Eur Respir J 2013; 41: 1101–1106
DOI: 10.1183/09031936.00065612

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

Emilie Catherinot*,#,¶, Anne-Laure Roux*,#, Marie-Anne Vibet+,§, Gil Bellis[f], Lydie Lemonnier, Evelyne Le Roux**, Claire Bernède-Bauduin+,§, Muriel Le Bourgeois##, Jean-Louis Herrmann*,#, Didier Guillemot+,§,¶¶ Jean-Louis Gaillard*,# for the OMA group++**

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 nontuberculous mycobacteria-negative CF controls matched for age, sex 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 4 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 *M. abscessus* complex lung disease and inhaled therapies or low-dose azithromycin in patients with CF.

KEYWORDS: Macrolides, *Mycobacterium bolletii*, *Mycobacterium massiliense*, nontuberculous mycobacteria lung disease, risk factor

Nontuberculous mycobacteria (NTM) are increasingly recognised 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 *Mycobacterium abscessus* complex (MABSC) (also called *Mycobacterium abscessus sensu lato*, comprising the closely related species *Mycobacterium asbcessus sensu stricto*, *Mycobacterium massiliense* and *Mycobacterium bolletii*) and *Mycobacterium avium* complex. MABSC accounts for 16–51% of the species isolated [1–5]. In addition, MABSC fulfills the American Thoracic Society (ATS) criteria for pulmonary NTM infection in 60–80% 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 and 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

AFFILIATIONS
*Laboratoire de Microbiologie, Hôpital Raymond Poincaré et Hôpital Ambroise Paré, Assistance Publique-Hôpitaux de Paris, Paris,
#EA3647, Université de Versailles-Saint-Quentin-en-Yvelines, Guyancourt,
¶Service de Pneumologie, Hôpital Foch, Suresnes,
+INSERM U657, Paris,
§PhEMI, Institut Pasteur, Paris,
fInstitut National d'Etudes Démographiques, Paris,
**Association Vaincre la Mucoviscidose, Paris,
##Service de Pneumologie Pédiatrique, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, and
¶¶Service de Médecine Aiguë, Hôpital Raymond Poincaré, Assistance Publique-Hôpitaux de Paris, Garches, France.
++A list of members of the Observatoire des mycobactéries atypiques (OMA) is given in the Acknowledgements section.

CORRESPONDENCE
J-L. Gaillard
Laboratoire de Microbiologie
Hôpital Ambroise Paré
9 Avenue Charles de Gaulle
92104 Boulogne
France
E-mail: jean-louis.gaillard@apr.aphp.fr

Received:
April 22 2012
Accepted after revision:
Aug 10 2012
First published online:
Aug 30 2012

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

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, sex 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 January 1, 2004 and December 31, 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 *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 ± 2, 5 and 10 years of the birth date for cases aged 0–15, 16–29 and $\geqslant 30$ years, respectively), sex 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 standardised questionnaire. The chief medical officer from each centre filled in patient data sheets during an annual check up. Information about the long-term ($\geqslant 3$ months) use of inhaled therapies (antibiotics, rhDnase and 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), nine 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 with 95% confidence intervals by conditional logistic regression analysis [28]. 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, USA). We considered p-values $\leqslant 0.05$ 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 forced expiratory volume in 1 s 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, 95% CI 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 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 either 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 internalised 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 ''nonmycobacterial'' factors relevant to the infection of CF patients is the Dnd phenotype 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 alkalinisation of the mycobacterial phagosome, to pH levels inhibiting most lysosomal proteases in RAW 264.7 cells, and to prevent basal intracellular killing and the interferon (IFN)-γ- and tumour necrosis factor (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 interleukin-12, 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, sex and centre. Age and sex 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 12% to 72.5% in adult centres and 6.5% to 50.5% 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 centres. However, the difference in inhaled steroid use among different paediatric centres was huge, ranging from 8.3% to 70.3%.

TABLE 1 Characteristics of cases and controls at inclusion

Characteristics	Cases	Controls	p-value
Males	15/30 (50)	30/60 (50)	
Mean age years	17.3 (14.7–19.9)[¶]	17.4 (15.6–19.2)[+]	0.8
ΔF508/ΔF508	16/27 (59.26)	34/52 (65.38)	0.3
Mean BMI	18.4 (17.1–19.7)[¶]	18.3 (17.6–9.1)[§]	1.0
Mean FEV_1 % pred	54.4 (46.7–62.1)[f]	64.4 (57.6–71.3)[##]	0.07
Use of pancreatic enzymes[#]	29/29 (100)	57/60 (95.0)	
Mean concentration of chloride in sweat mmol	110.5 (97.7–123.4)[¶¶]	104.7 (94.2–115.3)[++]	0.5

Data are presented as n/N (%) or mean (95% confidence interval), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; % pred: % predicted. [#]: the log-likelihood ratio test was not applicable because there were no nonexposed cases matched to exposed controls; [¶]: n=30; [+]: n=60; [§]: n=59; [f]: n=27; [##]: n=53; [¶¶]: n=26; [++]: n=42.

