Lectin pathway of complement activation and relation with clinical complications in critically ill children

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BACKGROUND: Critically ill children are susceptible to nosocomial infections, which contribute to adverse outcomes. Deficiencies in the innate immunity lectin pathway of complement activation are implicated in a child's vulnerability to infections in conditions such as cancer, but the role during critical illness remains unclear. We hypothesized that low on-admission levels of the pathway proteins are, in part, genetically determined and associated with susceptibility to infectious complications and adverse outcomes.

METHODS: We studied protein levels of mannose-binding lectin (MBL), H-ficolin and M-ficolin, three MBL-associated-serine proteases (MASPs) and MBL-associated protein (MAP44), and relation with functional genetic polymorphisms, in 130 healthy children and upon intensive care unit (ICU) admission in 700 critically ill children of a randomized study on glycemic control.

RESULTS: Levels of MASP-1, MASP-2, MASP-3, and MAP-44 were lower and the levels of M-ficolin were higher in ICU patients on admission than those in matched healthy controls. Only a low on-admission MASP-3 level was independently associated with risk of new ICU infections and prolonged ICU stay, after correcting for other risk factors. On-admission MASP-3 varied with age, illness severity, and genetic variation.

CONCLUSION: Low on-admission MASP-3 levels in critically ill children were independently associated with subsequent acquisition of infection and prolonged ICU stay. The biological explanation needs further investigation.

Children are admitted to the intensive care unit (ICU) most often after major or life-threatening surgery, extensive trauma, or serious infection. Clinically diagnosed nosocomial infection is a substantial problem occurring in ~30% of these patients (1). Systemic inflammation, evoked by insults such as infection, trauma, surgery, and ischemia–reperfusion injury, is another serious complication associated with high morbidity (2). The question why some patients are more susceptible to developing these complications than others is intriguing and raises the possibility of genetic predisposition. Certain genetic

variants of innate immunity genes would thus orchestrate different responses to apparently similar insults (3,4).

The innate immune system represents the immediate response toward injury, before amplification of the adaptive immune system. Part of innate immunity is started when membrane-bound, soluble, or intracellular receptor proteins recognize highly conserved pathogen- or danger-associated molecular patterns (5). Mannose-binding lectin (MBL), H-ficolin, and M-ficolin are examples of soluble pattern recognition molecules, able to bind certain patterns of chemical structures on microorganisms or altered cells in the body. These molecules are found in complexes with MBL-associated serine proteases (MASP-1, MASP-2, or MASP-3) and with MBL-associated proteins (e.g., MAP-44). Binding of MBL/MASP or ficolin/MASP complexes to a fitting pattern activates and regulates inflammation through initiation of the lectin pathway of the complement system (6,7).

Both insufficient and excessive activation of the lectin pathway may be detrimental. Whereas deficiency of some of these components may be clinically irrelevant in healthy individuals due to redundancy of the immune system, an increased susceptibility to secondary infections or complications was shown, e.g., deficiency in MBL or ficolins, in children with cancer or neonates with necrotizing enterocolitis (NEC) (8–10). In contrast, increased levels of innate immunity proteins, possibly in part explained by polymorphisms in the corresponding genes, may contribute to exaggerated inflammatory responses as observed in acute respiratory distress syndrome (11,12).

Data on the lectin pathway of complement activation in critically ill children are scarce. We measured serum levels of different proteins of this pathway on admission in a large cohort of critically ill children ($n = 700$), compared with those in healthy children ($n = 130$), and assessed their association with baseline characteristics and clinical outcomes (new ICU infections, duration of ICU stay, and mortality). We hypothesized that baseline deficiencies in these proteins, i.e., on admission to the ICU, may be associated with more infectious complications and poor outcome. In addition, we investigated the impact of functional polymorphisms on circulating protein levels and outcomes of critical illness.

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RESULTS

Lectin Pathway Proteins in Serum From Critically Ill Children and Healthy Controls

In healthy children, MASP-1 and MASP-3 varied with age, with the highest MASP-1 and lowest MASP-3 levels in

Figure 1. Serum levels of the lectin pathway proteins according to age. (a) Healthy children. (b) Critically ill children. Protein levels were compared among children aged less than 1 y (white boxes, $n = 9$ and $n = 303$, respectively, for healthy and critically ill children), between 1 and 4 y (hatched white boxes, $n = 44$ and $n = 166$, respectively), between 4 and 8 y (grey boxes, $n = 52$ and $n = 89$, respectively), and older than 8 y (hatched grey boxes, $n = 25$ and $n = 113$, respectively). Data are expressed as box plots, where the central lines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers represent the 10th and 90th percentiles. $*0.05 < P < 0.1$; $**P < 0.001$; $†P < 0.0001$ in Kruskal–Wallis test. MASP, MBL-associated serine protease; MBL, mannose-binding lectin.

children younger than 1 y of age (Figure 1a). Due to the inherent difficulty of recruiting healthy children in this youngest group, there was an imbalance between the age of the critically ill (1.3 (0.3–5.0) y) and healthy children (4.6 (2.2–6.8) y; $P < 0.0001$). To avoid bias due to this age difference, we selected two matched cohorts of critically ill children and controls through propensity score matching (Table 1). In the matched population, on-admission levels of MASP-1, MASP-2, MASP-3, and MASP-44 in critically ill patients were significantly lower than the levels in healthy controls (Table 1). Conversely, M-ficolin levels were higher in critically ill patients. MBL and H-ficolin levels were comparable in both groups.

Lectin Pathway Proteins on ICU Admission in Relation to Baseline Characteristics

In critically ill children, on-admission levels of the proteins increased with age, except for an inverse relation for MASP-1 and MASP-44 (Figure 1b and Table 2). A history of malignancy was associated with higher levels of MASP-2 (0.36 (0.17–0.55) vs. 0.17 (0.12–0.24) $\mu\text{g/ml}$; $P < 0.0001$) and H-ficolin (26.29 (21.75–36.15) vs. 21.09 (17.05–26.40) $\mu\text{g/ml}$; $P = 0.0001$). Patients with diabetes mellitus had lower MBL levels (0.26 (0.02–0.32) $\mu\text{g/ml}$) than those without diabetes (1.10 (0.43–1.94) $\mu\text{g/ml}$; $P = 0.003$). Cardiac patients had lower levels of MASP-2 (0.15 (0.11–0.21) vs. 0.24 (0.16–0.41) $\mu\text{g/ml}$; $P < 0.0001$) and H-ficolin (20.66 (16.60–24.98) vs. 25.70 (19.59–34.85) $\mu\text{g/ml}$; $P < 0.0001$) than the other patients. Admission levels of all proteins correlated inversely with severity of illness on ICU admission, as reflected in the Pediatric Risk of Mortality (PRISM) score (Table 2) (13,14).

