Some Historical perspectives
The Pharaoh Menes-Copper to Bronze
Britannicus vs Nero: The burning of Rome
Mithridates-Hyposensitization
King Richard III- A fatal case of urticaria

Take home-Allergies have been around for a long time and have actually played a significant role in history.

IgE-Observations & Discovery
• 1873 - Charles Blackley-Pollens/Skin Test
• 1919 - Ramirez Transfer by blood transfusion
• 1920 – Prausnitz/Kustner – PK Test
• 1921 – Arent de Besche – Large studies with PK
• 1964 – Loveless/Perelmutter Not A,G,M, D.
• 1966 – Teruko & Kimishige Ishizaka – IgE (erythema)
• 1966 – Bennich/Johansson – IgND myeloma
• WHO – Consolidated data – IgE represented a new class of immunoglobulin.

Age of the Radioallergosorbant Test (RAST) 1968-1992
• Finally something related to allergy that we could measure.
  – Allergens linked to a solid phase (modular)
  – Serum Incubation and unbound washed away
  – Tagged detection (anti-IgE) antibodies ¹²⁵I
  – Unbound detection antibodies washed away
  – Results expressed compared to a calibrator or as a percent of control.

The Phadbas™ RAST
• Pharmacia -1st test to the market 1974
• CNBr coupled allergens to paper discs
• Detection by radiolabeled anti-IgE
• Results equivalent to Birch s-IgE calibrator
• Very stable
• Market leader until 1992

Age of Expansion
• Different allergen sources & preparations
• Different coupling chemistries introduced
• Different solid phases(string/plastic/beads)
• Detection using enzyme labeled antibodies
• Monoclonal anti-IgE’s were introduced
• 100’s of allergens introduced
All these assays worked to some extent
All referred to as RAST tests reported out in log related scoring systems (classes I-V).
Age of Skepticism

- Discrepant results among different assays
- Variable results with clinical standards
- Quantitation was questionable-nonlinear
- Variability was considerable (technique?)
- Few allergens studied- good and bad
- Sensitivity claimed below skin testing
- Politics and economics – U.S. Boycott!

Immunoochemistry: A closer look

- 1 ng of IgE = 3 x 10^9 molecules (1 KU/L = 2.42 ng), thus Law of mass action applies
- AB + AG ≥ AGAB Ka = 10^5-10^8
- When KaRf/1 + KaRf > 10 assay is affinity independent
  10^5 x 10^-5 = 10/11 = 91% bound
  10^6 x 10^-5 = 100/1 = 100% bound
  10^7 x 10^-5 = 1000/0.1 = 10000% bound
- Note: Only when KaRf/1 + KaRf >10 can you measure concentration
- Take Home – Rf must be adequate for an assay to work, independent of concentration.

Consequences of Affinity Dependence

Problem solved-ImmunoCAP 1992

- Pharmacia introduced the ImmunoCAP
- Highly increased binding capacity (Rf)
- Cleaned up and characterized allergens
- Washing steps improved
- Calibrated with a total IgE assay curve
- Automated to eliminate errors
  – Resulted in a quantitative, precise, accurate reliable, assay.

Binding Capacities of Various Solid Phases

Evaluating how well these tests for specific IgE work?

Analytical Performance
- Precision
  – Reproducibility (%CV)
- Accuracy
  – Compared to standard
- Quantitative ability
  – Parallelism, Linearity, dilutional analysis, range
- Specificity
- Sensitivity-LOD

Clinical Performance
- Sensitivity-TP
- Specificity-TN
- Concordance
  – Usually dichotomous
- Positive/Negative predictive values
- Probability/Risk ratios
Analytical Performance Compared to an ideal standard

- Study outline:
  - Blinded samples containing variable levels of s-IgE were sent on 3 different occasions to 6 different laboratories via normal channels
  - Six samples presented in dilution series (5)
  - Analyzed s-IgE to 17 common aeroallergens
  - N = 12,708 results analyzed for precision, accuracy, quantitative ability for each allergen
  - Ann Allergy Asthma Immunol 2001;86:373-381

Precision and Accuracy

- Precision is the ability to make repetitive measurements
- Accuracy is the ability to compare to the true value
- A given assay can be precise without being accurate but can’t be accurate without precision.
- Can an assay be both accurate and precise?

Precision Study Results

- Precision measured by % CV of triplicates of positive responses (SD/mean as a percent)
- Method A: Average 27.5% Range 17-49%
- Method B: Average 14.7% Range 9-31%
- Method C: Average 9.8% Range 7-13%
- Precision profiles across range published Ann Allergy Asthma Immunol 2001;86:373-381.

Why precision is important?

Source: EuroEQAS

Why precision is important
Summary of Precision Studies

- Two of the three assays had very poor precision for a number of different specimens.
- This was allergen dependent
- One system was below the NCCLS/CLSI recommended % CV of 15% in all cases
- It is the laboratory’s responsibility to verify precision of chosen method.

Agreement of Methods A, B, & C on Dichotomous Basis (peanut)

- Cutoff = 0.35 KU/L
  - Method A = 94% agreement with C
  - Method B = 78% agreement with C
- Cutoff = 0.1 KU/L
  - Method A = 98% agreement with C
  - Method B = 87% agreement with C
- Thus, on a dichotomous basis these methods agree fairly well.

Analysis of peanut IgE antibody results of three different systems. Logarithmic Bland-Altman plot. Dotted lines are indicating +/-20% which is accepted deviation limits for results that are considered similar.

If all three methods disagree which is giving the true answer?

