Diagnostic significance of *Aspergillus* species isolated from respiratory samples in an adult pneumology ward

Takayoshi Tashiro[a], Koichi Izumikawa[b], Masato Tashiro[b], Takahiro Takazono[b], Yoshitomo Morinaga[b], Kazuko Yamamoto[b], Yoshifumi Imamura[b], Taiga Miyazaki[b], Masafumi Seki[b], Hiroshi Kakeya[b], Yoshihiro Yamamoto[b], Katsunori Yanagihara[c], Akira Yasuoka[d] and Shigeru Kohno[b]

[a]Department of Health Sciences, [b]Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, [c]Department of Laboratory Medicine, and [d]Nagasaki University Infection Control and Education Center, Nagasaki University Hospital, Nagasaki, JAPAN

Keywords: CNPA, CCPA, Aspergilloma, IPA, colonization

Running head: Significance of *Aspergillus* isolation

Corresponding author:

Koichi IZUMIKAWA, M.D., Ph.D.

Nagasaki University Graduate School of Biomedical Sciences

Department of Molecular Microbiology and Immunology

1-7-1 Sakamoto

Nagasaki 852-8501, JAPAN

Phone: +81-95-819-7273

Fax: +81-95-849-7285

E-mail: koizumik@nagasaki-u.ac.jp

Summary

Background. Although the diagnostic significance of isolating *Aspergillus* spp. from respiratory cultures has been studied in immunocompromised hosts with invasive pulmonary aspergillosis (IPA), little is known in immunocompetent patients with other forms of aspergillosis. In this study of adult pneumology ward patients, we examined the association between *Aspergillus* spp. and disease prevalence.

Methods. Laboratory records from April 1998 to March 2009 were reviewed to identify patients with *Aspergillus* spp. in respiratory samples. Correlations between the isolated species and clinical characteristics of patients were evaluated.

Results. During the study period, 165 *Aspergillus* spp. isolates were detected in the respiratory cultures of 139 patients. Of these patients, 62 (45%) were colonized with *Aspergillus* spp. and displayed no clinical symptoms of aspergillosis, while 77 (55%) had a form of pulmonary aspergillosis, characterized as either chronic necrotizing pulmonary aspergillosis (CNPA) (48%), aspergilloma (29%), IPA (13%), or allergic bronchopulmonary aspergillosis (ABPA) (10%). The dominant species were *A. fumigatus* (41%), *A. niger* (32%), and *A. versicolor* (12%). *A. fumigatus* was most commonly isolated in patients with IPA, aspergilloma, and CNPA, whereas *A. niger* was the dominant species in colonized patients and those with ABPA.

Conclusions. Isolation of an *Aspergillus* sp. from respiratory samples does not confirm it is the etiologic pathogen, because airway colonization by *Aspergillus* spp. is a common feature in several chronic lung diseases. Repeated isolation of the identical *Aspergillus* sp. and detection of anti-*Aspergillus* antibodies and/or *Aspergillus* antigens in sera are needed to determine the isolate that represents the etiologic organism.

INTRODUCTION

Members of the genus *Aspergillus* are ubiquitous saprophytic fungi, and due to their widespread nature in the environment, the average person may inhale hundreds of *Aspergillus* conidia per day [1]. Conidia are typically removed from the respiratory tract by mucociliary clearance and phagocytosis by alveolar macrophages, while germinating spores and hyphae are attacked by polymorphonuclear neutrocytes through degranulation and the release of oxidants [2]. Despite these effective clearance mechanisms for the elimination of inhaled conidia from the respiratory tracts of healthy individuals, *Aspergillus* conidia are capable of colonizing injured lung tissue and epithelia. Although such colonization often has no clinical consequences, *Aspergillus* conidia can cause a variety of clinical manifestations depending on the immune status of the host [3, 4]. For example, allergic bronchopulmonary aspergillosis (ABPA), which encompasses hypersensitivity reactions to *Aspergillus* antigens, can develop in atopic patients [5, 6], while aspergilloma is mainly observed in patients with chronic cavitary lung disease. Other forms of aspergillosis include chronic necrotizing pulmonary aspergillosis (CNPA), which is seen in mildly immunocompromised patients and those individuals with chronic lung diseases [7], and invasive pulmonary aspergillosis (IPA), which is the most serious lung infection due to *Aspergillus* spp. and typically occurs only in severely immunocompromised patients [8].

