Journal of Clinical Immunology (2019) 39:668–677
https://doi.org/10.1007/s10875-019-00675-8

ORIGINAL ARTICLE

Pattern Recognition Molecules of the Lectin Pathway—Screening of Patients with Suspected Immunodeficiency

Clara Mistegård Jørgensen[1,2] · Lisbeth Jensen[1] · Mette Christiansen[3] · Mette Bjerre[4] · Jens Magnus Bernth Jensen[3] · Steffen Thiel[1]

Received: 31 March 2019 / Accepted: 22 July 2019 / Published online: 3 August 2019

Abstract

Purpose To compare plasma concentrations of all lectin pathway (LP) pattern recognition molecules (PRMs) in patients referred for laboratory evaluation due to recurrent infections with healthy individuals.

Methods Patients were divided into categories according to referral: recurrent airway infections (RAI), recurrent abscesses, common variable immunodeficiency (CVID), lung transplantation candidates (LTX), and 'other causes'. LP PRMs (mannose-binding lectin (MBL), collectin liver 1 (CL-L1), H-ficolin, L-ficolin, M-ficolin) and C-reactive protein (CRP) were determined in 332 patients and 150 healthy blood donors using time-resolved immunofluorometric assays.

Results None of the LP PRMs was found in lower concentration in the patient categories; however, several PRMs were detected in higher concentrations. M-ficolin was found in higher concentrations in all patient categories. Patients suffering from RAI had higher concentrations of CL-L1 and H-ficolin. Patients suffering from abscesses exhibited higher concentrations of MBL and CL-L1, whereas LTX had higher concentrations of MBL. Patients with other causes of referral had higher concentrations of MBL and CL-L1. Prevalence of combined deficiencies of PRMs in patient categories and controls did not differ. CRP was used as a marker of ongoing inflammation and was significantly higher among all patient categories. Furthermore, CRP was found to correlate with both M-ficolin and L-ficolin.

Conclusion The results suggest that neither single nor combined deficiencies of LP PRMs are more frequent among patients referred for an immunological evaluation than in healthy individuals. Future studies are needed and should focus on deficiencies of LP PRMs combined with deficiencies in other parts of the immune system.

Keywords Immunodeficiency · Complement · Lectin pathway · Immunological evaluation · Screening

Introduction

The complement system, comprising more than 30 plasma proteins, is a cornerstone in our first line of defense against invading microorganisms [1, 2]. Intruding microorganisms can activate the complement system to undergo a proteolytic cascade reaction, ultimately killing the intruder either directly or by enhancing phagocytosis [3]. The biological importance of complement is highlighted by the medical history of patients without functional complement who are prone to suffer from recurrent, devastating bacterial infections [4, 5].

Activation of the complement system may proceed through three distinct pathways: the alternative, the classic, and the lectin pathways. All three pathways converge in a common terminal pathway, resulting in opsonization of the target, recruitment of immunological effector cells or ultimately generation of the membrane attack complex (MAC), and cell lysis.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10875-019-00675-8) contains supplementary material, which is available to authorized users.

✉ Clara Mistegård Jørgensen
clarjo@rm.dk

[1] Department of Biomedicine, Aarhus University, Wilhelm Meyers Allé 4, 8000 Aarhus, Denmark

[2] Department of Rheumatology, Aarhus University Hospital, Aarhus, Denmark

[3] Department of Clinical Medicine - Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark

[4] Department of Clinical Medicine - Medical Research Laboratory, Aarhus University, Aarhus, Denmark

The purpose of the complement system is thus essentially clearing of intruding microorganisms.

The alternative pathway is permanently active and functions as an amplification loop of complement activation in general. Complement factor I and factor H control the activity of the system and prevent potential host-related harmful effects of this amplification [6].

Activation of the classical and the lectin pathways, is based on the ability of soluble pattern recognition molecules (PRMs) to bind to specific molecular structures found on microorganisms [1]. The PRMs of the lectin pathway are comprised of collectins, defined by a collagenous structure and a C-type carbohydrate recognition domain, and ficolins, defined by collagen-like and fibrinogen-like sequences. They recognize specific clusters of carbohydrates or acetyl groups present on the surface of microorganisms [7–9]. The human collectins are mannan-binding lectin (MBL), collectin liver 1 (CL-L1, also named collectin 10), and collectin kidney 1 (CL-K1, or collectin 11). These proteins are described in more detail in supplementary table S1. In plasma, CL-L1 and CL-K1 are primarily present as the heterocomplex, CL-LK [10]. The human ficolins are H-ficolin, L-ficolin, and M-ficolin (also termed ficolin-3, ficolin-2, and ficolin-1, respectively). Collectins and ficolins circulate in plasma in complex with five other proteins. These are named MBL-associated serine proteases (MASP-1, MASP-2, and MASP-3) or MBL-associated proteins (MAp19 and MAp44). The binding of collectins and ficolins to their complementary ligands activates the complexed MASPs to initiate the downstream proteolytic cascade through generation of the C3 convertase, C4bC2a [11].

Contrary to deficiencies in the alternative or classical pathway, most deficiencies in the lectin pathway have only been sparsely investigated. MBL deficiency has been studied extensively, but still, its significance remains controversial [12]. MBL deficiency was originally found to cause an opsonin defect among infants [13] and later also to be associated with increased risk of chronic necrotizing pulmonary aspergillosis [14]. Other studies have however not confirmed significance of MBL deficiency [15]. The remaining PRMs of the lectin pathway have primarily been investigated in highly selected patient populations or single cases [16, 17]. A limitation in previous studies may be that only single or a few of the lectin pathway PRMs were investigated. This could mask a potential significance of the constellation of PRMs; e.g., deficiency of one PRM may be compensated by sufficient amounts of the others, whereas deficiency of multiple PRMs may increase infection risk. Recognition of this possible dependency demands that all the PRMs are investigated in the same study population. Until now, no such comprehensive studies have been reported.