TABLE 2 Inhaled therapies and low-dose azithromycin in cases and controls

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

Data are presented as n/N (%), unless otherwise stated. [#]: at least three consecutive months; [¶]: the log-likelihood ratio test was not applicable because there were no nonexposed cases matched to exposed controls.

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 4 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 and careful disinfection of nondisposable 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 immunemodulators, 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

TABLE 3 Use of inhaled therapies and low-dose azithromycin during 2003, at the participating cystic fibrosis centres[#] and at the centres with the highest prevalences of *Mycobacterium abscessus* complex (MABSC) infection

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

Data are presented as mean (95% CI), range, unless otherwise stated. ND: not determined. [#]: only centres with at least 20 patients with complete data were taken into account; [¶]: for at least 3 consecutive months; [+]: 7.8% prevalence of MABSC infection at the centre; [§]: 4.7% prevalence; [f]: 4.9% prevalence.

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.

SUPPORT STATEMENT

This work received financial support from Legs Poix (Chancellerie des Universités de Paris; grant number X157) and from the association ''Vaincre La Mucoviscidose'' (grant numbers RF201106000446 and RF201106000566).

STATEMENT OF INTEREST

Conflict of interest information can be found alongside the online version of this article at www.erj.ersjournals.com

ACKNOWLEDGMENTS

The members thank Isabelle Sénégas (Assistance Publique - Hôpitaux de Paris) for assistance and Polly Gobin (Hôpital Foch) for the English revision.

Members of the OMA are as follows: M. Mahaza, M. Pihet, F. Troussier and T. Urban (Centre Hospitalier Universitaire d'Angers, Angers); G. Couetdic, J-C. Dalphin, M-L. Dalphin and F. Grenouillet (Centre Hospitalier Universitaire de Besancon, Besancon); P. Domblides, M. Fayon and J. Texier Maugein (Centre Hospitalier Universitaire de Bordeaux, Bordeaux) ; M-L. Abalain, M-R. Munck, J-J. Quiot, G. Rault and J. Thubert (Centre Hospitalier Universitaire de Brest, Breast); P. Gautry and A. Sommabère (Centre Hospitalier de Brive la Gaillarde, Brive la Gaillarde); J-F. Duhamel and R. Leclercq (Centre Hospitalier Universitaire de Caen, Caen); S. Aberrane, L. Bassinet and P. Reinert (Centre Hospitalier de Créteil, Créteil); G-A. Loeuille, V. Montagne, A. Vachée and A. Verhaeghe (Centre Hospitalier de Dunkerque. Dunkerque); G. Grise and K. Lahsinat (Centre Hospitalier d'Elbeuf, Elbeuf); J-P. Chazalette (Centre Hospitalier de Giens, Giens); C. Cracowski, M. Maurin and I. Pin (Centre Hospitalier Universitaire de Grenoble, Grenoble); M.C. Jaffar-Bandjee, J-F. Lesure, A. Michault and M. Renouil (Centre Hospitalier de La Réunion, La Réunion); F. Evreux and B. Le Luyer (Centre Hospitalier de Le Havre, Le Havre); M-C. Chevalier and A. Marmonier (Centre Hospitalier de Le Mans, Le Mans); J-P. Canone, J. Clais and A. Sardet (Centre Hospitalier de Lens, Lens); M-O. Husson, N. Lemaître, B. Wallaert, and N. Wizla (Centre Hospitalier Universitaire de Lille, Lille); M. Guillot and C. Paris (Centre Hospitalier Universitaire de Lisieux, Lisieux); J. Languepin and C. Martin (Centre Hospitalier Universitaire de Limoges, Limoges); G. Bellon, I. Durieu, M. Pérouse de Montclos and F. Vandenesch (Centre Hospitalier Universitaire de Lyon, Lyon); M. Drancourt, M-J. Gévaudan, M. Reynaud-Gaubert and J. Sarles (Centre Hospitalier Universitaire de Marseille, Marseille); C. Carriere and R. Chiron (Centre Hospitalier Universitaire de Montpellier, Montpellier); M. Dailloux†, J. Derelle and P. Scheid (Centre Hospitalier Universitaire de Nancy, Nancy); P. Bemer, V. David and A. Haloun (Centre Hospitalier Universitaire de Nantes, Nantes); M. Albertini, L. Landraud and D. Sicard (Centre Hospitalier Universitaire de Nice, Nice); D. Dusser, B. Fauroux, A. Ferroni, M. Le Bourgeois, G. Lenoir, P. Mariani, P. Morand, C. Offredo, G. Paul, I. Sermet-Gaudelus and H. Vu Thien (Assistance Publique - Hôpitaux de Paris, Paris); B. Desrues, P-Y. Donnio and M. Roussey (Centre Hospitalier Universitaire de Rennes, Rennes); C. Marguet and M. Pestel-Caron (Centre Hospitalier Universitaire de Rouen, Rouen); G. Aubert and I. Rayet (Centre Hospitalier Universitaire de St Etienne, St Etienne); J-Y. Leberreb and D. Siret (Centre Hospitalier de St Nazaire, St Nazaire); L. Donato, R. Kessler, A. Kiesler, Y. Piemont and V. Rosner (Centre Hospitalier Universitaire de Strasbourg, Strasbourg); D. Grenet and P. Honderlick (Hôpital Foch, Suresnes); R. Bauriaud, F. Bremont, M. Murris-Espin, B. Sablayrolles and M. Thouvenot (Centre Hospitalier Universitaire de Toulouse, Toulouse); B. Cattier, P. Lanotte, S. Marchand and F. Varaigne (Centre Hospitalier Universitaire de Tours, Tours); F. Moreau-Benaoudia and B. Soto (Centre Hospitalier de Troyes, Troyes); and F. Doucet-Populaire and P. Foucaud (Hôpital de Versailles, Versailles).

