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Effect of patient immunodeficiencies on the diagnostic performance of serological assays to detect *Aspergillus*-specific antibodies in chronic pulmonary aspergillosis

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ABSTRACT

Background: Prevalence of chronic pulmonary aspergillosis (CPA) is ~3 million patients worldwide, and detection of *Aspergillus*-specific antibody is a critical diagnostic component. Some patients with CPA have subtle immune deficits possibly contributing to poor *Aspergillus* antibody production and false negative results.
Materials/methods: We analyzed patient data from 167 cases of clinically confirmed CPA previously evaluated by ImmunoCAP *Aspergillus*-specific IgG EIA, Bordier ELISA and LDBio *Aspergillus* IgG/IgM ICT lateral flow assay, to identify deficiencies in: mannose binding lectin (MBL), IgG, IgA, IgM, IFN gamma, IL12 or IL17 production, and/or low cell marker counts (CD4, CD19, CD56). We defined patients as 'sero-negative' if ImmunoCAP *Aspergillus* IgG was consistently and repeatedly negative (<40 mg A/L). 'Sero-positive' was defined as all other CPA cases.
Results: We found the rate of false negatives by ImmunoCAP *Aspergillus* IgG EIA (n = 23) to be more prevalent in patients with immunodeficiency markers, especially multiple defects. MBL deficiency combined with low CD19 cells ($p < 0.001$), pneumococcal antibody levels ($p = 0.043$), IgM ($p = 0.047$) or three combined ($p = 0.001–0.018$) or all four together ($p = 0.018$) were significant. The performance LDBio *Aspergillus* IgG/IgM ICT appears to be relatively unaffected by immunodeficiency (92.7% of ImmunoCap sero-negatives were positive). The Bordier assay performed significantly better than the ImmunoCAP assay ($P = 0.0016$) for sero-negative CPA cases.
Conclusions: In select cases of CPA, ImmunoCAP EIA yields a false negative result, making serological diagnosis difficult. ImmunoCAP false negatives are more prevalent in patients with multiple immunological defects, who may still be positive with the LDBio *Aspergillus* ICT or Bordier EIA.

1. Introduction

Invasive aspergillosis occurs primarily in patients with profound, but sometimes temporary, immunodeficiency, in contrast to chronic pulmonary aspergillosis (CPA) which occurs in people with no discernible immunodeficiency state. Over the last few years, several subtle immune defects have been found in some, but not all, CPA patients including mannose binding lectin deficiency [1], poor encapsulated bacterial vaccine responses [2], low circulating T- and natural killer cells [3], and interleukin-12 and gamma interferon defects [4]. We and others [5] have used the term 'subtle immunodeficiency' to describe the status of these patients, to distinguish them from those with major deficits, usually termed immunocompromised.

The cornerstone of laboratory diagnosis of CPA is detection of anti-*Aspergillus fumigatus* IgG antibody [6]. Some patients also produce specific IgM and IgE antibody, occasionally without specific IgG antibody [7], and further, there is a group of patients with low or undetectable *Aspergillus* specific antibody [8]. There could be several reasons for this. First the antibody test may have an inappropriately high cut-off value. We and others [8] have expended considerable efforts to define such cut-offs, but they are inevitably arbitrary. Second, patients do not generate antibody to the selected antigens used in a particular test. For

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example, we found in one study that 3.7% of patients had negative assays with the routine *Aspergillus*-specific IgG test used [8], but some had detectable antibody in another assay. Third, there may be a group of patients who do not generate *Aspergillus* specific antibody due to an unrecognised immune dysfunction. This may result in delays in diagnosis and progression of disease.

We have identified a group of patients with negative results in the ImmunoCap assay (an automated fluorescent singleplex enzyme immunoassay) and assessed their responsiveness to the new *A. fumigatus* lateral flow assay (LD Bio ICT) which detects both IgG and IgM and appears to be more sensitive than the ImmunoCap assay [9]. We used a third *Aspergillus* IgG manual ELISA (Bordier) to assist in definition of CPA in some of the cases as a comparator. We asked the question whether these 'unresponsive' patients had a higher degree of immune dysfunction by correlating with immunological markers.

