Inhaled therapies, azithromycin and *Mycobacterium abscessus* in cystic fibrosis patients

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† In memoriam

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Abstract

Cystic fibrosis (CF) patients are at particularly high risk of developing lung disease caused by *Mycobacterium abscessus* complex (MABSC). Over the last 10 years, changes in CF treatment, with increasing use of inhaled therapies and low-dose azithromycin, have been accompanied by an increase in the prevalence of MABSC infections in CF patients. There is therefore some concern about the role of new CF treatments in the emergence of MABSC infections.

We addressed this issue by means of a case-control study including 30 MABSC-positive cases and 60 NTM-negative CF controls matched for age, gender and centre. We also compared practices at the CF centres with the highest prevalence of MABSC with those at the other centres.

No positive association was found between MABSC lung disease and the use of inhaled therapies or low-dose azithromycin in the four years preceding MABSC isolation. These treatments were not significantly more frequently used at the CF centres with the highest MABSC prevalence rates.

In conclusion, there is no evidence for a link between *Mycobacterium abscessus* complex lung disease and inhaled therapies or low-dose azithromycin in patients with cystic fibrosis.
Introduction

Non tuberculous mycobacteria (NTM) are increasingly recognized as common respiratory pathogens in cystic fibrosis (CF) patients, with an overall NTM prevalence ranging between 6.6% and 13% in large prospective studies [1, 2]. The NTM most frequently isolated from CF patients are *M. abscessus* complex (MABSC) (also called *M. abscessus sensu lato*, comprising the closely related species *M. abscessus sensu stricto, M. massiliense* and *M. bolletii*) and *Mycobacterium avium* complex. MABSC accounts for 16 to 51% of the species isolated [1-5]. In addition, MABSC fulfills the ATS criteria for pulmonary NTM infection in 60 to 80% of patients, far more than for other mycobacteria [1, 6]. The isolation of MABSC from CF patients — mostly from teenagers — is always a cause for concern [7]. Chronic MABSC infection is associated with a greater decline of lung function [6]. MABSC can cause severe, pseudotuberculous lung disease [8], in some cases after following an indolent course for a number of years [9]. It may cause disseminated, sometimes fatal infections in lung transplant recipients [10, 11]. These mycobacteria are intrinsically resistant to most of the antibiotics currently available [12] and treatment failure is frequent despite aggressive antibiotic therapy.

The emergence of MABSC in the context of CF has been linked to improvements in surveillance and microbiological methods for the recovery and identification of NTM from respiratory samples from CF patients [6, 13-15]. However, the increase in MABSC isolation from CF patients since the early 1990s has coincided with the development and massive use of “new” therapeutic approaches for CF, including inhaled therapies based on various drugs (steroids, rhDnase, antibiotics) and low-dose azithromycin maintenance treatment [16, 17].

Inhaled steroids were found to be associated with an increase in the risk of pneumonia in COPD patients in several studies [18, 19]. The physiopathological explanation put forward to account for this effect is that these drugs induce a “local” immunosuppressed state. A rationale can also be proposed for an increase in MABSC infection due to inhaled rhDnase treatment.
Neutrophils have recently been shown to generate bactericidal extracellular traps consisting of DNA and cytotoxic granule proteins [20]. The sputum of CF patients consists predominantly of a high-density meshwork of these neutrophil extracellular traps (NETs) and NETosis-derived material [21]. The liquefaction of sputum by inhaled rhDnase results from the degradation of these NETs. *M. abscessus* (*sensu stricto*) has a large number of cysteine desulfurases, which are involved in the Dnd (DNA degradation) phenotype observed in MABSC [22, 23]. Thus, inhaled rhDnase treatment may promote infection with dnd-positive MABSC strains, which could use the DNA from degraded NETs as a nutrient.

The increasing use of oral and inhaled antimicrobial agents may also favour the emergence of multidrug-resistant organisms, such as MABSC [24]. Most of *M. abscessus sensu stricto* isolates from CF patients are azithromycin resistant *in vivo*, through induced expression of the ribosomal methyltransferase *erm(41)* gene [25]. Thus, low-dose azithromycin maintenance treatment cannot prevent colonisation and further infection with MABSC in most cases. In addition, the anti-inflammatory effects of this treatment may impair the immune response to mycobacteria, as suggested in a recent study [26].

We therefore investigated whether inhaled steroids, inhaled rhDnase, inhaled antibiotics and low-dose azithromycin were associated with an increase in the risk of MABSC infection in CF patients. We carried out a case-control study of 30 MABSC-positive cases and 60 NTM-negative CF controls matched for age, gender and centre. We also compared practices between the CF centres with the highest prevalence of MABSC infection and the other centres.

