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Mannose-binding lectin and L-ficolin polymorphisms in patients with community-acquired pneumonia caused by intracellular pathogens

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Summary

Community-acquired pneumonia (CAP) is the leading infectious disease requiring hospitalization in the western world. Genetic variability affecting the host response to infection may play a role in susceptibility and outcome in patients with CAP. Mannose-binding lectin (MBL) and L-ficolin (L-FCN) are two important activators of the complement system and they can enhance phagocytosis by opsonization. In a prospective cohort of 505 Dutch patients with CAP and 227 control participants we studied whether polymorphisms in the MBL (*MBL2*) and FCN (*FCN2*) genes influenced susceptibility and outcome. No difference in frequency of these genotypes was found between patients with CAP in general and controls. However, the +6424G>T single nucleotide polymorphism (SNP) in *FCN2* was more common in patients with a *Coxiella burnetii* pneumonia ($P = 0{\cdot}014$). Moreover, the haplotypes coding for the highest MBL serum levels (YA/YA and YA/XA) predisposed to atypical pneumonia (*C. burnetii*, *Legionella* or *Chlamydia* species or *Mycoplasma pneumoniae*) compared with controls ($P = 0{\cdot}016$). Furthermore, patients with these haplotypes were more often bacteraemic ($P = 0{\cdot}019$). It can therefore be concluded that *MBL2* and *FCN2* polymorphisms are not major risk factors for CAP in general, but that the +6424G>T SNP in the *FCN2* gene predisposes to *C. burnetii* pneumonia. In addition, patients with genotypes corresponding with high serum MBL levels are at risk for atypical pneumonia, possibly caused by enhanced phagocytosis, thereby promoting cell entry of these intracellular bacteria.

Keywords: community-acquired pneumonia; complement system; ficolin-2; intracellular bacteria; mannose binding lectin.

Introduction

Community-acquired pneumonia (CAP) is the leading infectious disease requiring hospitalization in the western world. It is associated with significant morbidity and mortality and related health-care costs are proportionally high.[1,2] There is a large variation in causative micro-organisms with *Streptococcus pneumoniae* isolated most often, followed by *Haemophilus influenzae* and influenza viruses.[3,4] Although well-established risk factors for CAP, like chronic obstructive pulmonary disease, are well known, many patients develop CAP without previous illnesses or significant co-morbidities. In these patients it is conceivable that genetic variability affecting the host response to infection may play a role.[5]

Mannose-binding lectin (MBL) is synthesized in the liver as part of the acute-phase response to infection. It is a calcium-dependent lectin, belonging to the group of

Abbreviations: CAP, community-acquired pneumonia; *FCN2*, gene coding for L-ficolin; ICU, intensive care unit; L-FCN, L-ficolin; *MBL2*, gene coding for mannose-binding lectin; MBL, mannose-binding lectin; PCR, polymerase chain reaction; SNP, single nucleotide polymorphisms

collectins. MBL has the ability to bind carbohydrates, especially *N*-acetylglucosamine, on the surface of a variety of micro-organisms through its carbohydrate-rich recognition domain. After binding, MBL activates the lectin pathway of the complement system, independently of antibodies, by binding to MBL-associated serine proteases. In addition MBL opsonizes micro-organisms, thereby promoting phagocytosis. Deficiency of MBL may increase the risk of infection in adults because of impaired phagocytosis by polymorphonuclear leucocytes secondary to defective opsonization and by lack of complement-mediated lysis.[6–9] The level of MBL and its functionality is mainly regulated by three single nucleotide polymorphisms (SNPs) in exon 1 and one SNP in the promoter region of the MBL gene (*MBL2*). The effect of different combinations of these SNPs are well studied and result in either normal, intermediate or low/absent MBL-levels in serum.[6,10]

L-Ficolin (L-FCN) is a serum protein comparable to MBL in structure and function. After binding to a micro-organism, it too can activate complement and enhance phagocytosis by opsonization. Various polymorphisms in the FCN gene (*FCN2*) have been described, with two coding SNPs in the fibrinogen-like domain responsible for binding to micro-organisms. These coding SNPs lead to, respectively, decreased or increased binding affinity for its substrates.[11–14]

