The Deficiency of Serum Mannose Binding Lectin in Early Onset Idiopathic Pulmonary Fibrosis and Familial Cases

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Abstract

Background: Idiopathic pulmonary fibrosis (UIP/IPF) is increasingly recognized that siblings & close blood relatives suggesting a genetic predisposition. Serum mannose binding lectin levels (MBL) forms part of the innate immune system with deficiency produces an opsonisation and phagocytosis defect. Serum levels are genetically determined.

Aims & method: We examined the serum MBL in healthy controls (HC), frequently exacerbating COPD, pulmonary TB & Sarcoidosis along with UIP/IPF patients including those with and without an affected family member.

Results: Mean serum MBL levels were not statistically different in HC, COPD or TB. Sarcoid had statistically higher mean levels. Those with IPF onset at <55 yrs old & those with affected blood relatives (FH) had significantly reduced levels compared with IPF onset >55 yrs and no other affected relative.

Chi squared analysis of these patterns showed no differences for HC, COPD & IPF>55 yrs. TB & Sarcoid had higher frequencies of normal MBL levels compared with HC (p=0.001 & 0.024 respectively). IPF <55 yrs & IPF & FH showed higher frequencies of moderate & severe deficiency compared with HC (p=0.001 & 0.001 respectively).

In early onset IPF and IPF & FH, the odds ratio for severe MBL deficiency is 4.32 (95% CI 1.45, 12.83) p=0.0078, and for moderate or severe deficiency was OR 3.309 (95% CI for OR 1.38, 7.91) p=0.0071.

Conclusion: The data suggests that MBL deficiency is common in early onset disease UIP/IPF and cases with an affected relative. The other groups no not show such a defect and their levels are consistent with published data. The action of MBL is central to much of the described histology changes and this observation needs expanding to further cases to gain a fuller understanding of its likely role in the disease process.

Keywords: Mannose binding lectin; UIP/IPF; Family history; Inheritance; Macrophages; Macrophage mannose receptor

Abbreviations: UIP/IPF: Usual Interstitial Pneumonia/Idiopathic Pulmonary Fibrosis; MBL: Mannose Binding Lectin; FH: Family History; COPD: Chronic Obstructive Lung Disease; TB: Tuberculosis; MBL-2 gene: Mannose Binding Lectin Gene; HC: Healthy Controls; MMR: Macrophage Mannose Receptor; MASP-2: Mannose Associated Serine Protease-2

Introduction

The incidence of idiopathic pulmonary fibrosis (IPF) has doubled between 1991-2003 and is now set to rise by 12% per decade [1]. This change is not explained by an ageing population or an increase in diagnosis. Familial cases account for 0.5-2% of the total and affect siblings and close family relatives, suggesting an unknown genetic predisposition suggested to be autosomal dominant with variable penetrance [2,3]. UK data shows a mean age for familial onset to be 55 yrs [4]. These cases are identical to the sporadic cases clinically, and to date no candidate gene has been consistently identified. A surfactant protein C gene has been described in one large family, but has rarely been found in other cases [5,6].

Currently there is a large collaboration between centres in the US trying to identify the genetics of familial cases to improve understanding of this disease [7-10]. Epidemiological studies have failed to link usual interstitial pneumonitis/idiopathic pulmonary fibrosis (UIP/IPF) to prescription drugs, despite large UK studies of GP prescribing [11]. It has however demonstrated an association of the disease with diabetes (odds ratio 2.36), deep vein thrombosis (odds ratio 3.39), myocardial infarction (odds ratio 3.14), and the use of ulcer drugs (Odds ratio 2.20). The prevalence of gastro-oesophageal reflux maybe above 90% in patients with UIP/IPF and have links to the cause of the lung injury [12,13]; with dietary, genetic and environmental factors interacting to create the disease.

