ULN of ALT definition when excluding the subjects with metabolic disorders. The adoption of an updated ULN of ALT activity in a subsequent investigation raised the sensitivity of the diagnosis of potential liver disease with acceptable specificity decrease [47, 107, 110, 114, 117].

Table 1. Updated Upper Limit of Normal Serum Alanine Aminotransferase Value in Reported Studies.

Authors, country, year [reference]	Number of enrolled participants (M/F)	Age of enrolled participants [year, mean \pm SD or mean (age range)]	Exclusion of viral hepatitis (No/Yes)	Exclusion of excessive alcohol consumption (No/Yes)	Exclusion of medication (No/Yes)	Exclusion of metabolic abnormality (No/Yes)	Exclusion of fatty liver disease by imaging tools (No/Yes)	Liver biopsy (No/Yes)	Statistical methods	ULN of ALT value (U/l)
Piton et al, France, 1998, [84]	487/546	30 \pm 0.36	Yes	No	No	No	No	No	95th percentile one sided	BMI \leq 23: 42 for men 31 for women BMI>23: 66 for men 44 for women
Prati et al, Italy, 2002, [107]	3865/2970	29.8 \pm 9.5	Yes	No	Yes	Yes	No	No	95th percentile one sided	30 for men 19 for women
Kariv R et al, Israel, 2006, [109]	6124/11374	31.91 \pm 17.07	Yes	No	Yes	Yes	No	No	95th percentile one sided	37.5 for all 44.9 for men 31.8 for women
Poorten et al, Australia, 2007, [110]	206/0	16.8 \pm 1.4	Yes	Yes	Yes	Yes	No	No	95th percentile one sided	28
Jamali et al, Iran, 2008, [111]	628/1300	40.7 \pm 14.7	Yes	Yes	No	No	No	No	95th percentile one sided	BMI<25 and non-diabetics: 36.1 for all 37.5 for men, 36 for women BMI>25: 51 for all 59 for men 45.25 for women
Kibaya et al, Kenya, 2008, [112]	1020/521	30(18-55)	No	No	No	No	No	No	97.5th percentile one sided	52 for all 53.9 for men 47 for women
Lee et al, Korea, 2010, [108]	643/462 for pathological normality 346/313 met for Prati criteria	29.1 \pm 9.0 for all 27.2 \pm 8.4 for men 31.6 \pm 9.3 for women	Yes	Yes	No	Yes	No	Yes	97.5th percentile one sided	Normal histological liver donor: 35 for men 26 for women Subjects met the Prati criteria: 33 for men 25 for women
Schwimmer et al, USA, 2010, [114]	548/434	14.5 \pm 1.8 for boys 15.1 \pm 1.8 for girls	Yes	Yes	Yes	Yes	No	No	95th percentile one sided	25.8 for boys 22.1 for girls
Kang et al, Korea, 2011, [113]	704/1041	41.8 \pm 12.5	Yes	Yes	Yes	Yes	No	No	95th percentile one sided	28 for all 31 for men 23 for women
Poustchi et al, IRAN, 2011, [10]	186/185	12.87 \pm 3.13 for all 12.58 \pm 3 for boys 13.17 \pm 3.41 for girls	Yes	Yes	No	Yes	No	No	95th percentile one sided	30 for boys 21 for girls
Volzke et al, Germany, 2011, [115]	1180/1423	20-79	Yes	Yes	No	No	Yes	No	95th percentile one sided	60 for men 45 for women 20-49 yr: 65 for men and 42 for women 50-79 yr: 42 for men and 35 for women
Park et al, Korea, 2012, [116]	880/836	14.4 (10-19)	Yes	Yes	No	Yes	No	No	97.5th percentile one sided	33 for boys 25 for girls
Ruhl et al, USA, 2012, [47]	1607/2140	42.0 \pm 16.9	Yes	Yes	Yes	Yes	No	No	95th percentile one sided	24 for men 18 for women
Wu et al, Taiwan, 2012, [117]	1237/1657	52.4 \pm 13.1	Yes	No	No	Yes	Yes	No	95th percentile one sided	21 for men 17 for women

