Impact of mannose-binding lectin insufficiency on the course of cystic fibrosis: A review and meta-analysis

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Received on August 31, 2010; revised on October 6, 2010; accepted on October 6, 2010

Mannose-binding lectin (MBL) is an innate immune protein produced by the liver. MBL binds to glycoconjugates containing mannose, fucose or N-acetylglucosamine that are present in a wide variety of bacteria, viruses and fungi. Upon binding, MBL may activate the lectin pathway of complement or directly opsonize organisms to enhance phagocytosis. MBL is primarily a serum protein but accumulates in the lung during acute inflammation. Recent evidence suggests an important role for MBL in a variety of infectious disorders. Cystic fibrosis (CF) is a multisystem disease caused by mutations in the gene encoding the CF transmembrane regulator (CFTR). The course of CF lung disease is highly variable even in patients with the same CFTR genotype, suggesting that other modulator genes are important for prognosis. MBL has been proposed as a possible modulator of clinical severity in CF. In this review and meta-analysis, we found that MBL2 genotypes associated with MBL insufficiency were associated with earlier acquisition of Pseudomonas aeruginosa (P < 0.0001), reduced pulmonary function among adult patients (P < 0.0001 for forced expiratory volume), and an increased rate of death or requirement for lung transplantation (odds ratio 3.69; P = 0.02). The available evidence therefore suggests that MBL insufficiency is associated with the severity of CF lung disease. The possible future prophylactic or therapeutic application of MBL replacement is discussed.

Keywords: cystic fibrosis / mannose-binding lectin / mannan-binding lectin / meta-analysis

Mannose-binding lectin

Mannose-binding lectin (also called mannan-binding lectin, MBL) is a serum protein produced by the liver and encoded by the MBL2 gene on chromosome 10 (Kilpatrick 2003). Disease association studies (Kilpatrick 2002) and experimental work in murine models (Shi et al. 2004; Moller-Kristensen et al. 2006) in vivo suggest a crucial role for MBL in innate immune responses to microorganisms (Neth et al. 2000; Moller-Kristensen et al. 2006). MBL is a pattern recognition receptor that distinguishes self from non-self by binding glycoconjugates containing mannose, fucose or N-acetylglucosamine that are present on a wide variety of bacteria, viruses and fungi (Moller-Kristensen et al. 2006). Upon binding, MBL may activate the lectin pathway of complement (Figure 1) via MBL-associated serine protease 2 (MASP2) leading to complement activation, with subsequent opsonization or direct lysis of the target (Wallis and Lynch 2007). MBL also interacts independently of MASPs with receptors on phagocytes and stimulates phagocytosis by “bridging” between phagocytes and microorganisms or apoptotic cells (Shiratsuchi et al. 2008; Hodge et al. 2010).

The concentration and activity of serum MBL is genetically determined to a major extent. Three single-nucleotide polymorphisms (SNPs) in exon 1 of the MBL2 structural gene (at codons 52, 54 and 57, referred to as the D, B and C alleles, and collectively designated “O”) have a profound effect on serum MBL levels. The normal or the wild-type codons at those loci are termed “A”. The inheritance of a B, C or (to a lesser extent) D allele results in impaired multimer formation and therefore insufficiency of a functional MBL protein. In addition, three variants in the promoter region of MBL2 may influence the serum levels, with the X/Y dimorphism (low and high, respectively) having a marked effect. The influence of the X variant on serum MBL is qualitatively similar to that of the D structural allele. The difference between the H (high) and the L (low) promoter dichotomy is more modest, and any difference between P and Q promoter variants is uncertain. There is a strong linkage disequilibrium between the promoter and the structural gene variants. Consequently, only seven haplotypes (out of a possible 64) are commonly found combining to form 28 genotypes (Garred et al. 2009).

In disease association studies, these genotypes are usually grouped into assumed low (YO/YO and YO/XA), medium (YA/YO and XA/XA) and high (YA/YA and YA/XA) conferring categories (Wallis and Lynch 2007). Most, but not all, individuals with A/A genotypes have serum MBL...
>600 ng/mL and those with O/O genotypes generally have serum MBL below 200 ng/mL (Swierzko et al. 2009). The A/O groups, however, are highly heterogeneous with respect to serum MBL values, despite average values being reported at \( \approx 400 \) ng/mL and perhaps a majority having concentrations <600 ng/mL.

