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Mannose-binding lectin deficiency and disease severity in non-cystic fibrosis bronchiectasis: a prospective study

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Summary

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Background Mannose-binding lectin (MBL) is a key component of innate immunity. MBL deficiency is common (10–30% of the general population depending on the definition used) and has been associated with disease progression in cystic fibrosis. We aimed to assess the effect of MBL deficiency on disease severity in non-cystic fibrosis bronchiectasis.

Methods We recruited patients with non-cystic fibrosis bronchiectasis and age-matched and sex-matched controls at a specialist bronchiectasis clinic in Edinburgh, UK. We assessed MBL function with genotyping (low-expressing genotype [deficiency] defined as homozygosity for exon 1 mutations [YO/YO] or compound heterozygosity [XA/YO]; YA/YO and XA/XA genotypes were defined as intermediate-expressing with all other genotypes defined as high-expressing) and serum measurements (deficiency defined with two parameters: <500 ng/mL or <200 ng/mL). We assessed rates of exacerbation, chronic bacterial colonisation, and lung function during 4 years of follow-up.

Findings We included 470 patients with bronchiectasis and 414 controls. MBL genotype frequencies and MBL serum concentrations did not differ between patients and controls. 55 (12%) patients with bronchiectasis had low-expressing genotypes. These patients had a mean of 2.7 exacerbations per year (SD 1.8), compared with 1.9 per year (1.2) for 135 patients with intermediate-expressing genotypes and 1.9 per year (1.3) for 280 patients with high-expressing genotypes ($p < 0.0001$). Chronic colonisation with bacteria was most frequent in patients with low-expressing genotypes (47 [85%] patients vs 82 [61%] patients with intermediate-expressing genotypes and 183 [65%] patients with high-expressing genotypes; $p = 0.0041$); especially *P aeruginosa* colonisation (19 [35%] patients vs 13 [10%] patients and 36 [13%] patients; $p < 0.0001$). Patients with low-expressing genotypes were more likely to be admitted to hospital for severe exacerbations during follow-up (27 [49%] patients vs 42 [31%] patients and 87 [31%] patients; $p = 0.032$). Patients with low-expressing genotypes also had increased scores for radiological severity and worse quality of life compared with the other two groups. MBL serum deficiency (<200 ng/mL) was associated with increased exacerbations, hospital admissions, and radiological severity. When <500 ng/mL was used as the definition of deficiency, the associations with exacerbation frequency and radiological severity were no longer significant.

Interpretation MBL might be an important modifier of disease severity in non-CF bronchiectasis.

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Introduction

Bronchiectasis is a chronic inflammatory lung disease characterised by permanent dilatation of the bronchi.¹ Patients with the disease have daily cough, sputum production, and recurrent respiratory infections.¹ Central to the pathogenesis of bronchiectasis is a cycle of failed bacterial clearance, airway inflammation, and airway structural damage.² Patients become chronically colonised with pathogens owing to a failure of host immune defences.³ The cause of adult bronchiectasis for most patients is unknown.⁴

Mannose-binding lectin (MBL) is a soluble pattern-recognition molecule of the innate immune system.⁵ MBL binds to glycoconjugates containing mannose, fucose or N-acetylglucosamine on the surface of a wide range of clinically important bacteria, viruses and fungi, activating the lectin pathway of complement.^{6,7} Through complement activation, MBL promotes phagocytosis and leucocyte chemotaxis and activation.^{8,9} MBL might also

have a role in the resolution of inflammation in the lung through the clearance of apoptotic cells and in suppression of proinflammatory cytokine secretion.^{10,11}

MBL deficiency is one of the most common immune defects, affecting 10–30% of people depending on the definition of deficiency used.¹² MBL deficiency has been associated with recurrent respiratory infections, and is associated with the presence of bronchiectasis in patients with common variable immunodeficiency.¹³

Although MBL deficiency is not the cause of cystic fibrosis, several studies have shown that such a status modifies the course of disease in cystic fibrosis, leading to a more rapid decline in forced expiratory volume in 1 s (FEV₁), early acquisition of *Pseudomonas aeruginosa*, infection with *Burkholderia cepacia*, and death.^{14–16} In this study, we aimed to assess whether mannose-binding lectin deficiency was also associated with disease severity and clinical outcomes in adults with non-cystic fibrosis bronchiectasis.

Methods

Study design and patients

We recruited patients with non-cystic fibrosis bronchiectasis from a regional specialist bronchiectasis clinic at the Royal Infirmary of Edinburgh (Edinburgh, UK). Bronchiectasis was defined as presence of bronchial dilatation on high-resolution CT scanning with a compatible clinical history of daily cough with sputum production and recurrent respiratory infections.

We excluded patients with primary immunodeficiency (eg, common variable immunodeficiency), active malignant disease, cystic fibrosis, active allergic bronchopulmonary aspergillosis (as defined elsewhere;⁴ current treatment with corticosteroids or itraconazole was also an exclusion), interstitial lung disease, active mycobacterial disease, current smoking (within 2 years), HIV infection, or current chronic liver disease.

We recruited age-matched and sex-matched healthy volunteers from the spouses and partners of patients attending the outpatient clinics at the Royal Infirmary of Edinburgh.