- Chimeric antibody studies
- Humanized (IgE constant portion) mouse monoclonals for Der p 2 and Birch Bet v 1
- Presented samples 3X blinded and in dilution to laboratories running different methods
- Measure total IgE in each sample
- Measure specific IgE in each sample
- Specific IgE = Total IgE in each sample

Observed vs actual total IgE values of chimeric antibody samples

Chimeric IgE results of three systems compared with the actual values of undiluted and diluted samples
Chimeric IgE results of three systems compared to actual values of undiluted and diluted samples

What about the quantitative ability of these assays?

- All three methods claim and are approved by the FDA as being quantitative.
- Thus:
  - Are dilutions of samples parallel to calibrator?
  - Does this occur over the stated range?
  - If so, is this true for all allergens?
  - What is the standard used for comparison?
  - Can an imprecise assay be quantitative?
- The ideal immunoassay as a standard

Parallelism-27 samples/9 Different Allergens-Method C

Standard for Comparison

Accuracy & Quantitative Ability via Dilutional Analysis

- The average slope coefficients for each dilution series compared to the ideal assay by OLS regression analysis. Target = 1

  Ave Slope C Ave R² Not significant

  - Method A = 0.82 45% 3
  - Method B = 0.77 62% 5
  - Method C = 0.98 93% 0


What is Analytical Specificity?

The ability to measure what you think you are measuring.

Immunoblots/inhibition
Different Patients same Allergen
Specificity

Removal of s-IgE by Solid Phase

What is analytical sensitivity?

The minimal detectable amount that can be accurately measured (LOD)

Assays are linear and reproducible below 0.35 KU\textsubscript{a}/L cutoff

A Word About Cutoffs

Summary of Analytical Studies

- Different assays do not give comparable or interchangeable results
- Studies on clinical interpretations depend upon the method used
- Accurate, precise, and quantitative results for s-IgE for many allergens are available
- Analytical sensitivity and specificity are different from clinical sensitivity and spec.
Why Quantitation is Important

Clinical Judgment of Test Performance

- Clinical Sensitivity = + Test in positive patient  \( \frac{TP}{TP + FN} \)
- Clinical Specificity = - Test in negative patient  \( \frac{TN}{TN + FP} \)
- Determined by comparison to a standard, usually clinical history and other tests
- Are there problems here?

Problems in Judging Test Clinical Performance Characteristics

- Is there a “Gold Standard”?
  – History, Skin and challenge testing
- Dichotomous versus quantitative analysis
  – Cutoffs, applicability to individual patients
- Variables in expression of symptoms
  – Age, gender, infection, exposures, allergen/s, genetics, psychological status, etc.
- Common symptoms with other causes

How to “Load” a Study

- To show test works well
  – Select a good allergen
  – Pick high positives and negative negatives and correlations will be high
- To show test is poor
  – Select a poor allergen
  – Pick low positives and questionable negatives and correlations will be low
- Galen and Gambino-How to Lie with statistics

Accuracy of the Clinical History

- Protocol: Use history to classify patients as positive, indeterminate, negative and compare to concordant test results. (n = 1036 diagnoses)
- Results:
  – Over-all Positive bias of 22%
  – Rarely exceeded 50% accuracy
  – Allergen and physician dependent
- Conclusions: History is not a good standard

Problems with the History and Physical Exam

- Heuristic errors
  – Anchoring: Initial impression determines outcome
  – Representativeness: Predisposition
  – Availability: Use of patterns, experience, literature to predict disease
  – Fear of regret allows unlikely causes to be considered.
  – Lancet: When Drs meet numbers
Is Skin Testing a good Standard?

- Patient variables (age, gender, exposures, allergen, psychological status, drugs, etc.)
- Extract variables—not all equal potency
- Testing variables—device, technique, etc.
- Interpretation variables?
- Quantitative?
- Standardization?

SKT vs S-IgE Standardization

<table>
<thead>
<tr>
<th>Method</th>
<th>S-IgE</th>
<th>SKT</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA clearance</td>
<td>Yes</td>
<td>Grandfathered</td>
</tr>
<tr>
<td>Proficiency Test</td>
<td>CAP Survey</td>
<td>None</td>
</tr>
<tr>
<td>CLIA regs</td>
<td>Inspections, etc</td>
<td>None</td>
</tr>
<tr>
<td>NCCLS/CLSI</td>
<td>Consensus Doc</td>
<td>None</td>
</tr>
<tr>
<td>Scoring</td>
<td>Quant/semi</td>
<td>None (1+ to 4+)</td>
</tr>
<tr>
<td>Standardization</td>
<td>By assay</td>
<td>Few (crude)</td>
</tr>
<tr>
<td>Specificity</td>
<td>Immunoblotting</td>
<td>Not known</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Analytical</td>
<td>Not known</td>
</tr>
</tbody>
</table>

Why do we run Tests?

- To gain reliable information relevant to disease discrimination
- A test does not diagnose but rather serves to confirm or deny a particular possibility. Changes the pretest odds.
- Due to the variable expression of symptoms results must be interpreted within the context of each patient.

Caveats

- CCD, alpha gal: Are these important?
- Relationship of sensitization to sensitivity
- Relevant allergens (i.e. Buckwheat)
- Crossreactivity – foods/related pollens
- Generations of tests are meaningless
- Most studies using clinical sens/spec are not accurate

Where are we going?

- Getting away from absolute cutoffs
- Gaining an understanding of interpreting results in lieu of influential variables
- Beginning to look at individual components
- Constructing logistic relationships with clinical expression and levels of s-IgE
- Understanding use of prevalence in testing
- Speed of test is increasing