The histology of UIP/IPF is that of epithelial damage with apoptosis of alveolar epithelial cells, generating oxidative stress but little inflammation [14]. This suggests that a good candidate gene would need to be involved or associated with abnormalities that affect alveolar function.

Mannose binding lectin (MBL) is a blood protein (polypeptide) synthesised by the liver and forms the first line of defence after physical barriers have been breached. It is part of the innate immune response and also functions as an acute phase protein [15,16], with elevation in levels by 1.5-3 fold in systemic inflammation associated with elevation of C-reactive protein. It is a member of the collectin family

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and as a C-lectin is in the same family as surfactant proteins A and D. MBL works as a PAMP (Pathogen Associated Molecular Patterns), recognising repeating sugars on the surface of bacteria, fungi, viruses & parasites resulting in its immune system activation [17].

Within the lung MBL protects against specific micro-organisms that carry a mannose sugar coat to which it avidly binds. Common pathogens include Pneumocystis jiroveci, Stapellococcus, Streptococcus, HIV, Candida, Chlamydia, Meningococcus and Influenza virus [18-20].

Within the alveoli MBL has 4 main actions [18,21]. It promotes opsonisation and phagocytosis of micro-organisms. It activates complement via the direct and alternate pathway. It promotes clearance of apoptotic cells (which expose mannose in the late stages of apoptosis) and clears necrotic dying cells via the macrophage mannose receptor (MMR). Finally it modulates pro and anti-inflammatory cytokine production from macrophages via their mannose receptor (MMR).

MBL production from the liver is genetically determined by MBL2 genes on chromosome 10 [18,22-25]. The Inheritance of 2 wild type genes (A/A) gives serum levels of MBL>650 ng/ml (Usual mean 1300 ng/ml). A single defective gene designated (A/O) gives levels between 100-600 ng/ml which is considered a moderate deficiency. Two defective genes (O/O) gives levels <100 ng/ml which is severe deficiency.

There are also promoter polymorphisms recognised on the MBL2 gene which affects translocation of MBL (H/L or X/Y) but they generally cause minor reduction in levels compared with the A/O gene defects [18,22,26,27]. Studies show MBL levels to be fairly constant after childhood remaining stable throughout adulthood with no influence of gender [27]. There is no recorded data on MBL levels in IPF patients.

In this study we examined our patients with UIP/IPF to see if MBL deficiencies were a feature of the disease and whether it was associated with familial cases amongst the patients. We included healthy controls and patients with treated TB and Sarcoid (both conditions can show fibrotic changes in the course of their disease) and COPD as comparisons groups.

**Methods**

### Study design

**The patient groups**: A total of 326 subjects were studied including healthy controls, COPD, TB, sarcoid and UIP/IPF. MBL levels were measured to examine whether patients with UIP/IPF had MBL deficiencies compared with other respiratory diseases and controls including those with conditions that can progress to fibrosis of the lung such as TB and Sarcoid. Ethical permission was given by the London – Surrey Borders, National research Ethics Service REC 4. All patients gave written informed consent. The study was funded by the Peel medical Trust funds, who were not involved in the study design nor the analysis or interpretation of the data.

**Healthy controls**: (111 subjects) with no prior history of chest diseases, frequent infections or pneumonia were selected. They were non smokers with no regular medication or other conditions that could produce a systemic inflammation.

**Pulmonary Tuberculosis (TB)**: 47 patients were selected from our TB clinic. These patients had proven tuberculosis infections from standard investigations including sputum culture, bronchoalveolar lavage or lymph node biopsy, supported by γ-interferon gamma testing and chest X rays etc. The blood samples were not taken until at 2-3 months into treatment, in order to avoid elevation in the CRP due to the infection and inflammation. Despite this one further patients sample (No 48) had to be eliminated due to significantly raised CRP value.

**Sarcoid**: 38 patients with lymph node proven sarcoid were included in the study. Many had a prior diagnosis >5 yrs and were well & stable without the need for treatment.

