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LETTER TO THE EDITOR

Metabolic dysfunction-associated steatotic liver disease: The question of long-term high-normal alanine aminotransferase as a screening test

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Abstract

The growing prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) is being driven by the obesity epidemic. The quest for solutions continues particularly with regard to early detection. This editorial comments on the utility of long-term high-normal alanine aminotransferase (ALT) in screening for MASLD. Chen *et al* found that new onset MASLD can be detected by repetitively high normal ALT. Implicit in this concept is the question of what should be the accepted upper limit of normal (ULN) for ALT. It was previously set at 40 IU/L based on studies that included people with subclinical liver disease but the new consensus is 30/19 U/L in healthy males/females. Thus, when Chen *et al* defines the ULN as 40 U/L, others may view it as excessively high. It is important to recognize the variables affecting ULN *e.g.* instrumentation, diurnal variations, exercise and ageing. These variables matter when the distinctions are subtle *e.g.* normal *vs* high-normal. In this regard, the utility of long-term high normal ALT as a disease marker could be enhanced by combining it with other biomarkers, imaging and MASLD genetics to create machine learning classifiers. All in all, Chen *et al*'s work on long-term high normal ALT as a marker of new-onset MASLD deserves merit.

Key Words: Metabolic dysfunction-associated steatotic liver disease; Alanine aminotransferase; Pathophysiology; Screening; Upper limit of normal; Biomarkers; Predictive models

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Core Tip: High normal alanine aminotransferase (ALT) has potential utility in screening for new-onset metabolic dysfunction-associated steatotic liver disease (MASLD). In view of its biochemistry, the upper limit of normal for ALT has to be interpreted with caution since it can be influenced by various methodologic and physiologic factors. Its utility as a screening tool could be enhanced by combining it with other serum biomarkers, cardiometabolic risk factors, imaging techniques and MASLD genetics to create predictive nomograms or machine learning classifiers.

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TO THE EDITOR

Metabolic dysfunction-associated steatotic liver disease (MASLD) is one of the most common liver diseases world-wide and its prevalence is steadily rising. This is largely being driven by lifestyle changes (overnutrition, food composition and physical underactivity) leading to the obesity epidemic[1,2]. Consequently, it is a growing cause of morbidity and mortality. The quest for solutions continues. One approach is early detection and timely intervention and in this regard Chen *et al*[3] investigated the utility of long-term high-normal alanine aminotransferase (ALT) as a screening tool for this condition[3]. There is a fairly long history of ALT as a surrogate marker for MASLD[4-7]. This editorial comments on the role of this enzyme in screening for MASLD.

Nomenclature

The nomenclature for nonalcoholic fatty liver disease (NAFLD) was recently changed to MASLD[8]. The main reasons driving the change were: (1) The terms "nonalcoholic" and "fatty" were considered to be stigmatizing; (2) NAFLD was quite limiting as a diagnosis because some such patients consume alcohol to a greater or lesser extent *i.e.* an overlap of MASLD with alcoholic liver disease (ALD) resulting in MetALD; and (3) The term failed to recognize the root cause of the disease, namely insulin resistance (IR)[1,2,8]. In fact, one of the major changes in moving from the old to the new terminology is that the definition of the disease now includes at least one of 5 cardiometabolic risk factors (CMRFs) pertaining to body mass index (BMI), type 2 diabetes mellitus (T2DM), elevated blood pressure, high plasma triglycerides and low high-density lipoprotein-cholesterol[8]. In light of the epidemiology of the disease as highlighted by Chen *et al* [3], it is also important that these CMRFs be adjusted according to ethnicity *e.g.* Hispanics, Europeans, Africans and Asians[3,9,10]. At the same time, it should be noted that the nomenclature used by Chen *et al*[3], even in the title, does not quite reflect the new terminology.

The entities comprising MASLD

MASLD represents a dynamic spectrum of liver disease[1,2,8]. At one end is metabolic dysfunction-associated steatotic liver (MASL) or simple steatosis, which is defined as the presence ≥ 5% steatosis with no evidence of hepatocellular injury. Further along the spectrum is metabolic dysfunction-associated steatohepatitis (MASH) which is indicative of the onset of hepatocyte membrane damage and the inflammatory cascade with or without some degree of fibrosis. At the other end is cirrhosis characterized by advanced fibrosis and its attendant complications. Along the spectrum, fibrosis is the dominant morphologic feature that predicts morbidity and mortality[1,2,8]. Screening protocols such as those advocated by Chen *et al*[3] and McGinty and Przemioslo[11] aim to detect the disease before it gets to cirrhosis.

