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Non-tuberculous mycobacterial disease & aspergillus-related lung disease in bronchiectasis

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Abstract

Objective: To determine if patients with bronchiectasis and non-tuberculous mycobacteria (NTM) have a higher prevalence of aspergillus-related lung disease.

Methods: A series of 30 consecutive patients with bronchiectasis and NTM (cases) were compared with 61 patients with bronchiectasis and no evidence of NTM (controls). Aspergillus serology and computerised tomography (CT) of thorax were used to identify aspergillus-related lung diseases including aspergilloma, allergic bronchopulmonary aspergillosis (ABPA) and chronic necrotizing pulmonary aspergillosis (CNPA).

Results: The rate of positive aspergillus serology was higher in cases with NTM disease compared to controls (10/30 v 4/61, p=0.005). The radiological features of aspergillus-related lung disease were also commoner among patients with NTM disease than in controls (6/30 v 0/61, p=0.003). This association between NTM disease and aspergillus-related lung disease remained significant after adjustment for confounding effects of age and lung function (adjusted odds ratio 5.1, 95% confidence interval 1.5-17.0, p=0.008).

Conclusion: Patients with bronchiectasis and NTM disease have a higher prevalence of coexisting aspergillus-related lung disease than patients with bronchiectasis without NTM. Identification of aspergillus-related lung disease is important as prognosis amongst undetected cases is invariably poor.

Introduction

Non-tuberculous mycobacterial (NTM) pulmonary disease, particularly that due to *Mycobacterium xenopi, M. kansasii* and *M. malmoense,* has increased in recent years.[1;2] Patients infected with NTM may have co-existing lung disease like bronchiectasis or chronic obstructive pulmonary disease, which can interfere with accurate differential diagnosis at presentation and with assessment of response to subsequent treatment. Patients with chronic lung disease are also prone to aspergillus colonisation and infection.[3] Cystic fibrosis (CF) and NTM infection may be associated with allergic bronchopulmonary aspergillosis (ABPA) and steroid therapy.[4]

Diagnosis of aspergillus-related lung disease is based on tests like serology, respiratory specimen (sputum or bronchoalveolar lavage) examination and radiology, but none of these is a satisfactory gold standard. In addition, sputum examination for *Aspergillus fumigatus* is often unreliable.[5;6] Pulmonary disease caused by *A. fumigatus* is traditionally classified into three distinct groups namely aspergilloma, ABPA and invasive aspergillosis. There may be clinical and radiological overlap between these groups as more than one aspergillus-related lung disease can co-exist.[7] Invasive aspergillosis has been further categorised as angio-invasive and acute or chronic airway invasive aspergillosis (chronic necrotising pulmonary aspergillosis (CNPA) or semi-invasive aspergillosis). [7;8] The former is an aggressive form of aspergillosis seen in severe immunosuppression, whereas the latter is an indolent form of invasive aspergillosis, which has been associated with NTM infection.[9]

NTM disease itself may prove difficult to diagnose due to its non-specific clinical and radiographic presentations, although characteristic radiographic features of some NTM species have been described.[10-12] NTM infection and concomitant aspergillus-related lung disease have been reported previously. For example, aspergillomas may originate in large cavity lesions of inactive *M. kansasii* infection,[13] and they may complicate pulmonary disease due to *M. xenopi.*[14] Patients with *M. malmoense* or *M. avium intracellulare complex* (MAC) infection may have concomitant CNPA.[9] There is also potential for confusion in the diagnosis of NTM infection and aspergillus-related lung disease because both entities can give radiological features of tree-in-bud, nodules and cavities.

It is not clear if in patients with bronchiectasis, whether NTM infection predisposes to aspergillus-related lung disease. This background prompted us to examine in cases of bronchiectasis whether serological markers and radiological features for aspergillus-related lung disease are more often positive in patients with NTM disease compared to those without.

