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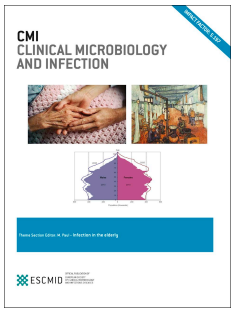

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Aspergillus-specific IgG antibodies for diagnosing chronic pulmonary aspergillosis compared to the reference gold standard

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Abstract

Objectives

To evaluate the performance of *Aspergillus*-specific IgG antibodies for diagnosing chronic pulmonary aspergillosis (CPA) by using a cohort of patients with histologically proven CPA as a reference gold standard.

Methods

We collected *Aspergillus*-specific IgG antibody titres from histologically proven CPA patients in collaboration with CPAnet study sites in Denmark, Germany, Belgium, India, Moldova, and Pakistan (N=47). Additionally, sera from diseased- and healthy controls were prospectively collected at the Medical Clinic of the Research Center Borstel, Germany (n=303). *Aspergillus*-specific IgG antibody titres were measured by the ImmunoCAP® assay (Phadia 100, Thermo Fisher Scientific, Uppsala, Sweden). An *Aspergillus*-specific IgG antibody titre ≥50 mgA/L was considered positive.

Results

Using histologically proven CPA patients as the reference standard, the ImmunoCAP® Aspergillus specific IgG antibody test had a sensitivity and specificity of 85.1% (95-% CI: 71.7 - 93.8%) and 83.6% (95-% CI: 78.0 – 88.3%) respectively. Patients with histologically proven CPA had significantly higher Aspergillus-specific IgG antibody titre with a median of 83.45 mgA/L (IQR 38.9-115.5) than all other cohorts ($p<0.001$). False-positive test results occurred in one-third of 79 healthy controls.

Discussion

Our study results confirm a high sensitivity of the *Aspergillus*-specific IgG antibody test for the diagnosis of CPA when using histologically proven CPA patients as a reference standard. However, positive test results should always match radiological findings as false-positive test results limit the interpretation of the test.

Introduction

Chronic pulmonary aspergillosis is a severe fungal infection due to moulds of the *Aspergillus* species complicating other respiratory diseases. Morbidity is high with a 5-year mortality of up to 80% [1, 2]. The estimated incidence is 22 cases per 100,000, ranging between 1.4 and 127 cases per 100,000 depending on the regional prevalence of predisposing respiratory diseases, particularly pulmonary tuberculosis [3, 4]. CPA is usually seen in immunocompetent or 'subtle immunodeficient' individuals with underlying respiratory diseases [5]. Any devastation of the lung parenchyma such as residual cavities after pulmonary mycobacterial infection or emphysema and bronchiectasis in patients with chronic obstructive pulmonary disease is a risk factor for developing CPA [1, 6].

The diagnosis of CPA needs compatible imaging, proof of mycological evidence, a chronic course of the disease >3 month, and exclusion of alternative diagnosis [7, 8]. A bronchoscopy for the detection of *Aspergillus* species from lower respiratory tract samples is resource-intensive, it needs a risk/benefit evaluation, and it is often unavailable, particularly in resource-constraint settings. In contrast, detection of *Aspergillus*-specific IgG antibodies from blood is simple, inexpensive, and can be applied to most settings [9]. Previous studies reported a high sensitivity and specificity of up to 93% and 99% respectively, depending on the manufacturer and the cut-off used [7, 10, 11]. However, the performance evaluations of *Aspergillus*-specific IgG antibody assays have never included histologically proven CPA patients as a reference gold standard to ensure an accurate diagnosis.

We aimed to evaluate the performance of *Aspergillus*-specific IgG antibodies for diagnosing CPA by using a cohort of patients with histologically proven CPA as a reference standard. The results were compared to larger groups of diseased controls, including patients with pulmonary tuberculosis (PTB), and patients with other respiratory diseases (ORD), and healthy controls.

Methods

We defined histologically proven CPA as the detection of hyphae in lung tissue (histopathologic, cytopathologic, or direct microscopic) obtained by needle aspiration or biopsy [12]. We collected *Aspergillus fumigatus*-specific IgG antibody titres from histologically proven CPA patients in collaboration with CPAnet study sites in Denmark (patients included; n=20), Germany (n=10), Belgium (n=8), India (n=6), Moldova (n=2), and Pakistan (n=1). We also prospectively collected sera from diseased- (high- and moderate CPA risk) and healthy- controls (low CPA risk) at the Medical Clinic of the Research Center Borstel, Germany (n=303). Diseased controls included a cohort of patients with PTB (high CPA risk) and a cohort of ORD (moderate CPA risk) defined as all other respiratory diseases than CPA and PTB. Healthy controls had no medical history of any respiratory- or allergic disease, respectively. *Aspergillus*-specific IgG antibody titres were measured by the ImmunoCAP® assay (Phadia 100, Thermo Fisher Scientific, Uppsala, Sweden). An *Aspergillus*-specific IgG antibody titre ≥50 mgA/L was considered positive as recommended by previous publications [11].

