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Deficiency of Mannose Associated Serine Protease2 (MASP-2) in Patients with Idiopathic Pulmonary Fibrosis

Varney VA[1*], Bansal A[2], Parnell H[1], Nicholas A[2], Quirke G[1], Evans J[3] and Sumar N[2]

[1]*Department of Medicine, St Helier Hospital, Wrythe Lane, Carshalton, Surrey SM5 1AA, UK*

[2]*Department of Immunology, St Helier Hospital, Wrythe Lane, Carshalton, Surrey SM5 1AA, UK*

[3]*Department of Medicine, St Georges Hospital, Blackshaw Road, Tooting, London SW17, UK*

[*]**Corresponding author** Dr VA Varney, Dept of Medicine, St Helier Hospital, Wrythe Lane, Carshalton, Surrey SM5 1AA, UK, Tel 004420 8296 2401; Email veronica.varney@btinternet.com

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Abstract

Background: Idiopathic pulmonary fibrosis (UIP/IPF) is increasing. The condition occurs both sporadically and in close blood relatives suggesting a genetic predisposition. Histology suggests that the fibrosis is linked to disordered apoptosis, but the causal agent is so far unrecognised. We previously published evidence of mannose binding lectin deficiency (MBL) in early onset disease and in those with a family history of UIP/IPF. MBL is part of the innate immune system with levels genetically determined. MBL activation is impaired if the mannose associated serine protease2 (MASP-2) is severely deficient in the serum (levels<100 ng/ml). The MBL/MASP-2 complex is involved in phagocytosis, complement activation and clearance of apoptotic cells. Levels of MASP-2 are genetically determined and not influenced by inflammation.

Aims and Method: We examined plasma MASP-2 levels in healthy controls (HC) and in patients with frequently exacerbating COPD, pulmonary TB and sarcoid along with UIP/IPF including those with or without a family history.

Results: Mean serum MASP-2 levels were lowest in those with UIP/IPF (304 ng/ml) compared with healthy controls (537 ng/ml), COPD (1090 ng/ml), TB (659 ng/ml) and sarcoid (385 ng/ml). Chi square analysis for the frequency of severe MASP-2 deficiency (<100 ng/ml versus >100 ng/ml) showed no differences between Healthy Controls, COPD, TB and sarcoid. UIP/IPF showed a significant increase in the frequency of severe deficiency. This was seen for early onset disease<55yrs (33% p=0.0001), late onset>55yrs (20% p=0.0001) and those with a family history (19% p=0.0143). The expected frequency for the Caucasian population is <1%.

Conclusion: The data shows that severe MASP-2 deficiency is seen in our UIP/IPF patients and is highest in those with early onset disease. For TB and Sarcoid there is an increased frequency of deficiency (6-7%) compared with healthy controls (3%): and these diseases can also give fibrotic scarring to the lung. Since MASP-2 levels<100 ng/ml are considered to cause impaired function of the lectin pathway it could have relevance to complement activation, apoptosis and fibrosis in this disease.

Keywords: MASP-2 Mannose binding lectin; UIP/IPF; Family history; COPD; TB; Sarcoid; Healthy controls

Abbreviations

UIP/IPF: Usual Interstitial Pneumonia/Idiopathic Pulmonary Fibrosis; MBL: Mannose Binding Lectin; FH: Family History; COPD: Chronic Obstructive Pulmonary Disease; TB: Tuberculosis; HC: Healthy Controls; MASP-2: Mannose Associated Serine Protease-2; <55 yr: early onset UIP/IPF under 55 yrs old; >55 yr: Later onset UIP/IPF over 55 yrs of age

Introduction

Idiopathic pulmonary fibrosis/usual interstitial pneumonia (UIP/IPF) is a disease of significant morbidity and mortality [1]. The exact cause is as yet unrecognised but is believed to involve genetic and environmental factors [2]. The genetic basis is under investigation, and recently the mucin gene (Muc5b), associated with fibroblastic foci in the lung has been identified as a risk allele for sporadic and familial cases [3]. The histology of UIP/IPF is that of epithelial damage with apoptosis of alveolar epithelial cells and progressive fibrosis [4].