The study was approved by the local research ethics committee and all participants provided written informed consent.

Procedures

At the time of inclusion, all patients were clinically stable with no antibiotic use in the preceding 4 weeks. We followed up patients for 4 years with review every 6 months. Patients provided blood samples for genomic DNA extractions, serum for measurement of MBL, and spontaneous sputum samples for bacteriological analysis and markers of airway inflammation.

We assessed severity of bronchiectasis by scoring high-resolution CT scans with a modified Reiff score.^{4,17} At every visit, all patients underwent clinical assessments including spirometry FEV₁, forced vital capacity with the highest of three technically satisfactory measurements recorded,¹⁸ and chest radiography. Patients completed the St George's respiratory questionnaire (minimum clinically important difference 4 units)¹⁹ and the Leicester cough questionnaire (minimum clinically important difference 1.3 units)²⁰ as measures of quality of life and cough severity.

We recorded unscheduled hospital admissions in the previous year for severe exacerbations from patient histories and verified reports by use of an administrative database that recorded all regional hospital admissions. We quantified outpatient antibiotic use for exacerbations of bronchiectasis from patient histories and verified findings against primary-care prescription records. Such clinical databases are widely used in clinical research in the UK.²¹ We classified patients as chronically colonised if they isolated in sputum culture a potentially pathogenic microorganism on two occasions at least 3 months apart in 1 year while clinically stable.

We undertook quantitative and qualitative bacteriological analysis as previously described.²² For measure-

	Abbreviated genotype	Abbreviated combined genotypes	MBL expression
HYPD/LYQC/LYPB haplotype	O	YO/YO	Low
LXPA haplotype	XA	XA/YO	Low
LYPE/LYQA haplotype	YA	YA/YO	Intermediate
HYPA haplotype			
Type 1	YA	XA/XA	Intermediate
Type 2	YA	YA/XA	High
Type 3	YA	YA/YA	High

MBL=mannose-binding lectin.

Table 1: Haplotypes and abbreviated genotypes according to serum MBL expression

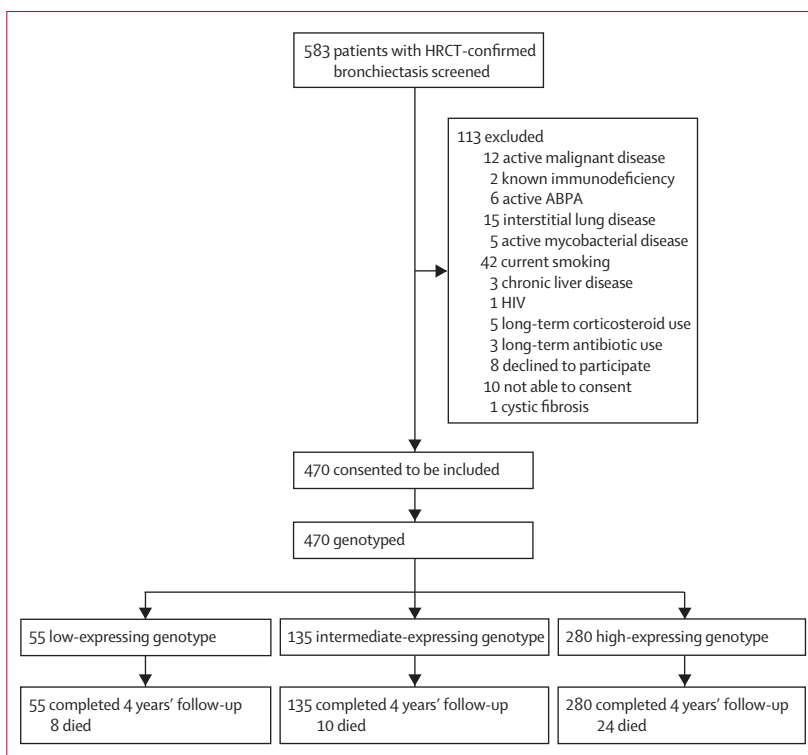


Figure 1: Trial profile

HRCT=high-resolution CT. ABPA=allergic bronchopulmonary aspergillosis.

ment of markers of airway inflammation, sputum was ultracentrifuged at 50 000×g for 90 min at 4°C. The sol phase was removed and immediately frozen at -70°C. We measured markers of airway inflammation as previously described.²³

We did serum measurement of MBL as described elsewhere.²⁴ Briefly, diluted sera were incubated in mannan-coated ELISA plates and bound MBL was detected with a specific monoclonal antibody to MBL (HYB131-01) followed by anti-mouse immunoglobulin conjugated to alkaline phosphatase with p-nitrophenyl phosphate as a substrate. Previous validation of this

assay showed an intra-assay coefficient of variation of 3·5% and inter-assay coefficient of variation of 7%.²⁴

To assess the effects of changes in MBL on disease severity over time, we measured serum MBL at study baseline, midpoint, and at the end of the study in all patients. In addition, to determine the effect of exacerbations on serum MBL, we recruited 68 patients attending the Royal Infirmary of Edinburgh bronchiectasis service for treatment of exacerbations.²³ Serum samples were obtained at day 1 (start of exacerbation) and day 14 (end of exacerbation). We treated patients with intravenous antibiotic therapy on the basis of their previous sputum microbiological results for 14 days. Repeat measurements were then made at least 3 months after exacerbation to determine return of MBL concentrations to baseline levels.