The rationale for ALT in screening for MASLD

ALT occurs abundantly in the cytosol of hepatocytes where it catalyzes the transfer of amino groups to form oxaloacetate [7,12]. Its activity in the liver is 3000 times greater than in the serum and as such, it is a sensitive marker of hepatocyte injury/loss of membrane integrity. Its plasma half-life is 47 hours which is significantly longer than that of serum aspartate aminotransferase (AST) (17 hours). In acute liver cell injury, AST usually rises immediately but within a day or 2, ALT will become higher due to its longer half-life[7,12]. Thus as a general rule, with ongoing liver damage, ALT is more commonly elevated than AST though exceptions occur (*e.g.* ALD).

Pathogenesis of liver damage in MASLD leading to changes in ALT

A key feature of MASLD is the impaired response of tissues to insulin *i.e.* IR[1,2,8]. The metabolic consequences of IR are hyperglycemia, dyslipidemia and visceral adiposopathy with the latter typically manifesting as abdominal fat. A vicious cycle occurs resulting in decreased uptake of glucose and release of free fatty acids which further aggravates hepatic fatty infiltration. Accumulation of fat in the liver can cause oxidative stress, release of cytokines and lipid peroxidation culminating in hepatocyte injury and/or death *i.e.* lipotoxicity[1,2,13,14]. This triggers the inflammatory process that can lead to MASH and, if left unchecked to fibrosis. Traditionally, MASL has been associated with normal ALT levels and MASH abnormal levels. The postulate by Chen *et al*[3] is that long-term high normal ALT is associated with new onset MASLD. It does not specifically distinguish between MASL and MASH. However, it could be argued that their cohort with abnormally high ALT (as opposed to high-normal) most likely represented MASH.

Fibrosis remodeling and regression

Fibrosis starts appearing at the MASH stage of the disease due in part to the activation of stellate cells and microvascular injury[1,15]. It begins as fine filmy wisps of fibrosis, so-called chicken-wire fibrosis that can be pericellular, perisinusoidal or perivenular. This is a very important stage in the disease process because the fibrosis is still quite delicate and amenable to regression[1,15-17]. Due to the concurrent activation of the inflammatory process during MASH, this is the period when ALT is most likely to be elevated thus offering the prospect of timely intervention. With cirrhosis, ALT can be quite variable depending on the balance between inflammatory activity and fibrosis *e.g.* burnt out disease with shrunken liver due to hepatocyte loss. Notwithstanding the timely concepts of remodeling and regression, it is important to note that regression as currently envisaged is a fairly modest process and does not imply resolution of cirrhosis since large areas of parenchymal extinction cannot be repopulated by hepatocytes[15,17,18].

DISCUSSION

Chen *et al*[3] defined MASLD based on abdominal ultrasound (US) findings and at least one of several CMRFs which is similar to what is envisaged in the new terminology[8]. Their study found that long-term high normal ALT could identify cases of new-onset MASLD. This is very helpful because such individuals can then be targeted for lifestyle changes/intervention. However, based on pathophysiology and natural history of MASLD, a number of discussion points come up with respect to the validity of long-term high normal ALT as a screening tool for this condition.

ALT as a screening test for MASLD

Screening is defined as the presumptive identification of unrecognized disease by tests which can be carried out easily and conveniently[12,19], and as emphasized by McGinty and Przemioslo[11], should be cost-effective. A screening test is not intended to be diagnostic but to classify individuals with a high probability of disease from those with a low probability. Based on the pathophysiology of MASLD and the biochemistry of ALT, changes in ALT would not be expected to occur until there is loss of hepatocyte membrane integrity[1,2,13,14,20]. Most studies do indeed reaffirm this but others show that the levels can be normal in subjects across the entire spectrum of MASLD[21,22]. Since physicians tend to pay more attention to those patients with elevated ALTs, it may be useful to devise a way for them to look at ALT levels in a different light. Essentially, this is part of the message being conveyed by Chen *et al*[3]. They are postulating that new onset MASLD can be detected by repetitively high normal ALT, and that this should be the trigger point for intervention/action rather than waiting for a truly abnormal level. Implicit in this concept is the question of what should be the accepted upper limit of normal (ULN) for ALT.