In recent years, the association between genetic variations in *MBL2*, and to a lesser extent *FCN2*, and susceptibility to disease have been the subject of various studies.[15–31] An association between *MBL2* polymorphisms and clinical outcome has been described in children with recurrent infections[22] and in adult patients with meningococcal sepsis.[17] *FCN2* SNPs have been associated with a number of clinical conditions, including rheumatic fever,[29] Behçet's disease,[20] peritonitis in patients undergoing peritoneal dialysis[28] and recurrent respiratory infections in children.[16,18] More recently, the role of *MBL2* polymorphisms in invasive pneumococcal disease[25,27,30,31] and CAP[23,24] has been studied but with conflicting results. In addition, several studies show that MBL dysfunction is associated with a worse clinical outcome in patients with CAP, sepsis and bacteraemia.[21,24,26] Only one study investigated the role of *FCN2* polymorphisms in pneumococcal disease and showed no apparent relation.[19]

We studied whether *MBL2* and *FCN2* polymorphisms contribute to susceptibility and outcome in two large cohorts of Dutch patients with CAP. We furthermore analysed the potential relationship between causative CAP pathogens and *MBL2* and *FCN2* status.

Materials and methods

Samples and data from a large, prospective clinical study, with a total number of 505 patients with CAP, were included. Inclusion and exclusion criteria, definition of CAP and pathogen identification in these cohorts were described previously.[23,32] The control group comprised 227 healthy blood bank donors from the same geographical region. All participants gave written informed consent and this study was approved by the local medical ethics committee.

Genomic DNA was extracted from 200 μl of EDTA blood with a MagNa Pure LC robot (Roche Diagnostics, Mannheim, Germany), using the MagNa Pure DNA isolation kit according to the manufacturer's protocol. Extracted DNA was amplified using a previously described PCR assay.[23] Primer sequences are shown in Table 1.

Genotyping of *MBL2* was done for the X/Y promoter SNP (rs7096206) and the exon 1 SNPs on codons 52 (+153C>T, rs5030737, variant 'D'), 54 (+160G>A, rs1800450, variant 'B') and 57 (+169G>A, rs1800451, variant 'C'). The promoter SNP is denoted as 'X/Y', with 'Y' being the wild-type. The three variants 'B', 'C' and 'D' in exon 1 are collectively coded as '0' and the wild-type is denoted as 'A'. These SNPs are combined into six different haplotypes: YA/YA, YA/XA, XA/XA, YA/0, XA/0 and 0/0. MBL serum levels are known to be deficient in the XA/0 and 0/0 haplotypes, intermediate in YA/0 and XA/XA and highest in wild-type (YA/YA) and YA/XA haplotypes.[6,10] The amplified DNA fragments were analysed on a polyacrylamide gel with a linear denaturing gradient of formamide and urea, as described previously.[23] All *MBL2* exon 1 genotypes could be distinguished by their different patterns of migration.

Genotyping of *FCN2* was performed for two coding SNPs in exon 8.1, with the wild-type being denoted as 'A'

Table 1. Primer sequences used for genotyping the *MBL2* promoter region, *MBL2* exon 1 and *FCN2* exon 8.1

MBL2 and *FCN2* exons	PCR primer sequences
MBL2 exon 1 forward (235)	5′-TCC ATC ACT CCC TCT CCT TCT C-3′ (with gc-clamp)
MBL2 exon 1 reverse (236a)	5′-GAG ACA GAA CAG CCC AAC ACG-3′
MBL2 promoter X forward (311)	5′-ATT TGT TCT CAC TGC CAC C-3′
MBL2 promoter Y forward (312)	5′-TTT GTT CTC ACT GCC ACG-3′
MBL2 promoter reverse (254)	5′-GAG CTG AAT CTC TGT TTT GAG TT-3′
FCN2 exon 8.1 forward (302c)	5′-GCC AGG CCT CAG GTA TAA A-3′ (with gc-clamp)
FCN2 exon 8.1 reverse (303c)	5′-CCA CCA AGC TCC CTG AAA C-3′

MBL2, gene coding for mannose binding lectin; *FCN2*, gene coding for L-ficolin.

and the mutant alleles as 'B' (+6359C>T, rs17549193) and 'C' (+6424G>T, rs7851696), respectively. This results in six possible haplotypes: AA (wild-type), AB/AC (heterozygous mutant) or BB/CC/BC (homozygous mutant). Previously, no association has been described between *FCN2* genotype and L-FCN serum levels. DNA amplification and subsequent genotype analysis were performed using the same techniques as described above. Primer sequences are shown in Table 1.