Isolation of *Aspergillus* spp. from respiratory samples of a patient with suspected aspergillosis does not necessarily indicate that the identified species is the etiologic agents of disease. Although the diagnostic significance of isolation of *Aspergillus* spp. from respiratory cultures has been extensively examined in immunocompromised hosts who develop IPA [9–13], little is known with respect to the presence of *Aspergillus* spp. in the respiratory tracts of immunocompetent or mildly immunocompromised patients with other forms of aspergillosis [14–16]. Here, we conducted a retrospective study to evaluate the clinical significance of *Aspergillus*-positive culture results of respiratory samples from adult patients in a pneumology ward.

MATERIALS AND METHODS

The microbiology laboratory records of the Pneumology Department of Nagasaki University Hospital, Japan from April 1998 to March 2009 were reviewed to identify all patients who had *Aspergillus* spp. isolated from cultures of respiratory samples. A total of 350-500 patients including 150-200 new patients per year admitted to this department and most common lung disease is lung cancer. The respiratory samples, which included sputum, endotracheal aspirates, and bronchoalveolar lavage fluid, were obtained as a part of a diagnostic work-up for respiratory disease. Samples were immediately cultured in sabouraud dextrose agar (SDA) 10 days at 30 °C. Filamentous fungi in SDA was then transferred to potato dextrose agar and incubated for 2-3 days at 30 °C. The isolates were identified based upon macroscopic and microscopic morphological characteristics following standard mycological procedures. For each patient with a positive sample for *Aspergillus*, the species and clinical characteristics of the patient were recorded. When an identical species of *Aspergillus* was isolated on more than one occasion from the same patient, it was counted only once.

Aspergilloma was diagnosed when a characteristic fungus ball was detected within a lung cavity by chest radiograph or computed tomography and a positive serological test (antibody detected) was obtained or *Aspergillus* spp. was isolated from respiratory samples. CNPA was diagnosed when chest radiographs showed a cavitary pulmonary lesion with evidence of paracavity infiltrates or cavitary change developing over a time-frame of several weeks to several months in patients with chronic pulmonary symptoms, elevated inflammatory markers, and positive serological tests (antigen/antibody detected) or isolation of *Aspergillus* spp. from respiratory samples [17]. IPA was diagnosed when chest radiographs showed acute and extensive pneumonia or nodular pneumonia resistant to broad-spectrum antibiotics in immunocompromised patients, and respiratory samples yielded *Aspergillus* spp. without pathogenic bacteria. ABPA was diagnosed according to the criteria of Rosenberg [5]. Serological tests including *Aspergillus* antibody and antigen tests were performed when the patients were suspected to have pulmonary aspergillosis. Patients were diagnosed as having airway colonization when *Aspergillus* spp., were isolated from respiratory samples without clinical or histological evidence of aspergillosis. The all of charts were reviewed by T.T., K.I., M.T., T.T., T.M., and H.K. as chart review committee. All of chart review committee discussed each case if there is incorrect or unclear diagnosis of aspergillosis. All data was reviewed by all authors.

RESUTLS

During the 11-year study period, 165 isolates of *Aspergillus* spp. were detected from the cultures of respiratory samples of 139 patients. Of these patients, 62 (45%) were colonized with *Aspergillus* spp. but displayed no clinical symptoms of aspergillosis, while the remaining 77 (55%) had a form of pulmonary aspergillosis, which could be classified as CNPA (48%), aspergilloma (29%), IPA (13%), or ABPA (10%). The clinical characteristics of the patients are summarized in Table 1.

The patients with CNPA or aspergilloma displayed a high occurrence of associated chronic lung disease, including chronic obstructive pulmonary disease (COPD), non-tuberculous mycobacteriosis (NTM), pulmonary tuberculosis, lung cancer, and bullae or a history of pneumothorax, interstitial pneumonia, and pneumoconiosis. In addition, the majority of patients with CNPA suffered from a mild systemic immunosuppressive condition, such as diabetes mellitus, solid-organ cancer, chronic liver disease, and corticosteroid or cytotoxic drug use. In patients with IPA, systemic immunosuppression due to hematologic malignancy, corticosteroid therapy, or chemotherapy were the major predisposing factors, while patients with ABPA often displayed bronchial asthma (88%) and/or other atopic diseases (63%). Of the 62 patients with *Aspergillus* colonization, nearly all had an associated chronic lung disease, such as tuberculosis, COPD, lung cancer, NTM, interstitial pneumonia, and/or an immunosuppressive condition induced by corticosteroid use, collagen vascular disease, solid-organ cancer other than lung, hematologic malignancy, or diabetes mellitus. In-hospital mortality was highest in patients with IPA (80%), whereas it was relatively low in patients with CNPA (16%), aspergilloma (5%), and *Aspergillus* colonization (2%). None of the patients with ABPA died during their hospitalization period.

