Infection and sepsis in severely injured patients is associated with single nucleotide polymorphisms in *MBL2, MASP2* and *FCN2* within the lectin pathway

Maarten W.G.A. Bronkhorst[1], Miranda A.Z. Lomax[1], Rolf H.A.M. Vossen[2], Jan Bakker[3], Peter Patka[1,4], Esther M.M. Van Lieshout[1]

[1] Department of Surgery–Traumatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[2] Leiden Genome Technology Center, Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

[3] Department of Intensive Care Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

4 Department of Emergency Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Corresponding author:

Esther M.M. Van Lieshout, MSc PhD

Erasmus MC, University Medical Center Rotterdam

Department of Surgery-Traumatology

P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

Phone: +31107030150; Fax : +31107032396; E-mail: e.vanlieshout@erasmusmc.nl

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ABSTRACT

Background. Infectious complications remain a serious threat to polytraumatized patients. Susceptibility and response to infection is, in part, heritable. The lectin pathway plays a major role in innate immunity. The aim of this study was to assess if single nucleotide polymorphisms (SNPs) in three key genes within the lectin pathway affect susceptibility to infectious complications in severely injured patients.

Methods. A prospective cohort of trauma patients admitted to a Level I Trauma Center between January 2008 and April 2011 was genotyped for SNPs in *MBL2* (Mannose-Binding Lectin 2), *MASP2* (MBL-Associated Serine Protease 2), and *FCN2* (Ficolin 2). Association of genotype with prevalence of infection was tested with chi-square and logistic regression analysis.

Results. A total of 219 patients were included, of which 112 (51%) developed a positive culture in either sputum, wounds, blood, or urine. Systemic Inflammatory Response Syndrome (SIRS) developed in 140 patients (64%), sepsis in 79 (36%), and septic shock in 37 (17%). Patients with a *MBL2* exon 1 variant allele were more prone to positive wound cultures (OR 2.50, p=0.025). A *MASP2* Y371D DD genotype predisposed for SIRS (OR 4.78; p=0.042) and septic shock (OR 2.53; p=0.003). A *FCN2* A258S AS genotype predisposed for positive wound cultures (OR 3.37; p=0.005) and septic shock (OR 2.18 p=0.011).

Conclusion. Severely injured patients with SNPs in *MBL2*, *MASP2 Y371D* and *FCN2 A258S* of the lectin pathway of complement activation are significantly more susceptible to positive culture findings and to infectious complications, SIRS and septic shock than patients with a common (wildtype) genotype.

INTRODUCTION

Trauma is the leading cause of death for young people. Early mortality due to exsanguination seems to have decreased [1], probably as a result of improvements in pre-hospital and early in-hospital care. Sepsis (3.1-17.0%) and multiple organ dysfunction syndrome (1.6-9.0%) remain unchanged as important causes of late trauma-related mortality [1]. Severe sepsis and septic shock are responsible for 30-50% of all deaths in Intensive Care Units [2]. Moreover, sepsis, multiple organ dysfunction syndrome, and infectious complications such as pneumonia and wound infection increase hospital costs [3]. Prevention of infections and identifying patients at risk deserves full attention.

The lectin pathway of complement activation plays a critical role in innate immunity (Figure 1). Severe injury poses a serious challenge to the immune system [4]. Dysregulation of the systemic inflammatory response to injury1–3 is central to the development of acute respiratory distress syndrome, multiple organ failure and sepsis [5]. Levels of complement factor C3a are already markedly increased at the scene of trauma. Moreover, complement function decreases dramatically within four hours after trauma, and does not return to normal in as much as five days. Complement activation correlates with injury severity, reduced tissue hypoperfusion as measured with base deficit, and adverse clinical outcomes [6]. The pathological basis for these conditions is attributed to immune dysfunction [7] An understanding of the mechanisms of innate immune activation and alarmins following trauma may lead to new therapeutic agents and improved patient survival [7].

The ability of the complement system to be activated depends on the genetic integrity of the coding genes [6]. Genetic variation, particularly Single Nucleotide Polymorphisms (SNPs), in innate immunity genes may play a central role in development of posttraumatic complications [8]. Key genes in the lectin pathway are *MBL2* (Mannose-Binding Lectin 2),

MASP2 (MBL-Associated Serine Protease 2), and *FCN2* (Ficolin 2). SNPs in all three genes and their promoter regions, have been associated with infectious diseases.

SNPs in exon 1 (codons 52, 54, and 55) of the *MBL2* gene, which encodes Mannose-Binding Lectin, cause transition from an A to a D, B, or C allele (which are all three known as 0 alleles). An A0 or 00 genotype as well as the presence of an YX promoter SNP results in a dramatic reduction in MBL concentration compared with the common AA genotype [9]. MBL deficiency has been associated with an increased risk of infectious disease in, among others, pediatric and ICU patients. [10].