Serum levels of the MBL and L-FCN proteins were measured using commercially available ELISAs (Sanquin, Amsterdam, the Netherlands and Hycult Biotech, Uden, the Netherlands, respectively).

The contribution of clinical characteristics and SNPs in the *MBL2* and *FCN2* genes to the development and outcome of CAP were analysed using the Pearson's chi-squared test or the Fischer's exact test, as appropriate. For continuous variables the *t*-test or one-way analysis of variance was used or the Mann–Whitney *U*-test or the Kruskal–Wallis test when needed. Two-tailed *P* values $< 0{\cdot}05$ were considered significant. All data were analysed using SPSS software version 21.0 (SPSS, Chicago, IL).

Results

Clinical data (Table 2) and blood samples for DNA extraction of 505 patients and 227 controls was available. In some cases DNA quantity or quality was insufficient for proper genotyping. Eventually, we were able to determine an *MBL2* haplotype in 493 patients and all controls. *FCN2* genotypes were constructed in 474 patients and all controls. All genotype frequencies were in Hardy–Weinberg equilibrium.

In 295 cases (58%) the causative micro-organism was identified. As expected, *S. pneumoniae* was the most frequently identified micro-organism. A relatively high number of atypical micro-organisms were isolated, especially *Legionella* species (20 cases) and *Coxiella burnetii* (28 cases). The high number of *Coxiella* infections is explained by a regional outbreak of Q fever in the Netherlands in 2007–2009.[33]

As reported previously, there is a strong relationship between *MBL2* haplotype and MBL serum concentration.[6,10] The YA/YA and XA/YA haplotypes result in the highest serum concentrations, XA/0 and 0/0 in the lowest and the XA/XA and YA/0 haplotypes correspond with an intermediate concentration of MBL in serum (data not shown). No relationship between *FCN2* genotype and L-FCN serum concentration was observed, as was shown previously by Hummelshoj *et al.*[12]

Table 3 shows the different *MBL2* and *FCN2* allele frequencies, genotypes and haplotypes, where the A/A and YA/0 haplotypes are considered sufficient and the 0/0 and XA/0 haplotype are considered deficient. There was no difference in frequency of *MBL2* or *FCN2* genotypes between patients with CAP in general and controls. Nor was there a statistical significant difference when patients with CAP caused by *S. pneumoniae*, *H. influenzae* or viral agents were compared with controls.

However, the *MBL2* haplotypes corresponding with the highest serum levels (YA/YA and YA/XA) were significantly over-represented in the group of patients with CAP caused by an atypical micro-organism (*C. burnetii*, *Legionella* or *Chlamydia* species or *Mycoplasma pneumoniae*) compared with controls ($P = 0{\cdot}016$). In addition, the +6424G>T SNP, coding for an Ala258Ser amino acid substitution in the *FCN2* gene, was more common in patients with a *C. burnetii* ($P = 0{\cdot}014$) pneumonia. Three patients (11%) with a *C. burnetii* infection were homozygous for this mutation. In contrast, only five control patients (2%) had this genotype.

Table 2. Baseline characteristics of 505 patients with CAP and 227 healthy control participants

	CAP (%)	Controls (%)
Number	505	227
Male gender	295 (58)	133 (59)
Age (years, mean)	63 ± 18	50 ± 12
Chronic obstructive pulmonary disease	98 (19)	n.a.
Causative micro-organism		
Unidentified	210 (42)	
Bacterial agent	259 (51)	
Streptococcus pneumoniae	124 (25)	
Haemophilus influenzae	26 (5)	
Staphylococcus aureus	9 (2)	
Legionella species	20 (4)	
Mycoplasma pneumoniae	9 (2)	
Chlamydia species	16 (3)	
Coxiella burnetii	28 (6)	
Viral agent	36 (7)	
Influenza virus A/B	14 (3)	
Herpes simplex virus	7 (1)	
FINE class		
I	71 (14)	
II	97 (19)	
III	110 (22)	
IV	153 (30)	
V	74 (15)	
Outcome		
Hospital mortality	24 (5)	
30-day mortality	27 (5)	
1-year mortality	73 (14)	
Intensive care unit admission	38 (8)	
Median length of hospital stay (days, range)	9 (1–144)	
Bacteraemia	49 (10)	

CAP, community-acquired pneumonia; FINE, scoring system reflecting pneumonia severity; n.a., not available.