In 2012, we reported both a moderate or severe deficiency of serum mannose binding lectin (MBL) in patients with UIP/IPF with a family history and also those with early onset disease (<55yrs): while late onset disease cases (>55yrs) had largely normal levels [5]. Within the alveoli, MBL promotes opsonisation and phagocytosis of micro-organisms and activates complement via the lectin pathway [6,7]. 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.

MBL works as a PAMP (pathogen associated molecular patterns) that distinguishes between self, non-self and altered self by recognising patterns of ligands on the surface of microorganisms or aberrant cells [8-10]. Once stimulated, Mannose–Associated Serine Protease-2 (MASP-2) is activated: the MBL/MASP-2 complex cleaves C4 and C2

leading to C3 convertase and downstream complement activation [11] as shown in Figure 1.

MASP-2 belongs to a subfamily of serine proteases MASP 1-3 that are produced by liver hepatocytes. MASP-1 has much higher plasma levels than MASP-2 and may co-complex with MASP-2 to protect it against auto-activation [12]. When the lectin pathway activity is stimulated, MASP-2 activation via MBL binding will occur and MASP-1 will aid MASP-2 by auxiliary C2 cleavage and associate with the MBL/MASP-2 complex [12,13]. In human studies, a deficiency of MASP-1 and MASP-3 from nonsense gene mutations does not affect the lectin pathway which is observed to function normally [12]. Studies show differences between animal and human pathway activation and this is an area of ongoing research [14-16].