2. Materials and methods

2.1. Patients

We performed a retrospective review of secondary data obtained from 167 CPA patients identified at the National Aspergillosis Centre (NAC) (Manchester, UK). Clinical and laboratory data was not available for all patients. The NAC is a nationally commissioned service providing long-term specialist care for patients with CPA throughout the UK. There are currently approximately 500 CPA patients on active follow-up, with approximately 130 new referrals annually. For each patient, CPA diagnosis was confirmed by an experienced specialist clinician. Using ERS/ESCMID guidelines [6], diagnosis required a combination of features: at least three months of relevant symptoms, characteristic radiological features, and positive 'microbiological evidence'. The latter primarily consisted of a positive serological result using the Ouchterlony 'double-diffusion' method to detect *Aspergillus* precipitins [10] or measurement of *Aspergillus*-specific IgG level by ImmunoCAP (positive result $\geq$40 mg A/L IgG). Also accepted as 'microbiological evidence' were: histological evidence following biopsy or resection of lung tissue, strongly positive *Aspergillus* antigen or DNA in respiratory fluids, microscopy of respiratory fluids showing hyphae or *Aspergillus* grown from sputum culture [6]. For the CPA cases included in this study, detection of *Aspergillus* antigen was determined by galactomannan EIA (Bio-Rad Laboratories, Marnes la Coquette, France), with an optical density (OD) $>$ 1.0 accepted as 'strongly' positive for bronchoalveolar lavage (BAL) samples and OD $>$ 6.5 for sputum [11]. *Aspergillus* DNA in respiratory fluid (sputum) was detected using a commercially available real-time PCR diagnostic assay (ELITech, Puteaux, France), with a strong positive PCR (ie transformed Ct value $>$ 2.0) denoting a positive result [11]. In rare cases (estimated 3.7% [8] - 7%), patients presented with clear clinical and radiological evidence of CPA as well as repeatedly positive sputum *Aspergillus* culture or PCR (ELITech, Puteaux, France), despite the absence of an antibody response (negative *Aspergillus* serology). A PCR, culture, or galactomannan result was considered positive if any result in up to 3 years of patient history was positive, even if other results over that period for the same test were negative. These were also accepted as CPA cases. *Aspergillus* precipitins (Microgen Bioproducts, Surrey, UK) were tested for in most patients at least once. Patients diagnosed with *Aspergillus* nodules were excluded [12].

2.2. Serological samples

Patient sera were obtained by convenience sampling. Patient sera were acquired at NAC as part of routine clinical care and all samples were also used for measurement of *Aspergillus*-specific IgG at the time of collection. Residual sera from these routine samples were collected between September 2016 and January 2019 ($>$90% of samples collected after January 01, 2018) and stored at -80 °C until use. A total of 167 CPA cases were identified and sampled – 154 were collected and used for evaluation of a novel diagnostic test [9] including 10 'sero-negative' CPA cases. For the purpose of our evaluation, a 'sero-negative' CPA case is defined for patients with a consistent and repeated negative result by ImmunoCAP *Aspergillus*-specific IgG over (up to) 3 years of patient history, with CPA confirmed by other diagnostics methods (radiology, clinical features, PCR, culture, etc). An additional 13 sero-negative cases were identified for evaluation inclusion in this study (Fig. 1).

2.3. Serological analysis

Of the 167 CPA patient samples, 166 were tested using the *Aspergillus* ICT IgG-IgM (LDBio, Diagnostics, Lyon, France) lateral flow assay (154 previously tested, 13 additional tested for this study), and 102 were previously tested by *Aspergillus fumigatus* IgG ELISA (Bordier Affinity Products, Crissier, Switzerland) [13]. Both assays were run in accordance with the manufacturers' guidelines, with manual pipetting and for the ELISA, washing. To run the *Aspergillus* ICT assay, 15 μl of sera was dispensed onto the ICT sample application pad, followed by four drops of eluting solution (provided with kit). The test was read after 20–30 min and a positive result was determined positive by the appearance of two lines: a blue positive control ("C") line, and a black positive test ("T") line. The appearance of any black line at the "T" marker was considered positive, as recommended in the manufacturer's guidelines. To interpret the *Aspergillus* IgG ELISA, an OD index value was calculated as: *[OD of sample]/[OD of cut-off provided by kit]*. OD index values $\geq$ 1.0 were considered 'positive', values $<$ 0.8 were considered 'negative'. According to the manufacturer, values between 0.8 and 1.0 are considered 'borderline', however, for the purposes of this analysis we considered any such value as a 'negative' as it would be a clinically indeterminate result.

