# Advanced Laboratory Medicine

## Post-Analytic Issues:

## Reference Ranges and Interpretation

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## Learning objectives

- What is a reference range and how is determined?
- What is the "sensitivity" and "specificity" of a diagnostic test? How are they calculated?
- Understand how disease prevalence affects the predictive power of a diagnostic test

## Types of Tests

- Screening
- Diagnostic
- Monitoring

## Reference Range

- A value or set of values used to interpret a laboratory test result
- The term "Normal Range" should be avoided because it implies health and/or a normal distribution and neither might represent the significance of a test result or the distribution of test results

## Interpretive Guidelines

- Screening – "Most" sensitive discriminator for detecting the possible presence of disease
- Diagnosis – "Best" discriminator for the presence of disease
- Monitoring – Range in which the therapy is safe and/or effective

## Reference Values

- Reference values should be determined on a representative sample from the patient population on which the test will be used
- The reference values can be a single cut-off, a set of cut-offs, or a range of values containing 95% of the results from a reference population
- May vary with age, sex, and other physiologic factors

## Factors Affecting Reference Range

- Test method utilized
- Test Equipment utilized
- Population Tested
- Experimental variability inherent to reference range studies
- Judgment

## Reference Values

- The most common definition is the range of values containing the central 95% of the "healthy" population, i.e. the Reference Limits are the values at 2.5% and 97.5%
- This definition results in 5% of the "healthy" population being classified as "abnormal" or "positive"

## How a Reference Range Study is Performed

1. Select a reference group representative of the population that will be tested
2. The reference group should be free of disease and conditions that might cause an "abnormal" result
3. Establish criteria for excluding individuals with factors that may impact the test
4. Screen and test reference group
5. Calculate reference range

## Analysis of Reference Range Study Data

- N = 120 (allows 3 subjects/each 2.5%)
- First, eliminate Outliers
- Determine 2.5% and 97.5% reference values
  - Parametric: Calculate mean +/- 2 SD
  - Non-Parametric: rank order the results and find the 2.5% and 97.5% values

## Frequency Distribution for Calcium Reference Range Study

| Ca mg/dL | Women | Men | Combined |
|---|---|---|---|
| 8.8 | 1 | 0 | 1 |
| 8.9 | 2 L | 0 | 2 |
| 9.0 | 1 | 0 | 1 |
| 9.1 | 3 | 2 | 5 L |
| 9.2 | 11 | 1 L | 12 |
| 9.3 | 11 | 8 | 19 |
| 9.4 | 8 | 6 | 14 |
| 9.5 | 16 | 11 | 27 |
| 9.6 | 16 | 12 | 28 |
| 9.7 | 26 | 13 | 39 |
| 9.8 | 8 | 16 | 24 |
| 9.9 | 7 | 14 | 21 |
| 10.0 | 3 | 7 | 10 |
| 10.1 | 2 | 10 | 12 |
| 10.2 | 3 H | 11 | 14 |
| 10.3 | 2 | 7 H | 9 H |
| 10.4 | 0 | 1 | 1 |
| 10.5 | 0 | 0 | 0 |
| 10.6 | 0 | 1 | 1 |
| N | 120 | 120 | 240 |

## Non-Parametric Reference Value Cut-offs

- 2.5 %
  - rank value #3; N = 120
  - rank value #6; N = 240
- 97.5%
  - rank value #118; N = 120
  - rank value #235; N = 240

## Frequency Distribution for Calcium Reference Range Study

| Ca mg/dL | Women | | Men | | Combined | |
|---|---|---|---|---|---|---|
| 8.8 | 1 | | 0 | | 1 | |
| 8.9 | 2 | L | 0 | | 2 | |
| 9.0 | 1 | | 0 | | 1 | |
| 9.1 | 3 | | 2 | | 5 | L |
| 9.2 | 11 | | 1 | L | 12 | |
| 9.3 | 11 | | 8 | | 19 | |
| 9.4 | 8 | | 6 | | 14 | |
| 9.5 | 16 | | 11 | | 27 | |
| 9.6 | 16 | | 12 | | 28 | |
| 9.7 | 26 | | 13 | | 39 | |
| 9.8 | 8 | | 16 | | 24 | |
| 9.9 | 7 | | 14 | | 21 | |
| 10.0 | 3 | | 7 | | 10 | |
| 10.1 | 2 | | 10 | | 12 | |
| 10.2 | 3 | H | 11 | | 14 | |
| 10.3 | 2 | | 7 | H | 9 | H |
| 10.4 | 0 | | 1 | | 1 | |
| 10.5 | 0 | | 0 | | 0 | |
| 10.6 | 0 | | 1 | | 1 | |
| N | 120 | | 120 | | 240 | |

