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ADVANCES IN DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS (O MORRISSEY, SECTION EDITOR)

Diagnostic Aspects of Chronic Pulmonary Aspergillosis: Present and New Directions

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Abstract

Purpose of Review Diagnosis of chronic pulmonary aspergillosis (CPA) is important since many diseases have a similar appearance, but require different treatment. This review presents the well-established diagnostic criteria and new laboratory diagnostic approaches that have been evaluated for the diagnosis of this condition.

Recent Findings Respiratory fungal culture is insensitive for CPA diagnosis. There are many new tests available, especially new platforms to detect *Aspergillus* IgG. The most recent innovation is a lateral flow device, a point-of-care test that can be used in resource-constrained settings. Chest radiographs without cavitation or pleural thickening have a 100% negative predictive value for chronic cavitary pulmonary aspergillosis in the African setting.

Summary Early diagnosis of CPA is important to avoid inappropriate treatment. It is our contention that these new diagnostics will transform the diagnosis of CPA and reduce the number of undiagnosed cases or cases with a late diagnosis.

Keywords Chronic pulmonary aspergillosis · Diagnostics · Serological test · Lateral flow device · Lateral flow assay · Resource-constrained settings

Introduction

Chronic pulmonary aspergillosis (CPA) is a fungal infection disease characterised by cough, haemoptysis and weight loss present for at least for 3 months in non-immunocompromised people [1••]. This disease is caused by *Aspergillus* spp. that can reach the respiratory tract by airborne transmission [2]. *Aspergillus* spp. have been known as a cause of human infection for centuries. In 1842, Bennett observed fragments of 'tubes', somewhat larger, more or less matted together, which appeared distinctly jointed in microscopic examinations of sputa and the lining membrane of tubercular cavities in the lungs of a man initially thought to have tuberculosis [3]. Despite being first described > 100 years before invasive aspergillosis, more attention has been focussed on acute invasive aspergillosis in recent years, but now with the development of new (and welcome) antifungal agents, attention has refocused on CPA. The first report of amphotericin B use for any form of aspergillosis was for a case of CPA [4]. In the 1960s, most efforts related to aspergillosis were focussed on the therapy of invasive aspergillosis with amphotericin B and allergic aspergillosis. The one exception was a large Medical Research Council study in the UK published in 1968 which found an aspergilloma associated with *Aspergillus* precipitating antibody in 17% of pulmonary tuberculosis survivors who had residual lung cavities [5].

A watershed study in 1983 retrospectively compared medical and surgical treatment of patients with 'pulmonary aspergilloma'. The authors suggested that medical therapy (which did not include antifungal agents) was not as effective as surgical resection [6]. A small study of low dose itraconazole (200 mg daily) published in 1991 was found to be ineffective [7], whereas the current recommended dose is 400 mg daily. These findings lead to medical passivity around

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CPA, and only surgical resection in those with resectable aspergillomas was offered for therapy. The publication in 2003 of a case series of 18 patients with full diagnostic criteria and antifungal therapy responses generated renewed interest in the problem by respiratory physicians [8]. The subsequent publication in 2011 of the magnitude of the problem after pulmonary tuberculosis globally (estimated at 1.17 million) has further focussed minds on addressing the problem [9].

CPA Burden of Disease

CPA cases after tuberculosis can be found worldwide, from an estimated 11,400 cases in Europe to 145,372 cases in South-East Asia in 2007 and these numbers are likely to keep increasing each year [9]. In most countries, the number of CPA cases correlates with the number of tuberculosis (TB) patients because the fungus can colonise and infect cavities left in the lungs after TB infection [5, 10]. There are an estimated 1,170,000 cases of CPA after TB and at least the same number or more cases from other pulmonary disorders namely chronic obstructive pulmonary disease (COPD), sarcoidosis, non-tuberculous mycobacterial infection (NTM), post-pneumothorax, allergic bronchopulmonary aspergillosis (ABPA) and rheumatoid arthritis [9, 11]. Untreated CPA has a high mortality rate, causing around 450,000 deaths every year [12]. Several factors are associated with a higher mortality risk for CPA including NTM infection, COPD, pleural involvement, cavitary disease, presence of an aspergilloma, shortness of breath, low physical activity, and low body mass. Those with multiple pulmonary infections such as NTM infection may be linked to subtle immunodeficiency, such as poor vaccine response rates and abnormally low CD4, CD19, and CD56 (natural killer) cell counts, as well as either gamma interferon or interleukin 12 deficiency [13–15]. The 1-, 5- and 10-year survival rates are 86%, 62% and 47%, respectively [16•].

CPA can manifest in different forms, which are sometimes overlapping (Table 1). Some patients will be diagnosed with only a single fungal ball or aspergilloma. CPA can also appear as one or more nodules in the lung although this is unusual. Other patients can have one or pulmonary cavities that can develop extensive lobar fibrosis over weeks or months after chronic fibrosing pulmonary aspergillosis (CFPA). Two entities that can precede CPA include community acquired pulmonary aspergillosis and subacute invasive pulmonary aspergillosis in moderately immunocompromised patients [17].

Diagnosis of CPA

The diagnosis of CPA is made with a combination of clinical symptoms (recognising that the early phases of the disease are often asymptomatic or clinically subtle), characteristic radiological findings and laboratory tests indicating infection with *Aspergillus* spp. [1••]. To distinguish the disease from sub-acute invasive aspergillosis, an arbitray duration of at least 3 months is required, and no, or only subtle, immunocompromise. With respect to corticosteroids, we usually take $\leq$ 10 mg of prednisolone long term or equivalent as that threshold to define immunocompromise, but again this is arbitrary. Acute invasive, sub-acute invasive and community acquired *Aspergillus* pneumonia can all persist beyond 3 months and sometimes it it helpful to re-categorise such patients as having CPA in terms of ongoing management [1••, 17]. Besides diagnosing CPA, co-infection such as NTM/TB (these infections can occur concurrently), other bacterial infections and endemic fungal infections including histoplasmosis and coccidioidomycosis should be excluded [16•, 19].