**Frequently exacerbating COPD (Chronic Obstructive Pulmonary Disease)**: 33 patients with >3 hospital admissions within 12 months for COPD were selected. Two further patients (subjects 34 & 35) had elevated C-reactive protein levels despite completion of treatment and were therefore removed from the study reducing the number from 35 patients to 33.

All patients had been diagnosed on conventional criteria (smoking history, lung function, chest symptoms) and had chest X rays showing hyperinflation and other features of COPD, but no evidence of interstitial lung disease. The samples were taken only after the patients had recovered from their exacerbation. All patients were on maximal treatment for COPD with samples collected after the exacerbation when CRP was expected to normalise.

**UIP/IPF patients**: 97 patients with UIP/IPF were included. We had detailed knowledge of these patients, including their age at symptom onset, affected relatives, history, examination, lung function, shuttle walking test and HRCT scans scores. They had been diagnosed according to the 2008 ATS/ERS guidelines (Thorax 2008) using major and minor criteria at our joint chest and radiological meetings. For most patients our lung function did not represent baseline, as most were symptomatic for up to 3 yrs on different treatments ± oral steroids before the lung fibrosis was accurately diagnosed.

**Measurement of samples**: MBL levels ng/ml and CRP mg/L levels were measured in all groups from a single serum sample. Samples were frozen (-20°C) and measured as one batch at St Helier Hospital.

**Measurement of MBL**: MBL Oligomer were measured by Elisa (kit 029, Biopporto Diagnostics, Denmark). This measurement was performed in duplicate for each sample. The samples had been stored for up to 6 months at -20°C. MBL samples are reported to remain stable long-term if stored at -20°C so long as repeat freezing and thawing of the samples are avoided and haemolysis is not present. The co-efficient of repeat measurement for this kit is 3.6% for MBL levels >2279 ng/ml and 3.8% for MBL levels <30 ng/ml. The lower limit of detection is 5 ng/ml, with a normal range >650 ng/ml, as quoted in the kit and published in the scientific literature.

**Measurement of C-reactive protein**: A single measurement was made of all samples (Siemens Healthcare diagnostics, USA). Normal range CRP 0-10.0 mg/L. Subjects were excluded if their C-reactive protein (CRP) level was >25 mg/l due to possible effects on artificial elevation of the MBL. This led to the removal of 2 patients with COPD and one case of TB reducing the cases to 33 and 47 respectively.

**Statistical analysis**

MBL, age and CRP (mean ± SEM) were calculated for all groups. The data was shown to be Gaussian in distribution and therefore analysis was made between the groups using unpaired t-test and also checked by one-way ANOVA. Statistical significance was taken at the 5% level. Multivariant analysis (MANOVA) of the data was also performed to assess the relationship between age, CRP and MBL levels for all groups.
The group patterns for MBL levels were examined by Chi-square analysis: For each group the number of cases with severe (MBL<100 ng/ml), moderate deficiency (100-600 ng/ml), normal levels (>650 ng/ml) were analysed.

This allowed comparison with the healthy controls. The MBL levels of "normal", "moderate" and "severe" deficiency levels were well defined and no result fell between 600-650 ng/ml.

Sub analysis of the MBL within the UIP/IPF group: Within the IPF group, it was noticed that extremely low MBL values were present amongst subjects with early onset disease <55 yrs and those with a known family history (FH) for the disease. Those with onset >55 yrs and no FH had a pattern similar to the healthy controls. This led to the sub-analysis in the group and calculation of the odds ratio (with significance at 5%) for the presence of moderate or severe deficiency.

Statistical Analysis of MBL risk: Analysis for MBL risk for 1. Early onset disease (<55 yrs IPF) including cases with a family history (IPF& FH) 2. Compared to late onset disease (>55 yrs) as the controls

Analysis using the frequency pattern of MBL in the IPF groups (defined as normal MBL >650, moderate MBL 100-600, Severe MBL <100), where cases are those with early onset & FH, controls are those with late onset >55 yrs and no FH.