Our aim of the present study was to compare plasma concentrations of all lectin pathway PRMs in a cohort of patients referred for laboratory evaluation due to recurrent infections compared with a group of healthy individuals. Secondly, we wanted to test if the cause for the referral (e.g., type of infection) was associated with different plasma concentrations of the lectin pathway PRMs. We hypothesized that single PRM deficiencies as well as constellations of PRM deficiencies would be more prevalent in the patients.

Materials and Methods

Study Population

Heparin plasma from patients referred for laboratory evaluation for immunodeficiency at Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark, was collected. Collection was performed over 2.5 years (February 2011–October 2013), and samples from 332 patients were obtained, corresponding to approximately 70% of referred patients (suitable material was not available for the remaining). Patients were referred due to frequent infections, and based on information in referrals, we categorized patients into five groups: recurrent airway infections (RAI); recurrent abscesses; common variable immunodeficiency (CVID) according to criteria set by the American Academy of Allergy, Asthma & Immunology and the American College of Allergy, Asthma & Immunology [18]; lung transplantation candidates (LTX); and ‘other causes’.

Patients referred for an immunological evaluation prior to lung transplantation ($n = 25$) suffered from severe chronic obstructive pulmonary disease (COPD) ($n = 10$), sarcoidosis ($n = 3$), cystic fibrosis ($n = 2$), lung fibrosis ($n = 3$), or unlisted primary disease ($n = 8$). We expect that this mixed population per se is healthy in terms of their innate immune system. The population, with severe structural lung damage to a degree necessitating lung transplantation, will have suffered from many more infections than the general population. Thus, the LTX group was included to control for the effect of numerous previous infections on the levels of the PRMs.

The category ‘other causes’ includes a mixture of many different referral causes: recurrent episodic fever ($n = 13$), recurrent herpes simplex infections ($n = 12$), hypogammaglobulinemia ($n = 7$), low IgA and IgM but not CVID ($n = 4$), IgG subclass defect ($n = 5$), recurrent warts ($n = 4$), severe recurrent candidiasis ($n = 4$),), sarcoidosis ($n = 2$), low IgG ($n = 2$), low CD4 cells and HIV-negative ($n = 2$), isolated IgA deficiency ($n = 2$), recurrent meningitis ($n = 2$), further one of each of *Listeria* sepsis, recurrent neuroborreliosis, 22q11 deletion, systemic scleroderma, primary ciliary dyskinesia, McKusick–Kaufman syndrome, tuberculosis spondylitis, recurrent pneumococcal meningitis, severe atopic dermatitis, adenosine deaminase deficiency, myeloperoxidase deficiency, neonatal herpes encephalitis,

suspected IFNGR1-mutation, HIV positive and numerous recurrent granulomas, recurrent panaritium, neutropenia, severe rheumatoid arthritis and lung fibrosis, hyper-IgE syndrome, severe eosinophilia, and juvenile psoriatic eczema. For the remaining 53 patients, a primary disease was not listed in referral.

The genetic deficiencies determined for some patients are shown in supplementary table S2.

Heparin plasma from 150 (75 females and 75 males) anonymized blood donors was included as healthy control material from the Blood Bank, Aarhus University Hospital, Aarhus, Denmark.

Methods

Heparinized blood samples were centrifuged at 1849×*g* for 5 min, and plasma was collected. Each sample was frozen at − 80 °C after collection, thawed, diluted 1/4 in Tris-buffered saline (10 mM Tris, 145 mM NaCl, pH 7.4) (TBS), and stored at − 80 °C. Freeze and thaw cycles were kept at a minimum.

Immunoassays were used for the measurements of the proteins involved in the lectin pathway: MBL, H-ficolin, L-ficolin, M-ficolin, and CL-L1. All proteins, except for L-ficolin (measured using a commercial enzyme-linked immunosorbent assay (Hycult Biotechnology, Uden, the Netherlands)), were measured by time-resolved immunofluorometric assays (TRIFMAs). The specific antibodies used in TRIFMAs were developed and produced in-house. The assays have been described in detail elsewhere [19–26]. Sample dilution and loading on microtiter plates were automated using a pipetting robot (JANUS, PerkinElmer, Hamburg, Germany). All analyses were performed in duplicate. Measurements were repeated if coefficient of variation (CV) of the duplicate analyses was above 15%. Inter-assay CV based on internal controls was also determined for each protein, and CV was below 15% for all proteins, except for H-ficolin (18%). Plasma high-sensitive C-reactive protein (hs-CRP) was determined by in-house time-resolved immunofluorometric assay based on commercially available monoclonal antibodies (MAB17071 and BAM17072; R&D Systems, Minneapolis, MN, USA) and calibrated against WHO 85/506 (National Institute for Biological Standards and Control). Limit of detection was 5 ng/L, intra- and interassay CVs were < 5% and < 6%, respectively. Protein measurements were performed blinded to patient data.

Statistics

MBL was analyzed by a gamma distribution generalized linear model with a robust variance estimator (means were compared with a Wald test). The remaining plasma protein concentrations (CL-L1, H-ficolin, L-ficolin, M-ficolin, and CRP) in patient categories and healthy controls were compared using a multiple linear regression model and *t* tests. Gaussian distributions were approximated by logarithmical transformation of the measured concentrations. Model validation after logarithmical transformation of data gave no reason to reject the model. Frequencies of low concentrations of PRMs (defined as below the 10th percentile of healthy controls) in the five patient categories were compared with healthy controls using Fisher's exact test. Correlations between concentrations of PRMs and CRP were performed using Spearman correlation.