MBL deficiency or insufficiency is an imprecise concept with cut-off values used in the literature ranging from <100 to <1000 ng/mL serum MBL. Similarly, the A/A genotypes are sometimes compared with O/O or O/O + O/XA, but at times both the homozygous and heterozygous (O/O + A/O) genotypes are regarded as insufficient. Some studies use both genotype grouping and serum MBL values to compare patients with healthy controls, but usually either one or the other is analyzed on its own.

The MBL2 gene products form a basic subunit consisting predominantly of a collagen-like triple helix with three globular heads. The basic subunit is capable of multimer formation up to hexamers. Only the higher multimers (trimers and above) bind to glycoconjugates with high affinity or activate the lectin pathway of complement (Figure 2). The gravimetric values used for serum MBL are based on the multimeric protein from A/A homozygotes; the effect of variant alleles of the MBL2 gene is to impair multimer formation from the basic subunits. The exon 1 variants, B and C, cause the disruption of Gly-X-Y repeats in the collagen-like domain leading to altered interchain disulfide bonding in the N-terminal cross-linking region. The D variant, resulting in the substitution of a cysteine residue for an arginine, also leads to aberrant disulfide bond formation (Wallis and Cheng 1999).

### Cystic fibrosis and MBL

Cystic fibrosis (CF) is a chronic, multisystem disease arising from mutations in the gene encoding the CF transmembrane regulator (CFTR). Over 1000 mutations in the CFTR gene are recognized but the \( \Delta F508 \) mutation accounts for \( \approx 70\% \) of cases in Caucasian populations (Andersen 1938; Riordan et al. 1989).

The phenotypic defect in CF is in epithelial chloride transport and is responsible for abnormally thick mucus production manifesting itself as pancreatic and pulmonary insufficiency. Progressive lung disease is the major cause of morbidity and mortality. Airway damage leads to severe bronchiectasis (permanent dilation of the airways leading to fluid-filled “cysts”) and chronic colonization of the normally sterile airway with bacteria. Colonization with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia cepacia* and other opportunistic pathogens leads to recurrent pulmonary exacerbations, airway inflammation and progressive loss of lung function (Zemanick et al. 2010). This vicious cycle leads ultimately to death from respiratory failure.

There are, however, large variations in the progression of pulmonary disease in CF that are not accounted for by the CFTR genotype. Consequently, it has been postulated that other “modifier genes” may account for the variable phenotype observed in CF (Collaco and Cutting 2008).

A potential role for MBL as a modifier of severity in CF is suggested by the finding that MBL binds *P. aeruginosa*, *S. aureus* and *B. cepacia* along with other clinically relevant bacteria (Neth et al. 2000; Moller-Kristensen et al. 2006).
MBL-deficient mice are susceptible to more severe infections with *P. aeruginosa* (Møller-Kristensen et al. 2006) and *S. aureus* (Shi et al. 2004) than the wild-type animals. Furthermore, although MBL is a serum protein, it accumulates in the lung during acute inflammation in 

**Fig. 2.** Structure of MBL. A representation of the gene product, triplet subunit and typical MBL multimer (n = 3) is shown. Multimer formation is a property of the cysteine-rich region, but is influenced by the amino acid changes in the collagen-like domain. Only the higher multimers are able to bind to glycoconjugates with high affinity and activate the lectin pathway of complement. The mutant alleles of the structural gene (B, C and D) result in fewer higher-order multimers and therefore cause functional MBL insufficiency.

MBL-deficient mice are susceptible to more severe infections with *P. aeruginosa* (Møller-Kristensen et al. 2006) and *S. aureus* (Shi et al. 2004) than the wild-type animals. Furthermore, although MBL is a serum protein, it accumulates in the lung during acute inflammation in CF in quantities sufficient to promote phagocytosis and cause complement activation (Reading et al. 1997; Fidler et al. 2009). MBL replacement therapy has been developed and has been suggested as a new therapeutic avenue for a disease with few effective treatments (Garred et al. 2002).

Studies of MBL as a disease modifier in CF have given inconsistent results. The first two studies to examine this topic (Gabolde et al. 1999; Garred et al. 1999) were in agreement that impaired lung function was associated with homozygosity for variant alleles (O/O). Although this relationship was confirmed and extended to A/O heterozygotes in some studies (Yarden et al. 2004; Trevisiol et al. 2005), it was not confirmed in others (Drumm et al. 2005; Carlsson et al. 2005). Davies et al. (2004) investigated age non-overlapping adult and pediatric groups and found that adults homozygous for variant alleles had worse lung function than patients with wild-type alleles. This relationship was not evident in adult heterozygotes or in children. This apparent relationship with age was given some support from Muhlebach et al. (2006) who found that low-serum MBL (<200 ng/mL) was linked to better lung function in patients under 15 years of age but poorer lung function in adult patients. Muhlebach et al. (2006) suggested that low circulating MBL concentrations accelerate the age-related decline in lung function and that future studies should address that aspect carefully. This was convincingly done by Dorfman et al. (2008) in a large series, showing that MBL insufficiency (YO/YO and YO/XA genotypes) was associated with a more rapid decline in pulmonary function, especially in patients who also had high-producing genotypes of transforming growth factor β1.

