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Invasive pulmonary aspergillosis with spontaneous resolution and the diagnostic utility of PCR from tissue specimens

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Summary This case describes a 61-year-old apparently immunocompetent female with invasive pulmonary aspergillosis (IPA) and eosinophilia who demonstrated spontaneous clinical and radiological recovery. The patient had a history of asthma and had been corticosteroid dependent until 2 months prior to her presentation. This report explores the role of PCR in confirming the diagnosis of invasive aspergillosis in circumstances where only histological data are available and highlights the fact that invasive infections with *Aspergillus* spp. can occur without profound immunological deficiency. The case also documents the resolution of IPA without specific therapy.

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Introduction

Invasive aspergillosis (IA) has been documented to occur in a wide range of scenarios, which are most commonly associated with defective host immunity.¹ In many instances the predisposing condition is not reversible, but in circumstances where host immunity is restored, the natural history of IA may be completely different. The following case of invasive pulmonary aspergillosis (IPA) occurred in the context of recent corticosteroid therapy and documents the natural history and resolution without specific antifungal therapy. PCR was used to

confirm that *Aspergillus* spp. was the causative pathogen since culture results were unavailable.

Case report

A 61-year-old woman with asthma, who had until the preceding 2 months, required regular courses of oral prednisolone was admitted to hospital for investigation after the identification of an elevated cancer antigen 125 (CA125) level of 48.7 IU/L (normal 0-35 IU/L), and concerns of gynaecological malignancy. Her symptoms consisted of vomiting, vague abdominal pain, anorexia and bloating. There had been a similar presentation 2 months previously, at which time a specific diagnosis could not be established and her symptoms resolved with

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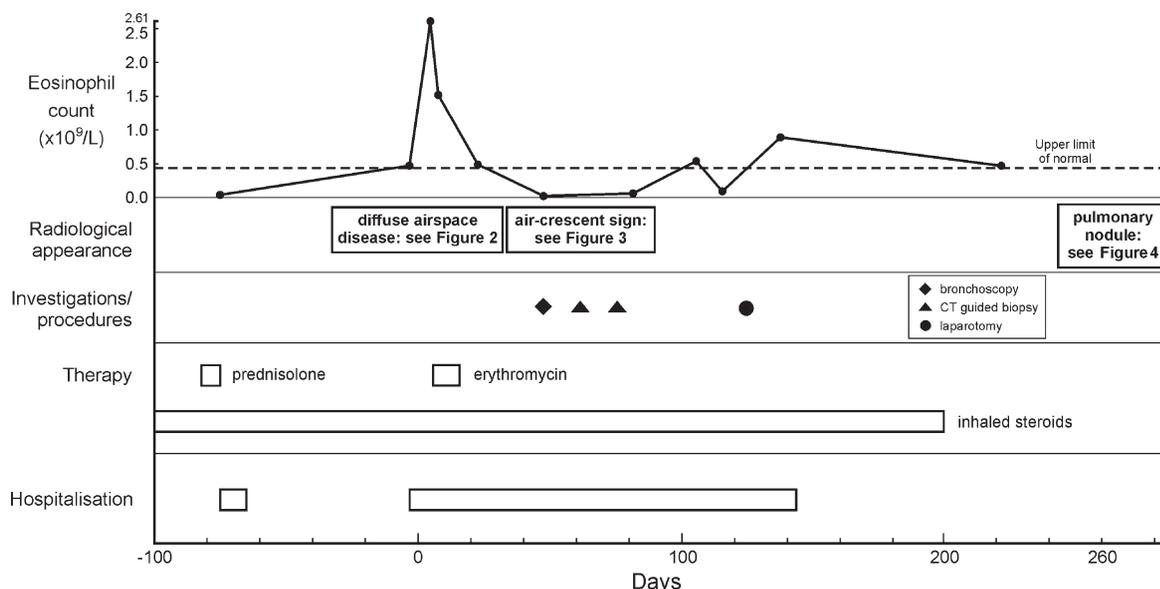


Figure 1 The time course of the illness. Day 0 refers to the initial identification of the radiological abnormality.

conservative management. At the time of the second admission, it was noted the patient was breathless at rest. She had a low grade fever and oxygen saturation was 83% while breathing air.