All tests were two-sided. p values < 0.05 were defined as significant. Ninety-five percent confidence intervals (95%CI) are presented in brackets in both figures and tables. Analyses were performed using Stata 12 software (StataCorp, TX, USA). Graphical representations were performed using GraphPad Prism software (GraphPad Software, CA, USA).

Ethics

The study was conducted under the approval by the Central Denmark Region Committees on Health Research Ethics (1-10-72-127-12) and the Danish Data Protection Agency (1-16-02-40-12). The study was performed according to the Declaration of Helsinki.

Results

Demographics of the Participants

The demographics of the study participants are presented in Table 1. The Danish population was in 2013 approx. 5.6

Table 1 Demographics of the study population. Controls were healthy blood donors. Patients were referred for immunological evaluation due to recurrent airway infection (RAI), common variable immunodeficiency (CVID), pre-lung transplantation assessment (LTX), recurrent abscesses, or other causes

		Females		Age (years)		
	n	%	*p value**	Median	IQR[a]	*p value*[§]
Controls	150	50.0		29.0	23.0–41.0	
Patients, total	332	55.7	0.24	39.2	11.3–55.7	0.02
RAI	122	63.9	0.02	42.4	10.7–61.5	< 0.01
CVID	29	51.7	0.87	42.8	33.0–60.5	< 0.01
Abscess	25	52.0	0.85	37.3	30.4–50.6	0.16
LTX	25	56.0	0.58	53.6	46.9–56.1	< 0.01
Other causes	131	49.6	0.95	33.9	7.8–51.2	0.16

*Compared with healthy controls by chi^2 test

§ Compared with healthy controls by Mann-Whitney *U* test

[a] *IQR*: interquartile range

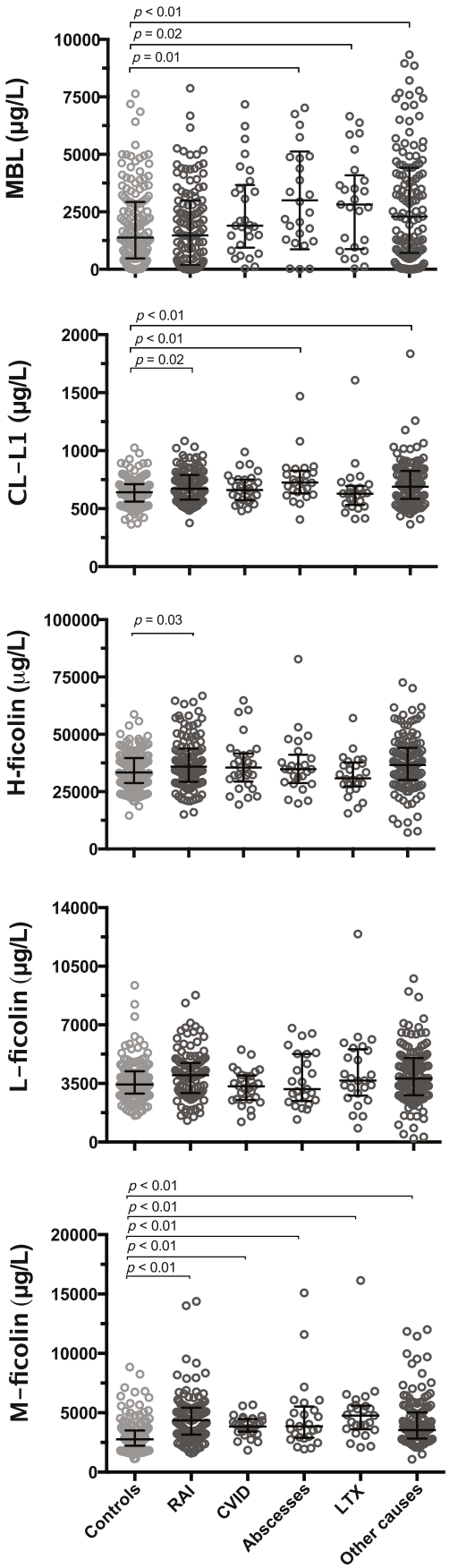


Fig. 1 Plasma concentrations of lectin pathway recognition molecules (PRMs) in patients and controls. Bars indicate median and interquartile range or mean and standard deviation (MBL). Controls were compared with single patient categories by *t* tests or Wald test (MBL)

million and quite homogenous in terms of ethnicity with approximately 90% of Danish descendant (Western European). We expect this reflected in the study population. Patients were divided according to reason of referral in the following categories: common variable immunodeficiency (CVID, $n = 29$), lung transplantation candidates (LTX, $n = 25$), recurrent airway infection without CVID or LTX (RAI, $n = 122$), abscesses ($n = 25$), and 'other causes' ($n = 131$). No difference in gender distribution was found between the patient cohort and the healthy controls ($p = 0.24$) whereas median age was 10 years older in the patients ($p = 0.02$).

Single patient categories' distribution of gender was indifferent from controls except for the RAI category ($p = 0.02$). Compared with controls, age differed in RAI (13 years older, $p < 0.01$), in LTX (24 years older, $p < 0.01$), and in CVID (14 years older, $p < 0.01$). Patients suffering from abscesses and 'other causes' did not differ from controls by gender or age.

Single PRM Concentrations

PRMs were quantified by TRIFMA (MBL, CL-L1, H-ficolin, and M-ficolin) or ELISA (L-ficolin) in plasma of patients and controls. The data are depicted in graphs in supplementary figure S1. MBL concentrations were below the quantification range in 7 of the 332 patients. This was not observed in any of the controls. Remaining PRMs were detectable in all patients and controls.