Table 1. Comparison between propensity score–matched patients and healthy controls

	Patients ($n = 81$)	Healthy controls ($n = 81$)	P value
Demographics			
Median age (IQR), y	3.3 (1.6–4.7)	3.6 (1.6–5.3)	0.74
Male gender, n (%)	53 (65.4)	46 (56.8)	0.26
Mean BMI (95% CI), kg/m^2	15.8 (15.3–16.3)	16.2 (15.7–16.6)	0.21
Median protein levels (IQR), $\mu\text{g/ml}$			
MBL	1.35 (0.42–2.19)	1.62 (0.58–2.16)	0.53
H-ficolin	22.03 (19.19–28.54)	24.00 (20.04–26.84)	0.41
M-ficolin	0.91 (0.64–1.39)	0.55 (0.40–0.72)	<0.0001
MASP-1	6.46 (4.79–8.06)	11.23 (7.89–13.93)	<0.0001
MASP-2	0.19 (0.13–0.27)	0.26 (0.19–0.37)	0.001
MASP-3	3.73 (3.05–4.70)	6.86 (5.80–7.71)	<0.0001
MASP-44	1.48 (1.16–1.80)	1.92 (1.72–2.22)	<0.0001

CI, confidence interval; IQR, interquartile range; MASP, MBL-associated serine protease; MBL, mannose-binding lectin.

Table 2. Association of on-admission levels of the lectin pathway proteins with parameters of disease severity

Proteins (μg/ml)	Rho (P)	
	Age (y)	PRISM score ^a
MBL	0.102 (0.008)	−0.090 (0.02)
H-ficolin	0.138 (0.0003)	−0.283 (<0.0001)
M-ficolin	0.260 (<0.0001)	−0.096 (0.01)
MASP-1	−0.344 (<0.0001)	−0.146 (0.0001)
MASP-2	0.226 (<0.0001)	−0.249 (<0.0001)
MASP-3	0.344 (<0.0001)	−0.251 (<0.0001)
MAp-44	−0.164 (<0.0001)	−0.144 (0.0002)

MAp, MBL-associated protein; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; PRISM, Pediatric Risk of Mortality.

^aModified PRISM score: PRISM score (13) calculated with only the admission value of blood glucose, which is unaffected by the study intervention. Similar associations were observed with the Risk Adjustment in Congenital Heart Surgery (RACHS) score for patients undergoing cardiac surgery for congenital heart disease, with a higher score indicating a higher risk of death (14).

Association of the Lectin Pathway Proteins on ICU Admission With Outcome

In a univariate analysis, patients who acquired an infection at any time in ICU had lower on-admission MASP-3 levels than those who did not acquire an infection ($P < 0.0001$; **Figure 2a**). The other proteins were not associated with infection (data not shown). After correcting for baseline characteristics, risk factors, and the other lectin pathway proteins in multivariate logistic regression analysis, low MASP-3 levels remained independently associated with acquisition of infection (**Table 3**). Indeed, the corresponding odds ratio and 95% confidence interval of 0.809 (0.675–0.959) per μg/ml MASP-3 added ($P = 0.02$) illustrate a lower risk of infection for a higher on-admission MASP-3 level. Performance of on-admission MASP-3 levels in the prediction of infection (area under the curve of 0.64) was somewhat better than that of C-reactive protein (CRP; area under the curve of 0.58; **Figure 3**). MASP-3 and CRP levels did not correlate (data not shown).

In a univariate analysis, lower on-admission MASP-3 levels were observed in patients needing a prolonged ICU stay (at least 3 d) than in patients with a shorter ICU stay ($P < 0.0001$; **Figure 2b**). A similar relation was found between lower levels of on-admission MASP-2 (0.16 (0.11–0.24) vs. 0.18 (0.13–0.27) μg/ml; $P = 0.002$) or H-ficolin (20.73 (16.60–25.75) vs. 22.08 (18.20–28.77) μg/ml; $P = 0.002$) and prolonged ICU stay. There was no association between the levels of the other proteins and ICU stay. In multivariate Cox proportional hazard analysis, correcting for baseline risk factors and the other lectin pathway proteins, a low on-admission MASP-3 (but not MASP-2 or H-ficolin) level remained an independent risk factor for prolonged time to ICU discharge (**Table 4**). The hazard ratio and 95% confidence interval were 1.088 and 1.020–1.157, respectively ($P = 0.01$), indicating a higher likelihood of earlier alive ICU discharge at any time with a higher on-admission MASP-3 level. The predictive value of MASP-3 for the likelihood of early alive discharge was maintained when acquisition

Figure 2. Univariate analysis for MASP-3 levels in relation to risk of new infection and prolonged need of intensive care. (a) Risk of new infection. Of the patients with an on-admission serum sample, 218 acquired a new infection (downward hatched box), whereas 453 did not (upward hatched box). (b) Prolonged need of intensive care. Of the patients with an on-admission serum sample, 375 patients needed intensive care for at least 3 d (downward hatched box), whereas 296 had a shorter ICU stay (upward hatched box). Data are expressed as box plots, where the central lines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers indicate the 10th and 90th percentiles. * $P < 0.0001$. ICU, intensive care unit; MASP, mannose-binding lectin–associated serine protease.

Table 3. Multivariate regression analysis for new infection

	Odds ratio (95% CI) ^a	P value
Age (per year added)	0.896 (0.846–0.946)	0.0001
Gender (male)	1.162 (0.786–1.720)	0.45
BMI (per kg/m ² added)	0.990 (0.951–1.026)	0.55
Randomized intervention (IIT)	0.637 (0.433–0.934)	0.02
Cardiac surgery	0.225 (0.126–0.397)	<0.0001
Malignancy	0.212 (0.066–0.608)	0.006
Modified PRISM score (per unit added)	1.154 (1.110–1.203)	<0.0001
CRP (per mg/l added)	1.009 (1.004–1.015)	0.0005
MBL (per μg/ml added)	1.133 (0.951–1.352)	0.16
H-ficolin (per μg/ml added)	1.006 (0.977–1.037)	0.68
M-ficolin (per μg/ml added)	1.095 (0.969–1.388)	0.36
MASP-1 (per μg/ml added)	0.978 (0.910–1.049)	0.54
MASP-2 (per μg/ml added)	1.006 (0.187–5.359)	0.99
MASP-3 (per μg/ml added)	0.809 (0.675–0.959)	0.02
MAp-44 (per μg/ml added)	1.258 (0.962–1.663)	0.10

CI, confidence interval; CRP, C-reactive protein; ICU, intensive care unit; IIT, intensive insulin therapy; MAp, MBL-associated protein; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; PRISM, Pediatric Risk of Mortality.