2.4. Routine diagnostics

Aspergillus-specific IgG levels were measured on all CPA patient samples as part of routine clinical care. Testing was carried out by the Manchester University NHS Foundation Trust, Department of Clinical Immunology, using the automated ImmunoCAP Phadia 1000 System (Thermo Scientific, Waltham, MA). Where a sample produced a result of $>$200 mg A/L, a 1:10 dilution was performed and the sample was re-tested [14].

2.5. Immunology data

Patient data was obtained from the National Aspergillosis Centre database, encompassing a period of three years prior to sample collection. We collected all available results for the following immunological biomarkers: mannose binding lectin (MBL), IgG, IgA, IgM, pneumococcal antibody titre (Pn Ab), *Haemophilus* antibody titre (Hm Ab), gamma interferon (IFNg) production, interleukin 12 (IL12) production, interleukin 17 (IL17) production, CD3, CD4, CD8, CD19, and CD56 (Fig. 1). Cytokine production assays were only ordered in patients failing antifungal therapy or those with dual mycobacterial and *Aspergillus* infections.

CD3 (reference range (700–2100) $\times$ 10^9/L), CD4 (reference range (300–1400) $\times$ 10^9/L), CD8 (reference range (200–900) $\times$ 10^9/L), CD19 (reference range (100–900) $\times$ 10^9/L), and CD56 (reference range (90–600) $\times$ 10^9/L) markers were done by the Manchester University NHS Foundation Trust, Department of Clinical Immunology. This facility is accredited (IS0 15189:2012) for CD enumeration using FC500 MPL flow cytometer equipment (Beckman Coulter Ltd., Buckinghamshire, UK).

2.6. Statistical analyses

McNemar's test was used for pairwise comparison of sensitivity between LDBio *Aspergillus* ICT, Bordier ELISA, and ImmunoCAP tests.

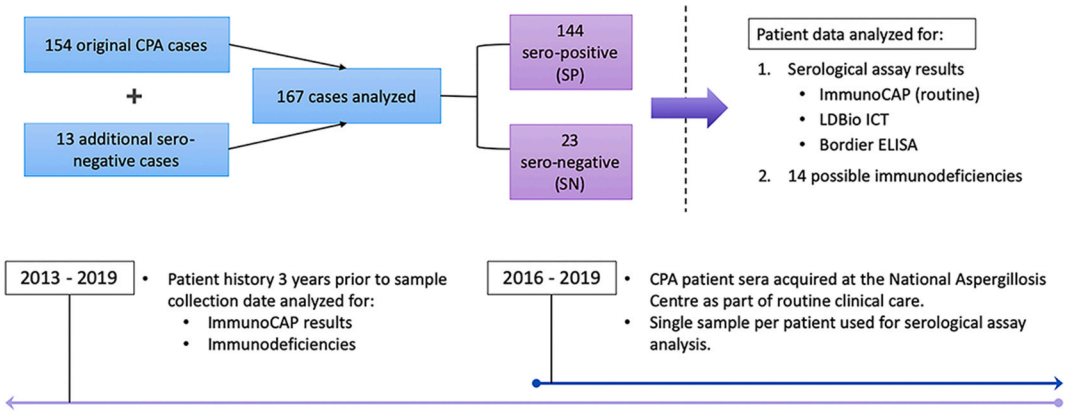

Fig. 1. Patient disposition and analyses.

Fisher's exact test was used for comparisons of the immunological markers between subgroups. Chi square analysis was used to compare assay sensitivity between subgroups ('low' vs. 'normal' reference values). For all results, a two-tailed *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Patients and serological assays

Patient characteristics for all 167 CPA patients are shown in Table 1, for all patients as well as 'sero-positive' (SP) and 'sero-negative' (SN) subgroups, with SN defined by the ImmunoCap assay. Age and sex did not differ between these groups.