Genomic DNA was isolated from EDTA (edetic acid)-anticoagulated whole-blood samples with the Nucleon BACC-3 kit (Gen-Probe, MA, USA). Isolated DNA was quality-tested and genotyping was done at the Wellcome Trust Clinical Research Facility Genetics Core (Edinburgh, UK). Validated Taqman allele specific PCR primers were purchased from Applied Biosystems (CA, USA) and PCR done on the Applied Biosystems 7900HT according to the manufacturer's instructions. We studied six single-nucleotide polymorphisms (SNPs) known to have the greatest effect on MBL serum concentrations: the exon-1 polymorphisms B-rs1800450, C-rs1800451, and D-rs5030737, the promoter polymorphisms H/L, rs11003125, X/Y, and rs7096206, and the 5'-untranslated

region SNP P/Q, rs7095891. These SNPs comprise seven well-characterised "secretor haplotypes" (HYPA, LYPA, LYQA, LXPA, HYPD, LYPB, and LYQC) which strongly influence circulating MBL concentrations²⁴ (table 1).

No universally agreed upon definition of MBL deficiency exists. MBL function can be assessed by genotype, serum concentrations, or functional activity in complement activation assays. Although these assessments are strongly correlated, they do not provide identical results. To account for this, we present data with three definitions of MBL deficiency determined a priori as genotypes associated with MBL deficiency, serum concentrations of less than 500 ng/mL, and serum concentrations of less than 200 ng/mL.

Statistical analysis

Normally distributed data are presented as mean (SD) and non-normally distributed data are presented as median (IQR). We analysed deviation from the normal distribution with the D'Agostino and Pearson omnibus K² test. For comparisons of more than two groups of continuous data, we used one-way ANOVA or the Kruskal-Wallis test as appropriate. We used the χ^2 test to analyse more than two groups of categorical data. To adjust for confounders of the relation between MBL deficiency and chronic colonisation, we used multi-variable logistic regression analysis. To investigate the relation between MBL genotype and survival, we used the Cox's proportional hazard model to estimate survivor functions with survival during 4 years of follow-up as the dependent variable. We adjusted for variables associated with mortality ($p < 0\cdot05$) in univariate analysis. For all analyses, $p < 0\cdot05$ was regarded as significant. Analyses were done with SPSS version 21 and Graphpad Prism software.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We included 470 patients with non-cystic fibrosis bronchiectasis and 414 healthy controls (figure 1). No patients were lost to follow-up. Table 2 shows the characteristics of the study population. None of the patients included in the study was treated with long-term (>28 days) oral or nebulised antibiotics or oral corticosteroids. We noted no significant differences in demographics or non-respiratory comorbid illnesses between patients with bronchiectasis and controls. In addition to a diagnosis of bronchiectasis, 55 patients had previously been diagnosed with asthma and 17 patients had chronic obstructive pulmonary disease.

	Bronchiectasis cohort (n=470)	Control cohort (n=414)	p value
Age, years	64 (53-72)	65 (53-74)	0·22
Sex, female	259 (55%)	211 (51%)	0·22
Body-mass index, kg/m ²	25·4 (5·2)	Not recorded	..
Ex-smokers	177 (38%)	180 (43%)	0·079
Comorbidities			
Chronic cardiac disease	108 (23%)	99 (24%)	0·74
Cerebrovascular disease	46 (10%)	42 (10%)	0·86
Chronic renal impairment	32 (7%)	33 (8%)	0·51
Diabetes mellitus	58 (12%)	41 (10%)	0·25
COPD	17 (4%)	0	<0·0001
Asthma	55 (12%)	0	<0·0001
Cause of bronchiectasis			
Idiopathic or after infection	378 (80%)
Previous ABPA	41 (9%)
Connective tissue disease	28 (6%)
Inflammatory bowel disease	14 (3%)
Others	9 (2%)
Inhaled corticosteroid use	196 (42%)	0	<0·0001

Data are median, n (%), or mean (SD). COPD=chronic obstructive pulmonary disease. ABPA=allergic bronchopulmonary aspergillosis.

Table 2: Clinical characteristics of the study population

378 (80%) of 470 patients had idiopathic or post-infective bronchiectasis. No controls had a history of chronic respiratory disease and none had been prescribed inhaled corticosteroids (table 2).

All studied SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$). Patients with high-expressing genotypes had a mean serum MBL concentration of 2300 ng/mL (SD 1300), compared with 950 ng/mL (SD 870) with intermediate-expressing genotypes, and 130 ng/mL (SD 220) in patients with low-expressing genotypes (defined as MBL deficient).

Genotype frequencies and serum MBL concentrations did not differ between patients with bronchiectasis and controls (table 3). The frequency of MBL deficiency did not vary according to cause of bronchiectasis (data not shown).

361 (77%) of 470 patients had chronic bacterial colonisation. *Haemophilus influenzae* was the most frequently isolated pathogen (141 patients [30%]), followed by *P aeruginosa* (68 patients [14%]), *Moraxella catarrhalis* (54 patients [11%]), enteric gram-negative organisms (46 patients [10%]), *Staphylococcus aureus* (43 patients [9%]), and *Streptococcus pneumoniae* (30 patients [6%]).

