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Siemens Immulite *Aspergillus*-specific IgG assay for chronic pulmonary aspergillosis diagnosis

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Abstract

Chronic pulmonary aspergillosis (CPA) complicates underlying lung disease, including treated tuberculosis. Measurement of *Aspergillus*-specific immunoglobulin G (IgG) is a key diagnostic step. Cutoffs have been proposed based on receiver operating characteristic (ROC) curve analyses comparing CPA cases to healthy controls, but performance in at-risk populations with underlying lung disease is unclear. We evaluated optimal cutoffs for the Siemens Immulite *Aspergillus*-specific IgG assay for CPA diagnosis in relation to large groups of healthy and diseased controls with treated pulmonary tuberculosis. Sera from 241 patients with CPA attending the UK National Aspergillosis Centre, 299 Ugandan blood donors (healthy controls), and 398 Ugandans with treated pulmonary tuberculosis (diseased controls) were tested. Radiological screening removed potential CPA cases from diseased controls (234 screened diseased controls). ROC curve analyses were performed and optimal cutoffs identified by Youden *J* statistic. CPA versus control ROC area under curve (AUC) results were: healthy controls 0.984 (95% confidence interval 0.972–0.997), diseased controls 0.972 (0.959–0.985), screened diseased controls 0.979 (0.967–0.992). Optimal cutoffs were: healthy controls 15 mg/l (94.6% sensitivity, 98% specificity), unscreened diseased controls 15 mg/l (94.6% sensitivity, 94.5% specificity), screened diseased controls 25 mg/l (92.9% sensitivity, 98.7% specificity). Results were similar in healthy and diseased controls. We advocate a cutoff of 20 mg/l as this is the midpoint of the range of optimal cutoffs. Cutoffs calculated in relation to healthy controls for other assays are likely to remain valid for use in a treated tuberculosis population.

Key words: *Aspergillus fumigatus*, aspergillosis, CPA, aspergilloma, immulite 2000.

Introduction

Chronic pulmonary aspergillosis (CPA) is a serious disease that complicates pulmonary disorders including treated tuberculosis. It leads to severe disability and death but can be treated effectively with antifungal drugs or surgery.[1–7] The estimated global five-year point prevalence of CPA secondary to pulmonary tuberculosis is 0.8 to 1.37 million cases.[8]

International CPA guidelines state that evidence of *Aspergillus* infection is required for diagnosis in addition to radiological and clinical features.[9,10] In addition to positive cultures, raised levels of *Aspergillus*-specific immunoglobulin G (IgG) are normally present and are accepted as evidence of infection.[2,3,11–15]

Exposure to *Aspergillus* is ubiquitous. Most adults have some degree of antibody response. The identification of an optimal cutoff to define raised levels is critical.[16,17]

Aspergillus-specific IgG levels can be measured on the Immulite 2000 (Siemens, Munich, Bavaria, Germany) automated immunoassay system. Analysis of Dutch healthy controls suggests an upper reference value of 19.3 mg/L.[18] Our recent study

used receiver-operating curve (ROC) analysis comparing untreated CPA cases at the UK National Aspergillosis Centre to the healthy Dutch controls and identified a similar optimal cutoff of 25 mg/L.[19]

However, our prior study comparing the same CPA cohort to Ugandan healthy controls identified a cutoff of 10 mg/l.[14] The specificity of a 10 mg/l cutoff in relation to the European healthy control cohort was 5.3%.[19] The cause of this marked variation is unclear as multiple factors such as age and environment varied between the two studies.

CPA occurs almost exclusively in patients with underlying lung disease,[20] but to our knowledge no study has assessed optimal cutoffs for CPA diagnosis in relation to controls suffering from a relevant underlying disease. The most common underlying disease in CPA is tuberculosis.[2,3,5,20] Human immunodeficiency virus (HIV) coinfection is common in tuberculosis,[21] but the impact of this on *Aspergillus*-specific IgG levels is unclear.

We have now performed ROC analyses comparing *Aspergillus*-specific IgG levels in our cohort of CPA patients to additional healthy and diseased control groups with treated tuberculosis to identify the best possible cutoff. We also assess batch-to-batch variation and the impact of HIV infection.