Besides the main diagnostic criteria for CPA, there are other findings that can distinguish other disorders from CPA. Chest radiographs remain the first imaging modality for the suspicion and diagnosis of CPA, but CT of the thorax offers much additional value, especially in identifying aspergillomas. Chest radiographs without cavitation or pleural thickening had a 100% negative predictive value for chronic cavitary pulmonary aspergillosis in the African setting [20••], such work has yet to be done rigorously in high-income countries. The imaging features of CPA result from a combination of the findings related to underlying lung disorders and changes secondary to Aspergillus infection itself, reflecting the chronic inflammatory and immune response to *Aspergillus* spp. [1••]. The presence of anti-*Aspergillus* antibodies differentiates between infected and colonised patients with a positive predictive value of 100% for detecting infection, but it does not distinguish which form of aspergillosis it is.

Clinical Symptoms

Aspergillosis is generally clinically silent with symptoms appearing late in the course of disease. There are many clinical symptoms that can be found in CPA patients such as weight loss, productive cough, haemoptysis, fever, sputum production, fatigue and shortness of breath. Fever is unusual and may suggest SAIA (subacute invasive aspergillosis) or an alternative infection such as tuberculosis. CPA patients must have one or more symptoms with a duration at least for 3 months and absence of overt immunosuppression [2, 8, 21, 22]. These symptoms are not specific, hence the need for additional data to confirm or refute the diagnosis. Some patients have very few symptoms but do have progressive disease, so reliance on symptoms alone to decide on therapy is sub-optimal clinical practice.

Table 1 Description of different forms of CPA [1••, 17, 18]

Term	Definition
Simple aspergilloma	Fungal ball inside single pulmonary cavity with minor or no symptoms and no radiological progression over > 3 months in a non-immunocompromised patient. Serological or microbiological evidence of *Aspergillus* spp. present in the material.
Aspergillus nodule	An unusual form of CPA which may mimic tuberculoma, carcinoma of the lung, coccidioidal nodule and other diagnoses. One or more nodules are present, with or without cavitation, without tissue invasion, although necrosis is frequent. They can only be definitively diagnosed on histology.
Chronic cavitary pulmonary aspergillosis (CCPA)	Significant pulmonary and/or systemic symptoms and overt radiological progression (new cavities, increasing pericavitary infiltrates or increasing fibrosis) over at least 3 months. One or more pulmonary cavities can be found (with either a thin or thick wall) possibly containing one or more aspergillomas or irregular intraluminal material.
Chronic fibrosing pulmonary aspergillosis (CFPA) ‘Destroyed lung’	Major loss of lung function because of severe fibrotic destruction of at least two lobes of lung complicating CCPA. Usually, the fibrosis is manifest as consolidation, but large cavities with surrounding fibrosis may be seen.
Community acquired pulmonary aspergillosis	Onset of respiratory symptoms within 2 weeks with hyphal elements on lung parenchyma and culture positive for *Aspergillus* spp. on non-immunocompromised patients. May follow massive exposure. Probably rare. Antibody seroconversion may allow diagnosis.
Subacute invasive aspergillosis/chronic necrotising/semi-invasive (SAIA)	Variable radiological features including cavitation, nodules, progressive consolidation with ‘abscess formation’ occurring over 1–3 months that usually present in mildly immunocompromised patients and lead to invasive aspergillosis. Biopsy shows hyphae in invading lung tissue and microbiological investigations reflect those in invasive aspergillosis, notably positive aspergillus galactomannan antigen in the blood (or respiratory fluids).

Radiological Findings

The unique radiological appearance that Deve described in 1938 as ‘mycotic lung tumor’ or what we now called a fungal ball or aspergilloma [23] is highly distinctive. Aspergilloma usually can be found in upper lobe, with air crescent and pleural thickening around it (see Fig. 1). Radiological features of CPA vary, but most authorities require at least one of two radiological appearances [1••]. First is a cavitary lesion with paracavitary fibrosis. Second is a new or expanding cavity on serial imaging [8, 22]. One or more pulmonary cavities with paracavitary infiltrates, including pleural thickening or parenchymal infiltrates, were the most common CT abnormalities detected [22]. Progression was usually characterised by coalescence of cavities to form larger ones, or fibrosis [8].

There are other manifestations of CPA that present as single or multiple nodule(s) without cavitation; this presentation can be missed as these findings are often associated with negative *Aspergillus* IgG or precipitins, or no biopsy to secure the diagnosis. Growing usage of computed tomography (CT) scanning has increased the chance of finding non-calcified pulmonary nodules, especially with lung cancer screening [24]. Many nodules found on lung cancer screening are resected or if impossible will be treated with radiotherapy which is not ideal for CPA [25••]. CCPA if left untreated may progress to CFPA. The appearance is no different from other forms of fibrosis but cavitation and fungal balls may be seen around the areas of fibrosis, and progression over time may provide a clue to the aetiology of the fibrosis.

Laboratory Investigations

Confirmation of CPA by laboratory testing normally consists of either detecting an elevated *Aspergillus* precipitin titre or an abnormally high *Aspergillus* IgG level, the precise cutoff depending on the actual test used. Conidia of *Aspergillus* are covered in rodlet proteins which are ‘invisible’ to the immune system. Once the conidia swell and germinate, multiple antigenic proteins are released and generate inflammatory responses, including dendritic cell and T cell activation [26]. The cell wall of *A. fumigatus* is mainly composed of polysaccharides, with many linked to proteins as glycoproteins. The majority of the antigens secreted by the fungus during its *in vitro* and *in vivo* growth are in or adherent to the cell wall and stimulate antibody production. [27].