First, we examined PRM concentrations as continuous data. Each patient category was compared with controls (Fig. 1). We found RAI patients to have higher geometric mean concentrations of CL-L1 (107% [101%; 112%]), H-ficolin (108% [101%; 117%]), and M-ficolin (145% [131%; 159%]) than healthy controls, but no difference in MBL and L-ficolin levels was found. Patients with CVID had higher geometric mean concentration of M-ficolin (132% [112%; 155%]) whereas MBL, CL-L1, H-ficolin, and L-ficolin levels did not differ. Patients suffering from abscesses had higher geometric mean concentrations of CL-L1 (115% [105%; 125%]) and M-ficolin (144% [121%; 171%]) and a higher mean MBL concentration (1120 μg/L [258 μg/L; 1982 μg/L]). H-ficolin and L-ficolin levels did not differ. Lung transplantation candidates had higher concentrations of M-ficolin (geometric mean 159% [134%; 188%]) and MBL (1014 μg/L [185 μg/L; 1843 μg/L] higher mean), whereas, CL-L1, H-ficolin, and L-ficolin levels did not differ. We expect that the patients in the LTX category per se are healthy in terms of their innate immune system. The population, with severe structural lung damage to a degree necessitating lung transplantation, will have suffered from many more infections than the general population. Thus, the LTX group was included to control for the effect of numerous previous infections on the levels of the PRMs.

The remaining patients in the category 'other causes' had higher geometric mean concentrations of CL-L1 (110% [104%; 115%]), M-ficolin (133% [121%; 147%]), and MBL (875 μg/L [384 μg/L; 1366 μg/L]). H-ficolin and L-ficolin levels did not differ.

We adjusted for age and gender in the linear regression, and it only had marginal impact on the estimates and did not change conclusions; hence, non-adjusted data are presented.

The PRMs were not found to be present in lower concentration in any patient category compared with healthy controls. Contrarily, several of the PRMs were actually found in higher concentration in certain patient categories.

We subsequently examined whether the prevalence of single PRM deficiency differed between patients and controls. For each PRM, deficiency was defined as samples with concentrations below the 10th percentile of the controls. The remaining samples were defined as non-deficient. We found that only M-ficolin deficiency displayed difference (Table 2). Deficiency was significantly less common in RAI and the 'other causes' category compared with controls (2.5% [0.5%; 7.0] and 1.5% [0.2%; 5.4%], respectively). The analyses were also performed using 5th and 1st percentiles as cut-off levels, respectively. When 5th percentile was used as cut-off, no differences were observed between patients and healthy controls (data not shown). When analyses were

Table 2 Prevalence of deficiencies of single PRMs in patients relative to healthy controls

	Controls		RAI		CVID		Abscess		LTX		Other causes	
	10th % (μg/L)	*n* (%)	*n* (%)	*p value*	*n* (%)	*p value*	*n* (%)	*p value*	*n* (%)	*p value*	*n* (%)	*p value*
MBL	38	15 (10)	17 (14)	n.s	1 (3.5)	n.s	3 (12)	n.s	1 (4)	n.s	10 (7.6)	n.s
CL-L1	508	15 (10)	9 (7.4)	n.s	2 (4.9)	n.s	1 (4)	n.s	4 (16)	n.s	16 (12)	n.s
H-ficolin	23,724	15 (10)	11 (9.0)	n.s	4 (14)	n.s	3 (12)	n.s	4 (16)	n.s	13 (9.9)	n.s
L-ficolin	2322	15 (10)	11 (9.0)	n.s	4 (14)	n.s	5 (20)	n.s	4 (16)	n.s	15 (11)	n.s
M-ficolin	1748	15 (10)	3 (2.5)	0.01	0 (0)	n.s	0 (0)	n.s	0 (0)	n.s	2 (1.5)	< 0.01

Deficiency definition: PRM concentrations below controls' 10th percentile

Patient categories were compared with controls by Fisher's exact test. *n.s* non-significant

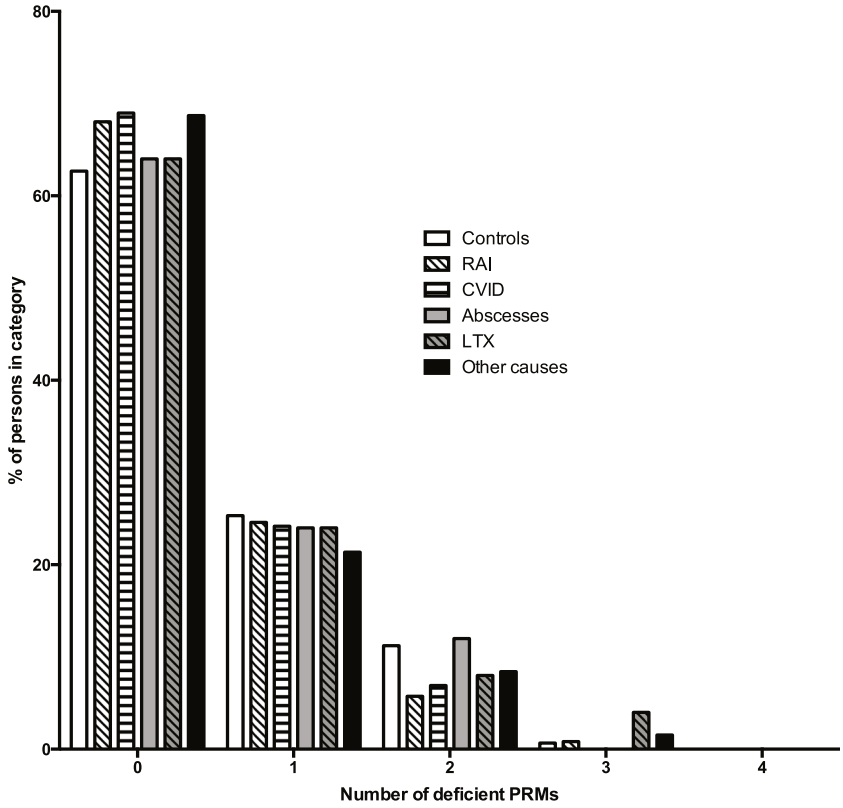


Fig. 2 Prevalence of combined deficiencies of PRMs in patient groups and controls. The prevalence did not differ between controls and any patient category for any number of deficient PRMs (Fisher's exact test)

