Serodiagnosis of *Mycobacterium abscessus* complex infection in cystic fibrosis

Tavs Qvist[1], Tania Pressler[1], David Taylor-Robinson[2], Terese L. Katzenstein[1] and Niels Høiby[3]

Affiliations:
[1]Copenhagen Cystic Fibrosis Centre, Dept of Infectious Diseases, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark.
[2]Dept of Public Health and Policy, University of Liverpool, Liverpool, UK.
[3]Dept of Clinical Microbiology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark.

Correspondence:
Tavs Qvist, Copenhagen Cystic Fibrosis Centre, Dept of Infectious Diseases section 8632, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark.
E-mail: tavs.qvist@gmail.com

ABSTRACT   Early signs of pulmonary disease with *Mycobacterium abscessus* complex (MABSC) can be missed in patients with cystic fibrosis (CF). A serological method could help stratify patients according to risk. The objective of this study was to test the diagnostic accuracy of a novel method for investigating IgG activity against MABSC.

A prospective study of all patients attending the Copenhagen CF Centre was conducted by culturing for MABSC during a 22-month period and then screening patients with an anti-MABSC IgG ELISA. Culture-positive patients had stored serum examined for antibody kinetics before and after culture conversion.

307 patients had 3480 respiratory samples cultured and were then tested with the anti-MABSC IgG ELISA. Patients with MABSC pulmonary disease had median anti-MABSC IgG levels six-fold higher than patients with no history of infection (434 *versus* 64 ELISA units; $p<0.001$). The test sensitivity was 95% (95% CI 74–99%) and the specificity was 73% (95% CI 67–78%). A diagnostic algorithm was constructed to stratify patients according to risk.

The test accurately identified patients with pulmonary disease caused by MABSC and was suited to be used as a complement to mycobacterial culture.

@ERSpublications
Measuring antibodies against *Mycobacterium abscessus* can identify patients who harbour this emerging threat to health http://ow.ly/LlRYw

Introduction

Lung infection with nontuberculous mycobacteria (NTM) is a diagnostic and therapeutic challenge in cystic fibrosis (CF) patients [1]. *Mycobacterium abscessus* complex (MABSC) is the dominant form of NTM in European patients with CF and new evidence of increasing incidence rates, human-to-human transmission and a detrimental effect on lung function has reinvigorated interest in these ubiquitous mycobacteria [2–4]. The pathogenesis of MABSC is not well understood but is a research priority, as there might be a window of opportunity for eradication [5] or suppression [6] at an early stage of infection.

While there is now consensus that *Mycobacterium tuberculosis* serology is best avoided in resource-constrained settings [7, 8], serology has found a place in other parts of tuberculosis (TB) research, for example in elucidating the immune response to mycobacterial infection. Most serological TB studies have focused on assays using purified antigens, such as bacille Calmette–Guerin (BCG) antigen [9, 10] or other single antigens [11], but increasingly the recommended approach is to use a combination of different antigens [12–14].

Patients with CF are followed closely for their entire lives and there might be a benefit in using serodiagnosis as a complement to routine culture, in the same way that antibodies against *Pseudomonas aeruginosa* and *Aspergillus* are used successfully in many CF centres [15–19]. Limitations in the predictive values of such assays have been reported [20], and currently antibody measurements for *P. aeruginosa* are used clinically in combination with culture results, rather than as stand-alone tests [21]. Measuring anti-MABSC IgG across whole CF populations could guide clinicians in deciding how vigilant they have to be in pursuing mycobacterial cultures. Two studies have indeed shown proof of concept for a diagnostic potential of mycobacterial serodiagnosis in CF populations using the antigen A60 [22, 23].

The objective of our study was to develop and test the diagnostic accuracy of a multi-antigen ELISA for measuring anti-MABSC IgG in serum. Our setting is a homogenous and well-screened cohort of patients with CF. We hypothesised that anti-MABSC IgG levels would be correlated with disease severity and that a useful clinical application could be developed. The study proceeded in two parts. First, we described antibody serology in patients with CF prospectively screened for MABSC by sputum culture at the Copenhagen CF Centre (Copenhagen, Denmark) in 2012–2014. Secondly, we followed the MABSC cases, describing antibody kinetics before and after onset of MABSC culture conversion.

Methods

Patients and setting

The Copenhagen CF Centre cares for 100 children and 216 adults with CF. Since the establishment of the centre in 1968, patients with CF have been seen for microbiological and clinical examinations in the outpatient clinic every 4 weeks. During visits, clinical parameters are registered in a clinical database. Serum samples for antibody determination are collected at least once a year and stored at −20°C for further investigation. The current study was approved by the Committee on Health Research Ethics in the Capital Region of Denmark (H-3-2012-098) and the Danish Data Protection Agency (2007-58-0015). Exemption from the requirement of written patient consent was granted by the Committee on Health Research Ethics in the Capital Region of Denmark.

Design and inclusion

All patients with CF registered at the Copenhagen CF Centre in May 2012 were eligible. Patients were consecutively enrolled during visits at the adult and paediatric outpatient clinics and admissions to the CF wards from May 2012 until February 2014. Between October 2013 and February 2014, a serum sample was collected from each patient for anti-MABSC IgG determination. Patients who had died or were lung transplanted before this time were excluded. By February 2014, the patients were divided into three groups on the basis of NTM culture results and clinical data captured from patient records: group A were patients with MABSC pulmonary disease at the time of serum sampling (MABSC-PD); group B consisted of patients with previous but not present MABSC-PD and patients with past or present infection with another NTM; and group C were patients with no known history of NTM infection, including patients with negative culture results as well as those with no NTM culture results prior to the current study. A group of healthy non-CF subjects (adults and children) was included as normal reference persons (group D).