## Calcium Reference Limits and 90% Confidence Intervals

Non Parametric Reference Limits

- Women: 8.9 - 10.2 mg/dL
- Men: 9.2 - 10.3 mg/dL
- Combined: 9.1 - 10.3 mg/dL

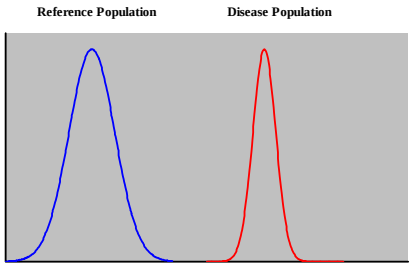
Confidence Interval for Reference Limits

- Women: 8.8 – 9.1 10.1 – 10.3 mg/dL
- Men: 9.1– 9.3 10.3 – 10.6 mg/dL
- Combined: 8.8 – 9.1 10.3 – 10.6 mg/dL

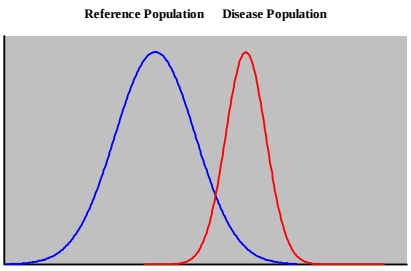
## CUMC Coagulation Crossover Study

| Nov-2004 | PT CORE | PT ALLEN | PTT CORE | PTT ALLEN | FIBRINOGEN CORE | FIBRINOGEN ALLEN |
|---|---|---|---|---|---|---|
| **Number** | 117 | 105 | 117 | 105 | 117 | 69 |
| **Min** | 12.8 | 12.3 | 26.0 | 25.3 | 208 | 225 |
| **Max** | 16.4 | 15.9 | 39.5 | 44.8 | 553 | 593 |
| **Median** | 13.9 | 13.4 | 30.4 | 30.9 | 337 | 374 |
| **Mean** | 14.0 | 13.5 | 30.7 | 31.6 | 339 | 370 |
| **Std Dev** | 0.7 | 0.7 | 2.9 | 3.7 | 61 | 72 |
| **Mean - 2SD** | **12.7** | **12.2** | **25.0** | **24.1** | **217** | **226** |
| **Mean + 2SD** | **15.4** | **14.8** | **36.5** | **39.0** | **461** | **514** |
| **2.50%** | **13.0** | **12.6** | **26.3** | **26.6** | **232** | **244** |
| **97.50%** | **16.0** | **15.3** | **37.5** | **40.2** | **447** | **512** |

## Ideal World Reference and Disease Groups do not Overlap




## Real World Overlapping Distributions




## Types of Tests

- Screening
- Diagnostic
- Therapeutic Monitoring

## Sensitivity and Specificity

Sensitivity: The probability that a patient who is disease positive will test positive

Specificity: The probability that a patient who is disease negative will test negative

## Positive and Negative Predictive Values

Positive Predictive Value: The probability that a patient who is test positive is disease positive

Negative Predictive Value: The probability that a patient who is test negative is disease negative

## Predictive Value Table

| | **Prevalence** | **1-Prevalence** | |
|---|---|---|---|
| | **Patients with disease** | **Patients without disease** | |
| **Test positive** | **TP** | **FP** | → **Positive Predictive Value** |
| **Test negative** | **FN** | **TN** | → **Negative Predictive Value** |
| | ↓ **Sensitivity** | ↓ **Specificity** | |

## Predictive Values as a Function of Sensitivity, Specificity, and Prevalence

$$PVp = \frac{TP}{TP + FP} = \frac{p*Se}{p*Se + (1-p)*(1-Sp)}$$

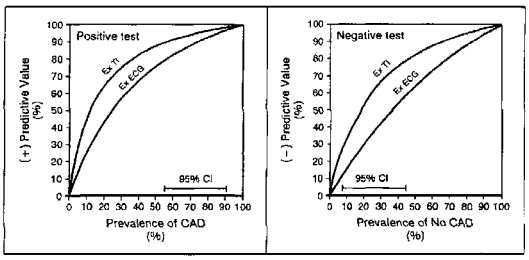
$$PVn = \frac{TN}{TN + FN} = \frac{(1-p)*Sp}{(1-p)*Sp + p*(1-Se)}$$