We noted a higher frequency of bacterial colonisation for patients with low-expressing genotypes (table 4) and deficiency for both serum concentrations (table 5). Rates of bacterial colonisation did not differ between patients with intermediate-expressing genotypes and those with high-expressing genotypes ($p = 0.36$). For both serum concentration cutoffs, we noted a higher frequency of bacterial colonisation with *H influenzae*, and *P aeruginosa* in the MBL-deficient group than in the non-deficient group. However, this effect was not evident when patients with the low-expressing genotype were excluded, suggesting that this difference was wholly attributable to these patients. After adjustment for age, radiographic severity, and percentage predicted FEV₁, the logistic regression analysis did not show a significant effect of low-expressing genotypes on chronic colonisation (adjusted odds ratio 1.85, 95% CI 0.96–3.59; $p = 0.063$). The relation between low-expressing genotypes and *P aeruginosa* was not significant after adjustment for age, radiographic severity, and percentage predicted FEV₁ (1.25, 0.55–2.83; $p = 0.65$).

Rates of colonisation with other bacterial species did not correlate with MBL deficiency as defined by genotype, although the numbers of cases in each group were small. One patient was chronically colonised with *B cepacia* and this patient had YO/YO genotype.

MBL deficiency defined by genotype or serum concentration was not related to percentage predicted FEV₁ or FVC (tables 4, 5). Patients with the low-expressing genotype had more severe radiological bronchiectasis with the modified Reiff score (table 4), as did patients with serum deficiency defined as less than 200 ng/mL (table 5). This effect was not evident when serum deficiency was defined as less than 500 ng/mL.

	Bronchiectasis cohort (n=470)	Control cohort (n=414)	p value
Low-expressing groups	55 (12%)	42 (10%)	0.46
YO/YO	18 (4%)	14 (3%)	0.72
XA/YO	37 (8%)	28 (7%)	0.53
Intermediate-expressing groups	135 (29%)	145 (35%)	0.06
YA/YO	111 (24%)	117 (28%)	0.12
XA/XA	24 (5%)	28 (7%)	0.30
High-expressing groups	280 (60%)	227 (55%)	0.15
YA/XA	119 (25%)	95 (23%)	0.41
YA/YA	161 (34%)	132 (32%)	0.45
Serum levels			
<500 ng/mL	128 (27%)	122 (29%)	0.46
<200 ng/mL	88 (19%)	79 (19%)	0.89

Data are n (%). MBL=mannose-binding lectin.

Table 3: Genotype frequencies and serum concentrations in patients with non-cystic fibrosis bronchiectasis and controls

	Low-expressing group (n=55)	Intermediate-expressing group (n=135)	High-expressing group (n=280)	p value
Age, years	63 (53–70)	65 (55–71)	63 (52–72)	0.96
Bacteriological findings				
Chronic colonisation	47 (85%)	82 (61%)	183 (65%)	0.0041
<i>Haemophilus influenzae</i>	26 (47%)	36 (27%)	79 (28%)	0.011
<i>Pseudomonas aeruginosa</i>	19 (35%)	13 (10%)	36 (13%)	<0.0001
Pulmonary function				
FEV ₁ , percentage predicted	68.9% (25.5)	70.0% (24.4)	74.3% (24.8)	0.096
FVC, percentage predicted	81.6% (24.2)	84.1% (22.4)	85.4% (24.6)	0.54
Exacerbations and quality of life				
Annual exacerbation frequency*	4.2 (3.4)	2.6 (2.5)	2.7 (2.8)	0.0017
Hospital admission*	19 (35%)	29 (21%)	63 (23%)	0.12
SGRQ score	55.4 (21.9)	45.3 (23.7)	44.3 (21.9)	0.0069
LCQ score	12.7 (4.9)	14.4 (4.4)	14.3 (4.4)	0.029
HRCT score	4 (3–15)	3 (2–6)	3 (2–6)	0.0011
Body-mass index, kg/m ²	26.9 (7.2)	25.8 (5.2)	25.8 (5.4)	0.43
Inflammatory markers				
Myeloperoxidase (units per mL)	15.4 (24.3)	6.3 (11.7)	9.5 (16.1)	0.0022
Neutrophil elastase (units per mL)	27.0 (39.8)	14.9 (36.6)	13.9 (32.8)	0.038
Interleukin 8 (ng/mL)	48.5 (24.4)	45.3 (31.1)	38.0 (24.1)	0.0076
TNF α (pg/mL)	2718 (3089)	1383 (2022)	1458 (1968)	0.0003
Interleukin 1 β (ng/mL)	3.1 (2.8)	2.1 (2.5)	2.5 (2.7)	0.015

Data are median (IQR), n (%), or mean (SD), unless otherwise stated. Data show results at baseline; data during prospective follow-up are presented elsewhere in the report. MBL=mannose-binding lectin. FEV₁=forced expiratory volume in 1 s. FVC=forced vital capacity. SGRQ=St George's respiratory questionnaire. LCQ=Leicester cough questionnaire. HRCT=high-resolution CT. *In the year before the study.