Results

Patient demographics

The study group demographics are shown in Table 1. This includes mean age for the various groups. Differences in ages or sex are not known to affect the levels of MBL which are genetically determined and remain largely stable after adolescence [27]. The table shows a lower mean age amongst the healthy controls, TB and sarcoid and IPF<55, with a greater mean age for COPD, IPF>55 yrs and IPF & FH. This was confirmed by one-way ANOVA analysis. This age difference suggests that for our group those with early onset IPF<55 are separate groups confirmed by one-way ANOVA analysis. This age difference suggests with a greater mean age for COPD, IPF>55 yrs and IPF& FH. This was consistent with the literature [28,29]. The TB group showed higher mean levels than HC, but this failed to reach significance (p=0.098) [18,30]. The literature on Tuberculosis show consistently that low MBL levels confer protection against TB, while higher levels are a risk for active disease [31-33]. There is no prior data in the literature for MBL levels in IPF.

IPF sub analysis

When the IPF patients were divided into 3 groups (Table 2) 1. Onset of disease >55 yrs of age (IPF>55) 2. Onset of disease <55 yrs of age (IPF<55) 3. IPF with affected family member (IPF & FH) a different pattern emerged

This showed significantly lower mean MBL levels in IPF<55 and those with a family history, compared with onset >55 yrs IPF (p=0.012 with an affected brother, 2 cases with an affected identical twin, 3 cases with an older father currently with the same disease and 2 families with 3 or more affected siblings.

IPF shows a higher level of male gender which is recognised for this condition.

C-reactive protein levels for the groups

These values are shown with the demographics (Table 1). One way ANOVA analysis showed significant differences between the TB, the all IPF group (and sub-groups) and Sarcoid for CRP (p=0.001, 0.001 and 0.01 respectively).

Mean MBL levels

Table 2 shows mean MBL levels with standard errors for the groups, and the results of the t-test analysis subdivisions of UIP/IPF group.

One-way ANOVA testing showed significant differences between TB and IPF<55 and FH and also between sarcoid and IPF<55 and FH at the 5% level.

Mean serum MBL levels were not statistically different between healthy controls (HC), COPD, TB and all UIP/IPF patients and >55 yrs, which was also confirmed by ANOVA. The patients with Sarcoid had higher mean levels than the healthy control group (p=0.02), consistent with the literature [28,29]. The TB group showed higher mean levels than HC, but this failed to reach significance (p=0.098) [18,30]. The literature on Tuberculosis show consistently that low MBL levels confer protection against TB, while higher levels are a risk for active disease [31-33]. There is no prior data in the literature for MBL levels in IPF.

Table 1: Shows the study group demographics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean MBL ± SEM ng/ml</th>
<th>P value VS HC</th>
<th>P value VS IPF&gt;55 yrs</th>
<th>P value VS IPF&lt;55 yrs</th>
<th>P value VS IPF &amp; FH</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC n=111</td>
<td>1315 ± 136</td>
<td>0.48</td>
<td>0.035</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>COPD n=33</td>
<td>1492 ± 257</td>
<td>0.58</td>
<td>0.90</td>
<td>0.05</td>
<td>0.022</td>
</tr>
<tr>
<td>TB n=47</td>
<td>1945 ± 268</td>
<td>0.98</td>
<td>0.24</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>Sarcoid n=38</td>
<td>2040 ± 275</td>
<td>0.02</td>
<td>0.12</td>
<td>0.002</td>
<td>0.0012</td>
</tr>
<tr>
<td>All IPF N=97</td>
<td>1164 ± 147</td>
<td>0.28</td>
<td>0.11</td>
<td>0.135</td>
<td>0.06</td>
</tr>
<tr>
<td>IPF&gt;55 yrs n=60</td>
<td>1475 ± 203</td>
<td>0.48</td>
<td>0.012</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>IPF&lt;55 yrs n=19</td>
<td>632 ± 213</td>
<td>0.03</td>
<td>0.012</td>
<td>0.007</td>
<td>0.59</td>
</tr>
<tr>
<td>IPF with FH n=18</td>
<td>688 ± 279</td>
<td>0.01</td>
<td>0.007</td>
<td>0.59</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Shows mean MBL levels with standard errors for the groups, and the results of the t-test analysis subdivisions of UIP/IPF group.
& 0.007 respectively). IPF patients with onset >55 yrs showed normal levels similar to the HC groups (p=0.48).