Methods

Stored sera from 100 healthy Ugandan blood donors and 241 CPA patients attending the UK National Aspergillosis Centre, Manchester, who were not on long-term azole therapy at the time of sampling, were tested for *Aspergillus*-specific IgG by the Immulite 2000 assay in Manchester, United Kingdom, in 2014 as previously described.[14]

Sera from 300 Ugandan blood donors (healthy controls), sequentially recruited in November 2012 were tested in Manchester, United Kingdom, in 2016 with test kits from a different manufacturing batch to that used in 2014. This included repeat testing of the 100 previously tested controls.

Sera from 398 persons treated for pulmonary tuberculosis (TB) within the previous 7 years (diseased controls) were collected between November and December 2012 in Gulu, Uganda, and tested in Manchester, United Kingdom, in 2014. Symptoms were recorded, and chest X-ray performed on all persons. Recurrent tuberculosis was excluded in those with productive cough by GeneXpert testing.

Subgroups were identified: 89 asymptomatic persons, 333 with no cavities on chest X-ray, 267 patients without chronic (≥1 month) cough or hemoptysis, 235 with neither chest X-ray cavities, chronic cough, or hemoptysis, 65 with cavities on chest X-ray, and 32 with cavities on chest X-ray, but without chronic cough or hemoptysis.

Additional sera were collected and chest X-ray repeated on 285 of the treated tuberculosis cohort in 2015. Computed tomography (CT) thorax scan was additionally performed on 73 persons who had suspicion of aspergilloma on chest X-ray or an *Aspergillus*-specific IgG of >10 mg/l Also, 234 persons were identified without radiological features suggestive of possible CPA (screened diseased controls). Each had no progressive cavitation on repeat chest X-ray and no fungal ball or paracavitary fibrosis on CT thorax (or chest X-ray where no CT thorax performed). Sera were tested in Manchester, United Kingdom, in 2015 and repeat tested in 2017 using test kits from another manufacturing batch. Subgroups with and without HIV were identified, based on testing performed as part of routine care at the time of tuberculosis treatment.

Batch-to-batch variation in test performance is displayed on a Bland-Altman chart. Median levels between batches and in HIV-positive and negative diseased controls are compared by Mann–Whitney *U* test. Correlation co-efficient and 95% limits of agreement for batch-to-batch testing are reported.

ROC analyses were performed comparing results in CPA cases to each control group. ROC area under the curve (AUC) is shown with 95% confidence intervals (95% CI). Optimal diagnostic cutoffs in relation to each control group were calculated using Youden *J* statistic (sensitivity + specificity − 1). Sensitivity and specificity are described for each cutoff, with 95% confidence intervals calculated by Wilson's method. Statistical analyses were performed using SPSS version 20 (IBM, USA) under license to the University of Manchester, United Kingdom.

Our previously published results from European healthy controls are also shown here for comparison.[19]

Results

Patient characteristics are shown in Table 1, where available. Antibody levels in cases and control groups and results of ROC AUC analyses are shown in Table 2. On repeat testing of healthy Ugandan controls with a different batch of test kits the median *Aspergillus*-specific IgG level increased by 1.87 mg/l ($P = .000$), with a correlation coefficient of .937($P = .000$) and 95% limits of agreement of −1.65 (1.02 to −4.32). Median levels in screened diseased controls increased by 1.88 mg/l ($P = .000$) on repeat testing with a correlation coefficient of .965 ($P = .000$) and 95% limits of agreement of −1.52 (5.5 to −8.53). Bland-Altman charts for batch-to-batch repeat testing are shown in supplementary material along with ROC AUC outcomes and optimal cutoffs for each batch.

The CPA case median level was 30 times higher than that of any control group. Median levels in prior tuberculosis diseased controls were slightly lower than Ugandan healthy controls, but 97·5th results were 10 times higher in the prior tuberculosis group. 97.5th centile results for screened diseased controls were similar to healthy controls. HIV infection was associated with 2.62 mg/l reduction in median antibody levels in screened diseased controls ($P = .000$). Antibody levels in different groups are shown in Figure 1.

Table 1. Patient characteristics.

