Comparison of Five Different Methods to Detect ANCA and Associated MPO & PR3 Antigens
OXFORD UNIVERSITY HOSPITALS
CLINICAL IMMUNOLOGY DIAGNOSTICS

> 150,000 assays per annum

Know and work with clinicians & GPs in all departments

Audit and Review clinical needs for patients diagnoses/monitoring of treatments

Clinical Laboratory Immunology:

Comprehensive diagnostic immunology service.

One of largest repertoire of clinical immunology assays in UK
ANCA Testing in Oxford

- ANCA testing in our laboratory is restricted to patients with a high pre-test probability of small vessel vasculitis to avoid misleading false positives in a range of non-vasculitic disorders.

ANCA Testing is Restricted to Patients Presenting With:

- Chronic necrotising large airways disease
- Cavitating pulmonary nodules
- Subglottic stenosis
- Pulmonary renal syndrome
- Rapidly progressing glomerulonephritis
- Cutaneous vasculitis with systemic symptoms
- Mononeuritis multiplex
Outline

• Why did we do the comparison

• How was the comparison conducted

• What do the results show

• What conclusions can be drawn
Use of Wieslab a-GBM, ANCA Screen Kit

• The Wieslab a-GBM, ANCA Screen Kit is used in our laboratory as a rapid screening method with results available within an hour

• It is used during out of hours due to its ease of use and rapid availability of results, as ANCA, MPO, PR3, and GBM take 2 hours
Why

- 6 years since last audit between ELISA Vs Wieslab a-GBM, ANCA Screen kit (rapid ANCA screen)


- Introducing new MPO/PR3 assay to laboratory and needed to compare to rapid ANCA Screen

- Many new PR3 and MPO assays introduced since first used rapid ANCA screen in our laboratory
How

• Patient samples collected retrospectively after IIF ANCA and divided into the following groups:
100 different ANCA negative samples

108 different ANCA positive samples

   divided into 3  groups of:

1.  34 ANCA positive MPO/PR3 negative

2.  33 ANCA/MPO positive

3.  41 ANCA/PR3 positive
Assays Performed

• **208** samples were then tested on rapid ANCA screen

• **ANCA negative samples which were PR3 or MPO positive** on rapid ANCA screen were tested on Phadia PR3 ELIA, Phadia MPO Varelisa, Phadia PR3/ MPO sensitive caps and Eurodiagnostica PR3/ MPO capture ELISA

• **ANCA positive MPO/PR3 negative samples** were tested on Phadia PR3 ELIA, Phadia MPO Varelisa, Phadia PR3/ MPO sensitive caps and Eurodiagnostica PR3/ MPO capture ELISA
Assays Performed

- **ANCA PR3 positive samples** were tested on Phadia PR3 ELIA, Phadia PR3 sensitive caps and Eurodiagnostica PR3 capture ELISA

- **ANCA MPO positive samples** were tested on Phadia MPO Varelisa, Phadia MPO sensitive caps and Eurodiagnostica MPO capture ELISA
ANCA vs Rapid ANCA Screen
Results: ANCA vs Rapid ANCA Screen

<table>
<thead>
<tr>
<th></th>
<th>P-ANCA POS</th>
<th>C-ANCA POS</th>
<th>NEGATIVE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid ANCA Screen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPO</td>
<td>22</td>
<td>4</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>PR3</td>
<td>6</td>
<td>37</td>
<td>3</td>
<td>46</td>
</tr>
<tr>
<td>Neg</td>
<td>16</td>
<td>14</td>
<td>93</td>
<td>123</td>
</tr>
<tr>
<td>EQ MPO</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>EQ PR3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>56</td>
<td>101</td>
<td>204</td>
</tr>
</tbody>
</table>

Concordance = 82.9 %
Results: ANCA Negative vs Rapid ANCA Screen

- **100 ANCA negative**: Tested on Rapid ANCA Screen

<table>
<thead>
<tr>
<th>MPO/PR3 neg</th>
<th>MPO</th>
<th>PR3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pos</td>
</tr>
<tr>
<td>92</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

- The 8 positive and equivocal samples were subsequently tested on the routine methodologies
<table>
<thead>
<tr>
<th>Concordance</th>
<th>Rapid ANCA screen and ANCA IIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordance</td>
<td>92%</td>
</tr>
<tr>
<td>Equivocal</td>
<td>3%</td>
</tr>
<tr>
<td>Non-concordance</td>
<td>8%</td>
</tr>
<tr>
<td>Rapid ANCA screen Positive but MPO/PR3 negative by other methods</td>
<td>6%</td>
</tr>
<tr>
<td>Rapid ANCA screen MPO Positive and weakly MPO positive only by Phadia sensitive cap</td>
<td>1%</td>
</tr>
<tr>
<td>Rapid ANCA screen MPO positive and MPO positive by all the methods</td>
<td>1%</td>
</tr>
</tbody>
</table>
ANCA Negative Vs Rapid ANCA screen MPO/PR3 Positive