Table 4: Baseline markers of severity in patients with MBL deficiency, according to genotype

Pulmonary function did not differ between patients with intermediate-expressing and high-expressing genotypes.

Frequency of exacerbations in the year before the study was higher in the low-expressing genotype group than it

was in the non-deficient groups (table 4). Frequency of exacerbations did not differ between non-deficient groups. Patients with serum concentrations of less than 200 ng/mL had more frequent exacerbations compared with those with higher MBL concentrations (table 5). Our

	MBL deficiency serum cutoff <500 ng/mL			MBL deficiency serum cutoff <200 ng/mL		
	Deficient (n=128)	Non-deficient (n=342)	p value	Deficient (n=88)	Non-deficient (n=382)	p value
Age, years	63 (53–72)	64 (52–72)	0.78	63 (56–71)	64 (52–72)	0.95
Bacteriological findings						
Chronic colonisation	94 (73%)	218 (64%)	0.047	67 (76%)	245 (64%)	0.032
<i>Haemophilus influenzae</i>	47 (37%)	94 (27%)	0.052	35 (40%)	106 (28%)	0.026
<i>Pseudomonas aeruginosa</i>	29 (23%)	39 (11%)	0.0020	22 (25%)	46 (12%)	0.0018
Pulmonary function						
FEV ₁ , percentage predicted	68.8% (25.5)	73.3% (24.7)	0.093	69.9% (26.1)	72.6% (24.7)	0.41
FVC, percentage predicted	84.8% (21.2)	85.3% (24.0)	0.85	81.5% (22.1)	85.5% (24.0)	0.60
Exacerbations and quality of life						
Annual exacerbation frequency	3.3 (2.8)	2.7 (2.8)	0.062	3.5 (3.2)	2.7 (2.8)	0.032
Hospital admission	39 (30%)	72 (21%)	0.032	32 (36%)	79 (21%)	0.0018
SGRQ score	48.4 (24.1)	44.7 (22.5)	0.14	49.6 (21.1)	45.0 (23.2)	0.13
LCQ score	13.1 (4.8)	14.5 (4.4)	0.0050	13.2 (4.7)	14.4 (4.4)	0.021
HRCT score	3 (2–9)	3 (2–6)	0.46	4 (2–12)	3 (2–6)	0.036
Body-mass index, kg/m ²	26.7 (5.8)	25.7 (5.3)	0.094	26.9 (6.0)	25.8 (5.3)	0.091

Data are median (IQR), mean (SD), or n (%), unless otherwise stated. MBL=mannose-binding lectin. FEV₁=forced expiratory volume in 1 s. FVC=forced vital capacity. SGRQ=St George's respiratory questionnaire. LCQ=Leicester cough questionnaire. HRCT=high-resolution CT.

Table 5: Markers of severity in patients with MBL deficiency, according to serum concentration

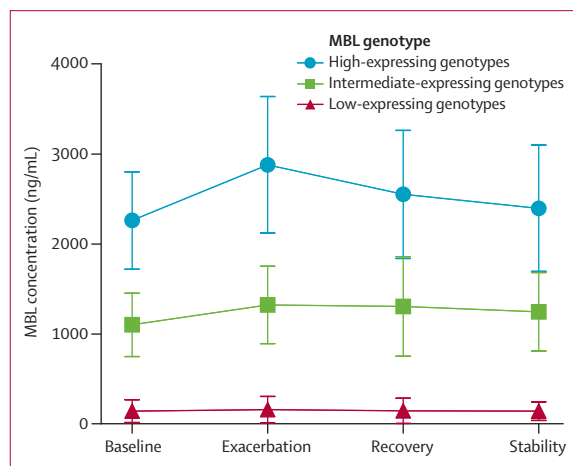


Figure 2: MBL serum concentrations
Datapoints are means and bars are 95% CIs. Blood samples were obtained at recruitment (baseline), day 1 (onset of exacerbation), and day 14 (end of exacerbation), and repeated when clinically stable at least 3 months after exacerbation (stability). Increase in MBL during exacerbation was only different for the high-expressing haplotypes ($p=0.01$ by ANOVA). MBL=mannose-binding lectin.

secondary-care population of patients with bronchiectasis had strikingly impaired quality of life. St George's respiratory questionnaire scores were higher for patients with low-expressing genotypes (table 4), but we noted no differences between non-deficient MBL genotypes ($p=0.35$). With the Leicester cough questionnaire, patients with low-expressing genotypes had severe cough symptoms compared with non-deficient genotypes (table 4); severity of cough symptoms did not differ between these non-deficient groups ($p=0.49$). Serum concentrations did not relate to St George's respiratory questionnaire scores but were associated with Leicester cough questionnaire scores (table 5).