Characteristic	UK CPA patients $n =$ 241 (%)	Ugandan healthy controls $n =$ 299 (%)	Previously tested Ugandan healthy controls $n = 100$ (%)	Prior tuberculosis diseased controls $n = 398$ (%)	Screened diseased controls $n =$ 234 (%)	HIV-negative screened diseased controls $n = 118$ (%)	HIV-positive screened diseased controls $n = 116$ (%)
Female sex	101 (41.9)	87 (29.1)	55 (55)	155 (38.9)	91 (38.9)	31 (26.3)	60 (51.7)
Mean age in years (range)	65 (2 –92)	20 (17–39)	19 (17–39)	43 (16–83)	42 (16–77)	45 (16–77)	40 (16–73)
HIV infection	0	4 (1.3)	2 (2)	199 (50)	116 (49·6)	0	116 (100)
Median CD4 count in HIV positive persons - cells/μl (range)	-	-	-	424 (14–1400)	420 (59–1400)	-	420 (59–1400)
Positive sputum smear at TB diagnosis	-	-	-	303 (76.1)	179 (76.5)	101 (85.6)	78 (67.2)
Traditional 'grass-thatch' home	0	-	-	371 (93.2)	220 (94)	112 (94.9)	108 (93.1)
Subsistence farmer	0	-	-	373 (93.7)	221 (94.4)	117 (99.2)	104 (89.7)
Chronic cavitary pulmonary aspergillosis	238 (98.8)	-	-	-	-	-	-
Aspergillus nodule disease	3 (1.2)	-	-	-	-	-	-
Positive precipitins	138 (57.3)	-	-	-	-	-	-
Aspergillus fumigatus growth in sputum culture	89 (36.9)	-	-	-	-	-	-
Aspergillus niger growth in sputum culture	3 (1.2)	-	-	-	-	-	-
Prior tuberculosis	37 (15.4)	-	-	398 (100)	234 (100)	118 (100)	116 (100)
Nontuberculous mycobacterial infection	28 (11.6)	-	-	-	-	-	-
Chronic obstructive pulmonary disease	85 (35.3)	-	-	-	-	-	-
Bronchiectasis	60 (24.9)	-	-	-	-	-	-
Allergic bronchopulmonary aspergillosis (ABPA)	35 (14.5)	-	-	-	-	-	-
Sarcoidosis	9 (3.7)	-	-	-	-	-	-
Malignancy (active or in remission)	33 (13.7)	-	-	-		-	-
Autoimmune disease	33 (13.7)	-	-	-	-	-	-
Diabetes	7 (2.9)	-	-	-	-	-	-

CPA, chronic pulmonary aspergillosis; HIV, human immunodeficiency virus; TB, tuberculosis.

Table 2. Antibody testing results.

Group	Mean (mg/l)	Median (mg/l)	Range (mg/l)	97.5th centile	IQR (mg/l)	ROC AUC	95% CI	Optimal diagnostic cutoff (mg/l)	Sensitivity (%)	95% CI	Specificity (%)	95% CI
UK CPA cases ($n = 241$)	678.86	392	3–7660	3474.5	84.8–1000.5	-	-	-	-	-	-	-
Previously tested Ugandan healthy controls run 1 ($n = 100$)	4.63	3.83	0.1–35.3	13.91	3.05–4.81	0.991	0.982–1	10	95.9	92.5–97.7	98	93.0–99.4
Previously tested Ugandan healthy controls run 2 ($n = 100$)	6.28	5.7	3.43–38.6	14.47	5–6.62	0.986	0.974–0.998	10	95.4	92.0–97.4	97	91.5–99.0
Screened diseased controls run 1 ($n = 234$)	7.32	5.24	0·1–200	19·24	3·8–7.81	0.98	0·967–0·992	2.5	92.9	89.0–95.5	98.7	96.3–99.6
Screened diseased controls run 2 ($n = 234$)	8.84	7.12	2.35–200	21·5	5·57–9.01	0.973	0.958–0.989	2.5	92.5	88.5–95.2	98.3	95.7–99.3
European healthy controls ($n = 152$)	13.71	13.2	8·75–25·5	19.34	11·92–15·17	0.948	0·921–0·975	2.5	92.9	89.0–95.5	99.3	96.4–99.9
Ugandan Healthy controls ($n = 299$)	6.43	5.77	3·22–38·6	13.65	4·89–7·02	0.984	0·972–0·997	1·5	94·6	91.0–96.8	98	95.7–99.1
Prior TB diseased controls ($n = 398$)	19.76	4.21	0.1–1060	149.97	3·17–6·26	0.972	0·959–0·985	1·5	94·6	91.0–96.8	94..5	91.8–96.3
HIV-positive screened diseased controls ($n = 116$)	6.69	5.85	2.35–37	16.57	4·49–7·72	0.982	0·97–0·995	2.5	92.5	88.5–95.2	99.1	95.3–99.8
HIV-negative screened diseased controls ($n = 118$)	10.95	8.47	4.88–200	28.19	6·68–10·6	0.964	0·944–0·984	2.5	92.5	88.5–95.2	97.5	92.8–99.1