MBL-associated serine-proteases (MASPs) are activated upon binding of MBL or ficolin to pathogen-associated molecular patterns located on the surface of bacteria or viruses. MASPs circulate in plasma in complexes with MBL and with ficolins 2 and 3 [11]. Of the known MASPs MASP2 is the main initiator of the lectin complement pathway [12] [13]. The *MASP2* D120G and Y371D SNPs have been associated with pneumococcal disease and to increase the risk of infectious complications after liver transplantation [14] [15] [16].

The *FCN2* gene encodes L-ficolin (Ficolin-2), a pattern recognition molecule. The *FCN2* T236M and A258S SNPs have been shown to influence infectious outcome after liver transplantation [16], peritoneal dialysis [17], perinatal infection in babies and respiratory infections [18], but largely the functional impact of these SNPs is unknown [19].

There are many reports on MBL levels, genetic variations in immune genes and susceptibility to infections in different kinds of populations such as surgical patients, burn patients, or ICU/sepsis patients [20-22], but the relevance of SNPs in the lectin complement pathway coding genes (*MBL2, MASP2* and *FCN2*) in severely injured patients is unknown. The aim of this study was therefore to determine the influence of lectin complement pathway SNPs on the susceptibility and course of infectious complications in severely injured patients.

PATIENTS AND METHODS

Patients and Clinical Data

A prospective cohort of consecutive severely injured patients admitted to a Level I Trauma Center between January 2008 and April 2011 was studied. Eligibility criteria were an Injury Severity Score (ISS) [23] of 16 or higher and age 18-80 years. Patients with a known immune disorder or those taking immunosuppressive medication were excluded. Patients with a neutrophil count of $<1x10^9$ cells/L before the onset of sepsis, infections associated with burns, or lack of commitment to full life-support measures by the primary physician were also excluded. After obtaining written informed consent from patient or proxy a blood sample was taken in an EDTA tube and stored at -80°C. The study was approved by the local medical research ethics committee and was registered in the Dutch Trial Registry under NTR1625.

Cultures were taken on a standardized routine basis or at the discretion of the physician based upon clinical signs. Endpoints used were: positive cultures during hospital stay, SIRS within the first 24 hours of admission and developing sepsis (primary endpoint), septic shock and mortality during hospital admission. SIRS, sepsis, and septic shock were defined using international criteria [24]. The following data were retrieved from electronic files: age at trauma, trauma mechanism, ISS score, length of stay in the ICU and the hospital, all positive cultures from blood, urine, sputum, wounds, or other positive cultures during hospital stay, if patients developed SIRS within 24 hours of hospital admission and sepsis or septic shock during the hospital stay, and if applicable the cause of death.

DNA Isolation

Genomic DNA was isolated from 300 µL EDTA-treated peripheral blood using the QIAamp® DNA Blood Mini kit (QiaGen Benelux, Venlo, The Netherlands), according to the

manufacturer's instructions. The purity (A260nm/A280nm index) and concentration of the isolated DNA samples were determined with the Thermo Scientific Nanodrop TM1000 spectrophotometer (Isogen Life Science, De Meern, The Netherlands). Samples were diluted to 10 ng/µL using Milli-Q and were stored at 4°C until use.

PCR Oligonucleotides

Details on the SNPs studied are given in Supplementary Table 1. SNP data were retrieved from Ensembl.org, National Center for Biotechnology Information (NCBI) GenBank, and NCBI SNP Database. All oligonucleotides (see Supplementary Table 2) were purchased from Eurogentec (Seraing, Belgium). Primers for *MBL2* amplify a 103-bp fragment overlapping the SNPs in exon 1. For the High Resolution Melting Analysis (HRMA) of all other SNPs 18-20 nucleotides upstream and downstream of the SNPs were used as forward and reverse primers. Oligonucleotides for direct sequencing were designed using the Oligo 6.22 software (Molecular Biology Insight, Cascade, CO), and resulted in 400-555 base pair amplicons. Oligonucleotides had melting temperatures (Tm) between 65.0-66.5°C (Tm calculated using the nearest neighbor method at a salt concentration of 50mM KCl and 4mM $MgCl_2$ (303mM of Na^+ equivalent) and 300nM oligonucleotides).

