Definitions of Clinical Laboratory Reference Limits

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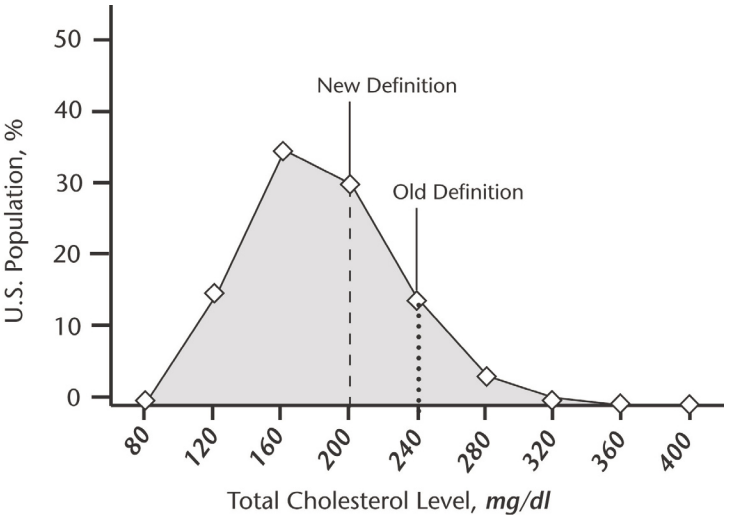
Introduction

It is clearly established that macro and micronutrient factors play important roles in the etiology of most diseases that are the major causes of morbidity and mortality in modern society.[1] Clinical laboratories now have reliable methods for measuring a patient's status of all essential and many conditionally essential nutrients, thus allowing the assessment of metabolic disorders amenable to nutrient interventions.[2] Laboratories that perform testing of nutrient depletion markers have a new challenge with regard to the definition of reference limits. Since early detection of nutrient insufficiency is the best way to prevent associated diseases, the challenge is not only to identify the presence or absence of a frank deficiency disease, but also to identify patients who are candidates for nutrient interventions to correct depleted body stores. This challenge requires a new look at the use of clinical laboratory reference limits. A similar situation applies to measures of toxicants and cell regulators. Clinical significance is derived from laboratory data when a result is compared to a reference limit and the laboratory provides interpretive comments. Variations in reference limits are common, even for tests that are used for confirmation of disease diagnosis. A survey of biochemical markers of myocardial damage recently found numerous different reference limits.[3] Reference ranges are more variable for tests of disease risk factors placing this class of testing particularly in need of definitions regarding how to set limits.

Laboratory Values as Definitions of Disease vs. Disease Risk

Measurement of cholesterol represents a well-known example of a laboratory test that is used to define as well as to prevent a disease. The disease in this case is the pathognomonic, hypercholesterolemia. Defining diseases as states of abnormal laboratory values has several undesirable outcomes, the greatest of which is the misunderstanding of human diseases. Early studies using statistical procedures placed the upper normal reference limit for serum total cholesterol in the 300-320 mg/dL interval. In Japanese populations, however, the same procedures gave lower limits of 240-250 mg/dL, leading to frustrations when the U.S. Framingham data caused reevaluation and suggestion of lowering the limit to 220 mg/dL.[4] Standard reference books for clinical chemistry acknowledge the disparity of definition. One published in 1987 lists 90 percent limits of 300 for males and 320 for females, and then states that "Although the ranges shown have been established for apparently normal,

Figure 1. Distribution of total serum cholesterol levels for the U.S. adult population and two definitions for hypercholesterolemia. From "Changing Disease Definitions: Implications for Disease Prevalence. Analysis of the Third National Health and Nutrition Examination Survey, 1988-



healthy adults, these may not necessarily be "desirable" values. "[5] Another, more current book warns that "Sharp inconsistencies are obvious when one studies published normal ranges of serum cholesterol in the United States through the 1970s and much of the 1980s."[6] They go on to cite a limit of 240 mg/dL for adults that is "excessive for young adults" for whom 220 mg/dL is somewhat high, depending on LDL, HDL, family history, and other factors." Most tables currently list 200 mg/dL as a desirable blood cholesterol level, even though approximately 40% of the general population has levels above that point, as shown in Figure 1. The area under the curve to the right of 200 mg/dL is defined as abnormal.

