Voorbij de grenzen van een kwalitatieve test
(Sectie HIM)

SKML jaarcongres
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Clinical significance of autoantibodies

- Pathology
  - Organ involvement
- Diagnosis
  - Cancer risk
- Stratification
  - Refractory disease
- Prognosis
  - Treatment response
Autoantibody testing... what do we expect?

Overall reliability

- Meaningful results
- No false positives, no false negatives
- Reproducibility:
  - same result day to day, month to month, year to year
  - same result if the sample is analysed in another lab
Autoantibody testing... reality check

Anti dsDNA Kit Control

Anti dsDNA Pt Control

(Radio)antibody

Patient

(Auto)antibody

(Auto)antigen

(Radio)antigen

Method

anti dsDNA titers (AU)

Time (days)

Batch #1: mean titer 62.2 AU, CV 10.7%
Batch #6: mean titer 82.5 AU, CV 5.8%
Batch #7: mean titer 76.8 AU, CV 4.5%
Standardisation of autoantibody testing... the challenges

Patient-variation
- Pre-clinical/Diagnostic/Follow-up
- Heterophillic Ab interference
- Treatment interference

Antibody-variation
- Isotype
- Subclass
- Affinity/avidity

Antigen-variation
- Human/xeno
- Purified/synthetic
- Complex/protein/subunit
- Stability
- Co-factor needed
- Lot-to-lot variation

Absence of robust reference materials

Method-variation
- Different immuno-assays
  - Dilution
  - Diluent
  - Capture/direct
  - Qualitative vs (semi) quantitative
- Different detection systems
  - Conjugate
  - Manual/automated
  - Qualitative vs (semi) quantitative
The dilemma of choosing your reference material...

Test Black: IgM ab’s

Test Red: High affinity ab’s

Test Blue: anti-human-Ag

Test Purple: anti-domain-X

\[ R_{\text{individual samples}} = 0.56 \]
\[ R_{\text{pooled samples}} = 0.96 \]

Falkenburg et al. CCLM 2018
Jacobs and Bossuyt. CCLM 2018
Practical reality of autoimmune diagnostics: quantitative elements

AID diagnosis = Clinical Features (score) AND Laboratory results (score)

'Strong positive' (>titer, ×ULN)

'Negative-test' 'Positive-test'

Frequency

Test Results

TN
TP

A 100% sensitivity
B 100% specificity
C most accurate

Δ
Introduction of ‘upper limit of normal’ for RA classification

Table 3. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis

<table>
<thead>
<tr>
<th>Classification criteria for RA (score-based algorithm: add score of categories A–D; a score of ≥6/10 is needed for classification of a patient as having definite RA)‡</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Joint involvement§</td>
<td>0</td>
</tr>
<tr>
<td>1 large joint¶</td>
<td>0</td>
</tr>
<tr>
<td>2–10 large joints</td>
<td>1</td>
</tr>
<tr>
<td>1–3 small joints (with or without involvement of large joints)#</td>
<td>2</td>
</tr>
<tr>
<td>4–10 small joints (with or without involvement of large joints)</td>
<td>3</td>
</tr>
<tr>
<td>&gt;10 joints (at least 1 small joint)**</td>
<td>5</td>
</tr>
<tr>
<td>B. Serology (at least 1 test result is needed for classification)††</td>
<td>0</td>
</tr>
<tr>
<td>Negative RF and negative ACPA</td>
<td>0</td>
</tr>
<tr>
<td>Low-positive RF or low-positive ACPA</td>
<td>2</td>
</tr>
<tr>
<td>High-positive RF or high-positive ACPA</td>
<td>3</td>
</tr>
<tr>
<td>C. Acute-phase reactants (at least 1 test result is needed for classification)‡‡</td>
<td>0</td>
</tr>
<tr>
<td>Normal CRP and normal ESR</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal CRP or abnormal ESR</td>
<td>1</td>
</tr>
<tr>
<td>D. Duration of symptoms§§</td>
<td>0</td>
</tr>
<tr>
<td>&lt;6 weeks</td>
<td>0</td>
</tr>
<tr>
<td>≥6 weeks</td>
<td>1</td>
</tr>
</tbody>
</table>

ACR Arthritis & Rheumatism 2010

≤1 ULN
>1≤3 ULN
>3 ULN
Performance assays have impact on ACR/EULAR classification of RA

WHO IgM RF standard
= 25 IU/mL

CDC ACPA reference
= 100 IU/mL
Reality of RF measurements in SKML EQA

Legenda:
- Onbekend
- EIA IgM (FEIA)
- Turbidimetrisch
- Nefelometrisch
- ELISA
- Overig

negatief             dubieus             zwak pos             sterk pos

Radboudumc
Child/adolescent with symptoms suggestive of CD

Anti-TG2/tTG IgA and total IgA

Anti-TG2/tTG positive

- Anti-TG2/tTG, >10x ULN
  - EMA (or DGPA) & HLA DQ8/DQ2*
    - EMA pos HLA pos: CD
      - GFD & FU
    - EMA pos HLA neg: Consider: False neg HLA biosies
    - EMA neg HLA neg: Consider: False pos anti-TG2/tTG