Genotyping

SNPs were detected using HRMA[25]. Polymerase chain reactions (PCRs) were performed in a total volume of 10µL, containing 20ng genomic DNA, 5pmol of both gene-specific oligonucleotides, 2pmol dNTPs (Promega, Madison, WI), 1µL LC-green (Bioké, Leiden, The Netherlands), 0.5U of Taq DNA polymerase (Roche Diagnostics, Almere, The Netherlands), and 1µl 10x PCR buffer containing 20mM $MgCl_2$. Two pmoles of calibrator oligonucleotides were added for calibration of melting curves. Reaction mixtures for the detection of *MBL2*

exon 1 SNPs contained 1pmol forward and 5pmol reverse oligonucleotide and 5pmol probe. The PCR was run on a Biometra Thermocycler (Biometra GmbH, Göttingen, Germany). The thermocycling program included denaturation at 95°C for 10 min, followed by 55 cycles of 20 sec at 95°C, 30 sec at 60°C and 40 sec at 72°C, and a final extension step of 5 min at 72°C. HRMA was performed using a LightScanner® (HR-96, Idaho Technology, Salt Lake City, USA). Melting was done from 55°C to 98°C at 0.1°C/sec. Melting curves were analyzed with the LightScanner® Software using Call-IT 1.5. Typical examples are shown in Figure 2.

All variant genotypes were confirmed by direct sequencing. PCR were performed in a total volume of 25µL, containing 50ng genomic DNA, 4mM $MgCl_2$, 3pmol of both oligonucleotides, 2pmol dNTPs, 1.0U Taq DNA polymerase, and 1µl 10x PCR buffer without $MgCl_2$. The thermocycling program included denaturation at 95°C for 5 min, followed by 35 cycles of 30 sec at 95°C, 1 min at 60°C, and 1 min at 72°C, and a final step of 7 min at 72°C. Amplicon purification and sequencing was performed by BaseClear (Leiden, The Netherlands). Results were analyzed using the SeqMan® analysis software.

Statistical Analysis

Allele frequencies for each SNP were determined by gene counting. The genotype distribution of each SNP was tested for departure from the Hardy-Weinberg equilibrium. The Hardy-Weinberg equilibrium is a principle stating that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors. In the χ^2 analysis the observed genotype frequencies are tested against the expected genotype based on the Hardy-Weinberg principle. A p-value <0.05 indicates deviation from theHardy-Weinberg equilibrium.

Data were analyzed using the Statistical Package for the Social Sciences, version 16.0 (SPSS, Chicago, Ill., USA). Binary logistic regression models were developed in order to model the

relation between different covariates and the occurrence of an infectious complication. Herein, age, gender, injury severity score, and trauma mechanism (*i.e.*, blunt or penetrating injury) were added as covariates. A p-value of <0.050 was considered to be statistically significant.

RESULTS

Patient Demographics

During the study period, 219 patients were included. Of all patients 177 (77.6%) were male, the median age was 44 years (P_{25}-P_{75} 27-56) and the median ISS score was 25 (P_{25}-P_{75} 18-29). A total of 13 patients (5.9%) sustained penetrating trauma and 159 (72.6%) were admitted to the ICU with a median stay of 3 (P_{25}-P_{75} 0-14) days. Trauma mechanisms are shown in table 1.

Genotype Frequencies

HRMA showed that 131 (59.8%) of all patients were wildtype for the *MBL2* exon 1 polymorphism, 72 (32.9%) patients carried the heterozygote A0 genotype, and 16 (7.3%) patients the homozygote 00 genotype. Of the A0 patients, 34 (15.5%) had an AB genotype, 13 (5.9%) AC, and 25 (11.4%) AD. The 00 group consisted of five BB, three BC, three BD, four CD, and one DD genotype. Seventy-four patients (33.8%) were heterozygous for the *MBL2* YX SNP, all other patients had the common YY genotype. For the *MASP2* D120G SNP 207 (94.5%) patients had the common DD genotype; the remaining 5.5% percent were DG. For the *MASP2* Y371D SNP 145 (66.2%) were YY, 60 (27.4%) YD, and 14 (6.4%) DD. For the *FCN2* T236M SNP 106 (48.4%) had a TT, 97 (44.3%) a TM and 16 (7.3%) a MM genotype. For the *FCN2* A258S SNP 168 (76.7%) had an AA, 47 (21.5%) and AS, and 4 (1.8%) a SS genotype. Linkage disequilibrium was noted for the *MBL2* YX (χ^2=9.05; P=0.003) and *MASP* Y371D (χ^2=4.72; P=0.030); the genotype distribution of the other SNPs was in agreement with the Hardy-Weinberg equilibrium (χ^2=0.11-1.86; P=0.173-0.736).

Effect of Genotype on Infectious Complications

Table 2 shows an overview of infectious complications encountered separated by genotype. Overall, Gram-positive bacteria were detected in 79 patients (36%), Gram-negative bacteria in 84 (38%) and fungi in 46 (21%). Approximately half of the patients, 112 (51.1%), developed a positive culture; this was mostly in sputum in70 patients, but positive cultures were also found in wounds in 36 patients, blood (N=32), and urine (N=32). A total of 139 patients (63%) developed SIRS within 24 hours, of which 79 (57%) developed sepsis and 37 (27%) septic shock. Thirteen (5.9%) patients died.

