E EA CH A C E A

Prospective study of the serum *A* - specific IgG, IgA and IgM assays for chronic pulmonary aspergillosis diagnosis

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A

Background: Chronic pulmonary aspergillosis (CPA) is an underdiagnosed and misdiagnosed disease and now increasingly recognised. However, the diagnosis of CPA remains challenging. In this study, we aimed to investigate the diagnostic values of serum *Aspergillus*-specific IgG, IgA and IgM antibodies in patients with CPA.

Methods: The prospective study was performed at Chinese People's Liberation Army General Hospital in Beijing, from January 2017 to December 2017. Adult patients with lung lesions presented as cavity, nodule, mass, bronchiectasis or severe fibrotic destruction with at least two lobes in CT imaging were enrolled. One hundred healthy persons were also enrolled as additional controls. The serum levels of *Aspergillus*-specific IgG, IgA and IgM antibodies and galactomannan (GM) levels were measured simultaneously by plate ELISA kit.

Results:

B

Ch ic , a a e gi i (CPA) , a cc i i , c e e i di id a i h e-e i i g ch ic , a di ea e, e ecia h e eadi g a , c- , a , g da age , ch a , a , be c i (PTB), ch ic b , c i e , a di ea e (COPD), a d b chiec a i [1–4]. He ce CPA ca be ea i eg ec ed i diag ed d e i a di idi , g e i . I i e i a ed ha he e a e 3 i i a- ie i h CPA d ide a d he 5- ea , i a a e f CPA i 60% e e he ea ed [5]. I Chi a, he e i- a ed a , a CPA ca e af e PTB a e e ha 67,000, b f he ca e a e , diag ed d e ac f efficie diag ic e h d i , i e ac ice [1]. The ef e, CPA ha a high bidi a d a i .

CPA diag i e , i e a c bi a i f cha ac e i ic c i ica a d adi gica fi di g ge he i h de- a i f *Aspergillus* i fec i i h g h f *Aspergillus* . c , e he e e ce f e e a ed e e f *Aspergillus* a ib d . Bi i he f i h e i h d e b i , e a di g i ca i a di ea e a ch ic i f a i a d fib i i ica ee [6]. O he he ha d, he c i ica a d adi- gica fi di g a e f e ecific d e he c- e i f a ie ' bac g , d , a d he e i i i f *Aspergillus* . c , e f b ch a e a a age f , id (BALF) , , i e [7]. The e i i i a d ecifici f e , ga ac a a *Aspergillus* a ige (GM) a a i a be 43.3 a d 80.8%, e ec i e [8]. C e , he diag i f CPA i cha e gi g.

I 2016 he I fec i , Di ea e S cie f A e ica (IDSA) a d e a a e he E a ea S cie f C i ica Mic bi g a d I fec i , Di ea e (ESCMID) i c- e a i i h he E a ea Re i a S cie (ERS) , b i hed g ide i e f he diag i f CPA [9, 10]. B h g ide i e ec e d ea , e e f *Aspergillus* IgG a ib d a a e diag ic e . H e e, he c- ff a , e f *A. fumigatus*- ecific IgG e ai be e ab i hed i Chi a a d a ie f each a a f a. Addi i a , he diag ic a , e f he he *As- pergillus*- ecific a ib die IgA a d IgM e ai , c ea [11].

I hi , d e ai ed e ab i h he i a c - ff a , e f e , *Aspergillus*- ecific IgG, IgA a d IgM a ib d a a a d i , a e , i e iga ed he diag- ic a , e f he e h ee a ib die f CPA diag i .

