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

To the Editor:

Nontuberculous mycobacteria (NTM) cause difficult-to-treat opportunistic infections, most frequently of the lungs. Patients with chronic obstructive pulmonary diseases, cystic fibrosis or bronchiectasis are prone to NTM pulmonary disease (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]. The 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 centre.

In this retrospective, single-centre 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 *Mycobacterium abscessus* with *A. fumigatus*.

We selected all patients who met the American Thoracic Society diagnostic criteria for NTM-PD between January 2015 and 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' computed tomography scan reports and re-analysed by two expert pulmonologists (C. Magis-Escurra and W. Hoefsloot). Positive IgG serology for *Aspergillus* was defined as >39 mg·L^{-1}, 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, Armonk, NY, USA); we applied Student's t-test or Fisher's exact test for comparative statistics.

In vitro symbiosis of *A. fumigatus* with *M. avium* ATCC700898 and *M. abscessus* CIP104536 was assessed using CAMH medium supplemented with culture supernatant of the other genus. Cultures of *A. fumigatus* were supplemented with the supernatant of *M. abscessus* incubated for 30 and 72 h, and of *M. avium* incubated for 72 and 168 h, to reflect mid-logarithmic phase and stationary phase, respectively. NTM growth was measured by colony-forming unit counting. Growth of liquid *A. fumigatus* cultures was evaluated by optical density monitoring [5].

47 patients met the inclusion criteria, of whom 53.2% were female, with a mean age of 64.4±9.7 years. 30 (63.8%) patients had positive *Aspergillus* IgG serology (21 out of 34 with *M. avium* complex (MAC) and five out of six with *M. abscessus*), with a mean level of 67.2±56.1 mg·L^{-1}. Baseline characteristics did not differ between the *Aspergillus* IgG-positive and -negative patients for age, sex, history of smoking, comorbidities or radiological presentation. 16 (59.3%) of the 27 patients with fibrocavitary disease and 12 (66.7%) out of 18 patients with nodular–bronchiectatic disease were *Aspergillus* IgG-positive; two had other NTM-PD manifestations.

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Out of 37 patients with sputum cultures for fungi, *Aspergillus* cultures were positive in 19 patients (51.4%; 13 by sputum only, four by bronchoalveolar lavage (BAL) only, and two by both BAL and sputum). *A. fumigatus* was most frequently isolated (n=18, 94.7%); one patient had a single *Aspergillus niger* isolate in sputum culture. 14 (37.8%) of these 37 patients had positive cultures and simultaneous elevated *Aspergillus* IgG levels. *Aspergillus* culture results did not differ between fibrocavitary and nodular–bronchiectatic disease groups. Six patients received azole therapy (four voriconazole, one itraconazole and one posaconazole) 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%) out of 47 NTM-PD patients were treated for their NTM-PD, of which 33 (70.2%) for >6 months (26 MAC, three *M. abscessus*, two *Mycobacterium kansasii*, one *Mycobacterium simiae* and one *Mycobacterium xenopi*). 22 (85%) MAC-PD patients were treated with a rifamycin–ethambutol–macrolide-based regimen. Four (15%) patients received a clofazimine–ethambutol–macrolide-based regimen. 16 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 (six out of 21, 28.6%) than in those with negative *Aspergillus* IgG (eight out of 12, 66.7%; p=0.039). Microbiological cure rates were lower in patients treated for >6 months who had positive *Aspergillus* IgG (three out of 21, 14.3%) compared to patients with negative *Aspergillus* IgG (six out of 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 (three out of 17) than in IgG-negatives (five out of nine; p=0.063), as was microbiological cure (one out of 17 *versus* four out of nine; p=0.034).

Treatment outcomes also differed between fibrocavitary and nodular–bronchiectatic disease, as treatment more frequently failed in patients with fibrocavitary disease manifestation (12 out of 21 (57.1%) for fibrocavitary and two out of 12 (16.7%) for nodular–bronchiectatic disease, p=0.058) and microbiological cure was more common in nodular–bronchiectatic disease (six out of 12, 50.0%) than in fibrocavitary disease (three out of 21, 14.3%; p=0.036).

A. 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.

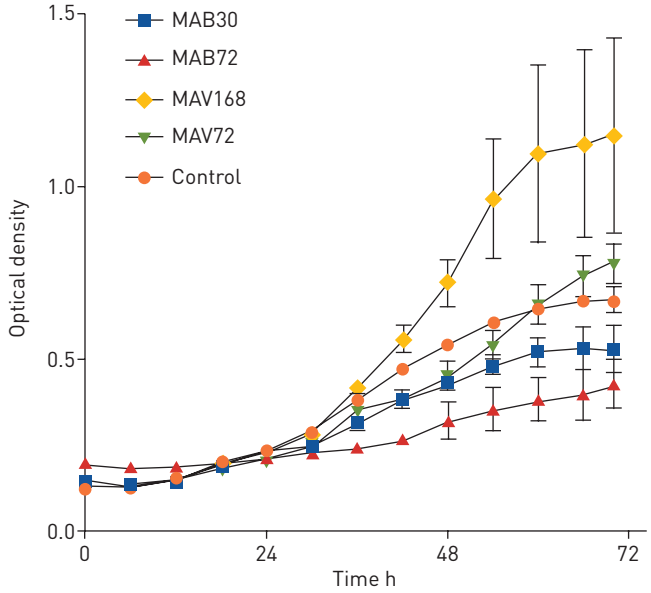

FIGURE 1 *Aspergillus fumigatus* growth rate in RPMI1640 medium with and without nontuberculous mycobacterial culture supernatants. Control: RPMI1640 without supernatants; MAB30: with *Mycobacterium abscessus* logarithmic growth phase supernatant; MAB72: with *M. abscessus* stationary phase supernatant; MAV72: with *Mycobacterium avium* logarithmic growth phase supernatant; MAV168: with *M. avium* stationary phase culture supernatant.

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 fibrocavitary NTM-PD cases in our cohort. Fibrocavitary disease was reported as a risk factor for developing CPA [3, 6, 8]. Still, fibrocavitary disease was equally frequent in the *Aspergillus* IgG-positive and IgG-negative group in our cohort (53% *versus* 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 to be 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 $mg{\cdot}L^{-1}$ 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 (38.3%) out of 47 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* colonisation 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 randomised clinical trials.

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