Table 3 shows the results of the multivariable logistic regression models. Patients with the *MBL2* exon 1 heterozygous A0 variant were at significantly increased risk for developing positive wound cultures (Odds Ratio, OR, 2.51; 95% CI 1.12-5.62; p=0.025). Twenty-five percent of A0 patients developed a positive wound culture versus 11.5% of patients with the common AA genotype (Table 2). The risk of Gram-positive or Gram-negative organisms was unaltered by the *MBL2* exon 1 genotype. Patients with an A0 or 00 genotype had a consistently higher rate of SIRS, sepsis, and septic shock than patients with an AA genotype (Table 2); however, no statistically significant associations between *MBL2* exon 1 genotype and these endpoints were found in the multivariable analysis.

The presence of a *MBL2* YX promoter SNP was a significant risk factor for a fungal culture (OR 2.32; 95% CI 1.08-4.96; p=0.030; Table 3); in 27.0% of YX patients a fungus was cultured (mainly *Candida albicans* from pulmonary aspirates) versus 17.9% of YY patients. No other significant associations were found for the YX genotype.

For the *MASP2* D120G genotype polymorphism we found no statistically significant differences for all endpoints although, strikingly, fungi, positive blood cultures and septic shock were only found in DD patients (22.2%, 15.5%, and 17.9%, respectively). Another

striking, yet non-significant, finding was that only 8.3% of DG patients developed sepsis versus 37.7% in DD patients (p=0.060).

Carrying a *MASP2* Y371D DD variant genotype was a risk factor for developing SIRS within 24 hours after hospital admission (OR 4.78; 95% CI 1.06-21.59; p=0.042); SIRS developed in 78.6% of homozygous DD patients versus 58.6% of patients with a common YY genotype. Patients with the *MASP2* Y371D DD variant were also at significantly increased risk for developing septic shock (OR 2.53; 95%CI 1.12-4.33; p=0.003; Table 3); 35.7% of patients with a DD genotype developed a septic shock versus 23.3% in the DG and 12.4% in the YY group (Table 2). The prevalence of Gram-positive culture findings in patients with a *MASP2* Y371D DD genotype was 50% (versus 33% in the YY group), however this was not statistically significantly different (OR 3.19; 95% CI 0.95-10.71; p=0.060).

No significant associations between the *FCN2* T236M genotype and infectious events were found. A noteworthy finding was that positive blood cultures developed in 16.5% of patients with a TM variant genotype and in 25.0% of patients with a variant MM genotype, versus only 11.3% of patients with the common TT genotype; both were not statistically significant in the multivariate model (p=0.560 and p=0.092, respectively).

Heterozygosity for the *FCN2* A258S SNP was associated with increased risk of positive wound cultures (OR 3.37; 95%CI 1.45-7.85; p=0.005); positive culture findings were seen in 29.8% of AS patients versus 12.5% in AA patients. In addition, a *FCN2* A258S AS genotype significantly increased the risk of developing septic shock (27.7% in heterozygous AS patients versus 14.3% of AA patients; OR 2.18; 95%CI 1.30-4.78; p=0.011). Strikingly, the prevalence of Gram-negative bacteria in *FCN2* A258S AS patients was 48.9% (versus 34.5% in the AA group), yet this did not reach statistical significance (OR 1.87; 95% CI 0.93-3.77; p=0.079).

DISCUSSION

In this study the association between single nucleotide polymorphisms (SNPs) in three important genes (*MBL2, MASP2* and *FCN2*) of the innate immune system and the susceptibility of severely injured patients to positive culture findings and infectious complications has been investigated. The *MBL2* exon 1 A0 genotype was associated with increased rates of positive wound cultures, whereas the YX genotype of the *MBL2* promotor region increased the rates of colonization with fungi. The *MASP2* Y371D DD genotype put the patient at increased risk for SIRS and septic shock, and the *FCN2* A258S AS genotype was a risk factor for positive wound cultures and septic shock.

Trauma is the leading cause of death among young adults and infection is one of the major causes of late deaths among trauma patients. Prevention of infection in trauma patients is therefore of great clinical importance. Finding the association between different SNPs and infection risk is a first step in creating a genetic risk profile for infection susceptibility. In the future this may lead to the development of preventive measures for patients who are found to be at risk. The development of infectious complications is multifactorial in origin but genetic variation in the innate immune system may be one such important factor

The genotype distributions of *MBL2* [21 26 27], *MASP2* D120G [14], *MASP2* Y371D [16], *FCN2* T236M [16], and *FCN2* A258S [16 28] were in agreement with published data on European patients. All five genotypes were in agreement with the Hardy-Weinberg equilibrium, indicating that the allele and genotype frequencies of all SNPs in the population remain in equilibrium from generation to generation.