Serological Tests

Immunodiffusion (ID) is one of the earliest methods to detect antibodies in serum samples [28], also known as agar gel double diffusion, precipitation in gel or the precipitin test. The principle of this test is the formation of an antigen-antibody complex that is visible in an agar gel. As the name implies, both antigen and antibody diffuse through the gel. It is used extensively to check antisera for the presence and specificity of antibodies to a particular antigen or antigens [29]. It was first used in the diagnosis of CPA in 1964 when the presence of specific precipitins in patients’ serum against antigenic extracts of cultures of *A. fumigatus* were considered

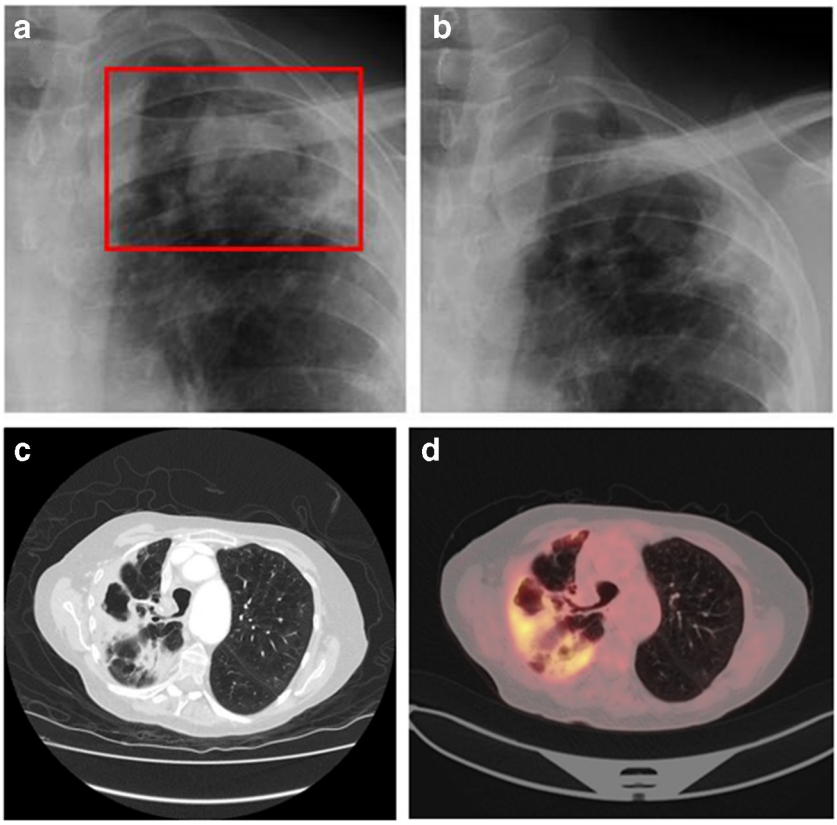

Fig. 1 **a** An example of a left upper lobe cavity containing a fungal ball (aspergilloma) with an air crescent above it (1:00 to 3:00) and pleural thickening together with some other parenchymal infiltrates separate from the area of aspergillosis. **b** Chest x-ray from same patient in panel **a**, the fungal ball has gone (it was coughed up) leaving only pleural thickening and slightly more peri-cavitary infiltrate infero-laterally, most consistent with progressive CPA, even though the fungal ball has gone. **c** Typical appearance of CCPA on CT scan with a thick walled cavity in the right upper lobe, probably containing a very small amount of fungal material laterally with marked pleural thickening, and an area of cavitating consolidation posteriorly, on a background of marked emphysema. **d** The corresponding PET scan of panel **c** that shows how much inflammation there is in the areas of pleural thickening and consolidation, showing up the small area of cavitation within the area of consolidation. (source: National Aspergillosis Centre)

to have value in the diagnosis of pulmonary aspergillosis [30]. Although no complex equipment is needed, the method takes a couple of days to perform, is labour intensive and relies on subjective interpretation of results [31].

Counterimmunoelectrophoresis (CIE) is a modification of immunodiffusion. This method produce precipitin lines faster than immunodiffusion because it applies electrical fields to make the movement process of antigen-antibody faster [32]. CIE is preferred over ID in most laboratories not only for its increase rapidity but also because it can reduce the amount of reference sera and antigen extract needed for testing [33]. Although the sensitivity of CIE is similar to ID [34], some laboratories still use ID even now, as it has been an established method for > 40 years. This is because there are a number of troublesome disadvantages of CIE. Since reagents migrate as finite zones, with essentially no concentration gradients, precipitins will form only if antigen:antibody equivalence becomes established in the area of the gel between the wells. Virtually, no time is available for second-stage antigen:antibody complexes to accumulate. Nowadays, both assays are less sensitive than other antibody detection methods and slower.

Enzyme-linked immunosorbent assay (ELISA) has been used to detect antibody in aspergillosis patients for decades [35]. There are several different types of ELISA, but the most commonly known are competitive and sandwich type. In competitive ELISA, labelled antigen competes with unlabelled antigen for binding to a limited quantity of antibody. In sandwich ELISA, antigen reacts with excess solid-phase antibody, and the bound antigen is treated with excess labelled antibody [36].

Most ELISA for CPA diagnosis must be performed manually, but it can be fully automated too [37, 38, 39•]. Bio-Rad (Marnes-la-Coquette, France) and Bordier Affinity Products SA (Crissier, Switzerland) are examples of diagnostics companies that produce ELISA assays for detecting *Aspergillus* antibody IgG. Phadia ImmunoCAP (Thermo Fisher Scientific Inc., Uppsala, Sweden) and Immulite (Siemens AG, Munich, Germany) are an automated variant of ELISA that being used in many large hospital laboratories. Both of them use a similar

principle where *Aspergillus* antigen that is coupled to the solid phase will react with specific IgG antibodies in the patient sample. Enzyme labelled antibodies against IgG are added after washing away non-specific IgG antibodies. The bound complex formed is then incubated with a developing agent before stopping the reaction and measurement of fluorescence [37]. The performance of ELISA tests that already available commercially are varied, but the recommended assay preferably have 90% sensitivity and 85% specificity [40]. The sensitivity and specificity of the Bio-Rad, Bordier, ImmunoCAP and Immulite were 86–97.4% and 89.6–98.2%, 97.4 and 90.3%, 83.8–97.9% and 98%, 92.9–96% and 98–99.3%, respectively [37, 38, 39•, 41–43].