performed by using 1st percentile as cut-off, L-ficolin deficiency was observed more frequent among certain patient categories: three patients (12%) in LTX and nine patients (7%) in 'other causes' (data not shown).

Combined PRM Concentrations

Each of the PRMs recognizes only a narrow range of structures. The combined arsenal of PRMs however recognizes a far wider range of structures. We therefore examined whether the prevalence of multiple PRM deficiencies differed between patients and controls. We found no difference in the prevalence of multiple PRM deficiencies in any patient category compared with controls (Fisher's exact $p > 0.05$) (Fig. 2).

Correlations Between Concentrations of PRMs and CRP

As presented above, we found higher concentrations of several PRMs in the patients compared with the controls. Transient increased concentrations of some of the PRMs may be caused by recent infection [27]. Infections are likely to be more frequent in the patient categories. We therefore performed measurements of CRP, which is often used as a biomarker for systemic inflammation, and investigated whether the PRM concentrations were correlated with the concentration of CRP and hence inflammation.

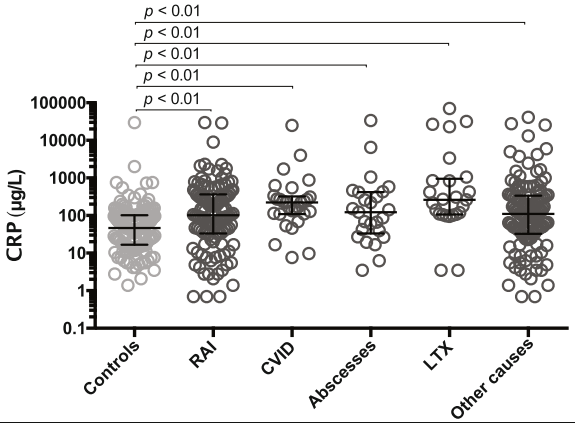
We found that all patient categories had higher concentrations of CRP than controls (Fig. 3a). Relative to controls, geometric mean of CRP was 216% (138%; 338%) for RAI, 292% (132%; 646%) for abscesses, 887% (400%; 1960%) for LTX, 443% (211%; 934%) for CVID, and 231% (149%; 358%) for 'other causes'.

We subsequently examined a potential correlation between concentrations of PRMs and CRP in both patients and controls (Fig. 3b). CRP correlated positively with L- and M-ficolin (Fig. 3b, d). The correlation between CRP and M-ficolin was observed in patents ($r = 0.51$, $p < 0.01$) and controls ($r = 0.41$, $p < 0.01$). The correlation between CRP and M-ficolin was observed in all patient categories ($r \geq 0.41$, $p \leq 0.01$) except for CVID ($r = 0.11$, $p = 0.58$).

The correlation between CRP and L-ficolin was observed in patients ($r = 0.14$, $p < 0.01$) and controls ($r = 0.21$, $p = 0.01$). The correlation between CRP and L-ficolin was only observed in patients suffering from RAI ($r = 0.21$, $p = 0.02$); no correlation was observed in patients suffering from abscesses, CVID, LTX, or 'other causes' ($p \geq 0.19$).

Finally, we examined pairwise correlations between the various PRMs in both patients and controls (Fig. 3b). A strong correlation was observed between H-ficolin and L-ficolin among patients ($r = 0.66$, $p < 0.01$) as well as controls ($r = 0.62$, $p < 0.01$) (Fig. 3c). The correlation between H-ficolin and L-ficolin was also observed in all patient categories ($r \geq 0.53$, $p \leq 0.01$).

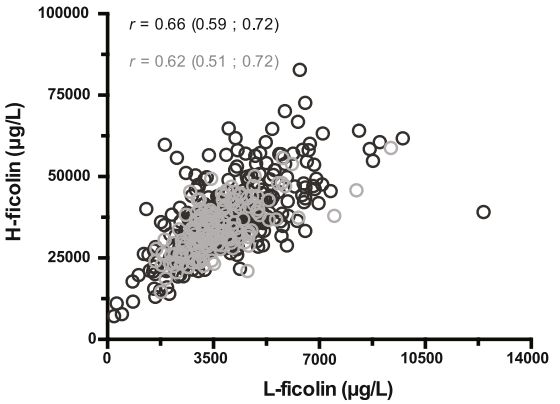
a



b

Correlations	MBL	CL-L1	H-ficolin	L-ficolin	M-ficolin
CRP	r = -0.07 p = 0.22 r = 0.06 p =0.50	**r = -0.14** **p = 0.01** r = -0.02 p = 0.83	r = -0.01 p = 0.93 r = 0.14 p = 0.10	**r = 0.14** **p <0.01** **r = 0.21** **p = 0.01**	**r = 0.51** **p <0.01** **r = 0.41** **p <0.01**
M-ficolin	r = -0.07 p = 0.20 r = 0.00 p = 0.99	r = -0.06 p = 0.29 r = 0.02 p = 0.83	r = 0.04 p = 0.44 r = -0.01 p = 0.86	**r = 0.17** **p <0.01** r = 0.10 p = 0.24	
L-ficolin	r = 0.01 p = 0.84 r = 0.04 p = 0.62	**r = -0.12** **p = 0.03** **r = -0.28** **p <0.01**	**r = 0.66** **p <0.01** **r = 0.62** **p <0.01**		
H-ficolin	r = -0.05 p = 0.40 r = 0.05 p = 0.51	**r = -0.11** **p < 0.05** r = -0.14 p = 0.08			
CL-L1	r = 0.08 p = 0.17 r = 0.03 p = 0.69				