Zheng et al, China, 2012, [118]	4765/8872	35.3(19-44)	Yes	Yes	Yes	Yes	Yes	No	95th percentile one sided	35 for men 23 for women
Alhamoudi et al, Saudi Arabia, 2013, [119]	41/93 for pathological normality 52/11 for Prati criteria	28.9±7.3 for all 28.5±7.3 for men 30.0±7.1 for women	Yes	Yes	Yes	Yes	No	Yes	95th percentile one sided	Normal histological liver donor: 42 for men 31.1 for women Subjects met the Prati criteria: 33 for men 22 for women
Kabir et al, Iran, 2013, [120]	688/621	61.5 ± 7.9 for men 61.4 ± 7.9 for women	Yes	Yes	Yes	Yes	No	No	95th percentile one sided	21.4 for men 18.8 for women
Park et al, Korea, 2013, [121]	1355/1961	36.3(≥20)	Yes	Yes	Yes	Yes	No	No	95th percentile one sided	42 for men 25 for women
Sohn et al, Korea, 2013, [122]	297 663/113 577	24.6±6.4 for men 22.2±5.2 for women	Yes	Yes	Yes	No	No	No	95th percentile one sided	34 for men 24 for women 15-19 yr: 27 for men, 20 for women 20-24 yr: 31 for men, 21 for women 25-29 yr: 35 for men, 22 for women 30-34 yr: 36 for men, 23 for women 35-39 yr: 36 for men, 24 for women 40-44 yr: 34 for men, 28 for women 45-49 yr: 37 for men, 24 for women ≥50 yr: 33 for men, 29 for women
Tanaka et al, Japan, 2013, [123]	1462/2046	50.6±19.8 for men 48.8±19.4 for women	Yes	No	Yes	Yes	Yes	No	95th percentile one sided	36 for men 27 for women

Abbreviation: ALT: alanine aminotransferase; BMI: body mass index; F: female; M: male; SD: standard deviation; ULN: upper limit of normal; U/l: unit per liter; yr: year.
^a: the unit of BMI is kg/m².