Longitudinal study of MABSC cases

We applied the ELISA method on previously stored serum samples from all patients with previous or ongoing MABSC from 1987 onwards. Lung transplanted patients were not excluded in this analysis of antibody kinetics before, during and after MABSC infection.

NTM disease classification

The CF Centre uses the criteria of the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) to classify NTM patients [24]. In this study, we use the term MABSC-PD to describe patients who fulfilled the ATS/IDSA's clinical, radiological and microbiological criteria for pulmonary disease. We defined clearing MABSC-PD as 12 months of culture negativity after culture conversion to negative (based on at least four separate MABSC-negative cultures).

Respiratory samples and isolates

Sputum samples or laryngeal suction samples were collected and *Burkholderia cepacia* selective agar was used as growth medium according to a previously described method [25]. The first positive samples were transferred to the International Reference Laboratory of Mycobacteriology at Statens Serum Institut (Copenhagen, Denmark), where identification of MABSC was carried out either by InnoLipa species (InnoGenetics, Ghent, Belgium) and/or by 16S DNA sequencing using primers directed at hypervariable region A. After first isolate species determination, subsequent isolates were controlled locally with MALDI-TOF (matrix-assisted laser desorption/ionisation time-of-flight) and antibiotic susceptibility testing was performed. In cases of suspected new NTM species, isolates were again forwarded to the Reference Laboratory. The longitudinal study also relied on previous NTM culture data from 1987 to 2012, which were collected according to methods previously described in detail [26].

ELISA method

Mycobacterial antigen preparation was performed according to CLOSS *et al.* [27] with minor modifications and is described in detail in the online supplementary material. Anti-MABSC IgG levels were determined by ELISA, modified from a previously described method from our laboratory [15, 28], and expressed in ELISA units. The test positivity threshold was determined using a receiver operating curve (ROC). Intraplate, plate-to-plate and day-to-day variations were performed by double determinations (blinded) on samples from 10 patients, covering the spectrum of high to low antibody titres, and were expressed using coefficient of variation. Clinical data were extracted from patient files and the Danish CF registry.

Statistical methods

Baseline data were reported as median and interquartile range (IQR) for non-normally distributed continuous variables, and as percentages for categorical variables. Group comparisons were made using Kruskal–Wallis non-parametric tests and Dunn's multiple comparisons test. A p-value $\leqslant 0.05$ was considered statistically significant. SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA) were used for data analysis.

Results

A flow diagram of the study design is shown in figure 1. The Copenhagen CF cohort consisted of 316 patients, of which 307 were included. From these patients, 3480 NTM cultures were performed between May 2012 and February 2014. The average number of cultures per patient was 11 (range 1–23). All 307 patients had one serum sample collected between October 2013 and February 2014. 19 patients were found to have ATS/IDSA-defined pulmonary disease with MABSC (6.2%), so were classified as group A. Group B comprised 36 patients who had either a history of previous MABSC-PD (n=11) or current infection with MABSC but without fulfilling ATS/IDSA criteria (n=11), and 14 had past or present *Mycobacterium avium* complex. The characteristics of the patients and healthy non-CF controls in February 2014 are presented in table 1.

Antibody levels in different groups

The median anti-MABSC IgG was four times higher in patients with MABSC-PD than in patients in group B who had either *M. avium* complex or non-PD MABSC (p=0.03), and six times higher than in patients with CF without any NTM history (p<0.001) (fig. 2). Healthy, non-CF controls had the lowest IgG levels, with 95% of subjects demonstrating values <77 ELISA units. No MABSC subspecies differences were observed within the MABSC-PD group (data not shown). Differences within group B were not statistically significant.

Test performance

For the purpose of evaluating test performance, patients with MABSC-PD were defined as cases and all other patients with CF as controls (groups B and C combined; n=288). A ROC was created based on different thresholds of test positivity (fig. 3). A cut-off of 125 ELISA units was chosen based on its proximity to the upper left corner of the ROC (perfect classification point) and its high sensitivity, given the intended clinical use of the test (designed to be used in conjunction with culture, which gives high specificity). Table 2

CF patients at Copenhagen
CF Centre, May 2012
(n=316)

Monthly MABSC
culture screening from
May 2012 to Feb 2014

Excluded due to lung
transplantation or death
(n=9)

Included CF patients
(n=307)

Anti-MABSC antibody
measurement from
Oct 2013 to Feb 2014

MABSC-PD
(n=19)

Other NTM or
non-PD MABSC
(n=36)

No history
of NTM
(n=252)

Healthy non-CF
controls
(n=532)

Longitudinal case study

All patients with previous or
current MABSC from primary study
(n=33)

All MABSC patients dead or lung
transplanted prior to primary study
(n=20)

Data spanning
the years
1987–2014

Excluded due
to missing
longitudinal data
(n=17)