As this was a retrospective laboratory study to correlate patient immune markers with *Aspergillus* serological assay results, not all patients had a complete set of data for the 14 markers and/or 3 serological assays evaluated in this study. The number of patients with data for each of the 14 immunological assays, categorized by serological assay (including number of patients with results for each assay), is summarized in Table 2.

3.2. Sero-negative patient data

We evaluated patient data for a period of three years prior to sample collection. The individual profiles of all 23 sero-negative patients (diagnostic test results + immunodeficiencies) is shown in Table 3.

3.3. Sero-negative vs. sero-positive patients

We found sero-negative patients more likely to be mannose-binding lectin (MBL), IgG, IgA, or IgM deficient, or to have low levels of pneumococcal antibodies (Pn Ab), interferon gamma (IFN), CD8, CD19, or CD56. Using Fisher's exact test to compare the number of 'low' or 'normal' reference values versus patient classification as sero-negative or sero-positive, the difference between patient groups neared significance for MBL ($P = 0.088$), IgM ($P = 0.071$), and Pn ($P = 0.070$) (Fig. 2).

With regards to assay performance in sero-negative versus sero-positive cases, we observed a lower sensitivity in the sero-negative group for ImmunoCAP and Bordier assays (not significant) compared to the sero-positive group (Table 4). LDBio performance remained consistent for all groups. Comparing the three assays for each patient group, we found the LDBio assay performed significantly better than both the Bordier ($P = 0.0006$) and ImmunoCAP ($P < 0.0001$) assays in all CPA cases, as well as in the sero-negative group ($P = 0.046$ and $P < 0.0001$, respectively); and significantly better than the Bordier assay ($P = 0.0045$) in the sero-positive group. The Bordier assay performed significantly better that the ImmunoCAP assay ($P = 0.0016$) for sero-negative CPA cases, and the ImmunoCAP assay performed significantly better than the Bordier assay ($P = 0.0075$) for sero-positive cases (Fig. 3). Overall, of the 102 samples tested by all 3 assays, 4 sera (3.9%) were negative for all three tests and 13 (12.7%) for two of the three.

Table 1
Patient characteristics.

Characteristic	All CPA (n = 167)	ImmunoCAP SN[a] (n = 23)	ImmunoCAP SP[a] (n = 144)
Age Range (Mean)	32-87 (64)	32-84 (67)	33-87 (63)
% Male (n)	57.1% (101)	56.5% (13)	59.0% (85)
% Female (n)	42.9% (76)	43.5% (10)	41.0% (59)

[a] ImmunoCAP result used to define 'sero-negative' (SN) and 'sero-positive' (SP) groups.

Table 2
Immunodeficiency analysis: Patients (n) with immunodeficiency data per serological assay.

Assay [patients tested, (n)]	ImmunoCAP SN/SP[a]	MBL[b]	IgG	IgA	IgM	Pn[c,f]	Hm[d,f]	IFN[e]	IL12	IL17	CD3	CD4	CD8	CD19	CD56
ImmunoCAP EIA (167)	23/144	150	155	156	155	156	153	49	39	33	63	63	63	63	64
LDBio LFA (166)	22/144	149	154	155	154	155	152	49	39	33	63	63	63	63	64
Bordier ELISA (102)	16/86	92	97	97	96	101	95	27	21	16	37	37	37	37	37

[a] ImmunoCAP result used to define 'sero-negative' (SN) and 'sero-positive' (SP) groups.
[b] MBL: Mannose-binding lectin.
[c] Pn: Pneumococcal antibodies.
[d] Hm: Haemophilus antibodies.
[e] IFN: Interferon gamma.
[f] Determination of deficiency based on most recent result, irrespective of vaccination status.

Table 3
Sero-negative[a] patient data profile (3-year history).