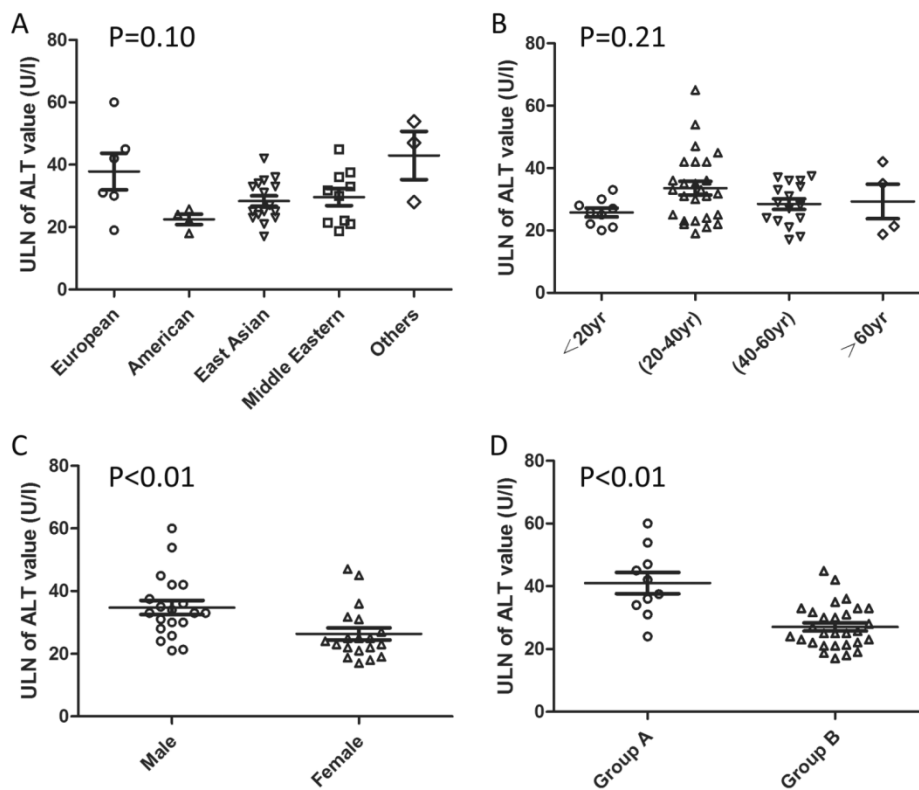


Figure 2. Pooled reported ULN of ALT values by categories. ^a the others is represented as Australian and African. ^b Group A includes the studies without exclusion of subjects with metabolic abnormality when evaluating the ULN of ALT value. ^c Group B includes the studies with exclusion of subjects with metabolic abnormality when evaluating the ULN of ALT value. *P*-value is based on the comparison of ALT ULN classified by different categories (by Mann-Whitney U test).

Although controversy exists in the definition of the ALT normal range for the debate on the focus of risk-benefit or cost-effectiveness [124], the metabolic disorders should be considered when defining the normal range of the ALT level. And the ULN of ALT level should be re-defined individually with more specific ethnicity to make best use of ALT in related disease detection. In addition, these updated thresholds in the general population should be validated in the follow-up studies to make the best balance between sensitivity and specificity.

7.3 ALT-cardiovascular disease (CVD) association as a plausible issue

Some scholars have summarized the previous studies and referred to the existing controversy on the association between ALT elevation and CVD incidence [125-127]. Some scholars have found a positive link between ALT elevation and CVD-related incidence in their own studies [128-130], while others did not [131-134]. Wang et al [135] attributed the unobserved significant ALT-CVD association to the presence of viral hepatitis infections and alcohol abusers amongst the enrolled participants, in agreement with the opinion of Stefano et al [125]. Otherwise, age, gender distribution, and ethnicity may also contribute to the heterogeneity of the ALT-CVD association in specific populations [99, 127, 136].

Inferior to gamma glutamyltransferase (GGT) [127], the ALT level is not the best indicator to screen for the incidence of CVD events in the general population. More evidence-based studies focused on ALT-CVD association are needed to disclose their inner relationship.

7.4 Key limitation of serum ALT assay in health check-ups

Although available in detecting underlying disease status, some limitation for the ALT assay should be noted. First, the histologic severity of NAFLD does not correlate with the ALT elevation. Patients with non-alcoholic steatohepatitis (NASH) were also observed in patients with normal ALT levels [63]. No significant difference on histologic severity was found between NAFLD patients with or without ALT elevation after matching gender and age of respective subgroups [137]. Second, a significant percentage of liver lesions were observed in patients with viral hepatitis and persistently normal ALT (PNALT). The percentage with corresponding liver fibrosis amongst patients infected with HCV/HBV with PNALT was 16% and 37%, respectively, while the percentage with corresponding cirrhosis was higher in patients infected with HBV (27%) [45,138]. Third, the ALT assay is a continuous variable and even the fluctuation in

the normal range also indicates the potential risk for metabolic disorders or cardiovascular disease in a given population [139, 140]. Therefore, clinicians should interpret the sole ALT abnormality carefully, and multi-biomarker evaluation might enhance the diagnostic efficiency further [141].

7.5 What should clinicians do when faced with an ALT elevation

In spite of the limitations referred earlier, what appropriate measure should be taken for a clinician when faced with subjects who have ALT abnormalities? A flow diagram has been created to aid clinicians in treating adults with ALT elevations (Figure 3). When the ALT value is in normal range, annual routing test is recommended. When the ALT level is decreased to an extremely low level, especially in older adults, the accelerated process of aging and frailty, followed by reduced liver size and lowered liver blood flow, is suggested. Therefore, imaging measurements should include ultrasonography and CT scan to evaluate metabolic function of the liver. When the ALT level is elevated and exceeds the normal range, the common cause of ALT elevation, including viral hepatitis indicators, metabolic covariates, alcohol abuse, long-term medication history, and other liver functional indicators, should be investigated. Less common causes of ALT elevation, including hemochromatosis, autoimmune hepatitis, celiac disease, and muscle injury, should be identified when the inspection results referred before are negative. A liver biopsy might be the final instrument when the ALT abnormality cannot be explained. However, the clinicians should carefully evaluate and balance the patients' effect and risk they should afford when a liver biopsy is performed.