**Methods**

The 2004 French NTM prevalence study included 1582 CF patients from 41 of the 49 French CF centres (17 adult centres, 20 paediatric centres and four mixed adult and paediatric centres) [1]. At least three sputum samples (or other respiratory specimens) were obtained from
each patient included for NTM analysis between 1\textsuperscript{st} January 2004 and 31\textsuperscript{st} December 2004. We included all patients from this study who were positive for MABSC and met the ATS 2007 microbiological criteria for pulmonary NTM infection [27]. We excluded two of the 40 patients fulfilling these criteria because they also tested positive for \textit{M. avium} complex and eight patients for whom no data were available for the year preceding MABSC isolation. Thus, 30 cases were included in the study and each was matched with two controls. Controls were NTM-negative patients included in the NTM prevalence study matched with the cases for age (controls born within $\pm$ 2, 5 and 10 years of the birth date for cases aged 0-15, 16-29 and $\geq$ 30, respectively), gender and centre. If more than two eligible controls were identified for a given case, the two controls actually used were selected at random. All patients, or their parents if they were children, gave their informed consent, and an internal review board approved the study.

Since 1992, a national database, the French cystic fibrosis registry has collected the individual medical and social data annually from CF patients attending the 49 national CF centres in France using a standardized questionnaire. The chief medical officer from each centre filled in patient data sheets during an annual check up. Information about the long-term ($\geq$ 3 months) use of inhaled therapies (antibiotics, rhDNase, steroids) and azithromycin maintenance treatment in cases and controls was obtained from this registry. We also collected the frequency of use of inhaled therapies and low-dose azithromycin in 34 of the 41 centres that participated in the French 2004 NTM prevalence study: 15 paediatric centres (1032 subjects), 9 mixed centres (559 subjects) and 10 adult centres (633 subjects).

Cases and controls were compared in likelihood ratio tests. We assessed the association of infection with inhaled therapies and low-dose azithromycin by estimating univariate and multivariate odds ratios (ORs) with 95\% confidence intervals (95\% CI), by conditional logistic regression analysis [28]. \textit{p}-values were determined for likelihood ratio tests. Exact tests were used
when necessary (SAS/STAT software, version 9.1, SAS Institut Inc, Cary, NC). We considered \( p \)-values \( \leq 0.05 \) to be statistically significant.

**Results**

We included 30 MABSC-positive cases and 60 NTM-negative controls in this study. The cases and controls had similar baseline characteristics. The controls tended to have better \( \text{FEV}_1 \) values than the cases, but this difference was not statistically significant (Table 1). The use of inhaled therapies and low-dose azithromycin by cases and controls in the year preceding MABSC isolation is shown in Table 2. No significant association was found between these treatments and infection. Inhaled steroid treatment tended to be more frequent among the controls (OR = 0.4 [0.1–1.2]), although this difference was not significant (\( p=0.08 \)). A similar analysis was performed with data collected in years -2, -3, and -4. Again, no significant association was found with either inhaled therapies or low-dose azithromycin.

The use of inhaled therapies and low-dose azithromycin at the 34 French CF centres that participated in the 2004 NTM prevalence study is shown in Table 3. The treatments administered differed considerably between centres, even within the paediatric, mixed and adult subgroups. However, the centres with the highest prevalence of MABSC infection in each subgroup (centres A, B and C, respectively) did not seem to use inhaled therapies or low-dose azithromycin with a higher frequency than expected, except for a slightly higher frequency of inhaled steroids at the paediatric centre (centre A) than at other paediatric centres (47%; 95% CI for paediatric centres: 24-42.6%). The frequency of inhaled rhDnase use at the paediatric centre and the mixed centre with the highest prevalence of MABSC infection was also particularly low (Table 3).

**Discussion**
Unlike non-CF bronchiectasis patients, CF patients are particularly susceptible to MABSC infection. This susceptibility may result from CF related treatments or from CF itself. The genetic defect underlying CF was identified in 1989 (mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR)), but the mechanisms by which CFTR defects cause lung disease remain unclear. Various mechanisms have been proposed, such as defective submucosal gland secretion in the airways, abnormal airway surface liquid composition and volume and intrinsic inflammation [29]. In addition, CFTR was found to be involved in phagosomal pH control in alveolar macrophages in one study [30]. Indeed, lysosomes from CFTR-null macrophages displayed acidification defects and were unable to kill internalized bacteria. However, CFTR-dependent lysosomal acidification in alveolar macrophages was not confirmed by another group [31]. Therefore, there is no clear evidence of an intrinsic immune defect of bacterial killing in CF patients.