- 6/8  Negative for MPO and PR3

  **Clinical Details:** All non vasculitis patients

- 1/8  Weakly positive  [Phadia MPO sensitive caps]  
       (6.1u/ml > 5 pos)

  **Clinical Details:** Neurology patient with seizures and post encephalitis

- 1/8  Positive for MPO on all three methods,

  **Clinical Details:** Patient is a known ANCA associated vasculitis
Results: ANCA Positive Vs Rapid ANCA Screen

- 108 ANCA positive: Tested on Rapid ANCA Screen

<table>
<thead>
<tr>
<th>MPO/PR3 pos</th>
<th>MPO/PR3 neg</th>
<th>MPO/PR3 equivocal</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>30</td>
<td>6</td>
</tr>
</tbody>
</table>

- Concordance = 70.9%
ANCA Positive Concordance Vs Rapid ANCA Screen

- 70.9% Concordance  $n = 73$
- 5.8% Equivocal  $n = 6$
- 29.1% Non-concordance  $n = 30$
Results: ANCA Positive Vs Rapid ANCA Screen

• 30 ANCA positive samples were negative for MPO and PR3 on rapid ANCA screen

• 26/30 were non vasculitis patients

• 3/30 were known ANCA associated vasculitis patients which were all negative for MPO/PR3 assays

• 1/30 was a known Granulomatous Polyangiitis (GPA) patient which was PR3 positive on all PR3 assays
## Rapid ANCA Screen Sensitivity & Specificity

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO</td>
<td>91.3 %</td>
<td>94 %</td>
</tr>
<tr>
<td>PR3</td>
<td>97 %</td>
<td>88.2 %</td>
</tr>
</tbody>
</table>
Rapid ANCA Screen Vs MPO/PR3 Methods
## Rapid ANCA Screen Vs MPO/PR3 Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Concordance</th>
<th>No of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO Capture ELISA Eurodiagnostica</td>
<td>97.1%</td>
<td>70</td>
</tr>
<tr>
<td>MPO Sensitive Caps Phadia</td>
<td>97.0 %</td>
<td>67</td>
</tr>
<tr>
<td>MPO Varelisa Phadia</td>
<td>91.7%</td>
<td>60</td>
</tr>
<tr>
<td>PR3 ELIA Phadia</td>
<td>96.3%</td>
<td>80</td>
</tr>
<tr>
<td>PR3 Sensitive Caps Phadia</td>
<td>92.5%</td>
<td>80</td>
</tr>
<tr>
<td>PR3 Capture ELISA Eurodiagnostica</td>
<td>90.9%</td>
<td>77</td>
</tr>
</tbody>
</table>
MPO Capture Vs Rapid ANCA Screen

- 97.1% Concordance n= 68
- 2.9% Discordance n= 2
- 2/2 Negative on capture, positive on rapid ANCA screen

Clinical Details: Known ANCA associated vasculitis patients
MPO Sensitive Caps Vs Rapid ANCA Screen

- 97% Concordance n=65
- 3% Discordance n=2
- 1/2 = Positive on rapid ANCA screen, negative on sensitive caps, MPO positive on all other methods

**Clinical details: Non ANCA associated vasculitis**

- 1/2 = Negative on rapid ANCA screen, positive on sensitive caps

**Clinical details: Known ANCA associated vasculitis**
MPO Varelisa Vs Rapid ANCA Screen

- 91.7% Concordance n=55
- 8.3% Discordance n=5
- 2/5 Positive on rapid ANCA screen, negative on varelisa

**Clinical Details: Known ANCA associated vasculitis**

- 3/5 rapid ANCA screen negative, weak positives on varelisa

**Clinical Details: No ANCA associated vasculitis**
PR3 ELIA Vs Rapid ANCA Screen

- 96.3 % Concordance n = 77
- 3.7 % Discordance n = 3
- 1/3 Negative on rapid ANCA screen, positive on ELIA

Clinical Details: Known GPA

- 2/3 Positive on rapid ANCA screen negative on ELIA

Clinical Details: No ANCA associated vasculitis
PR3 Sensitive Caps Vs Rapid ANCA Screen

- 92.5% Concordance n=74
- 7.5% Discordance n=6
- 1/6 Negative on rapid ANCA screen, positive on sensitive caps

**Clinical details: Known GPA**

- 5/6 Positive on rapid ANCA screen, negative on sensitive caps

**Clinical Details: 3/5 No ANCA associated vasculitis, 2/5 known GPA patients**
PR3 Capture Vs Rapid ANCA Screen

- 90.9% Concordance n=72
- 9.1 % Discordance n=5
- 1/5 Negative on rapid ANCA screen, positive on capture ELISA