Substudy population
(n=32)
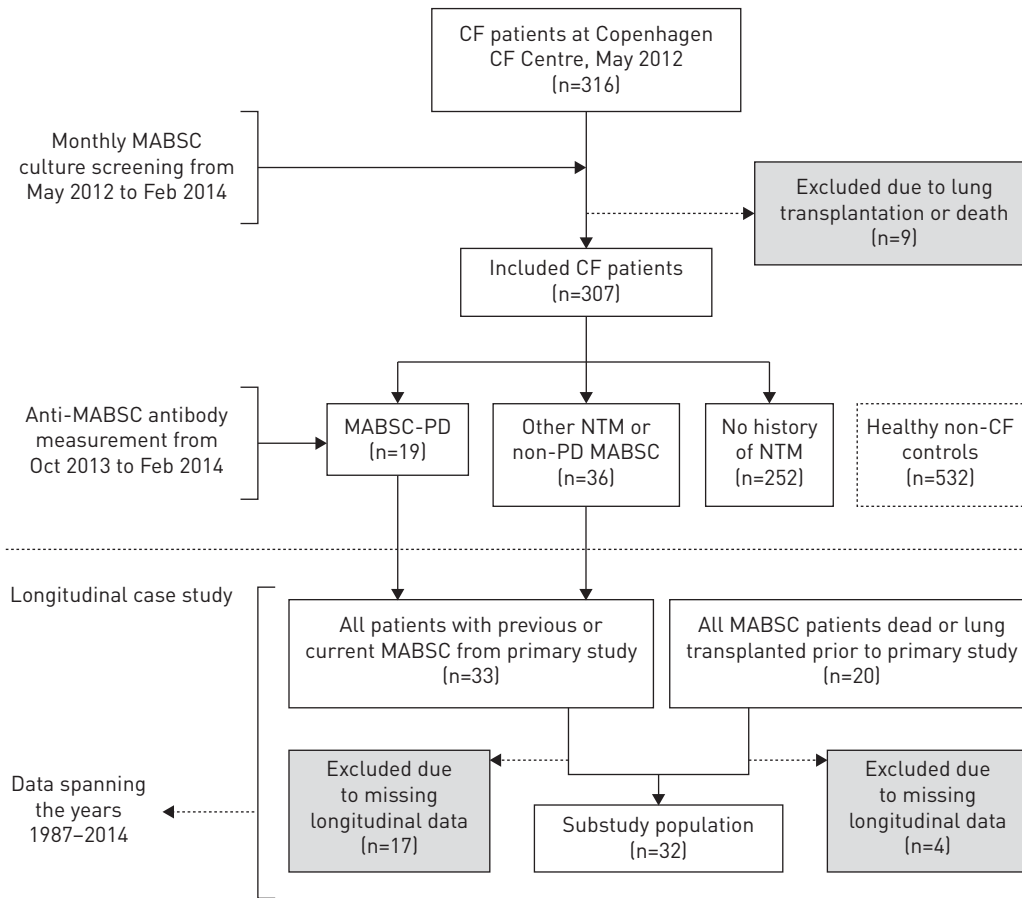
Excluded due
to missing
longitudinal data
(n=4)

FIGURE 1 Study design. CF: cystic fibrosis; MABSC: *Mycobacterium abscessus* complex; PD: pulmonary disease (as defined by the American Thoracic Society/Infectious Diseases Society of America criteria); NTM: nontuberculous mycobacteria.

shows test characteristics for patients with MABSC-PD (n=19) compared with all other CF patients (n=288). Supplementary test performance is summarised in online supplementary table S1.

Reproducibility of the assay

Intraplate, day-to-day and interplate variation measurements were 5%, 16% and 11%, respectively (IQR 3–9%, 10–27% and 5–14%, respectively).

Diagnostic algorithm

A diagnostic algorithm was constructed based on the principle of risk stratification (fig. 4). The premise of the algorithm was one routine serum sample for anti-MABSC IgG measurement per patient per year, and compatibility with current ATS/IDSA screening recommendations [24]. Raw data, multilevel predictive values and likelihood ratios are shown in table 3.

Longitudinal study of MABSC cases

From 1987 to 2010, the NTM incidence among patients with CF was stable in Copenhagen, with 1.1 new MABSC culture-positive cases annually (range 0–3). From 2010 to 2014 the number of MABSC cases rose to 6.7 per year (range 4–9). Figure 5 shows anti-MABSC IgG levels before and after first positive culture in 26 MABSC cases that fulfilled criteria for MABSC-PD and six patients who only had one positive MABSC culture.

11 out of the 26 patients became IgG positive prior to first positive culture. The majority (nine) of these 11 patients were found to be MABSC culture positive before 2012 when screening was less frequent. Noticeably, for four of these cases, their first positive MABSC culture was also their first ever NTM culture. Seven (27%) patients had increases in antibody titres prior to culture conversion, despite previous negative NTM cultures, suggesting various degrees of diagnostic delay. Overall, 85% of patients that went on to develop MABSC-PD had sharp increases in antibodies either before or after culture positivity,

TABLE 1 Characteristics of 307 patients stratified by nontuberculous mycobacteria (NTM) status and 532 healthy non-cystic fibrosis (CF) controls

	CF patients[#]			D: healthy non-CF controls[¶]
	A: MABSC-PD	B: other NTM or non-PD MABSC	C: no history of NTM	
Subjects n	19	36	252	532
Age years	21 (15–24)	28 (23–37)	24 (14–37)	30 (15–46)[§]
Age <18 years	32	17	36	53
Female	53	47	49	52[f]
Homozygote for ∆F508[+]	68	89	63	NA
FEV1 % pred	80 (63–84)	71 (62–80)	76 (53–94)	
Chronic Gram infection	58	42	34	NA
Received NTM treatment	58	61	0	NA
Positive NTM cultures	7 (4–16)	4 (1–10)	0	NA
Time positive for NTM years	2 (1–5)	2 (1–6)	0	NA

