RESEARCH LETTER

Influence of Local Reference Populations on Upper Limits of Normal for Serum Alanine Aminotransferase Levels

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hronic liver disease can progress to cirrhosis if not detected early and appropriate interventions taken when possible. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase are commonly measured during routine health care examinations to detect such unsuspected liver disease. Care providers often use the reporting laboratory’s ALT upper reference limit (upper limit of normal [ULN]) or a multiple thereof (eg, 1.5 \times ULN) to trigger further evaluation. Such evaluation can be expensive and invasive, yet ignoring aminotransferase elevations can allow life-threatening liver disease to progress if not recognized and treated appropriately. Therefore, how clinical laboratories define their own ALT ULN values is critically important in determining the risk-benefit ratio of further evaluation.

For technical reasons related to sample stability, validated standards are not used to establish a ULN for ALT and aspartate aminotransferase. Instead, laboratories use locally defined reference populations to establish their own reference ranges for these tests. The criteria used to include and exclude individuals from this important cohort directly determine the value of the test in identifying the presence of disease. As obesity increases in the general population, such reference populations could increasingly include individuals with unsuspected nonalcoholic fatty liver disease (NAFLD), which would skew the upper reference limit to inappropriate high levels. One recent population study excluded people at risk for NAFLD and concluded that the “healthy range” for serum ALT level should be up to 30 U/L (to convert to microkatal per liter, multiply by 0.0167) for men and 19 U/L for women. Although some have argued that this would lead to unnecessary medical expenditures and further burden our health care system, large population studies in Korea have demonstrated increased prevalence of NAFLD and increased liver-related mortality in middle-aged adults with ALT levels between 20 and 40 U/L compared with those with ALT levels lower than 20 U/L.

The Nonalcoholic Steatohepatitis (NASH) Clinical Research Network (CRN), a group of 8 academic institutions assembled by and in collaboration with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to study fatty liver disease, began to design clinical studies in 2002 and considered using an ALT value greater than the ULN as an entry criterion for its major pediatric treatment trial. However, variability was found in the ALT ULN values reported by clinical laboratories at CRN clinical centers that could confound the inclusion of homogeneous patient cohorts in this and other studies. The study described herein was undertaken to establish the causes of the variability in laboratory-defined ULN using results of analyses of samples distributed to clinical laboratories as part of annual accreditation by the College of American Pathologists (CAP).

Methods. Eleven clinical laboratories were identified as the primary laboratories to be used by the CRN clinical centers. The directors of these laboratories were queried in March 2003 using a written questionnaire regarding their results for ALT determinations of 5 samples analyzed as part of their CAP accreditation in 2002, the standard deviations of their analysis of these samples, the make and model of the analyzer used, and how they defined their own laboratory’s ULN. The CAP external proficiency samples for ALT activity measurement are shipped as liquid serum or plasma maintained at 4°C in a thermally insulated container. Directors were asked whether people with the following criteria were excluded from the reference population in order to establish the ALT ULN: known medical conditions (eg, hypertension, diabetes, heart disease, arthritis), current medication use, history of intravenous drug use, obesity (body mass index [BMI; calculated as weight in kilograms divided by height in meters squared] >30), overweight (BMI >25), or other factors. Data were collated by the NASH CRN Data Coordinating Center.

Results. Responses to the surveys were provided by all 11 clinical laboratories queried. The upper reference ranges defined using local reference populations (Table) varied from 35 U/L to 79 U/L for men and 31 U/L to 55 U/L for women (Figure 1). Known disease states were typically used as exclusion criteria, but overweight and obesity were not taken into account by any of the laboratories.

The samples were distributed to laboratories by CAP for analysis, and the results of sample C15, a sample with an ALT level near the upper reference range value reported by most laboratories, were compared with the national mean from all laboratories that used the same analyzer. Intralaboratory variability in measuring sample C15 was small (Figure 2, SD <3 U/L). Interlaboratory variability was also small, with laboratories reporting mean C15 results in close agreement with other laboratories using the same analyzer. The maximum variability in C15 analysis attributable to differences among analyzers used by CRN laboratories was 18 U/L. Whereas this difference based on the particular machine used is not trivial, it did not completely explain the wide variation in ULN
Most of the variation of the ULN reported by these 11 laboratories can only be explained by differences in their reference populations. The impact of the variable ULN on interpretation of the normalcy of the 11 laboratories’ C15 results is shown in Figure 3.

Figure 1. Reference ranges for serum alanine aminotransferase (ALT) reported by the 11 Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) laboratories. The bars indicate the reference ranges; the upper limit for women varied from 31 U/L in laboratory B to 55 U/L in laboratory E; the upper limit for men varied from 35 U/L in laboratory A to 79 U/L in laboratory K. The lower limit ranged from 0 to 12 U/L. To convert ALT to microkatals per liter, multiply by 0.0167.