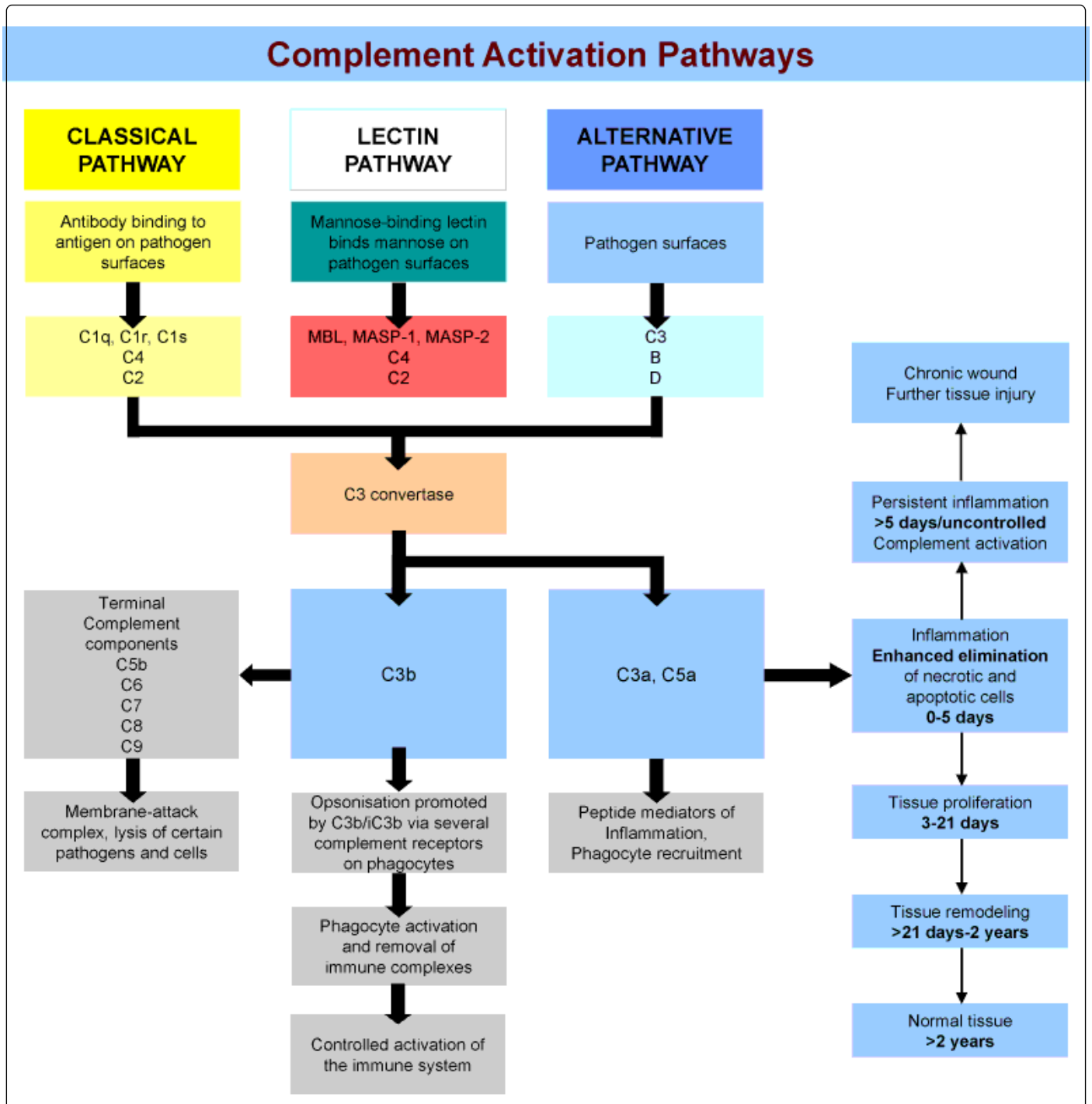

Figure 1: Diagram of complement activation for classical, lectin and alternate pathway and its function.

Deficiency of MASP-2 levels (<100 ng/ml) leads to malfunction of complement activation via MBL/MASP-2 complex regardless of the MBL levels [17]. An understanding of MASP-2 function in health and disease is limited but growing: overall it is thought to be a disease modifier [18-21]. Dysfunction of the lectin complement pathway may give susceptibility to infections or auto inflammatory conditions such as systemic lupus erythematosis and rheumatoid arthritis [12,22]. MASP-2 deficiency is described in Chagas disease, leprosy and in cystic fibrosis with more rapidly deteriorating lung function [23-26].

Polymorphisms of the MASP-2 genes resulting in reduced MASP-2 levels are reported to increase mortality in critically ill patients on intensive care unit [23,27]. Normally, plasma levels of MASP-2 are very low at birth and increase slowly to become stable after childhood and thereafter are unaffected by age, gender or physical exercise and unchanged by an inflammatory state, although current smoking can raise levels [28-31].

The genes for MASP-2 are located on chromosome 1p36, with different functional polymorphisms recognised in different ethic groups [18]. MASP-2 levels are lowest in Africans (mean 196 ng/ml), followed by Hong Kong Chinese (mean 262 ng/ml), Brazilian-Amer-Indian (mean 290 ng/ml) and highest in Caucasian (mean 416 ng/ml) [18].

Caucasians and Europeans show rare but consistent and clinically important polymorphisms in the CUB1 domain of the MASP-2 gene, which is the most critical region for the function of MASP-2. Here aspartic acid is exchanged for glycine at position 120 (D120G) due to a single nucleotide mutations. This polymorphism (D/G) of the MASP-2 gene is not seen in other ethic groups unless they share ancestry [18,32-34]. 92-96% of all Caucasian have the DD (wild type) genes with mean plasma of 543 ng/ml (SD ± 213) shown in large European studies [33,35]. 4-8% are heterozygous (D/G) with mean levels of 197 ng/ml. <1% are homozygous (G/G) with MASP-2 levels<100 ng/ml [34]. The frequency of the homozygous mutation (G/G) is 1 in 1000 Caucasians. It significantly lowers MASP-2 levels. This possible influence on survival which may reduce its frequency in society [32,35]. In Danish and Spanish populations a second rarer polymorphism is described D105G, where glutamic acid is exchanged for glycine at position 105 of the protein, again due also a single nucleotide mutations which like D120G, affects MASP-2 levels and complement activation [33].

In Caucasians and Europeans a third polymorphism is described V377A (V/A)that has little effect on MASP-2 levels and no functional impairment of complement activation [18,36]. Inheritance is 98% wild type (V/V) with mean levels 428 ng/ml: 2% heterozygosity (V/A) mean levels 384 ng/ml: homozygosity is rare (A/A) mean levels 290 ng/ml [18]. This is in contrast to homozygosity (G/G) of D120G or D105G polymorphisms, which in Caucasians is the primary cause of severe MASP-2 deficiency (<100 ng/ml), with effects on MBL activity and binding regardless of normal MBL levels [18,32].

In this study we examined our patients with UIP/IPF to see if plasma MASP-2 deficiency was present: and whether it was associated with familial cases, early or late onset disease amongst the patients. We included healthy controls and patients with treated TB and Sarcoid and chronic obstructive pulmonary disease (COPD) as comparisons groups. We also examined the relationship between their prior MBL levels and MASP-2 levels to determine if deficiency of one or both was a common finding for any of the groups.