Study design

Thi , d , a f a ec i e , ice e c i ica ia (C i ica T ia . g : NCT03027089), a f Ja , a 2017 Dece be 2017 a Chi e e Pe e' Libe a i A Ge e a H i a , Bei i g, Chi a. A a ici a ided i e i f ed c e a d he , d a a ed b he e hic c i ee (S2017–015-01). I- c , i c i e ia e e a ie aged be ee 18 85 ea , i h , g e i e e i g a e e ca- i ie , d e , a e , b chiec a i e e e fib ic de , c i i h a ea be f , g ee c- , ed g a h (CT) i agi g. E c , i c i e ia e e a f :

(1) A if ga ea e f e 2 ee i hi 3 h f e e (2) I c e e ec d f c i ica da a e a i a i ; (3) P eg a c ac a i ; (4) Se e e i , c i ed a ie , i c , di g a) ece hi f e , e ia (<0.5 10^9 e , hi /L f >10 da); b) ecei f a a ge ei c e ce a- a , c) ged , e f c ic e id (e c , di g a ie i h a e gic b ch , a a e gi i) a a ea i i , d e f 0.3 g/ g/da f ed i e e , i a e f >3 ee , d) ea e i h he ec g- i ed T ce i , , e a , , ch a c c i e, TNF-a b c e , ecific c a a ib die (, ch a a e , , ab), , ce ide a a g e d , i g he a 90 da , e) i he i ed e e e i , deficie c (, ch a ch ic g a , a , di ea e e e e c bi ed i- , deficie c) a d f) ac , i ed i , deficie c- d e (AIDS).

Data and samples collection

D , i g he fi i i he de g a hic a d c i ica da a f a ie , i c , di g , de i g di ea e , ch ic , - a e ic , de ai f ea e a d e , f CT e e ec ded. Mea hi e, e , a e e e c ec ed a d i edia e ef ige a ed a 4 °C f *Aspergillus*- ecific IgG a ib d a a. The e id a e , a e e e f e a −80 °C f GM e , *Aspergillus*- ecific IgA a d IgM a ib d a a . C- e- ac i e ei (CRP) e , he e i he a a d diffe e ia hi e b d ce (WBC) c , , , , di ec ic c f f , gi a d acid fa baci i, a d f- ga , bac e ia a d c bac e ia c , e e e b ai ed. A a ie , de e b ch c a d b cha e- a a age f , id (BALF) a d e i e a b chia bi eci e e e b ai ed. The BAL ced , e i acc di g ATS d c , e [12]. N a a i e i i- i ed h , gh he b ch c e, i h a a , e ha i be ee 100 a d 300 a d di ided i h ee fi e a i , . I h e i h d , e a e, a e c- a e , a i a e a b ai ed, a d di ec ic c a d c , e e e , de a e i hi 5 da f h ia- i a i . C e , a e e e c ec ed f 100 hea h Chi e e e e i h a CT, CRP, a d WBC c , . A e , a e e e i edia e ef ige a ed a 4 °C f *Aspergillus*- ecific IgG a a . The e id a e , a e e e a f e a −80 °C f *Aspergil- lus*- ecific IgA a d IgM a a a d GM e .

Definition of CPA diagnosis

CPA diag i a de e i ed acc di g e i ed ESC-MID/ERS CPA diag i c i e ia i hich e , *Aspergil-lus-* ecific IgG e , GM a a e e c ide ed a ic bi gica e ide ce. Diag i f CPA e , i ed he f i g c i e ia: 1) e e a ha e bee e e f a ea 3 h (, , , c , gh, he i , fe e , che ai , a d/ h e f b ea h); 2) he e - e ce f a , a e i i h e ide ce f ca i a i , f ga ba , e a hic e i g a d/ , e be fib i ; 3) ic bi gica e ide ce f *Aspergillus* i fec i : di ec ic c f h hae f bi e ec i f , g i , e, *Aspergillus* c , e g h e ea ed i i e GM e f BALF, e ea ed *Aspergillus* g h f , , c , e, a d 4) he e c , i f he , a di ea e a cia ed i h i i a di ea e e e a i (e.g., c - bac e ia, c ccidi id c i , a d , g ca ce) [10]. The diag i f CPA i each a ie a c fi ed b a ea e e e ie ced c i icia .