Table 3. (a) *MBL2* and (b) *FCN2* genotype and haplotype distribution across all patients with CAP, certain causative pathogens and controls

	Controls (%) $n = 227$	CAP (%) $n = 505$	*Streptococcus pneumoniae* (%) $n = 124$	Atypical spp. (%) $n = 73$	*Coxiella burnetii* (%) $n = 28$
(a) *MBL2* genotypes					
Structural alleles					
Total A	333 (73)	761 (77)	194 (80)	116 (81)	45 (80)
Total 0	121 (27)	225 (23)	50 (20)	28 (19)	11 (20)
B	60	124	29	15	6
C	15	28	5	2	0
D	46	73	16	11	5
Structural genotypes					
A/A (wild-type)	120 (53)	287 (57)	77 (62)	45 (62)	18 (65)
A/0	93 (41)	187 (37)	40 (32)	25 (34)	9 (32)
0/0	14 (6)	19 (4)	5 (4)	2 (3)	1 (4)
Missing	0	12 (2)	2 (2)	1 (1)	0
Promoter alleles					
Y	357 (79)	765 (77)	179 (73)	117 (81)	46 (82)
X	97 (21)	223 (23)	65 (27)	27 (19)	10 (18)
Promoter genotype					
YY (wild-type)	142 (63)	296 (59)	66 (53)	45 (62)	18 (64)
XY	73 (32)	173 (34)	47 (38)	27 (37)	10 (36)
XX	12 (5)	25 (5)	9 (7)	0	0
Missing	0	11 (2)	2 (2)	1 (1)	0
Structural and promoter haplotypes					
YA/YA (wild-type)	62 (27)	148 (29)	34 (27)	27 (37)[1]	12 (43)
YA/XA	46 (20)	113 (22)	34 (27)	18 (25)[1]	6 (21)
XA/XA	12 (5)	25 (5)	9 (7)	0	0
YA/0	66 (29)	128 (25)	27 (22)	16 (22)	5 (18)
XA/0	27 (12)	59 (12)	13 (11)	9 (13)	4 (14)
0/0	14 (6)	20 (4)	5 (4)	2 (3)	1 (4)
Missing	0	12 (2)	2 (2)	1 (1)	0
Sufficient haplotypes (A/A, YA/0)	186 (82)	414 (82)	104 (84)	61 (84)	23 (82)
Deficient haplotypes (XA/0, 0/0)	41 (18)	79 (16)	18 (15)	11 (15)	5 (18)
(b) *FCN2* genotypes					
+6359C>T structural alleles					
Total C	326 (72)	687 (72)	165 (71)	111 (80)	44 (79)
Total T	128 (28)	261 (28)	69 (29)	27 (20)	12 (21)
+6359C>T structural genotypes					
C/C (wild-type)	121 (53)	251 (50)	62 (50)	46 (63)	17 (61)
C/T	84 (37)	185 (37)	41 (33)	19 (26)	10 (36)
T/T	22 (10)	38 (8)	14 (11)	4 (6)	1 (4)
Missing	0	31 (6)	7 (6)	4 (6)	0
+6424G>T Structural alleles					
Total G	395 (87)	826 (87)	208 (89)	120 (87)	48 (86)
Total T	59 (13)	122 (13)	26 (11)	18 (13)	8 (14)
+6424G>T Structural genotypes					
G/G (wild-type)	173 (76)	359 (71)	91 (73)	55 (75)	23 (82)
G/T	49 (22)	108 (21)	26 (21)	10 (14)	2 (7)
T/T	5 (2)	7 (1)	0	4 (6)	3 (11)[2]
Missing	0	31 (6)	7 (6)	4 (6)	0
Structural haplotypes					
AA (wild-type)	80 (35)	177 (35)	46 (37)	33 (45)	13 (46)
AB	71 (31)	144 (129)	31 (25)	18 (25)	9 (32)
AC	36 (16)	67 (13)	16 (13)	9 (12)	1 (4)
BB	22 (10)	40 (8)	14 (11)	4 (6)	1 (4)
BC	13 (6)	39 (8)	10 (8)	1 (1)	1 (4)