Patients with MBL deficiency were more likely to have exacerbations or be admitted to hospital during the 4 years of follow-up. Patients with low-expressing genotypes had a mean exacerbation rate of 2.7 per patient per year (SD 1.8), compared with patients with intermediate-expressing genotypes (mean 1.9 per patient per year [SD 1.2]) or high-expressing genotypes (mean 1.9 per patient per year [SD 1.3]; $p<0.0001$). The difference in the mean incidence of exacerbations between patients with low-expressing genotypes and non-deficient genotypes was 0.72 per patient per year (95% CI 0.34–1.10). A greater proportion of patients with low-expressing genotypes were admitted to hospital on at least one occasion during follow-up (27 [49%] of 55 patients in the low-expressing group compared with 42 [31%] of 135 patients in the intermediate-expressing group and 87 [31%] of 280 patients in the high-expressing group; $p=0.032$). Patients with serum MBL deficiency (<200 ng/mL cutoff) had an increased frequency of exacerbations (mean 2.6 [SD 1.7] for deficiency vs 1.8 [1.3] for non-deficiency; $p<0.0001$) and an increased frequency of hospital admission during follow-up (38 [42%] vs 118 [31%]; $p=0.027$). The increased frequency of exacerbations was also noted with a cutoff of 500 ng/mL (mean 2.1 [SD 1.6] for deficiency vs 1.8 [1.3] for non-deficiency; $p=0.044$) but rates of hospital admission did not persist (49 [38%] vs 107 [31%]; $p=0.15$). Patients with low-expressing genotypes had higher rates of airway inflammation than did patients without this deficiency (table 4). These differences were mainly attributable to increased bacterial loads in sputum for patients with low-expressing genotypes compared with non-deficient genotypes (mean log₁₀ bacterial load 6.5 [SD 2.8] in the deficient group vs 5.1 [3.4] in the intermediate-expressing genotype group and 5.0 [3.3] in the high-expressing genotype groups; $p=0.014$ by ANOVA).

As reported previously,²⁴ MBL serum concentrations are largely genetically determined and we noted no significant variation in MBL serum concentrations at the start, middle, or end of the study ($p>0.05$ for all comparisons; figure 2). In subanalyses, changes in serum MBL over time did not correlate with deteriorating lung function or changes in bacterial

colonisation (data not shown) but MBL serum concentrations were increased at the onset of exacerbations (figure 2). We assessed 24 patients with high-expressing genotypes, 26 patients with intermediate-expressing genotypes, and 18 patients with MBL-deficient genotypes who attended for blood and sputum sampling at the onset of exacerbations and repeat measurement at completion of 14 days antibiotics. In the high-expressing MBL group, MBL concentrations increased by a mean of 27·3% at the start of the exacerbation ($p=0\cdot007$). Although not statistically significant, we noted a similar pattern in the intermediate-expressing group (mean increase 20·2%; $p=0\cdot37$). Patients with low-expressing genotypes ($p=0\cdot82$) did not upregulate MBL serum concentrations during exacerbation (figure 2).

42 deaths occurred during follow-up, with 25 (60%) of these deaths deemed to be related to bronchiectasis. In an exploratory analysis, we noted that eight (15%) deaths occurred in 55 patients with low-expressing genotypes, ten (7%) deaths occurred in 135 patients with intermediate-expressing genotypes, and 24 (9%) deaths occurred in 280 patients with high-expressing genotypes. Figure 3 shows the Kaplan-Meier analysis of all-cause mortality. Mortality did not differ significantly between groups (log-rank $p=0\cdot25$). A Cox-proportional hazard regression analysis adjusting for age, FEV₁, bacterial colonisation, and radiological severity of bronchiectasis did not establish higher mortality in the low-expressing genotype group (hazard ratio 1·58, 95% CI 0·73–3·4; $p=0\cdot44$). No increase in mortality was associated with serum MBL levels of less than 200 ng/mL (hazard ratio 1·43, 95% CI 0·65–3·12; $p=0\cdot37$) and no increase in mortality associated with serum MBL levels of less than 500 ng/mL (1·07, 0·54–2·12; $p=0\cdot84$).

Discussion

To our knowledge, this study is the first to describe a genetic modifier of disease severity in non-cystic fibrosis bronchiectasis. We showed that MBL deficiency (defined by genotype) was related to severity of disease, including quality of life and frequency of exacerbations and admission to hospital (panel).

Deficiency of MBL arises from variants in exon-1 and in the promoter region of the *MBL2* gene. Exon-1 alleles designated B, C, and D have a profound dominant effect on MBL serum concentrations. Patients homozygous for exon-1 mutations or compound heterozygous for an exon-1 mutation and the X/Y promoter polymorphism have very low serum MBL concentrations, typically less than 200 ng/mL.^{5,12,16}

Our study showed that these patients, with low serum MBL concentrations, have an increased frequency of chronic bacterial colonisation and an increased incidence of *H influenzae* and *P aeruginosa* compared with patients with higher serum MBL concentrations.