Methods

We conducted a case-control study including consecutive patients with bronchiectasis and NTM seen in our unit during 1995-2003. Cases with NTM were identified by sputum examination as part of routine screening, because of suggestive symptoms or if radiology was suggestive of NTM disease. We routinely check patients with bronchiectasis for

NTM disease annually, however if symptoms or radiology change we test patients more frequently.

Sputum samples were processed for mycobacteria using the modified Petroff's 4% sodium hydroxide method.[15] The processed samples were then inoculated onto two Lowenstein-Jensen slopes (one with pyruvate and one with glycerol). AFB positive smear samples were also inoculated into Middlebrook >H12 Bactec vials. Positive cultures were identified by biochemical and temperature tests and where appropriate Accuprobe.[16] A group of newly referred consecutive patients assessed in our unit who underwent a planned protocol of investigations of their condition and who did not have evidence of NTM served as controls. Cases with NTM were culture positive on three or more occasions for a single NTM species whereas controls were culture negative.

Patients' characteristics, the underlying cause of bronchiectasis, the presence of peripheral blood eosinophilia, and the severity of bronchiectasis using lung function tests including forced expiratory volume in the first second (FEV1) were recorded. Aspergillus serology data including *A. fumigatus* RAST (radioallergosorbent test), total serum immunoglobulin E (IgE) and aspergillus precipitins were collected and sputum was examined for presence of *A. fumigatus*. Positive aspergillus serology was classified as one or more positive results of either aspergillus RAST and/or aspergillus precipitins. The cut-off used for aspergillus RAST was 10 IU/l. Aspergillus RAST and precipitins are specific tests for *A. fumigatus,* the most important species for lung disease.

High resolution computed tomography (HRCT) scans were examined for evidence of aspergilloma, consisting of an upper-lobe, mobile, intracavitary mass with an air crescent in the periphery. [17] Since radiological features of ABPA, including central bronchiectasis and pulmonary infiltrates, are difficult to differentiate in patients with bronchiectasis and NTM infection, clinical and serological criteria including episodic bronchial obstruction (asthma), peripheral blood eosinophilia and positive aspergillus serology were used to diagnose ABPA.[18] HRCT-scans were evaluated for features of CNPA: multiple nodules, focal consolidation, tree-in-bud appearance and mycetoma formation.[7]

For statistical analysis, characteristics and findings of patients with NTM were compared with those without, initially in a univariate analysis using Chi-squared or Fisher's exact test for contingency tables and t-test for means. A multivariate logistic regression model was constructed to examine the association between NTM and aspergillus-related lung disease with adjustment for confounding of effect of other variables like underlying lung function. This allowed us to estimate the association using odds ratios and confidence intervals.

Results

There were 34 cases with bronchiectasis and NTM, 4 with missing data. The NTM species isolated were *M. avium intracellulare* in 18 patients, *M. kansasii* in 5 patients, *M. chelonae* in 2 patients, *M. malmoense* in 2 patients, and *M. fortuitum in* 2 patients and *M. simii* in 1 patient. All patients met the American Thoracic Society (ATS) criteria for NTM disease [19] with more than three positive cultures. Ten out of 30 patients with NTM had radiological evidence of cavities, whereas none of the controls had cavitary lesions.

The characteristics of patients with univariate analyses are shown in table 1. Cases with NTM were characterised by older age (p=0.002) and lower FEV1 levels (p=0.02). The predominant aetiologies of underlying bronchiectasis were different in the two groups. Moreover, lung function was worse in both cases and controls with positive aspergillus markers: the mean FEV1 was 1.30 l among cases with positive aspergillus markers and 1.82 l among those without (p=0.09), whereas among controls the FEV1 was 1.47 l with positive markers and 2.07 l among those without (p=0.08). Overall, the difference in FEV1 between those with and without aspergillus markers was significant (p=0.01) in a multiple linear regression analysis controlling for NTM disease status.