		Diagnostic Tests					Known immunodeficiencies													
Case	NAC[b] Entry	LDBio ICT	Bordier ELISA	PPT[c]	PCR	*Aspergillus* spp. (culture)	MBL[d]	IgG	IgA	IgM	Pn[e]	Hm[f]	IFN[g]	IL12	IL17	CD56	CD3	CD4	CD8	CD19
1	2009	+		-	+	*fumigatus*	✓				✓	<1.0								✓
2[h]	2006	+	+	-	+	-		✓			✓		✓		✓					
3	2005	+	+	-	-	*fumigatus*	✓				✓				✓					✓
4[h]	2017	+	-		-	-				✓										✓
5	2009	+	-	-		-	✓			✓	✓	<1.0								
6	2009	+	+	-	+	-					✓									
7[h]	2010	+	+	-	+	*fumigatus, versicolor*	✓				✓		✓	✓	✓					
8[*]	2010	+	-	-	+	*fumigatus*					✓	✓	✓		✓					
9	2011	+	+	-	-	*fumigatus*	✓			✓	✓	✓								✓
10	2011	+		-	-	-				✓	✓	<1.0	✓		✓	✓	✓	✓	✓	✓
11	2011	+		-	+	*fumigatus*					✓						✓	✓		✓
12[g]	2012	+	+	-	-	-	✓				✓	<1.0	✓		✓			✓		
13	2013	+	+	-	-					✓	✓	✓	✓							
14	2016	+	+	+	-	-	✓	✓	✓	✓	✓	✓								✓
15	2018	+	+	-	-	-	✓				✓	✓								
16	2017	-	-	-	-	*fumigatus*					✓									
17	2017	+		-	+	*hiratsukae*					✓									
18	2017			-	+	*fumigatus, niger*	✓			✓	✓	<1.0								
19	2017	+	-	-		-	✓													
20	2017	-		+	+	*fumigatus, creber*	✓				✓									✓
21	2018	+	+	+	-	*fumigatus*					✓									
22	2018	-	-	-	-	-					✓	✓								
23	2013	+	-	-	-	*fumigatus*														

+ = positive result, - = Negative result, ✓ = Deficiency present
Blank cells = Normal level, **Shaded cells** = unknown/no results in 3-year patient history
[a]ImmunoCAP-negative result used to define 'sero-negative' (SN) group
[b]NAC: National Aspergillosis Centre (year of entry into NAC service), [c]PPT: precipitins assay
[d]MBL: Mannose-binding lectin, [e]Pn: Pneumococcal antibodies, [f]Hm: Haemophilus antibodies, [g]IFN: Interferon gamma
[h]These patients had ImmunoCAP-positive results >3 years prior (all positive results <100 mgA/L)

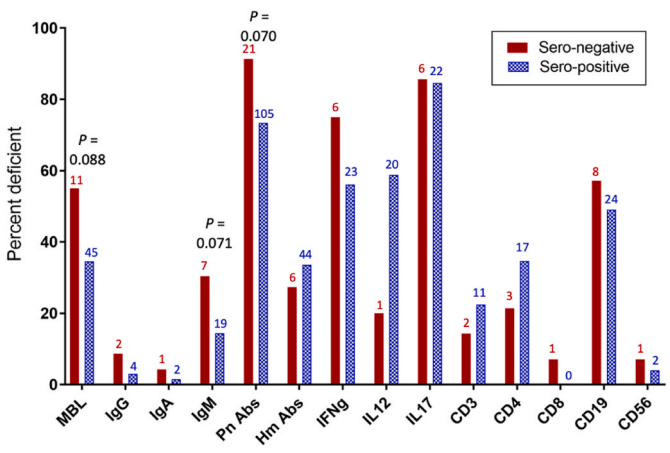

Fig. 2. Percent of sero-negative or sero-positive cases (as defined by ImmunCap values (±40 mg A/L)) with "low" reference value (with 'n' noted for each bar).

3.4. Correlation of immunological markers with serological assay performance

After comparing serological assay performance between sero-negative and sero-positive CPA patient groups, we then examined the effect of individual immunodeficiencies on assay sensitivity. We first determined the sensitivity of each assay for individual immunodeficiencies, comparing assay sensitivity between 'low' and 'normal' reference value groups for each. We then eliminated from further evaluation any immunodeficiency for which (1) there was greater or equivalent incidence of deficiency in the sero-positive group compared with the sero-negative group, or (2) there were fewer than 5 cases in the sero-negative group with data for a specific immunodeficiency. The remaining five (5) immunodeficiencies to be evaluated further were: MBL, IgG, IgM, Pn, and CD19.