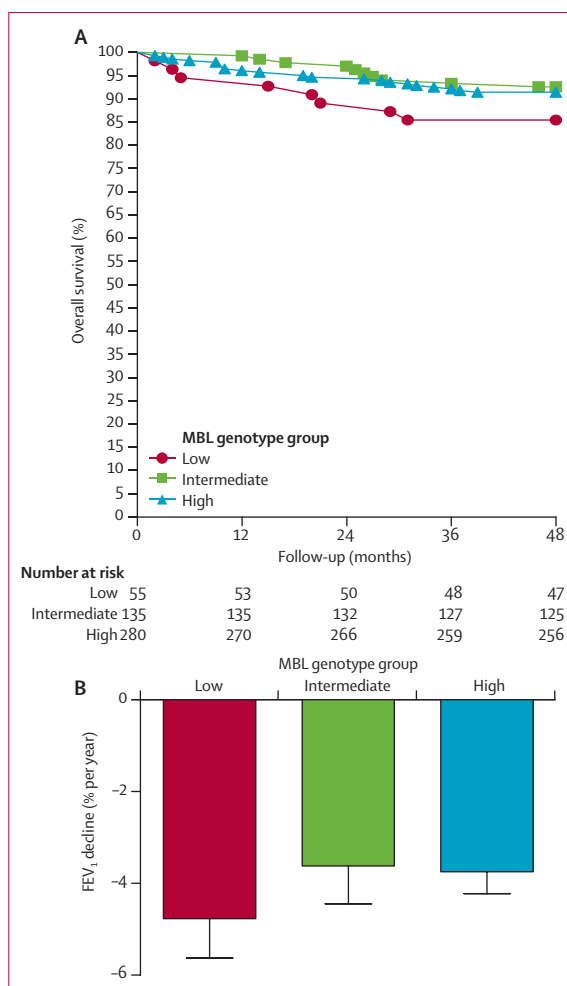


Figure 3: Long-term prognosis in patients with bronchiectasis according to MBL genotype group (A) and mean reduction in percentage predicted forced expiratory volume in 1 s (B). Error bars in (B) show standard error of the mean; $p=0\cdot3$ for comparison between groups.

Compared with patients with non-deficient MBL expression, patients with low-expressing genotypes had severe bronchiectasis as assessed by radiological scoring, and an increased frequency of outpatient exacerbations and hospital admissions for severe exacerbations during 4 years of follow-up. These patients also had increased impairment in quality of life and worse cough severity as assessed by the Leicester cough questionnaire. Patients with low-expressing genotypes had significantly higher measures of neutrophil mediated airway inflammation, primarily related to higher bacterial loads. Our study was not powered to assess mortality and larger multicentre studies are needed to address this outcome.

Despite our finding of increased severity of disease in patients with MBL deficiency, measurements of FEV₁ and FVC did not differ between MBL groups. This finding might seem inconsistent, but adds to a growing body of

literature suggesting that spirometric values are not as useful for prediction of outcome in non-cystic fibrosis bronchiectasis as they are in cystic fibrosis. For example, FEV₁ was not identified as an independent predictor of outcome in a study of long-term survival in bronchiectasis,²⁴ and other trials^{22,25} have shown FEV₁ does not improve significantly in response to treatment in bronchiectasis.

Notably, we did not identify any evidence for an effect of intermediate-expressing MBL genotypes on disease severity, with no significant differences noted in these markers of disease severity between intermediate-expressing and high-expressing MBL genotypes.

When we used definitions of serum MBL deficiency determined a priori (serum concentration <500 ng/mL or <200 ng/mL), we noted a significant association between serum MBL and bacterial colonisation, hospital admission, exacerbations, and radiological severity. These differences were, however, wholly attributable to the presence of patients with the most severe MBL deficiency in this group, because exclusion of patients with low-expressing MBL genotypes

removed the association. We therefore conclude that patients with intermediate-expressing MBL genotypes are not at increased risk of severe disease. Our study suggests that genotype is the most useful method of defining MBL deficiency, rather than serum levels, for future studies of MBL in bronchiectasis. Although serum levels are genetically determined, they can also be influenced by hormones, drugs, and the acute phase response.²⁸ We previously reported²⁹ no differences between patients with bronchiectasis and controls in serum MBL concentrations and have now confirmed these findings in a larger cohort. One other study³⁰ investigated the effect of serum MBL on disease severity in 133 patients with non-cystic fibrosis bronchiectasis. The study measured serum MBL concentrations but did not do genotyping, and reported no difference in disease severity between patients with serum concentrations of less than 600 ng/mL compared with those with more than 600 ng/mL. Although the study³⁰ was underpowered to show significant differences, a subgroup analysis of 13 patients suggested that serum concentrations of less than 100 ng/mL were associated with severe disease with more *P aeruginosa* and *H influenzae* infections and a higher frequency of exacerbations. These results seem therefore to support the findings of our present study, showing that only patients with severe MBL deficiency have a worse phenotype. However, several differences existed between MacFarlane and colleagues' study³⁰ and our own, including that their study obtained MBL measurements for clinical reasons and therefore included a much higher frequency of patients colonised with *P aeruginosa* than is typically reported in the literature.

Several large studies have now confirmed a relation between low-expressing MBL genotypes and disease severity in cystic fibrosis.¹⁴⁻¹⁶ Meta-analysis of studies¹⁶ in adults with cystic fibrosis showed that MBL deficiency was associated with early acquisition of *P aeruginosa*, increased infections with *B cepacia*, and increased mortality. Notably, the largest studies suggest that the poor prognosis associated with variant MBL alleles is only evident in those with low-expressing MBL genotypes (YO/YO and XA/YO).¹⁴ The meta-analysis also reported no significant effect on lung function or markers of cystic fibrosis severity with the intermediate-expression genotypes.¹⁶ This finding supports the results of the present study that suggested poor prognosis is associated with the low-expressing genotypes but that intermediate MBL expression is not associated with greater disease severity.