Lateral Flow Device

Lateral flow devices (LFD) are inexpensive, simple to use disposable devices that can identify a substance in a clinical sample effortlessly. It has developed from the principle of latex agglutination assays that were used as a test to diagnose rheumatoid arthritis more than half century ago [44]. Serological lateral flow tests then emerged 30 years ago [45]. A good example is the well-known human pregnancy LFD that was derived from the development of a hCG beta–subunit radioimmunoassay [46]. It did not take a long time for commercial rapid lateral flow tests to be used in other fields besides clinical diagnostics particularly in veterinary, food, environmental applications, biodefense and drug abuse [47].

LFD strips contain four main components as shown in Fig. 2. The sample application pad is the location where the sample is applied. It is made of cellulose and/or glass fibre. The conjugate pad is the place where labelled biorecognition molecules are dispensed. The nitrocellulose membrane is where test and control lines are located. An adsorbent pad is at the end of the strip that maintains the flow rate of the liquid and stops backflow of the sample [48]. An LFD test is now available for *Aspergillus* IgG detection.

The principle is to let sample flow in one direction which will bind to test line if it contains the protein of interest and the result can be interpreted with a naked eye. Some tests have a reader which is slightly more sensitive and allows the result to be captured for laboratory reporting using format reporting procedures. The only commercially available lateral flow assay available for detecting *Aspergillus-specific* antibodies currently is the LDBio *Aspergillus* ICT lateral flow assay. This assay has 88.9–91.6% sensitivity and 96.3–98% specificity that is comparable with ELISA tests that already being used widely above [49•, 50•]. This assay has not been used quantitatively.

Serology Test in Resource-Constrained Settings

Based on the epidemiology data, most of CPA cases are most probably located in low-middle income countries [9]. The current guideline for the diagnosis of CPA includes *Aspergillus* IgG detection to prove microbiological evidence of *Aspergillus* spp. infection in resource constrained settings [40]. Although many serology tests for CPA available now, not all healthcare systems have access to them [51]. As described before, many CPA cases in resource-limited settings are left untreated because of the lack of diagnostics. The most suitable solution for this problem is lateral flow device as point-of-care test. Lateral flow device meets the World Health Organization's ASSURED criteria for being affordable, sensitive, specific, user-friendly, rapid and robust, equipment free, and deliverable to end-users [52].

Other Tests

Direct microscopy is a well-established diagnostic test that is still used today to identify fungi, especially filamentous fungi in clinical samples. This method is used for fungal diagnosis because the morphology of many fungi is quite different. The presence of fungi in samples is usually determined by direct microscopy of the clinical specimen on a microscopic glass slide with 10% of potassium hydroxide (KOH) for wet mount examination or enhanced by brightener agent like Calcoflour white. Direct microscopy in the diagnosis CPA can help the laboratory to decide which culture media and procedures to use if fungal hyphae are seen on sample [53].

Fungal culture and identification can detect fungi from many kinds of samples with low to medium sensitivity for all fungi (except *Pneumocystis* and *Microsporidia* which are not readily culturable), and have 3–14 days turnaround time [54]. *Aspergillus* species are ubiquitous saprophytic fungi that are distributed widely in the environment. From hundreds of known species, less than 40 cause disease in humans. The most common pathogens are *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger* which are also the most commonly reported species of *Aspergillus* in nature [55]. The most common cause of CPA is *A. fumigatus*, although *A. flavus* and *A. niger* also can cause CPA [2, 56]. The presence of *A. fumigatus* in a bronchoscopic specimen is far more common in infection compared to colonisation and is consistent with infection, including CPA. A recent study showed that high volume culture has good sensitivity compared to conventional culture; it also increase the recovery of cryptic species from sample [57, 58].

Polymerase chain reaction (PCR) is a molecular method that has a higher sensitivity since it allows the detection of small amounts of deoxyribonucleic acid (DNA) in clinical samples by amplification. Extraction of DNA from hyphae

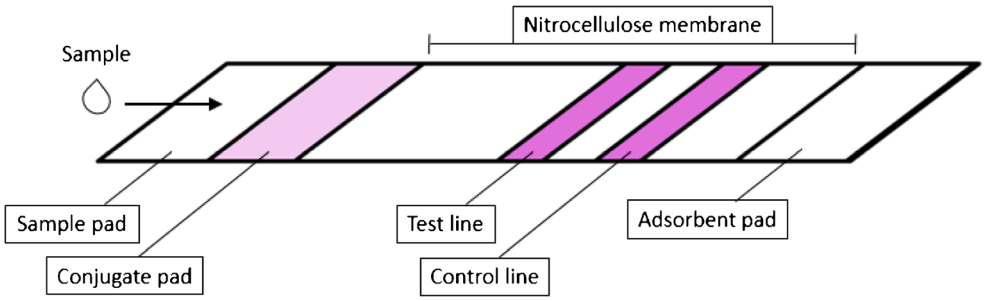

Fig. 2 Schematic diagram of lateral flow device strip. Sample will move from sample pad to adsorbent pad. The direct format gives colour appearance at both of test and control lines when a sample with target analytes is applied

and/or conidia is an important step for the detection of fungi using PCR. The structure of the fungal cell wall is highly complex and no single universal fungal DNA extraction method has been adopted [59]. There are various studies describing PCR for the detection of *Aspergillus* spp. in the context of CPA; the average sensitivity and specificity is around 80% [60, 61, 62•, 63].