We determined the distribution of *Aspergillus* spp. among the 165 isolates from the respiratory cultures of 139 patients (Table 2). The two dominant species of *Aspergillus* were *A. fumigatus* (41%) and *A. niger* (32%), with *A. versicolor* (12%), *A. terreus* (6%), *A. flavus* (5%), *A. nidulans* (2%), *A. sydowii* (1%), and unidentifiable *Aspergillus* spp. (0.6%) accounting for the remaining isolates. When the patient data between 1998 and 2004 were compiled, *A. fumigatus* was the most commonly isolated species (58.2%), followed by *A. niger* (20.9%), *A. flavus* (10.4%), *A. versicolor* (6.0%), and *A. terreus* (4.5%). However, between 2005 and 2009, the incidence of *A. fumigatus* isolation decreased by approximately half (28.6%), whereas that of *A. niger* isolation increased approximately two fold (39.8%), with *A. versicolor* (16.3%) and *A. terreus* (7.1%) again being the next most common isolates (Figure 1).

To gain a better understanding of the diagnostic significance of the presence of *Aspergillus* spp. in respiratory samples, we examined the correlation between the identified species and the clinical characteristics of patients with respect to disease. It was revealed that *A. fumigatus* was the most commonly isolated species in patients with IPA (82%), aspergilloma (68%), and CNPA (54%), while *A. niger* was the second most prevalent isolate in patients with these diseases. In patients with ABPA, *A. niger* was the most common isolate (40%), followed by *A. fumigatus* (30%). *A. niger* was also the most common isolate (48%) in patients asymptomatic for aspergillosis, followed by *A. versicolor* (20%) and *A. fumigatus* (16%). Interestingly, with respect to colonization of *Aspergillus* species, 70% (14/20) and 62% (33/53) of cases of *A. versicolor* and *A. niger* isolation, respectively, were considered as asymptomatic colonization, whereas only 16% (11/67) of cases of *A. fumigatus* isolation were not associated with disease.

Finally, we examined the respiratory tract colonization profiles and patterns among the 139 patients positive for *Aspergillus* spp. In most patients (83%), only a single *Aspergillus* spp. was isolated from respiratory tract samples, whereas two or more species were isolated in the remaining patients (17%). In 17 of these 23 patients, a synchronous isolation pattern was observed, which was characterized by isolation of two or more species in the same sample or different samples obtained within a 1-month period (Table 3). The remaining six patients displayed a metachronous isolation pattern, in which different samples obtained with an interval of over 1 month was revealed (Table 4). The combination of *A. fumigatus* and *A. niger* was the most common profile for both the synchronous and metachronous isolation patterns of the observed species combinations.

DISCUSSION

Although the genus *Aspergillus* consists of approximately 200 species [2, 18], only a few are considered human pathogens and generally require immunocompromised hosts to cause disease. Among *Aspergillus* spp., *A. fumigatus* is the primary causative agent of human infections, followed by *A. flavus*, *A. terreus*, *A. niger*, and *A. nidulans* [8, 19–26]. In the present retrospective study, *A. fumigatus* was the most commonly isolated species from respiratory samples, followed by *A. niger*, *A. versicolor*, *A. terreus, A. flavus*, *A. nidulans,* and *A. sydowii.* However, our analysis of laboratory records and clinical disease revealed that the isolation of a particular *Aspergillus* spp. does not confirm it is the etiologic pathogen of the patient. The diagnostic value of *Aspergillus* spp. in respiratory samples is not straightforward, principally because of difficulties distinguishing colonization from disease. In our series, 42% (69 of 165) of the isolated *Aspergillus* spp. was considered to represent colonization, and the most common colonized species was *A. niger*, followed by *A. versicolor*, *A. fumigatus*, *A. terreus*, *A. flavus*, *A. sydowii*, and *A. nidulans*. Although *A. niger* is less virulent than *A. fumigatus* and *A. flavus*, it can occasionally cause IPA, CNPA, or ABPA, and may colonize respiratory tracts of patients with chronic lung diseases [8, 23, 24]. From our analyses, *A. niger* was most frequently associated with patients diagnosed with ABPA (40%), but for all other forms of pulmonary aspergillosis, *A. fumigatus* was the dominant isolate. Although *A. fumigatus* is the most pathogenic *Aspergillus* spp., it may also colonize respiratory tracts without leading to clinical symptoms of aspergillosis, as was observed in 16% of cases.