ULN for ALT

Ideally, the ULN should be determined in individuals without definite liver disease but such subjects are difficult to identify in population-based studies. It was previously set at 40 IU/L regardless of sex or BMI based on studies that were carried out before the introduction of viral hepatitis C testing and prior to the concept of MASLD[23-26]. Inevitably, this included a proportion of people with subclinical liver disease (*e.g.* viral hepatitis carriers and MASLD) thereby limiting the sensitivity of ALT as a tool for assessing liver damage[6,7,12]. In light of the changing epidemiology of liver disease, this called for a re-evaluation of the ULN after excluding individuals with subclinical liver disease. There appears to be convergence towards a new ULN of 30/19 U/L in healthy males and females (M/F) though there is no unanimity[6,25-28]. Thus, when Chen *et al*[3] define their ULN as 40 U/L, others may view it as excessively high.

Variables affecting ALT values

Methodology: Chen *et al*[3] rightly considered some of the factors affecting the ULN for ALT *e.g.* variability in methods and instrumentation depending on geographic location[28,29]. For example: (1) There is progressive ALT activity loss the longer the time interval from collection of the blood sample to centrifugation[27,28]; (2) Freezing the serum specimens after collection followed by thawing prior to assay can cause a 46% loss of ALT activity compared to only a 6% loss with refrigeration at 4 °C[27]; (3) Conducting the test at 37 °C instead of 30 °C raises enzyme activity and results in higher ALT values[28] and (4) Laboratories using different instruments and reagents may report different ALT levels[29]. These are well-known issues in clinical chemistry and efforts have been made to standardize analyzer and assay measurements but with limited success[28,30-32]. In this regard, recommendations have been made to use a reference interval rather than a fixed cutoff until such a time as there are internationally harmonized ALT methods[32,33].

Physiology of ALT: (1) It is also found in the kidney and to a lesser extent in cardiac and skeletal muscle. This may not matter as much when ALT is significantly elevated *e.g.* × 5, × 10 or × 15 normal as can occur in certain conditions[12,31]. However, in high-normal or borderline cases, it may matter; (2) ALT activity varies day to day, by as much as 10% to 30%; (3) Within a given day, there is significant diurnal variation, with ALT activities being up to 45% higher in the afternoon than early morning[12,34]; (4) Seasonal variations also occur[35]; (5) ALT activity can vary quite substantially depending on exercise: No exercise, customary exercise levels and strenuous activity[12,36]; (6) Fasting has a bearing on ALT levels; hence the recommendation that any mildly increased ALT should be re-evaluated in the fasting state[37]; and (7) ALT activity is substantially higher in adult males than females, and also increases with age up to a certain point[38]. Chen *et al*'s study rightly recognizes the M/F differences and establishes a lower end cut-off of 18 years[3]. However, it is not clear to what extent Chen *et al*[3] took into account all of the above physiologic variables.

Ageing and osteoporosis can alter ALT activity in the serum: In fact, after age 60, the reference limits change in women [39]. Furthermore, other investigators have suggested that elevations in ALT in middle-aged and elderly men and women with MASLD could be related to reduced bone mineral density as a result of curtailed osteoblast activity[40-42] rather than being an indication of MASLD.

Non-invasive tests for MASLD

While liver biopsy is the gold standard for diagnosing MASLD, it has limitations due to its invasiveness, inherent complications (*e.g.* pain, bleeding, perforation), spatial variability in sampling and impracticality for serial monitoring[43-45]. Recent approaches using endoscopic US-guided biopsies have some advantages over the traditional percutaneous samples but do not completely mitigate these drawbacks[46,47], hence the need for non-invasive tests. They can be classified as: (1) Serum biomarkers; and (2) Imaging techniques.

