

**Title:** Association of lectin pathway proteins with intra-abdominal *Candida* infection in high-risk surgical intensive-care unit patients. A prospective cohort study within the fungal infection network of Switzerland.

**Running title:** Lectin pathway proteins and intra-abdominal *Candida* infection

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## **Abstract:**

**Objectives:** Human studies on the role of mannose-binding lectin (MBL) in patients with invasive candidiasis have yielded conflicting results. We investigated the influence of MBL and other lectin pathway proteins on *Candida* colonization and intra-abdominal candidiasis (IAC) in a cohort of high-risk patients.

**Methods:** Prospective observational cohort study of 89 high-risk intensive-care unit (ICU) patients. Levels of lectin pathway proteins at study entry and six *MBL2* single-nucleotide polymorphisms were analysed by sandwich-type immunoassays and genotyping, respectively, and correlated with development of heavy *Candida* colonization (corrected colonization index (CCI)  $\geq 0.4$ ) and occurrence of IAC during a 4-week period.

**Results:** Within 4 weeks after inclusion a CCI $\geq 0.4$  and IAC was observed in 47% and 38% of patients respectively. Neither serum levels of MBL, ficolin-1,-2,-3, MASP-2 or collectin liver 1 nor *MBL2* genotypes were associated with a CCI $\geq 0.4$ . Similarly, none of the analysed proteins was found to be associated with IAC with the exception of lower MBL levels (HR 0.74,  $p=0.02$ ) at study entry. However, there was no association of MBL deficiency ( $<0.5 \mu\text{g/ml}$ ), *MBL2* haplo- or genotypes with IAC.

**Conclusion:** Lectin pathway protein levels and *MBL2* genotype investigated in this study were not associated with heavy *Candida* colonization or IAC in a cohort of high-risk ICU patients.

## Introduction:

Invasive candidiasis is a frequent complication after recurrent gastrointestinal tract (GIT) perforation or acute necrotizing pancreatitis with significant morbidity and a mortality of up to 50% in intensive-care unit (ICU) patients(1, 2). It includes candidemia with or without deep-seated infection and intra-abdominal candidiasis (IAC) with negative blood cultures, the latter being responsible for the majority of cases in high-risk surgical ICU patients(2, 3). Broad-spectrum antibiotic treatment favors proliferation and colonization of body sites by *Candida species (spp.)*, and disruption of natural barriers (GIT perforation, anastomotic leaks, intravascular catheters) enables their translocation into tissues and the bloodstream(4). Apart from clinical risk factors, host innate immunity including pattern recognition receptors (PRR) seems to influence the risk of colonization and infection with *Candida spp* (5-7).

The lectin pathway of complement is activated after binding of one or more of the soluble PRRs mannose-binding lectin (MBL), collectin liver 1 (CL-L1) and ficolins (ficolin (FCN)-1, -2, and -3) to carbohydrate patterns, acetyl groups or immunoglobulin M (IgM) bound to antigens on pathogens and dying cells, with subsequent activation of MBL-associated serine protease (MASP)-1 and -2 and assembly of the C3 convertase(8). Inter-individual serum concentrations of these PRR and proteases vary to a considerable degree with the greatest distribution observed in MBL levels (from undetectable to about 10 µg/mL)(9). Well-characterized exon and promoter polymorphisms in the *MBL2* gene on chromosome 10 are responsible for the remarkable variation in serum MBL levels(10). Consequently, low MBL levels may be observed in approximately a third of the population worldwide(10). For the ficolins and MASPs a number of major polymorphisms have been described with a much weaker genotypic/phenotypic relationship compared with MBL(11-13).

MBL has been shown to bind to *Candida albicans* augmenting complement activation(14) and facilitating phagocytosis(15) *in vitro*. In addition, intravenous administration of MBL increased

survival in a mouse model of disseminated candidiasis(16). However, human studies have yielded conflicting results in patients with invasive candidiasis(17, 18), and a recent genome-wide association study did not identify *MBL2* polymorphisms as genetic risk factors for candidemia(19). Whereas ficolins seem to influence infections with *Aspergillus fumigatus*(20, 21), data are lacking with regard to *Candida* infections.