Method

Study design

The patient groups: A total of 414 subjects were studied including healthy controls, COPD, TB, sarcoid and UIP/IPF. Plasma MASP-2 levels were measured to examine whether healthy controls and patients with the above diseases had abnormalities in their MASP-2 levels.

Ethical permission was granted 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 (HC): 150 subjects with no prior history of chest diseases, nor infections or pneumonia were selected. They were non smokers on no regular medication.

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 ± lymph node biopsy, supported by interferon-gamma testing and chest X-rays. The blood samples were not taken until 2-3 months into treatment when CRP was normalised.

Sarcoid (S): 38 patients with lymph node biopsy proven sarcoid were included in the study. Many had been diagnosed 1-20yrs earlier and were stable without the need for treatment.

Chronic obstructive pulmonary disease (COPD): 34 patients with >3 hospital admissions within 12 months for COPD were selected. All patients were ex-smokers and 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 between exacerbations.

Pulmonary fibrosis (UIP/IPF): 145 patients with UIP/IPF were included. We had detailed knowledge of these patients, including their age at symptom onset, affected relatives, history, examination and HRCT scans. They had been diagnosed according to the ATS/ERS 2011 guidelines using major and minor criteria at our joint chest and radiological meetings. They were sub-divided into UIP/IPF with family history (n=21: IPF and FH), Early onset<55yrs (n=30) and late onset>55yrs (n=94).

Measurement of MASP-2 levels in plasma: Human plasma MASP-2 levels were measured using quantitative ELISA (Kit HK326 Hycult Biotech purchased from Cambridge Bioscience Ltd UK). The plasma samples had been stored at <-26°C. MASP-2 has high stability at room temperature and below [37]. It is reported stable indefinitely if re-freezing and thawing of the sample is avoided and the plasma is free from haemolysis, lipaemia or other contamination. The coefficient of variation of repeat measurement for the kit is quoted as <10%, with sensitivity down to 1.6 ng/ml. Their quoted normal range was 170-1196 ng/ml [37]. We had an in-house variation of <2 % from our duplicated samples using this kit. All samples were measured over 2 separate days and standards for each kit gave a perfect curve.

Serum mannose binding lectin levels (MBL): Had been measured and reported previously [5]. This utilized the ELISA kit produced by Bioporto Diagnostics, Denmark . The normal range for serum MBL is 650-1300 ng/ml, with severe deficiency <100 ng/ml and moderate

deficiency 100-600 ng/ml. MBL is increased by inflammation and a raised C reactive protein.

Statistical analysis

Our data was analysed by a statistician.

MASP-2 levels were calculated and examined for normality. This showed a bimodal distribution. Means with standard deviations, medians with interquartile ranges were therefore determined for all groups to examine the data.

Comparisons were made between the MASP-2 levels and disease category using Kruskal-wallis one-way analysis of variance. Significance was at the 5% level (0.05) with the Bonferroni correction applied for multiple group comparisons (0.05 ÷ 10=0.005).

The frequency of severe MASP-2 deficiency (<100 ng/ml) was determined for each disease group including UIP/IPF sub-groups (early and late onset and FH). The frequency of levels<100 ng/ml were compared by chi-squared testing with the frequency of levels>100 ng/ml. Significance was taken at 5% level with a Bonferroni correction for 5 groups comparison with significance at<0.01.

Individual patient MASP-2 levels and MBL levels (severe, moderate deficiency or normal MBL) were compared by Kruskal-wallis one-way analysis of variance: to find any association between the 2 measures in the various disease groups.

Results

Patient demographics

The study group demographics are shown in Table 1. This includes mean age, sex and ethnicity for the various groups and UIP/IPF subgroups. Analysis of variance showed no evidence that MASP-2 levels differed by age (p=0.19).

Table 1 shows a lower mean age amongst the healthy controls, TB and sarcoid groups with a greater mean age for COPD and late onset UIP/IPF as expected for these diseases. UIP/IPF showed the expected male predominance. Our UIP/IPF-family history group included- 5 cases with an affected sister, 6 cases 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.