Several other recent MBL genotyping studies are consistent with some aspects of previous studies. In agreement with Muhlebach et al. (2006) and Davies et al. (2004), Olesen et al. (2006) found that lung function was actually better in patients (mostly children) with insufficient MBL2 genotypes. Choi et al. (2006) reported that variant alleles were associated with poor lung function in adult patients. Buranawuti et al. (2007) found O/O genotypes (compared with A/A or A/O) to be associated with nonsurvival in adults.

Although lung function impairment is the outcome most frequently studied with regard to MBL and disease severity, microbiological features have also been extensively investigated. Following Garred et al. (1999), the relationship with earlier acquisition of *P. aeruginosa* was confirmed by Trevisiol et al. (2005). Subsequently, a number of studies examined the frequency of colonization with *P. aeruginosa* in MBL insufficient vs. MBL sufficient groups. The majority of those studies found no association with MBL insufficiency (including Davies et al. 2004; Trevisiol et al. 2005; Olesen et al. 2006; Buranawuti et al. 2007). Carlsson et al. (2005) even found that *P. aeruginosa* was significantly less frequent in MBL insufficient individuals. *Pseudomonas aeruginosa* colonization is highly age-dependent and the majority of CF patients become colonized over time. Age at acquisition is potentially a more useful outcome as earlier age at acquisition correlates with poor prognosis. This was subsequently investigated in two large series by Dorfman et al. (2008) and McDougal et al. (2010), both of which confirmed the previous observation of earlier acquisition of *P. aeruginosa* in MBL insufficient individuals.
Colonization with *B. cepacia* is less common, but is also associated with poor outcome in CF. Following Garred et al. (2009), five other studies examined the frequency of *B. cepacia* colonization (Davies et al. 2004; Carlsson et al. 2005; Olesen et al. 2006; Buranawuti et al. 2007; McDougal et al. 2010), but none found a statistically significant association.

Studies of survival have also given conflicting results. In support of Garred et al. (2009), Buranawuti et al. (2007) found O/O genotypes (compared with A/A or A/O) to be associated with nonsurvival in adults. Carlsson et al. (2005), Drumm et al. (2005) and most recently McDougal et al. (2010), however, found no significant association with survival or lung transplantation.

The discussion above focuses entirely on the pulmonary manifestations of CF. Three studies have also investigated the role of MBL in CF liver disease. The study by Gabolde et al. (2005) found a strong association between MBL insufficient genotypes and liver disease, but this was not confirmed by subsequent studies by Tomaiuolo et al. (2009) or Bartlett et al. (2009).

Therefore, the literature on MBL as a disease modifier in CF has given conflicting results. We therefore sought to investigate the role of MBL insufficiency on several clinically important markers of disease severity by performing a systematic review and meta-analysis of the gene association studies.

**Meta-analysis methodology**

**Search criteria**

A search of PUBMED (1980 to January 2010) was conducted for the following search terms: “cystic fibrosis” or “CF” AND “mannose-binding lectin” OR “mannan-binding lectin” OR “MBL” OR “MBL2” OR “MBL-2”.

The search was repeated in EMBASE and Google scholar to identify any manuscripts missed by the original PUBMED search. No language restrictions were applied. In addition, reference lists of retrieved manuscripts, bibliographies and the authors’ personal files were reviewed. Full articles of all potentially relevant studies were reviewed in detail. Only peer-reviewed data were included therefore conference abstracts were not included.

**Study inclusion, data extraction and study quality assessment**

All studies were considered eligible if they fulfilled the following criteria: (1) original publications; (2) inclusion of a population of patients with CF confirmed by CFTR genotype; (3) determination of MBL genotype or serum levels; (4) report of a recognized endpoint associated with severity of CF. Case reports were not included. Studies without the Hardy–Weinberg equilibrium for a given SNP were excluded.

Two investigators independently assessed articles to determine study eligibility. Nonrelevant studies were excluded based on title and abstract review only. Potentially relevant studies were reviewed by at least two researchers who carried out data extraction and quality assessment in a blinded fashion. Any disagreement between abstractors was resolved independently by a third abstractor. We contacted all of the authors of included manuscripts to request additional data, to clarify or obtain any missing data and to request unpublished data if available. Study quality was assessed blindly by two reviewers using criteria defined by Hayden et al. (2006).

