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Early View

Research letter

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Nontuberculous mycobacterial pulmonary disease and *Aspergillus* co-infection: Bonnie and Clyde?

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Take Home Message: Forty percent of patients diagnosed with nontuberculous mycobacterial lung disease also meet diagnostic criteria for chronic pulmonary aspergillosis and *Mycobacterium avium* stimulates *Aspergillus* growth, *in vitro*

Nontuberculous mycobacteria (NTM) cause difficult-to-treat opportunistic infections, most frequently of the lungs. Patients with COPD, cystic fibrosis or bronchiectasis are prone to non-tuberculous mycobacterial pulmonary disease (NTM-PD) and other opportunistic infections, including by *Aspergillus fumigatus*. Co-infections are difficult to identify as diagnostic criteria for NTM-PD and chronic pulmonary aspergillosis (CPA) overlap [1,2]. Literature suggests that NTM and *Aspergillus* co-infections are associated with higher mortality [3]. Therefore, *Aspergillus* serology is part of NTM-PD diagnostic work up in our reference center.

In this retrospective single-center cohort study, we assessed the frequency of *Aspergillus* IgG seropositivity and its relation to disease outcome in patients with NTM-PD. Furthermore, we studied symbiosis of *Mycobacterium avium* and *M. abscessus* with *A. fumigatus*.

We selected all patients who met American Thoracic Society (ATS) diagnostic criteria for NTM-PD between January 2015-January 2018 and had *Aspergillus* IgG serology results available from the time of NTM-PD diagnosis or referral (+/- 3 months) [1]. Patients with cystic fibrosis were excluded from the analysis.

For all included patients, we registered clinical, microbiological and radiographic features; the latter were recorded from radiologists' CT scan reports and re-analyzed by two expert pulmonologists (CM-E, WH). Positive IgG serology for *Aspergillus* was defined as >39 mg/l, as recommended by the manufacturer (ImmunoCAP, Phadia/ThermoFisher, Landsmeer, the Netherlands).

Treatment outcomes were defined according to the NTM-net consensus [4]. Statistical analyses were performed using SPSS version 25 (IBM, New York, USA); we applied Student's *t* or Fisher-exact tests for comparative statistics.

In vitro symbiosis of *A. fumigatus* with *M. avium* ATCC700898 and *M. abscessus* CIP104536 was assessed using CAMH medium supplemented with supernatant of the other genus. Cultures of *A. fumigatus* were supplemented with supernatant of *M. abscessus* incubated for 30 and 72 hours and of *M. avium* incubated for 72 and 168 hours, to reflect mid-log phase and stationary phase. NTM

growth was measured by colony forming unit counting. Growth of liquid *A. fumigatus* cultures was evaluated by optical density (OD) monitoring [5].

Forty-seven patients met the inclusion criteria of which 53.2% were female, with a mean age of 64.4±9.7 years. Thirty (63.8%) patients had positive *Aspergillus* IgG serology (21/34 with *M. avium* complex [MAC], 5/6 with *M. abscessus*), with a mean level of 67.2±56.1 mg/l. Baseline characteristics did not differ between the *Aspergillus* IgG-positive and -negative patients, considering age, sex, history of smoking, comorbidities, radiological presentation. Sixteen of the twenty-seven (59.3%) patients with fibro-cavitary disease and 12/18 (66.7%) patients with nodular-bronchiectatic disease were *Aspergillus* IgG-positive; two had other NTM-PD manifestations.

Out of 37 patients with sputum cultures for fungi, *Aspergillus* cultures were positive in 19 patients (51.4%; 13 sputum only, 4 broncho-alveolar lavage (BAL) only, 2 BAL and sputum); *A. fumigatus* was most frequently isolated (n=18, 94.7%), one patient had a single *A. niger* isolate in sputum culture. Fourteen of these 37 patients (37.8%) had positive cultures and simultaneous elevated *Aspergillus* IgG levels. *Aspergillus* culture results did not differ between fibro-cavitary and nodular bronchiectatic disease groups. Six patients received azole (4 voriconazole, 1 itraconazole, 1 posaconazole) therapy on basis of positive serology and culture; antifungal treatment had no significant effect on either culture conversion (p=0.587) or microbiological cure (p=0.678) of NTM-PD.