^aAn odds ratio >1 indicates an increased risk of infection. Hence, patients with a higher level of MASP-3 on ICU admission have a lower risk of acquiring a new infection in ICU.

of a new infection was added to the model (hazard ratio: 1.080 (1.009–1.152); $P = 0.03$). The corresponding hazard ratio for infection was 0.238 (0.191–0.296; $P < 0.0001$). There was no association between the levels of any of the proteins measured on ICU admission and ICU mortality (data not shown).

Association of Genetic Polymorphisms in the MASP1 Gene With Protein Levels and Outcomes of Critical Illness

Because only MASP-3 levels were related to outcomes, we studied only single-nucleotide polymorphisms (SNPs) in

Figure 3. Receiver operating characteristic (ROC) curves for prediction of infection with on-admission MASP-3 and CRP levels. **(a)** ROC curve for MASP-3: AUC = 0.64. **(b)** ROC curve for CRP: AUC = 0.58. Black lines show the ROC curves. The point of intersection between the black and the grey lines shows the cutoffs for on-admission MASP-3 and CRP levels, respectively, with the best combined specificity and sensitivity for prediction of infection. AUC, area under the curve; CRP, C-reactive protein; MASP, mannose-binding lectin–associated serine protease.

the *MASP1* gene, encoding MASP-1, MASP-3, and MAP-44. For all SNPs, allele frequencies showed a similar distribution in patients and controls (Table 5) and were consistent with Hardy–Weinberg equilibrium. However, for five SNPs, more than 5% of the samples could not be attributed to a genotype. Of the remaining SNPs, 18 were monomorphic or near monomorphic, with less than 1% attributed to a genotype different from the ancestral allele homozygosity. These 23 SNPs were not further considered.

In univariate analysis, only rs710469 (C>T, intron SNP) and rs850312 (G>A, synonymous SNP in coding region) were associated with MASP-3 levels. More specifically, rs710469 variant allele (T) homozygosity and presence of a variant allele (A) in rs850312 were associated with higher MASP-3

Table 4. Cox proportional hazard analysis for likelihood of early alive discharge from ICU

	Hazard ratio (95% CI) ^a	P value
Age (per year added)	1.039 (1.017–1.061)	0.0005
Gender (male)	0.939 (0.799–1.104)	0.45
BMI (per kg/m ² added)	0.999 (0.973–1.018)	0.90
Randomized intervention (IIT)	1.201 (1.022–1.412)	0.03
Cardiac surgery	1.917 (1.498–2.473)	<0.0001
Malignancy	1.007 (0.644–1.512)	0.97
Modified PRISM score (per unit added)	0.915 (0.899–0.932)	<0.0001
CRP (per mg/l added)	0.999 (0.997–1.001)	0.29
MBL (per µg/ml added)	1.037 (0.960–1.118)	0.36
H-ficolin (per µg/ml added)	1.001 (0.990–1.012)	0.82
M-ficolin (per µg/ml added)	1.010 (0.950–1.049)	0.71
MAp-44 (per µg/ml added)	0.994 (0.965–1.023)	0.70
MAp-44 (per µg/ml added)	0.992 (0.498–1.890)	0.98
MAp-44 (per µg/ml added)	1.088 (1.020–1.157)	0.01
MAp-44 (per µg/ml added)	0.937 (0.822–1.059)	0.30

CI, confidence interval; CRP, C-reactive protein; ICU, intensive care unit; IIT, intensive insulin therapy; MAp, MBL-associated protein; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; PRISM, Pediatric Risk of Mortality.

^aA hazard ratio >1 indicates a higher likelihood of earlier alive discharge from the ICU at any time. Hence, higher on-admission MASP-3 levels decrease the risk of an extended stay in the ICU.

levels (Figure 4a,b). Taking into account the relationship between higher MASP-3 and better outcome, we considered the rs710469 TT and rs850312 AA or AG genotypes as “protective.” Combining the two SNPs as one endpoint, patients having maximally one protective genotype had lower MASP-3 levels than patients having both protective genotypes (Figure 4c). Similarly, patients without any protective SNP had lower MASP-3 levels than patients having at least one protective SNP (Figure 4d). In multiple linear regression analysis using age, PRISM score, and genotype, having an ancestral allele in rs710469 (CC or CT) or homozygosity in the ancestral allele for rs850312 (GG)—i.e., having maximally one protective SNP—determined low MASP-3 levels, independent of—and without any significant interaction with—younger age and higher severity of illness (Table 6). Similar results were obtained for patients having no protective SNP (data not shown). rs710469 and rs850312 were not associated with the acquisition of infection or time in ICU in univariate or multivariate analysis with or without the MASP protein levels present in the model.

DISCUSSION

Critically ill children with varying underlying pathology show altered serum profiles of several lectin pathway proteins, with low MASP and high M-ficolin levels, on ICU admission compared with healthy children. Low MASP-3 levels, in particular, were robustly associated with a higher risk of acquiring a new infection in the ICU and prolonged need for intensive care. Age, severity of illness, and genetic variation in the *MASP1* gene independently explained the variation in MASP-3 levels