c



d

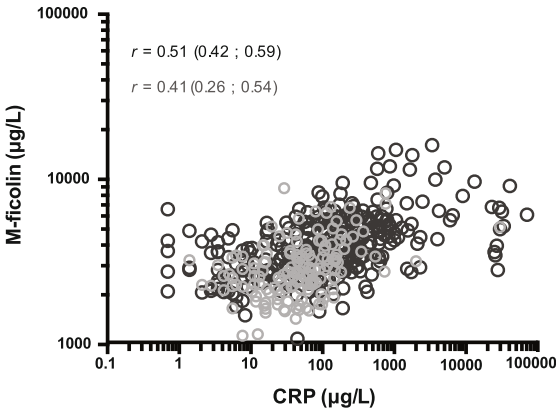


Fig. 3 Association of CRP and PRMs in heparin plasma samples. **a** CRP in controls and patient categories. **b** Spearman's rho with *p* value for all pairwise correlations. **c** Data points of H-ficolin versus L-ficolin and **d** M-ficolin versus CRP. In **c** and **d**, controls are colored light gray and patients in darker color

Discussion

In the present study, we compared plasma concentrations of all PRMs of the lectin pathway in patients with suspected immunodeficiency with healthy controls. The value of measuring these PRMs in clinical settings is somewhat controversial, in particular with respect to MBL.

We included patients examined prior to lung transplantation in one category, LTX, and they served as extra controls. Patients in the LTX category have suffered from numerous infections because of severe structural lung damage, but are per se expected healthy in regard to their innate immune system. Thus, the LTX category was included to control for the effect of numerous previous infections on the levels of the PRMs.

Contrary to our hypotheses, none of the PRMs displayed lower concentration in any patient category. Most of the PRMs were actually found in higher concentration in certain patient categories. Only L-ficolin concentrations did not differ in the patient categories and healthy controls.

We found concentrations of M-ficolin to be higher in all five patient categories compared with controls. M-ficolin is produced by monocytes and neutrophils [28]. The blood concentration of these cells correlates with M-ficolin in both healthy blood donors and persons with ongoing inflammation [29]. We therefore suspect that the higher concentration of M-ficolin in patients is caused by ongoing inflammation. In accordance, we found that all patient categories had more than twofold higher CRP concentration than healthy controls. This was most pronounced for patient categories LTX and CVID. Interestingly, concentrations of CRP and M-ficolin correlated strongly among patients and healthy controls, as well as in four of the patient categories and controls, with the only exception being CVID ($p = 0.58$). This could suggest that not only presence of inflammation is of importance but also which specific immunological components participate in the inflammatory response.

We observed higher concentrations of MBL in some patient categories (abscesses, LTX, and 'other causes'). No patient category had lower MBL concentrations. Despite being the most well-described PRM of the lectin pathway, the biological importance of MBL is not entirely clear, and MBL-deficiency is quite common among Caucasians with an estimated prevalence of 5–15% [4]. Some studies have reported lower concentrations in patients with recurrent or severe infections [30] whereas others [31], including the present study, have failed in verifying this. In addition, MBL deficiency (< 50 ng/mL) is not more prevalent among patients with IgG subclass deficiency [32]. MBL is however, to some extent, an acute-phase reactant and may increase up to 1.5-fold during inflammation, even though the increase is much less rapid than CRP [27]. The patients included in our study did demonstrate signs of more inflammation based on the CRP measurements discussed above; however, active, severe infections were presumably rare based on the relatively low levels of CRP. Hence, it is plausible that MBL concentrations were higher in the patients due to inflammation, ongoing infection, or increased colonization at the time when blood samples were collected.

The estimated differences in concentrations of CL-L1 and H-ficolin between patients and healthy controls were minor. CL-L1 was found in higher concentrations in patient categories RAI, abscesses, and 'other causes', and estimated differences were < 15% higher among all patient categories. H-ficolin was found in higher concentration only in RAI and was 8% higher in patients than controls. We believe the biological significance of such small differences is questionable.

L-ficolin did not differ between patients and controls, albeit it correlated weakly with CRP, which was higher in patients than controls as described above.

We also compared frequencies of single PRM deficiency between patients and controls. The concentration of various PRMs required for full functional capacity of the lectin pathway remains uncertain for most involved proteins. In lack of established limits for deficiency, we defined the 10th percentile concentrations of healthy controls as the limit for low concentrations of PRMs in the present study. We found that deficiency of single PRMs was not more frequent in any patient category. M-ficolin deficiency was actually less frequent in the patient categories RAI and 'other causes'. This may be explained by generally increased level of M-ficolin caused by increased inflammation as argued above. In contrast to our findings, Hoeflich et al. [30] found significantly higher frequency of low concentrations (defined as < 50 μg/L) of MBL in patients suffering from recurrent or severe infections, which was also supported by Holdaway et al. (low levels defined as < 75 μg/L) [31]. The limits used in these studies are higher than ours of 38 μg/L. However, applying an identical cut-off level does not change any conclusions of our study. The differences of the study results might be explained by differences in selections of patients or to some degree ethnicity and consequently genotypes.