The reported frequency of *Aspergillus* spp. colonization ranges from 36–91% of clinical cases, depending on the patient population studied [9–16]. Perfect et al. [11] reported that approximately 50% of *Aspergillus*-positive cultures represented colonization, with *A. fumigatus* (63%) representing the dominant colonizing species, followed by *A. niger* (14%), *A. flavus* (9%), *A. nidulans* (1%), *A. terreus* (1%), and other species (4%). Although the general trend for the colonizing species in our data was similar, the prevalence of *A. fumigatus* (41%) and *A. niger* (32%) was much more equal. The difference in the incidence of colonizing species between the two studies is considered to be mainly a result of the study periods and patients. Our data were obtained between 1998 and 2009, whereas Perfect et al. [11] conducted their surveillance in 1995. As our data between 1998 and 2004 more closely resembled that of Perfect et al. [11] with respect to the distribution of *A. fumigatus* (58.2%) and *A. niger* (20.9%), it appears that *A. niger* is becoming more prevalent in respiratory tract samples, a speculation that is consistent with a reported

increase in the frequency of *A. niger* isolation in recent years [27–29]. Colonization may represent transient passage in the airway, as long-term benign carriage is typically observed in patients with localized structural or functional pulmonary deficits; however, it may be a warning sign preceding overt *Aspergillus* infection [14, 15].

CNPA, which was originally reported by Binder et al. [7], has been widely used to characterize a syndrome complex consisting of slowly progressive cavitary lung disease, chronic respiratory symptoms, and the presence of precipitating antibodies to *Aspergillus* spp. A few reports related to CNPA have described direct invasion of pulmonary parenchyma by hyphal elements [7, 23, 30]; however, the majority of reports have not found clear evidence of parenchymal invasion despite progressive tissue damage. Denning et al. [31] proposed that chronic cavitary pulmonary aspergillosis (CCPA) accounts for cases in which there is formation and expansion of multiple cavities over time. Furthermore, they proposed that the term CNPA be reserved for cases in which hyphal invasion of tissue is demonstrated, such as the subacute form of IPA. The majority of our patients with CNPA may have been diagnosed with CCPA, because the pathological appearance of hyphal invasion had not been examined. In our patients with CNPA, *A. fumigatus* was the most commonly isolated species (54%), followed by *A. niger* (24%), *A. terreus* (10%), *A. versicolor* (6%), *A. flavus* (4%), and *A. nidulans* (2%). This result is consistent with that of Perfect et al. [11] who reported that the most common *Aspergillus* species identified in patients with CNPA was *A. fumigatus* (80%), followed by *A. niger* (10%), *A. flavus* (2%), and other species (8%).

The profile of *Aspergillus* spp. isolated from patients with aspergilloma was similar to that from patients with CNPA, and consisted of *A. fumigatus* (68%), *A. niger* (12%), *A. terreus* (12%), *A. versicolor* (4%), and *A. nidulans* (4%). Perfect et al. [11] reported that the most common *Aspergillus* species identified in patients with aspergilloma was *A. fumigatus* (69%), followed by *A. niger* (13%), *A. flavus* (2%), and other species (5%).

The majority of IPA is caused by *A. fumigatus*, with the second most frequent pathogenic species being *A. flavus* and, to a lesser extent, *A. niger* and *A. terreus* [8, 32]. In our retrospective study, the most common *Aspergillus* species identified in patients with IPA was *A. fumigatus* (80%), followed by *A. niger* (9%) and *A. flavus* (9%). Perfect et al. [11] reported that *A. fumigatus* (67%) was most commonly isolated, followed by *A. flavus* (16%), *A. niger* (5%), *A. terreus* (3%), and *A. nidulans* (1%).