Her asthma was long standing and managed with inhaled bronchodilators and oral theophylline. Until the preceding 2 months, the patient had been prescribed intermittent courses of oral prednisolone at a dose averaging around 300 mg/month. The patient was an ex-smoker of 6 months duration and had smoked between 15 and 25 cigarettes per day for many years.

The clinical data spanning the two hospital admissions are summarised in Fig. 1. The chest X-ray at the time of the patient’s initial hospitalisation (75 days before the onset of the initial radiological changes) was normal. The radiological appearances documenting the evolution of her pulmonary illness are shown in Figs. 2-4. These demonstrate initial airspace disease (day 0, Fig. 2)

followed by the development of an air-crescent sign at day 62 (Fig. 3) and finally a pulmonary nodule at day 264 (Fig. 4), which persisted despite other evidence of disease resolution. An elevated eosinophil count was observed at a time concordant with the initial radiological abnormality and the temporal course and relationship with the other features of her illness are illustrated in Fig. 1.

A diagnosis of community acquired pneumonia was made and the patient was prescribed erythromycin since she reported an allergy to β-lactam antibiotics.

Given the persistence of the radiological changes despite antibiotic therapy, a bronchoscopy was performed on day 48 (see Fig. 1). Endobronchial disease was excluded and there was no evidence of malignancy on cytological examination of bronchial washings. Unfortunately specimens were not sent for culture. Two weeks later (day 62), a CT-guided biopsy of the chest lesion was undertaken but no



Figure 2 Airspace disease identified at day 0.



Figure 3 Air-crescent sign identified at day 62.



Figure 4 Pulmonary nodule identified at day 264.

diagnostic material was obtained. A second biopsy was organised on day 76 and histology demonstrated a septate filamentous fungus with branching at 45° consistent with *Aspergillus* spp. with chronic inflammation and necrosis. Again, no material was sent for culture. No therapy was commenced, presumably because the fungus was thought to be a contaminant or non-significant. There were no new symptoms referable to the chest.

The patient developed increasing abdominal pain, vomiting and an abdominal X-ray consistent with a small bowel obstruction. A laparotomy was performed which showed an ileal stricture and this was resected. Histological examination was non-specific with no evidence of granulomas or fungal elements. The patient remained well after her surgery and continued to improve 16 months after her initial presentation. A test for *Aspergillus* precipitins (Microgen Bioproducts Ltd. Surrey, UK) was negative when performed after specialist consultation, 10 months after her initial presentation. At no time did she receive antifungal therapy.

In an attempt to confirm the diagnosis of IPA retrospectively, PCR was performed on tissue embedded within the paraffin block. A fragment from the block was placed in 1 ml of octane to which 75 µl of methanol was added and mixed until the wax dissolved. The mixture was centrifuged at 14 000 rpm and the supernatant removed. The tissue was washed in 75 µl of ethanol and centrifuged at 14 000 rpm for 1 min. Ethanol was removed and the resulting pellet dried. The pellet was resuspended in Magnapure PK buffer and proteinase K, incubated for 1 h at 60 °C, then heated at 95 °C for 10 min before processing on Magnapure using the total nucleic acid extraction kit. DNA was eluted in 50 µl ready for use in the PCR reaction mix. Genus specific primers adopted from Kami et al.² were used. A 5 µl volume of DNA was added to the TaqMan reaction mix achieving final

concentrations of 0.3 µM of each forward and reverse primer and 0.2 µM of the FAM labelled probe. The PCR conditions were 2 min at 50 °C, 10 min at 95 °C and then 45 cycles of 95 °C for 15 s followed by 60 °C for 1 min. The sample was positive for *Aspergillus* spp. after 32 cycles.

In retrospect, while very unusual it appears as if the respiratory illness and all of the radiological changes are attributable to *Aspergillus* spp. This hypothesis is supported by a histological picture consistent with *Aspergillus* spp. as well as the positive PCR result.

Discussion

This case highlights two important points. The first is the possibility of using PCR to 'upgrade' the diagnosis from an invasive filamentous fungal infection to proven IPA, and the second is the spontaneous recovery from IPA without any specific antifungal therapy.