Table 3 *(Continued)*

	Controls (%) $n = 227$	CAP (%) $n = 505$	*Streptococcus pneumoniae* (%) $n = 124$	Atypical spp. (%) $n = 73$	*Coxiella burnetii* (%) $n = 28$
CC	5 (2)	7 (1)	0	4 (6)	3 (11)
Missing	0	31 (6)	7 (6)	4 (6)	0
Wild-type (AA)	80 (35)	177 (35)	46 (37)	33 (45)	13 (46)
Heterozygous (AB, AC)	107 (47)	211 (42)	47 (38)	27 (37)	10 (36)
Homozygous (BB, CC, BC)	40 (18)	86 (17)	24 (19)	9 (12)	5 (18)

MBL2, gene coding for mannose-binding lectin; *FCN2*, gene coding for L-ficolin; CAP, community-acquired pneumonia.
[1]$P = 0{\cdot}016$ for the combined haplotypes YA/YA and YA/XA in patients with atypical pneumonia versus controls.
[2]$P = 0{\cdot}014$ for genotype T/T in patients with *Coxiella burnetii* pneumonia versus controls.

Table 4. FINE class and outcome in patients with CAP with different *MBL2* and *FCN2* genotypes and haplotypes

MBL2 and *FCN2* genotypes/haplotypes	Number	FINE class 4–5 ($n = 227$)	Bacteraemia ($n = 49$)	ICU admittance ($n = 38$)	Hospital mortality ($n = 23$)	Median length of hospital stay (days, range)
MBL2 Structural and promoter haplotypes						
YA/YA (%) (wild-type)	148	70 (47)	19 (13)[1]	14 (9)	9 (6)	8 (1–84)
YA/XA (%)	113	55 (49)	14 (12)[1]	8 (7)	2 (2)	9 (2–73)
XA/XA (%)	25	11 (44)	3 (12)	3 (12)	1 (4)	9 (3–26)
YA/0 (%)	128	52 (41)	8 (6)	7 (5)	7 (5)	8 (2–71)
XA/0 (%)	59	25 (42)	4 (7)	3 (5)	2 (3)	8 (3–144)
0/0 (%)	20	8 (40)	0 (0)	2 (10)	2 (10)	8 (5–25)
MBL2-sufficient haplotypes (A/A, YA/0) (%)	414	188 (45)	44 (11)	32 (8)	19 (5)	9 (1–84)
MBL2-deficient haplotypes (XA/0, 0/0) (%)	79	33 (42)	4 (5)	5 (6)	4 (5)	8 (3–144)
Missing (%)	12	6 (50)	1 (8)	1 (8)	0 (0)	0 (0)
FCN2 +6359C>T Structural genotypes						
C/C (%) (wild-type)	251	115 (46)	21 (8)	19 (8)	9 (4)	8 (3–144)
C/T (%)	185	80 (43)	22 (12)	12 (6)	13 (7)	9 (2–84)
T/T (%)	38	16 (42)	5 (13)	4 (11)	1 (3)	8 (1–73)
FCN2 +6424G>T Structural genotypes						
G/G (%) (wild-type)	359	168 (47)	36 (10)	28 (8)	17 (5)	9 (1–144)
G/T (%)	108	40 (37)	12 (11)	7 (6)	5 (5)	8 (3–46)
T/T (%)	7	3 (43)	0 (0)	0 (0)	1 (14)	8 (3–14)
FCN2 Structural haplotypes						
Wild-type (AA) (%)	177	87 (49)	14 (8)	16 (9)	6 (3)	8 (3–144)
Heterozygous (AB, AC) (%)	211	90 (43)	24 (11)	11 (5)	12 (6)	9 (2–84)
Homozygous (BB, CC, BC) (%)	86	34 (40)	10 (12)	8 (9)	5 (6)	8 (1–73)
Missing (%)	31	16 (52)	1 (3)	3 (10)	0 (0)	0 (0)

FCN2, gene coding for L-ficolin; CAP, community-acquired pneumonia; *MBL2*, gene coding for mannose-binding lectin; FINE, scoring system reflecting pneumonia severity.
[1]$P = 0{\cdot}019$ for the combined haplotypes YA/YA and YA/XA versus other haplotypes in patients with CAP and bacteraemia.