Data are presented as median (interquartile range) or %, unless otherwise stated. MABSC: *Mycobacterium abscessus* complex; PD: pulmonary disease (as defined by the American Thoracic Society/Infectious Diseases Society of America criteria); FEV1 % pred: forced expiratory volume in 1 s as percentage of predicted for age, height and sex; NA: not applicable. [#]: at the Copenhagen CF Centre in February 2014; [¶]: from frozen sera left from a previous study; [+]: mutation in the cystic fibrosis transmembrane conductance regulator gene; [§]: age missing for 64 out of 250 adult controls and 184 out of 282 paediatric controls; [f]: sex data missing for 184 out of 282 paediatric controls.

validating the diagnostic potential of the test. Three had slowly rising antibody levels and did not reach the cut-off of 125 ELISA units within 3 years of being classified as having MABSC-PD. The majority of patients with only one positive MABSC culture did not become IgG positive (fig. 5b).

Clearing infection

Seven patients received extensive antimycobacterial treatment prior to clearance and two of the seven were also lung transplanted. Five (56%) patients had a convincing overlap (±12 months) of falling IgG levels and time of clearance, defined as 12 months of culture negativity in at least four cultures. Two remained antibody positive, despite consistently being culture negative and having clinical and radiological signs of arrested disease progression (fig. 5c).

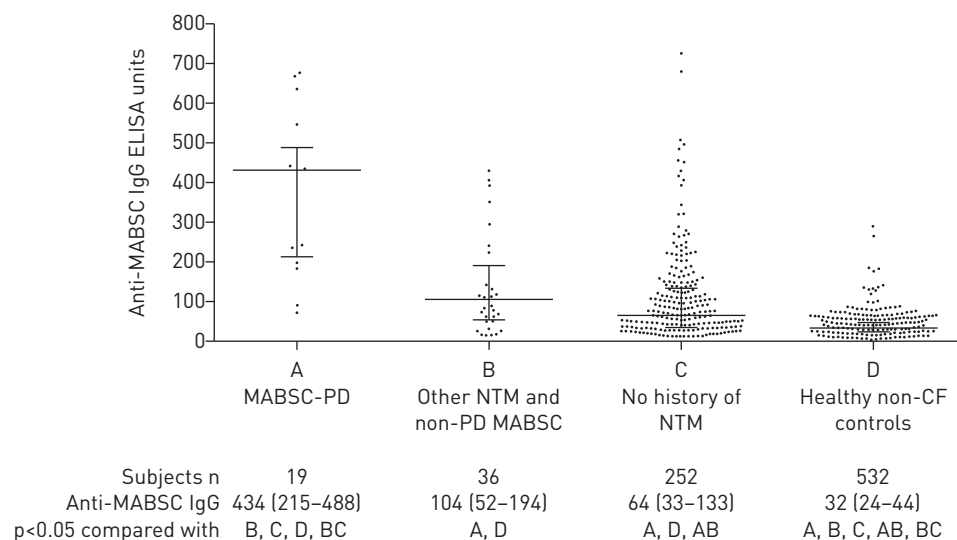|	A MABSC-PD	B Other NTM and non-PD MABSC	C No history of NTM	D Healthy non-CF controls
Subjects n	19	36	252	532
Anti-MABSC IgG	434 (215–488)	104 (52–194)	64 (33–133)	32 (24–44)
p<0.05 compared with	B, C, D, BC	A, D	A, D, AB	A, B, C, AB, BC

FIGURE 2 Anti-*Mycobacterium abscessus* complex (MABSC) IgG levels among three groups of patients with cystic fibrosis (CF) (groups A, B and C) and healthy non-CF controls (group D). Wide horizontal lines represent the median, short horizontal lines represent the interquartile range (IQR). Anti-MABSC IgG values given below the graph are presented as median (IQR) ELISA units. PD: pulmonary disease (as defined by the American Thoracic Society/ Infectious Diseases Society of America criteria); NTM: nontuberculous mycobacteria; BC: combination of groups B and C; AB: combination of groups A and B. The only nonsignificant comparison was group B *versus* group C (p=0.66).
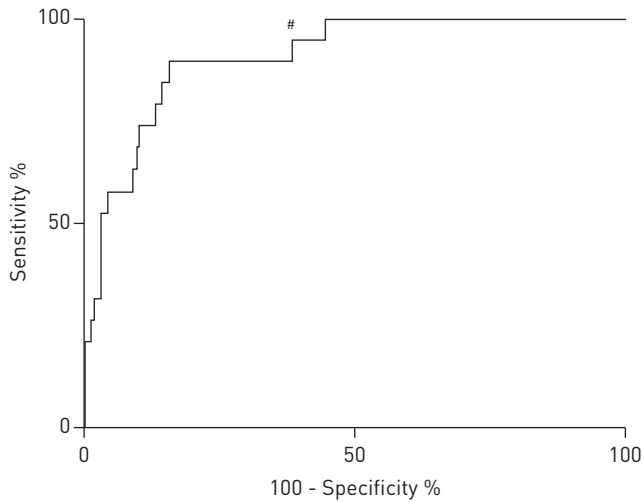
FIGURE 3 Receiver operating curve for anti-*Mycobacterium abscessus* complex IgG ELISA. [#]: chosen cut-off of 125 ELISA units.