Differential Diagnosis of Positive Aspergillus Antibody and Aspergillosis Syndromes

Invasive aspergillosis is a life-threatening disease that commonly occurs mostly in immunocompromised person, such as people with acute leukaemia, stem cell and other transplants (especially lung) [64, 65]. *Aspergillus* IgG antibody can be detected in invasive aspergillosis although it is less important than *Aspergillus* antigen detection [65, 66]. *Aspergillus* bronchitis is chronic superficial infection of the lower airways (characterised by bronchial cast containing mucus and mycelia of any *Aspergillus* pathogenic species) that commonly occurred in non-immunocompromised patients. *Aspergillus*-specific IgG may be positive or negative in *Aspergillus* bronchitis cases [67]. *Aspergillus* rhinosinusitis characterised by a mass within one or more paranasal sinuses with evidence of invasion of contiguous structures such as the base of the skull, orbit and brain [68]. Species specific precipitating antibodies are present in > 90% of cases in chronic invasive disease and correlates well with disease progression, usually to *A. flavus* [69, 70]. Similarly, precipitating antibodies to *Aspergillus* spp. are present in around two-thirds of cases of chronic granulomatous sinusitis, especially a positive IgG to *A. flavus* [71–73]. Chronic pulmonary histoplasmosis presents in a similar way to CPA with cough, weight loss, fever, chest pain, fatigue and haemoptysis the most frequent symptoms in decreasing order of frequency. *Histoplasma* antibody is usually detectable although there is possible cross-reaction with *Aspergillus* (assuming not a dual infection) [19, 74]. Coccidioidomycosis may present with acute pneumonia, nodules or chronic cavitation. Sputum culture is usually positive in cavitary disease. Anti-coccidioidal antibody detection is important for diagnosis with probably little cross-reaction with *Aspergillus* [75–77]. Fungus ball as a result of superinfection with other fungi including *Aspergillus* sp. can be formed when cavities are present.

Conclusion

CPA has been neglected for a long time despite its high morbidity and mortality, because it has similar clinical symptoms and radiological appearance to other lung diseases. Laboratory diagnostics, especially *Aspergillus*-specific IgG assays are very valuable in the diagnosis of CPA. There are many different options of serological tests available, including ELISA, with differing performance characteristics [37]. The ELISA capability to measure IgG titre quantitatively is important to monitor CPA progress and treatment [78, 79]. The lateral flow assay is an option as a simple diagnostic test for CPA, particularly in a resource-constrained setting. It is inexpensive and simple to run and accessible to many users. The current lateral flow assay for the detection *Aspergillus*-specific IgG that are commercially available can only produce a qualitative result, but it has potency to become a semi-quantitative or quantitative test [80, 81]. We hope that the availability of new tests will translate into the earlier diagnosis of CPA allowing clinicians to start treatment earlier resulting in a better prognosis. People from resource-constrained settings where probably have higher CPA prevalence will get more benefit from point-of-care tests such as a lateral flow device.

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Compliance with Ethical Standards

Conflict of Interest David W. Denning 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, Zambon, iCo Therapeutics, Roivant, Biosergen and Fujifilm. In the last 3 years, he has been paid for talks on behalf of Dynamiker, 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 and the British Society for Medical Mycology Standards of Care committee. Malcolm D. Richardson has received personal fees for talks and consultancy from