Table 5. Distribution of the SNPs in critically ill and healthy children

SNPs in MASP-1/ MASP-3	Genotype	Critically ill children, n (%)	Healthy children, n (%)	P value
rs3105782 T>C	CC	42 (6.9)	12 (9.4)	0.36
	CT	232 (38.0)	54 (42.2)	
	TT	322 (52.8)	60 (46.9)	
rs113938200 C>T ^a	CC	595 (97.5)	127 (99.2)	0.95
	CT	5 (0.8)	1 (0.8)	
rs710469 C>T	CC	169 (27.7)	34 (26.6)	0.86
	CT	294 (48.2)	66 (51.6)	
	TT	134 (22.0)	27 (21.1)	
rs874603 A>G	AA	502 (82.3)	106 (82.8)	0.96
	AG	91 (14.9)	20 (15.6)	
	GG	6 (1.0)	1 (0.8)	
rs710456 T>C ^a	CT	2 (0.3)	0 (0.0)	0.51
	TT	598 (98.0)	128 (100)	
rs3774268 G>A	AA	12 (2.0)	3 (2.3)	0.90
	AG	148 (24.3)	30 (23.4)	
	GG	419 (68.7)	93 (72.7)	
rs6799834 C>T ^{a,b}	CC	562 (92.1)	116 (90.6)	0.99
rs16861801 G>C ^{a,b}	CG	10 (1.6)	0 (0.0)	0.13
	GG	518 (84.9)	121 (94.5)	
rs35384947 G>A ^a	AG	1 (0.2)	0 (0.0)	0.64
	GG	595 (97.5)	127 (99.2)	
rs28945068 G>A	AG	14 (2.3)	5 (3.9)	0.30
	GG	591 (96.9)	123 (96.1)	
rs28945071 A>G ^a	AA	597 (97.9)	127 (99.2)	0.30
	AG	5 (0.8)	0 (0.0)	
rs28945069 C>T	CC	602 (98.7)	126 (98.4)	0.26
	CT	6 (1.0)	0 (0.0)	
rs115022399 C>A ^a	CC	601 (98.5)	127 (99.2)	0.99
rs850312 G>A	AA	56 (9.2)	17 (13.3)	0.37
	AG	276 (45.2)	54 (42.2)	
	GG	259 (42.5)	54 (42.2)	
rs115961221 G>A	AG	31 (5.1)	5 (3.9)	0.58
	GG	566 (92.8)	120 (93.8)	
rs7652842 A>G	AA	594 (97.4)	121 (94.5)	0.23
	AG	13 (2.1)	5 (3.9)	
rs111908734 G>T ^a	GG	603 (98.9)	127 (99.2)	0.99
rs116384821 C>A ^a	CC	599 (98.2)	127 (99.2)	0.99
rs72549155 C>G ^a	CC	605 (99.2)	127 (99.2)	0.99
rs73068950 C>T ^a	CC	602 (98.7)	126 (98.4)	0.43
	CT	3 (0.5)	0 (0.0)	
rs114365879 C>T ^a	CC	603 (98.9)	127 (99.2)	0.52
	CT	2 (0.3)	0 (0.0)	

Table 5. Continued

SNPs in MASP-1/ MASP-3	Genotype	Critically ill children, n (%)	Healthy children, n (%)	P value
rs116763673 C>T ^a	CC	603 (98.9)	127 (99.2)	0.64
	CT	1 (0.2)	0 (0.0)	
rs116595107 C>T ^{a,b}	CC	526 (86.2)	118 (92.2)	0.92
	CT	5 (0.8)	1 (0.8)	
rs73886128 C>T ^a	CC	603 (98.9)	126 (98.4)	0.43
	CT	3 (0.5)	0 (0.0)	
rs116001173 C>T ^a	CC	600 (98.4)	127 (99.2)	0.65
	CT	1 (0.2)	0 (0.0)	
rs115241263 C>T ^a	CC	593 (97.2)	126 (98.4)	0.99
rs78137557 A>G ^a	AA	592 (97.0)	126 (98.4)	0.99
rs72549252 T>C ^a	TT	606 (99.3)	127 (99.2)	0.99
rs115647447 G>A ^a	GG	602 (98.7)	127 (99.2)	0.99
	CT	4 (0.7)	0 (0.0)	
rs74784100 C>T ^a	CC	604 (99.0)	127 (99.2)	0.99
	CT	7 (1.1)	4 (3.1)	
rs77189011 C>T ^b	CC	523 (85.7)	110 (85.9)	0.10
	CT	7 (1.1)	4 (3.1)	
rs1062049 C>T ^{a,b}	CC	589 (96.6)	119 (93.0)	0.37
	CT	4 (0.7)	0 (0.0)	

MASP, mannose-binding lectin–associated serine protease; SNP, single-nucleotide polymorphism.

No blood cell sample was available for DNA extraction from 2 healthy children and 90 critically ill patients. For three other patients, the DNA yield was too low for use on the Open Array platform. The percentages of the genotypes are thus calculated relative to 610 critically ill and 128 healthy children. Other missing SNPs could not be reliably attributed to a specific genotype.

^aSNPs not further considered for reason of being monomorphic or near monomorphic, with less than 1% attributed to a genotype different from homozygosity for the ancestral allele. ^bSNPs not further considered because > 5% of the samples could not be attributed to a certain genotype.

on ICU admission. However, genetic variation did not by itself contribute to the risk of infection and prolonged critical illness.

Critically ill children showed lower levels of MASP-1, MASP-2, MASP-3, and MASP-44 and higher M-ficolin levels on ICU admission than age-matched healthy children, whereas levels of MBL and H-ficolin were comparable. Thus, critical illness has an acute effect on most of these proteins. Matching for age appeared crucial because of the important changes with age reported for most of these proteins (10,15–18) and largely confirmed in our cohorts of healthy and critically ill children.

We observed higher levels of MASP-2 and H-ficolin in critically ill patients with a history of malignancy than in those without. Elevated levels of MASP-2 and MBL, but not of M-ficolin, have been reported for children with cancer (19,20). Low levels of MASP-2 and H-ficolin have been associated with fever, neutropenia, bacteremia, prolonged hospitalization, and intravenous antimicrobial therapy (9,21). Data on the lectin pathway in pediatric critical illness are scarce and available only for small cohorts of neonates. Low MBL and high M-ficolin levels in umbilical cord blood have been associated

Figure 4. Impact of genetic variation on the on-admission MASP-3 levels. Data are expressed as box plots, where the central lines indicate the medians, the boxes indicate the interquartile ranges, and the whiskers indicate the 10th and 90th percentiles. (a) rs710469 protective single-nucleotide polymorphism (SNP) ($n = 131$) vs. no protective SNP ($n = 446$), (b) rs850312 protective SNP ($n = 246$) vs. no protective SNP ($n = 324$), (c) rs710469/rs850312 maximally one protective SNP ($n = 459$) vs. both protective SNPs ($n = 101$), and (d) rs710469/rs850312 no protective SNP ($n = 219$) vs. at least one protective SNP ($n = 341$). * $P = 0.0003$; ** $P = 0.004$; † $P = 0.001$. MASP, mannose-binding lectin–associated serine protease.