We used Kruskal-Wallis Test and Chi-squared tests to compare the continuous and categorical variables between the groups. Receiver operating characteristics (ROC) curve analyses were performed comparing results in histologically proven CPA patients with each control group. A cut-off of 50 mgA/L was used for the analysis of sensitivity and specificity, while the exact *Aspergillus*-specific IgG antibody levels were used for the ROC curve and determination of the optimal cut-off. For patients with PTB, additional clinical parameters were tested for their influence on the *Aspergillus*-specific IgG antibody level using Pearson's correlation analysis. The study protocol was approved by the Ethics Committee of the University of Luebeck, Germany (approval number, 17-160).

Results

We enrolled 340 patients, including 110 patients with PTB, 104 patients with ORD, 47 patients with histologically proven CPA, and 79 healthy controls. Among these patients, 64% were men. CPA subtypes included 30 patients with a chronic cavitary pulmonary aspergillosis, eight patients with a

single aspergilloma, seven patients with aspergillus nodules, while two patients were not classified. Patients with PTB were significantly younger (median 39 years, interquartile range (IQR) 28-52) compared to patients with ORD (median 70, IQR 62-77), and patients with histologically proven CPA (median 58, IQR 47-69) (p<0.001).

The median *Aspergillus*-specific IgG antibody titre was 22.3 mg/l (IQR 8.5-43.1), 19.3 mgA/L (IQR 9.0-35.3), and 26.2 mgA/L (IQR 16.0-62.7) in patients with PTB, with ORD, and in healthy controls, respectively. Patients with histologically proven CPA had significantly higher *Aspergillus*-specific IgG antibody titre with a median of 83.45 mgA/L (IQR 38.9-115.5) than all other cohorts (p<0.001).

Forty out of 47 patients with histologically proven CPA (85%) had an *Aspergillus*-specific IgG antibody titer >50 mgA/L, while seven (14.9%) patients had a negative test result. The frequency of positive test results was significantly lower in the diseased and the healthy controls including 24/110 (22%) of patients with PTB, 11/104 (11%) of patients with ORD, and 25/79 (31.6%) of healthy controls (p<0.001). Weight correlated indirectly with *Aspergillus*-specific IgG antibody score in PTB patients (r=-0.27). Smokers had a lower *Aspergillus*-specific IgG antibody score (r=-0.41), whereas dyspnea (r=0.35) and COPD Assessment Test (CAT) score (r=0.21) correlated directly with *Aspergillus*-specific IgG antibody.

Using histologically proven CPA patients as the reference standard, the ImmunoCAP® *Aspergillus*-specific IgG antibody test had a sensitivity and specificity of 85.1% (95-% CI: 71.7 - 93.8%) and 83.6% (95-% CI: 78.0 – 88.3%,)respectively. The performance of a ROC analysis revealed an optimal cut-off of *Aspergillus*-specific IgG antibody titer of 58 mgA/L (Fig. 1). Under the assumption of a prevalence of CPA of 5 per 100.000 hospitalized patients from Western Europe the positive and negative predictive values of the ImmunoCAP® *Aspergillus*-specific IgG antibody test would be 21.5% and 99.0%, respectively [1].

Discussion

The present data support previous study results showing a high sensitivity of the *Aspergillus*-specific IgG antibody test to prove mycological evidence in CPA. It contributes substantially to a better understanding since performance evaluations of *Aspergillus*-specific IgG antibody assays have never included histologically proven CPA patients as a reference standard so far. This was repeatedly supposed to be a possible limitation of previous studies influencing sensitivity and specificity. However, even in this unique cohort of histology-confirmed CPA patients, 15% of patients were not identified by the *Aspergillus*-specific IgG antibody test. The possible reasons might be an immunologically controlled aspergilloma, encapsulated lesion that is not accessible by the host immune system. Also, the use of antifungals or any severe immunosuppression may affect the *Aspergillus*-specific IgG titre (6/47 received antifungal treatment at the time of CPA diagnosis).