Gilead Sciences Europe, Pfizer, MSD and Basilea, Dynamiker, Mylan and Lupin Pharmaceuticals. He is currently a member of the European Society for Clinical Microbiology and Infectious Diseases Aspergillosis and Rare Moulds Guidelines groups. Bayu Wilopo declares no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.•• Denning DW, Cadranel J, Beigelman-Aubry C, Ader F, Chakrabarti A, Blot S, et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management Task Force Report ESCMID/ERS Guidelines. Eur Respir J. 2016;47:45–68. https://doi.org/10.1183/13993003.00583-2015 **The world's first guidelines for the diagnosis and treatment of chronic pulmonary aspergillosis, with many illustrative radiology examples**.
2. Ohba H, Miwa S, Shirai M, Kanai M, Eifuku T, Suda T, et al. Clinical characteristics and prognosis of chronic pulmonary aspergillosis. Respir Med. 2012;106:724–9. https://doi.org/10.1016/j.rmed.2012.01.014.
3. Bennett JH. XVII. On the parasitic vegetable structures found growing in living animals. Trans R Soc Edinburgh. 1844;15:277–94. https://doi.org/10.1017/S0080456800029963.
4. Kelmenson VA. Treatment of pulmonary aspergillosis. Dis Chest. 1959;36:442–3. https://doi.org/10.1378/chest.36.4.442.
5. Research Committee of the British Tuberculosis Association. *Aspergillus* in persistent lung cavities after tuberculosis: a report from the Research Committee of the British Tuberculosis Association. Tubercle. 1968;49:1–11. https://doi.org/10.1016/S0041-3879(68)80002-9.
6. Jewkes J, Kay PH, Paneth M, Citron KM. Pulmonary aspergilloma: analysis of prognosis in relation to symptoms and treatment. Thorax. 1983;38:572–8. https://doi.org/10.1016/j.jtcvs.2009.01.019.
7. Campbell JH, Winter JH, Richardson MD, Shankland GS, Banham SW. Treatment of pulmonary aspergilloma with itraconazole. Thorax. 1991;46:839–41. https://doi.org/10.1136/THX.46.11.839.
8. 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. https://doi.org/10.1086/376526.
9. Denning D, Pleuvry A, Cole D. Global burden of chronic pulmonary aspergillosis as a sequel to pulmonary tuberculosis. Bull World Health Organ. 2011;89:864–72. https://doi.org/10.2471/BLT.11.089441.
10. Chakaya J, Kirenga B, Getahun H. Long term complications after completion of pulmonary tuberculosis treatment: a quest for a public health approach. J Clin Tuberc Other Mycobact Dis. 2016;3:10–2. https://doi.org/10.1016/j.jctube.2016.03.001.
11. Denning DW. The ambitious "95-95 by 2025" roadmap for the diagnosis and management of fungal diseases. Thorax. 2015;70:613–4. https://doi.org/10.1136/thoraxjnl-2015-207305.
12. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012;4:1–9. https://doi.org/10.1126/scitranslmed.3004404.
13. Bongomin F, Harris C, Foden P, Kosmidis C, Denning DW, Bongomin F, et al. Innate and adaptive immune defects in chronic pulmonary aspergillosis. J Fungi. 2017;3:26. https://doi.org/10.3390/jof3020026.
14. Kosmidis C, Powell G, Borrow R, Morris J, Alachkar H, Denning DW. Response to pneumococcal polysaccharide vaccination in patients with chronic and allergic aspergillosis. Vaccine. 2015;33:7271–5. https://doi.org/10.1016/j.vaccine.2015.10.114.
15. Doffinger R, Harris C, Lear S, Newton P, Alachkar H, Kumararatne D, Barcenas-Morales G, Denning D. Reduced gamma interferon (gIFN) production in chronic pulmonary aspergillosis (CPA). 5th Advances Against Aspergillus Conference; 2012.
16.• Lowes D, Al-Shair K, Newton PJ, Morris J, Harris C, Rautemaa-Richardson R, et al. Predictors of mortality in chronic pulmonary aspergillosis. Eur Respir J. 2017;49:1601062. https://doi.org/10.1183/13993003.01062-2016 **An overview of CPA mortality highlighting that co-infection with NTM is a poor prognostic feature**.
17. Denning DW. Community acquired *Aspergillus* pneumonia and/or pneumonitis. *Aspergillus* & Aspergillosis Website. 2015. https://www.aspergillus.org.uk/content/community-acquired-aspergillus-pneumonia-andor-pneumonitis. Accessed 7 Jul 2019.
18. Farid S, Mohamed S, Devbhandari M, Kneale M, Richardson M, Soon SY, 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. https://doi.org/10.1186/1749-8090-8-180.
19. Unis G, Severo LC. Chronic pulmonary histoplasmosis mimicking tuberculosis. J Bras Pneumol. 2005;31:318–42.
20.•• Page ID, Byanyima R, Hosmane S, Onyachi N, Opira C, Richardson M, et al. Chronic pulmonary aspergillosis commonly complicates treated pulmonary tuberculosis with residual cavitation. Eur Respir J. 2019;53:1801184. https://doi.org/10.1183/13993003.01184-2018 **Key study showing that over 2 years follow up the annual rate of CPA development in those with a residual cavity 2-7 years after TB was 6.5%, regardless of HIV status and only 0.2% in those without a cavity**.
21. Kohno S, Izumikawa K, Ogawa K, Kurashima A, Okimoto N, Amitani R, et al. Intravenous micafungin versus voriconazole for chronic pulmonary aspergillosis: a multicenter trial in Japan. J Infect. 2010;61:410–8. https://doi.org/10.1016/j.jinf.2010.08.005.
22. Jhun BW, Jeon K, Eom JS, Lee JH, Suh GY, Kwon OJ, et al. Clinical characteristics and treatment outcomes of chronic pulmonary aspergillosis. Med Mycol. 2013;51:811–7. https://doi.org/10.3109/13693786.2013.806826.
23. Deve F. Une nouvelle forme anatomo-radiologique de mycose pulmonaire primitive, Le mega-mycetome intrabronchectasique. Arch Med Chir Appl Resp. 1938;13:337–61.
24. van Klaveren RJ, Oudkerk M, Prokop M, Scholten ET, Nackaerts K, Vernhout R, et al. Management of lung nodules detected by volume CT scanning. N Engl J Med. 2009;361:2221–9. https://doi.org/10.1056/NEJMoa0906085.
25.•• Muldoon EG, Sharman A, Page I, Bishop P, Denning DW. *Aspergillus* nodules; another presentation of chronic pulmonary aspergillosis. BMC Pulm Med. 2016;16:123. https://doi.org/10.

1186/s12890-016-0276-3 **First comprehensive report of pulmonary nodules as a subset of CPA**.