In a previous whole-genome analysis of M. abscessus (sensu stricto), we were surprised to discover that this mycobacterium had a large series of specific genes in common with the other two major CF pathogens Pseudomonas aeruginosa and Burkholderia cepacia complex. Among the “non-mycobacterial” factors relevant to the infection of CF patients is the Dnd phenotype (DNA degradation) conferred by a large number of cysteine desulfurases [22, 23]. We speculated that rhDnase treatment permitted the use of sputum DNA as a nutrient by dnd–positive MABSC strains in CF patients. However, we did not find any such association. Whether an underlying host mechanism explains the striking susceptibility of CF patients to MABSC, P. aeruginosa and B. cepacia complex infection remains to be determined.

Azithromycin has been shown to improve lung function of CF patients, particularly those colonised with P. aeruginosa [32]. These benefits are believed to result from the attenuation of inflammation and the prevention of bacterial biofilm formation [33]. As MABSC isolates are usually resistant to azithromycin, MABSC may be selected by long-term azithromycin treatment in
CF patients. In addition, a recent study also suggested that azithromycin may increase susceptibility to mycobacterial disease by inhibiting the autophagy-dependent killing of mycobacteria [26]. Prior treatment with azithromycin was shown to result in significant alkalisation of the mycobacterial phagosome, to pH levels inhibiting most lysosomal proteases in RAW 264.7 cells; and to prevent basal intracellular killing and the IFN-γ- and TNF-dependent killing of *M. abscessus* in primary human macrophages. The authors also demonstrated that azithromycin treatment was associated with persistent lung infection and lower levels of IL12, IFN-γ and TNF production in their mouse model of *M. abscessus* lung infection. Thus, the potential favouring role of azithromycin on MABSC infection is a matter of concern.

Recent studies suggest a relationship between MABSC infection and long term low-dose azithromycin treatment [4, 26]. However, we did not find a significant positive association between the presence of MABSC in sputum and the use of the other “new” therapeutic approaches to CF such as inhaled therapies or long-term azithromycin. These discrepancies may result from differences in the methods used in the other studies. Indeed, these studies are subject to several limitations —small study populations and unsatisfactory control groups— which probably biased the findings. We overcame these problems, by using an appropriate control group established by matching cases with controls for age, gender and centre. Age and gender must be taken into account because they are significantly associated with NTM in CF patients [1, 34]. Moreover, ‘Centre’ has been overlooked as a factor in previous studies. However, our analysis of data from the French CF registry showed that the therapeutic management of patients differed considerably between participating centres. For example, the frequency of azithromycin use varied from 30 to 56% in adult centres and 13 to 28% in paediatric centres. Similarly, the frequency of inhaled steroid use varied from 8 to 55% in adult centres, and from 8 to 70% in paediatric centres. It is therefore important to control for any potential ‘centre effect’.
There was also no evidence that centres with the highest prevalence of MABSC infection differed substantially in their treatment practices from other centres of the same type (paediatric, mixed or adult). Indeed, the centres with the highest prevalence of MABSC infection did not use more low-dose azithromycin, inhaled antibiotics or inhaled rhDNAse than the others. We only found a slightly higher frequency of inhaled steroids at paediatric centre A than at other paediatric centers. However, the difference in inhaled steroid use among different paediatric centres was huge, ranging from 8.3 to 70.3%.

Thus, contrary to our expectations, we found no evidence of a significant association between MABSC infection and the administration of inhaled therapies or long-course, low-dose azithromycin, whether at the individual or CF centre level. However, we cannot formally exclude the existence of such a link. Indeed, our study was retrospective and we were able to trace the use of these treatments only for the four years preceding the first MABSC isolation. It therefore remains theoretically possible — given the frequently indolent or slowly progressive nature of MABSC infection — that the MABSC was acquired years earlier, at a time at which the patient was treated with inhaled therapies and/or long-term azithromycin. However, this is unlikely, because treatment escalations are much more frequent than treatment reductions during the progression of the disease in a given patient. It is unlikely, for example, that a patient not treated with an aerosol at the time of the study would have received such a treatment in any significant manner several years previously. However, this residual doubt should incite us to remain vigilant concerning the possible impact of these treatments on the bacterial populations circulating in patients. Of course, it is out of the question that we should “let our guard down” concerning inhaled therapies or call into question the absolute necessity to respect a certain number of rules aimed at preventing contamination (e.g. the use of sterile solutes, careful disinfection of non-disposable items).
The emergence of MABSC in CF patients is estimated to have occurred in the mid-1990s [35] and was probably multifactorial. This period saw major improvements in the microbiological methods for isolating MABSC and other NTM [13-15]. It also coincided, not only with the widespread use of inhaled therapies and long-course macrolides as immune-modulators, but also with more systematic intravenous antibiotic treatment, greatly increasing antibiotic pressure and the selection of organisms naturally resistant to antibiotics commonly used to treat CF patients. *Stenotrophomonas maltophilia*, which belongs to an entirely different bacterial group, provides a good example of the selection of multiresistant organisms by broad-spectrum antibiotic treatments in CF patients in the last 20 years [36, 37]. However, this link between antibiotic selection pressure and MABSC has yet to be demonstrated formally.