ABPA is an allergic pulmonary disorder caused by hypersensitivity to *Aspergillus* spp., and *A.*

fumigatus is the primary causal organism. In our series, the isolated *Aspergillus* spp. from patients with ABPA were *A. niger* (40%), *A. fumigatus* (30%), *A. versicolor* (20%), and *A. terreus* (10%). Since we did not test allergic reactions to non-*fumigatus Aspergillus* spp., isolates other than *A. fumigatus* could not be confirmed as the etiologic antigen to ABPA. However, ABPA due to *A. flavus, A. nidulans, A. terreus* or *A. niger* has been reported [33–35]. Recently, Benndorf et al. [36] identified IgE antibodies that exclusively recognize spore extracts from *A. versicolor* in sera from patients, and also found a relationship between increased spore concentration in indoor air or visible mould affection and a positive reaction of sera to *A. versicolor*. As the isolation of *A. versicolor* in respiratory samples is generally considered as colonization [37–40], the possibility that it is an etiologic agent for aspergillosis, including ABPA, cannot be denied.

Two limitations of this study warrant mention. First, as histopathological observations were not available for most patients, we could not differentiate between CNPA and other forms of chronic pulmonary aspergillosis, including CCPA. Second, since immunological tests, such as IgG or IgE antibody titer and antigen tests for non- *fumigatus Aspergillus* spp. were not available, isolates other than *A. fumigatus* could not be confirmed as the etiologic agent of disease in chronic pulmonary aspergillosis including ABPA.

In conclusion, the results of our study show that isolation of *Aspergillus* spp. from respiratory samples does not confirm that they represent the etiologic pathogen, because airway colonization by *Aspergillus* spp. is a common feature in patients with chronic lung disease. Even if *Aspergillus* spp. is isolated in a patient with clinically diagnosed aspergillosis, the colonizing species may not be associated with mycotic disease. The examined clinical, radiological, and microbiological data from 139 patients positive for *Aspergillus* spp. isolates did not allow differentiation between infection and colonization in several cases. It is therefore considered that repeated isolation of the identical *Aspergillus* sp. and detection of anti-*Aspergillus* antibodies and/or *Aspergillus* circulating antigens in sera are needed to conclude that an isolated species represents the etiological organism in immunocompetent or mildly immunocompromised individuals.

Acknowledgements

Potential conflicts of interest

All authors: No conflicts.

References

1. Hospenthal DR, Kwon-Chung KJ, Bennett JE. Concentrations of airborne *Aspergillus* compared to the incidence of invasive aspergillosis: lack of correlation. *Med Mycol* 1998; **36**:165–8.

2. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. *Clin Microbiol Rev* 2009; **22**: 447–65.

3. Walsh TJ, Anaissie EJ, Denning DW, *et al*. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008; **46**: 327–60.

4. Hope WW, Walsh TJ, Denning DW. The invasive and saprophytic syndromes due to *Aspergillus* spp. *Med Mycol* 2005; **43(Suppl 1)**: S207–38.

5. Rosenberg M, Patterson R, Mintzer R, *et al*. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. *Ann Intern Med* 1977; **86**: 405–14.

6. Agarwal R: Allergic bronchopulmonary aspergillosis. *Chest* 2009; **135**: 805–26.

7. Binder RE, Faling LJ, Pugatch RD, Mahasaen C, Snider GL. Chronic necrotizing pulmonary aspergillosis: a discrete clinical entity. *Medicine* 1982; **61**:109–24.

8. Denning D W. Invasive aspergillosis. *Clin Infect Dis* 1998; **26**: 781–803.

9. Ascioglu S, Rex JH, de Pauw B, *et al*. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; **34**: 7–14.

10. Horvarth JA, Dummer S. The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. *Am J Med* 1996; **100**: 171–8.

11. Perfect JR, Cox GM, Lee JY, *et al*. The impact of culture isolation of *Aspergillus* species: A hospital-based survey of aspergillosis. *Clin Infect Dis* 2001; **33**: 1824–33.

12. Levy H, Horak DA, Tegtmeier BR, Yokota SB, Forman SJ. The value of bronchoalveolar lavage and bronchial washings in the diagnosis of invasive pulmonary aspergillosis. *Respir Med* 1992; **86**: 243–8.

13. Treger TR, Visscher DW, Bartlett MS, *et al*. Diagnosis of pulmonary infection caused by *Aspergillus*: usefulness of respiratory cultures. *J Infect Dis* 1985; **152**: 572–6.