26. Green BJ, Mitakakis TZ, Tovey ER. Allergen detection from 11 fungal species before and after germination. J Allergy Clin Immunol. 2003;111:285–9. https://doi.org/10.1067/mai.2003.57.
27. Latge JP, Debeaupuis JP, Sarfati J, Diaquin M, Paris S. Cell wall antigens in *Aspergillus fumigatus*. Arch Med Res. 1993;24:269–74.
28. Ouchterlony O. Antigen- Antibody Reactions in Gels. Acta path microbiol scand. 1953;32:230–40. https://doi.org/10.1111/j.1600-0463.2007.apm_678a.x.
29. Bailey GS. Ouchterlony double immunodiffusion. In: Protein Protocol Handbook. Totowa: Humana Press; 1996. p. 749–52.
30. Longbottom JL, Pepys J. Pulmonary aspergillosis: diagnostic and immunological significance of antigens and C-substance in *Aspergillus fumigatus*. J Pathol Bacteriol. 1964;88:141–51. https://doi.org/10.1002/path.1700880119.
31. Page ID, Richardson M, Denning DW. Antibody testing in aspergillosis–quo vadis? Med Mycol. 2015;53:417–39. https://doi.org/10.1093/mmy/myv020.
32. Dee TH. Detection of *Aspergillus fumigatus* serum precipitins by counterimmunoelectrophoresis. J Clin Microbiol. 1975;2:482–5.
33. Bernstein RM, Bunn CC, Hughes GRV. Identification of antibodies to acidic antigens by counterimmunoelectrophoresis. Ann Rheum Dis. 1982;41:554–5. https://doi.org/10.1136/ard.41.5.554.
34. Malo JL, Longbottom J, Mitchell J, Hawkins R, Pepys J. Studies in chronic allergic bronchopulmonary aspergillosis. 3. Immunological findings. Thorax. 1977;32:269–74. https://doi.org/10.1136/thx.32.3.269.
35. Richardson M, Stubbins JM, Warnock D. Rapid enzyme-linked immunosorbent assay (ELISA) for *Aspergillus fumigatus* antibodies. J Clin Pathol. 1982;35:1134–7. https://doi.org/10.1136/jcp.35.10.1134.
36. Wisdom GB. Enzyme-immunoassay. Clin Chem. 1976;228:1243–55.
37. Page ID, Richardson MD, Denning DW. Comparison of six *Aspergillus*-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis (CPA). J Infect. 2016;72:240–9. https://doi.org/10.1016/j.jinf.2015.11.003.
38. 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. https://doi.org/10.1111/1469-0691.12133.
39.• Dumollard C, Bailly S, Perriot S, Brenier-Pinchart MP, Saint-Raymond C, Camara B, et al. Prospective evaluation of a new *Aspergillus* IgG enzyme immunoassay kit for diagnosis of chronic and allergic pulmonary aspergillosis. J Clin Microbiol. 2016;54:1236–42. https://doi.org/10.1128/JCM.03261-15 **Description of a new Aspergillus IgG ELISA with good performance**.
40. Denning DW, Page ID, Chakaya J, et al. Case definition of chronic pulmonary aspergillosis in resource-constrained settings. Emerg Infect Dis. 2018;24:e1–e13. https://doi.org/10.3201/eid2408.171312.
41. Page ID, Richardson MD, Denning DW. Siemens immulite *Aspergillus*- specific IgG assay for chronic pulmonary aspergillosis diagnosis. Med Mycol. 2019;57:300–7. https://doi.org/10.1093/mmy/myy024.
42. 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;50:762–5. https://doi.org/10.1128/JCM.01257-11.
43. Page ID, Baxter C, Hennequin C, Richardson MD, Van Hoeyveld E, Van Toorenenbergen AW, et al. Receiver operating characteristic curve analysis of four *Aspergillus*-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis. Diagnostic Microbiol Infect Dis. 2018;91:47–51. https://doi.org/10.1016/j.diagmicrobio.2018.01.001.
44. Singer JM, Plotz CM. The latex fixation test. Am J Med. 1956;21:888–92. https://doi.org/10.1016/0002-9343(56)90103-6.
45. Hawkes R, Niday E, Gordon J. A Dot-Immunobinding assay for monoclonal and other antibodies. Anal Biochem. 1982;119:142–7.
46. Seppälä M, Ranta T, Tontti K, Stenman UH, Chard T. Use of a rapid hCG-beta-subunit radioimmunoassay in acute gynaecological emergencies. Lancet. 1980;315:165–6.
47. Yetisen AK, Akram MS, Lowe CR. Paper-based microfluidic point-of-care diagnostic devices. Lab Chip. 2013;13:2210–51. https://doi.org/10.1039/c3lc50169h.
48. Sajid M, Kawde A-N, Daud M. Designs, formats and applications of lateral flow assay: A literature review. J Saudi Chem Soc. 2015;19:689–705. https://doi.org/10.1016/j.jscs.2014.09.001.
49.• Thérèse Coste A, De Carolis E, Araujo R, et al. Multicenter evaluation of a novel immunochromatographic test for anti-aspergillus IgG detection. Front Cell Infect Microbiol. 2019;2019. https://doi.org/10.3389/fcimb.2019.00012 **The first description of a lateral flow assay for Aspergillus IgG and IgM antibody detection**.
50.• Stucky Hunter E, Richardson MD, Denning DW. Evaluation of LD Bio *Aspergillus* ICT lateral flow assay for IgG and IgM antibody detection in chronic pulmonary aspergillosis. J Clin Microbiol. 2019. https://doi.org/10.1128/JCM.00538-19 **Detailed diagnostic performance characteristics for the new LFD for CPA**.
51. Richardson MD, Page ID. *Aspergillus* serology: have we arrived yet? Med Mycol. 2017;55:48–55. https://doi.org/10.1093/mmy/myw116.
52. Klauser JD, Vijayan TCT. Sensitivity and specificity of a new cryptococcal antigen lateral flow assay in serum and cerebrospinal fluid. MLO Med Lab Obs. 2013;45:16–20.
53. Hedayati MT, Azimi Y, Droudinia A, Mousavi B, Khalilian A, Hedayati N, et al. Prevalence of chronic pulmonary aspergillosis in patients with tuberculosis from Iran. Eur J Clin Microbiol Infect Dis. 2015;34:1759–65. https://doi.org/10.1007/s10096-015-2409-7.
54. GAFFI. Fungal Disease Diagnosis and portfolio of diagnostic tests in Mycology Reference Laboratories. In: ambitious '95–95 by 2025' roadmap diagnosis Manag. fungal Dis. 2015; http://www.gaffi.org/wp-content/uploads/Appendices-4-V3.pdf.
55. Klich MA. Identification of clinically relevant aspergilli. Med Mycol. 2006;44:127–31. https://doi.org/10.1080/13693780600796546.
56. Carlos Severo L, Resin Geyer G, da Silva Porto N, Bernardes Wagner M, Thomaz Londero A. Pulmonary *Aspergillus niger* intraca-vitary colonization. Report of 23 cases and a review of the literature. Rev Iberoam Micol. 1997;14:104–10.
57. Vergidis P, Moore C, Rautemaa-Richardson R, Richardson M. High-volume sputum culture for the diagnosis of pulmonary aspergillosis. Open Forum Infect Diseases. 2017:S609–9.
58. Bongomin F, Moore CB, Masania R, Rowbotham E, Alastruey-Izquierdo A, Frazer LN, et al. Sequence analysis of isolates of *Aspergillus* from patients with chronic and allergic aspergillosis reveals a spectrum of cryptic species. Future Microbiol. 2018. https://doi.org/10.2217/fmb-2018-0178.
59. Karakousis A, Tan L, Ellis D, Alexiou H, Wormald PJ. An assessment of the efficiency of fungal DNA extraction methods for maximizing the detection of medically important fungi using PCR. J Microbiol Methods. 2006;65:38–48. https://doi.org/10.1016/j.mimet.2005.06.008.
60. Imbert S, Meyer I, Palous M, Brossas JY, Uzunov M, Touafek F, et al. *Aspergillus* PCR in bronchoalveolar lavage fluid for the diagnosis and prognosis of aspergillosis in patients with hematological and non-hematological conditions. Front Microbiol. 2018;9:1–9. https://doi.org/10.3389/fmicb.2018.01877.