Ethnicity does affect MASP-2 levels as discussed in the introduction. In our study Caucasian ethnicity was 88-100% for HC, COPD and UIP/IPF. As expected, the TB and Sarcoid groups showed an increased number of afro-Caribbean, Indian and Chinese patients.

Groups	HC n=150	COPD n=33	TB n=47	Sarcoid n=38	All UIP/IPF n=145	UIP/IPF >55yrs n=94	UIP/IPF <55yrs n=30	UIP/IPF & FH n=21
Mean age at time of study (range)	50 (16-85)	72 (46-89)	46 (20-85)	48 (28-78)	70 (38-93)	76 (55-93)	50 (38-55)	68 (49-84)
% male	51	57	68	42	77	78	73	76
% Caucasian	93	100	58	74	88	86	84	100
% Afro-Caribbean	1.5	0	8	16	0	0	0	0
% Chinese	0.5	0	4	10	0	0	0	0
% Indian	5	0	30	0	12	14	16	0

Table 1: Study group demographics.

Groups	Mean MASP-2	SD	Median MASP-2	Lower IQR	Upper IQR	* Versus HC	* Versus UIP/IPF	* Versus Sarcoid	* Versus TB
Healthy Controls (HC)	537	390	415	250	690	-	0.0062	0.0275	0.3347
COPD	1090	355	1245	850	1350	0.0001	0.0001	0.0001	0.0004
TB	659	506	486	228	1250	0.3347	0.0119	0.0335	-
Sarcoid	385	287	335	200	470	0.0275	0.4101	-	0.0335
UIP/IPF (all)	304	191	230	190	400	0.0062	-	0.4101	0.0119
UIP/IPF>55yrs	299	221	260	110	440	-	-	-	-
UIP/IPF<55yr	251	233	200	90	290	-	-	-	-
UIP/IPF & FH	295	200	230	180	440	-	-	-	-
*Kruskal-Wallis one-way analysis of variance with significance at <0.005									

Table 2: MASP-2 levels (ng/ml) with Mean ± SD, medians and IQR for the study groups and results from the one-analysis of variance.

MASP-2 levels for the groups

Table 2 shows the means, standard deviation, medians and interquartile ranges for the 5 main groups and sub-groups with the accompanying statistical analysis.

Kruskal-Wallis one-way analysis of variance testing for the groups was applied to determine whether the samples came from the same distribution. A difference in MASP-2 levels by disease category was found (p=0.0001). This was investigated further by inter-group comparisons with the results of the analysis shown in Table 2. The Bonferroni correction for multiple group comparisons was applied which gave significance at p=0.005.

Mean and median MASP-2 levels were highest in COPD (1090 ng/ml) and lowest for all UIP/IPF groups (304 ng/ml). HC, TB and Sarcoid all fell within the expected normal MASP-2 range for Caucasians.

The COPD group had statistically higher MASP-2 levels compared with the other 4 groups (Table2). TB and sarcoid were similar to HC (p=0.3347 and 0.0275). UIP/IPF did not show a significance difference from HC despite the lower levels (p=0.0062).

MASP-2 deficiency pattern for the groups

Table 3 shows the pattern of MASP-2 deficiency for the 5 study groups and 3 sub-groups including the number of cases and percentage. MASP-2 levels<100 ng/ml represent severe deficiency and could influence the downstream complement activation. Normal levels are those above 200 ng/ml, with moderate deficiency at 100-200 ng/ml which is not reported to cause a functional effect [18,32].

Groups ;- numbers of cases in each group	MASP-2<100 ng/ml	MASP-2 100-200 ng/ml	MASP-2 Normal>200 ng/ml	Chi square analysis* with HC	Chi square analysis* All IPF patients
Healthy controls n=150	5 (3%)	12 (8%)	133 (89%)	-	0.0001
COPD n=34	1 (2%)	0	33 (98%)	1	0.008
TB n=47	3 (6%)	8 (17%)	36 (77%)	0.399	0.0124
Sarcoid n=38	3 (8%)	6 (16%)	29 (76%)	0.2042	0.0402
IPF>55yrs n=94	19 (20%)	22 (24%)	53 (56%)	0.0001	0.5297
IPF<55yrs n=30	10 (33%)	5 (17%)	15 (50%)	0.0001	0.2207
IPF & FH n=21	4 (19%)	4 (19%)	13 (62%)	0.0143	0.7023
All fibrosis n=145	33 (23%)	31 (21%)	81 (56%)	0.0001	-
*number of cases with MASP-2<100 ng/ml versus number of cases with MASP-2>100 ng/ml TB versus sarcoid p=1.0 Significance at p<0.01					

Table 3: Pattern of MASP-2 deficiency in the study groups with results of chi-square analysis for frequency of severe MASP-2 deficiency (<100 ng/ml versus >100 ng/ml).