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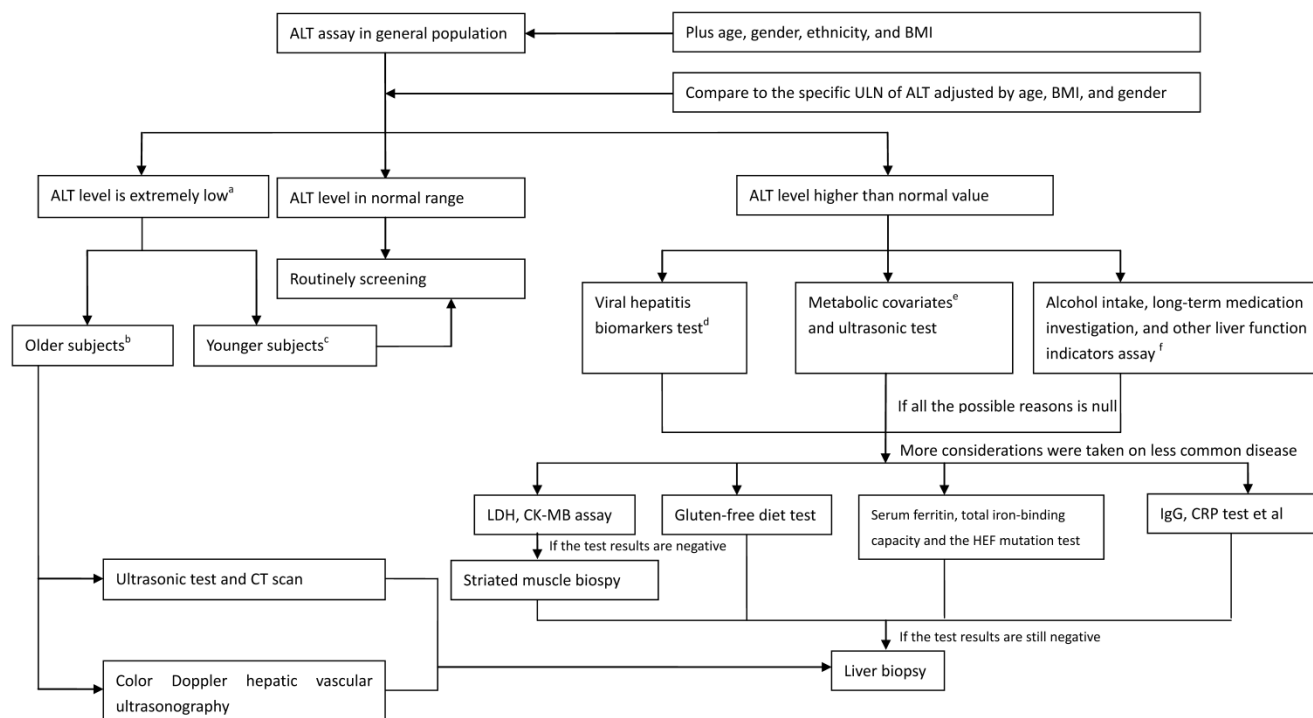


Figure 3. Strategy diagram of checking the subjects with ALT assay (on the ideal status). ^a the extremely low ALT value is represented as lower than the median value. ^b the Older subjects are indicative of the subjects aged > 70 years. ^c the Younger subjects are indicative of the subjects aged ≤ 70 years. ^d Viral biomarkers includes the hepatitis B virus surface antigen, and the anti-HCV. ^e Metabolic covariates includes the BMI, triglyceride, high density lipoprotein cholesterol, glucose, and blood pressure et al expressed as a series of metabolic status.

Conflict of interest

The authors declare no conflict of interest.

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