**Multi-variant analysis of age, CRP and MBL for all 7 groups**

MANOVA testing after log transformation concluded that HC, COPD, Sarcoid, TB and IPF >55 yrs differences between the groups, were attributable to age and CRP rather than MBL.

For IPF subgroups, MANOVA supports a difference in the subgroups not attributed to age or CRP. With support for MBL values being lower for <55yr and FH (p=0.005 & p=0.001 respectively) compared with >55 yrs.

**Patterns of MBL levels in the groups**

Figure 1 shows the pattern of MBL deficiency for the groups. Our HC group findings of 15% severe deficiency, 32% moderate & 53% normal levels are close to the normal UK population data. UK data shows 12% severe MBL deficiency <100 ng/ml, 34% moderate MBL deficiency 100-600 ng/ml, 54% normal MBL >650 ng/ml.

Chi-square analysis of frequency distribution for normal, moderate and severe deficiency patterns compared with HC (Figure 1) shows no difference for HC, COPD and IPF >55 yrs. TB and sarcoid have a higher frequency of normal MBL levels (p=0.001 & 0.024 respectively) compared with HC. For IPF<55 yrs and IPF & FH there is a higher frequency of both moderate and severe deficiencies compared with HC and all other groups.

**Individual results of MBL in the IPF subgroups**

Figure 2 shows individual results for each IPF subject in the sub-group in a scatter plot.

Analysis showed that those who experienced early onset disease <55 or had a FH were 3.3091 times more likely than late onset IPF to exhibit moderate or severe MBL deficiency (compared to normal MBL). Odds ratio=3.3091 (95% CI for 1.3836, 7.9145) p=0.0071.

For severe MBL deficiencies, those who experienced early onset IPF or had a FH were 4.32 times more likely than late onset IPF to exhibit severe MBL deficiency (compared to moderate and/or normal deficiency). Odds ratio=4.3200 (95% CI 1.4544, 12.8318) p=0.0078.

These findings suggest that MBL levels are different for IPF patients with early onset disease<55 yrs of age and those with an affected family member irrespective of age of onset or CRP level. Despite the TB and Sarcoid having known fibrotic disease processes in some affected patients, there was no suggestion of MBL deficiency. The HC group, COPD group and late onset IPF (>55 yrs) showed MBL patterns and means similar to the normal UK population data. This finding raises the question as to whether the MBL could play a role in both familial disease and those with early onset.

**Discussion**

The lung is constantly exposed to pathogenic particles and microorganisms where the innate immune system is the first line of defence [15,18]. The literature shows MBL to consistently have an important central role in lung defences via complement activation, apoptosis and phagocytosis with cytokine modulation via the macrophage mannose receptor (MMR) [17]. Studies show that low MBL levels impair MMR function reducing the innate immune response. Studies of Australian blood donors examined the relationship between MBL genotype, MBL levels and function [24]. This showed A/O genes to be the main determinant of plasma levels apart from one haplotype (LXA) which are rare in the European/Caucasian population. We have DNA samples for all our study patients, but analysis would appear to be worthwhile only for those with moderately severe deficiency states. MASP-2 (mannose associated serine binding protease-2) measurement would also be worthwhile as polymorphisms of MASP-2 can inactivate MBL function despite normal MBL levels [22,34].