CI, confidence interval; CPA, chronic pulmonary aspergillosis; IQR, interquartile range; ROC, receiver operating characteristic.

Sensitivity and specificity at various points on the ROC curve in relation to each control group are shown in Table 3. ROC curves in relation to different control groups and HIV status are shown in Figure 2.

Optimal cutoffs calculated in relation to Ugandan healthy controls and prior tuberculosis diseased controls ranged from 10 mg/l to 20 mg/l. European healthy controls and screened diseased controls both produced a cutoff of 25 mg/l. Optimal cutoffs did not vary with manufacturing batch or HIV status.

Results for five alternatively screened diseased control groups were similar to those displayed here and are described in supplementary material Tables S1–S4.

Discussion

Our study provides the first comparison of *Aspergillus*-specific IgG levels in healthy and prior tuberculosis diseased control groups and the first description of the impact of both HIV status and screening to remove CPA cases from diseased control cohorts on antibody levels. We compared optimal diagnostic cutoffs through ROC curve analysis and consider whether an overall optimal cutoff can be identified.

Statistically significant increases in median test results were observed between batches (1.87 and 1.88 mg/l), demonstrating that *Aspergillus*-specific IgG levels do not fall with 2 years storage at −80°C. This variation between batches was much smaller than that seen between cases and Ugandan healthy controls (392 mg/l) but was similar to that seen between Ugandan healthy controls and prior tuberculosis diseased controls (1.56 mg/l). The overall variation in median levels between control groups was 9.27 mg/l.

The assay has a within-run co-efficient of variation of 3.6% ($n = 20$) at a level of 62.6 mg/l.[14] Antibody levels do not rise with age in adults, as median results were lower in prior tuberculosis diseased controls (median age 43 years) than Ugandan healthy controls (median age 20 years). Taken together, these findings suggest that the small differences in levels observed between control groups represent expected variation in line with the performance characteristics of this assay.

The 97.5th centile results were raised in prior tuberculosis diseased controls. Around 5% of cases of pulmonary tuberculosis are thought to be complicated by CPA.[8] Median antibody levels were 30 times higher in our cases than controls. High 97.5th centile results therefore probably reflect inclusion of CPA cases in the prior tuberculosis diseased controls group. This was corrected by radiological screening, which should therefore be incorporated into future studies using diseased controls in this context.

Median levels and 97.5th centiles were slightly higher in the HIV-negative group, but optimal cutoffs from ROC analyses were unaffected. This demonstrates that the same cutoffs can be used in HIV-positive and -negative populations. Median CD4