Table. Characteristics of the Reference Populations Used to Define the Serum Alanine Aminotransferase Upper Limit of Normal by 11 Clinical Laboratories

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>No. of People</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Unknown</td>
<td>Reference range was determined long ago; as new instruments have been added, results always correlate, and we continue to use this range.</td>
</tr>
<tr>
<td>B</td>
<td>94</td>
<td>Excluded people with known medical conditions (e.g., hypertension, diabetes, heart disease, and arthritis) or current medication use.</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>Selected blood donors with no clinical symptoms.</td>
</tr>
<tr>
<td>D</td>
<td>Unknown</td>
<td>Normal values have been the same for 25 years using the same instrument manufacturer; findings from comparison studies between upgrades have remained the same.</td>
</tr>
<tr>
<td>E</td>
<td>Unknown</td>
<td>Excluded people with known medical conditions (e.g., hypertension, diabetes, heart disease, and arthritis), current medication use, and history of intravenous drug use.</td>
</tr>
<tr>
<td>F</td>
<td>222</td>
<td>None.</td>
</tr>
<tr>
<td>G</td>
<td>222</td>
<td>None.</td>
</tr>
<tr>
<td>H</td>
<td>Unknown</td>
<td>Unknown criteria.</td>
</tr>
<tr>
<td>I</td>
<td>&gt;120</td>
<td>Excluded people with known medical conditions (e.g., hypertension, diabetes, heart disease, and arthritis), current medication use, history of intravenous drug use, and whose medical history would affect test results.</td>
</tr>
<tr>
<td>J</td>
<td>1029</td>
<td>Excluded people with known medical conditions (e.g., hypertension, diabetes, heart disease, and arthritis).</td>
</tr>
<tr>
<td>K</td>
<td>1354</td>
<td>Selected people with normal aspartate aminotransferase, alkaline phosphatase, and bilirubin levels.</td>
</tr>
</tbody>
</table>

Figure 2. Mean alanine aminotransferase (ALT) values for the College of American Pathologists (CAP) sample C15. The gray bars indicate the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) laboratory-specific means of replicate analyses and the thin line error bars indicate ±1 SD in each direction; 4 laboratories (A, D, J, and K) did not provide standard deviations of their results. The national machine-specific mean ±1 SD in each direction for the analyzer used in each laboratory is indicated by overlying the thick line error bars. The results from most laboratories were within 2 U/L of the national mean for their specific analyzer; laboratory A deviated the most from the national mean, yet the difference was still only 4.9 U/L, indicating that laboratory performance was generally excellent. To convert ALT to microkatals per liter, multiply by 0.0167.

Figure 3. The alanine aminotransferase (ALT) activity of 1 standardized sample (C15) reported by 11 different laboratories is expressed as a multiple of the sex-specific upper limit of normal (ULN) for those laboratories. The disparate ULN values reported by different laboratories causes the results of this 1 sample to be variably interpreted as 0.66 to 1.75 ULN if it came from a man and 0.87 to 1.73 ULN if it came from a woman. If this sample was from a woman, it would be reported as normal by 3 laboratories (E, F, and G) and elevated by the other 8. If it was from a man, it would be reported as normal in 7 laboratories and elevated in 4 (A, B, C, and D).

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chines were proportional to the values obtained or if the difference among machines was relatively constant. A relatively constant difference becomes less significant at higher ALT levels, whereas a proportional difference amplifies the variability among analyzers and becomes relevant for clinical or study criteria that use high levels such as 500 U/L as a cutoff to trigger an intervention or evaluation. An analysis of the CAP samples with lower and higher ALT levels revealed that the analyzer-specific differences were relatively constant and not proportional to the ALT result. Thus, the variability among analyzers becomes less significant when measuring samples with higher ALT levels.

Comment. The analysis of the CAP data for ALT from the 11 clinical laboratories with respect to each laboratory's reported reference range demonstrates that the primary factor contributing to the widely divergent ALT ULN values is not variability within a laboratory or variability caused by the use of different analyzers but must be related to the characteristics of the cohorts used by individual laboratories to define their own reference ranges. This unsettling observation suggests that the reported ULN values provided by laboratories with their test reports may not be reliable in identifying patients with unsuspected chronic liver disease.

Especially germane to this issue is the common use of multiples of reported ALT levels as criteria for clinical trial entry or initiating clinical interventions such as stopping medication use or further evaluating the abnormality with blood tests, imaging, and even a liver biopsy. Multiplying inaccurate ULN values only multiplies the errant value created by using the local reference population. For example, if therapy with a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (ie, a statin) is to be discontinued if the ALT rises above 3 × ULN, then clinical management will vary considerably depending on where a patient obtains a blood test.