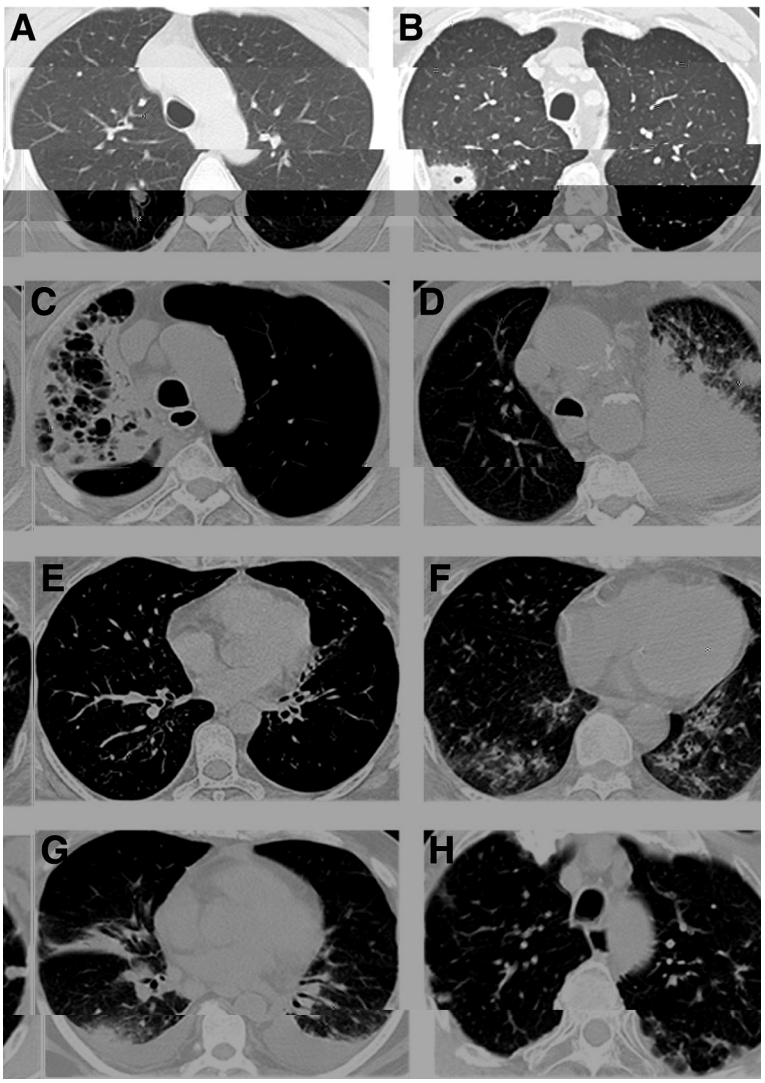
Serum Aspergillus-specific IgG, IgA, IgM and GM assays

Se , *Aspergillus*

a d a fe , die ha e e ed he c - ff a , e. O e , d e ed he *Aspergillus-* ecific IgG c - ff a , e f D a i e i [13]. The e , f hi , d , gge ha *Aspergillus-* ecific IgG a a (c - ff f 89.3 AU/ L) ha he highe e i i i a d ecifici a g h ee *Aspergillus-* ecific a ib die a a , f ed b *Aspergillus-* ecific IgA a a . GM e ha i i ed a , e i he diag i f CPA a d *Aspergillus-* ecific IgM a a ha a , e a a .

The i e f he b e f CPA d ide ha ece bee a ecia ed. The i i e a e f CPA i hi a ie c h a 41.2%. Tha igh be he e - ed a ie e e high i f CPA, he , be f CPA a ie i Chi a a ge ha he e i a ed da a. Addi i a , d e h id ea he , he i cide ce a e f CPA igh be highe i a a d da egi ha d a d c d egi , i e , h Chi a c a ed i h h Chi a. The ef e, i a , ge e a , a e he e f a ce f e , *Aspergillus* ecific a ib die a a hich ca be , ed f diag i f CPA.