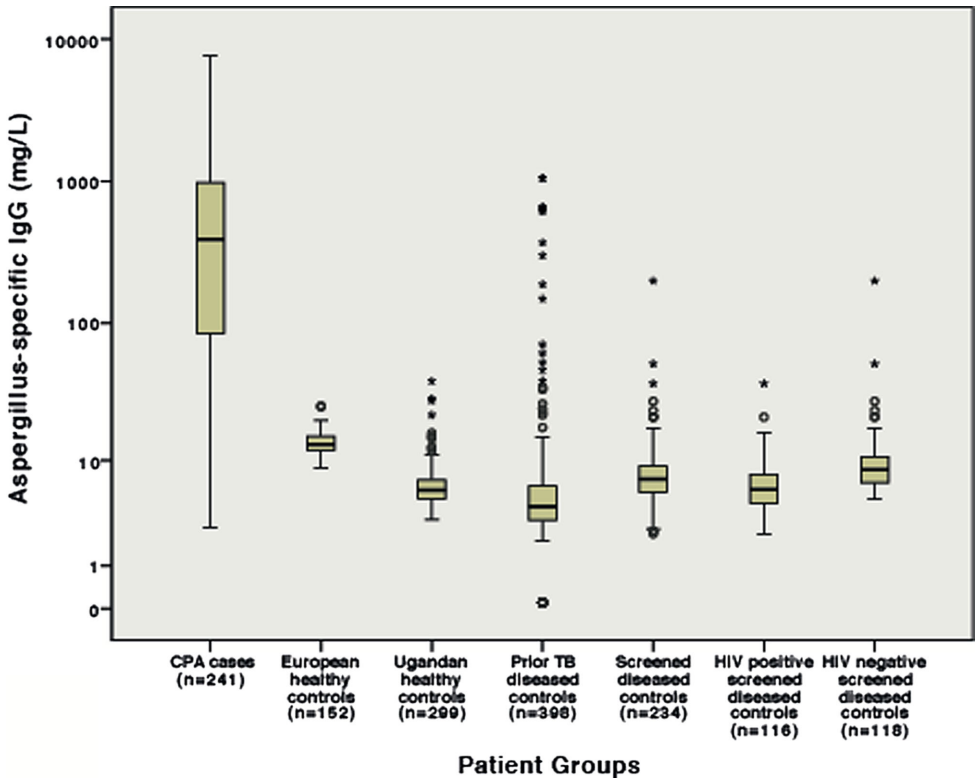

Figure 1. Antibody levels in cases and controls. This Figure is reproduced in color in the online version of *Medical Mycology*.

count in HIV patients was 420 cells/μl. Test performance may differ in a more immunosuppressed population.

The cutoff calculated in relation to Ugandan healthy controls increased from 10 mg/l to 15 mg/l with the inclusion of 199 additional sera from the same recruitment event. Youden *J* statistic was very similar for the two cutoffs. The variation in cutoff between these groups is probably a chance finding.

Cutoffs below 20 mg/l demonstrated unacceptably poor specificity in relation to some groups, especially European controls. Cutoffs of 20 and 25 mg/l had good performance in relation to all groups. We suggest adopting 20 mg/l as it is in the center of the range of optimal results identified and is within the range of 97.5th centile results seen in healthy controls and screened diseased controls (13.65–21.5 mg/l).

Previous studies have varied in their use of healthy[14,18,19,22,23] or diseased controls.[15] In allergic aspergillosis diseased controls have produced higher *Aspergillus*-specific IgG cutoffs than healthy controls.[24–26] However, in our study ROC analysis with healthy or diseased controls produced similar outcomes. This suggests that optimal cutoffs identified in relation to healthy controls can be used with confidence in CPA diagnosis.[19]

We describe the first attempt to our knowledge to screen diseased controls to remove CPA cases. As radiological findings are required for CPA diagnosis,[9] removing persons that meet these criteria from the control cohort ensures that no CPA cases are included.

We used the most robust radiological screening method available to us. This did not exclude all persons with cavities. Only those with progressive cavitation between X-rays, paracavitary fibrosis, or fungal ball were excluded. However, excluding controls with cavitation on a single chest X-ray produced very similar results and might be a useful strategy for future studies.

The assay might be of greatest clinical use in persons with residual cavities on chest X-ray following tuberculosis. However, 20% of this group is predicted to develop CPA,[8,27] which is consistent with the 97.5th centile result of 1053 mg/l in this study. An ideal diseased control group might be those with residual cavities but no fungal ball and no symptoms consistent with CPA. We identified 32 such patients and an optimal cutoff of 25 mg/l in relation to these controls, but this may be unreliable due to the small number of controls.

No practical screening system can exclude all cases of aspergillosis. Symptomatic screening would not exclude simple aspergilloma, as evidenced by the raised 97.5th centile for asymptomatic diseased controls. Chest X-ray screening is unlikely to identify cases of *Aspergillus* nodules[28] or *Aspergillus* bronchitis.[29] However, the similarity between antibody levels in screened

Table 3. Performances at various points on the ROC AUC curve for other groups.