61. Urabe N, Sakamoto S, Sano G, Suzuki J, Hebisawa A, Nakamura Y, et al. Usefulness of two Aspergillus PCR assays and *Aspergillus* galactomannan and β-D-glucan testing of bronchoalveolar lavage fluid for diagnosis of chronic pulmonary aspergillosis. J Clin Microbiol. 2017;55:1738–46. https://doi.org/10.1128/JCM.02497-16.
62.• Fayemiwo S, Moore CB, Foden P, Denning DW, Richardson MD. Comparative performance of *Aspergillus* galactomannan ELISA and PCR in sputum from patients with ABPA and CPA. J Microbiol Methods. 2017;140:32–9. https://doi.org/10.1016/j.mimet.2017.06.016 **The limited value of galactomannan detection in sputum for CPA and ABPA**.
63. Denning DW, Park S, Lass-Florl C, Fraczek MG, Kirwan M, Gore R, et al. High-frequency triazole resistance found in nonculturable *aspergillus fumigatus* from lungs of patients with chronic fungal disease. Clin Infect Dis. 2011;52:1123–9. https://doi.org/10.1093/cid/cir179.
64. Matthews R, Burnie JP, Fox A, Tabaqchali S. Immunoblot analysis of serological responses in invasive aspergillosis. J Clin Pathol. 1985;38:1300–3. https://doi.org/10.1136/jcp.38.11.1300.
65. Herbrecht R, Letscher-Bru V, Oprea C, Lioure B, Waller J, Campos F, et al. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. J Clin Oncol. 2002;20:1898–906. https://doi.org/10.1200/JCO.2002.07.004.
66. Richardson M, Page I. Role of Serological Tests in the Diagnosis of Mold Infections. Curr Fungal Infect Rep. 2018;12:127–36. https://doi.org/10.1007/s12281-018-0321-1.
67. Prats JAGG, Denning DW. Aspergillus bronchitis. *Aspergillus* & Aspergillosis Website. 2016. https://www.aspergillus.org.uk/content/aspergillus-bronchitis. Accessed 5 Jul 2019
68. Goncer IR, Denning DW. Chronic *Aspergillus* sinusitis. Aspergillus & Aspergillosis Website. 2015. https://www.aspergillus.org.uk/content/chronic-aspergillus-sinusitis. Accessed 7 Jul 2019
69. Hedayati MT, Bahoosh M, Kasiri A, Ghasemi M, Motahhari SJ, Poormosa R. Prevalence of fungal rhinosinusitis among patients with chronic rhinosinusitis from Iran. J Mycol Med. 2010;20:298–303. https://doi.org/10.1016/j.mycmed.2010.09.002.
70. Chakrabarti A, Sharma SC, Chander J. Epidemiology and pathogenesis of paranasal sinus mycoses. Otolaryngol Neck Surg. 1992;107:745–50. https://doi.org/10.1177/019459988910700606.1.
71. Yagi HI, Gumaa SA, Shumo AI, Abdalla N, Gadir AA. Nasosinus aspergillosis in Sudanese patients: Clinical features, pathology, diagnosis, and treatment. J Otolaryngol. 1999;28:90–4. https://doi.org/10.1016/j.jaci.2012.05.050.
72. Currens J, Hutcheson PS, Slavin RG, Citardi MJ. Primary paranasal *Aspergillus* granuloma: case report and review of the literature. Am J Rhinol. 2002;16:165–8.
73. Hope WW, Walsh TJ, Denning DW. The invasive and saprophytic syndromes due to *Aspergillus* spp. Med Mycol. 2005;43:207–38. https://doi.org/10.1080/13693780400025179.
74. Wheat J, French ML, Kamel S, Tewari RP. Evaluation of cross-reactions in Histoplasma capsulatum serologic tests. J Clin Microbiol. 1986;23:493–9.
75. Galgiani JN, Ampel NM, Blair JE, Catanzaro A, Geertsma F, Hoover SE, et al. 2016 Infectious Diseases Society of America (IDSA) Clinical practice guideline for the treatment of coccidioidomycosis. Clin Infect Dis. 2016;63:e112–46. https://doi.org/10.1093/cid/ciw360.
76. Donovan FM, Zangeneh TT, Malo J, Galgiani JN. Top questions in the diagnosis and treatment of coccidioidomycosis. Open Forum Infect Dis. 2017;4:1–4. https://doi.org/10.1093/ofid/ofx197.
77. Jude CM, Nayak NB, Patel MK, Deshmukh M, Batra P. Pulmonary coccidioidomycosis: pictorial review of chest radiographic and CT findings. RadioGraphics. 2014;34:912–25. https://doi.org/10.1148/rg.344130134.
78. Felton TW, Baxter C, Moore CB, Roberts SA, Hope WW, Denning DW. Efficacy and safety of posaconazole for chronic pulmonary aspergillosis. Clin Infect Dis. 2010;51:1383–91. https://doi.org/10.1086/657306.
79. Bongomin F, Harris C, Hayes G, Kosmidis C, Denning DW. Twelve month outcomes of 206 patients with chronic pulmonary aspergillosis. PLoS One. 2018;13:e0193732. https://doi.org/10.1371/journal.pone.0193732.
80. Zangheri M, Cevenini L, Anfossi L, Baggiani C, Simoni P, Di Nardo F, et al. A simple and compact smartphone accessory for quantitative chemiluminescence-based lateral flow immunoassay for salivary cortisol detection. Biosens Bioelectron. 2015;64:63–8. https://doi.org/10.1016/j.bios.2014.08.048.
81. Yin HY, Chu PT, Tsai WC, Wen HW. Development of a barcode-style lateral flow immunoassay for the rapid semi-quantification of gliadin in foods. Food Chem. 2016;192:934–42. https://doi.org/10.1016/j.foodchem.2015.06.112.