Table 6. Multiple linear regression analysis for factors determining MASP-3 levels

	β coefficient (95% CI)	Standardized β	P value
Age (/y)	0.053 (0.017–0.090)	0.153	0.004
PRISM score ^a (/unit)	–0.070 (–0.099 to –0.042)	–0.227	<0.0001
Low-MASP-3 genotype ^b	–0.249 (–0.411 to –0.087)	–0.119	0.003
Interactions			
Age $\times$ low-MASP-3 genotype	0.032 (–0.004 to 0.069)	0.094	0.08
PRISM score $\times$ low-MASP-3 genotype	0.000 (–0.028 to 0.029)	0.000	0.99

CI, confidence interval; MASP, mannose-binding lectin–associated serine protease; PRISM, Pediatric Risk of Mortality.

^aModified PRISM score: PRISM score calculated with only the admission value of blood glucose, which is unaffected by the study intervention. ^brs710469 CC or CT genotype or rs850312 GG genotype.

with Gram-negative and early-onset sepsis, and low H-ficolin levels have been associated with susceptibility to Gram-positive and late-onset sepsis (22,23). Low MBL levels in blood from neonates were related to an increased risk of neonatal sepsis and pneumonia (24,25). Cord blood MASP-2 levels tended to be higher for babies with infections (26) and were higher for neonates who developed NEC (17). Risk of death and need for

mechanical ventilation for neonates with NEC were higher when M-ficolin levels were low (10). Major surgery was shown to lead to an initial decrease in MASP-1, MASP-3, and MAP-44 levels, followed by gradual recovery (15,16).

In our cohort of critically ill children, low on-admission MASP-3 levels were robustly associated with an increased risk of acquiring a new infection, even after correcting for baseline characteristics, risk factors, and the other lectin pathway proteins. The likelihood for an earlier alive discharge from ICU was also much lower for patients with low on-admission levels of MASP-3 in multivariate analysis. The latter association was only partially attributable to the higher infection rate in these patients because the effect of MASP-3 was somewhat attenuated but remained significant when acquisition of a new infection was added to the statistical model. The biological activity of MASP-3 remains incompletely understood. Recombinant MASP-3 has some activity on insulin-like growth factor-binding protein-5 and some synthetic substrates (27). A critical role of MASP-3 in insulin-like growth factor-binding protein-5 availability during craniofacial, muscle, and neural crest development was suggested based on specific mutations (28–30). In mice, MASP-1 and MASP-3 play a role in the alternative pathway of complement activation and thus might act as a backup system when the lectin pathway is deficient (31). However, the alternative complement activation pathway functioned normally in a patient deficient in MASP-1 and MASP-3 due to a nonsense mutation in the common part of the *MASP1* gene (32). Our findings suggest that MASP-3 plays an important role in the host defense against infections in the ICU and may also be of much broader importance as low MASP-3 was associated with prolonged need for intensive care irrespective of infection.

We observed an inverse correlation between on-admission MASP-3 levels and severity of illness, suggesting a more severe acute depression of MASP-3 for sicker patients. Interest in the functional consequences of genetic variation in determining susceptibility and response to disease is increasing, also in critical illness (3,4,33,34). Our study of several SNPs spread throughout the *MASP1* gene encoding MASP-3 (7,35) identified two SNPs for which the variant allele was associated with higher on-admission MASP-3 levels. These “protective” genotypes were equally distributed among patients and controls; thus, not having a protective genotype did not predispose to critical illness. Carrying one protective SNP variant appeared sufficient for having higher MASP-3 levels because having both the protective SNPs was not more protective than having only one. The variation in MASP-3 levels was only partially explained by this genetic variation because age and illness severity were additional independent determinants of these protein levels, without interaction with genetic variation. The actual protein level of MASP-3 appeared more important for outcome than genetic variation in the *MASP1* gene. Indeed, whereas low on-admission protein levels were clearly associated with risk of infection and prolonged ICU stay, carrying a protective

SNP variant obviously was not sufficient to compensate for the impact of the acute insult of critical illness that reduces MASP-3 levels and was not associated with risk of infection or ICU stay.

The major strength of our unique study is that it evaluated a wide array of lectin pathway proteins in large cohorts of healthy children and critically ill children with varying underlying pathology and of all ages, in addition to combining this with genetic analyses. However, a first limitation of our study is that although we found robust associations between on-admission MASP-3 levels and clinical outcomes, our study does not provide hard evidence for causal involvement. This would require artificial interference with MASP-3 levels in a randomized study. A second limitation relates to the number of SNPs analyzed. The selected SNPs covered exons, introns, and the 5'- and 3'-untranslated regions of the *MASPI* gene. Nevertheless, we did not study all genetic variations in the *MASPI* gene, and hence, we cannot exclude the importance

of any other SNPs for the outcome of critically ill children. Moreover, several SNPs did not show any, or showed insufficient, variation in our cohorts, hampering the study of any relation with outcome.

In conclusion, in a large unselected heterogeneous cohort of critically ill children, we observed remarkable disturbances in several proteins of the lectin pathway of complement activation on ICU admission. We identified a low circulating MASP-3 protein level as an independent predictor of subsequent acquisition of infection and prolonged dependency on intensive care. The impact of critical illness appeared to overwhelm any impact of protective SNPs on MASP-3 protein levels. These intriguing results open the way for further investigation of the role of MASP-3 in general and in critical illness in particular. Obviously, additional pathophysiological research will be needed to further elucidate the role of the lectin pathway of complement activation during critical illness.