Cutoff (mg/l)	Sensitivity (%)	Specificity (%)	Youden *J* statistic
European healthy controls			
5	99.2	0	0
10	95.4	5.3	.007
15	94.6	73	.676
20	93.4	98.7	.921
25	**92.9**	**99.3**	**.922**
30	90.9	100	.909
Ugandan healthy controls			
5	99.2	27.8	.27
10	95.4	94.6	.9
15	**94.6**	**98**	**.926**
20	92.9	98.7	.916
25	92.5	99	.915
30	90.9	99.7	.906
Prior TB diseased controls			
5	99.2	59.8	.59
10	95.4	90.5	.859
15	**94.6**	**94.5**	**.891**
20	93.4	94.7	.881
25	92.9	95.5	.884
30	90.9	95.7	.866
Screened diseased controls			
5	99.2	47.4	.466
10	95.4	85.5	.809
15	94.6	95.3	.899
20	93.4	97.9	.913
25	**92.9**	**98.7**	**.916**
30	90.9	99.1	.9
HIV-positive screened diseased controls			
5	99.2	35.3	.345
10	95.4	87.9	.833
15	94.6	96.6	.912
20	92.9	98.3	0.912
25	**92.5**	**99.1**	**.916**
30	90.9	99.1	.9
HIV-negative screened diseased controls			
5	99·2	1.7	.009
10	95·4	72.9	.683
15	94·2	94.1	.883
20	92·9	94.9	.878
25	**92·5**	**97.5**	**.9**
30	90·9	98.3	.892

HIV, human immunodeficiency virus; TB, tuberculosis. Optimal cut offs are highlighted in bold for each control group.

diseased and healthy controls demonstrates the effectiveness of our radiological screening method.

CPA complicating tuberculosis is a global public health issue, estimated to afflict one to three million persons worldwide.[8,30,31] Access to *Aspergillus* serology in resource poor settings is limited but improving and can, in principle, be delivered in any basic hospital laboratory.[32] Our study allows *Aspergillus*-specific IgG to be interpreted with greater confidence. More work is now needed to confirm that our conclusions are applicable in other countries and other underlying conditions. Above all, marked improvements in access to testing are now required.

Supplementary material

Supplementary data are available at *MMYCOL* online.

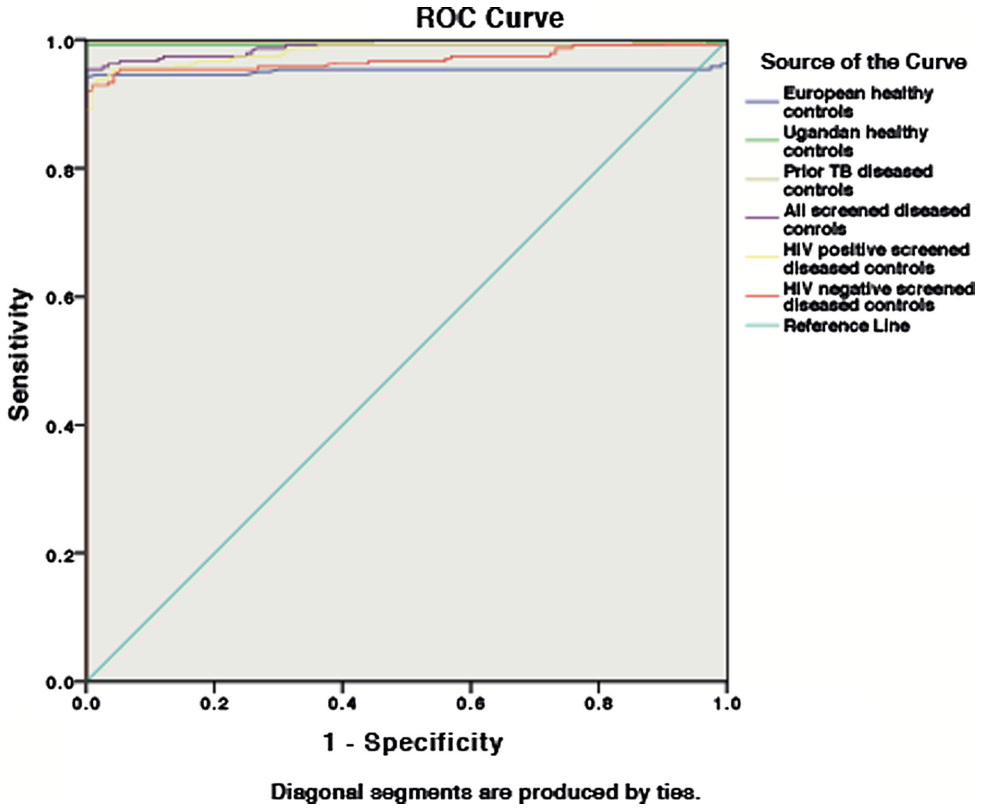

Figure 2. ROC curves comparing CPA cases with control groups. This Figure is reproduced in color in the online version of *Medical Mycology*.