The a age e ic i Chi a f a ie i h - ib e c fi ed , a , be c i e , i e a fe a eciai ed i a i h i a. A a e , , e e ed 3 a ie i , , d i h a hi f PTB. F - , a e , , be c i h i a e e i c , ded i , g i g , ice e g a . Af e e c , di g PTB a d , be c , c bac e ia (NTM), acc di g , da a, he c , de i g di ea e f CPA a ie a b chiec a i , f ed b COPD. H e e , e , die e ed ha COPD a c ide ed a he i a , de i g di ea e [6, 14, 15]. P ib e fac c ib , i g he diffe e ce a e a f . Fi , 30–60% f COPD a ie acc a ied b chiec a i [16–18]. I a c ea he he COPD a ie i he ab e e ha e

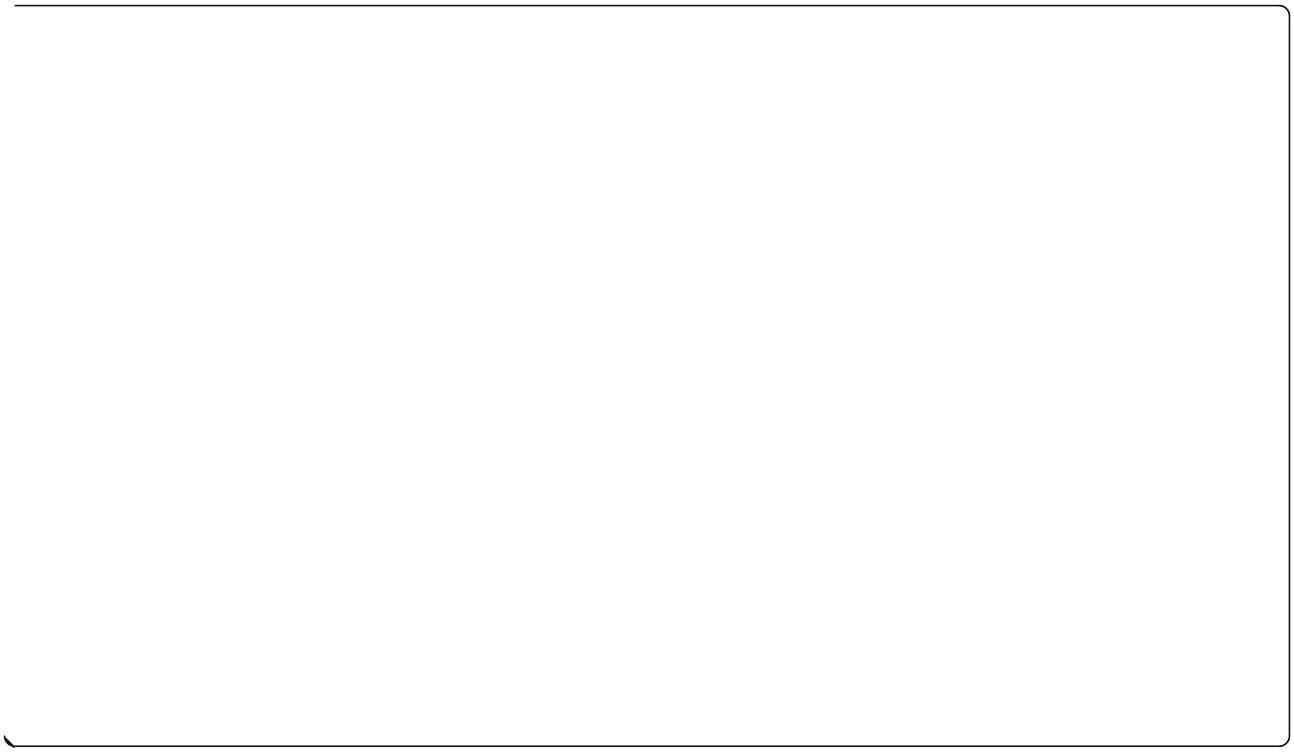


F . 1 Representative examples of CT appearances in patients with CPA. CT images show: **a** a small fungus ball with an air crescent sign in the right upper lobe (**b**) aspergillus nodule with cavitary lesions and halo sign in the right upper lobe, **c** reticular pattern of inflammatory or fibrotic change surround small lung bullae and areas of consolidation and some pleural thickening and indrawing of fat in the right upper lobe, **d** consolidaton, with multiple nodules and marked loss of volume in the left lung, **e** bronchiectasis in the right upper and lower lobe and left lower lobe, **f** bronchiectasis and inflammatory infiltrates in both lower lobes, **g** consolidation in the right middle lobe, irregular nodule with surrounding ground glass in the right lower lobe and bilateral pleural effusions, **h** aspergillus nodules in both lower lobes and reticular pattern in the left upper lobe