We first evaluated the sensitivity of each serological assay for 'low' and 'normal' reference values groups for these markers (Fig. 4) compared to overall assay performance (sensitivity for all CPA cases: sero-negative + sero-positive), and found ImmunoCAP assay sensitivity to be impacted (lowered compared to overall sensitivity) for cases with low levels of MBL, IgG, IgM, Pn, and CD19. Bordier ELISA sensitivity was lowered for cases with low levels of MBL, IgM, and CD19. Underlying patient immunodeficiencies appeared to have little-to-no impact on LDBio ICT assay performance. There was little-to-no variation compared to overall sensitivity for the 'normal' reference value groups, across all three assays.

Having observed the effect of underlying immunodeficiencies on assay performance, we then sought to determine whether false negative results for each assay were associated with increased frequency of immunodeficiency. We evaluated the percent of positive or negative assay results with a 'low' reference value for each deficiency, and determined significance using Chi square analysis to compare assay sensitivity between 'low' and 'normal' reference groups (Fig. 5). We found increased potential for false negative results by ImmunoCAP in cases of MBL ($P = 0.062$), IgM ($P = 0.006$), and Pn ($P = 0.071$) deficiencies, and by Bordier ELISA in the case of MBL deficiency ($P = 0.092$). There was no statistically significant difference for any other comparisons.

Given our finding for this sample group that immunodeficiencies occur more frequently in sero-negative versus sero-positive CPA patients individually, we examined whether there was an increased frequency of

Table 4
Serological assay performance in sero-negative (SN) vs. sero-positive (SP)[a] CPA.

			Sensitivity (%)		
Assay (total CPA tested, n)	ImmunoCAP SN (n)	ImmunoCAP SP (n)	All CPA	ImmunoCAP SN	ImmunoCAP SP
ImmunoCAP (167)	23	144	74.3	0	86.1
LDBio (166)	22	144	91.6	90.9	91.7
Bordier (102)	16	86	75.5	62.5	77.9

[a] ImmunoCAP result used to define 'sero-negative' (SN) and 'sero-positive' (SP) groups.

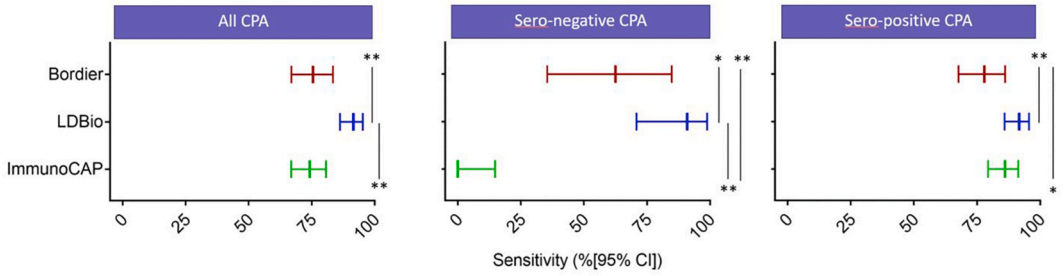

Fig. 3. Comparison of assay sensitivity for all CPA, ImmunoCAP sero-negative CPA cases, and ImmunoCAP sero-positive CPA cases. McNemar's test was used for pairwise comparisons (* = $P < 0.05$, ** = $P < 0.005$).