As seen in Table 2, 10 of 30 patients had positive serological markers for aspergillus and six had the radiological features of aspergillus-related lung disease. All 10 patients with positive serology had positive aspergillus precipitins, three had positive aspergillus RAST and four had positive sputum in addition. One patient with an aspergilloma also had *A. fumigatus* in sputum. Among 61 controls, there were six patients with positive serology, of which only one had positive precipitins and RAST. *A. fumigatus* in sputum was isolated from one patient without serological or radiological features of aspergillus-related lung disease. There was no radiological evidence of CNPA or aspergilloma among control patients.

Positive aspergillus serology was more prevalent in cases with NTM disease than in controls (10/30 v 4/61, p=0.005), and this finding persisted after adjustment for FEV1 levels (adjusted odds ratio 3.6, 95% confidence interval 1.1-11.6, p=0.03). The

radiological features of aspergillus-related lung disease were also more frequent in patients with NTM disease (6/30 v 0/61, p=0.003). As shown in table 3, the association of positive aspergillus serology and radiology was more prevalent in NTM disease than in controls (odds ratio 7.01, 95% confidence interval 2.3-21.1, p= 0.0005), and this finding persisted after adjustment for age and lung function (adjusted odds ratio 5.1, 95% confidence interval 1.5-17.0, p= 0.008).

Discussion

This case-control study showed that positive aspergillus serology and radiological features of aspergillus-related lung disease were more common in bronchiectasis with NTM compared to controls. The observed association of NTM and positive aspergillus markers was moderately strong with the odds of positive aspergillus serology being five times higher in cases with NTM exposure. There are several possible explanations as to why the association exists. Patients with NTM have multiple broad spectrum antibiotics before the diagnosis is made and this drives culture findings to fungal rather than bacterial infection. There is a common milieu factor favouring the organisms just as organisms are associated in CF.[20] A common host defence abnormality, steroid therapy or immunosuppression due to chronic disease may predispose to both NTM and aspergillus-related lung disease. There may be a severity association and as FEV1 is an imperfect measure of disease severity, aspergillus colonisation becomes a surrogate for disease severity associated with NTM. The lung disease of NTM is unusually destructive causing cavities, independent of the level of airflow obstruction, and this provides a favourable environment for *A. fumigatus*.

The validity of our findings depends on the strength of our study methods. Case-control design falls below cohort studies in the hierarchy of epidemiological evidence. However, it is the most suitable approach for rare diseases and it is a significant improvement over case series without control groups. The features of good case-control studies relate to minimisation of bias and confounding. We verified case and control status using robust tests for diagnosis thereby reducing bias due to the risk of misclassification as much as possible. The key deficiency of our study is the inability to confirm the diagnosis of aspergillus-related lung disease with confidence due to its ubiquitous nature, a problem that is inherent in all such studies. Our case-control design is best suited to deal with this issue as the diagnostic errors are likely to affect both groups studied. Moreover our multivariate analytic approach aimed to adjust for confounding factors that could lead to diagnostic imbalances between groups.

Further support for our analytic approach comes from the clinical imperative to investigate patients with available tests, despite their deficiencies. ABPA is usually diagnosed by a variety of clinical, serological and radiological criteria.[18] With regard to radiological criteria, pulmonary infiltrates and central bronchiectasis are usually suggestive of ABPA. However they may be encountered in severe bronchiectasis such as CF as a result of chronic bacterial infection and may therefore not be appropriate as selective criteria for diagnosis.[21] NTM infection especially MAC infection can give rise to bronchiectasis and pulmonary infiltrates as seen in ABPA[10;11;22] In view of this we did not attempt to review CT-scans for evidence of ABPA, but relied on combined clinical

and serological criteria as recommended for patients with CF.[21] Depending on severity of ABPA the serum IgE and IgG antibodies to *A. fumigatus* may be low or high.[17] Furthermore, patients with mild ABPA may have abnormal serological markers without suggestive CT features.[23] ABPA may be associated with an increased risk of invasive aspergillosis, especially when treated with corticosteroids.[24] In our study, cases with NTM compared to controls had an increased rate of clinical and serological evidence of ABPA, however this was not statistically significant.