When clinical reference limits are used to define disease, changes of the limits take on great significance for national health policy because new definitions can dramatically inflate disease prevalence and national healthcare costs. Although laboratory reference ranges are the focus of this article, the issue of definitions applies to all types of quantitative measurements. Blood pressure is one of the most reliable clinical markers of cardiovascular health. Even here, debate over the definition of normal arises from reports such as one showing that middle-aged women have significantly greater impairment of psychomotor

Test	Old Limit	New Limit	Disease	New Cases millions
Fasting serum glucose	140 mg/dL	126 mg/dL	Diabetes	1.7
Diastolic/Systolic bp	100/160 mm Hg	90/140 mm Hg	Hypertension	13
Serum cholesterol	240 mg/dL	200 mg/dL	Hypercholesterolemia	42
Body mass index	27	25	Obesity	29

Table 1. Proposed changes in disease risk factor reference limits. Redefining abnormal results in large numbers of individuals moving from negative to positive status for respective diseases.

speed when blood pressure is in the high "normal" range.[7] A 1999 evaluation of the NHANES III database revealed that the overall impact of just the reference limit changes shown in the Table 1 would be to define 75% of the adult U.S. population as diseased.[8] More recent recommendations have further lowered definitions of normal blood pressure to 120/80.[9] Since healthcare policy calls for treatment of patients with abnormal laboratory values, and the treatments generally call for drugs that are covered under healthcare plans, the economic impact can be staggering. As one might guess, there has been resistance to such redefinitions of normality.

A more useful view of laboratory evaluation is to use the tests listed in Table 1 to define risk factors for disease rather than defining a disease. Some challenges to the lowered limits propose a lower range of "abnormality" where diet and lifestyle advice is the standard of care, while therapeutic drugs would be prescribed at more abnormal levels. Thus blood pressures of 120 to 159/90 to 99 would call for diet and lifestyle modification, while those above 159 are to be treated for the disease of hypertension.[10] Similar splitting of cutoff values could be done for many tests in an attempt to identify candidates for early intervention while restricting the population for whom prescription medications are mandated. This approach may have merit in changing the way we think about the use of laboratory data. The focus of primary care should be on the early identification of modifiable risk factors that can result in real reduction of disease incidence, not just improved survival for the diseased population. For example, the more narrow definition of normal for blood pressure is attractive because it allows the identification of endothelial dysfunction that is a precedent in the etiology of cardiovascular disease.

The use of laboratory evaluations to reveal nutrient insufficiencies as underlying causes of disease opens the question more broadly. Interventions for correcting abnormalities are generally presumed to be nutrition and lifestyle-based modifications that are not paid for by insurance providers. The interventions differ fundamentally also in their very high degree of safety, so there is little cause for liability concern. As intervention costs drop and their inherent safety rises, the acceptability of narrowing reference ranges increases. In other words, we are more willing to accept higher numbers of false positives for the advantages of identifying more true positives at the risk of treating some patients unnecessarily with moderate levels of nutrients.

The experimental design for assigning limits has generally involved establishing criteria that define positive and negative subject populations. The ideal negative population for a nutrient sufficiency test is a set of individuals who will never develop diseases related to that nutrient. The obvious difficulty of determining such a population inhibits progress in defining nutrient testing reference limits. For example, cardiovascular disease is clearly related to endothelial function. Elevated serum homocysteine has negative impact on endothelial function[11], and homocysteine concentration, in turn, is increased by folic acid deficiency.[12] Elevated urinary formiminoglutamic acid (FIGLU) is a sensitive marker for folic acid deficiency, but, heart disease is only one of many potential outcomes of folic acid insufficiency, and that outcome may be separated by years from the initial FIGLU elevation. Reference ranges for FIGLU testing cannot be based solely on a population with the end-stage deficiency outcome of heart disease because that approach would miss the goal of early detection. A more realistic goal is to derive risk factors for various folate-dependent degenerative conditions at various levels of FIGLU elevation. The higher a given patient FIGLU result falls in the range of general population values, the greater the risk of all folate-deficiency diseases. Similar arguments apply to testing for all essential nutrients and for

toxicants such as lead, mercury, or measures of intestinal bacterial and yeast overgrowth.

A new approach is needed to allow the clinical laboratory to clearly communicate to physicians the significance of a patient result. Some laboratories have attempted to arbitrarily redefine normality by arbitrarily narrowing reference ranges. The question that necessarily arises from this approach is what process or rationale was used to reach the new definition. Clinicians can be left wondering about the significance of a result. The best approach is one that clearly shows how the reference limit is established and where a given result lies relative to the general patient population.

Defining Abnormality with Population Percentile Cutoffs

The closer one tries to approach defining optimal wellness, the greater the challenge of finding a "normal" cohort. One method used for discerning small differences is to simply divide a study population into lower and higher segments of a measured parameter. This approach alleviates the requirement to select a normal and diseased population. Thus, in order to study the early manifestations of hypertension, children in the upper quintile of blood pressure were found to have higher left ventricular hypertrophy relative to those in the lower quintile.[13] Here the population of 264 school children was divided into 5^{ths}, or quintiles, according to their blood pressure readings. For a sense of the extent of application of this approach, a search of reports using quintile comparisons to draw conclusions yields 91 titles in the first six months of 2003. The measured parameters range from calcium intake effects on heart disease[14] and prefrontal cortex monoamine oxidase in Alzheimers disease[15] to the relationship between residential proximity to traffic and adverse birth outcomes[16].