**MBL genotypes and definition of MBL insufficiency**

As discussed above, the structural polymorphisms in exon 1 (A/O) and the ~221 promoter polymorphism (X/Y) exert the strongest effects on serum MBL levels and functional activity. Individuals who are A/A (no variant alleles in exon 1) or YA/O (1 variant allele in exon 1 but without a promoter polymorphism) have normal serum levels and MBL activity. Individuals with genotype O/O (two variant alleles in exon 1) or with genotype XA/O (compound heterozygous with one structural and one promoter polymorphism) are functionally deficient. In addition, Eisen et al. (2008) reported that a serum MBL level of <500 ng/mL is a reliable indicator of MBL insufficient genotypes; therefore, the studies of serum MBL levels using a cut-off of <500 ng/mL or lower were included.

**End-points**

A review of the literature identified the following as potentially important clinical endpoints: pulmonary exacerbation rates; health-related quality of life; pulmonary function tests [forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC)]; measures of airway inflammation; presence of CF liver disease; radiographic severity by high-resolution computed tomography; colonization with *P. aeruginosa* and *B. cepacia*; age at acquisition of *P. aeruginosa*; survival; growth during childhood; death or requirement for lung transplantation. These final two endpoints are often grouped together as “end-stage” CF (Rosenfeld 2007).

**Statistical analysis**

For meta-analysis of categorical outcomes, odds ratios (ORs) across the studies were weighted by the Mantel–Haenszel pooled using a random-effects model to account for between-study heterogeneity. For meta-analysis of lung function (a continuous variable), data were converted to mean ± standard deviation and were weighted by the inverse of their variance and pooled using a random-effects model with the results expressed as mean difference. Where data were not available in this format, it was requested from the authors directly or converted from median using a previously described method (Hozo et al. 2005). Publication bias was assessed by visual inspection of funnel plots (Egger et al. 1997).

Statistical heterogeneity was assessed using Cochran’s *Q* (chi-squared) test and Higgins’s *I*² tests (Higgins et al. 2003). For the Cochran’s *Q* test, *P* < 0.1 was considered to represent significant heterogeneity. For the Higgins test, *I*² < 25% indicates low heterogeneity, 25–50% moderate and >50% severe heterogeneity.

A priori the authors decided to conduct subgroup analyses to explore the sources of heterogeneity in the main analysis. Age is a major potential confounder for some markers of severity. Preplanned sensitivity analyses therefore included analysis of studies including predominantly adults vs. studies...
including predominantly children and exclusion of studies where age was significantly different between the MBL insufficient and the MBL sufficient groups.

Analyses were conducted using SPSS version 13 for windows (SPSS inc., Chicago, IL) and Review manager version 5 (Cochrane collaboration).

**Systematic review**

The systematic review yielded 42 titles from which 19 were immediately excluded as irrelevant following title and abstract review. There were a further 7 exclusions from the 23 studies examined in depth. The remaining 16 studies satisfied the inclusion criteria and are shown in Table I.

**Microbiological outcomes**

_Pseudomonas aeruginosa_ and _B. cepacia_ are opportunistic pathogens. The presence of these organisms in the sputum of patients with CF is associated with poor outcome and deterioration in lung function (Ledson et al. 2002). We examined the association between MBL insufficient genotypes and the presence of these organisms.

_Pseudomonas aeruginosa_

Seven studies reported the frequency of _P. aeruginosa_ between MBL insufficient and sufficient groups. Reporting of data was inconsistent, with two studies combining the A/O and O/O groups into a single cohort and one study combining the A/A and A/O groups into a single cohort. This limited our ability to perform a pooled analysis. The acquisition of _P. aeruginosa_ is age-dependent, and insufficient data were available to account for this in the analysis. The limited analysis did not suggest that the O/O genotype was associated with _P. aeruginosa_ colonization [OR: 1.42; 95% confidence interval (CI): 0.43–4.72, _P_ = 0.6].