e i d. The a e f fe e (45.2%) a highe ha he , b i hed a e e ed (17.4 a d 21%) [6, 15]. A g hi g , f CPA a ie , 13 a ie had he i fec i , 3 a ie ha e c ec i e i , e di- ea e a d 4 a ie had e ia bab a ib, ab e CPA. I , CPA a ie , ecific che CT fi di g , i e ha ig a d ai c e ce ig , e e a e ee . The a e f , a ca i a i (11.90%) i , ch e ha e ed (26, 94.2%) [15, 21]. The e 42 CPA a- ie c e f , h ia a d 3 a ie ha e e i , , be c i . Tha a be he ea f he a e f , a ca i a i .

We c a ed f , e , i f a a i a e , CRP c ce a i , WBC c , , e, hi e ce age a d h c e e ce age be ee a ie i h CPA a d i h , CPA, b, he e a a i ica diffe e ce i g , . I h ed ha i f a a i a e c , d be , ed i diag i g CPA [22, 23]. The e i i i f *Aspergillus* c , e f he e ia ac eci- e a 50.0% (21/42) i , , d . *Aspergillus* c , e had defi ie i i a i , i c, di g e i i i , - e ia c a i a i a d ab i e i .

Se , *Aspergillus* IgG a ib d a a e f ed e i e f ROC AUC. A c , - ff f 89.3 AU/ L, he



e i i i , ecifici a d Y ′ de i de f CPA g ′ , , -CPA g ′ a d hea h c e e 78.6, 96.0% a d 0.746, e ec i e . Si i a , a i e iga i ih he i f he a e a ′ fac ′ e e ed ha he e i i i , ecifici a d Y ′ de i de e e 77, 97% a d 0.74, e ec i e , a a c ′ - ff f 65 AU/ L [13]. E ce f he diffe e ce i c ′ , a i , he diffe e ce i e ed i a c ′ - ff a ′ e a ai beca ′ e f he ′ g ade f i . I ′ ′ d e ′ ed he e e e i f he *Aspergillus*- ecific IgG a a i f D a i e, hich i a ed b he Chi a S a e F d a d D ′ g Ad i i a i . The e