Overall, 43 (91.5%) of 47 NTM-PD patients were treated for their NTM-PD, of which 33 (70.2%) for >6 months (26 MAC, 3 *M. abscessus*, 2 *M. kansasii*, 1 *M. simiae* and 1 *M. xenopi*). Twenty-two MAC-PD patients (85%) were treated with a rifamycin-ethambutol-macrolide based regimen. Four (15%) patients received a clofazimine-ethambutol-macrolide based regimen. Sixteen received additional amikacin and/or clofazimine.

NTM culture conversion, in patients treated for >6 months, was less frequent in patients who had positive *Aspergillus* IgG (6/21, 28.6%) than in those with negative *Aspergillus* IgG (8/12, 66.7%;

p=0.039). Microbiological cure rates were lower in patients treated for >6 months who had positive *Aspergillus* IgG (3/21, 14.3%) compared to patients with negative *Aspergillus* IgG (6/12, 50%; p=0.036). Time to NTM sputum culture conversion did not differ significantly between the *Aspergillus* IgG-positive and negative groups (8.7±5.4 and 14.1±13.1 weeks, p=0.315). In MAC-PD patients, culture conversion was also less frequent in *Aspergillus* IgG-positives (3/17) than in IgG-negatives (5/9; p=0.063) as was microbiological cure (1/17 vs. 4/9; p=0.034).

Treatment outcomes also differed between fibro-cavitary and nodular-bronchiectatic disease, as treatment more frequently failed in patients with fibro-cavitary disease manifestation (12/21 (57.1%) for fibro-cavitary and 2/12 (16.7%) for nodular-bronchiectatic disease, p=0.058), and microbiological cure was more common in nodular-bronchiectatic disease (6/12, 50.0%) than in fibro-cavitary disease (3/21, 14.3%; p=0.036).

Aspergillus fumigatus showed a strongly decreased growth rate in medium supplemented with *M. abscessus* supernatant; *M. avium* supernatant increased the *A. fumigatus* growth rate. The effect was strongest for stationary phase supernatants (Figure 1). Mycobacterial growth was not influenced by *A. fumigatus* supernatants.

In this NTM-PD cohort, co-infection with *A. fumigatus* was common in NTM-PD as 63.8% of the patients had positive *Aspergillus* IgG and 37.8% had positive IgG and *Aspergillus* cultures. Previous studies have reported IgG-positivity rates of 7-12% and *Aspergillus* culture positivity rates of 6-12%, lower than in our cohort [3,6-8]. This difference may be partly explained by the high percentage of fibro-cavitary NTM-PD cases in our cohort. Fibro-cavitary disease was reported as a risk factor for developing CPA [3,6,8]. Still, fibro-cavitary disease was equally frequent in the *Aspergillus* IgG-positive and IgG-negative group in our cohort (53% vs 65%; p=0.851).

Establishing a diagnosis of NTM-PD and *Aspergillus* co-infection or CPA is difficult; diagnostic criteria for NTM-PD state that alternative diagnoses need to be excluded [1], while diagnosing mycobacterial

infections does not exclude CPA, as radiological progression prior to starting antimycobacterial or antifungal therapy was accepted as evidence of CPA [2]. Ignoring this caveat, 37.8% of our patients with sufficient data met diagnostic criteria for both NTM-PD and CPA.

Aspergillus IgG positivity seems of clinical importance as microbiological cure rates were lower in both IgG positive patients with NTM-PD (p=0.036) and for MAC species specifically (p=0.034). While in accordance with the observed poor prognosis in patients with NTM and fungal co-infections [3,6-9], this specific association has not been previously observed; it might hint at subtle immunodeficiencies increasing susceptibility to mycobacterial and fungal infection. Screening NTM patients for *Aspergillus* co-infection seems clinically relevant, even if the implications of positive results do not yet extend beyond associations with poorer treatment outcomes. The effect of CPA treatment on the prognosis and course of NTM-PD should be established in clinical trials.