**Age at first acquisition of Pseudomonas aeruginosa**

Age of first acquisition is potentially a more useful outcome since earlier acquisition of _P. aeruginosa_ is associated with poor outcome (Emerson et al. 2002). Data reporting for this outcome was inconsistent. Garred et al. (1999) and Trevisiol et al. (2005) combined the O/O and A/O groups into a single cohort, whereas Dorfman et al. (2008) and McDougal et al. (2010) considered the O/O or XA/O group separately. Defining MBL insufficiency as reported in the papers [O/O or A/O for Garred et al. (1999) and Trevisiol et al. (2005), O/O or XA/O for Dorfman et al. (2008) and McDougal et al. (2010)] showed that all four studies indicated that MBL insufficiency was associated with earlier acquisition of _P. aeruginosa_: mean difference, 2.83 years; 95% CI, 1.63–4.03 years, _P_ < 0.0001 (Figure 3A). However, the results show significant heterogeneity (I^2 = 73%) that may be due to the different definitions of insufficient used in each study.

**Chronic colonization with Burkholderia cepacia**

Seven studies reported the frequency of colonization with _B. cepacia_. The analysis includes six separate cohorts as Davies et al. (2004) reported pediatric and adult cohorts separately. Only one study found a statistically significant association with _B. cepacia_ colonization and MBL insufficiency (Garred et al. 1999). When comparing MBL insufficient (O/O) patients with MBL sufficient patients, there was a significant association with _B. cepacia_ colonization: OR, 3.46; 95% CI, 1.14–10.52 (Figure 3B). There were however only nine patients with _B. cepacia_ colonization across the seven studies in the O/O group. Combining the O/O and the A/O groups into a single cohort, we found no significant association between variant MBL alleles and _B. cepacia_ colonization (OR: 1.26; 95% CI: 0.79–2.01, _P_ = 0.3).

**MBL insufficiency and lung function in CF**

**Forced expiratory volume in 1 s**

FEV₁ is an important marker of disease severity in CF. In total, nine studies contained valid data for FEV₁ as an outcome. Seven studies presented data for the exon 1 mutations (A/O), whereas one study presented a combination of exon 1 and the promoter –221 X/Y polymorphisms. One study used serum levels of <200 ng/mL. In two studies, the adult and the pediatric cohorts were presented separately.

The initial pooled analysis (Figure 4A) suggested no significant effect of MBL insufficiency (defined as O/O or XA/O or a serum level of <200 ng/mL) on reduced FEV₁ % predicted (mean difference –7.10%; 95% CI: –15.59 to 1.38, _P_ = 0.1). There was significant heterogeneity in the analysis, however. Visual inspection of the forest plots revealed the majority of the heterogeneity was due to three studies of exclusively pediatric cohorts (Davies et al. 2004; Muhlebach et al. 2006; Dorfman et al. 2008) and the study by Olesen et al. (2006), in which the median age was 14 years. The MBL insufficient group in the study by McDougal et al. (2010) was significantly younger compared with the control group and hence was excluded.

**FEV₁ subanalysis in predominantly adult cohorts**

Excluding these six studies containing a majority of adult patients. This subanalysis revealed a correlation between MBL insufficiency and reduced FEV₁ % predicted: mean difference, 19.65; 95% CI, –10.83 to –28.48, _P_ = 0.001, with nonsignificant heterogeneity (Figure 4B).

The intermediate expressing MBL genotypes (A/O or XA/XA) were next compared with the wild-type or “high”-expressing MBL genotypes. Seven studies contained sufficient data for this analysis. Given the findings above, the pediatric cohorts were excluded leaving five studies for analysis. In the pooled analysis, there was no statistically significant relationship between intermediate expressing genotypes and FEV₁: mean difference, –4.31; 95% CI, –9.25 to 0.64, _P_ = 0.09, with no significant heterogeneity (P = 0.5).