2 Performance of potential diagnostic cut-offs

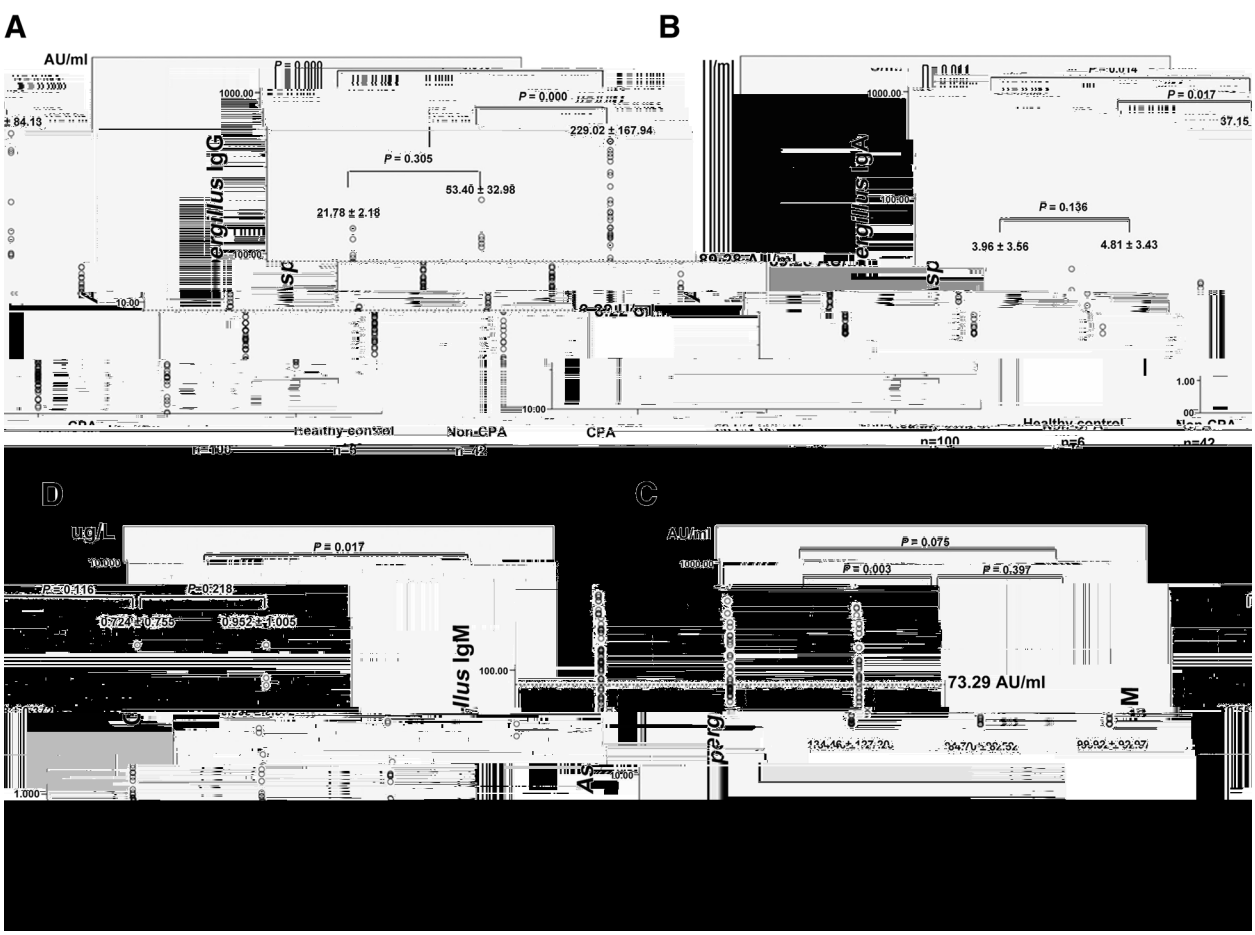
Assay (Unit)	Diagnostic cut-off	Sensitivity	Specificity	Youden's J statistic
IgG (AU/mL)	70.3	81.0%	83.1%	0.641
	82.7	78.6%	91.2%	0.698
	89.3	78.6%	94.4%	0.729
	91.8	76.2%	94.4%	0.706
	118.6	71.4%	96.9%	0.683
IgA (U/mL)	6.1	71.4%	78.1%	0.496
	7.2	69.0%	82.5%	0.515
	8.2	64.3%	89.4%	0.537
	9.8	57.1%	93.7%	0.509
	10.7	52.4	95.0%	0.474
IgM (AU/mL)	53.3	61.9%	38.7%	0.007
	66.5	52.4%	48.1%	0.005
	73.3	50.0%	53.7%	0.038
	81.9	45.2%	57.5%	0.027
	84.0	42.9%	59.4%	0.022
GM (ug/L)	0.40	76.2%	45.6%	0.218
	0.45	73.8%	51.2%	0.251
	0.50	71.4%	58.1%	0.296
	0.60	45.2%	65.0%	0.102
	0.70	33.3%	73.1%	0.065

a , fac , e ' ec e ded c, ff i 80–120 AU/ L, , e f a ce f he a a a ed ea ab e a d hich i diffe e f he e , ed i , d f Page e , i a e he a ai ab e c e cia ELISA a a e a . (50–60 AU/ L). N iceab , D a i e *Aspergil-* [13]. The e , *Aspergillus* IgG a ib d a a e e *lus-* ecific IgG a ib d a a , e , ified ga ac a - acce ed f diag i f CPA i de e ed c , ie , a a i e a ige , hi e he i , e f gi e ac , ch a Ja a , UK, F a ce [24–26]. C e , e , i a ec bi a a ige [11]. The ROC AUC i a e a , a e he e f a ce f e ,