As shown in Table 4, patients with an *MBL2* haplotype coding for high MBL serum levels (YA/YA and YA/XA) were significantly more often bacteraemic than patients with other haplotypes ($P = 0{\cdot}019$). There was no association between *MBL2* or *FCN2* genotypes and other clinically relevant endpoints or markers for more severe illness (Table 4). Also, no correlation was found between *MBL2* or *FCN2* genotypes and C-reactive protein, interleukin-6 or interleukin-8 serum levels at the day of hospital admission (data not shown).

Discussion

The interest in the potential role of MBL in the pathophysiology of CAP was aroused by the finding that patients with an *MBL2*-deficient genotype (0/0) were at risk for invasive pneumococcal disease.[30] Although several later studies failed to confirm this result,[25,27,31] a meta-analysis of all these studies did show a significant association between *MBL2*-deficient genotypes and susceptibility to invasive pneumococcal disease.[25] Two subsequent studies focused

on the role of MBL in susceptibility to pneumococcal CAP but were not able to reproduce this finding.[23,24] Garcia-Laorden *et al.*[24] did show that MBL deficiency predisposes patients with CAP to a more severe course of disease and worse outcome, but this was not confirmed by Endeman *et al.*[23] In our study we also did not find an association between MBL deficiency and CAP susceptibility. In fact, there was a non-significant trend towards a protective effect of MBL deficiency in the pneumococcal CAP subgroup. This phenomenon was described earlier in two Spanish studies,[24,34] adding to the controversy of MBL deficiency as an important factor in susceptibility to pneumococcal CAP.

There are several explanations for the lack of association between MBL deficiency and pneumococcal CAP. First, *in vitro* studies show that *S. pneumoniae* does not have a strong binding affinity for MBL.[7] In addition, the immune system has several other, maybe more effective, ways to eliminate *S. pneumoniae*.[13,35,36] In fact, the classical and alternative complement pathways have been shown to play a more important role in complement activation in pneumococcus-infected mice.[37]

Finally, an interesting explanation might be found in the pivotal role of MBL in the modulation of infection. Takahashi *et al.*[38] showed that MBL-deficient mice with septic peritonitis actually had a survival benefit compared with mice with the *MBL2* wild-type. Walsh *et al.*[39] found that MBL deficiency protected mice from reperfusion injury after myocardial ischaemia. It is hypothesized that MBL serves as an important pro-inflammatory molecule and that deficiency could result in a less pronounced state of inflammation and, as a consequence, less collateral damage to the host.[40]

To our knowledge, this is the first cohort of patients with CAP in which susceptibility in relation to *FCN2* genotypes is studied. One previous study investigated the role of *FCN2* polymorphisms in a group of patients with invasive pneumococcal disease and did not find any association.[19] Our study also failed to show an association between *FCN2* genotypes and susceptibility to CAP in general or pneumococcal CAP specifically.