The analysis of single PRM deficiency was also performed using 5th and 1st percentiles as cut-off levels. When using the 5th percentile as cut-off, no differences were observed between patients and healthy controls. When using 1st percentile as cut-off, L-ficolin deficiency was observed more frequent among certain patient categories: three patients (12%) in LTX and nine patients (7%) in 'other causes'. This cut-off level resulted in very few patients in each category. As described earlier, patients in the LTX category are expected to have a healthy innate immune system and the deficiencies observed using the 1st percentile cut-off level might be caused by consumption due to frequent and persistent lung infections.

We also examined correlations between single PRMs. The strongest correlation was observed for H-ficolin and L-ficolin (Fig. 3c). Such a correlation has also recently been observed in a different study investigating concentrations of lectin pathway PRMs in healthy individuals [26], but the biological significance has yet to be determined, potentially providing new information regarding the lectin pathway.

A great strength of our study is the large patient population, comprising a large proportion of patients with clinically suspected immunodeficiency based on recurrent or severe infections and referred to a highly specialized health department. The Danish health care system provides free-of-charge health care, clinical evaluation, and treatment of all Danish citizens, and hence, the patient population in our study represents the part of the Danish population where immunological evaluation is considered relevant by immunological specialists. The heterogeneity of the patients and the relatively large number of patients in the category 'other causes' composes a great strength. This reflects the clinical situation eminently and allows the transfer of results regarding screening of lectin pathway PRMs as a clinical tool. Furthermore, all five PRMs of the lectin pathways have been determined in all patients. To our knowledge, this is the first comprehensive study of all lectin pathway PRMs among patients with suspected immunodeficiency.

Our study has some limitations to be considered. A possible limitation is the wide age distribution among the patients in contrast to the age distribution among the healthy controls. Age-related influence on the concentration of the PRMs of the lectin pathway has only been sparsely investigated, but their plasma levels seem to stabilize around 6 months of age without significant fluctuations afterwards in healthy persons [26, 33]. As only four of the 332 examined patients in our study less than 6 months of age, we expect the difference in age to be of minor importance.

Gender-related differences in PRM concentration (H-ficolin, L-ficolin, and M-ficolin) have been reported [26]. Of the patient categories in our study, only RAI differed from controls by gender; i.e., among these patients, 63.9% were

female. In the present study, we adjusted for age and gender in linear regression, and it only had marginal impact on the estimates and did not change conclusions; hence, the non-adjusted data are presented.

In the present study, 50% hemolytic complement (CH50) activity of serum was not determined, as it is not a specific measurement for lectin pathway activity, but rather a measurement for general consumption or lack of complement factors.

The results of our study support that neither single nor combined deficiencies of lectin pathway PRMs are more frequent among patients in general with suspected immunodeficiency compared with healthy blood donors. The study is performed in a clinical setting where access to health care, clinical evaluations, and treatment is free-of-charge, and hence, the patient population in our study represents the part of the Danish population where specialized immunological evaluations are considered relevant.

Based on our results, we suggest there is no indication of performing screening of lectin pathway PRMs as standard in an immunological evaluation. We cannot rule out that deficiency of one or more of the lectin pathway PRMs may be of importance in more selected populations and that screening in such populations might be beneficial. To clarify this, further studies among more selected patient populations are needed.

Eleven different proteins are known to be involved in the lectin pathway, and clinical relevance might also be due to combined deficiencies of PRMs and/or associated proteases (MASP-1, MASP-2, and MASP-3) or proteins (MAp19 and MAp44), and further studies are needed to clarify this.

In the present study, parameters such as CH50, lymphocyte subpopulations, and full genetic testing were not available for all patients and could thus not be included in the analyses of data. Future studies may benefit from including additional parts of the immune system, such as antibodies and cellular components.

Acknowledgements We thank Karen Mathiassen, the Medical Research Laboratory, Aarhus University, for the technical assistance.

Compliance with Ethical Standards

The study was conducted under the approval by the Central Denmark Region Committees on Health Research Ethics (1-10-72-127-12) and the Danish Data Protection Agency (1-16-02-40-12). The study was performed according to the Declaration of Helsinki.

Conflict of Interest The authors declare that they have no conflict of interest.

Abbreviations LP, lectin pathway; PRM, pattern recognition molecule; RAI, recurrent airway infections; CVID, common variable immunodeficiency; LTX, lung transplantation candidates; CRP, C-reactive protein; TRIFMA, time-resolved immunofluorometric assay; CV, coefficient of variation

