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.

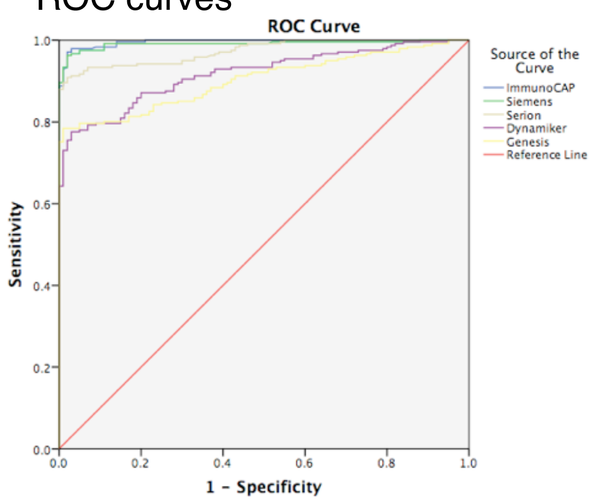
METHODS

- 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

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

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 specificty.
- 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.

Denning DW, Riniotis K, Dobrashian R, Sambatakou H. Chronic cavitary and fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature change, and review. *Clin Infect Dis*. 2003;37 Suppl 3:S265–80. doi:10.1086/376526

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