However, we did find a significant association between the +6424G>T SNP in exon 8.1 of the *FCN2* gene and the susceptibility to CAP caused by *C. burnetii*. The two known coding SNPs in exon 8.1 of the *FCN2* gene alter the affinity of L-FCN for its substrates. The +6424G>T SNP (rs7851696) codes for an Ala258Ser amino acid substitution and increases the binding capacity of L-FCN to GlucNac, whereas the +6359C>T SNP (rs17549193) codes for a Thr236Met amino acid substitution and decreases binding capacity.[12] Interestingly, we also found that the *MBL2* genotypes known to result in the highest serum levels of MBL, are associated with susceptibility to CAP caused by obligate intracellular pathogens, such as *C. burnetii*, *M. pneumoniae* and *Legionella* species. This finding is in line with the observation that MBL deficiency can confer protection against infection with mycobacteria, whose pathogenicity also relies on their capacity to grow and/or survive within cells.[41,42] It has been suggested that this mechanism is the main reason why *MBL2* polymorphisms are well preserved throughout evolution. In regions where the prevalence of these polymorphisms is particularly high, the potential harmful effect of MBL deficiency might be counterbalanced by the protective effect against certain infectious diseases such as tuberculosis, malaria and leishmaniasis.[42,43] One can hypothesize that the same holds true for the *FCN2* +6424G>T SNP. Increased binding capacity is favourable in situations where this ability creates extra protection against pathogens, but it can be harmful in scenarios where these pathogens benefit by entering the cell or favour an intracellular life cycle. If both MBL and L-FCN would bind to the same micro-organism this could have an additive, synergistic, or antagonistic effect on complement activation and the ultimate fate of the micro-organism, depending on the nature, density and distribution of the respective ligands on a given micro-organism. The ligands for MBL have been relatively well defined. The spectrum of ligands for Ficolin 2 includes not only *N*-acetyl groups (including GlucNac) but also sulphate- and phosphate-containing carbohydrates,[44] as well as lipoteichoic acid, a cell wall constituent of all Gram-positive bacteria.[45] The balance between MBL- and L-FCN-mediated complement activation therefore can have a major effect on elimination or survival of intracellular pathogens.

Obviously, larger studies that focus specifically on this topic are needed to substantiate this hypothesis.

We found no association between *MBL2* or *FCN2* polymorphisms and clinical outcome of CAP. In the study of Garcia-Laorden *et al.*[24] MBL deficiency was associated with a higher incidence of severe sepsis and death in patients with CAP. This finding has not been reproduced so far and our study adds to these conflicting results.

Surprisingly, we observed a higher incidence of bacteraemia in patients carrying the *MBL2*-sufficient haplotype. A similar finding was previously reported by Perez-Castellano *et al.*,[34] but not by other studies in patients with CAP.[23,24] A possible explanation might be that MBL can inhibit peptidoglycan-induced production of pro-inflammatory cytokines and enhance phagocyte recruitment, hypothetically favouring bacterial entrance to the bloodstream.[46] Another hypothetical explanation might be that a pro-inflammatory state, associated with high MBL serum levels, damages the vessel wall and interstitium, making it easier for microbes to enter the bloodstream. The clinical importance of this finding needs to be established, since bacteraemia is associated with more intensive care unit admissions and deaths (data not shown).

This is the first study in which the relation of both *MBL2* and *FCN2* genotypes with susceptibility to CAP have been investigated. Patient data were prospectively

collected, which is an advantage compared with other studies with often a retrospective design or that only included a specific group of patients. The study population as well as the control population comprises almost only Caucasians, which minimizes the risk of population stratification. Because of the extensive diagnostic procedures used in these studies, we were able to identify the causative micro-organism in the majority of cases, including a relatively large proportion of atypical micro-organisms.

Several aspects of our study should be kept in mind when interpreting the results. Although our sample size was reasonable, larger groups would have been beneficial to the robustness of our data. Our control population was significantly younger (mean age 50 years) than the total group of patients with CAP (mean age 63 years). However, patients with a *C. burnetii* infection were significantly younger than other patients with CAP. The mean age of these patients was 50 years and so comparable to the control population. Moreover, *MBL2* and *FCN2* genotypes did not differ between different age groups (data not shown).

In summary, our study shows that MBL deficiency is not a major risk-factor for CAP in general and pneumococcal CAP specifically. *FCN2* polymorphisms are not associated with CAP in general or pneumococcal CAP. However, the +6424G>T SNP in exon 8.1 coding for an Ala258Ser amino acid substitution, which increases the binding capacity of L-FCN to micro-organisms, is associated with *C. burnetii* pneumonia. Also *MBL2* genotypes coding for the highest serum levels of MBL are more frequently observed in patients with atypical pneumonia. The role of MBL and L-FCN in CAP caused by intracellular micro-organisms therefore warrants further investigation.

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Disclosure

The authors state that they have no disclosures to declare.

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