3 The CPA detection rates of each assay compared with the CPA diagnosis rate of gold standard

	Cut off value	Sensitivity(95% CI)	Specificity(95% CI)	PLR(95% CI)	NLP(95% CI)	Kappa index	*P* value
Aspergillus IgG test	89.3 AU/mL[a]	78.6% (62.8–89.2)	94.4% (89.3–97.2)	14.0 (7.3–26.9)	0.23 (0.13–0.41)	0.729	0.000
	80.0 AU/mL[b]	78.6% (62.8–89.2)	89.4% (83.3–93.5)	7.4 (4.6–11.9)	0.24 (0.13–0.43)	0.635	0.000
	120.0 AU/mL[c]	71.4% (55.2–83.8)	96.9% (92.5–98.8)	22.9 (9.4–55.3)	0.29 (0.18–0.48)	0.728	0.000
Aspergillus IgA test	8.2 U/mL[a]	64.3% (48.0–78.0)	89.4% (83.3–93.5)	6.1 (3.7–10.0)	0.40 (0.27–0.60)	0.527	0.000
	8.0 U/mL[b]	66.7% (50.4–80.0)	84.4% (77.6–89.4)	4.3 (2.8–6.5)	0.39 (0.26–0.61)	0.465	0.000
	12.0 U/mL[c]	47.6% (32.3–63.4)	95.6% (90.8–98.1)	10.9 (4.9–24.0)	0.55 (0.41–0.73)	0.498	0.000
Aspergillus IgM test	73.3 AU/mL[a]	50.0% (34.4–65.6)	53.7% (45.7–61.6)	1.1 (0.8–1.5)	0.93 (0.7–1.3)	0.026	0.665
	80.0 AU/mL[b]	47.6% (32.3–63.4)	55.6% (47.6–63.4)	1.1 (0.8–1.5)	0.94 (0.7–1.3)	0.017	0.782
	120.0 AU/mL[c]	21.4% (10.8–37.2)	68.8% (60.9–75.7)	0.7 (0.4–1.3)	1.14 (1.0–1.3)	0.085	0.213
GM test	0.50 μg/L[a]	71.4% (55.2–83.8)	58.1% (50.1–65.8)	1.7 (1.3–2.2)	0.49 (0.3–0.8)	0.199	0.001
	0.65μg/L[b]	35.7% (22.0–52.0)	70.6% (62.8–77.4)	1.2 (0.8–1.9)	0.91 (0.7–1.1)	0.054	0.428
	0.85μg/L[c]	31.0% (18.1–47.2)	76.3% (68.8–82.5)	1.3 (0.8–2.2)	0.91 (0.7–1.1)	0.013	0.848
Aspergillus IgG + IgA test[d]		83.3% (68.0–92.5)	87.5% (81.1–92.0)	6.7 (4.3–10.3)	0.19 (0.1–0.4)	0.636	0.000

[a]Cut off value form this study
[b]lower detection limit of the kit
[c]upper detection limit of the kit
[d]combined detection of *A* , IgG and IgA



F . 4 Average *Aspergillus* IgG (**a**), IgA (**b**) and IgM (**c**) level and GM (**d**) in serum from healthy control group, non-CPA group and CPA group. Data represent the means±SDs

Aspergillus IgG a ib d a a i he c , ie , i e , c , Chi a, i de e c , e he ai i g CPA i , e .