14. Khasawneh F, Mohamad T, Moughrabieh MK, *et al*. Isolation of *Aspergillus* in critically ill patients: a potential marker of poor outcome. *J Crit Care* 2006; **21**: 322–7.

15. Soubani AO, Khanchandani G, Ahmed HP. Clinical significance of lower respiratory tract *Aspergillus*

culture in elderly hospitalized patients. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 491–4.

16. Uffredi ML, Mangiapan G, Cadranel J, Kac G. Significance of *Aspergillus fumigatus* isolation from respiratory samples of nongranulocytopenic patients. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 457–62.
17. Kohno S, Masaoka T, Yamaguchi H, et al. A multicenter, open-label clinical study of micafungin (FK463) in the treatment of deep-seated mycosis in Japan. *Scand J Infect Dis* 2004; **36**: 372–9.
18. Geiser DM, Klich MA, Frisvad JC, *et al*. The current status of species recognition and identification in *Aspergillus*. *Stud Mycol* 2007; **59**: 1–10.
19. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiology* 2007; **153(Pt 6)**: 1677–92.
20. Krishnan S, Manavathu EK, Chandrasekar PH. *Aspergillus flavus*: an emerging non-*fumigatus* *Aspergillus* species of significance. *Mycoses* 2009; **52**: 206–22.
21. Hachem RY, Kontoyiannis DP, Boktour MR, *et al*. *Aspergillus terreus*: an emerging amphotericin B-resistant opportunistic mold in patients with hematologic malignancies. *Cancer* 2004; **101**: 1594–600.
22. Baddley JW, Pappas PG, Smith AC, Moser SA. Epidemiology of *Aspergillus terreus* at a University Hospital. *J Clin Microbiol* 2003; **41**: 5525–9.
23. Saraceno JL, Phelps DT, Ferro TJ, Futerfas R, Schwartz DB. Chronic necrotizing pulmonary aspergillosis: approach to management. *Chest* 1997;**112**:541-8.
24. Severo LC, Geyer GR, Porto NS, Wagener MB, Londero AT. Pulmonary *Aspergillus niger* intracavitary colonization. Report of 23 cases and a review of the literature. *Rev Iberoam Micol* 1997; **14**: 104–10.
25. Walsh TJ, Groll AH. Overview: non-*fumigatus* species of *Aspergillus*: perspectives on emerging pathogens in immunocompromised hosts. *Curr Opin Investig Drugs* 2001; **2**:1366–7.
26. Baddley JW, Marr KA, Andes DR, *et al*. Patterns of susceptibility of *Aspergillus* isolates recovered from patients enrolled in the Transplant-Associated Infection Surveillance Network. *J Clin Microbiol* 2009; **47**: 3271-5.
27. Fianchi L, Picardi M, Cudillo L, *et al*. *Aspergillus niger* infection in patients with haematological diseases: a report of eight cases. *Mycoses* 2004; **47**: 163–7.
28. Bulpa P, Dive A, Sibille Y. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease. *Eur Respir J* 2007; **30**: 782–800.
29. Person AK, Chudgar SM, Norton BL, Tong BC, Stout JE. *Aspergillus niger:* an unusual cause of

invasive pulmonary aspergillosis. *J Med Microbiol* 2010; **59**: 834–8.

30. Yousem SA. The histological spectrum of chronic necrotizing forms of pulmonary aspergillosis. *Hum Pathol* 1997; **28**: 650–6.
31. 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(Suppl 3)**: S265–80.
32. Patterson TF, Kirkpatrick WR, White M, *et al*. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. 13 Aspergillus Study Group. *Medicine (Baltimore)*. 2000; **79**:250–60.
33. Laham MN, Allen RC, Greene JC. Allergic bronchopulmonary aspergillosis (ABPA) caused by *Aspergillus terreus*: specific lymphocyte sensitization and antigen-directed serum opsonic activity. *Ann Allergy* 1981; **46**:74–80.
34. Patterson R, Greenberger PA, Halwig JM, *et al*. Allergic bronchopulmonary aspergillosis: natural history and classification of early disease by serologic and roentgenographic studies. *Arch Intern Med* 1986; **146**: 916–8.
35. Greenberger PA. Allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 2002; **110**: 685–92.
36. Benndorf D, Muller A, Bock K. Manuwald O, Herbarth O, von Bergen M. Identification of spore allergens from the indoor mould *Aspergillus versicolor*. *Allergy* 2008; **63**: 454–46.
37. Jussila J, Komulainen H, Kosma VM, Nevalainen A, Pelkonen J, Hirvonen MR. Spores of *Aspergillus versicolor* isolated from indoor air of a moisture-damaged building provoke acute inflammation in mouse lungs. *Inhal Toxicol* 2002; **14**: 1261–77.
38. Dean TR, Roop B, Betancourt D, Menetrez MY. A simple multiplex polymerase chain reaction assay for the identification of four environmentally relevant fungal contaminants. *J Microbiol Methods* 2005; **61**: 9–16.
39. Rydjord B, Hetland G, Wiker HG. Immunoglobulin G antibodies against environmental moulds in a Norwegian healthy population shows a bimodal distribution for *Aspergillus versicolor. Scand J Immunol*. 2005; **62**:281–8.
40. Bellanger AP, Reboux G, Roussel S, *et al*. Indoor fungal contamination of moisture-damaged and allergic patient housing analysed using real-time PCR. *Lett Appl Microbiol* 2009; **49**: 260–6.