A familiar example of this approach is found in standard serum chemistry reports. Clinical decisions regarding cardiac risk based on LDL cholesterol are guided by expressing the results in terms of relative risk. Commonly, four or more categories are defined starting at "normal" risk for results less than 150 mg/dL and increasing to "Very High" risk for values above 500 mg/dL. Similarly, LDL/HDL ratio results are routinely expressed as falling into one of several categories of increasing risk as the ratio increases. It is generally understood that the actual coronary risk is determined by a combination of various parameters such as smoking habits, obesity, and blood lipid data. Thus, the problem of overlapping normal and abnormal is solved by simply stating the position of a given patient in the continuum of results that correlate with increasing risk. Such an approach might be adopted as a general way of interpreting various factors that contribute to disease risk.

Finer population divisions become possible as the number of subjects becomes very large. Very large population data sets may be divided into one hundredth's and expressed in percentile units. When the 5th percentile for serum cholesterol has been defined as 115 mg/dL for young males and 119 mg/dL for young females one knows at a glance that a result below these cutoff values places the patient in the bottom 5% of the population.[6] For most clinical decisions about nutrient interventions, a patient who falls outside of the fifth quintile, meaning that 80% of the total population has greater reserve, might be considered a candidate for a repletion dose trial. As quantitative relative risk data accrues for a given test, then the population positions may be converted into risk factors as has been done for LDL/HDL ratios and several other measures.

A laboratory report of urinary metabolic markers of cofactor status based on quintile definitions of the analyte distributions is shown in Figure 3. All of the analytes shown are metabolic markers of vitamin insufficiency. They are biochemical intermediates that spill into urine in response to specific functional vitamin insufficiencies. When the patient status of vitamin B_6 is low, for example, the urinary concentration of xanthurenate becomes elevated. This relationship is due to slower conversion at the pyridoxal-5-phosphate-dependent step for clearance of the xanthurenic acid precursor, 3-hydroxykynurenin when intake of vitamin B_6 is low.[17] As the precursor accumulates, greater amounts are converted into xanthurenic acid that is excreted. In Figure 3, the reference limits for elevated levels (where an "H" is printed next to the result) are set at the 5th quintile. The patient result is plotted within decile or 10% population divisions. Thus, the methylmalonate result is in the first decile, and, while both xanthurenate and formiminoglutamate are in the fifth quintile, the results are in the tenth and ninth deciles, respectfully, as indicated by their shift to the left or right of the quintile section on the bar chart.

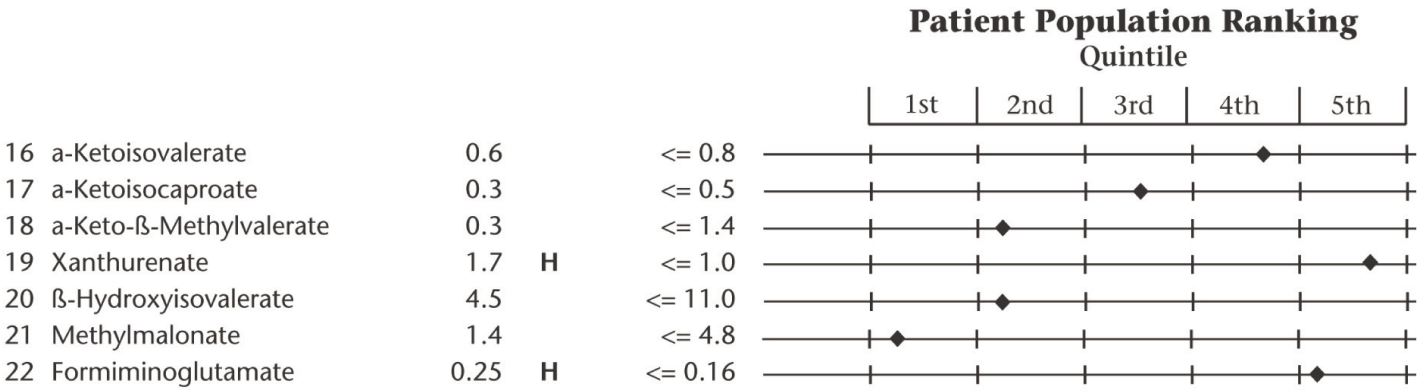


Figure 2. A laboratory report utilizing quintile definitions of abnormal. The bar charts show the position for each result relative to the general population distribution. The cutoff for the 5th quintile is defined as an abnormally high result that suggests the patient is a candidate for nutrient repletion. Points are plotted into the decile divisions of the population for each analyte, and hash marks are placed at each quintile division.