CL-L1 belong to a novel group of pattern recognition molecules comprising collectin-11 (CL-K1), CL-P1 and CL-L1(22), which are capable of activating the lectin pathway of complement(23). As the collectins have been shown to bind to different microorganisms including *C. albicans*(24) and altered/exposed structures in the body, this new group of PRR may also play a role in inflammatory conditions.

Hence, this study was designed to comprehensively assess the influence of MBL and other lectin pathway proteins on the predisposition to *Candida* colonization and IAC in a prospective cohort of high-risk surgical ICU patients.

## **Patients and methods:**

### *Participants:*

This analysis was conducted as part of the observational FUNGINOS cohort study in two Swiss University hospital ICUs(25). Consecutive, >18 years old surgical ICU patients with recurrent GIT perforation or acute necrotizing pancreatitis not receiving antifungal agents were included and prospectively studied until 2 weeks after ICU discharge. A complete description of the study including information about clinical workup, recorded clinical variables, microbiological sampling and treatment has been reported previously(25). Blood samples for genetic testing and determination of protein levels were drawn at study inclusion. During the study period, at least three of five nonsterile sites (mouth, urine, stool, skin, respiratory tract) were screened twice weekly for colonization with *Candida spp.* and colonization was graded as weak, moderate, or heavy according to semiquantitative cultures.

### *Ethics statement:*

The FUNGINOS study including this analysis had been approved by institutional ethical committees, and all participants or their legal representatives gave written informed consent for the study.

### *Definition of endpoints:*

The corrected colonization index (CCI) was developed as a prediction rule to differentiate between colonization and invasive infection in surgical patients(26). It is the product of the colonization index (calculated as number of colonized sites divided by the number of cultured sites) and the ratio of the number of heavily colonized sites to the total number of colonized sites, which was calculated at inclusion and twice weekly during the study period. A threshold of  $\geq 0.4$  indicating “heavy” colonization was used as endpoint as described previously(26).

Candidemia was defined as at least one positive blood culture with *Candida spp.*

Intra-abdominal candidiasis (IAC) was defined as a positive culture result from specimens obtained at surgery showing either monomicrobial growth of *Candida spp.*, growth of *Candida spp.* in a polymicrobial abscess or moderate/heavy growth of *Candida spp.* in polymicrobial peritonitis not responding to appropriate antibacterial therapy(25). Antifungal treatment was administered according to international guidelines with a strong recommendation against administration of antifungal prophylaxis regardless of presence/absence of *Candida* colonization.

In addition, 30-day mortality and elevated 1-3- $\beta$ -D-glucan levels (defined as  $\geq 80$  pg/mL) at inclusion and at diagnosis of IAC were analyzed as endpoints.

Association of lectin pathway proteins and SNPs with the above mentioned endpoints was assessed during a 4-week high-risk period after diagnosis of recurrent GIT perforation or acute necrotizing pancreatitis.

Patients with pre-emptive antifungal therapy were excluded from the analysis of IAC only (n=18).

#### *Determination of lectin protein serum levels:*

Sera sampled at study inclusion were stored at -80°C before being analyzed in batch with duplicate testing by two investigators blinded to any patient data. Quantification of MBL plasma levels was performed using a mannan-binding enzyme-linked immunosorbent assay as previously described (27, 28). Briefly, mannan-coated microtitre plates were incubated with samples at 1:25 and 1:100 dilutions for 90 min at room temperature followed by detection of bound MBL with a biotinylated monoclonal anti-MBL antibody (HYB 131-01, BioPorto Diagnostics, Denmark). Moderate MBL deficiency was defined as serum level  $< 0.5$   $\mu$ g/ml, and severe as  $< 0.1$   $\mu$ g/ml, respectively(29).

Levels of ficolin-1, -2, -3 and MASP-2 were determined using commercially available ELISAs (Hycult, the Netherlands). Levels of collectin liver 1 (CL-L1) were determined by using a sandwich-type time-resolved immunofluorometric assay (TRIFMA) as described in detail previously(23).