Most aspergillomas develop in pre-existing tuberculous cavities, which frequently occur in the upper lobes and superior segment of the lower lobes. However, all cavities in our study were found in the upper lobes. Lung cavities often become colonized with *A. fumigatus*, but it is their infrequent progression to aspergilloma formation or to invasive aspergillosis that leads to significant morbidity and mortality. The diagnosis of an aspergilloma is usually made on radiographic features, when an upper-lobe, mobile intracavitary mass with an air crescent in the periphery is present.[17] Patients usually have positive aspergillus precipitins, however some patients may be seronegative. [18]Although aspergillomas are often thought to be due to colonizations of the lung with *A. fumigatus*, the manifestations of aspergillus-related lung disease overlap with a continuum between colonization and tissue invasion. Invasive pulmonary aspergillosis may develop from an aspergilloma. In our study definite features of an aspergilloma occurred in two cases and probable features in three patients. Out of these one case had clinical and serological evidence of ABPA.

In invasive aspergillosis the use of antibodies against *A. fumigatus* has produced conflicting results, as patients in high risk groups are frequently falsely seronegative. With antibody or antigen testing, serial assays appear more valuable than isolated tests, but specific recommendations about frequency of testing have not been established. [25] When faced with a positive respiratory specimen for *A. fumigatus* or positive aspergillus precipitins the patient may be colonized or infected.[18] In immunosupressed patients e.g. post-transplantation, isolation of *A. fumigatus* from a respiratory specimen is considered to have a high positive predictive value of invasive aspergillosis.[5] In immune-competent patients with CNPA however reliable diagnostic criteria are missing and histopathological confirmation cannot always be obtained.[6;8]

Sputum examination often gives false-positive results in aspergillus-related lung disease and we therefore did not include an isolated positive *A. fumigatus* in sputum without positive radiology or serology in our analysis. It is not clear what positive predictive value should be attributed to positive aspergillus serology in patients with bronchiectasis and NTM. One third of our cases with NTM have large cavities, which might be colonized with *A. fumigatus* and give positive results of aspergillus precipitins. Also radiographic findings in aspergillus-related lung disease are not diagnostic, because other infections and conditions may sometimes produce similar pictures. [26] There is no gold standard for the diagnosis of ABPA and established diagnostic criteria can often only be partially fulfilled in certain patient groups.[27] In view of this difficulty and as the gold

standard to ascertain a diagnosis of CPNA and aspergilloma is based on open lung biopsy or post mortem examination, it is impractical to undertake test accuracy studies. This issue is best addressed by observational studies of the type we have reported here.

Combined serology, sputum and radiology for aspergillus-related lung disease will aid in reducing diagnostic uncertainty and inadequate treatment in NTM disease until new more accurate tests become commonly available. There are many examples of poor outcome in NTM disease and coexisting aspergillus-related lung disease: A case series of four patients with NTM infection who deteriorated or relapsed after treatment secondary due to concomitant aspergillus infection has been reported.[9] The radiological appearances of these patients were in keeping with CPNA. Other recent case reports have identified concomitant infection with aspergillus as a possible reason for failure to respond to antimycobacterial chemotherapy. Bollert *et al.* reported co-infection by *M. malmoense* and aspergillus in three patients, all of whom died despite antimycobacterial and antifungal treatment. Two of the three patients had evidence of an aspergilloma at post-mortem examination.[3] Similarly, Debieuvre *et al* reported a fatal case of *M. malmoense* complicated by co-infection with aspergillus.[28] Two other case series with poor outcome have described complex aspergillomas complicating *M. kansasii* and *M. xenopi* infections.[13;14]

We propose an association between NTM and aspergillus-related lung disease, that is important for diagnosis of the disease in clinical practice. Whether or not there is a causal

relationship needs to be determined by further studies. It would also be useful to know if routine screening and early treatment of aspergillus-related lung disease leads to an improvement in outcome compared to standard care. Due to rarity of the condition, it would be extremely difficult to launch a randomized trial, so more robust observational studies are required. The practical implication of our finding is that in patients with bronchiectasis and NTM infection it is important to investigate for coexisting aspergillus-related lung disease infection early at the time of starting treatment for NTM. This is because the outcome of such cases is generally poor,[3;28] and treatment of the fungal disease might improve outcome. If initially not diagnosed, aspergillus-related lung disease may be subsequently mistaken as treatment failure or as relapse of NTM infection.[9]

Table 1: Characteristics of patients with bronchiectasis according to non-tuberculous mycobacterial (NTM) lung disease.