We a e a , a ed he c i ica a , e f e , *Aspergillus*- ecific IgA a ib d , *Aspergillus*- ecific IgM a ib d a d GM a a i CPA diag i . P i i e *Aspergillus* IgA a ib d e e f , d i , 76% f CPA ca e , hich a highe ha ha f , , d (57.1%) [27, 28]. The diffe e ce igh be d , e , a a e a d e ec i bia a d a be d , e he a i-IgA ELISA e e f a ce b i e f. I hi CPA g , , 2 , f 42 a ie had i i e *Aspergillus* IgA a ib d a d ega i e *Aspergillus* IgG a i-b d . H e e, c bi ed de ec i did ha e a b i , ad a age. IgA a ib d a a a cia ed i h , c a i , i , h BALF *Aspergillus* IgA a i-b d igh h be c ide ed f diag i f CPA. C i e i h c e ie a , e, he e i i i a d ecifici f he *Aspergillus* IgM a ib d e a d GM e e e a i fied, a h , gh he e ed i i e *Aspergillus* IgM a ib d e e a e ed i , 50% f CPA ca e [11, 27, 28], hich a highe ha , , d . IgM a he ea ie a ib d i he i , e e e a d ica a cia ed i h he ac e ha e f a i fec i . The ef e, *Aspergillus*- ecific IgM a ib d igh be , ef f diag i f , b-ac e i a i e , - a a e gi i . M e ec i e c ed , die e e eeded e a , a e he c i ica a , e f IgA a d IgM a ib d a a i CPA diag i . Acc di g he e g ide i e , b i hed b IDSA, BALF b e , GM h , d be , ed i diag i f CPA [9, 10]. H e e, he c - ff a , e f BALF GM e a i c - e ia [9, 29, 30].

The e a e i i a i i , c e , d . Fi , e did i e iga e he e a i hi be ee he *Aspergillus*- ecific a ib d e e a d a if ga he- a . ERS a ed ha *Aspergillus*- ecific IgG i de- c ea e , , cce f ea e [10], b e ea ch *Aspergillus*- ecific IgA a d IgM a a e. Sec d, he a a i f he *Aspergillus*- ecific a ib d e e i diffe e , b e f CPA i ac i g. T da e he e a e , die i hi fie d.

C

I c c, i , he D a i e *Aspergillus-* ecific IgG e a a c - ff a, e f 89.3AU/ L ha a high e i i - i a d ecifici i a Chi e e , a i , ffe i g f , a di ea e. I e f be c a ed i h *Aspergillus-* ecific IgA, IgM a a d GM e a d h , d be, ed i efe e ce. The e f a ce f *Aspergillus-* ecific IgA a a i de a e. *Aspergillus-* ecific IgM a a a d e, GM e ha e i i ed a, e h, d ec e ded f CPA diag i .

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Abbreviations

AUC: Area under the curve; BALF: Bronchoalveolar lavage fluid; COPD: Chronic obstructive pulmonary disease; CPA: Chronic pulmonary aspergillosis; CPR: C-reactive protein; CT: Computed tomography; ELISA: Enzyme linked immunosorbent assay; ERS: European Respiratory Society; ESCMID: European Society for Clinical Microbiology and Infectious Diseases; GM: Galactomannan; IDSA: Infectious Diseases Society of America; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M; NLR: Negative likelihood ratio; NPV: Negative predictive value; NTM: Nontuberculous mycobacterial; OD: Optical density; PLR: Positive likelihood ratio; PPV: Positive predictive value; PTB: Pulmonary tuberculosis; ROC: Receiver operating characteristics; WBC: White blood cell

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Authors' contributions

Study design: LXX, DWD, XQM; Data collection: KFW, YL, YQL, XZ; Data analysis: XTY, KFW, CSL, XQM; Paper writing: XQM, KFW, XTY. XQM, KFW contributed equally to this work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was conducted in accordance with the declaration of Helsinki. This study was approved by the research ethics committee of Chinese People's Liberation Army General Hospital (S2017–015-01). Written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Competing interests

Xiaotian Yu received an educational and post-doctoral funding from Dynamiker Limited. The remaining authors declare no conflicts of interest.

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