Table 7. Characteristics of the critically ill patients and healthy volunteers

	CIT (n = 351)	IIT (n = 349)	Healthy children (n = 130)
Baseline characteristics			
Demography			
Male gender, n (%)	199 (56.7)	202 (57.9)	77 (59.2)
Median age (IQR), y	1.3 (0.3–4.6)	1.4 (0.3–5.5)	4.6 (2.2–6.8)
Mean BMI (95% CI), kg/m ²	15.4 (14.7–16.1)	15.4 (15.0–15.7)	16.4 (16.0–16.8)
Medical history			
Malignancy, n (%)	15 (4.3)	21 (6.0)	—
History of diabetes mellitus, n (%)	3 (0.9)	3 (0.9)	—
Diagnostic category			
Cardiac surgery for congenital heart defects, n (%)	265 (75.5)	261 (74.8)	—
Complicated/high-risk surgery or trauma, n (%)	38 (10.8)	36 (10.3)	—
Neurological medical disorders, n (%)	8 (2.3)	13 (3.7)	—
Infectious medical diseases, n (%)	16 (4.6)	16 (4.6)	—
Other medical disorders, n (%)	17 (4.8)	17 (4.9)	—
Solid organ transplants, n (%)	7 (2.0)	6 (1.7)	—
Median PELOD score (IQR) at first 24 h	11 (2–12)	11 (2–12)	—
Median PRISM score ^a (IQR)	10 (7–14)	10 (7–14)	—
Median CRP level on admission (IQR), mg/l	29 (14–48)	27 (17–51)	—
Median blood glucose on admission (IQR), mg/dl	136 (103–187)	128 (101–164)	—
Outcome parameters			
ICU mortality, n (%)	20 (5.7)	9 (2.6)	—
Median days in ICU (IQR)	3 (2–7)	3 (2–6)	—
Secondary infection, n (%)	129 (36.8)	102 (29.2)	—
Peak bilirubin >1.5 mg/dl ^c , n (%)	75 (22.0)	65 (19.0)	—
Median time on mechanical ventilation (IQR), d	2 (1–5)	2 (1–4)	—
Median time on vasoactive drug support (IQR), d	2 (0–4)	2 (0–3)	—

CI, confidence interval; CIT, conventional insulin therapy; CRP, C-reactive protein; ICU, intensive care unit; IQR, interquartile range; IIT, intensive insulin therapy; PELOD, Pediatric Logistic Organ Dysfunction; PRISM, Pediatric Risk of Mortality.

^aModified Pediatric Risk of Mortality score: PRISM score calculated with only the admission value of blood glucose, which is unaffected by the study intervention. ^bTo convert glucose to mmol/l, multiply by 0.05551. ^cTo convert bilirubin to μ mol/l, multiply by 17.1.

METHODS

Subjects

This is a preplanned analysis of all patients ($n = 700$) included in a randomized controlled study on tight glycemic control in the pediatric ICU (1). Patients had been allocated to conventional insulin therapy ($n = 351$, insulin started only when blood glucose exceeded 215 mg/dl, and the dose was tapered down and stopped when glycemia decreased to levels less than 180 mg/dl) or intensive insulin therapy ($n = 349$, insulin infused to achieve age-adjusted normal fasting glucose levels). Patient characteristics were comparable for both randomization groups (Table 7). Intensive insulin therapy reduced nosocomial infections, ICU stay, and ICU mortality (1). As controls, we included 130 healthy children (Table 7) scheduled for minor ambulatory surgery, allowing preoperative blood withdrawal

after catheterization required for induction and/or maintenance of anesthesia. The study was approved by the KU Leuven Institutional Review Board (ML2586 and amendment) and was registered with ClinicalTrials.gov (NCT00214916). The study was performed in accordance with the 1964 Declaration of Helsinki and its amendments. Written informed consent was obtained from the parents or legal guardians of patients and controls.

Biochemical and Genetic Analyses

CRP was determined by routine clinical chemistry. Whole arterial blood glucose was measured at 1- to 4-h intervals using the ABL700 blood gas analyzer (Radiometer Medical A/S, Copenhagen, Denmark). On ICU admission, serum and blood cell samples for DNA extraction were collected and stored at -80°C until analysis. For 29 and 90 patients, no baseline serum or blood cell sample was available, respectively.

Table 8. The SNPs determined in the patients and healthy children

SNP	Database ID	TaqMan assay	Probe 1/probe 2 or sequence surrounding SNP ^b
1	rs3105782	C___3289661_10	TTTCATCTTCATTCTCTCTTTTATA[A/G]TTTACAATGGCTCTTGACGTGGACA
2	rs113938200	AH89M1P ^a	CAGAAAATGAAATCGATCTGG/AGAAAATGAAATCAATCTGG
3	rs710469	C___8831704_10	GAGCTGGGAAGGCACATCTGATAAG[A/G] AGAAGCCAAGAGGCTTTGGTTTCTA
4	rs874603	AHBJ0DQ ^a	ACAGATGCAGCATT/CAGATGCGGCATT
5	rs710456	C___26425865_10	AGAACATGTTCTCCGTGACGCTGTA[A/G]TTGCCCGAGCGGGACTCATAGCTAG
6	rs3774268	C___27499239_20	GGCCCCAATGATCCTCTTGACCAG[A/G] CTTGGCAGGGAGCGGGAGGGCTGAC
7	rs6799834	C___29113676_20	TGTAAGGTTGAAGAAACCACAGAG[C/T]CAAGCTATGGGAAGCTGGTTTCTTT
8	rs16861801	C___33187189_10	TGTGGAGGATGTAGCCGAAGCGGCA[C/G]GAGCAGTAGTAGCCCAATGTAGT
9	rs35384947	C___58141611_10	CCTCCACCACTATCAGGGCCTGCCA[C/T]GGGAAGAGGCCAGGCTCAGCATTTCC
10	rs28945068	C___58141621_10	TGGAAGGCAGGTGGGTAGGCTTCTC[C/T]CCAATACTTTATTCATCCAGACTCC
11	rs28945071	C___58141663_10	TTGCAGGCTCACCTTGATGTAGTCA[C/T]AGGGGCAGGGCACCTCAGGATGGTC
12	rs28945069	C___58141664_10	CCCTCCTCCAGCTCGATGGTATACA[A/G]GCATTACAGAGCTCTGGGGTAAGGG
13	rs115022399	AHT98YZ ^a	CCCAGAGGGGACTC/CCGAGCGGGACTC
14	rs850312	AHV1647 ^a	TCAGATGTCTACAGTATG/AGATGTCTGCAGTATG
15	rs115961221	AHWR5BF ^a	CACACGGACTTTGTCA/CACACGGACCTTGTC
16	rs7652842	AHX03HN ^a	CATCAGCAGTGGCACAC/CAGCAGCGGCACAC
17	rs111908734	AHY91NV ^a	CCCCCACATGCT/CCCCAACATGCT
18	rs116384821	AH70OVH ^a	AAGCAGCCAACTGAAA/AAGCAGCCACCTGAAA
19	rs72549155	AH1RX0B ^a	CTGCAGGAGCCTGTGC/TGCAGGAGGCTGTGC
20	rs73068950	AH20V6J ^a	CATGTCACCGTCTACCT/CATGTCAACCTACCT
21	rs114365879	AH39UCR ^a	TTCTACTAATCCAGGTATATAT/TTCTACTAATCCAGATATATAT
22	rs116763673	AH5ISIZ ^a	AGGATGGGACGTGGAGTA/AAGGATGGGACATGGAGTA
23	rs116595107	AH6RQO7 ^a	AAAGTGCTGAAGGTGC/AAAGTGCTAAAGGTGC
24	rs73886128	AH70OVF ^a	CTCTCATACAGGGCTGC/CTCTCATACAAGGCTGC
25	rs116001173	AH89M1N ^a	CCAGCCCCGGTTCT/CCAGCCCTGGTTCT
26	rs115241263	AHAA17G ^a	CTACATTGGCGGCTACT/CTACATTGGCAGCTACT
27	rs78137557	AHBJ0DO ^a	TTGTGGCAGTAGTGGTCA/TGGCAGTGGTGGTCA
28	rs72549252	AHCSYJW ^a	CTCGTCCACGTCTGTAG/CTCGTCCACATCTGTAG
29	rs115647447	AHD1WP4 ^a	CCTGTGAAACACTCCTC/CTGTGAAACGCTCCTC
30	rs74784100	AHFAUWC ^a	AGATCCAGTCGCCTGGTT/CAGATCCAGTCACCTGGTT
31	rs77189011	AHGJS2K ^a	CCACACCGTGGAGCT/CCACACCATGGAGCT
32	rs1062049	AHHSQ8S ^a	CTCCACGGTGTGGGC/CTCCACGATGTGGGC