Serum biomarkers: Although ALT is the enzyme most commonly used for screening of MASLD, it's utility can be augmented by other biomarkers potentially analyzable from the same routine blood samples. They include but are not limited to AST, gamma-glutamyl transferase, fasting blood glucose, hemoglobin A1c, triglycerides and cholesterol. These biomarkers are increasing being used in conjunction with other parameters in various algorithms for assessing MASLD *e.g.* the fatty liver index[48-50], liver fat score[49], and hepatic steatosis index[49,51].

Imaging techniques: Various imaging techniques such as abdominal US, computed tomography (CT) and magnetic resonance imaging (MRI) can be used in the assessment of MASLD. Of these: (1) US is the fastest and least expensive and can detect hepatic fat by comparing liver hyper-echogenicity in relation to the kidney. However, it's utility is limited by low sensitivity for mild disease. Furthermore, accuracy decreases as BMI increases[52,53]. Vibration-controlled transient elastography (VCTE) (Fibroscan) is an US-based method that utilizes shear wave velocity to perform liver stiffness measurement. When equipped with a module called controlled attenuation parameter, it can be very useful for quantifying the levels of liver fat and fibrosis[44,54,55]. The test is instantaneous and reliable which makes it suitable for population-wide screening as well as point-of-care testing[44,55]; (2) On CT, fat reduces liver attenuation causing it to be hypodense and can provide detailed information, potentially averting needle biopsies[43]. However, it is more expensive than US especially when applied to the general population; and (3) Unlike US and CT which use proxies to assess liver fat, magnetic resonance can measure the proton density fat fraction which is highly accurate in quantifying liver fat and may be even more precise than liver biopsy[43,45]. A significant drawback is that MRI-based techniques are costly in terms of equipment/scanners and require technical and professional expertise that is usually only available in tertiary care centres. As such, they may not be suitable for large scale population screening.

Prediction models and machine learning classifiers in MASLD

Given all the variables that affect ALT measurements, it is prudent to consider other approaches for assessing risk in MASLD. This concept has been captured by using combined biomarker parameters. Scores such as Agile 3 + and Agile 4 combine laboratory data (*e.g.* ALT, AST, fasting glucose, triglycerides, high- and low density lipoproteins), demographic features (*e.g.* age, sex, BMI, waist-to-hip ratio, T2DM, hypertension) and liver stiffness measurements as determined by VCTE[43,56]. The data can also be used to create multiparameter predictive nomograms or machine learning classifiers. Such predictive models are promising to be effective not only as screening tools for assessing risk in MASLD but also for identifying patients for referral to specialists/hepatologists[1,57-59].

Future directions

While liver biopsy is the gold standard for diagnosing MASLD, it is not practical for population studies. A more useful and practical alternative would be serum biomarkers which in addition to ALT, could include AST/ALT ratio, Fibrosis-4, Hepascore, C-reactive protein, ferritin, serum hyaluronic acid and alpha-2-macroglobulin[1,60-62]. From a medical imaging perspective, US is gaining wider acceptance as a biomarker since it can be used in population-based studies. While CT and MRI are still quite expensive, continuing improvements in imaging technologies could improve accessibility, enhance accuracy and lower costs. A potentially exciting field is MASLD genetics: It is known that the risk of developing MASLD is quite variable among ethnic groups and individuals, and is determined by both environmental and genetic factors[10]. Candidate genes include *PNPLA3*, *TM6SF2*, *HSD17B13*, *MBOAT7* and *GCKR* which can be used for creating polygenic risk scores to gain further insights into MASLD prevalence and progression[10,63]. From a prognostic viewpoint, regression of disease from MASH to MASL does occur but whether remodeling of advanced fibrosis/cirrhosis can be achieved is still an open question.

CONCLUSION

The MASLD epidemic appears to be growing. There are ongoing efforts to reverse this trend particularly changes in lifestyle. Screening to identify people at risk can be helpful, but to be cost-effective, it has to be targeted. In this regard, individuals with CMRFs stand to benefit the most from ALT screening and this is part of the message conveyed by Chen *et al*[3] and McGinty and Przemioslo[11]. The question is what is the actionable level for ALT for professional intervention and whether it can be augmented by other approaches such as MASLD genetics, predictive nomograms and/or machine learning classifiers.

FOOTNOTES

Author contributions: As the sole author, Moyana TN is responsible for all aspects of the work, including conception, design, research, writing, and finalization of the manuscript.

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