Comparative efficacy of five Aspergillus-specific IgG ELISAs for the diagnosis of chronic pulmonary aspergillosis (CPA)

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INTRODUCTION

- Chronic pulmonary aspergillosis (CPA) is a fatal condition that is estimated to effect 2-3 million people worldwide.
- Measurement of Aspergillus-specific IgG is central to the diagnosis of CPA, alongside clinical and radiological features.
- In-house assays are in use in some referral centers, but cannot be easily reproduced in other laboratories.
- Various assays are commercially produced.
- There is currently very little published evidence regarding the efficacy of these assays for the diagnosis of CPA.
- Where available, diagnostic cut-offs are often based on results from acute invasive aspergillosis.
- Existing studies are often potentially biased by the use of antifungal agents at the time of sampling.
  - This reduces Aspergillus-specific IgG levels.
  - It is not known whether all assays are equally affected.
- The UK National Aspergillosis Centre has the world's largest known cohort of CPA patients.

METHODS

- The efficacy of five Aspergillus-specific IgG ELISAs and precipitins testing for the diagnosis of CPA is described.
- We tested stored sera from 250 patients at the UK National Aspergillosis Centre with untreated CPA and 100 healthy controls.
- CPA diagnosis was based on criteria by Denning et al.
- ROC AUC analysis was used to compare performance and sensitivity and specificity with optimal cut-offs stated.

RESULTS

<table>
<thead>
<tr>
<th>Aspergillus-specific IgG assay</th>
<th>Co-efficient of variation (20 repeats)</th>
<th>Median Aspergillus IgG level in healthy controls (n=100)</th>
<th>Median Aspergillus IgG level in CPA cases (n=250)</th>
<th>Maximum Aspergillus IgG level in CPA cases (n=250)</th>
<th>Receiver-Operator Curve Area Under Curve (95% CI)</th>
<th>New proposed diagnostic cut-off</th>
<th>Sensitivity with new cut-off (250 cases)</th>
<th>Specificity in healthy controls (100 controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIEMENS (Germany)</td>
<td>3.38%</td>
<td>4 mg/L</td>
<td>390 mg/L</td>
<td>7660 mg/L</td>
<td>0.991 (0.982 - 1)</td>
<td>10 mg/L</td>
<td>95.6%</td>
<td>98%</td>
</tr>
<tr>
<td>ImmunoCAP (ThermoFisher Scientific - multinational)</td>
<td>-</td>
<td>4.7 mg/L</td>
<td>126 mg/L</td>
<td>1707 mg/L</td>
<td>0.995 (0.991 – 0.999)</td>
<td>20 mg/L</td>
<td>96%</td>
<td>98%</td>
</tr>
<tr>
<td>SERION (Germany)</td>
<td>23.16%</td>
<td>6 U/ml</td>
<td>133 U/ml</td>
<td>3436 U/ml</td>
<td>0.972 (0.957 – 0.986)</td>
<td>35 AU/ml</td>
<td>90.4%</td>
<td>98%</td>
</tr>
<tr>
<td>DYNAMIKER (China)</td>
<td>12.1%</td>
<td>34 U/ml</td>
<td>126 U/ml</td>
<td>6118 U/ml</td>
<td>0.918 (0.890 - 0.946)</td>
<td>70 U/ml</td>
<td>75.2%</td>
<td>98%</td>
</tr>
<tr>
<td>GENESIS (UK)</td>
<td>11.1%</td>
<td>7 U/ml</td>
<td>60 U/ml</td>
<td>930 U/ml</td>
<td>0.902 (0.872 - 0.933)</td>
<td>16 U/ml</td>
<td>78.4%</td>
<td>97%</td>
</tr>
<tr>
<td>Precipitins with Microgen antigens (UK)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>59%</td>
<td>100%</td>
</tr>
</tbody>
</table>

ROC curves

CONCLUSIONS

- All ELISAs have substantially better performance than precipitins.
- The Siemens and ImmunoCAP assays have excellent performance and are statistically significantly superior to all other assays assessed.
- The automated Siemens assay has much better reproducability than the other ELISAs tested, which were performed manually.
- New optimal diagnostic cut-offs for the diagnosis of CPA result in excellent sensitivity, while maintaining high specificity.
- The commonly used ImmunoCAP cut off (40 mg/L) is sub-optimal for use in CPA (83% sensitivity) and should be replaced by the new cut off (20mg/L).
- The Siemens assay produces results around 3 X higher than ImmunoCAP – this must be taken into account when comparing results between systems.

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