Interestingly, the *Aspergillus*-specific IgG antibody test showed a false-positive test result in one-third of healthy controls in our study. The specificity contrasts with previous publications reporting almost no false positive test results in their healthy control groups [11]. We expected to see some false positive test results in healthy controls since IgG antibodies reflect an immunological long-term memory and exposure to aspergillus spores is frequent; however, we were surprised by the order of magnitude of false-positive test results, which substantially limits the clinical value for the application of this test in clinical practice. Risk factors for false-positive test results in healthy controls, such as allergies or asthma, could not be identified. The test performance of the *Aspergillus*-specific IgG antibody test for the diagnosis of CPA was optimal at a cut-off of 58 mgA/L with loss in sensitivity at higher cut-offs. However, calculated cut-off values always refer to the patient cohorts used in the respective study depending on *Aspergillus*-specific IgG antibody levels, severity of disease, distribution of CPA subtypes and characteristics of controls [13]. Exploring population specific cut-off values respecting differences in sex, age, immune status, CPA subtype, or geographical distribution could help to harmonize cut-off values.

Our study has some limitations. 1) data of histologically proven CPA patients were collected retrospectively. 2), patient characteristics including the CPA subtype, disease severity or antifungal treatment differed between study sites. 3) information of the specific *Aspergillus* species was not available for every CPA patient, which could possibly influence the test performance in case of a high number of non-*Aspergillus fumigatus* species. 4) we had no cohort of patients suspected of CPA. So, calculation of negative and positive predictive values was not directly possible. To provide an exemplary sensitivity analyses we used data from a large French cohort study [1] where a CPA prevalence of 5/100 000 hospital admissions was found to calculate PPVs and NPVs. These values are not representative for other populations and other geographic regions with a different CPA burden.

Positive *Aspergillus*-specific IgG antibody titre are also frequently reported in patients with PTB or with post tuberculosis lung disease. Reasons might be a higher rate of *Aspergillus* colonization due to structural lung damage, but also high rates of CPA infections. In a recent publication by Lakoh and colleagues the authors suggested that CPA probably is the most common tuberculosis misdiagnosis, while 37% of PTB patients were just clinically diagnosed as reported in the World Health Organization Global Tuberculosis Report 2022 [14, 15].

In conclusion, our study results confirm a high sensitivity of the *Aspergillus*-specific IgG antibody test for the diagnosis of CPA when using histologically proven CPA patients as a reference standard, however positive test results should be interpreted with caution and should match radiological findings as a high-rate of false-positive test results limit the interpretation of the test.

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Author contributions

All authors have made substantial contribution to the data collection and the analysis or interpretation. H.S., C.O. and C.L. designed the study; H.S. and M.R. drafted the manuscript; M.R. performed the statistical analysis; H.S., M.R., C.O., J.D., C.L., E.B., R.A., K.A., O.M., M.I. and C.L. contributed to the critical revisions, and the final approval of the manuscript.

Conflict of Interests

No author has any conflict of interest in relation to this publication.

R.A. reports receiving grants from Cipla Pharmaceuticals, India for research in allergic bronchopulmonary aspergillosis, all outside the submitted work. K.A. reports personal fees from Insmed and Astra Zeneca for attending meetings, all outside the submitted work. J.D. reports personal fees from Roche, Boehringer Ingelheim, and Chiesi for lectures, and has received support for congresses, attending meetings and travel, all outside the submitted work. C.L. reports speakers' honoraria from Insmed, Gilead and Janssen for lectures at sponsored symposia and fees for participating in an advisory board for Insmed, all outside the submitted work. H.S. reports personal fees from Insmed, Pfizer, Gilead, Menarini, and Chiesi for lectures, and has received support for attending meetings and travel, all outside the submitted work. M.I, C.L., O.M., C.O., M.R., E.B. have nothing to disclose.

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222 **Fig. 1** Receiver operating characteristics (ROC) curve analyses were performed comparing results in
223 histologically proven CPA patients with groups of diseased controls, including patients with
224 pulmonary tuberculosis, patients with other respiratory diseases (ORD), and healthy controls.
225 AUC, area under the curve; CPA, chronic pulmonary aspergillosis; HC, healthy controls; TB,
226 tuberculosis; TOD, to other diseases.
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References

[1] T. Maitre, J. Cottenet, C. Godet, A. Roussot, N. Abdoul Carime, V. Ok, A. Parrot, P. Bonniaud, C. Quantin, J. Cadranel, Chronic pulmonary aspergillosis: prevalence, favouring pulmonary diseases and prognosis, The European respiratory journal 58(2) (2021).

[2] D. Lowes, K. Al-Shair, P.J. Newton, J. Morris, C. Harris, R. Rautemaa-Richardson, D.W. Denning, Predictors of mortality in chronic pulmonary aspergillosis, Eur Respir J 49(2) (2017).

[3] F. Bongomin, S. Gago, R.O. Oladele, D.W. Denning, Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision, J Fungi (Basel) 3(4) (2017).