TABLE 2 Test characteristics for 19 patients with *Mycobacterium abscessus* complex (MABSC) pulmonary disease[#] compared with 288 other cystic fibrosis patients

	Value (95% CI)
Sensitivity %	95 (74–99)
Specificity %	73 (67–78)
Positive likelihood ratio	3.5 (2.8–4.3)
Negative likelihood ratio	0.07 (0.0–0.5)
PPV %	19 (11–28)
Adjusted[¶] PPV %	21 (13–32)
NPV %	100 (97–100)
Area under ROC	0.90 (0.84–0.96)
SE	0.03
p-value	<0.01

PPV: positive predictive value; NPV: negative predictive value; ROC: receiver operating curve. [#]: as defined by the American Thoracic Society/Infectious Diseases Society of America criteria; [¶]: adjusted analysis where patients with previous MABSC or other nontuberculous mycobacteria were excluded (n=252).

Discussion

This study is the largest examination of antibodies against NTM in CF to date. Its primary strength is the prospective design in a homogeneous CF cohort, screened on a monthly basis.

Main findings

Anti-MABSC IgG was found to be significantly elevated in patients with MABSC-PD and the test demonstrated good diagnostic accuracy. Longitudinal measurements of known MABSC cases revealed patterns of early antibody response indicative of significant diagnostic delay, suggesting a potential for earlier intervention. The potential for verification bias was reduced with an average of 11 cultures per patient and concomitant clinical evaluations [29]. Through the use of stored serum samples dating back 27 years, the MABSC case study could visualise kinetics of the antibody response longitudinally, providing a unique opportunity to retrospectively assess MABSC-PD pathogenesis. A diagnostic algorithm was constructed, exploiting the fact that assay cross-reactivity and low specificity is a negligible problem in a CF setting, where culture will always follow serology. Among study weaknesses are the changes in mycobacterial culture techniques and screening procedures in the period 1987–2011, which could confound the overall incidence rates of NTM during this period. Regarding validation, intra- and interplate variations were good (5% and 11%, respectively). Day-to-day variation was 16%, which can be classified as acceptable. All biomarker assays have inherent analytical variability, ranging from 5% to 20% [30]. There are also variances within a subject over time and a day-to-day ELISA variation <20% indicates adequate repeatability [31].

| Annual CF population anti-MABSC IgG screening |
IgG result <125 EU	IgG result 125–400 EU	IgG result >400 EU
Low risk	Intermediate risk	High risk
Continue with annual NTM culture	Consider NTM culture 2–4 times annually	Consider NTM culture 4–12 times annually

FIGURE 4 Diagnostic algorithm employing anti-*Mycobacterium abscessus* complex (MABSC) IgG levels to stratify patients with cystic fibrosis (CF) according to risk of MABSC infection. EU: ELISA units; NTM: nontuberculous mycobacteria.
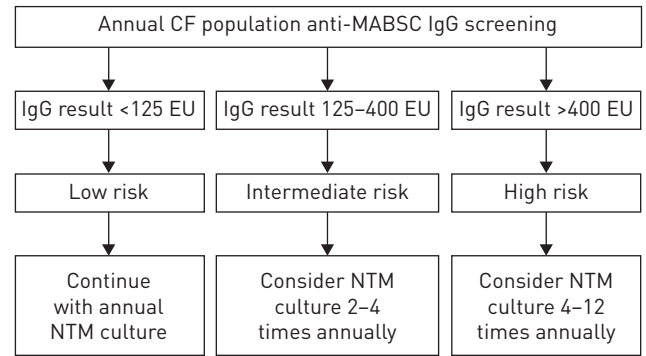
TABLE 3 Raw data, multilevel predictive values and likelihood ratios for 307 cystic fibrosis patients

	Culture results n			Predictive value (95% CI) %	Likelihood ratio (95% CI)
	MABSC+	MABSC−	Total		
Serum anti-MABSC IgG ELISA units					
>400	10	12	22	45 (24–68)	13 (6–25)
125–400	8	67	75	11 (5–20)	2 (1–3)
<125	1	209	210	0.5 (0.1–2.6)	0.1 (0.1–0.5)
Total	19	288	307		

MABSC: *Mycobacterium abscessus* complex.

Need for improved diagnostic measures

The rise of MABSC in the CF population is of concern and new diagnostic measures are in demand. Our group recently showed that less than a third of Scandinavian patients with CF with repeated MABSC cultures manage to clear infection, and as many as a quarter have lung transplants or die [26]. Despite an increased understanding of the pathogenic potential of MABSC-PD, diagnostic principles have remained largely unchanged, as have their inherent obstacles. Acquiring two consecutive positive sputum cultures can take considerable time and culture failure is common, typically due to insufficient material or overgrowth of Gram-negative bacteria or *Aspergillus*. Additionally, radiological pathology is nonspecific in CF patients [24].