SNP, single-nucleotide polymorphism.

^aCustom-made assay. ^bProbe sequences are given for custom-made assays.

Serum levels of MBL, H-ficolin, M-ficolin, MASP-2, MASP-3, and MAP-44 were measured by sandwich-type antibody-based time-resolved immunofluorometric assays (TRIFMAs) (15,36–38). MASP-1 was measured using an inhibition-type TRIFMA (16).

Genetic analyses were prospectively planned as a target gene approach, focused on SNPs in genes encoding proteins associated with outcome. Thus, 32 SNPs in the *MASP1* gene were selected (Table 8), based on frequencies in the SNP database and location (covering exons, introns, and the 5′- and 3′-untranslated regions). The *MASP1* gene encodes a transcript that may be spliced to form mRNAs encoding the three proteins MASP-1, MASP-3, and MAP-44. MASP-1 and MASP-3 share five structural domains but differ by having two different serine protease domains (28). MAP-44 shares the first four structural domains with MASP-1 and MASP-3 but has in addition a unique 17 amino acid C-terminal end. We thus analyzed SNPs in the areas encoding shared domains (included in exons 1–8, 10, and 11; exon 9 encodes the MAP-44-specific sequence) and in the area of the gene encoding the MASP-3-specific protease domains (exon 12).

DNA was extracted from the blood cells using the QIAasympyphony robot and the corresponding DNA extraction kit (QIAGEN, Venlo, The Netherlands). Samples and negative controls without DNA template were analyzed by the high-throughput TaqMan Open Array Genotyping Platform according to the manufacturer's protocol (ABI, Foster City, CA). First, samples were diluted to a final concentration of ~50 ng DNA/μl in 96-well plates. Negative controls were randomly distributed on all 96-well plates to estimate the quality of the samples. Subsequently, 384-well plates were prepared using a Biomek NX (Beckman Coulter, Fullerton, CA). Finally, 100 ng of DNA was mixed with 2 μl TaqMan Open Array Mastermix (ABI) in 384-well plates, and the samples were loaded with the Open Array NT Autoloader (ABI) onto custom-designed 32-SNP Open Array plates (ABI), containing the TaqMan assays described in Table 8. PCR was performed using a GeneAmp 9700 thermal cycler (ABI) according to the following conditions: 91 °C for 10 min + 50 cycles (51 °C for 23 s; 53.5 °C for 30 s; 54.5 °C for 13 s; 97 °C for 22 s; and 92 °C for 7 s) + 20 °C for 5 min, followed by storage at 4 °C until analysis. Fluorescence of the FAM and VIC reporters was read with the Open Array NT Imager (ABI). Data were analyzed with the Biotrove Open Array SNP Genotyping Analysis Software package, version 1.0.3 (Biotrove, Woburn, MA).

Statistical Analysis

Data were analyzed with JMP10 (SAS Institute, Cary, NC). Two-sided *P* values less than 0.05 were considered significant. Data are presented as mean (95% confidence interval), median (interquartile range), or proportion (percentage). Continuous variables were analyzed with the Wilcoxon test and proportions were studied with the χ^2 test. Associations between variables were calculated with Spearman's correlation coefficient. To evaluate the association of on-admission protein levels with risk of infection, independent of known risk factors, multivariate logistic regression analysis was performed. The classical baseline characteristics and risk factors (age, gender, BMI, randomization, CRP, history of malignancy, cardiac surgery, and modified PRISM score) were considered, together with the on-admission lectin pathway protein levels. Receiver operating characteristic curves were drawn, and area under the curve was calculated to assess the predictive power of on-admission lectin pathway protein and CRP levels for infection. To evaluate the predictive power for the likelihood of early alive discharge from ICU, Cox proportional hazard analysis was performed entering the same risk factors, with nonsurvivors censored beyond the longest staying survivor, ensuring the most stringent analysis and avoiding competing risk between death and earlier discharge (39). Multiple linear regression analysis was performed to assess independent determinants of levels of lectin pathway proteins that appeared important for clinical outcomes. SNP allele frequencies were tested against proportions expected from Hardy–Weinberg equilibrium using the χ^2 test.

Demographically comparable cohorts of healthy and critically ill children were selected through propensity score matching, using the SPSS R-menu (version R2.10.1, R Foundation for Statistical Computing) in IBM SPSS Statistics 19 (SPSS, Chicago, IL) (40,41). Propensity scores were estimated by logistic regression, with age,

gender, and BMI as continuous covariates. Next, one-to-one nearest-neighbor matching was performed by using a caliper of 0.004.