Finally, another disturbing finding is the extremely heterogeneous clinical presentation of MABSC disease, extending from totally asymptomatic infection to fulminant forms in some patients, whether or not they had undergone transplantation [7-10]. This observation suggests a particular susceptibility to MABSC infection in some patients. It could be a key point for the control and the prevention of the disease. We are currently investigating this issue.

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Table 1. Characteristics of cases and controls at inclusion

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malea</td>
<td>15/30 (50)</td>
<td>30/60 (50)</td>
<td></td>
</tr>
<tr>
<td>Mean age, yrs [95% CI]</td>
<td>17.3 [14.7-19.9]</td>
<td>17.4 [15.6-19.2]</td>
<td>0.8</td>
</tr>
<tr>
<td>n = 30b</td>
<td>n = 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔF508/ΔF508a</td>
<td>16/27 (59.26)</td>
<td>34/52 (65.38)</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean BMI [95% CI]</td>
<td>18.4 [17.1 – 19.7]</td>
<td>18.3 [17.6-19.1]</td>
<td>1.0</td>
</tr>
<tr>
<td>n = 30</td>
<td>n = 59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean FEV1, % predicted [95% CI]</td>
<td>54.4 [46.7-62.1]</td>
<td>64.4 [57.6-71.3]</td>
<td>0.07</td>
</tr>
<tr>
<td>n=27</td>
<td>n=53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of pancreatic enzymesa</td>
<td>29/29 (100)</td>
<td>57/60 (95.0)</td>
<td></td>
</tr>
<tr>
<td>Mean concentration of chloride in</td>
<td>110.5 [97.7-123.4]</td>
<td>104.7 [94.2-115.3]</td>
<td>0.5</td>
</tr>
<tr>
<td>sweat, mmol[95% CI]</td>
<td>n = 26</td>
<td>n=42</td>
<td></td>
</tr>
</tbody>
</table>

(a) No./ no. evaluated (%).
(b) n = no. evaluated.
(c) The log-likelihood ratio test was not applicable because there were no non-exposed cases matched to exposed controls.

Abbreviations: CI, confidence interval; Mabs, M. abscessus; BMI, body mass index; FEV1, forced expiratory volume in one second.
Table 2. Inhaled therapies and low-dose azithromycin in cases and controls