The HC group showed predominately normal MASP-2 levels (89%) suggestive of wild type genes, although the expected frequency for European Caucasians is 93%. MASP-2 levels below 100 ng/ml were higher at 3% compared with the expected 1%. Although 7% of the group were non-Caucasians, none had levels<170 ng/ml.

COPD followed the expected pattern with 98% having normal MASP-2 levels and 2% showing a deficiency, this group consisted solely of Caucasian patients.

The groups of TB, sarcoid and UIP/IPF all showed a higher frequency of severe MASP-2 deficiency. For TB and sarcoid this was 6-8% of cases, with these groups including a higher percentage of Chinese, Indian and Afro-Caribbean patients (Table 1).

The UIP/IPF (n=145) group had a much higher frequency of severe deficiency at 23% for the whole group (88% Caucasian). This was yet higher (33%) in those with early onset UIP/IPF (84% Caucasian), but similar for the FH (19%) and late onset (20%) disease.

MASP-2 Results from the Chi-square analysis (Table 3), showed significance differences for the UIP/IPF groups compared with HC. This was not seen for COPD, TB and sarcoid relative to HC. When all UIP/IPF was compared with the other groups, the sarcoid group showed no statistical difference (p=0.040), confirming the higher frequency of severe deficiency, while TB showed borderline significance (p=0.0124).

There is little data on expected MASP-2 levels for the Indian subcontinent although they may follow Brazilian-Amer-Indian levels [18].

Amongst our 5 study groups the MASP-2 range for Indian subjects were 10-1400(mean 565 ng/ml), Chinese 240-1470(mean 548 ng/ml) and afro-Caribbean's were 90-1320(mean 451 ng/ml).

In the HC and sarcoid groups all non-Caucasians patients had MASP-2 levels>170 ng/ml. In the TB groups, 3 non-Caucasian (2 Indian & 1 West-Indian) individuals had MASP-2 levels below 100

ng/ml. For the UIP/IPF group, 7 Indian patients had MASP-2 levels <100(mean 67 ng/ml).

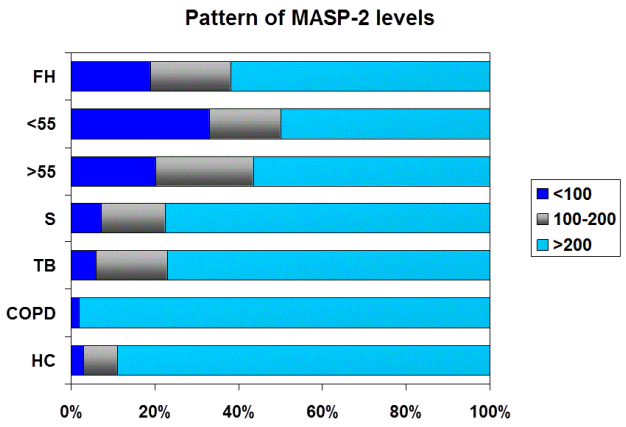

Figure 2: Pattern of MASP-2 deficiency shown as a percentage for the groups.

Pattern of MASP-2 levels in the groups

Figure 2 shows the pattern of MASP-2 deficiency for the 5 groups including the 3 subgroups demonstrating the differences between the groups.

Individual results of MASP-2 in the UIP/IPF Subgroups

Figure 3 shows the individual results for each UIP/IPF subject in the sub-groups of early onset (<55yrs), late onset (>55yrs) and FH as a scatter plot.

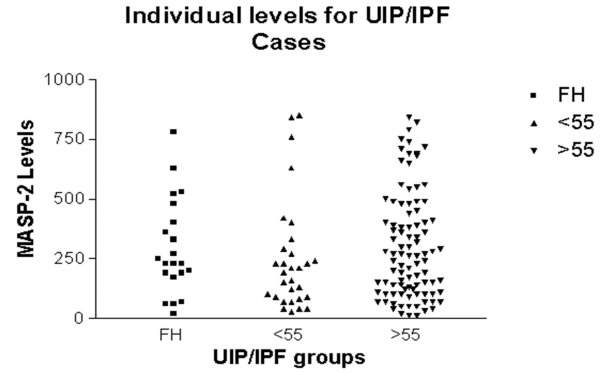

Figure 3: Individual MASP-2 levels as a scatter plot for the UIP/IPF sub-groups (>55yr, 55yrs, family history FH).