- Anti-TG2/tTG, <10x ULN
  - Not available

Anti-TG2/tTG negative

- Not CD
  - Consider further testing if: IgA deficiency, Low gluten intake, High suspicion

Gastroduodenoscopy with duodenal biopsies

- Marsh 0-1
  - Consider: False pos serology
  - False neg biopsy
  - Follow-up serology/biosy

- Marsh 2 or 3
  - CD
  - GFD & FU

Adapted from Husby et al JPGN 2012
Transglutaminase IgA tests are not standardized at xULN

Variability in x ULN for top 6 IgA anti-tTG testing methods in UK NEQAS

24/183 (13%) of samples with < 100 U/ml (<10x ULN) in Phadia FEIA test, were > 200 CU (>10x ULN) in QUANTAflash CIA IgA anti-tTG

Unpublished data Bontkes/Norman

Egner et al, JPGN, 2012; 55:733-735
xULN TGA cut-off should be established for each method

**Correlation between Marsh ≥2 and different ULN of CLIA assay**

<table>
<thead>
<tr>
<th>Anti-tTG IgA cut off</th>
<th>Marsh ≥2</th>
<th>PPV %</th>
<th>NPV %</th>
<th>LR+</th>
<th>LR−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 CU (10× ULN)</td>
<td>119/130</td>
<td>92</td>
<td>78</td>
<td>7.71</td>
<td>0.21</td>
</tr>
<tr>
<td>560 CU (28× ULN)</td>
<td>85/86</td>
<td>99</td>
<td>62</td>
<td>59.84</td>
<td>0.43</td>
</tr>
<tr>
<td>1000 CU (50× ULN)</td>
<td>78/78</td>
<td>100</td>
<td>55</td>
<td>+∞</td>
<td>0.58</td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 CU (10× ULN)</td>
<td>134/143</td>
<td>94</td>
<td>82</td>
<td>28.84</td>
<td>0.38</td>
</tr>
<tr>
<td>450 CU (17.5× ULN)</td>
<td>110/112</td>
<td>98</td>
<td>78</td>
<td>94.69</td>
<td>0.48</td>
</tr>
<tr>
<td>400 CU (20× ULN)</td>
<td>106/106</td>
<td>100</td>
<td>77</td>
<td>+∞</td>
<td>0.5</td>
</tr>
</tbody>
</table>

CU = chemiluminescent units; LR = likelihood ratio; NPV = negative predictive value; PPV = positive predictive value; ULN = upper limit of normal.

*Cut off suggested by ESPGHAN 2012 to avoid duodenal biopsy.
†One patient with anti-tTG IgA 960 CU, EMA 1:320; Marsh 1 on a small biopsy fragment.

Values CLIA:
202 CU
308 CU

When cut-off Previtali is used (>28x ULN): biopsies should be taken, which is in agreement with the FEIA assay at >10x ULN.

Previtali, JPGN 2018:66
Analytical issues of monoclonal FLC measurements

Variation in:
- AA sequence and size
- Charge (pI range 4.5 – 9)
- Glycosylation
- Polymerisation

Both assays report results in mg/L
Which result is correct?
Patients switching from hospital...

Tate et al. Clin Biochem Rev 2009
The importance of FLC standardisation/harmonisation

FLC ratio

N Latex
Freelite

Newly Added Criteria To Diagnose MM

<table>
<thead>
<tr>
<th>Clonal bone marrow plasma cells ≥10% or plasmacytoma plus one of these:</th>
<th>2-y Incidence of Organ Damage, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal marrow plasma cells ≥60%</td>
<td>95</td>
</tr>
<tr>
<td>Ratio of involved to uninvolved serum free light chain ≥100</td>
<td>80^a</td>
</tr>
<tr>
<td>≥2 focal bone lesions ≥5 mm on MRI</td>
<td>70-80</td>
</tr>
</tbody>
</table>

Rajkumar et al. Lancet Oncology 2014

Freelite FLC-ratio : N Latex FLC-ratio
100 ~ 30
Conclusions

• AID mostly developed/calibrated to be qualitative (Pos/Neg)
• Increasing number of ‘quantitative elements’ in guidelines
• AID mostly non-harmonized at these ‘quantitative cut-offs’
• EQA useful tool to create awareness of differences between methods
Thank you

Section Humoral Immunology
All board members, all coordinators.
Hetty Bontkes
Marco Schreurs
Cas Weykamp

Autoimmune diagnostics = Personalized diagnostics