The *MBL2* exon 1 A0 genotype was significantly associated with an increased risk of developing a positive wound culture. This has been demonstrated before in a murine model of burn wounds [29] but is a novel finding in human trauma patients. Also, heterozygosity in the *MBL2* YX promoter region conferred a risk for developing fungal colonization (mainly *C.*

albicans) with YX patients having a 10% higher frequency of fungi than patients with the common YY genotype. The *MBL2* exon 1 genotype seemed unrelated to Gram-positive or Gram-negative organisms as causative micro-organisms, while others found *MBL2* 0/0 to be associated with a higher incidence of Gram-positive bacteria [26]. Although the percentage of patients with a *MBL2* A0 or 00 genotype that developed SIRS, sepsis, or septic shock was higher than in wildtype AA patients, this difference was not statistically significant. In a Danish ICU cohort study, the MBL 00 and to a lesser extent MBL A0 genotypes were significantly associated with mortality after sepsis [26].

In the current study only twelve patients carried a *MASP2* D120G DG genotype, which prevalence is similar as published before [14]. In DG patients, a consistent trend was noted towards lower rates of infection, sepsis and septic shock as compared to DD patients, yet statistical power was too low to reach statistical significance. Lack of association with bacteraemia was also described for patients admitted to an ICU in Belgium [21]. In that study, however, mortality was increased in patients with a *MASP2* D120G DG or GG genotype. The *MASP2* D120G polymorphism has also been associated with pneumococcal disease and to increase the risk of infectious complications after liver transplantation [14 15 16]. As no relevant information is available, the importance of genetic variation in *MASP2* for injured patients yet has to be elucidated in a larger cohort.

In this study, the rare *MASP2* Y371D DD genotype increased the risk for developing SIRS and septic shock. in a univariate model in patients undergoing liver transplantation this association was not found [15]. No further data on trauma and intensive care patients are available for this polymorphism.

For the *FCN2* T236M SNP we found no statistically significant association with any of the endpoints. In previous studies *FCN2* T236M was found to predispose to bacterial

infection after liver transplantation [15], and to infection in patients on continuous ambulatory peritoneal dialysis [17], but no data on trauma or patients in the ICU are available.

The *FCN2* A258S AS genotype was significantly associated with positive wound cultures (13% to 25%) and with septic shock (6% to 25%) in our cohort. This SNP produces the lowest serum levels of ficolin-2 [30]. This SNP was found to be related to earlier onset of P. *aeruginosa* colonization in patients with cystic fibrosis [31] and to cytomegalovirus infection after liver transplantation [16], but largely the functional impact of this SNP is unknown [19].

The strength of this study is that we assessed the relevant SNPs in the lectin pathway as a whole. Some genotypes were found very infrequently. Although this study is among the largest studies assessing the contribution of genotype differences to infection susceptibility in trauma patients, the sample size is not large enough yet to answer all questions. For instance, it was not large enough to assess the roles of the genotypes studied for rare outcomes such as mortality. It also limits the evaluation of combinations of SNPs. Increasing the sample size will allow us to overcome these limitations. A final limitation of this study relates to the multitude of statistical tests applied in order to assess the relevance of several SNPs to multiple outcome measures. The authors acknowledge that not applying a multiple comparison correction may result in falsely accepting a spurious relation. However, a Bonferroni correction would be a too stringent correction and may result in falsely denying true effects. Presenting the 95% confidence intervals for all odds ratio's allows readers, who will mainly be interested in a specific gene or effect, to value the results.

In summary, the results of the current study clearly show that SNPs in *MBL2, MASP2* and *FCN2* influence the susceptibility to positive cultures and severity of infection in severely injured trauma patients. The development of infectious complications is multifactorial in origin but genetic variation in the innate immune system may be one such important factor. Finding the association between different SNPs and infection risk is a first step in creating a

genetic risk profile for infection susceptibility. In the future this may lead to the development of preventive measures for patients who are found to be at risk. Substitution therapy would be one way to overcome deficiencies caused by genetic variation. MBL substitution therapy phase I trials have already shown interesting results [32-36].