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Ethical approval

Stored sera from CPA patients were acquired from the ManARTS biobank and from samples provided for the purpose of *Aspergillus*-specific IgG testing as part of routine care at the National Aspergillosis Centre, Manchester, UK. The South Manchester Research Ethics Committee granted ethical approval in December 2013 (REC reference number 10/H1010/7). Control samples were acquired as part the 'Pulmonary aspergillosis in association with tuberculosis' study. Ethical approval was granted by Gulu University IRB (ref. GU/IRC/04/07/12), the Ugandan National Council for Science and Technology (ref. HS1253) and the University of Manchester (ref. 11424) in 2012. Testing of healthy European control samples was performed on leftover samples and analyzed in an anonymous fashion in line with Dutch regulations.

Declaration of interest

I.D.P. has received kit donations and grants from Serion, Genesis, OLM Medical, and Dynamiker in addition to the funding stated above. He has current grant support from the Medical Research Council and has received travel grants from the British Infection Association, British Society for Medical Mycology, Microbiology Society, International Society for Human and Animal Mycology, European Society for Clinical Microbiology and Infectious Diseases and the Conference on Retroviruses and Opportunistic Infections within the last 5 years. M.D.R. lectures on behalf of, and provides educational material and advice for Gilead Sciences Europe, Astellas Pharma. MSD, and Pfizer. D.W.D. and family hold Founder shares in F2G Ltd, a University of Manchester spin-out antifungal discovery company. He acts or has recently acted as a consultant to Astellas, Sigma Tau, Basilea, Scynexis, Cidara, Biosergen, Quintiles, Pulmatrix, Pulmocide and Zambon. In the last 3 years, he has been paid for talks on behalf of Astellas, Dynamiker, Gilead, Merck, and Pfizer. He is a longstanding member of the Infectious Disease Society of America Aspergillosis Guidelines group, the European Society for Clinical Microbiology and Infectious Diseases

Aspergillosis Guidelines group, and the British Society for Medical Mycology Standards of Care committee. The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of this paper.

