Clinical Chemistry 63:7
1196–1198 (2017)

Opinion

American Liver Guidelines and Cutoffs for "Normal" ALT: A Potential for Overdiagnosis

Mauro Panteghini,[1*] Khosrow Adeli,[2] Ferruccio Ceriotti,[3] Sverre Sandberg,[4] and Andrea Rita Horvath[5]

In 2 recent American clinical guidelines dealing with laboratory tests for evaluation of liver disease, key recommendations relate to alanine aminotransferase (ALT)[6] upper reference limits (URLs) to be used as action limits in interpreting abnormal results of this enzyme *(1, 2)*. In adults, the American College of Gastroenterology (ACG) recommends ALT URLs of 33 U/L for males and 25 U/L for females, respectively, and that individuals with enzyme catalytic activity concentrations above these URLs should be further investigated *(1)*. In children, experts of the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) state that the interpretation of ALT should be based upon gender-specific URLs (26 U/L for boys and 22 U/L for girls).

We have some reservations about the universal application of these ALT cutoffs and we are concerned that the implementation of these recommendations may lead to overdiagnosis and unnecessary further testing. The contentious issues and our opinion about those are summarized below.

The first problem is the analytical variation among commercial assays measuring ALT. The mentioned guidelines recommend the use of universal cutoffs for ALT without considering any differences between laboratory assays. Reassuring statements are rather reported as follows: *"interlaboratory differences for ALT levels have not been reported to differ significantly" (1)* or *"the* [*ALT*] *assay is standardized between facilities" (2)*. In principle, fixed limits, be they medical decision thresholds or URLs, can be used only if the laboratory test in question is standardized and if this standardization is implemented in laboratories using the same limits. Despite the availability of a reference measurement system (RMS) for standardizing ALT results in clinical samples, the current evidence is, however, that ALT is still measured by methods that give quite differing values *(3)*. Assay performance also varies considerably within users of instruments from the same manufacturer *(4)*. This is mainly due to the use on the same platforms of various reagents with different analytical selectivity for ALT. For measuring aminotransferases, almost all manufacturers still market assays with or without the addition of pyridoxal-5-phosphate (P-5′-P), and declare that both are traceable to the RMS. However, it is impossible to calibrate procedures for aminotransferases that do not incorporate P-5′-P using a procedure that does, such as the reference measurement procedure (RMP), because the ratio of preformed holoenzyme to apoenzyme differs among specimens *(5)*. A recent study confirmed that assays without P-5′-P activation give different results compared to assays with P-5′-P activation and are often unable to fulfill quality specifications when ALT results are compared to the RMP *(6)*. This is immediately evident when looking at the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) data *(7)*: in the age range 13 to 19 years, ALT URLs were 22 U/L and 24 U/L for females and males, respectively, becoming 24 U/L and 33 U/L when reagents including P-5′-P were used. Therefore, the still significant differences in measuring ALT carry the risk that many individuals might be misclassified when clinicians start using these universally recommended cutoffs.

Unfortunately a majority, if not all, of the studies reporting reference values for ALT fail to demonstrate the traceability of their assays to the RMS. In addition, very different or even inappropriate criteria have been used for the selection of a reference population and the statistical procedures used to define the reference intervals also have been variable. These methodological issues were raised in one of the international hepatology journals over 10 years ago *(8)*, but clinical journals have continued publishing

[1] Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy; [2] CALIPER program, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; [3] Central Laboratory, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy; [4] Norwegian Quality Improvement of Laboratory Examinations (Noklus), Haraldsplass Deaconess Hospital, Bergen, Norway; [5] Department of Clinical Chemistry and Endocrinology, Prince of Wales Hospital and School of Medical Sciences, University of New South Wales, Sydney, and Screening and Test Evaluation Program, School of Public Health, University of Sydney, Australia.
* Address correspondence to this author at: UOC Patologia Clinica, Ospedale Luigi Sacco, Via GB Grassi 74, 20157 Milan, Italy. Fax +39-02-3564018; e-mail mauro.panteghini@unimi.it.
Received April 5, 2017; accepted April 18, 2017.
Previously published online at DOI: 10.1373/clinchem.2017.274977

[6] Nonstandard abbreviations: ALT, alanine aminotransferase; URL, upper reference limit; ACG, American College of Gastroenterology; NASPGHAN, North American Society of Pediatric Gastroenterology, Hepatology and Nutrition; RMS, reference measurement system; RMP, reference measurement procedure; CALIPER, Canadian Laboratory Initiative on Pediatric Reference Intervals; NAFLD, nonalcoholic fatty liver disease; NICE National Institute for Health and Care Excellence.

papers without special attention being paid to these important aspects *(9, 10)*. In a multicenter study fulfilling the rigorous design required to produce accurate reference intervals *(11)* and using ALT assays traceable to the RMS, we obtained ALT URLs in adults clearly higher than those reported in the mentioned American liver guidelines *(12)*. By enrolling healthy individuals from 4 different ethnic groups (Nordic countries, Italy, Turkey and China), we confirmed a significant difference in ALT activities between adult males and females. Corresponding ALT URLs were 59 U/L and 41 U/L, respectively *(12)*. One could observe that the URLs in this study may have suffered from some bias in selecting the population because individuals with a body mass index (BMI) of up to 30 kg/m^2 were included. However, by excluding all individuals with BMI ≥25 kg/m^2, the resulting URLs were 49 U/L for men and 33 U/L for women, which are still higher than those proposed by ACG (33 U/L and 25 U/L). Applying the latter recommended cutoffs to our population with BMI <25 kg/m^2, 13.7% of males and 10.9% of females would have been classified as "abnormal," consequently deserving further studies by the clinicians.