Adoption of such a convention, allows the clinician to tell where a given patient falls relative to the general population for each analyte. For direct measures of nutrients in physiological fluids, the insufficient sign is a low level, and results in the 1st quintile identify a candidate for nutrient repletion. Abnormally high values might signify a different class of risk that becomes significant only at more extreme values above the 9th decile.

The example of plasma levels of the amino acid methionine illustrates the use of first quintile cutoff for high risk of essential nutrient deficiency disorders. The histogram for a representative population of 2972 outpatients is shown in Figure 2, where the number of occurrences within each concentration range is plotted against the plasma concentration in micromoles per liter. The curve shows near-gaussian distribution. Published ranges of 10-42 µM encompass nearly the entire range of values found.[18] Such ranges are useful only for severe methionine elevations as might occur from inborn errors of metabolism or the use of high-methionine TPN formulas.[19, 20]

A case may illustrate the clinical usefulness of assessments based on quintile definitions of reference limits. A 60-year-old college professor with extreme chemical sensitivity was treated for heavy metal toxicity with oral methionine and taurine. The initial plasma methionine value was near the 1st quintile limit, while the level after 6 months

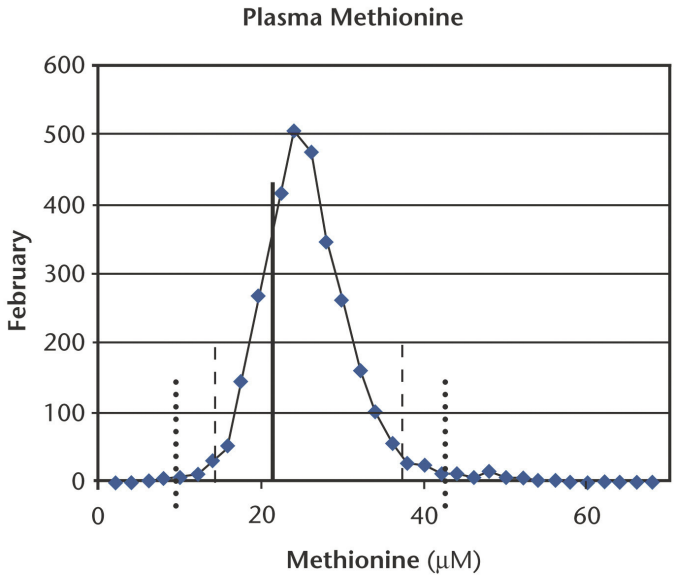


Figure 3. Plasma methionine population distribution. Dotted line, ranges published for testing of genetic defects; dashed line, +- 1.5 SD cutoffs; solid line, 1st quintile (20 percentile) cutoff.

of supplementation had risen to the 10th decile. At the same time his plasma arginine stayed at the upper part of the 1st quintile. Such a combination of markers presents a clear warning about possible nitric oxide deficit clinical effects such as local vasodilatory response failure. Arginine is the substrate for nitric oxide formation, and elevated methionine concentrations favor the formation of the nitric oxide synthase inhibitor, asymmetric dimethyl arginine. The patient had experienced a worsening of severe headaches, nasal allergies, and viral infections. All of these signs place the patient at high risk for the effects of poor nitric oxide responses.[21-23] Thus, the pattern of first quintile arginine with dramatic rise of methionine from second to tenth quintile status says much about how to manage this patient.

The difficulty with defining normality extends to many other tests. Waiting for extremely low erythrocyte selenium to institute selenium repletion is inappropriate. Even mild selenium deficiency can contribute to the development of autoimmune thyroid diseases.[24] Improved clinical assessment of thyroid status might result from clarification of individual shifts relative to population distributions for assays of T3, rT3, T4, and TSH. For example, numerous subtle shifts have been reported to occur associated with obesity and starvation.[25]

The concept of quintile definitions for results may also be extended to cellular indicators as well. Extreme hypereosinophilia (>2.052 x 10(9)/l) occurs rarely while values between 0.5 and 1.0 x 10(9)/l are indicative of bronchial asthma.[26] The number one suspect in patients with hypereosinophilia is parasitic infections, so eosinophil counts falling from 5th to 3rd quintile following antihelminth treatment is a simple criteria for positive diagnosis.[27] Eosinophilic conditions where values hover in the tenth decile may be due to immune up-regulation.[28]

Conclusion

The use of the clinical laboratory for disease risk evaluation is rapidly increasing. The ability to place significance on a reported value depends on clear communication between laboratories and clinicians. Old definitions based on non-overlapping ranges for diseased and non-diseased populations do not fit the need for markers of disease risk from chronic nutrient deficiency, toxicant exposures, or cell regulator levels. The adoption of reference limits based on population distribution such as quintiles affords a clear and meaningful approach to redefining reference limits.

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