Table 1. Clinical characteristics of patients with *Aspergillus*-positive cultures from respiratory samples

Characteristic [a]	No. (%) of patients, according to clinical condition				
	CNPA (n=37)	Aspergilloma (n=22)	IPA (n=10)	ABPA (n=8)	Colonization (n=62)
Age in years, range (mean)	19-83 (64.7)	46-84 (68.7)	18-83 (58.8)	27-80 (51.6)	20-85 (61.8)
Sex (male/female)	29/8	15/7	7/3	4/4	26/36
Underlying lung disease					
COPD	14 (38)	9 (41)	1 (10)		11 (18)
Non-tuberculous mycobacteriosis	13 (35)	1 (5)		1 (13)	10 (16)
Tuberculosis	9 (24)	9 (41)		1 (13)	14 (23)
Lung cancer survivor	5 (14)	2 (9)		1 (13)	11 (18)
Bullae or pneumothorax	4 (11)	3 (14)			4 (6)
Interstitial pneumonia	2 (5)	1 (5)	1 (10)		10 (16)
Pneumoconiosis	2 (5)	1 (5)			2 (3)
Bronchial asthma	1 (3)			7 (88)	1 (2)
Other lung diseases		2 (9)	2 (20)		8 (13)
Immunosuppressive condition					
Solid organ cancer	6 (16)		1 (10)		4 (6)
Hematologic malignancy	1 (3)		5 (50)		4 (6)
Diabetes mellitus	11 (30)		2 (20)		2 (3)
Chronic liver disease	3 (8)		2 (20)		2 (3)
Chronic kidney disease	1 (3)				3 (5)
Collagen vascular disease					10 (16)
Atopic disease				5 (63)	
Other diseases	2 (5)				
Corticosteroid use	3 (8)		5 (50)		12 (19)
Cytotoxic drug use			5 (50)		2 (3)
Bone marrow transplant			1 (10)		
In-hospital mortality	6 (16)	1 (5)	8 (80)	0 (0)	1 (2)

NOTE. CNPA, chronic necrotizing pulmonary aspergillosis; IPA, invasive pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis; COPD, chronic obstructive pulmonary disease

[a] A patient may have more than one characteristic

Table 2. *Aspergillus* species associated with disease based on positive culture results

Aspergillus species	No. (%) of positive culture results, according to clinical condition					
	CNPA (n=50)	Aspergilloma (n=25)	IPA (n=11)	ABPA (n=10)	Colonization (n=69)	Total (n=165)
A. fumigatus	27 (54)	17 (68)	9 (82)	3 (30)	11 (16)	67 (41)
A. niger	12 (24)	3 (12)	1 (9)	4 (40)	33 (48)	53 (32)
A. versicolor	3 (6)	1 (4)		2 (20)	14 (20)	20 (12)
A. terreus	5 (10)			1 (10)	4 (6)	10 (6)
A. flavus	2 (4)	3 (12)	1 (9)		3 (4)	9 (5)
A. nidulans	1 (2)	1 (4)			1 (1)	3 (2)
A. sydowii					2 (3)	2 (1)
Not identified					1 (1)	1 (0.6)

NOTE. CNPA, chronic necrotizing pulmonary aspergillosis; IPA, invasive pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis

Table 3. Synchronously isolated *Aspergillus* species according to clinical condition