Our data is consistent with the published literature for our healthy controls and COPD population. Our TB patients show an increased frequency of normal MBL levels, and this is considered a risk for infection due to competitive blocking of the MMR by MBL. While MBL deficiencies confer protection against TB infection [18,30-33]. Sarcoidosis is associated with normal MBL levels similar to the healthy population including promoter and structural gene variants [28,29].

To date there are no reported associations of MBL deficiency with UIP/IPF, which may be because the condition is not regarded as an infection and hence its treatment by immuno-suppression. The observed deficiency of MBL in early onset disease and FH is not explained by age or CRP differences and could be a potential genetic risk factor of interest. This difference was evident despite both of the UIP/IPF groups having an elevated mean C-reactive protein in
comparison to other groups. Could these patients be pre-disposed to an organism with mannose residues in the cell membrane? The action of MBL relates closely to the alveolus, the macrophage and links to IPF histology. The lack of an inflammatory milieu is well described, and the disease is thought to be a disordered apoptosis [35,36]. MBL binding to the MMR is known to suppress inflammatory cytokines and release Reactive Oxygen Species causing oxidative stress along with matrix Metalloproteases and IL-8 release. Interferon-γ reduces MMR binding, although this has shown little benefit therapeutically in IPF studies [37].

The 4-fold increase in IPF in the UK since 1987 cannot be related to a genetic change and makes it more likely to be an environmental trigger [1]. The increased prevalence of obesity, regular alcohol intake, smoking and a high fat diet of processed foods may explain the high level of reflux in society and particularly this disease [12,13]. Here reflux is estimated to have a prevalence of >90%, which may suggest a food component of relevance to the lung injury. Diabetes is increased in IPF (OR 2.36) and raised glucose levels and insulin deficiency reduce MMR function [38,39].

Our data suggests that severe MBL deficiency may show an association with early IPF and familial cases (OR 4.32). In IPF >55 yrs MBL levels show a normal pattern similar to the healthy controls, suggesting that “sufficiency of MBL” may confer a protection against the earlier onset of a lung insult. Expansion of this observation is important. Familial cases are only 2% of all IPF, in this analysis 22% of the cases fell into the FH group. Severe MBL deficiency requires 2 defective genes, and would be inherited as in autosomal recessive pattern with 25% of relatives having severe deficiency, 50% moderate deficiency and 25% normal levels [24,25].

Gallium-67 lung scans in familial idiopathic pulmonary fibrosis have shown 25% of unaffected family members to have both abnormal gallium scans and alveolar inflammation on bronchial lavage similar to those with overt disease [40]. A further 25% of family members showed abnormal bronchial lavage only. In total 50% of relatives were affected despite the individuals being clinically normal. Lavage demonstrated neutrophilia and activated macrophages that released high levels of fibronectin and growth factors for fibroblasts [3,5,40]. The authors concluded that the familial link must involve alleles involved in inflammation and the immune system, but shared environmental factors could not be ruled out.

MBL has a central role in the control of Pneumocystis jiroveci infection in the lung, by binding and removing the organisms via the MMR [41]. Pneumocystis injures the alveolar epithelium and invades the lung interstitium. Clearance from the alveolus needs MMR binding and fibronectin receptors [42,43]. Co-trimoxazole treatment in lung fibrosis has shown benefit in a small pilot study [44], and in a more recently presented study in UIP/IPF [45]. Although its mechanism of action is unknown, the drug can clear pneumocystis infections and in that way relates to MBL and the macrophage.

MBL is available as an infusion, but work on the benefit of infusions in different diseases is only just beginning [46,47].

Our findings warrant further studies for reproducibility and relevance to familial cases including the progression of IPF and any relation to lung function and MASP-2 serine proteases. PET scans are also gaining interest in IPF [48] and may be useful in familial cases including any link to MBL levels.

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**References**