REFERENCES

1 Roux AL, Catherinot E, Ripoll F, *et al.* Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in france. *J Clin Microbiol* 2009; 47: 4124–4128.
2 Olivier KN, Weber DJ, Wallace RJ Jr, *et al.* Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. *Am J Respir Crit Care Med* 2003; 167: 828–834.
3 Esther CR Jr, Henry MM, Molina PL, *et al.* Nontuberculous mycobacterial infection in young children with cystic fibrosis. *Pediatr Pulmonol* 2005; 40: 39–44.
4 Levy I, Grisaru-Soen G, Lerner-Geva L, *et al.* Multicenter cross-sectional study of nontuberculous mycobacterial infections among cystic fibrosis patients, Israel. *Emerg Infect Dis* 2008; 14: 378–384.
5 Sermet-Gaudelus I, Le Bourgeois M, Pierre-Audigier C, *et al. Mycobacterium abscessus* and children with cystic fibrosis. *Emerg Infect Dis* 2003; 9: 1587–1591.
6 Esther CR Jr, Esserman DA, Gilligan P, *et al.* Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis. *J Cyst Fibros* 2010; 9: 117–123.
7 Griffith DE. Emergence of nontuberculous mycobacteria as pathogens in cystic fibrosis. *Am J Respir Crit Care Med* 2003; 167: 810–812.
8 Tomashefski JF Jr, Stern RC, Demko CA, *et al.* Nontuberculous mycobacteria in cystic fibrosis. An autopsy study. *Am J Respir Crit Care Med* 1996; 154: 523–528.
9 Cullen AR, Cannon CL, Mark EJ, *et al. Mycobacterium abscessus* infection in cystic fibrosis. Colonization or infection? *Am J Respir Crit Care Med* 2000; 161: 641–645.
10 Sanguinetti M, Ardito F, Fiscarelli E, *et al.* Fatal pulmonary infection due to multidrug-resistant *Mycobacterium abscessus* in a patient with cystic fibrosis. *J Clin Microbiol* 2001; 39: 816–819.
11 Chalermskulrat W, Sood N, Neuringer IP, *et al.* Non-tuberculous mycobacteria in end stage cystic fibrosis: implications for lung transplantation. *Thorax* 2006; 61: 507–513.
12 Brown-Elliott BA, Wallace RJ Jr. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. *Clin Microbiol Rev* 2002; 15: 716–746.
13 Ferroni A, Vu-Thien H, Lanotte P, *et al.* Value of the chlorhexidine decontamination method for recovery of nontuberculous mycobacteria from sputum samples of patients with cystic fibrosis. *J Clin Microbiol* 2006; 44: 2237–2239.
14 Whittier S, Hopfer RL, Knowles MR, *et al.* Improved recovery of mycobacteria from respiratory secretions of patients with cystic fibrosis. *J Clin Microbiol* 1993; 31: 861–864.
15 Brown-Elliott BA, Griffith DE, Wallace RJ Jr. Diagnosis of nontuberculous mycobacterial infections. *Clin Lab Med 2002; 22*, 911–925: vi.
16 Equi A, Balfour-Lynn IM, Bush A, *et al.* Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. *Lancet* 2002; 360: 978–984.
17 Ramsey BW, Pepe MS, Quan JM, *et al.* Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. *N Engl J Med* 1999; 340: 23–30.
18 Singh S, Loke YK. Risk of pneumonia associated with long-term use of inhaled corticosteroids in chronic obstructive pulmonary disease: a critical review and update. *Curr Opin Pulm Med* 2010; 16: 118–122.
19 Torres A, Ewig S. The strange case of community-acquired pneumonia in COPD. *Chest* 2011; 139: 483–485.
20 Brinkmann V, Reichard U, Goosmann C, *et al.* Neutrophil extracellular traps kill bacteria. *Science* 2004; 303: 1532–1535.