## Comparison of the Predictive Power of 3 Hypothetical Diagnostic Tests

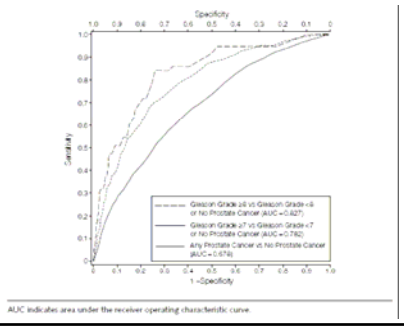
| Test | Sensitivity | Specificity |
|---|---|---|
| A | 0.95 | 0.81 |
| B | 0.85 | 0.83 |
| C | 0.75 | 0.85 |

It is impossible to compare the predictive power of these tests without knowing the prevalence and the predictive power will change as the prevalence changes.

## Predictive Values as a Function of Prevalence



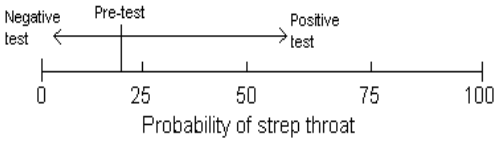
## Receiver Operating Characteristic for PSA




## Likelihood Ratios

- We take our initial assessment of the likelihood of disease ("pre-test probability"), do a test to help us shift our suspicion one way or the other, and then determine a final assessment of the likelihood of disease ("post-test probability").
- The likelihood ratio incorporates both the sensitivity and specificity of the test and provides a direct estimate of how much a test result will change the odds of having a disease.
- (+)LR, the likelihood ratio for a positive result, indicates how much the odds of the disease increase when a test is positive.
- (-)LR, the likelihood ratio for a negative result, indicates how much the odds of the disease decrease when a test is negative.

## How LR(+) and LR(-) Shift the Probability of Disease




The "Positive Likelihood Ratio" (LR+) tells us how much to increase the probability of disease if the test is positive.

The "Negative Likelihood Ratio" (LR-) tells us how much to decrease it if the test is negative.

## Definition of Likelihood Ratios

**(+)LR** =

$$\frac{\text{probability of a patient } \textbf{with} \text{ the disease having a + test}}{\text{probability of a patient } \textbf{without} \text{ the disease having a + test}}$$

$$= \textbf{Se/(1-Sp)}$$

**(-)LR** =

$$\frac{\text{probability of a patient } \textbf{with} \text{ the disease having a - test}}{\text{probability of a patient } \textbf{without} \text{ the disease having a - test}}$$

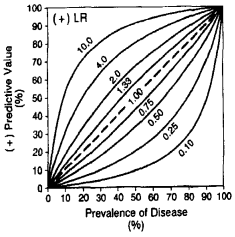
$$= \textbf{(1-Se)/Sp}$$

## Relationship of Predictive Values to Likelihood Ratios

$$PVP = \frac{TP}{TP + FP} = \frac{p*Se}{p*Se + (1-p)*(1-Sp)} = \frac{p}{p + (1-p)*(1-Sp)/Se}$$

$$PVN = \frac{TN}{TN + FN} = \frac{(1-p)*Sp}{(1-p)*Sp + p*(1-Se)} = \frac{(1-p)}{(1-p) + p*(1-Se)/Sp}$$

## Predictive Value as a Function of Prevalence for Different Likelihood Ratios



## Comparison of the Predictive Power of 3 Hypothetical Diagnostic Tests

| Test | Sensitivity | Specificity | (+)LR | (-)LR |
|---|---|---|---|---|
| A | 0.95 | 0.81 | 5.0 | 0.06 |
| B | 0.85 | 0.83 | 5.0 | 0.18 |
| C | 0.75 | 0.85 | 5.0 | 0.29 |

The Likelihood values clarify the relative rule-in (positive) and rule-out (negative) power of these tests for all levels of prevalence.

## Interpretation of Likelihood Results

| LR | Interpretation |
|---|---|
| **> 10** | Large and often conclusive increase in the likelihood of disease |
| **5 - 10** | Moderate increase in the likelihood of disease |
| **2 - 5** | Small increase in the likelihood of disease |
| **1 - 2** | Minimal increase in the likelihood of disease |
| **1** | No change in the likelihood of disease |
| **0.5 - 1.0** | Minimal decrease in the likelihood of disease |
| **0.2 - 0.5** | Small decrease in the likelihood of disease |
| **0.1 - 0.2** | Moderate decrease in the likelihood of disease |
| **< 0.1** | Large and often conclusive decrease in the likelihood of disease |

## Calculation of Post-Test Odds

- The likelihood ratio has an interesting property:

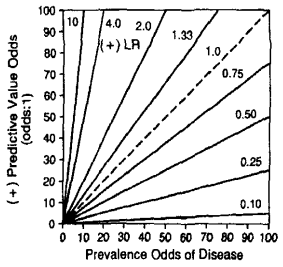
  Post-test odds of disease = likelihood ratio * pre-test odds of disease

- So, for positive and negative tests:

  odds of disease for (+) test = odds of disease before testing * (+)LR

  odds of disease for (-) test = odds of disease before testing * (-)LR

## Predictive Value versus Odds of Disease




## Probability versus Odds

- The terms "odds of disease" and "probability of disease" are not the same thing.
- Consider a group of 10 patients, 3 have strep and 7 don't have strep
- The **probability** that a patient in this group has strep is 3/10 or 0.3 or 30%.
- On the other hand, the **odds** of having strep in this group are 3 : 7

## Converting Probability to Odds

| Probability | Odds |
|---|---|
| 1% | 1:99 |
| 5% | 1:19 |
| 10% | 1:9 |
| 20% | 1:4 |
| 33% | 1:2 |
| 50% | 1:1 |
| 67% | 2:1 |
| 80% | 4:1 |
| 90% | 9:1 |
| 99% | 99:1 |

- for an odds of a : b, probability = a / (a + b)
- for a probability of x%, the odds are x : (100 - x)

## Calculation of Post-Test Odds

| Step | Description |
|---|---|
| 1. | Convert the pre-test probability to odds form |
| 2. | Multiply the pre-test odds by the LR to calculate the post-test odds |
| 3. | Convert the post-test odds back to a probability |

## Example Calculation

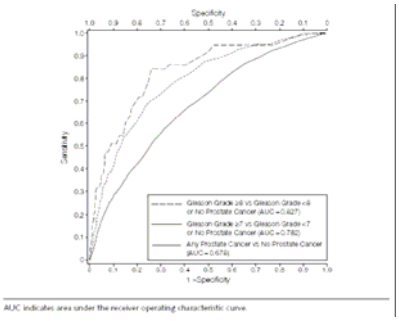
pre-test probability = 40%
(+)LR = 9

| Step | Description | Calculation |
|---|---|---|
| 1. | Convert the pre-test probability to odds form | **40%** = 40 / (100-40) = 40 : 60 = 4 : 6 |
| 2. | Multiply the pre-test odds by the LR to calculate the post-test odds | (4 : 6) x **9** = 36 : 6 |
| 3. | Convert the post-test odds back to a probability | 36 : 6 = 36 / (36 + 6) = 36/42 = 0.86 or **86%** |

## PSA Screening

| | Any Cancer (n = 1225) vs No Cancer (n = 4362) | | | | p=.28 | | p=.20 | |
|---|---|---|---|---|---|---|---|---|
| **PSA** | **Sensitivity** | **Specificity** | **(+) LR** | **(-) LR** | **PVP** | **PVN** | **PVP** | **PVN** |
| 1.1 | 83.4 | 38.9 | 1.36 | 0.43 | 0.35 | 0.86 | 0.25 | 0.90 |
| 1.6 | 67.0 | 58.7 | 1.62 | 0.56 | 0.39 | 0.82 | 0.29 | 0.88 |
| 2.1 | 52.6 | 72.5 | 1.91 | 0.65 | 0.43 | 0.80 | 0.32 | 0.86 |
| 2.6 | 40.5 | 81.1 | 2.14 | 0.73 | 0.46 | 0.78 | 0.35 | 0.85 |
| 3.1 | 32.2 | 86.7 | 2.42 | 0.78 | 0.49 | 0.77 | 0.38 | 0.84 |
| 4.1 | 20.5 | 93.8 | 3.31 | 0.85 | 0.56 | 0.75 | 0.45 | 0.83 |
| 6.1 | 4.6 | 98.5 | 3.07 | 0.97 | 0.54 | 0.73 | 0.43 | 0.81 |
| 8.1 | 1.7 | 99.4 | 2.83 | 0.99 | 0.53 | 0.72 | 0.41 | 0.80 |
| 10.1 | 0.9 | 99.7 | 3.00 | 0.99 | 0.54 | 0.72 | 0.43 | 0.80 |

## Receiver Operating Characteristic for PSA




AUC indicates area under the receiver operating characteristic curve.

## Learning objectives

- What is a reference range and how is determined?
- What is the “sensitivity” and “specificity” of a diagnostic test? How are they calculated?
- Understand how disease prevalence affects the predictive power of a diagnostic test

## Discussion