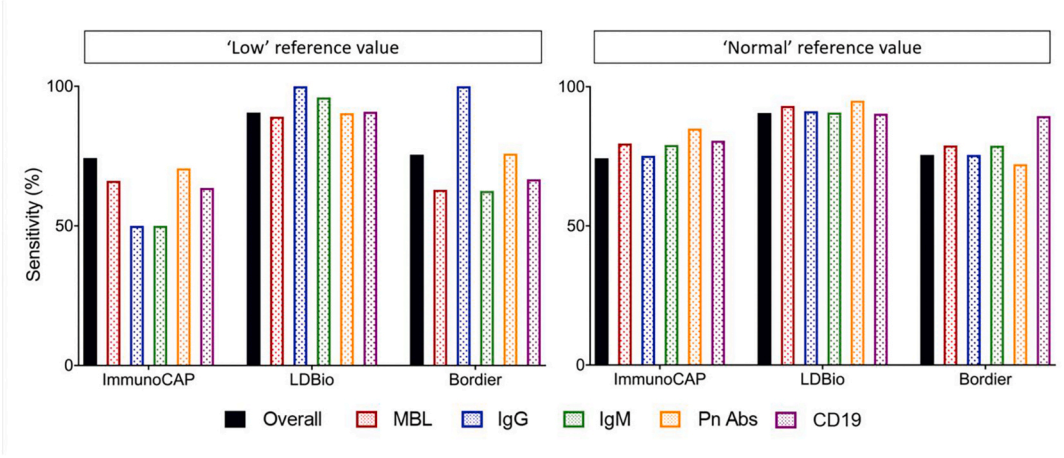

Fig. 4. Comparison of performance of assays in patients with low or normal concentrations of immunological markers, by Aspergillus IgG assay.

specific immunodeficiencies occurring in combination in sero-negative CPA patients. Fisher's exact test was used to analyse incidence of MBL, IgG, IgA, IgM, Pn, and/or CD19 deficiencies occurring together in sero-negative versus sero-positive patients. We found that in sero-negative patients MBL, IgM, Pn, and CD19 deficiencies were significantly more likely to be comorbid in sero-negative as compared to sero-positive patients (Table 5).

4. Discussion

This study suggests that the LDBio Aspergillus ICT assay has superior performance for the diagnosis of CPA compared to the ImmunoCAP and Bordier EIA assays in patients with evidence of impaired immunological function. The LD-Bio *Aspergillus* ICT assay exhibits consistent performance across all patient subgroups and little variation in the presence of evidence of deficiency in humoral immunity. A key difference of the LDBio assay is the ability to detect *Aspergillus*-specific IgM in addition to IgG, whilst both ImmunoCAP and Bordier assay only detect *Aspergillus*-specific IgG. The improved performance of the combination of IgG and IgM testing was also shown in a study from China, in which the sensitivity of the combination of IgM and IgG for the diagnosis of CPA was 95.1%, compared to 84.1% for IgG only testing and 43.9% for IgM only testing [15]. In contrast, *Aspergillus* precipitin testing, which detects IgG and IgM antibodies, has low sensitivity and has been supplanted by ELISA [16].

Patients with CPA usually have underlying chronic lung disease such as COPD or previous TB and therefore are not considered overtly immunocompromised. For this reason, the diagnosis of aspergillosis may be delayed due to lack of suspicion, especially in the setting of negative *Aspergillus* serology, if histological or microbiological confirmation is not obtained. Patients with CPA have evidence of impaired immune function as demonstrated particularly by non-protective pneumococcal titres, MBL levels, cytokine production defects and lower numbers of CD19 cells [2,3]. In particular, patients with a negative ImmunoCap test were more likely to have lower MBL levels, non-protective pneumococcal serology and lower IgM levels. Presence of immune dysfunction suggested by the above tests should alert physicians seeing patients with a chronic pulmonary process to consider CPA even with negative serology. The LD-Bio ICT assay appears to offer enhanced sensitivity for these patients and can be used in addition, or can replace, other serological tests, at least for screening for *Aspergillus* antibody.