21 Manzenreiter R, Kienberger F, Marcos V, *et al.* Ultrastructural characterization of cystic fibrosis sputum using atomic force and scanning electron microscopy. *J Cyst Fibros* 2012; 11: 84–92.
22 Zhang Y, Yakrus MA, Graviss EA, *et al.* Pulsed-field gel electrophoresis study of *Mycobacterium abscessus* isolates previously affected by DNA degradation. *J Clin Microbiol* 2004; 42: 5582–5587.
23 Ripoll F, Pasek S, Schenowitz C, *et al.* Non mycobacterial virulence genes in the genome of the emerging pathogen *Mycobacterium abscessus*. *PLoS One* 2009; 4: e5660.
24 Saiman L, Siegel J. Infection control in cystic fibrosis. *Clin Microbiol Rev* 2004; 17: 57–71.
25 Bastian S, Veziris N, Roux AL, *et al.* Assessment of clarithromycin susceptibility in strains belonging to the *Mycobacterium abscessus* group by *erm(41)* and *rrl* sequencing. *Antimicrob Agents Chemother* 2011; 55: 775–781.
26 Renna M, Schaffner C, Brown K, *et al.* Azithromycin blocks autophagy and may predispose cystic fibrosis patients to mycobacterial infection. *J Clin Invest* 2011; 121: 3554–3563.
27 Griffith DE, Aksamit T, Brown-Elliott BA, *et al.* An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007; 175: 367–416.
28 Hosmer DW, Lemeshow S. Applied logistic regression. Chichester, John Wiley and Sons, 2000.
29 Haggie PM, Verkman AS. Defective organellar acidification as a cause of cystic fibrosis lung disease: reexamination of a recurring hypothesis. *Am J Physiol Lung Cell Mol Physiol* 2009; 296: L859–L867.
30 Di A, Brown ME, Deriy LV, *et al.* CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. *Nat Cell Biol* 2006; 8: 933–944.
31 Haggie PM, Verkman AS. Cystic fibrosis transmembrane conductance regulator-independent phagosomal acidification in macrophages. *J Biol Chem* 2007; 282: 31422–31428.
32 Cai Y, Chai D, Wang R, *et al.* Effectiveness and safety of macrolides in cystic fibrosis patients: a meta-analysis and systematic review. *J Antimicrob Chemother* 2011; 66: 968–978.
33 Zarogoulidis P, Papanas N, Kioumis I, *et al.* Macrolides: from *in vitro* anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases. *Eur J Clin Pharmacol* 2012; 68: 479–503.
34 Pierre-Audigier C, Ferroni A, Sermet-Gaudelus I, *et al.* Age-related prevalence and distribution of nontuberculous mycobacterial species among patients with cystic fibrosis. *J Clin Microbiol* 2005; 43: 3467–3470.
35 Jönsson BE, Gilljam M, Lindblad A, *et al.* Molecular epidemiology of *Mycobacterium abscessus*, with focus on cystic fibrosis. *J Clin Microbiol* 2007; 45: 1497–1504.
36 Graff GR, Burns JL. Factors affecting the incidence of *Stenotrophomonas maltophilia* isolation in cystic fibrosis. *Chest* 2002; 121: 1754–1760.
37 Goss CH, Otto K, Aitken ML, *et al.* Detecting *Stenotrophomonas maltophilia* does not reduce survival of patients with cystic fibrosis. *Am J Respir Crit Care Med* 2002; 166: 356–361.