The diagnosis of invasive filamentous fungal disease is secured in any context by the demonstration of hyphal elements within tissue. The presence of additional morphological features typical for *Aspergillus* spp. such as dichotomously branching slender septate hyphal elements is not definitive evidence for IA, since other filamentous organisms have similar appearances. Despite this, some therapeutic studies have defined proven IA on the basis of the visualisation of hyphae which resemble *Aspergillus* spp. in histological sections,³ while others⁴ have labelled this entity as probable IA. According to the current EORTC/MSG criteria,⁵ this is a case of proven invasive fungal infection (IFI), but further sub-classification or comment regarding the likelihood of *Aspergillus* spp. as the causative organism is not possible. On the basis of histological data alone, perhaps the most accurate term for this case is proven IFI, probably due to *Aspergillus* spp., reflecting the fact that *Aspergillus* spp. are the commonest cause of invasive filamentous fungal infections.

A diagnosis of proven IA requires evidence which enables identification of the responsible organism to at least genus level. Traditionally at least, a positive culture result for *Aspergillus* spp., preferably obtained from the same site, has served this purpose. The difficulty with this approach is that the rate of positive cultures for *Aspergillus* spp. may be as low as 50%.⁶ In this situation it seems reasonable that other tests which provide genus specific information such as PCR, galactomannan and potentially even antibody detection or

immunohistochemistry could be used in a similar manner. The EORTC/MSG criteria state that an invasive mould infection should be annotated with the relevant genus if such information is available, although non-culture tests have not been specifically explored or evaluated in this context. PCR may be used to 'support a diagnosis' and it may be useful in future revisions of the EORTC/MSG to be more specific as to the precise manner in which this occurs.

While the achievement of high levels of diagnostic accuracy are ideal, the practical significance of upgrading the diagnosis from IFI to IA remains unclear. Specific fungal pathogens which mimic *Aspergillus* spp. in histological sections include *Fusarium* spp. and *Scedosporium* spp. and since their susceptibility to antifungal drugs may differ from *Aspergillus* spp., the inclusion of cases of non-*Aspergillus* infections in therapeutic trials of anti-*Aspergillus* agents at least has the potential to bias results. The multicentre trial of itraconazole conducted by the NIAID/MSG showed that the response rate of patients in whom the diagnosis was based on histology alone was 23% as compared with the overall response rate of 39% although the authors concluded that inclusion of non-*Aspergillus* moulds was an unlikely explanation for this discrepancy.³ Analysis of compassionate use data for itraconazole did not reveal any difference in outcome based upon the method of diagnosis.⁷ We think that it is reasonable that the positive PCR result in the context of the histological demonstration of hyphae in tissue enables the diagnosis of proven IPA to be established. The increased diagnostic accuracy this approach provides, would allow more definitive conclusions regarding the response to antifungal agents to be reached, and in our opinion is a worthy goal.

The case also raises some important issues pertaining to the natural history and outcome of IA. In this respect, the most impressive feature is the documentation of the recovery from IPA without specific antifungal therapy, which, to the best of our knowledge, has not been previously described. The frequent courses of corticosteroids prescribed for asthma probably predisposed to the development of invasive aspergillosis, although the temporal relationship between their administration and the onset of infection is not as close as one might expect. Undoubtedly, however, the fact that systemic steroids were not administered after the onset of overt clinical disease, contributed to the favourable outcome. This case adds to a small but well described group of patients with documented invasive aspergillosis occurring in the setting of corticosteroid therapy for asthma,

chronic obstructive pulmonary disease or other types of chronic lung disease. Not surprisingly, lung is the principal site of infection,⁸⁻¹⁶ although infection at other deep sites such as the vertebral discs has been described, which presumably represents haematogenous dissemination from the lungs.^{17,18} The interval development of eosinophilia is of interest, and might serve to confuse the clinician, as a diagnosis of allergic bronchopulmonary aspergillosis is more common than invasive aspergillosis in the context of asthma, prompting more corticosteroid therapy.

This case also elegantly illustrates the relationship between the degree of immunosuppression and the natural history of IA. The appearances of the CT scans are consistent with IPA, but the slow radiological progression in the context of the relatively indolent clinical course serves as a point of distinction from the fulminant and often fatal syndrome that is characteristic of more severely immunocompromised hosts.¹

In summary, this is a case of invasive pulmonary aspergillosis in a patient without severe immunocompromise, with clear documentation of the clinical and radiological sequence from the onset of disease to resolution. The positive PCR provided confirmation that *Aspergillus* spp. was the causative pathogen. The case illustrates that spontaneous recovery from IA is possible especially without profound immunocompromise.

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