Only three “adult” studies contained sufficient data to make a meaningful comparison between the O/O group and the intermediate expressing A/O group. These three studies,
### Table I. Characteristics of studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Location of study</th>
<th>Age of cohort</th>
<th>Definition of CF</th>
<th>N</th>
<th>Alleles investigated</th>
<th>Definition of deficiency</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlsson et al. (2005)</td>
<td>Lund, Sweden</td>
<td>Median 20.5 (range 4–54)</td>
<td>“Genetically verified CF”</td>
<td>112</td>
<td>Exon 1, promoter −550 (H/L), p promoter −221 (X/Y)</td>
<td>O/O or LXA/O</td>
<td>Spirometry; sputum microbiology: <em>P. aeruginosa, B. cepacia</em> and <em>S. aureus</em>; lung transplantation</td>
</tr>
<tr>
<td>Yarden et al. (2004)</td>
<td>Combined data from cohorts in Belgium and Czech Republic</td>
<td>Mean 13.4 (range 12–15)</td>
<td>ΔF508 homozygous</td>
<td>179</td>
<td>Exon 1, promoter −221 (X/Y)</td>
<td>O/O, XA/O, XA/XA</td>
<td>Spirometry; sputum microbiology: <em>P. aeruginosa</em>; age at first isolation of <em>P. aeruginosa</em></td>
</tr>
<tr>
<td>Gabolde et al. (1999)</td>
<td>France</td>
<td>Mean 19.0 (SD = 11.1)</td>
<td>ΔF508 homozygous</td>
<td>22</td>
<td>Not stated but presumed to be exon 1 and promoter −221 (X/Y)</td>
<td>0/0 or compound heterozygous (presumed to mean XA/O)</td>
<td>Spirometry; sputum microbiology: <em>P. aeruginosa</em></td>
</tr>
<tr>
<td>Dorfman et al. (2008)</td>
<td>National study, Canada</td>
<td>Mean 10.5 (limited to age &lt;18 years)</td>
<td>Mutations associated with CF</td>
<td>1393</td>
<td>Exon 1 and promoter −221 (X/Y)</td>
<td>O/O or XA/O</td>
<td>Spirometry; age of acquisition of <em>P. aeruginosa</em></td>
</tr>
<tr>
<td>Drumm et al. (2005)</td>
<td>Multicentre, USA and Canada</td>
<td>Severe group: mean 16.2 (4.1); mild group: mean 28.6 (±9.7)</td>
<td>Initial study- ΔF508 homozygous</td>
<td>808</td>
<td>Exon 1 and promoter −221 (X/Y)</td>
<td>O/O or XA/O</td>
<td>Spirometry; survival to death or lung transplantation</td>
</tr>
<tr>
<td>Garred et al. (1999)</td>
<td>Copenhagen, Denmark</td>
<td>Median 16.2 (range 7.2–40.7)</td>
<td>Mutations associated with CF</td>
<td>149</td>
<td>Exon 1, promoter −221 (X/Y)</td>
<td>O/O or XA/O</td>
<td>Death or lung transplantation after 10 years follow-up; sputum microbiology: <em>P. aeruginosa</em> and <em>B. cepacia</em>; Serum C-reactive protein; leukocyte count; number of inverse variance antibiotics</td>
</tr>
<tr>
<td>Davies et al. (2004)</td>
<td>London, UK</td>
<td>Adult cohort: mean 29.7 (SE ±0.5); pediatric cohort: 8.5 ± 0.3</td>
<td>Genetic mutation associated with CF</td>
<td>298 adults, 260 children</td>
<td>Exon 1 and promoter −221 (X/Y)</td>
<td>O/O or XA/O</td>
<td>Spirometry; annual decline in FEV1; oxygen saturations; sputum microbiology: <em>P. aeruginosa</em> and <em>B. cepacia</em>; Serum C-reactive protein; leukocyte count; number of inverse variance antibiotics</td>
</tr>
<tr>
<td>Gabolde et al. (2001)</td>
<td>France</td>
<td>Wild-type group: mean 14.5 (SD 8.7); deficient group: 17.9 (8.4)</td>
<td>ΔF508 homozygous</td>
<td>216</td>
<td>Exon 1</td>
<td>O/O</td>
<td>The presence of liver cirrhosis.</td>
</tr>
<tr>
<td>Trevisiol et al. (2005)</td>
<td>Trieste, Italy</td>
<td>Mean 17.0 (females) and 19.9 (males)</td>
<td>CFTR genotyping</td>
<td>47</td>
<td>Exon 1</td>
<td>O/O or A/O</td>
<td>Age at <em>P. aeruginosa</em> colonization; spirometry</td>
</tr>
<tr>
<td>Olesen et al. (2006)</td>
<td>Denmark</td>
<td>Median 14 (range 2–40)</td>
<td>Class I, II or III CFTR mutations</td>
<td>109</td>
<td>Exon 1, promoter −550 (H/L), promoter −221 (X/Y), promoter +4 (P/Q)</td>
<td>O/O or XA/O</td>
<td>Spirometry; sputum microbiology: <em>P. aeruginosa</em> and <em>B. cepacia</em>; Survival of bacteria</td>
</tr>
<tr>
<td>Buranawuti et al. (2007)</td>
<td>USA</td>
<td>Children: mean 9.4 (range 0–16); adults: 30.8 (17–66)</td>
<td>CFTR mutations</td>
<td>153 adults, 101 children</td>
<td>Exon 1</td>
<td>O/O</td>
<td>Spirometry; sputum microbiology: <em>P. aeruginosa</em> and <em>B. cepacia</em>; Survival of bacteria</td>
</tr>
<tr>
<td>Muhlebach et al. (2006)</td>
<td>NC, USA</td>
<td>Mean 12.8, range 0–45</td>
<td>Positive sweat test and at least 1 CFTR mutation</td>
<td>148</td>
<td>Serum MBL levels</td>
<td>Serum MBL &lt;200 ng/mL</td>
<td>Spirometry; sputum microbiology: <em>P. aeruginosa</em> and <em>S. aureus</em></td>
</tr>
</tbody>
</table>
FEV$_1$: subanalysis in predominantly pediatric cohorts