After extraction of genomic DNA using a QIAcube machine (Qiagen, Switzerland), six *MBL2* single-nucleotide polymorphisms (SNPs) were genotyped (Illumina Veracode genotyping platform (Illumina, U.S.A.) or KASP system (LGC Genomics, U.S.A.) chosen on the basis of their remarkable impact on MBL levels. Rs1800451 (A/C), rs1800450 (A/B), rs5030737 (A/D) denote SNPs in the exon 1 region, whereas rs11003125 (H/L), rs7096206 (Y/X), rs7095891 (P/Q) are variants in the *MBL2* promoter region. *MBL2* genotypes were classified as low (XA/YO, YO/YO), intermediate (XA/XA, YA/YO) or high (YA/YA, XA/YA) producing genotypes according to published literature(30) with exon 1 variant alleles collectively designated as O and the wild-type allele as A, and the promoter and the wild-type allele of rs7096206 designated as X and Y, respectively. *MBL2* SNPs were analyzed separately and combined as haplo- and genotype.

#### *Statistical analysis:*

We used the chi-square test for comparisons of categorical variables and allele and haplo-/genotype frequencies, and to check for Hardy-Weinberg equilibrium. Cox regression models (dominant model of inheritance) were used to analyze the association of clinical endpoints with lectin pathway protein levels and SNPs during a 4-week high-risk period considering the diagnosis of recurrent GIT perforation or acute necrotizing pancreatitis as the starting date with censoring at death or ICU discharge with and without adjustment for age, sex, and other relevant covariates. In addition, differences in clinical endpoints according to lectin pathway protein levels were analyzed using the Mann-Whitney U test (with reporting of median and interquartile range (IQR) due to the non-Gaussian distribution of MBL levels) and the Student's t test (with reporting of mean and standard deviation (SD) for all other proteins), respectively. All testing was two-tailed. Haplotype and linkage disequilibrium analysis was carried out with the haploview program (version 4.2). All other analyses were performed using SPSS statistics, version 17.0 (SPSS Inc., USA).



## Results:

### *Demographic and clinical characteristics:*

The cohort consisted of 89 Caucasian patients admitted to two surgical ICUs and at high risk for IAC, 68 with recurrent GIT perforation and 21 with acute necrotizing pancreatitis. Demographic and clinical characteristics are outlined in Table 1. The majority of patients had multiple risk factors for invasive candidiasis including presence of a central venous catheter, total parenteral nutrition and broad-spectrum antibacterial therapy. *C. albicans* was the dominant pathogen isolated in the majority of yeast-positive abdominal cultures (n=23, 79%). Eighteen (20%) of patients received pre-emptive therapy for suspected and 26 (29%) targeted therapy for confirmed invasive candidiasis. After four weeks of follow-up, a CCI>0.4 and IAC was observed in 42/89 (47%) and 27/71 (38%) of patients, respectively, and 30-day mortality was 8%.

### *Association of lectin pathway protein levels with clinical endpoints:*

Lectin pathway protein levels were successfully determined in 86/89 patients (sera not available in three patients), the exception being CL-L1, which was only measured in 83/89 patients due to lack of material. Overall, 28/86 (32.6%) and 11/86 (12.8%) patients demonstrated moderate and severe MBL deficiency, respectively. Neither serum levels of tested lectin pathway proteins nor moderate or severe MBL deficiency were associated with the development of heavy *Candida* colonization (CCI $\geq$ 0.4) (Table 2). Similar results were observed in patients who were diagnosed with IAC compared to patients without IAC with the exception of lower MBL levels, which were associated with the risk of IAC in time-dependent analysis only (Table 2). However, this difference was mainly driven by the presence of very high MBL levels in the control group (Figure 1), whereas there was no difference in the frequency of moderate or severe MBL deficiency (and of *MBL2* SNPs, see below for details) in both groups. In addition, lectin pathway protein levels were not associated with elevated 1-3- $\beta$ -D-glucan levels ( $\geq$ 80 pg/mL) at any time point or 30-day mortality (data not shown).

Including age, sex, disease severity and risk factors for invasive candidiasis as covariates in multivariate Cox regression models did not significantly alter the observed univariate results (data not shown).