Recently, a cut-off of 50 mg/L in the ImmunoCAP *Aspergillus* IgG assay has been proposed for the diagnosis of CPA, based on a European cohort [10]. Applying this new cut-off, 18/47 (38.3%) of the cohort tested positive; the trend towards poor outcomes remained but was no longer significant for culture conversion (p=0.289) or microbiological cure (p=0.239).

M. avium produces substances that significantly stimulate *Aspergillus* growth; if secreted in NTM-PD, these substances may directly increase the risk for *Aspergillus* colonization and ultimately CPA. It is striking that *M. abscessus* supernatants inhibit growth of *A. fumigatus*. This observation warrants further investigation.

In conclusion, a large proportion of patients diagnosed with NTM-PD had signs of *Aspergillus* infection or met diagnostic criteria for CPA. *Aspergillus* infection evidenced by IgG-positivity correlated with worse NTM-PD treatment outcomes. *M. avium* may aggravate *Aspergillus* infection by direct interaction. All patients with NTM-PD need to be screened for *Aspergillus* co-infection by

IgG serology and culture. The clinical relevance of *Aspergillus* co-infection and the optimal approach to treatment should be assessed through randomized clinical trials.

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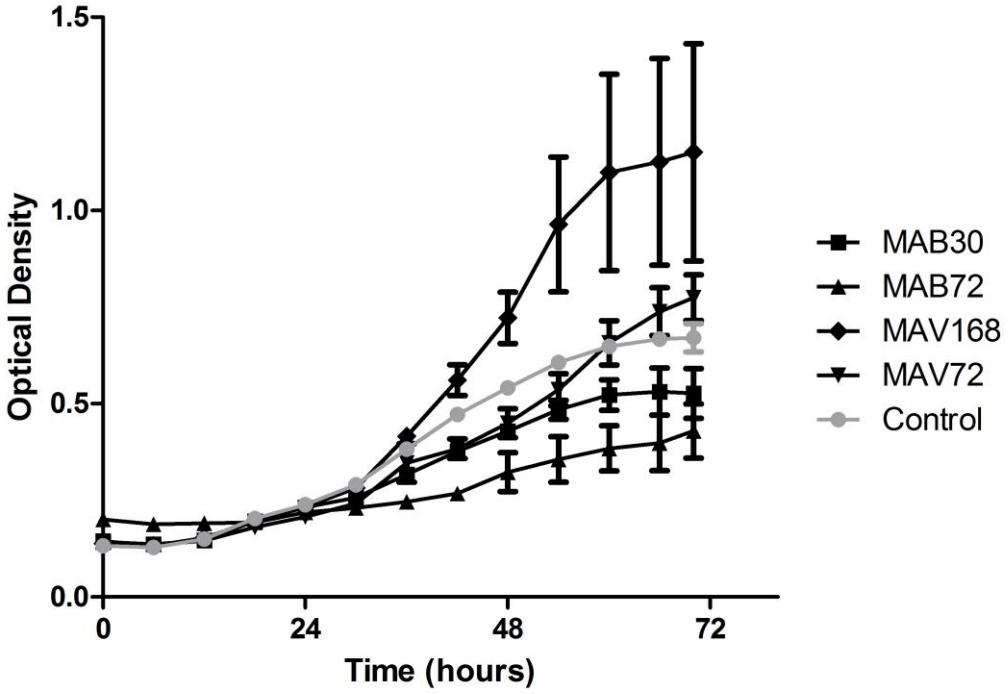
Figure: *Aspergillus fumigatus* growth rate in RPMI1640 medium with and without NTM culture supernatants

Note: growth rate is expressed by optical density. Control: RPMI1640 without supernatants; MAB30: with *M. abscessus* logarithmic growth phase supernatant; MAB72: with *M. abscessus* stationary phase supernatant; MAV72: with *M. avium* logarithmic growth phase supernatant; MAV168: with *M. avium* stationary phase culture supernatant


1.5
1.0
0.5
0.0
Optical Density
0
24
48
72
Time (hours)
MAB30
MAB72
MAV168
MAV72
Control