Online data supplement

Serodiagnosis of *Mycobacterium abscessus* complex infection in cystic fibrosis

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SUPPLEMENTARY METHODS

Antigen preparation

Mycobacterial antigen preparation was performed using a *M. abscessus sensu stricto* serovar obtained from a patient known to be infected with MABSC. Cells were grown in Souton's medium for 4 weeks and harvested by filtration and centrifugation, (12,000 G), X-press disrupted 5 times at maximal force (200 MPa at -20°C) and then sonicated 5 min. with a Sonopuls Ultrasonic Homogenizer (Bandelin, Berlin, Germany) 5 times and the disrupted cells pelleted by centrifugation (48,372 G). The supernatant was then used as antigen for the ELISA test. The protein concentration was set at 2.67 mg/ml as determined by refractometry (Atago, Tokyo, Japan). The antigen preparation was stored at -80° C.

Supplementary ELISA method

The measured (mean of double determinations) optical density (OD) values of sera were transformed to EU through the use of a standard curve plotting the titre of pooled sera from known MABSC cases in eight dilutions (1:500 to 1:64000). Outlying results were not excluded. All serum testing was performed by one laboratory technician, with expert level ELISA experience, who was blinded in regard NTM status.

Longitudinal case study setting 1987 - 2014

Patients attending the Copenhagen cystic fibrosis (CF) Centre account for approximately 70 % of the total Danish CF population. Population wide screening for nontuberculous mycobacteria (NTM) was performed once in 1988. The following 23 years, testing for NTM was only performed routinely on bronchoalveolar lavage (BAL) fluids. Sputum samples were cultured for NTM in case of clinical suspicion. In 2011, prior to the present study, systematic annual NTM screening was introduced, consisting of acid fast microscopy and mycobacterial culture performed at the International Reference Laboratory of Mycobacteriology at the State Serum Institute, Copenhagen, Denmark.

Criteria for disease

The American Thoracic Society and Infectious Disease Society of America (ATS/IDSA)'s criteria to classify NTM patients were: Pulmonary symptoms, nodular or cavitatory opacities on chest radiograph, or a HRCT scan that shows multifocal bronchiectasis with multiple small nodules after appropriate exclusion of other diagnoses. In addition, positive culture results from at least two separate sputum samples or positive culture results from at least one bronchoalveolar lavage. Information from patient files was used to confirm historical NTM cases and classify when patients fulfilled ATS/IDSA criteria for pulmonary disease (PD).

RESULTS

Values from CF patients with no known history of NTM were not normally distributed, but approximated normal distribution after log transformation. Geometric mean values and confidence intervals for this group and the non-CF controls were 66 EU (95 % CI: 59 – 75) and 32 EU (95 % CI: 31 – 34) respectively.

Supplementary Table

Table S1. Supplementary test characteristics of anti-*Mycobacterium abscessus* complex IgG antibody ELISA* using alternative combinations of patient groups

	Group AB vs. C	95 % CI	Group AB vs. D	95 % CI	Group A vs. D	95 % CI	Group A vs. BC	95 % CI
Sens, %	56	(42-70)	56	(42-70)	89	(67-98)	89	(67-98)
Spec, %	74	(68-79)	98	(96-99)	98	(96-99)	73	(67-78)
PLR	2.2	(1.6-2.9)	25.0	(13.6-45.8)	40.0	(22.2-70.9)	3.3	(2.6-4.2)
NLR	0.6	(0.4-0.8)	0.5	(0.3-0.6)	0.1	(0-0.4)	0.2	(0-0.5)
PPV, %	32	(23-42)	72	(56-85)	59	(39-76)	18	(11-27)
NPV, %	89	(83-93)	96	(94-97)	100	(99-100)	99	(97-100)

* = applying the 125 ELISA unit cut-off. Group A = cystic fibrosis (CF) patients with *M. abscessus* complex (MABSC) pulmonary disease (PD), Group B = CF patients with other nontuberculous mycobacteria (NTM) and patients with previous MABSC-PD, Group C = CF patients with no history of NTM disease, Group D = healthy non-CF controls, IgG = immunoglobulin, ELISA = enzyme-linked immunosorbent assay, CI = confidence interval, sens = sensitivity, spec = specificity, PLR = positive likelihood ratio, NLR = negative likelihood ratio, PPV = positive predictive value, NPV = negative predictive value.