<table>
<thead>
<tr>
<th>Treatment (a)</th>
<th>Cases</th>
<th>Controls</th>
<th>OR [95% CI]</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhaled antibiotics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year before</td>
<td>15/30 (50.0)(b)</td>
<td>35/58 (60.3)</td>
<td>0.7 [0.3-1.6]</td>
<td>0.4</td>
</tr>
<tr>
<td>2 years before</td>
<td>18/29 (62.1)</td>
<td>22/49 (44.9)</td>
<td>1.9 [0.7 - 4.9]</td>
<td>0.2</td>
</tr>
<tr>
<td>3 years before</td>
<td>12/24 (50.0)</td>
<td>15/41 (36.6)</td>
<td>1.8 [0.6 - 5.3]</td>
<td>0.3</td>
</tr>
<tr>
<td>4 years before</td>
<td>8/21 (38.1)</td>
<td>13/37 (35.1)</td>
<td>1.1 [0.4 - 3.2]</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Inhaled rhDnase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year before</td>
<td>18/30 (60.0)</td>
<td>32/58 (55.2)</td>
<td>1.3 [0.5-3.7]</td>
<td>0.6</td>
</tr>
<tr>
<td>2 years before</td>
<td>19/29 (65.5)</td>
<td>27/49 (55.1)</td>
<td>1.6 [0.6 - 4.6]</td>
<td>0.4</td>
</tr>
<tr>
<td>3 years before</td>
<td>13/24 (54.2)</td>
<td>19/41 (46.3)</td>
<td>1.3 [0.4 - 3.9]</td>
<td>0.6</td>
</tr>
<tr>
<td>4 years before</td>
<td>13/21 (61.9)</td>
<td>21/37 (56.8)</td>
<td>1.2 [0.3 - 4.6]</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Inhaled steroids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year before</td>
<td>8/30 (26.7)</td>
<td>26/58 (44.8)</td>
<td>0.4 [0.1-1.2]</td>
<td>0.08</td>
</tr>
<tr>
<td>2 years before</td>
<td>11/29 (37.9)</td>
<td>21/49 (42.9)</td>
<td>0.8 [0.3-2.2]</td>
<td>0.7</td>
</tr>
<tr>
<td>3 years before</td>
<td>11/24 (45.8)</td>
<td>18/41 (43.9)</td>
<td>1.1 [0.4-3.5]</td>
<td>0.6</td>
</tr>
<tr>
<td>4 years before</td>
<td>3/21 (14.3)</td>
<td>15/37 (40.6)</td>
<td>- (c)</td>
<td>- (c)</td>
</tr>
<tr>
<td><strong>Low-dose azithromycin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year before</td>
<td>10/28 (35.7)</td>
<td>23/58 (39.7)</td>
<td>0.9 [0.3-2.3]</td>
<td>0.8</td>
</tr>
<tr>
<td>2 years before</td>
<td>8/29 (27.6)</td>
<td>20/51 (39.2)</td>
<td>0.6 [0.2-1.7]</td>
<td>0.4</td>
</tr>
<tr>
<td>3 years before</td>
<td>3/23 (13.0)</td>
<td>9/41 (22.0)</td>
<td>0.4 [0.1-2.0]</td>
<td>0.4</td>
</tr>
<tr>
<td>4 years before</td>
<td>4/21 (19.0)</td>
<td>11/37 (29.7)</td>
<td>0.6 [0.2-1.9]</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\((a)\) At least three consecutive months.

\((b)\) No. /no. evaluated (%).

\((c)\) The log-likelihood ratio test was not applicable because there were no non exposed cases matched to exposed controls.

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio.
Table 3. Use of inhaled therapies and low-dose azithromycin during 2003, at the participating CF centres and at the centres with the highest prevalences of MABSC infection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paediatric centres (15 centres, 1032 subjects)</th>
<th>Centre A (n=102)</th>
<th>Mixed centres (9 centres, 559 subjects)</th>
<th>Centre B (n=64)</th>
<th>Adult centres (10 centres, 633 subjects)</th>
<th>Centre C (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhaled antibiotics(b)</td>
<td>36 [29.8-42.3], 13.2-54.3(d)</td>
<td>38</td>
<td>38.7 [28.4-49.0], 21.1-60.9</td>
<td>29</td>
<td>54.7 [44.9-64.4], 30.1-80.0</td>
<td>52</td>
</tr>
<tr>
<td>Inhaled rhDnase(b)</td>
<td>49.7 [37.8-61.6], 2.2-71.8</td>
<td>13</td>
<td>52 [36.2-67.8], 19.2-81</td>
<td>25</td>
<td>52.2 [35.0-69.3], 23.1-96</td>
<td>ND</td>
</tr>
<tr>
<td>Inhaled steroids(b)</td>
<td>33.3 [24-42.6], 8.3-70.3</td>
<td>47</td>
<td>32.1 [14-50.1], 7.7-72.5</td>
<td>16</td>
<td>30.25 [17.9-42.6], 8-55.1</td>
<td>39</td>
</tr>
<tr>
<td>Inhaled bronchodilators(b)</td>
<td>31.9 [25.1-38.8], 16.1-51.4</td>
<td>34</td>
<td>36.1 [16.6-55.5], 11.5-76.2</td>
<td>12</td>
<td>49.2 [35.0-63.4], 12.2-65.4</td>
<td>61</td>
</tr>
<tr>
<td>Azithromycin (b,e)</td>
<td>20.9 [13.6-28.1], 6.5-50.5</td>
<td>22</td>
<td>28 [17.1-38.8], 5.8-50</td>
<td>20.5</td>
<td>43.7 [30.9-56.4], 11.8-72.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

(a) Only centres with at least 20 patients with complete data were taken into account.
(b) At least 3 consecutive months.
(c) (): number of subjects in the centre, obtained through national registry; [:]: Prevalence of MABSC infection at the centre.
(d) Mean [95% CI] proportion (%), range.
(e) Low-dose maintenance treatment.
References