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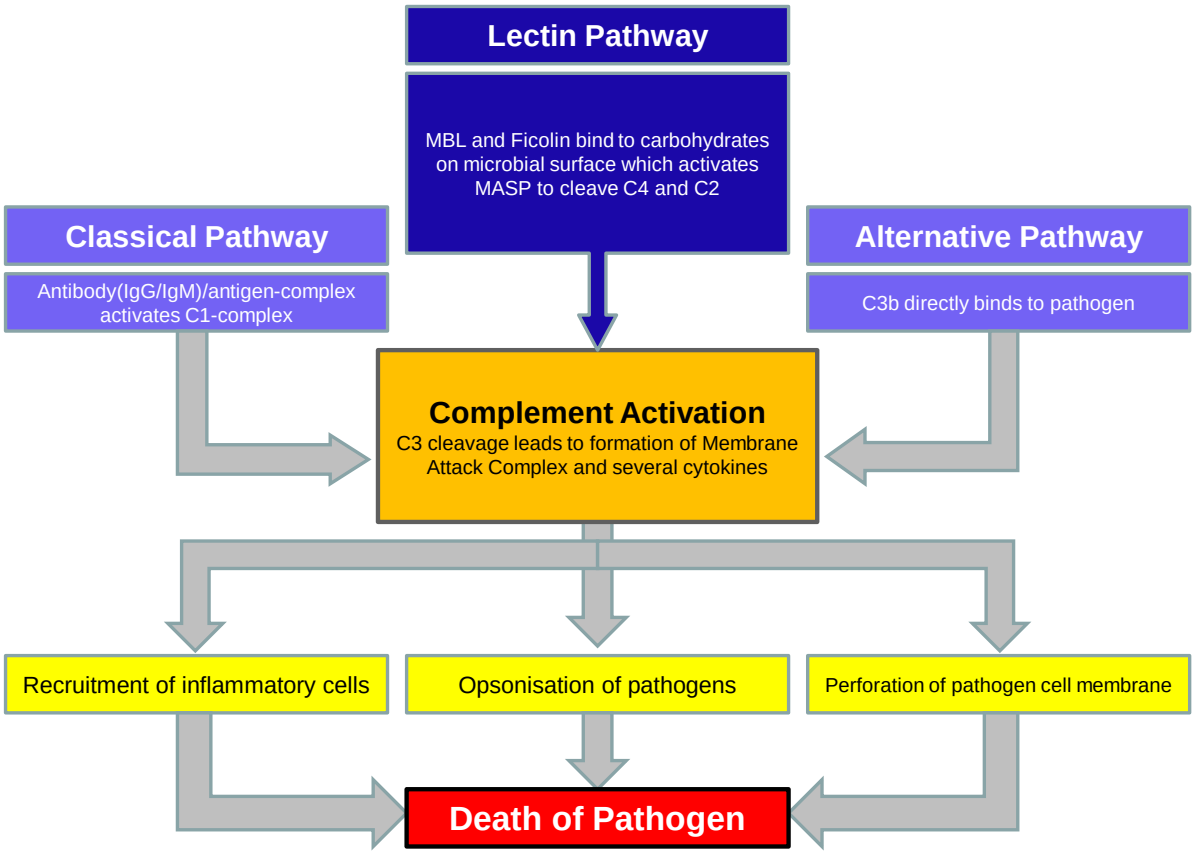
Figure 1. Schematic illustration of the Lectin pathway of complement activation



Genetic variation in *MBL2*, *MASP2*, and *FCN2* results in protein deficiency or diminished protein activity. This in turn reduces the ability to encounter pathogen attacks and increases the susceptibility to the onset and course of infections.

Supplementary **Table 1. Positions of SNPs and their corresponding nucleotide and amino acid transition**

Gene	SNP	dbSNP ID	Nucleotide transition (Accesion code)	Amino-Acid transition (Accesion code)	Location on gene / Domain
MBL2	D allele	rs5030737	5219C>T	Arg52Cys	Exon 1, collagenous region
	B allele	rs1800450	5226G>A	Gly54Asp	Exon 1, collagenous region
	C allele	rs1800451	5235G>A	Gly57Glu	Exon 1, collagenous region
	Y-221X	rs7096206	4776C>G	N.A.	Promoter, 5'untranslated regior
			(NG_008196.1)	(NP_000233.1)	
MASP2	D120G	rs72550870	5620A>G	Asp120Gly	Exon 3, CUB1
			(NG_007289.1)	(NP_006601.2)	
	Y371D	rs12711521	21370T>G	Tyr371Asp	Exon 9, CCP
			(NG_007289.1)	(NP_006601.2)	
FCN2	T236M	rs17549193	11369C>T	Thr236Met	Exon 8, fibrinogen-like domain
	A258S	rs7851696	11434G>T	Ala258Ser	Exon 8, fibrinogen-like domain
			(NG_011649.1)	(NP_004099.2)	

SNP, Single Nucleotide Polymorphism;

Amino acids: Ala (A), Alanine; Arg (R), Arginine; Asp (D), Asparctic acid; Cys (C), Cysteine; Gly (G), Glycine; Glu (E), Glutamic acid; Met (M), Methionine; Ser (S), Serine; Thr (T), Threonine; Tyr (Y), Tyrosine

Supplementary Table 2.