The five predominantly pediatric cohorts pooled together showed no significant effect of MBL2 genotype on FEV$_1$: mean difference, 4.17; 95% CI, −1.10 to 9.43, $P=0.1$.

Forced vital capacity

Four studies contained satisfactory data for analysis of forced vital capacity. As before, there was a marked difference between the predominantly pediatric and the predominantly adult cohorts (Figure 5). With the pediatric cohorts excluded, there was a significant association between MBL insufficiency (as defined above) with reduced FVC: mean difference, −13.31 (−2.97 to −23.66, $P=0.01$; Figure 5B).

The effect of age on lung function in CF

Lung function declines with time in both children and adults with CF. This is potentially a major confounder, if there are significant differences in age between MBL sufficient and insufficient groups. The studies included in this analysis variously attempted to adjust for the effects of age. Muhlebach et al. (2006) and Davies et al. (2004) divided their studies into adult and pediatric cohorts, and there were no significant differences in age between the insufficient and the sufficient adult cohorts. Garred et al. (1999) similarly showed no significant differences in age between groups. The patients in the study by Gabolde et al. (1999) were age-matched. We excluded data from the study by McDougal et al. (2010) as the MBL insufficient group was significantly younger than the control group, and therefore, would be expected to have better lung function. It therefore seems unlikely that the differences observed in this analysis are due to imbalances in age between the insufficient and the sufficient cohorts.

Death or end-stage lung disease requiring transplantation

In CF studies, the endpoint of death or transplantation is frequently combined as “end-stage CF”. Seven studies reported data for these endpoints. The methodology for assessing survival/end-stage lung disease varied in each study. Garred et al. (1999) followed their cohort for 10 years from 1989 to 1999. Similarly, the study by McDougal et al. (2010) was derived from the US CF twin and sibling study and provided follow-up data over several years. Buranawuti et al. (2007) selected 38 samples from nonsurviving adult patients for analysis. Carlsson et al. (2005) and Muhlebach et al. (2006) included patients attending a CF clinic who had undergone lung transplantation. Olesen et al. (2006) followed up patients for 2–3 years following CF genotyping and recorded death or requirement for lung transplantation. Follow-up periods were unclear in the other studies.

In the analysis, the low expressing MBL group (O/O or XA/O) was compared with the wild-type group (A/A or
high-expressing groups). This analysis consisted of four studies and just failed to reach statistical significance (OR, 3.96; 95% CI, 0.97–16.24; P = 0.06). Combining the intermediate- and high-expressing MBL genotypes into a single group, as was done in seven studies, revealed a significant association of low-expressing MBL genotypes with mortality or lung transplantation: OR 2.36 (1.06–5.22, P = 0.03) without significant heterogeneity (P = 0.14; Figure 6). This conclusion is, however, based on only 22 events in the MBL insufficient group across seven studies.
Data were available from four studies. Only two studies considered the X/Y promoter polymorphisms. In the pooled analysis, O/O was not associated with the presence of liver disease when compared with all other alleles (OR, 2.65; 95% CI, 0.60–11.78; P = 0.2) or to the A/A genotype only (OR, 2.29; 95% CI, 0.62–8.44; P = 0.21) but these three studies included only 51 patients with the O/O genotype. Similarly, the XA/O or the O/O genotypes combined did not increase the risk of liver disease (OR, 1.20; 95% CI, 0.73–2.01; P = 0.5). There was no significant heterogeneity in this analysis.

Similarly, in the pooled analysis, intermediate-expressing MBL genotypes were not associated with liver disease compared with wild-type: OR 0.95 (0.68–1.35), P = 0.8, with no significant heterogeneity (I² = 0%). Pooling the intermediate- and low-expressing MBL genotypes also produced no significant effect: OR 1.00 (0.72–1.39), P = 1.00, with no heterogeneity (I² = 0%).

Other outcomes

There were insufficient data regarding other outcomes identified in the CF literature, including decline in FEV₁ over time; pulmonary exacerbation rates; health-related quality of life; measures of airway inflammation; radiographic severity by high-resolution computed tomography or growth during childhood. Investigators may wish to consider these in future studies.