Immunological tests for mycobacteria

Immunological testing for mycobacteria is inappropriate in resource-constrained settings, where there is a considerable risk of overdiagnosing patients [7, 32]. The tuberculin skin test (TST) and later interferon-γ release assays have been widely used in high-income countries with a low TB incidence [33, 34], and have found a place in selecting patients for further examination by the gold standard (culture), keeping in mind that the TST has low sensitivity and is affected by BCG vaccination, and interferon-γ release assays are only sensitive in patients previously unexposed to TB. The situation in CF populations is fundamentally different from both of these settings. Patients with CF have a high risk of exposure to NTM, negligible TB risk, are followed closely for their entire lives, are routinely NTM cultured once a year, and have no use for commercially available interferon-γ release assays, which cannot detect CF-relevant NTM [35]. A central clinical challenge in CF is to avoid diagnostic delay of new MABSC-PD cases, as these are the patients most likely to benefit from prompt treatment. It is in this light that serodiagnosis for mycobacteria has re-emerged as a field of interest in CF. In 2001, OLIVER *et al.* [22] showed elevated IgG antibodies against the mycobacterial interspecies antigen A60 in four patients with CF and NTM. FERRONI *et al.* [23] used the same commercially available test in a study of 186 patients with CF and found that 15 patients with MABSC-PD had IgG titres six-fold higher than 144 patients without NTM. The results from our 307 subjects demonstrate the same convincing six-fold difference in IgG levels, although it should be noted that different units were applied and we used median IgG values, which were considered more appropriate when analysing skewed distributions in small groups.
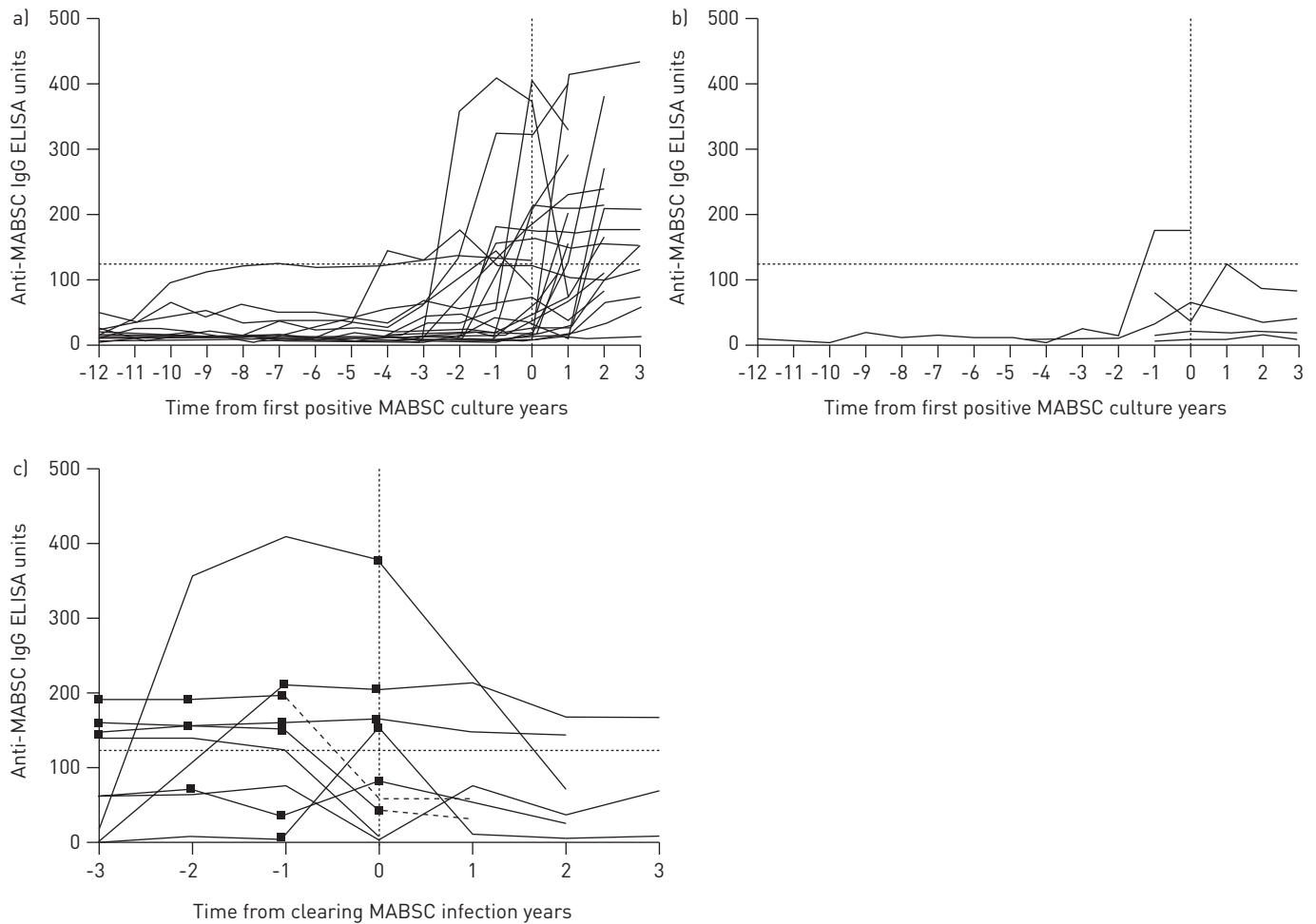
FIGURE 5 Anti-*Mycobacterium abscessus* complex (MABSC) antibody kinetics in serum from a) 26 cystic fibrosis (CF) patients who developed MABSC pulmonary disease, and b) six patients with CF with only one positive MABSC culture. c) Anti-MABSC IgG kinetics in nine CF patients who cleared active pulmonary MABSC infection. The dotted horizontal lines are at 125 ELISA units (test positivity threshold). Dotted vertical lines represent a, b) the date of first positive culture, and c) the date of last positive culture before clearance of MABSC pulmonary disease. Squares represent intravenous courses of amikacin combined with a carbapenem. Dashed lines are data after lung transplantation.