Oligonucleotide sequence for primers and probes used in *MBL2, MASP2* and *FCN2* genotyping

Name (Accession code)	SNP	Assay	Primer /probe	Start Position	Sequence
Calibrators	-	HRMA	Forward	-	5'- TTAAATTATAAAATATTTAT AATATTAATTATATA TATATAAATATAAT A-Amine-C6-3'
			Reverse	-	5'- TATTATATTTATATATATAT AATTAATATTATAAA TATTTTATAATTTAA-Amine-C6-3'
MBL2	B, C, D	HRMA	Forward	5163	5'-CTGCAGTGATTGCCTGTAGC-3'
(NG_008196.1)			Reverse	5246	5'-GCCCAACACGTACCTGGTTC-3'
			Probe	5207	5'-GGCAAAGATGGGCGTGATGGC ACCAAGGGA-Amine-C6-3'
		Seq	Forward	5033	5'-TTCCCTGAGTTTTCTCACA-3'
			Reverse	5430	5'-GGCTGGCAAGACAACTATT-3'
	YX	H	Forward	4755	5'-CCCATTTGTTCTCACTGCCA-3'
			Reverse	4777	5'-AAGACTATAAACATGCTTTC-3'
		Seq	Forward	4392	5'-CAGGGCCAACGTAGTAAG-3'
			Reverse	4886	5'-TTGTGACACTGCGTGACT-3'
MASP2	D120G	H	Forward	5600	5'-GGACATTACCTTCCGCTCCG-3'
(NG_007289.1)			Reverse	5621	5'-ACGGCTTCTCGTTGGAGTAG-3'
		Seq	Forward	5313	5'-ACCTCTGCGAGTACGACT-3'
			Reverse	5733	5'-TGCCTGGCCTAAGACA-3'
	Y371D	H	Forward	21350	5'-TTGACTGTGGCCCTCCTGAT-3'
			Reverse	21371	5'-CACTCGGCCACTGGGTAGAT-3'
		Seq	Forward	21171	5'-CTCGGCTTTTTAACCTTTC-3'
			Reverse	21695	5'-ACCATTTGGAATACATTGTGT-3'
FCN2	T236M	H	Forward	11349	5'-CTGCACAGGAGATTCCCTGA-3'
(NG_011649.1)			Reverse	11370	5'-AGGACTGGTTGTTGTGGAAC-3'
	A258S	H	Forward	11414	5'-ATCTTAACACCGGAAATTGT-3'
			Reverse	11435	5'-AGCTCCCTGAAACATCACAG-3'
	T236M, A258S	Seq	Forward	11186	5'-CAGCAGGGCAGTATTCAC-3'
			Reverse	11541	5'-TATCCTTTCCCCGACTTC-3'

HRMA, High-Resolution Melting Analysis.

Seq: Sequencing

Table 1. Demographics of trauma mechanisms

Traffic accidents	48%
Falls from height	32%
Crush injury to thorax	6%
Street fighting	3%
Gunshot wounds	2%
Stabbing	2%
Horseriding accidents	2%
Miscellaneous accidents	5%

Miscellaneous injuries include collapsing buildings, helicopter crash, ice skating, kitesurfing, fireworks.

Table 2: Demographic description of positive cultures and infectious complications by genotype

	Geno type	N (%)	Sputum Culture	Blood Culture	Urine Culture	Wound Culture	SIRS *	Sepsis *	Septic Shock *
Entire population		219	70 (32.0)	32 (14.6)	32 (14.6)	36 (16.4)	139 (63.5)	79 (36.4)	37 (16.9)
MBL2 Exon 1**	**AA**	131	44 (33.6)	17 (13.0)	21 (16.0)	15 (11.5)	81 (61.8)	44 (33.6)	19 (14.5)
	A0	72	21 (29.2)	15 (20.8)	11 (15.3)	18 (25.0)	49 (68.1)	31 (43.1)	15 (20.8)
	00	16	5 (31.2)	0 (0.0)	0 (0.0)	3 (18.8)	9 (56.2)	4 (25.0)	3 (18.8)
MBL2 YX	**YY**	145	47 (32.4)	21 (14.5)	22 (15.2)	26 (17.9)	88 (60.7)	54 (37.2)	27 (18.6)
	YX	74	23 (31.1)	11 (14.9)	10 (13.5)	10 (13.5)	51 (68.9)	25 (33.8)	10 (13.5)
	XX	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
MASP2 D120G	**DD**	207	68 (32.9)	32 (15.5)	30 (14.5)	35 (16.9)	132 (63.8)	78 (37.7)	37 (17.9)
	DG	12	2 (16.7)	0 (0.0)	2 (16.7)	1 (8.3)	7 (58.3)	1 (8.3)	0 (0.0)
	GG	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
MASP2 Y371D	**YY**	145	45 (31.0)	17 (11.7)	22 (15.2)	19 (13.1)	85 (58.6)	48 (33.1)	18 (12.4)
	YD	60	20 (33.3)	14 (23.3)	7 (11.7)	13 (21.7)	43 (71.7)	24 (40.0)	14 (23.3)
	DD	14	5 (35.7)	1 (7.1)	3 (21.4)	4 (28.6)	11 (78.6)	7 (50.0)	5 (35.7)
FCN T236M	**TT**	106	32 (30.2)	12 (11.3)	18 (17.0)	19 (17.9)	69 (65.1)	36 (34.0)	15 (14.2)
	TM	97	31 (32.0)	16 (16.5)	13 (13.4)	16 (16.5)	61 (62.9)	36 (37.1)	18 (18.6)
	MM	16	7 (43.8)	4 (25.0)	1 (6.3)	1 (6.3)	9 (56.2)	7 (73.8)	4 (25.0)
FCN2 A258S	**AA**	168	53 (31.5)	23 (13.7)	22 (13.1)	21 (12.5)	107 (63.7)	58 (34.5)	27 (14.3)
	AS	47	17 (36.2)	9 (19.1)	9 (19.1)	14 (29.8)	30 (63.8)	19 (40.4)	13 (27.7)
	SS	4	0 (0.0)	0 (0.0)	1 (25.0)	1 (25.0)	2 (50.0)	2 (50.0)	0 (0.0)