The factors contributing to the variability in reference cohorts used to establish ULN values reported by different laboratories are unknown. Certainly, obesity could be a major factor. Obesity is commonly associated with NAFLD, an increasingly common condition that causes progressive liver disease to the point of cirrhosis in some patients. Nonalcoholic fatty liver disease can cause elevations in ALT level, and as the current epidemic of obesity increases, reference populations may include increasing numbers of people with undiagnosed NAFLD. A recent study of adults enrolled in the Dallas Heart Study identified imaging evidence of NAFLD in 33% of the adult population, suggesting a high likelihood that obesity and associated liver disease contribute to the relatively high ALT levels in laboratory reference populations. Alternatively, anthropomorphic, clinical, or demographic differences other than obesity could be responsible for the variation among laboratories.

In summary, the ULN for serum ALT levels reported by clinical laboratories usually reflects characteristics of the local reference population, a population that was not screened for risk factors for fatty liver disease in our study. Differences among analyzers provided by different manufacturers play only a modest role in different ULN values from different laboratories, whereas laboratory profi-

ciency does not play a role. The implication of this finding for clinical practice is that the ULN for ALT levels reported by clinical laboratories that accompanies test results may not be reliable for identifying patients with unsuspected liver disease when the ULN is substantially above the proposed healthy range for ALT of 30 U/L for men and 19 U/L for women. With the increasing prevalence of obesity and the likely parallel increase in the prevalence of NAFLD, clinical laboratories need to anticipate how this disease might adversely alter the makeup of reference populations. Laboratories should consider identifying healthy subjects without risk factors for insulin resistance and fatty liver disease when establishing reference groups for testing serum ALT level. Until this is done, clinicians might identify patients with liver disease more consistently by using absolute values (such as 40 U/L) for the normal upper reference limit of ALT level rather than relying on laboratory-specified reference ranges.

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**COMMENTS AND OPINIONS**

**Risk of Psoriasis and Obesity in Women**

In a large prospective cohort study, Setty and colleagues found that multiple measures of adiposity, such as body mass index (BMI), waist and hip circumference, and waist to hip ratio, along with change in adiposity as assessed by weight gain since the age of 18 years, were substantial risk factors for the development of incident psoriasis. Analyses were performed with adjustment for comorbidities associated with increased adiposity such as type 2 diabetes mellitus, hypertension, and cardiovascular diseases.

To our knowledge, many studies have reported a significant association between increased adiposity and psychological morbidity. Sachs-Ericsson et al found that BMI was associated with depressive symptoms. Similarly, Pickering et al showed that obesity among women is related to episodes of major depression with atypical features.

On the other hand, several studies have demonstrated the association of psoriasis with severe psychological morbidity, in particular with depression. In a study examining the various psychological aspects of psoriasis, Devrimci-Ozguven et al reported that patients with psoriasis had significantly higher degrees of depression and more body cathexis problems than controls. Similarly, Esposito et al demonstrated that psoriasis is associated with profound psychological morbidity (mainly with depression) in a large number of patients. Albobelli et al also observed that psoriasis was significantly associated with depression (15.4%), while in cases with psoriatic arthritis, the percentage of patients with depression was even higher (30%).

Finally, it is important to mention that in their study Setty et al did not consider the family history of psoriasis, since this information was not available in their cohort. There is, however, solid evidence that positive family history of psoriasis is one of the most commonly seen patterns in a great number of patients, sometimes reaching very high rates (45.9%).

Although adiposity as assessed by Setty et al could be considered an individual risk factor for incident psoriasis, psychological comorbidity—mainly in the form of depression, which is undeniably related to and also caused by obesity—is a similarly contributing risk factor for the onset of psoriasis in a nonnegligible, large number of patients. In light of this current evidence, we firmly believe that psychological comorbidity should be kept under thoughtful consideration for a more precise estimation of the risk of psoriasis in obese populations.

**Pneumococcal Vaccination in Adults**

In their recent article, Johnstone et al demonstrated that for the years 2000 to 2002, hospitalized Canadian adults with community-acquired pneumonia who had previously received 23-valent polysaccharide pneumococcal vaccine (PPV) experienced lower mortality or less need for intensive care unit admission compared with unvaccinated patients. However, the investigators pointed out that only 215 of 2416 eligible patients (9%) were vaccinated at hospital discharge. A recent UK-based study showed that depending on the risk, vaccine uptake in at-risk individuals ranged from 13% to 69% but was substantially lower in populations where the proportion of nonwhites exceeds 10%. Marked ethnic disparities were also reported among American elderly adults. The uptake of PPV among Muslims attending the Haj pilgrimage in Mecca, Saudi Arabia, is not known, even though up to 12% of the