In addition, the improved performance of the LD-Bio ICT assay in

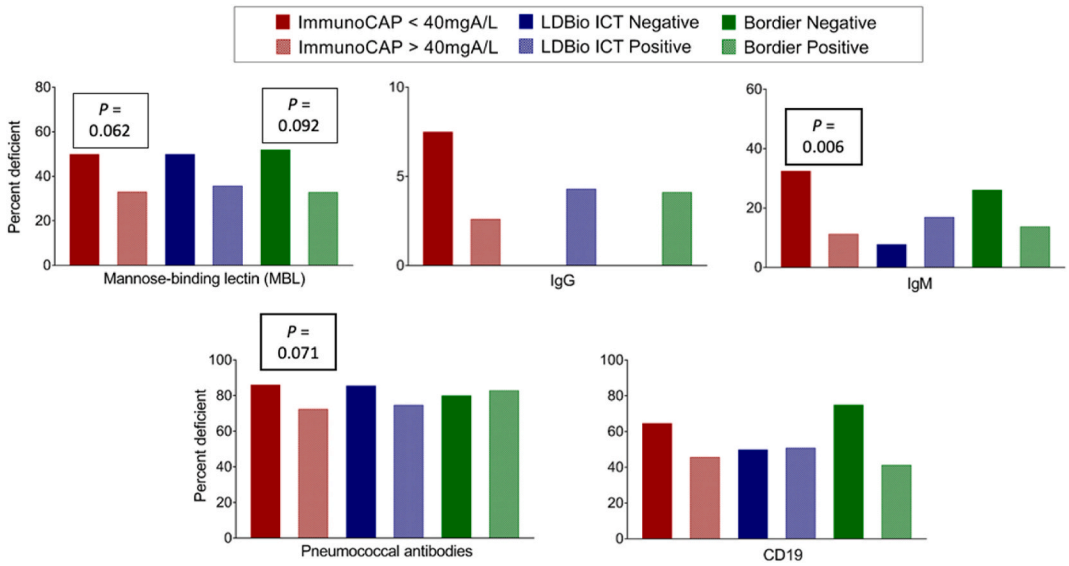

Fig. 5. Statistical comparison of low vs normal values for mannose binding lectin, total IgG, total IgM, pneumococcal antibody and CD19 positive cells.

Table 5
Frequency of immunodeficiencies occuring in sero-negative versus sero-positive CPA patients.

Immunodeficiency combination	*P*
MBL + IgM	0.047
MBL + Pn	0.043
MBL + CD19	<0.001
IgM + Pn	0.008
IgM + CD19	0.004
Pn + CD19	0.027
MBL + IgM + Pn	0.001
MBL + Pn + CD19	0.001
IgM + Pn + CD19	0.008
MBL + IgM + CD19	0.018
MBL + IgM + Pn + CD19	0.018

patients with some evidence of impaired immune response raises the question whether this test may be of some utility for the diagnosis of invasive aspergillosis (IA) which usually affects patients with profound immunodeficiency. Overall less than 50% of patients with IA have detectable *Aspergillus* antibody [7], but sero-conversion might be a useful means of retrospectively making the diagnosis [17]. A recent study evaluating an IgM test (Dynamiker) showed a suboptimal sensitivity in IA (30% when a cut-off of 50U/mL was used) [18]. Assessment of the LD-Bio ICT assay in the setting of IA has not been performed to our knowledge. This test has been assessed in allergic bronchopulmonary aspergillosis with almost equivalent sensitivity to testing for CPA [19].

Defects in IFNγ production were more common with seronegative CPA patients, however this was a common feature in CPA and may suggest a pathogenetic association. Recently, IFNγ replacement was shown to be beneficial in the prevention of exacerbations and hospital admissions, when used as salvage therapy in a cohort of patients with severe CPA [20].

Limitations of this study include the lack of complete data on the immunological parameters; it is possible that patients who had these tests were the ones experiencing the most severe disease. In addition, the cut-offs used are those suggested by local laboratories based on population studies in the UK, but the clinical significance of these cut-offs has not been confirmed. A different analysis of 114 residual UK sera found the optimal cut-off of the Bordier assay to be 0.9 [21] and that if a cut-off of 27 mg A/L is applied to the ImmunoCap assay (as suggested by a study in India) [22], then 11% more samples would be positive, with an unknown impact on specificity [21].

In conclusion, the LDBio Aspergillus ICT assay demonstrated superior performance compared to other serological tests in patients with CPA who appeared to have evidence of impaired immune function. This test may have a role in the routine diagnosis of CPA in both low- and high-resource settings.

Authorship contributions

This project was conceived by ESH and DWD. BAPW undertook the Bordier testing and contributed to analysis. MDR and CK contributed to writing the paper and general conceptual framework which lead to the investigation.

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Declaration of competing interest

These are our potential conflicts of interest. "Authors ESH, BAPW and CK declare no conflict of interests. MDR has received grant support from Gilead Sciences, Pfizer and MSD, and acts as a consultant and speaker for Gilead Sciences, Pfizer, Astellas and Schering-Plough. DWD 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 Scynexis, Pulmatrix, Pulmocide, Zambon, iCo Therapeutics, Mayne Pharma, Biosergen, and Fujifilm. In the last 3 years, he has been paid for talks on behalf of Hikma, Gilead, Merck, Mylan 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."

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