Characteristics	Non-tuberculous mycobacterial (NTM) lung disease		*P*-value*
	Yes (N = 30)	No (N = 61)	
Mean age (years)	62.2 (SD 14.6)	51.6 (SD 15.3)	0.002
Gender			
Male	9	23	0.46
Female	21	38	
Underlying aetiology of bronchiectasis+			
Idiopathic	10	27	0.002
Post Tuberculosis	4	0	
Post adulthood infection	1	10	
Post childhood infection	2	8	
Rheumatoid Arthritis	2	1	
Primary ciliary dyskinesia	3	7	
Diffuse pan-bronchiolitis	0	2	
Allergic bronchopulmonary aspergillosis	4	3	
Yellow nail syndrome	0	3	
Miscellaneous	4	0	
Pulmonary function tests			
FeV1 (l)	1.6 (SD 0.8)	2.0 (SD 0.8)	0.02
RV (% predicted)	147 (SD 35)	152 (SD 45)	0.62
TLCO (% predicted)	67 (SD 25)	77 (SD 15)	0.05

***** Univariable analysis using Chi-squared or Fisher's exact test for contingency tables and t-test for comparison of means.

+ The most predominant aetiology reported. Some patients had two aetiologies.

Table 2: Features of aspergillus-related lung disease in patients with bronchiectasis according to non-tuberculous mycobacterial (NTM) lung disease

Features	Non-tuberculous mycobacterial (NTM) lung disease		*P*-value*
	Yes (N = 30)	No (N = 61)	
Eosinophil count/ Total IgE			
Eosinophilia > 0.5	2	3	1.0
IgE > 150	4	14	0.27
Size of IgE among positives	2740 (SD 2709)	835 (SD 1520)	0.08
Positive serology for aspergillus-related lung disease +			
Total number of patients with positive serology	10	6	0.005
Positive Aspergillus fumigatus precipitins and RAST	3	1	0.10
Positive Aspergillus fumigatus precipitins	10	4	0.003
1 line	2	1	
2 lines	6	3	
3 lines	2	0	
Positive Aspergillus fumigatus RAST	3	2	0.32
Radiological features suggestive of aspergillus-related lung disease	6	0	0.003
Invasive aspergillosis (CNPA)	1		
Probable aspergilloma	3	0	
Definite aspergilloma	2	0	
Clinical and serological features consistent with ABPA			
ABPA	4	3	0.2
ABPA, aspergilloma	1	0	

* Univariable analysis using Chi-squared or Fisher's exact test for contingency tables and t-test for comparison of means.
CNPA= chronic necrotising pulmonary aspergillosis
ABPA= allergic bronchopulmonary aspergillosis
+ see method section for details

Table 3: Logistic regression estimates of the association between non-tuberculous mycobacterial (NTM) lung disease and aspergillus-related lung disease.

	Simple Regression		Multiple Regression*	
Independent Variable	**Odds Ratio (95% Confidence Intervals)**	**p value**	**Odds Ratio (95% Confidence Intervals)**	**p-value**
Non-tuberculous mycobacterial (NTM) lung disease (Yes / No)	**7.01** (2.3 - 21.1)	0.0005	**5.1** (1.5 - 17.0)	0.008
FEV1 (litres)	**0.25** (0.10 - 0.64)	0.003	**0.34** (0.13 - 0.89)	0.028

*Multiple logistic regression model with aspergillus-related lung disease (serology and radiology combined) as the binary dependent variable and NTM lung disease, age and FEV1 as independent variables. Age did not contribute significantly to the model. See methods for details.

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