* SIRS, sepsis and septic shock were only determined for patients admitted to the Intensive Care Unit.

** The wildtype is referred to as A, while the variant genotype is referred to as 0

Table 3: Association between genotype and infections outcome in polytraumatized patients

Covariate		Sputum Culture	Blood Culture	Urine Culture	Wound Culture	SIRS	Sepsis	Septic Shock
MBL2 Exon 1	**A0**	0.73 (0.38-1.42)	1.54 (0.67-3.54)	0.99 (0.43-2.31)	**2.51* (1.12-5.62)**	1.24 (0.65-2.39)	1.28 (0.66-2.47)	1.34 (0.59-3.05)
	00	1.19 (0.35-4.10)	N.D.	N.D.	2.29 (0.49-10.81)	0.97 (0.30-3.14	0.78 (0.20-3.05)	1.71 (0.35-8.46)
MBL2 Promoter	**YX**	1.04 (0.54-1.99)	1.13 (0.46-2.76)	0.72 (0.31-1.66)	0.86 (0.36-2.05)	1.61 (0.85-3.08)	0.92 (0.47-1.80)	0.85 (0.35-2.07)
MASP D120G	**DG**	0.35 (0.07-1.79)	N.D.	1.04 (0.19-5.75)	0.45 (0.05-4.35)	1.03 (0.29-3.66)	0.14 (0.01-1.35)	N.D.
MASP Y371D	**YD**	0.95 (0.47-1.90)	2.03 (0.83-4.97)	0.87 (0.33-2.30)	1.62 (0.70-3.77)	1.71 (0.85-3.44)	1.14 (0.57-2.31)	1.73 (0.73-4.12)
	DD	1.90 (0.54-6.64)	0.93 (0.11-8.26)	1.13 (0.25-5.09)	3.23 (0.80-13.04)	**4.78 * (1.06-21.59)**	2.82 (0.82-9.69)	**2.53 *** (1.12-4.33)**
FCN2 T236M	**TM**	0.91 (0.48-1.73)	1.31 (0.53-3.21)	0.87 (0.38-2.00)	0.85 (0.37-1.93)	0.81 (0.43-1.52)	1.12 (0.58-2.16)	1.05 (0.45-2.46)
	MM	1.71 (0.54-5.42)	3.49 (0.82-14.92)	0.32 (0.04-2.82)	0.44 (0.05-3.84)	0.67 (0.22-2.07)	1.80 (0.54-2.95)	3.09 (0.76-12.56)
FCN2 A258S	**AS**	1.34 (0.65-2.76)	1.70 (0.64-4.50)	1.23 (0.50-3.01)	**3.37*** (1.45-7.85)**	1.07 (0.51-2.22)	1.41 (0.67-2.99)	**2.18 * (1.30-4.78)**
	SS	N.D.	N.D.	1.36 (0.10-19.10)	1.65 (0.13-21.69)	0.31 (0.04-2.75)	0.71 (0.07-7.39)	N.D.

Multivariable logistic regression models were made for all individual outcome measure (*i.e.*, positive culture or infectious complication) as dependent variable. Age, gender, trauma mechanism, ISS, and individual SNPs were entered as covariate. Data are shown as odds ratio, with the 95% CI between brackets. For all SNPs the wildtype genotype was used as reference category. N.D., not determined due to insufficient numbers.

Statistically significant data are indicated in boldface. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.001$.