References

1. Schweer K, Bangard C, Hekmat K, Cornely O. Chronic pulmonary aspergillosis. *Mycoses*. 2013; 57: 257–270.
2. Ohba H, Miwa S, Shirai M et al. Clinical characteristics and prognosis of chronic pulmonary aspergillosis. *Respir Med*. 2012; 106: 724–729.
3. Jhun BW, Jeon K, Eom JS et al. Clinical characteristics and treatment outcomes of chronic pulmonary aspergillosis. *Med Mycol*. 2013; 51: 811–817.
4. Agarwal R, Vishwanath G, Aggarwal AN, Garg M, Gupta D, Chakrabarti A. Itraconazole in chronic cavitary pulmonary aspergillosis: a randomised controlled trial and systematic review of literature. *Mycoses*. 2013; 56: 559–570.
5. Chen QK, Jiang GN, Ding JA. Surgical treatment for pulmonary aspergilloma: a 35-year experience in the Chinese population. *Interact Cardiovasc Thorac Surg*. 2012; 15: 77–80.
6. Al-Shair K, Atherton GT, Harris C, Ratcliffe L, Newton PJ, Denning DW. Long-term antifungal treatment improves health status in patients with chronic pulmonary aspergillosis: A longitudinal analysis. *Clin Infect Dis*. 2013; 57: 828–835.
7. Farid S, Mohamed S, Devbhandari M et al. Results of surgery for chronic pulmonary aspergillosis, optimal antifungal therapy and proposed high risk factors for recurrence: a national centre's experience. *J Cardiothorac Surg*. 2013; 8: 180.
8. Denning D, Pleuvry A, Cole D. Global burden of chronic pulmonary aspergillosis as a sequel to pulmonary tuberculosis. *Bull World Health Organ*. 2011; 89: 864–872.
9. Denning DW, Cadranel J, Beigelman-Aubry C et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. *Eur Respir J*. 2016; 47: 45–68.
10. Patterson TF, Thompson GR, Denning DW et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016; 63: e1–e60.
11. 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: S265–80.
12. Baxter CG, Denning DW, Jones AM, Todd A, Moore CB, Richardson MD. Performance of two *Aspergillus* IgG EIA assays compared with the precipitin test in chronic and allergic aspergillosis. *Clin Microbiol Infect*. 2013; 19: E197–204.
13. Guitard J, Sendid B, Thorez S, Gits M, Hennequin C. Evaluation of a recombinant antigen-based enzyme immunoassay for the diagnosis of noninvasive aspergillosis. *J Clin Microbiol*. 2012; 50: 762–765.
14. Page ID, Richardson MD, Denning DW. Comparison of six *Aspergillus*-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis (CPA). *J Infect*. 2016; 72: 240–249.
15. Dumollard C, Bailly S, Perriot S et al. Prospective evaluation of a new *Aspergillus* IgG enzyme immunoassay kit for diagnosis of chronic and allergic pulmonary aspergillosis. *J Clin Microbiol*. 2016; 54: 1236–1242.
16. Page ID, Richardson M, Denning DW. Antibody testing in aspergillosis: quo vadis? *Med Mycol*. 2015; 53: 417–439.
17. Richardson MD, Page ID. *Aspergillus* serology: have we arrived yet? *Med Mycol*. 2017; 55: 48–55.
18. Van Toorenenbergen AW. Between-laboratory quality control of automated analysis of IgG antibodies against *Aspergillus fumigatus*. *Diagn Microbiol Infect Dis*. 2012; 74: 278–281.
19. Page I, Baxter C, Hennequin C, Richardson MD, van Toorenenbergen AW, Denning DW. Receiver operating characteristic curve analysis of four *Aspergillus*-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis. *Diagn Microbiol Infect Dis*. 2018; 91: 47–51.
20. Smith NL, Denning DW. Underlying conditions in chronic pulmonary aspergillosis including simple aspergilloma. *Eur Respir J*. 2011; 37: 865–872.
21. World Health Organization. *WHO Global tuberculosis report 2016*. http://www.who.int/tb/publications/global_report/en/. Published 2016. Accessed September 12, 2016.
22. Van Hoeyveld E, Dupont L, Bossuyt X. Quantification of IgG antibodies to *Aspergillus fumigatus* and pigeon antigens by ImmunoCAP technology: an alternative to the precipitation technique? *Clin Chem*. 2006; 52: 1785–1793.
23. Watkins ML, Benjamin RL, Kotzé E, Hawarden D. Reference range for specific IgG antibodies to *Aspergillus fumigatus* in the South African adult population. *Curr Allergy Clin Immunol*. 2012; 25: 212–214.
24. Barton RC, Hobson RP, Denton M et al. Serologic diagnosis of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis through the detection of immunoglobulin G to *Aspergillus fumigatus*. *Diagn Microbiol Infect Dis*. 2008; 62: 287–291.
25. Baxter CG, Dunn G, Jones AM et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. *J Allergy Clin Immunol*. 2013; 132: 560–566.
26. Agarwal R, Dua D, Choudhary H et al. Role of *Aspergillus fumigatus* -specific IgG in diagnosis and monitoring treatment response in allergic bronchopulmonary aspergillosis. *Mycoses*. 2017; 60: 33–39.
27. British Tuberculosis Association. Aspergilloma and residual tuberculous cavities: the results of a resurvey. *Tubercle*. 1970; 51: 227–245.
28. Muldoon EG, Sharman A, Page I, Bishop P, Denning DW. *Aspergillus* nodules: another presentation of chronic pulmonary aspergillosis. *BMC Pulm Med*. 2016; 16: 123.
29. Chrdle A, Mustakim S, Bright-Thomas RJ, Baxter CG, Felton T, Denning DW. *Aspergillus* bronchitis without significant immunocompromise. *Ann N Y Acad Sci*. 2012; 1272: 73–85.
30. Denning DW, Pleuvry A, Cole DC. Global burden of chronic pulmonary aspergillosis complicating sarcoidosis. *Eur Respir J*. 2013; 41: 621–626.
31. Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Med Mycol*. 2013; 51: 361–370.
32. Cole DC, Govender NP, Chakrabarti A, Sacarlal J, Denning DW. Improvement of fungal disease identification and management: combined health systems and public health approaches. *Lancet Infect Dis*. 2017; 17: e412–e419.