Diagnostic delay

Our longitudinal study of MABSC cases revealed patterns of early antibody response indicative of significant diagnostic delay. MARTINIANO *et al.* [6] have previously shown that a low forced expiratory volume in 1 s prior to first positive NTM culture is associated with whether the subsequent infection became clinically significant. The authors avoid speculating on whether the causality involved was either poor lung function leading to increased susceptibility to NTM or conversely nondetected NTM itself causing the lung function decline in the year leading up to its first isolation (diagnostic delay). Our study of anti-MABSC IgG kinetics supports the latter hypothesis that NTM itself is more likely to be the cause of poor lung function. The rise of antibody levels prior to culture positivity suggests significant infection, albeit clinically undetected.

Usefulness of risk algorithm

Serological screening can expedite diagnosis by selectively increasing diagnostic vigilance in high-risk patients. *P. aeruginosa* serology has proved a useful clinical tool in supporting clinical decision making [15, 17–19]. The principle has limitations [20, 21], and treatment decisions should not be based on *P. aeruginosa* antibody levels alone, since the antibody response to some antigens may be delayed [17]. Also, the induced antibody response may persist due to immunological memory, a principle well described in the field of vaccinology. In this study, we propose a risk algorithm that can selectively increase the mycobacterial culture frequency of patients with CF at highest risk of MABSC infection. The algorithm will need to be validated in a large multicentre cohort study before it can be recommended for routine clinical use. Conventional culture remains the gold standard and is required for the final diagnosis of

MABSC infection. However, an annual serum screening would allow patients to be stratified according to risk, permitting clinicians to readily differentiate between patients in need of intensified observation and those who can await routine annual culture. This sequence of first serum screening, then culture, allows for the optimal combination of high sensitivity (serology) and subsequent high specificity (culture). This explains our choice of a relatively low positivity threshold (125 ELISA units) for the IgG screening. While a higher positive predictive value could be achieved at the cut-off of 400 ELISA units, the cut-off of 125 ELISA units had the advantage of minimising the false negative rate (5%). The trade-off on positive predictive value is acceptable because antibody screening will always be followed by culture. Applying the algorithm to our MABSC-negative population, 73% were IgG negative, resulting in no clinical consequence, 23% had intermediate values between 125 and 400 ELISA units, warranting two to four annual NTM cultures, and just 4% were high-risk patients who would be subjected to monthly NTM culturing. With a positive likelihood ratio of 13 for patients with anti-MABSC IgG >400 ELISA units, the test fulfils the prerequisites of a useful diagnostic test for this purpose [36]. FERRONI et al. [23] showed that using the A60 assay manufacturer's recommended cut-off resulted in low sensitivity (80%), but this could be improved somewhat (to 86%) by applying their own cut-offs. We deliberately set the cut-off low, achieving a high sensitivity (95%) and the ability to rule out disease (low-risk patients). This is a rational approach in our centre, but the best use of antibody testing for MABSC might vary depending on the setting. Distinguishing genuine disease from mere colonisation has been declared one of the most important challenges in NTM management [1], and any objective marker of disease progression that would assist in making this distinction is valuable. Whether the test has other uses, such as for monitoring treatment effect in patients with MABSC disease, remains to be seen. Certainly, supplementary disease monitoring methods are in demand, as there are currently no good indicators of MABSC eradication. Culture conversion is necessary but in itself not sufficient to stop treatment, and relapses are known to occur upon cessation of therapy [37]. MABSC treatment regimens are exceedingly burdensome, with hearing loss and nephrotoxicity being the most feared adverse events [37]. Any indicator that could assist in reducing unnecessary long-term treatment is thus welcomed.

Conclusions

Antibody levels against MABSC were significantly elevated in patients with CF with MABSC-PD. Anti-MABSC IgG screening proved to be a useful diagnostic tool and can help clinicians identify patients with CF in need of more frequent mycobacterial culture, thus reducing the problem of diagnostic delay.

References

1   Leung JM, Olivier KN. Nontuberculous mycobacteria: the changing epidemiology and treatment challenges in cystic fibrosis. *Curr Opin Pulm Med* 2013; 19: 662–669.
2   Qvist T, Pressler T, Høiby N, et al. Shifting paradigms of nontuberculous mycobacteria in cystic fibrosis. *Respir Res* 2014; 15: 41.
3   Bryant JM, Grogono DM, Greaves D, et al. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. *Lancet* 2013; 381: 1551–1560.
4   Esther CRJr, Esserman DA, Gilligan P, et al. Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis. *J Cyst Fibros* 2010; 9: 117–123.
5   Griffith DE. Therapy of nontuberculous mycobacterial disease. *Curr Opin Infect Dis* 2007; 20: 198–203.
6   Martiniano SL, Sontag MK, Daley CL, et al. Clinical significance of a first positive nontuberculous mycobacteria culture in cystic fibrosis. *Ann Am Thorac Soc* 2014; 11: 36–44.
7   Pai M. Diagnosis of pulmonary tuberculosis: recent advances. *J Indian Med Assoc* 2013; 111: 332–336.
8   Dowdy DW, Steingart KR, Pai M. Serological testing *versus* other strategies for diagnosis of active tuberculosis in India: a cost-effectiveness analysis. *PLoS Med* 2011; 8: e1001074.
9   Cocito CG. Properties of the mycobacterial antigen complex A60 and its applications to the diagnosis and prognosis of tuberculosis. *Chest* 1991; 100: 1687–1693.
10 Bukhary ZA. Evaluation of anti-A60 antigen IgG enzyme-linked immunosorbent assay for serodiagnosis of pulmonary tuberculosis. *Ann Thorac Med* 2007; 2: 47–51.
11 Khurshid S, Afzal M, Khalid R, et al. Improving sensitivity for serodiagnosis of tuberculosis using TB16.3-echA1 fusion protein. *Tuberculosis* 2014; 94: 519–524.
12 Abebe F, Holm-Hansen C, Wiker HG, et al. Progress in serodiagnosis of *Mycobacterium tuberculosis* infection. *Scand J Immunol* 2007; 66: 176–191.
13 Okuda Y, Maekura R, Hirotani A, et al. Rapid serodiagnosis of active pulmonary *Mycobacterium tuberculosis* by analysis of results from multiple antigen-specific tests. *J Clin Microbiol* 2004; 42: 1136–1141.
14 Baumann R, Kaempfer S, Chegou NN, et al. Serologic diagnosis of tuberculosis by combining Ig classes against selected mycobacterial targets. *J Infect* 2014; 69: 581–589.
15 Pressler T, Karpati F, Granström M, et al. Diagnostic significance of measurements of specific IgG antibodies to *Pseudomonas aeruginosa* by three different serological methods. *J Cyst Fibros* 2009; 8: 37–42.