Relationship between MBL levels and MASP-2 levels for patients in the 5 groups and sub-groups is shown in Table 4.

This table shows the relationship between the MASP-2 patterns (<100, 100-200 and >200 ng/ml) and the previously measured MBL levels (severe deficiency, moderate or normal). Kruskal-Wallis one-way analysis of variance shows no evidence that MASP-2 levels in the 5 disease groups has any relationship with the MBL levels (p=0.2). For the UIP/IPF sub-groups analysis, there is also no evidence of a relationship (p=0.61) .This relationship has been previously studied and reported, including mono- and dizygotic twins, and confirms an independent relationship between MBL and MASP-2 which is genetically determined [33].

Groups	Severe MBL Deficiency<100			Moderate MBL Deficiency 100-600			Normal MBL>650 ng/ml		
	MASP-2	MASP-2	MASP-2	MASP-2	MASP-2	MASP-2	MASP-2	MASP-2	MASP-2
	<100	100-200	>200	<100	100-200	>200	<100	100-200	>200
HC n=150	0	0	18	2	5	40	3	13	69
COPD n=34	0	0	6	0	0	9	1	0	18
TB n=47	1	0	7	0	1	5	2	7	24
Sarcoid n=38	1	2	3	0	0	6	2	4	20
IPF>55yrs n=94	0	0	5	6	8	16	16	11	32
IPF<55yrs n=30	4	0	4	1	2	7	5	3	4
IPF & FH n=21	0	1	5	1	2	5	2	1	7

Table 4: Individual Relationship between MBL pattern (severe deficiency, moderate or normal) and MASP-2 levels (<100, 100-200 and >200 ng/ml) for the individuals in the study groups.

Discussion

Following our observations of a higher frequency of moderate and severe MBL deficiency in patients with early onset fibrosis or a family history [5], we measured MASP-2 levels in the plasma of all our previously studied patients along with some additional healthy controls and new UIP/IPF cases.

Although the innate immune system is central to immediate protective responses in man, there is little data on MASP-2 levels in disease and no published data on MASP-2 levels in pulmonary fibrosis. Yet this is an area of interest due to the known role of MBL in lung

defences, with MBL and MASP-2 involved in the lectin pathway of complement activation that deals with pathogenic particles, micro-organisms and clearance of apoptotic cells as outlined in Figure 1 [32,34]. Abnormal apoptosis is now believed to be central to UIP/IPF and under-pins the fibrotic scarring response. Equally, TB and sarcoid are conditions in which fibrotic scars may arise in a smaller number of patients, interestingly both our groups showed a higher than expected frequency of severe MASP-2 deficiency. TB and Sarcoid patients are reported to have high MBL levels which we also confirmed [5], but there is no data on MASP-2 levels and this may be an interesting area for further work.

From the literature, MASP-2 deficiency is linked to the inherited genotype with no evidence for low levels reflecting MASP-2 consumption [18,22]. There are reports that some disseminated tumours can chronically raise both MBL and MASP-2 along with inflammatory markers. Although this is not fully understood, it is believed to result from a perpetual inflammatory state and is associated with a poorer prognosis [38,39].

Our data for the healthy control group and COPD patients are close to the published data for expected deficiency patterns in Caucasians [35]. The few Chinese, Indian and West Indians patients present in these groups did not contribute to the borderline increase in deficiency seen.

The statistical analysis applied to our results utilised a Bonferroni correction as multiple analyses were conducted. Thus the level of statistical significance was set significantly higher than the normal 0.05 level. As such the mean and median levels of serum MASP-2 were not significantly different between several of the groups. However, it may be argued by some statisticians that differences were nevertheless present. As such we suggest that our data, while not reaching our pre-analysis level of statistical significance, does nevertheless show reduced levels of MASP-2 in those with UIP/IPF and that these differences were greatest in those <55years and those with a family history of the disease. Furthermore, differences were also evident in the COPD group who had a higher level than the HC and the UIP/IPF groups. This finding certainly warrants further investigation given the ability of the lectin pathway of complement activation to alter monocyte activation via Transforming Growth Factor beta-1 signalling [40].