Aspergillus species	No. of patients, according to clinical condition					
	CNPA (n=7)	Aspergilloma (n=1)	IPA (n=1)	ABPA (n=2)	Colonization (n=6)	Total (n=17)
A. fumigatus+ *A. niger*	3	1		1	2	7
A. niger+ *A. versicolor*	1				2	3
A. fumigatus+ *A. terreus*	1					1
A. fumigatus+ *A. flavus*			1			1
A. niger+ *A. flavus*					1	1
A. niger+ *A. terreus*				1		1
A. niger+ *A. sydowii*					1	1
A. terreus +*A. nidulans*	1					1
A. fumigatus+ *A. niger*+ *A. versicolor*	1					1

NOTE. CNPA, chronic necrotizing pulmonary aspergillosis; IPA, invasive pulmonary aspergillosis; ABPA, allergic bronchopulmonary aspergillosis

Table 4. Metachronously isolated *Aspergillus* species according to clinical condition

Aspergillus species	Clinical condition (No. of patients)
A. fumigatus → *A. niger*	CNPA (n=2)
A. niger → *A. fumigatus*	CNPA (n=1)
A. niger → *A. fumigatus* → *A. terreus* → *A. versicolor*	CNPA (n=1)
A. niger → *A. fumigatus* → *A. nidulans*	Aspergilloma (n=1)
A. terreus → *A. fumigatus*	Colonization (n=1)

NOTE. CNPA, chronic necrotizing pulmonary aspergillosis

Figure legends

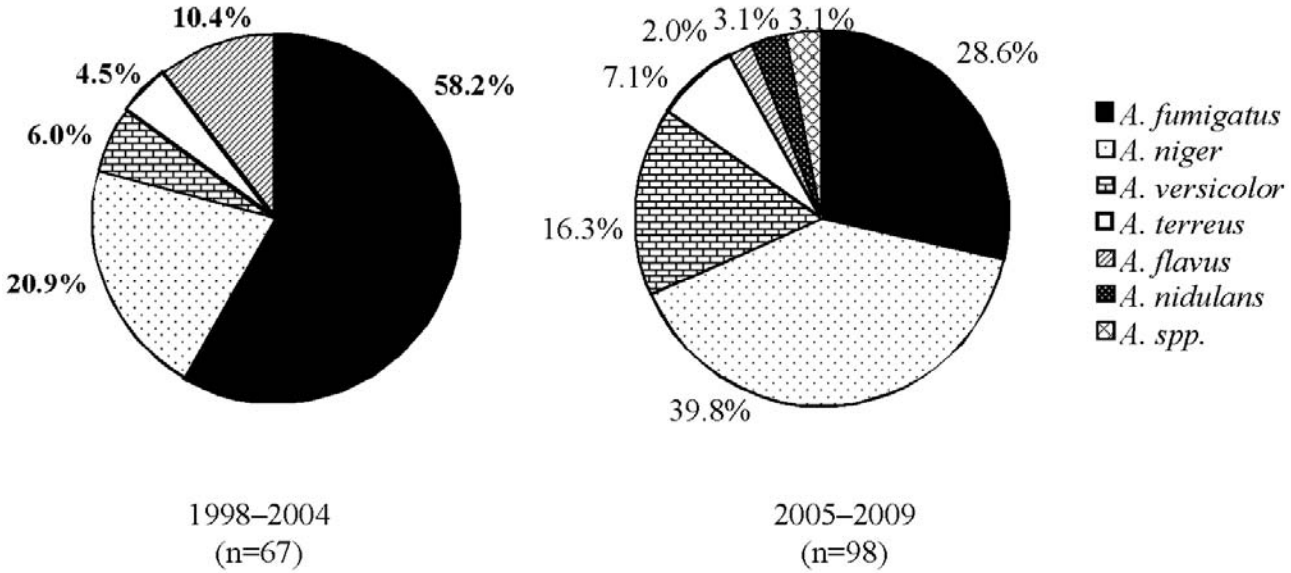

Figure 1. Comparison of *Aspergillus* spp. isolated during 1998–2004 and 2005–2009 from adults in a pneumology ward. *A. fumigatus* was the most commonly isolated species between 1998 and 2004; however, between 2005 and 2009 its frequency of isolation had decreased by approximately half, while *A. niger* increased approximately two fold to become the most commonly isolated species.