16   Skov M, Pandey JP, Pressler T, *et al.* Immunoglobulin allotypes and IgG subclass antibody response to *Aspergillus fumigatus* in cystic fibrosis patients. *J Cyst Fibros* 2004; 3: 173–178.

17   Döring G, Høiby N. Longitudinal study of immune response to *Pseudomonas aeruginosa* antigens in cystic fibrosis. *Infect Immun* 1983; 42: 197–201.

18   Mauch RM, Levy CE. Serum antibodies to *Pseudomonas aeruginosa* in cystic fibrosis as a diagnostic tool: a systematic review. *J Cyst Fibros* 2014; 13: 499–507.

19   Kappler M, Nagel F, Feilcke M, *et al.* Predictive values of antibodies against *Pseudomonas aeruginosa* in patients with cystic fibrosis one year after early eradication treatment. *J Cyst Fibros* 2014; 13: 534–541.

20   Daines C, VanDeVanter D, Khan U, *et al.* Serology as a diagnostic tool for predicting initial *Pseudomonas aeruginosa* acquisition in children with cystic fibrosis. *J Cyst Fibros* 2014; 13: 542–549.

21   Ratjen F, Walter H, Haug M, *et al.* Diagnostic value of serum antibodies in early *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *Pediatr Pulmonol* 2007; 42: 249–255.

22   Oliver A, Maiz L, Canton R, *et al.* Nontuberculous mycobacteria in patients with cystic fibrosis. *Clin Infect Dis* 2001; 32: 1298–1303.

23   Ferroni A, Sermet-Gaudelus I, Le Bourgeois M, *et al.* Measurement of immunoglobulin G against mycobacterial antigen A60 in patients with cystic fibrosis and lung infection due to *Mycobacterium abscessus*. *Clin Infect Dis* 2005; 40: 58–66.

24   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.

25   Esther CRJr, Hoberman S, Fine J, *et al.* Detection of rapidly growing mycobacteria in routine cultures of samples from patients with cystic fibrosis. *J Clin Microbiol* 2011; 49: 1421–1425.

26   Qvist T, Gilljam M, Jönsson B, *et al.* Epidemiology of nontuberculous mycobacteria among patients with cystic fibrosis in Scandinavia. *J Cyst Fibros* 2015; 14: 46–52.

27   Closs O, Harboe M, Axelsen NH, *et al.* The antigens of *Mycobacterium bovis*, strain BCG, studied by crossed immunoelectrophoresis: a reference system. *Scand J Immunol* 1980; 12: 249–263.

28   Pedersen SS, Espersen F, Høiby N, *et al.* Immunoglobulin A and immunoglobulin G antibody responses to alginates from *Pseudomonas aeruginosa* in patients with cystic fibrosis. *J Clin Microbiol* 1990; 28: 747–755.

29   Marras TK, Daley CL. Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clin Chest Med* 2002; 23: 553–567.

30   Wu AH. Biological and analytical variation of clinical biomarker testing: implications for biomarker-guided therapy. *Curr Heart Fail Rep* 2013; 10: 434–440.

31   Jacobson RH. Validation of serological assays for diagnosis of infectious diseases. *Rev Sci Tech* 1998; 17: 469–526.

32   Menzies D. Hammering the point home: serologic testing costs more and harms more patients than other strategies for the diagnosis of active tuberculosis in India. *Evid Based Med* 2012; 17: 58–59.

33   Hjelt K, Højlyng N, Howitz P, *et al.* The role of mycobacteria other than tuberculosis (MOTT) in patients with cystic fibrosis. *Scand J Infect Dis* 1994; 26: 569–576.

34   Hermansen T, Lillebaek T, Hansen AB, *et al.* QuantiFERON-TB Gold In-Tube test performance in Denmark. *Tuberculosis* 2014; 94: 616–621.

35   Hermansen TS, Thomsen VØ, Lillebaek T, *et al.* Non-tuberculous mycobacteria and the performance of interferon gamma release assays in Denmark. *PLoS One* 2014; 9: e93986.

36   Fischer JE, Bachmann LM, Jaeschke R. A readers' guide to the interpretation of diagnostic test properties: clinical example of sepsis. *Intensive Care Med* 2003; 29: 1043–1051.

37   Nessar R, Cambau E, Reyrat JM, *et al.* *Mycobacterium abscessus*: a new antibiotic nightmare. *J Antimicrob Chemother* 2012; 67: 810–818.