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REFERENCES

- Vlasselaers D, Milants I, Desmet L, et al. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. *Lancet* 2009;373:547–56.
- Robertson CM, Coopersmith CM. The systemic inflammatory response syndrome. *Microbes Infect* 2006;8:1382–9.
- Kumpf O, Schumann RR. Genetic variation in innate immunity pathways and their potential contribution to the SIRS/CARS debate: evidence from human studies and animal models. *J Innate Immun* 2010;2:381–94.
- Wong HR. Genetics and genomics in pediatric septic shock. *Crit Care Med* 2012;40:1618–26.
- Kumar H, Kawai T, Akira S. Pathogen recognition in the innate immune response. *Biochem J* 2009;420:1–16.
- Sørensen R, Thiel S, Jensenius JC. Mannan-binding-lectin-associated serine proteases, characteristics and disease associations. *Springer Semin Immunopathol* 2005;27:299–319.
- Degn SE, Hansen AG, Steffensen R, Jacobsen C, Jensenius JC, Thiel S. MAP44, a human protein associated with pattern recognition molecules of the complement system and regulating the lectin pathway of complement activation. *J Immunol* 2009;183:7371–8.
- Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. *Lancet* 2001;358:614–8.
- Schlapbach LJ, Aebi C, Hansen AG, Hirt A, Jensenius JC, Ammann RA. H-ficolin serum concentration and susceptibility to fever and neutropenia in paediatric cancer patients. *Clin Exp Immunol* 2009;157:83–9.
- Schlapbach LJ, Kessler U, Thiel S, et al. M-ficolin in the neonatal period: associations with need for mechanical ventilation and mortality in premature infants with necrotising enterocolitis. *Mol Immunol* 2009;46:2597–603.
- Vollmer-Conna U, Piraino BF, Cameron B, et al.; Dubbo Infection Outcomes Study Group. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. *Clin Infect Dis* 2008;47:1418–25.
- Gao L, Barnes KC. Recent advances in genetic predisposition to clinical acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2009;296:L713–25.
- Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. *Crit Care Med* 1996;24:743–52.
- Jenkins KJ, Gauvreau K, Newburger JW, Spray TL, Moller JH, Iezzoni LI. Consensus-based method for risk adjustment for surgery for congenital heart disease. *J Thorac Cardiovasc Surg* 2002;123:110–8.
- Degn SE, Jensen L, Gál P, 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;361:37–50.

16. Thiel S, Jensen L, Degn SE, et al. Mannan-binding lectin (MBL)-associated serine protease-1 (MASP-1), a serine protease associated with humoral pattern-recognition molecules: normal and acute-phase levels in serum and stoichiometry of lectin pathway components. *Clin Exp Immunol* 2012;169:38–48.
17. Schlappbach LJ, Aebi C, Fisch U, et al. Higher cord blood levels of mannose-binding lectin-associated serine protease-2 in infants with necrotising enterocolitis. *Pediatr Res* 2008;64:562–6.
18. Sallenbach S, Thiel S, Aebi C, 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;22:424–30.
19. Fisch U, Zehnder A, Hirt A, et al. Mannan-binding lectin (MBL) and MBL-associated serine protease-2 in children with cancer. *Swiss Med Wkly* 2011;141:w13191.
20. Schlappbach LJ, Thiel S, Aebi C, et al. M-ficolin in children with cancer. *Immunobiology* 2011;216:633–8.
21. Schlappbach LJ, Aebi C, Otth M, Leibundgut K, Hirt A, Ammann RA. Deficiency of mannose-binding lectin-associated serine protease-2 associated with increased risk of fever and neutropenia in pediatric cancer patients. *Pediatr Infect Dis J* 2007;26:989–94.
22. Schlappbach LJ, Mattmann M, Thiel S, et al. Differential role of the lectin pathway of complement activation in susceptibility to neonatal sepsis. *Clin Infect Dis* 2010;51:153–62.
23. Schlappbach LJ, Kjaer TR, Thiel S, et al. M-ficolin concentrations in cord blood are related to circulating phagocytes and to early-onset sepsis. *Pediatr Res* 2012;71(4 Pt 1):368–74.
24. Frakking FN, Brouwer N, van Eijkelenburg NK, et al. Low mannose-binding lectin (MBL) levels in neonates with pneumonia and sepsis. *Clin Exp Immunol* 2007;150:255–62.
25. de Benedetti F, Auriti C, D'Urbano LE, et al. Low serum levels of mannose binding lectin are a risk factor for neonatal sepsis. *Pediatr Res* 2007;61:325–8.
26. St Swierczko A, Cedzynski M, Domzalska-Popadiuk I, et al. Mannan-binding lectin-associated serine protease-2 (MASP-2) in a large cohort of neonates and its clinical associations. *Mol Immunol* 2009;46:1696–701.
27. Cortesio CL, Jiang W. Mannan-binding lectin-associated serine protease 3 cleaves synthetic peptides and insulin-like growth factor-binding protein 5. *Arch Biochem Biophys* 2006;449:164–70.
28. Degn SE, Jensenius JC, Thiel S. Disease-causing mutations in genes of the complement system. *Am J Hum Genet* 2011;88:689–705.
29. Sirmaci A, Walsh T, Akay H, et al. MASP1 mutations in patients with facial, umbilical, coccygeal, and auditory findings of Carnevale, Malpuech, OSA, and Michels syndromes. *Am J Hum Genet* 2010;87:679–86.
30. Rooryck C, Diaz-Font A, Osborn DP, et al. Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. *Nat Genet* 2011;43:197–203.
31. Iwaki D, Kanno K, Takahashi M, Endo Y, Matsushita M, Fujita T. The role of mannose-binding lectin-associated serine protease-3 in activation of the alternative complement pathway. *J Immunol* 2011;187:3751–8.
32. Degn SE, Jensen L, Hansen AG, 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;189:3957–69.
33. Lukaszewicz AC, Payen D. The future is predetermined in severe sepsis, so what are the implications? *Crit Care Med* 2010;38(10 Suppl):S512–7.
34. Henckaerts L, Nielsen KR, Steffensen R, et al. Polymorphisms in innate immunity genes predispose to bacteremia and death in the medical intensive care unit. *Crit Care Med* 2009;37:192–201, e1–3.
35. Thiel S. Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. *Mol Immunol* 2007;44:3875–88.
36. Ytting H, Christensen IJ, Thiel S, et al. Biological variation in circulating levels of mannann-binding lectin (MBL) and MBL-associated serine protease-2 and the influence of age, gender and physical exercise. *Scand J Immunol* 2007;66:458–64.
37. Krarup A, Sørensen UB, 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;73:1052–60.
38. 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;2:167–80.
39. Casaer MP, Mesotten D, Hermans G, et al. Early versus late parenteral nutrition in critically ill adults. *N Engl J Med* 2011;365:506–17.
40. Heinze G, Jüni P. An overview of the objectives of and the approaches to propensity score analyses. *Eur Heart J* 2011;32:1704–8.
41. Thoemmes F. Propensity score matching in SPSS. *ArXiv: 1201.6385 [online]*, 2012.