In the patients with TB and sarcoid there was a lower level of MASP-2 deficiency at 6% and 7% respectively, but the group size was much smaller than that of UIP/IPF. Only 3 non-Caucasian patients in the TB group contributed to the deficiency. For Sarcoid the literature does report a reduction in ficolin-3. This is another member of the Lectin pathway that showed an inverse relationship to the CD4+ and CD8+ lymphocytes counts in the disease [41].

Our findings show an unexpectedly high frequency of severe MASP-2 deficiency in all fibrosis patients, with early onset disease having the highest frequency. The fibrosis groups were largely Caucasians, and normally should affect only 1 in 1000 subjects. Genotyping these patients would be informative.

There is little data on complement activation in UIP/IPF, probably because the condition has been viewed as a type of auto-immunity and immune-suppression with steroids and azathioprine used. This treatment is now no longer advised following a randomised trial showing a 10 fold increased mortality in the prednisolone and azathioprine arm compared with placebo [42]. Treatment is now focused on anti-fibrotic approaches such as pirfenidone [43].

The literature describes a case report from the UK of a 28 yr old man, who developed ulcerative colitis at 13 yrs, followed by systemic lupus erythematosis, pneumococcal infection and progressive lung fibrosis diagnosed by transbronchial lung biopsy [44]. Detailed assessment of his immune system showed an inherited deficiency of MASP-2 with a plasma level of 20 ng/ml despite normal MBL levels in the plasma. The immune dysfunction was caused by absence of MASP-2 in the MBL-MASP-2 complex which was restored by recombinant MASP-2. Genetic studies including family members showed him to be homozygous for the MASP-2 gene mutation D120G preventing the formation of functional MBL-MASP-2 complex. His family and children were heterozygous for the defect and showed no disease. Since MASP-2 measurements are not routinely available, its significance maybe overlooked in many diseases and understanding of its function limited.

Genetic studies in fibrosis-susceptible and fibrosis-resistant mice strains given subcutaneous bleomycin identified genetic variations between them that may explain differences in their susceptibility to bleomycin lung injury [45]. Genome-wide linkage studies of the 2 strains identified 11 genes that predisposed to bleomycin-induced fibrosis. Two of these genes had recognised protein expression and included a gene for MASP-2 and one for Cep55 (an autophagy gene). The others were polymorphisms in areas of silent DNA that may regulate processes that are not yet understood. The Complement activation system and its downstream effects on apoptosis and inflammation have received little attention in UIP/IPF. Rodent studies of bleomycin induced lung fibrosis showed that complement C5a deficient mice had an exaggerated inflammatory response and higher mortality compared with C5 sufficient mice [46]. Complement C5 showed an early protective and anti-inflammatory effect on bleomycin induced injury. Since C5a and C5b aid the clearance of apoptotic cells then failure here could result in increased inflammation and autoimmunity. Data at 14 and 28 days however, showed C5 to be more deleterious in established injury, suggesting early and late opposing roles. However this original model of one off bleomycin exposure is being replaced by frequent low dose exposure which appears to produce a closer picture to the UIP/IPF human disease [46].

Rheumatoid arthritis is a condition in which 10-15% of patients may develop an associated interstitial lung disease with a UIP/IPF pattern being common. A recent study of MASP-2 levels and gene polymorphisms in rheumatoid patients and their relatives showed reduced levels in cases (median 181 ng/ml) and relatives with articular symptoms (285 ng/ml) compared with controls (median 398 ng/ml) [22]. High MASP-2 levels were associated with lower susceptibility to Rheumatoid arthritis and articular symptoms which were independent of age, gender, ethnicity, smoking and rheumatoid factor positivity. The MASP-2 levels related to genotype without evidence for MASP-2 consumption [22].

Our findings are of interest in fibrotic lung disease and lectin mediated apoptosis. Further studies are warranted for reproducibility and relevance to the diagnosis and progression of UIP/IPF along with TB and Sarcoid. Important questions would include the role of MASP-2 levels and genotype as a risk factor for abnormal fibrotic responses in lung injury and disease progression.

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