Evidence from studies of chronic obstructive pulmonary disease suggests that MBL deficiency might predispose patients to exacerbations.²⁷ Our study recruited patients from a specialist bronchiectasis clinic and therefore few patients in our cohort had a diagnosis of chronic obstructive pulmonary disease.

Panel: Research in context

Systematic review

We searched Pubmed, Embase, and Google scholar for articles published in any language between Jan 1, 1980, and Nov 30, 2012, with the search terms: "bronchiectasis", "cystic fibrosis", or "CF" AND "mannose-binding lectin" OR "mannan-binding lectin" OR "MBL" OR "MBL2" OR "MBL-2". This search updated a previous systematic review published in 2010.¹⁶ We identified two studies of mannose-binding lectin (MBL) in non-cystic fibrosis bronchiectasis: one study²⁵ that reported no difference in MBL serum concentrations between patients and controls and another UK-based study of 133 patients that reported no difference in disease severity between patients with serum concentrations of less than 600 ng/mL and those patients with serum concentrations of more than 600 ng/mL. We identified no genetic studies in non-cystic fibrosis bronchiectasis. For cystic fibrosis, meta-analysis of 18 studies suggested that MBL-deficient genotypes were associated with younger age of onset of colonisation with *Pseudomonas aeruginosa* (1713 patients; mean difference 3.7 years [95% CI 2.1-5.3 years]; p<0.0001), colonisation with *Burkholderia cepacia* (odds ratio 3.5 [95% CI 1.1-10.5]; p=0.028), lower forced expiratory volume in 1 s in adults (mean difference 19.7% [95% CI 10.8-28.0]; p<0.0001), and a higher risk of end-stage cystic fibrosis (death or lung transplantation; odds ratio 2.4 [95% CI 1.1-5.2]; p=0.034).

Interpretation

Our study shows that MBL deficiency defined by genotype is associated with increased severity of bronchiectasis as defined by exacerbation frequency, hospital admission, radiological findings, and health-related quality of life. Patients with an MBL deficiency also had a higher frequency of colonisation with bacteria including *Haemophilus influenzae* and *P aeruginosa*. After 4 years of follow-up, patients had more exacerbations and hospital admissions without significant association noted for survival or decline in lung function over time. MBL might be an important modifier of disease severity in non-CF bronchiectasis. This postulation should be confirmed in an independent cohort. Recent evidence that the antibiotic azithromycin, which is effective in bronchiectasis,²⁶ can reverse the basic defect associated with bronchiectasis²⁷ might lead to a new pharmacogenetic approach to treatment of this disease.

Bronchiectasis occurs commonly in patients with chronic obstructive pulmonary disease,³¹ so whether MBL deficiency also plays a part in chronic obstructive pulmonary disease and associated bronchiectasis will be important to understand.

The mechanism through which MBL predisposes to severe disease is not clear, although reports suggest that MBL binds *P aeruginosa*, *S aureus*, and *B cepacia* and other clinically relevant bacteria, leading to complement activation and enhanced clearance.^{6,7} MBL deficiency might therefore lead to deficient opsonophagocytosis; however, other mechanisms have also been proposed. MBL seems to be important in clearance of apoptotic cells, a key mechanism for the resolution of inflammation. Hodge and colleagues reported low concentrations of MBL in the airway of patients with chronic obstructive pulmonary disease and importantly, these low concentrations correlated with reduced apoptotic cell clearance.³²

To our knowledge, our report is the largest study to examine genetic modifiers of disease severity in non-cystic fibrosis bronchiectasis.^{33,34} Strengths of the study included the relative absence of confounders such as long-term antibiotic and steroid use and current smoking. We included a broad spectrum of patients from individuals with very mild disease to those with very severe disease who had verified hospital admissions and exacerbations using electronic data to reduce bias. Our data are from a single centre in the UK and are not necessarily generalisable to other health-care settings. Ideally our findings should now be replicated in a large independent, multicentre cohort.

MBL deficiency is a recognised immunodeficiency, with clinical testing available in many centres and guideline recommendations suggesting such testing should be done for patients with suspected primary immunodeficiency.³⁵ Identification of a group of patients at increased risk of exacerbations and poor outcome could be very useful to clinicians and we hope will stimulate further research to improve outcomes in bronchiectasis. Presently, few evidence-based treatments exist for bronchiectasis.¹ MBL-replacement therapy has been developed and might form a new therapeutic avenue for diseases associated with MBL deficiency.³⁶ Of more immediate clinical relevance to bronchiectasis, Hodge and colleagues showed that azithromycin could restore the failure of apoptotic-cell clearance associated with MBL deficiency, which raises the question of whether future trials should specifically target long-term macrolide treatment for patients with MBL deficiency to improve outcome.^{25,32}

Contributors

JDC wrote the report. BJM, CD, MPS, JRG, DCP, and ATH edited the report. All authors contributed to study design and data collection. JDC, DCP, and ATH analysed data. JDC, JRG, DCP, and ATH interpreted data.

Conflicts of interest

We declare that we have no conflicts of interest.

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