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or page down to review presentation
What’s Normal?

Daniele Prati,
Ospedale Alessandro Manzoni, Lecco
Guidance for Industry
Drug-Induced Liver Injury: Premarketing Clinical Evaluation

- ALT or AST >8xULN
- ALT or AST >5xULN for more than 2 weeks
- ALT or AST >3xULN and (TBL >2xULN or INR >1.5)
- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

July 2009
Drug Safety
Use of Hy’s Law and a New Composite Algorithm to Predict Acute Liver Failure in Patients With Drug-Induced Liver Injury


Figure 2. Prognostic algorithm for ALF/OLT to apply at DILI recognition. Cases with a higher risk of ALF/OLT are indicated in white squares (specificity, 82%; sensitivity, 80%; positive likelihood ratio, 4.4; negative likelihood ratio, 0.24).
Outline

• The concept of normality, and some considerations about the definition of normal values

• Normal ranges and other cutoffs for the diagnosis liver injury
“Every normal person, in fact, is only normal on the average.”

“His ego approximates to that of the psychotic in some part or other and to a greater or lesser extent.”

S. Freud

Analysis Terminable and Interminable, 1937
### Different aspects of the concept of normality

<table>
<thead>
<tr>
<th>Definition</th>
<th>Discipline</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Probabilistic</td>
<td>Statistics</td>
<td>Gaussian (or other)</td>
</tr>
<tr>
<td>2. Most common in its category</td>
<td>Descriptive sciences</td>
<td>Modal</td>
</tr>
<tr>
<td>3. Common in its category</td>
<td>Descriptive sciences</td>
<td>Habitual</td>
</tr>
<tr>
<td>4. Most suitable to survive</td>
<td>Genetics</td>
<td>Best adapted</td>
</tr>
<tr>
<td>5. Without any disease</td>
<td>Clinical medicine</td>
<td>Healthy</td>
</tr>
<tr>
<td>6. Best in its category</td>
<td>Moral philosophy</td>
<td>Ideal</td>
</tr>
</tbody>
</table>

Mod. From Murphy EA. Perspect Biol Med 1972
“Normal” values for continuous laboratory variables
The Gaussian Model

Mean ± 2 SD

2.5% 95% 2.5%
“Normal” values for continuous laboratory variables
The percentile approach

95th percentile
of the “normal population”
Probability for an individual to be called normal when subjected to different numbers of diagnostic tests

<table>
<thead>
<tr>
<th>Number of tests</th>
<th>Calculation</th>
<th>Probability of testing ”normal”</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.95</td>
<td>95%</td>
</tr>
<tr>
<td>2</td>
<td>0.95²</td>
<td>90%</td>
</tr>
<tr>
<td>20</td>
<td>0.95²⁰</td>
<td>35%</td>
</tr>
<tr>
<td>100</td>
<td>0.95¹⁰⁰</td>
<td>0.6%</td>
</tr>
</tbody>
</table>

Sackett and Haynes. The architecture of diagnostic research
“Normal” or “reference” values?

The concept of reference values was introduced as a replacement for the ambiguous term “normal values”. Reference values would apply to any type of reference individuals whether healthy or unhealthy, and assuming that the reference values were properly qualified.

Changing terminology

“Normal ranges”

_The old terminology:_

Should be replaced by

“Reference ranges”

_The new terminology:_

*When calculated from and/or referred to apparently healthy subjects*

“Health related reference ranges”

“Healthy ranges”
Normal values can be simplistic

- Healthy individuals
- At risk for complication
- Responders or cured
The Architecture of Diagnostic Research: From Bench to Bedside—Research Guidelines Using Liver Stiffness as an Example

Agostino Colli, Mirella Fraquelli, Giovanni Casazza, Dario Conte, Dimitrinka Nikolova, Piergiorgio Duca, Kristian Thorlund, and Christian Gluud

The diagnostic research process can be divided into five phases, designed to establish the clinical utility of a new diagnostic test—the index test. The aim of the present review is to illustrate the study designs that are appropriate for each diagnostic phase, using clinical examples regarding liver fibrosis diagnosed with transient elastography, when possible. Phase 0 is the preclinical pilot phase during which the validity, reliability, and reproducibility of the index test are assessed in healthy and diseased people. Phase I is designed to describe the distribution of the index test results in healthy people and its normal values. Phase IIA comprises studies designed to estimate the accuracy (sensitivity and specificity) of the index test in discriminating between diseased and non-diseased people in a clinically relevant population. Phase IIB studies allow the comparison of the accuracy of different index tests; Phase IIC studies aim to evaluate the possible harms of incorporating the index test in a diagnostic-therapeutic strategy. In phase III, diagnostic test-therapeutic randomized clinical trials aim to assess the benefits and harms of the new diagnostic-therapeutic strategy versus the present strategy. Phase IV comprises large surveillance cohort studies that aim to assess the effectiveness of the new diagnostic-therapeutic strategy in clinical practice. Conclusion: As common in clinical research, giving excessive weight to the results of single studies and trials is likely to divert from the totality of evidence obtained through the systematic reviews of these
Possible approaches:

✓ **Healthy ranges**: in a population at low risk for liver disease (clinical and subclinical)

✓ **Diagnostic thresholds**: in subjects at risk for liver disease
  – Thresholds to exclude complication
  – To confirm complication

✓ **Outcome studies**: (morbidity and mortality)
  – Among healthy subjects
  – Among subjects at risk for complications

✓ **Individual reference ranges**: the reference subject is the subject himself when he/she was healthy or uncomplicated ("reference change values")
Continuous variable

Risk of disease

“LOW” levels

THRESHOLDS

“HIGH” levels

Specificity

Sensitivity

“NORMAL”???
Distributions of test results

Changing the cutoff criterion from 1 to 2 (i.e., lowering the threshold)

- decreases the number of false negatives (increases sensitivity)
- increases the number of false positives (decreases specificity).
Outline

• The concept of normality, and some considerations about the definition of normal values

• Normal ranges and other cutoffs for the diagnosis liver injury
The healthy population for a liver disease targeting test

- Take a symptom free population (blood donors, general population etc)
- Exclude viral hepatitis carriers
- Exclude subjects at higher risk for metabolic syndrome/steatosis
  - High serum lipids/tryglicerides
  - High glucose levels
  - Overweight individuals
  - Heavy drinkers
  - ...
- Calculate reference intervals (95\textsuperscript{th} centile)
Different approaches to define new thresholds for alanine aminotransferase

1. “Low risk population”: values observed in subjects at low risk for liver disease (exclude those at high risk including HBV, HCV, alcoholic, NAFLD, etc)

2. “Mortality definition”: the values predicting mortality for chronic liver disease

3. “Therapeutic definition”: values observed in individuals cured from liver disease (eg, SVR for hepatitis C)
Different ALT thresholds for different clinical outcomes

ALT U/L

Outcomes

- Any histological disease in CLD pts
- Death for CLD in the general population
- Risk of decompensation or HCC in HCV pts
- Intracranial hemorrhage

?? in 10 yrs
?? in 30 yrs
Updated limits for ALT activity

6,835 blood donors

“Historical” upper limits

"Healthy" upper limits
(population at low risk for liver disease)*

40 U/L males
30 U/L females

30 U/L males
19 U/L females

(*) Normal BMI, triglycerides, cholesterol, and glucose levels; absence of concurrent medication use

"Receiver operating characteristic (ROC) curves of serum ALT for identification of people at risk of death from liver diseases"
“Therapeutic definition” of upper normal limit for ALT

- Population: 2881 patients included in 3 trials using pegylated IFN + Ribavirin
- Threshold: 95th percentile of ALT in sustained virological responders

Historical thresholds (Lab-specific) → 25% reduction

E.g. 40 U/L → 30 U/L

Prati D et al., J Hepatol 2006
### ALT cutoffs determined with different approaches

<table>
<thead>
<tr>
<th>Approach</th>
<th>New thresholds (U/L)</th>
<th>Reduction from to historical thresholds</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk population</td>
<td>30 in males, 19 in females</td>
<td>25-35%</td>
<td>Prati, Ann Int Med 2002</td>
</tr>
<tr>
<td>Mortality</td>
<td>30 in males</td>
<td>25%</td>
<td>Kim, BMJ 2004</td>
</tr>
<tr>
<td>Therapeutic efficacy</td>
<td>-- <em>(Expressed as xULN)</em></td>
<td>27%</td>
<td>Prati, J Hepatol 2006</td>
</tr>
</tbody>
</table>
Among the 665 individuals (346 men and 319 women) selected according Prati criteria, healthy ALT values were 33 IU/L for men and 25 IU/L for women.
- So, is everything definitely set?
- No!

- Upper reference limits in different labs are still variable.
- Expressing results as times x ULN does not solve the problem.
- What is the impact of the introduction of standardized IFCC assays on reference ranges?
- There is no «clinical» validation of standardized IFCC aminotransferase assays.
- Furthermore, most clinicians have no knowledge that assays have been changed.
- So far, attempts of setting cutoffs for liver tests focused mainly on ALT. Appropriate cutoffs for AST, ALP, and bilirubin (among others), still need to be identified.
- New studies are needed!

Presentation of Professor Dufour later in this session.
Messages

- Several studies conducted during the last decade suggest that the healthy ranges for ALT can be provisionally set around 30 U/L in males, and 20-25 U/L in females.
- However, the simplistic approach of a universal cutoff value valid for all clinical situations should be abandoned. Results should be interpreted flexibly, taking into account the scope of the test and the patient’s characteristics (gender, age, clinical history, specific risk factors, symptoms, signs, etc).
- Setting appropriate cutoffs needs close cooperation between hepatologists, clinical pathologists, statisticians, regulatory institutions.
- Much work is still needed.