Clinical Details: Known GPA

- 4/5 Positive on rapid ANCA screen, negative on capture

Clinical Details: 2/4 No ANCA associated Vasculitis 2/4 known GPA
Comparison of MPO Methods
## Comparison of MPO Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Concordance</th>
<th>No of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO Capture ELISA Eurodiagnostica vs MPO Sensitive Caps Phadia</td>
<td>97.2%</td>
<td>73</td>
</tr>
<tr>
<td>MPO Capture ELISA Eurodiagnostica vs MPO Varelisa Phadia</td>
<td>97.2%</td>
<td>70</td>
</tr>
<tr>
<td>MPO Varelisa Phadia vs MPO Sensitive Caps Phadia</td>
<td>95.2%</td>
<td>62</td>
</tr>
</tbody>
</table>
Correlation of MPO Methods

MPO Varelisa vs MPO Sensitive Caps

P = < 0.0001
R = 0.8770

Phadia MPO Varelisa U/mL vs Phadia MPO Sensitive Caps IU/mL
MPO Varelisa Vs MPO Capture ELISA

P =< 0.0001
R = 0.8919
MPO Sensitive Caps vs. MPO Capture ELISA

P = < 0.0001
R = 0.9066
MPO Capture Vs MPO Sensitive Caps

• Concordance 97.2% n =71
• Discordance 2.8% n = 2

• 1/2 Positive on the capture ELISA, negative on the sensitive caps

Clinical Details: No ANCA associated vasculitis

• 1/2 Negative on Capture ELISA, positive on sensitive caps

Clinical Details: Known MPO positive patient
MPO Capture Vs MPO Varelisa

• Concordance 97.2% n= 68

• Discordance 2.8% n=2

• 1/2 Positive on Capture ELISA, negative on Varelisa

• 1/2 Negative on Capture ELISA, positive on Varelisa

Clinical Details: No ANCA associated vasculitis
MPO Varelisa Vs MPO Sensitive Caps

- Concordance 95.2% n= 60
- Discordance of 4.8% n= 2
- 2/2 Positive on the sensitive caps and negative on the varelisa

Clinical Details: 1= Known MPO positive patient
1 = No ANCA associated vasculitis
# MPO Methods Sensitivity & Specificity

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO Varelisa</td>
<td>81.4 %</td>
<td>100 %</td>
</tr>
<tr>
<td>MPO Sensitive</td>
<td>91.3 %</td>
<td>100 %</td>
</tr>
<tr>
<td>MPO Capture</td>
<td>83.3 %</td>
<td>97.0 %</td>
</tr>
</tbody>
</table>
Comparison of PR3 Methods
Comparision of PR3 Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Concordance</th>
<th>No of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR3 Capture ELISA Eurodiagnostica vs. PR3 Sensitive Caps Phadia</td>
<td>98.8%</td>
<td>82</td>
</tr>
<tr>
<td>PR3 ELIA Phadia vs. PR3 Sensitive Caps Phadia</td>
<td>96.3%</td>
<td>81</td>
</tr>
<tr>
<td>PR3 Capture ELISA Eurodiagnostica vs. PR3 ELIA Phadia</td>
<td>95.2%</td>
<td>83</td>
</tr>
</tbody>
</table>
Correlation of PR3 Methods

ELIA PR3 vs PR3 Sensitive caps

\[
P = < 0.0001
\]

\[
R = 0.8662
\]
ELIA PR3 vs PR3 Capture ELISA

P = < 0.0001
R = 0.8565

Eurodiagnostica PR3 Capture ELISA IU/mL

Phadia ELIA PR3 IU/mL
PR3 Sensitive Caps Vs PR3 Capture ELISA

Phadia PR3 Sensitive cap IU/ml

Eurodiagnostica PR3 Capture Elisa IU/ml

P = < 0.0001
R = 0.9417
PR3 Capture ELISA Vs PR3 Sensitive Caps

- Concordance 98.8% n=81
- Discordance 1.2 % n=1
- 1/1 Positive on the sensitive caps but negative on the capture ELISA

Clinical Details: Known GPA
PR3 ELIA Vs PR3 Sensitive Caps

- Concordance = 96.3 % n=78
- Discordance = 3.7% n =3

- 3/3 Positive on ELIA, negative on the sensitive caps

Clinical Details: 1/3 = Known vasculitis
2/3 had no ANCA associated vasculitis
PR3 Capture ELISA Vs PR3 ELIA

- Concordance 95.2% n=79
- Discordance of 4.8% n=4
- 4/4 Positive on the ELIA, negative on capture ELISA

Clinical Details: 2/4 Known GPA
2/4 had no ANCA associated vasculitis
## PR3 Methods Sensitivity and Specificity

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR3 ELIA</td>
<td>92 %</td>
<td>100 %</td>
</tr>
<tr>
<td>PR3 Sensitive</td>
<td>87.2 %</td>
<td>100 %</td>
</tr>
<tr>
<td>PR3 Capture</td>
<td>86.1 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>
Conclusions

• After conducting the audit we are pleased with the performance of the rapid ANCA screen

• The results show an excellent concordance with MPO and PR3 methods

• There is also a good concordance and correlation between the quantitative methods available for MPO and PR3 detection
Thank You

- Thank you to Eurodiagnostica for providing the wieslab, MPO/PR3 capture ELISA kits

- Thank you to staff in Immunology Laboratory who helped with the testing