[4] D.W. Denning, A. Pleuvry, D.C. Cole, Global burden of chronic pulmonary aspergillosis as a sequel to pulmonary tuberculosis, Bull World Health Organ 89(12) (2011) 864-72.

[5] E.S. Hunter, B. Wilopo, M.D. Richardson, C. Kosmidis, D.W. Denning, Effect of patient immunodeficiencies on the diagnostic performance of serological assays to detect Aspergillus-specific antibodies in chronic pulmonary aspergillosis, Respir Med 178 (2021) 106290.

[6] H.J. Salzer, J. Heyckendorf, B. Kalsdorf, T. Rolling, C. Lange, Characterization of patients with chronic pulmonary aspergillosis according to the new ESCMID/ERS/ECMM and IDSA guidelines, Mycoses 60(2) (2017) 136-142.

[7] D.W. Denning, J. Cadranel, C. Beigelman-Aubry, F. Ader, A. Chakrabarti, S. Blot, A.J. Ullmann, G. Dimopoulos, C. Lange, M. European Society for Clinical, D. Infectious, S. European Respiratory, Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management, Eur Respir J 47(1) (2016) 45-68.

[8] H.J.F. Salzer, C. Lange, M. Honigl, [Aspergillus in airway material : Ignore or treat?], Internist (Berl) 58(11) (2017) 1150-1162.

[9] H.J.F. Salzer, I. Massango, N. Bhatt, E. Machonisse, M. Reimann, S. Heldt, C. Lange, M. Hoelscher, C. Khosa, A. Rachow, Seroprevalence of Aspergillus-Specific IgG Antibody among Mozambican Tuberculosis Patients, J Fungi (Basel) 7(8) (2021).

[10] D.W. Denning, Diagnosing pulmonary aspergillosis is much easier than it used to be: a new diagnostic landscape, Int J Tuberc Lung Dis 25(7) (2021) 525-536.

[11] I.D. Page, C. Baxter, C. Hennequin, M.D. Richardson, E. van Hoeyveld, A.W. van Toorenenbergen, D.W. Denning, Receiver operating characteristic curve analysis of four Aspergillus-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis, Diagn Microbiol Infect Dis 91(1) (2018) 47-51.

[12] J.P. Donnelly, S.C. Chen, C.A. Kauffman, W.J. Steinbach, J.W. Baddley, P.E. Verweij, C.J. Clancy, J.R. Wingard, S.R. Lockhart, A.H. Groll, T.C. Sorrell, M. Bassetti, H. Akan, B.D. Alexander, D. Andes, E. Azoulay, R. Bialek, R.W. Bradsher, S. Bretagne, T. Calandra, A.M. Caliendo, E. Castagnola, M. Cruciani, M. Cuenca-Estrella, C.F. Decker, S.R. Desai, B. Fisher, T. Harrison, C.P. Heussel, H.E. Jensen, C.C. Kibbler, D.P. Kontoyiannis, B.J. Kullberg, K. Lagrou, F. Lamoth, T. Lehrnbecher, J. Loeffler, O. Lortholary, J. Maertens, O. Marchetti, K.A. Marr, H. Masur, J.F. Meis, C.O. Morrisey, M. Nucci, L. Ostrosky-Zeichner, L. Pagano, T.F. Patterson, J.R. Perfect, Z. Racil, E. Roilides, M. Ruhnke, C.S. Prokop, S. Shoham, M.A. Slavin, D.A. Stevens, G.R. Thompson, J.A. Vazquez, C. Viscoli, T.J. Walsh, A. Warris, L.J. Wheat, P.L. White, T.E. Zaoutis, P.G. Pappas, Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium, Clin Infect Dis 71(6) (2020) 1367-1376.

[13] M.R. Lee, H.L. Huang, L.T. Keng, H.L. Chang, C.C. Sheu, P.K. Fu, J.Y. Wang, I.W. Chong, J.Y. Shih, C.J. Yu, Establishing Aspergillus-Specific IgG Cut-Off Level for Chronic Pulmonary Aspergillosis Diagnosis: Multicenter Prospective Cohort Study, J Fungi (Basel) 7(6) (2021).

[14] A.G. Loxton, Bcells and their regulatory functions during Tuberculosis: Latency and active disease, Molecular immunology 111 (2019) 145-151.

[15] S. Lakoh, J.B. Kamara, E. Orefuwa, D. Sesay, D.F. Jiba, O. Adekanmbi, G.F. Deen, J.B.W. Russell, A.B. Bah, M.J. Kargbo, E. Firima, G.A. Yendewa, D.W. Denning, Prevalence and predictors of Aspergillus seropositivity and chronic pulmonary aspergillosis in an urban tertiary hospital in Sierra Leone: A cross-sectional study, PLoS Negl Trop Dis 17(7) (2023) e0011284.