As a result of the inappropriate application of the above laboratory-related concepts, there is a danger that universally recommended URLs for ALT will lead to the "overdiagnosis" of many individuals, potentially generating unnecessary further investigations and healthcare costs. In the mentioned guidelines, ALT values above the URLs are used to trigger further clinical and laboratory investigations. For instance, the NASPGHAN panel recommends follow-up tests to explore reasons for increased ALT, including laboratory tests for excluding viral infections, endocrine disorders, autoimmune and genetic causes. Incorrect setting of the ALT URLs can result in costly and possibly invasive (e.g., liver biopsy) confirmatory evaluations. Although ALT screening seems to be a cheap intervention, subsequent costs and burdens to the healthcare system and to patients might be substantial.

A further concern is summarized by the question: what is the "normal" catalytic activity concentration for ALT? Apart from the above-mentioned problems related to the different assays for ALT measurement, there is also confusion in reported studies regarding when the concept of URL, as defined in the CLSI EP28-A3 standard *(11)*, should be applied and when decision limits or outcome-based thresholds should be recommended, for example, to exclude hepatitis C virus or other viral infections or nonalcoholic fatty liver disease (NAFLD) *(13)*. We advise that guideline developers do not use URLs and decision limits interchangeably and that they synthesize the evidence behind these values while considering the clear distinction of these terminologies and their clinical meaning.

Although we understand that the guideline panels' priority of lowering the ALT cutoffs is to avoid missing cases with disease present, we have not yet seen any outcome data on patients screened vs. not screened by ALT for NAFLD. In such cases, obesity is the underlying pathophysiology needing intervention, but ALT screening is not essential for guiding the management of patients in losing weight. A recent guideline by the National Institute for Health and Care Excellence (NICE) in the UK does not include a similar screening scenario for NAFLD, and its evidence summary is helpful in demonstrating that there is only very weak evidence to support the use of ALT as a screening tool for this clinical condition *(14)*.

We note in both American guideline panels that no laboratory professionals were involved to offer specialist advice on the critical appraisal of the evidence on ALT URLs published in the literature. The NICE guideline, however, did include a laboratory expert in its panel and that panel's conclusions and recommendations related to the clinical utility of ALT differed substantially from those of the American guidelines. We strongly believe that only a multidisciplinary approach can help to focus guideline panels on important laboratory-related items that can influence the implementation of recommendations and subsequent healthcare outcomes *(15)*. The issues we have raised highlight the critical need for laboratory expertise when drafting clinical guidelines involving the use of laboratory tests.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

References

1. Kwo PY, Cohen SM, Lim JK. ACG clinical guideline: evaluation of abnormal liver chemistries. Am J Gastroenterol 2017;112:18–35.
2. Vos MB, Abrams SH, Barlow SE, Caprio S, Daniels SR, Kohli R, et al. NASPGHAN clinical practice guideline for the diagnosis and treatment of nonalcoholic fatty liver disease in children. J Pediatr Gastroenterol Nutr 2017; 64:319–34.
3. Infusino I, Frusciante E, Braga F, Panteghini M. Progress and impact of enzyme measurement standardization. Clin Chem Lab Med 2017;55:334–40.
4. Jansen R, Jassam N, Thomas A, Perich C, Fernandez-Calle P, Faria AP, et al. A category 1 EQA scheme for comparison of laboratory performance and method performance: an international pilot study in the framework of the Calibration 2000 project. Clin Chim Acta 2014;432:90–8.
5. Panteghini M, Bais R. Serum enzymes. In: Rifai N, Horvath RH, Wittwer C, editors. Tietz textbook of clinical chemistry and molecular diagnostics. 6th ed. Elsevier Saunders: St. Louis; 2017. p 318–36.

6. Goossens K, Van Uytfanghe K, Thienpont LM. Trueness and comparability assessment of widely used assays for 5 common enzymes and 3 electrolytes. Clin Chim Acta 2015;442:44–5.
7. Colantonio DA, Kyriakopoulou L, Chan MK, Daly CH, Brinc D, Venner AA, et al. Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children. Clin Chem 2012;58:854–68.
8. Ceriotti F, Schumann G, Panteghini M. Redefining reference limits needs more attention to the analytical aspects. Liver Int 2006;26:1155–6.
9. Lee JK, Shim JH, Lee HC, Lee SH, Kim KM, Lim Y-S, et al. Estimation of the healthy upper limits for serum alanine aminotransferase in asian populations with normal liver histology. Hepatology 2010;51:1577–83.
10. Schwimmer JB, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. Gastroenterology 2010;138:1357–64.
11. CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline—third edition. CLSI document EP28-A3c, Wayne, (PA): CLSI; 2008.
12. Ceriotti F, Henny J, Queraltó J, Ziyu S, Özarda Y, Chen B, et al. Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in serum: results from an IFCC multicenter study. Clin Chem Lab Med 2010;48:1593–601.
13. Miller WG, Horowitz GL, Ceriotti F, Fleming JK, Greenberg N, Katayev A, et al. Reference intervals: strengths, weaknesses, and challenges. Clin Chem 2016;62:916–23.
14. National Institute for Health and Care Excellence. Nonalcoholic fatty liver disease: assessment and management. 2016. NICE guideline NG49. https://www.nice.org.uk/guidance/ng49 (Accessed May 2017).
15. Aakre KM, Langlois MR, Watine J, Barth JH, Baum H, Collinson P, et al. Critical review of laboratory investigations in clinical practice guidelines: proposals for the description of investigation. Clin Chem Lab Med 2013;51:1217–26.