References

1. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11(9):785–97.
2. Sunyer JO, Zarkadis IK, Lambris JD. Complement diversity: a mechanism for generating immune diversity? Immunol Today. 1998;19(11):519–23.
3. Morgan BP. The membrane attack complex as an inflammatory trigger. Immunobiology. 2015.
4. Skattum L, van Deuren M, van der Poll T, Truedsson L. Complement deficiency states and associated infections. Mol Immunol. 2011;48(14):1643–55.
5. Troldborg A, Steffensen R, Trendelenburg M, Hauser T, Winther KG, Hansen AG, et al. Ficolin-3 deficiency is associated with disease and an increased risk of systemic lupus erythematosus. J Clin Immunol. 2019;39:421–9.
6. Merle NS, et al. Complement system part I - molecular mechanisms of activation and regulation. Front Immunol. 2015;6:262.
7. Bajic G, Degn SE, Thiel S, Andersen GR. Complement activation, regulation, and molecular basis for complement-related diseases. EMBO J. 2015;34(22):2735–57.
8. Krarup A, Thiel S, Hansen A, Fujita T, Jensenius JC. L-ficolin is a pattern recognition molecule specific for acetyl groups. J Biol Chem. 2004;279(46):47513–9.
9. Matsushita M. Ficolins in complement activation. Mol Immunol. 2013;55(1):22–6.
10. Henriksen ML, Brandt J, Andrieu JP, Nielsen C, Jensen PH, Holmskov U, et al. Heteromeric complexes of native collectin kidney 1 and collectin liver 1 are found in the circulation with MASPs and activate the complement system. J Immunol. 2013;191(12):6117–27.
11. Degn SE, Jensen L, Hansen AG, Duman D, Tekin M, Jensenius JC, et al. Mannan-binding lectin-associated serine protease (MASP)-1 is crucial for lectin pathway activation in human serum, whereas neither MASP-1 nor MASP-3 is required for alternative pathway function. J Immunol. 2012;189(8):3957–69.
12. Heitzeneder S, Seidel M, Förster-Waldl E, Heitger A. Mannan-binding lectin deficiency - good news, bad news, doesn't matter? Clin Immunol. 2012;143(1):22–38.
13. Super M, et al. Association of low levels of mannan-binding protein with a common defect of opsonisation. Lancet. 1989;2(8674):1236–9.
14. Crosdale DJ, Poulton KV, Ollier WE, Thomson W, Denning DW. Mannose-binding lectin gene polymorphisms as a susceptibility factor for chronic necrotizing pulmonary aspergillosis. J Infect Dis. 2001;184(5):653–6.
15. Dahl M, Tybjærg-Hansen A, Schnohr P, Nordestgaard BG. A population-based study of morbidity and mortality in mannose-binding lectin deficiency. J Exp Med. 2004;199(10):1391–9.
16. Michalski M, Świerzko AS, Pągowska-Klimek I, Niemir ZI, Mazerant K, Domżalska-Popadiuk I, et al. Primary ficolin-3 deficiency–is it associated with increased susceptibility to infections? Immunobiology. 2015;220(6):711–3.
17. Schlapbach LJ, Thiel S, Kessler U, Ammann RA, Aebi C, Jensenius JC. Congenital H-ficolin deficiency in premature infants with severe necrotising enterocolitis. Gut. 2011;60(10):1438–9.
18. Bonilla FA, Khan DA, Ballas ZK, Chinen J, Frank MM, Hsu JT, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. J Allergy Clin Immunol. 2015;136(5):1186–205 e1-78.
19. Thiel S, et al. Assays for the functional activity of the mannan-binding lectin pathway of complement activation. Immunobiology. 2002;205(4–5):446–54.

20. Krarup A, Sorensen UBS, Matsushita M, Jensenius JC, Thiel S. Effect of capsulation of opportunistic pathogenic bacteria on binding of the pattern recognition molecules mannan-binding lectin, L-ficolin, and H-ficolin. Infect Immun. 2005;73(2):1052–60.
21. Wittenborn T, Thiel S, Jensen L, Nielsen HJ, Jensenius JC. Characteristics and biological variations of M-ficolin, a pattern recognition molecule, in plasma. J Innate Immun. 2010;2(2):167–80.
22. Axelgaard E, Jensen L, Dyrlund TF, Nielsen HJ, Enghild JJ, Thiel S, et al. Investigations on collectin liver 1. J Biol Chem. 2013;288(32):23407–20.
23. Degn SE, Jensen L, Gál P, Dobó J, Holmvad SH, Jensenius JC, et al. Biological variations of MASP-3 and MAp44, two splice products of the MASP1 gene involved in regulation of the complement system. J Immunol Methods. 2010;361(1–2):37–50.
24. Moller-Kristensen M, et al. Levels of mannan-binding lectin-associated serine protease-2 in healthy individuals. J Immunol Methods. 2003;282(1–2):159–67.
25. Degn SE, Thiel S, Nielsen O, Hansen AG, Steffensen R, Jensenius JC. MAp19, the alternative splice product of the MASP2 gene. J Immunol Methods. 2011;373(1–2):89–101.
26. Troldborg A, Hansen A, Hansen SWK, Jensenius JC, Stengaard-Pedersen K, Thiel S. Lectin complement pathway proteins in healthy individuals. Clin Exp Immunol. 2017;188(1):138–47.
27. Thiel S, et al. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol. 1992;90(1):31–5.
28. Liu Y, Endo Y, Iwaki D, Nakata M, Matsushita M, Wada I, et al. Human M-ficolin is a secretory protein that activates the lectin complement pathway. J Immunol. 2005;175(5):3150–6.
29. Ammitzboll CG, Thiel S, Ellingsen T, Deleuran B, Jorgensen A, Jensenius JC, et al. Levels of lectin pathway proteins in plasma and synovial fluid of rheumatoid arthritis and osteoarthritis. Rheumatol Int. 2012;32(5):1457–63.
30. Hoeflich C, Unterwalder N, Schuett S, Schmolke K, Boenisch O, Hammer M, et al. Clinical manifestation of mannose-binding lectin deficiency in adults independent of concomitant immunodeficiency. Hum Immunol. 2009;70(10):809–12.
31. Holdaway J, Deacock S, Williams P, Karim Y. Mannose-binding lectin deficiency and predisposition to recurrent infection in adults. J Clin Pathol. 2016;69:731–6.
32. Barton JC, Barton JC, Bertoli LF. Clinical and laboratory associations of mannose-binding lectin in 219 adults with IgG subclass deficiency. BMC Immunol. 2019;20(1):15.
33. Sallenbach S, Thiel S, Aebi C, Otth M, Bigler S, Jensenius JC, et al. Serum concentrations of lectin-pathway components in healthy neonates, children and adults: mannan-binding lectin (MBL), M-, L-, and H-ficolin, and MBL-associated serine protease-2 (MASP-2). Pediatr Allergy Immunol. 2011;22(4):424–30.