Limitations of the analysis

Meta-analyses are entirely dependent on the quality of the source studies. Although we contacted each author to request additional (including unpublished) data and assessed statistically for publication bias, it is possible, particularly in the smaller studies, that authors selectively report positive findings and do not report negative associations. We encountered
heterogeneity in the alleles genotyped, as some studies only examined the exon 1 mutations whereas others also studied the promoter variants. We encountered heterogeneity in methodology, such as the duration of follow-up for survival studies that significantly limit these analyses. In some cases, lung function data were not fully reported and had to be obtained from authors or converted to allow meta-analysis. This introduces a degree of error and bias into the assessment of lung function. Some studies were not limited to patients with the ΔF508 mutation. Other gene modifiers appear to influence the CF phenotype. Along with the CFTR genotype, TGFβ and MASP2 have been identified as severity modifiers and appear to interact with MBL2 in CF patients. This cannot be adjusted for in this analysis and requires further study.

The major findings from this meta-analysis are summarized in Figure 7.

**MBL replacement therapy**

If MBL insufficiency is associated with a poorer prognosis in patients with CF, can replacement of MBL by intravenous infusion be used to improve outcomes in these patients? The safety and tolerability of intravenous MBL therapy has been assessed by Valdimarsson et al. (2004) and Petersen et al. (2006), using plasma-derived and recombinant MBL, respectively, who showed administration of MBL could restore the ability to activate the lectin pathway of complement, and was well tolerated by patients. Frakking et al. (2009) and Brouwer et al. (2009) extended these observations to children with chemotherapy-induced neutropenia, a group of patients at higher risk of opportunistic infections. Twice weekly infusions of MBL in this patient group effectively restored the activity of the lectin pathway of complement. In addition, MBL replacement increased neutrophil phagocytosis of zymosan ex vivo. High doses of MBL were required to restore MBL pathway activity to normal levels in this patient cohort but the treatments were well tolerated with no evidence of systemic complement activation.

Although MBL replacement appears to be effective in restoring the MBL pathway in adults and children with MBL insufficiency, there is as yet no evidence from randomized clinical trials to show a beneficial effect in treatment or prevention of infectious disease. The only clinical evidence in support of MBL replacement in CF comes from a case report by Garred et al. (2002). In this case, the authors administered purified MBL to a patient with severe CF and *P. aeruginosa* infection. The infusions were well tolerated. Assessing the influence of MBL is impossible in an uncontrolled study but the authors report that the patient’s condition stabilized during treatment. On the basis of the available studies, a controlled trial of MBL replacement therapy in CF may be justified.

Several factors limit enthusiasm for the potential of MBL replacement therapy in CF. First, the results of the present analysis suggest any benefit may be limited to the small proportion (~10–15%) of patients with most severe (O/O, XA/0) insufficiency. Second, all studies so far have used intravenous infusions of MBL. In the Garred et al. (2002) report, the patient received twice weekly infusions. Given that MBL replacement may be required long term, there are practical and economic considerations to treating patients indefinitely with intravenous infusions. The potential cost of MBL replacement therapy would require careful consideration relative to the potential benefits.

On the other hand, a few patients seem to experience long-term benefits from short courses of MBL therapy (Valdimarsson 2003). The recent discovery that MBL can alter the maturation program of dendritic cells and thereby augment cytokine production by mononuclear cells supports the possibility that MBL can modify cellular immunity in addition to its established role as an opsonin (MacDonald et al. 2010).

**Conclusions**

The available evidence suggests that the MBL2 gene is a major modifier of lung disease in CF. MBL insufficiency is associated with reduced lung function, earlier infection with *P. aeruginosa*, colonization with *B. cepacia* and a higher rate of end-stage CF. Definitive confirmation of those conclusions requires a randomized controlled trial of MBL prophylaxis.

**Acknowledgements**

We thank Dr. Jane Davies, Dr. Hanne Vebert Olesen and Dr. Kathryn McDougal for kindly providing additional unpublished data from their previously published series.

**Conflict of interest**

None declared.
Funding
This work was supported by the award of a Medical Research Council Clinical Research Fellowship to J.D. Chalmers.

Abbreviations
CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane regulator; CI, confidence interval; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; MASP2, mannose-binding lectin-associated serine protease 2; MBL, mannose-binding lectin; OR, odds ratio; SNP, single-nucleotide polymorphism.

References


MBL insufficiency and cystic fibrosis