#### *Association of MBL2 SNPs with clinical endpoints:*

Successful genotyping was achieved for 6 loci in the *MBL2* gene in 94 (rs11003125) - 100% of cases, and allele frequencies at all positions were in agreement with the predicted Hardy-Weinberg equilibrium ( $p > 0.05$  for all analyses). Minor allele and haplotype frequencies of the study cohort were in accordance with those reported in the literature(31, 32) (Table 3). As expected, median MBL levels significantly correlated with *MBL2* genotypes (0.01  $\mu\text{g/ml}$  (interquartile range (IQR) 0-0.66) for low (XA/YO, YO/YO), 0.42  $\mu\text{g/ml}$  (IQR 0.21-0.87) for intermediate (XA/XA, YA/YO), and 2.69  $\mu\text{g/ml}$  (IQR 1.77-3.55) for high (YA/YA, XA/YA) producing *MBL2* genotypes,  $p < 0.001$ ).

Frequencies of *MBL2* exon 1 or promoter variant alleles and haplotypes did not differ significantly between subjects with and without heavy *Candida* colonization (Table 3) with the exception of the exon 1 variant rs1800451 (A/C) in time dependent analysis only (hazard ratio (HR) 3.85 (interquartile range (IQR) 1.18-12.58),  $p = 0.03$ ; not significant when using Fisher's exact test, 3/42 heterozygous/homozygous subjects with heavy *Candida* colonization vs. 0/47,  $p = 0.1$ ). Similarly, variant alleles and haplotypes were equally distributed in patients with and without IAC (Table 3) with the exception of the promoter variant rs7095891 (P/Q) (hazard ratio 2.25 (IQR 1.05-4.80),  $p = 0.04$ ; 14/27 (52%) hetero- or homozygous subjects with IAC vs. 12/44 (27%),  $p = 0.05$ , Fisher's exact test). In particular, there was no difference in *MBL2* geno- or haplotypes according to the presence or absence of IAC despite small differences in MBL levels in these groups (for details see above). This was also the case for the association of variant alleles or haplotypes with elevated 1-3- $\beta$ -D-glucan levels and 30-day mortality (data not shown).

## Discussion:

PRRs of the lectin pathway of complement have been implicated in the pathogenesis of fungal infections in several experimental models(14-16, 20, 21). This is the first human study designed to examine not only the role of MBL but also other lectin pathway PRR and its associated protease MASP-2 in the predisposition to *Candida* colonization and intra-abdominal candidiasis.

Despite convincing experimental data(14-16) we did not demonstrate any association of lectin pathway protein levels or *MBL2* SNPs/haplotypes with the presence of heavy *Candida* colonization or IAC in a prospective cohort of well-characterized high-risk surgical ICU patients. The notable exceptions are weak associations of lower MBL levels and of the promoter variant rs7095891 (P/Q) with IAC, and of the exon 1 variant rs1800451 (A/C) with heavy *Candida* colonization both in time dependent analysis only, whose significance are limited in the context of multiple comparisons investigated in this study and a lack of association of MBL deficiency, other *MBL2* variant alleles and *MBL2* low-producing genotypes with IAC or heavy *Candida* colonization.

Previous studies have yielded conflicting results with regard to invasive candidiasis in surgical or hematological patients. Van Till et al. investigated a similar cohort of 88 patients at risk for IAC(17). MBL levels were significantly lower in patients with IAC (28/88) compared to patients without IAC, and moderate MBL deficiency (<0.5 µg/ml) was encountered more frequently in the IAC group (71 vs. 42% compared with only 33% in our patients with IAC). The fact, that blood samples were drawn much earlier in the previous study (at first laparotomy for perforation or GIT necrosis in the majority of subjects) might partly explain the discrepant results between this and our study with regard to MBL levels and presence of MBL deficiency in IAC patients, as MBL levels are known to increase over time as a consequence of a mild acute-phase reaction(33, 34). This assumption is underscored by a lack of difference in *MBL2* variant genotypes according to the presence of IAC, and neither MBL levels nor genotypes were associated with *Candida* colonization similar to our results(17). In a second study, 68 patients with candidemia were compared to blood donors and hospitalized patients with different

degrees of *Candida* colonization(18). Interestingly, MBL levels were significantly higher in candidemia patients as compared to the latter two groups, whereas MBL levels were not associated with *Candida* colonization (in comparison to blood donors). Of note, MBL levels were significantly lower two days prior to the presence of *Candida spp.* in the bloodstream highlighting the importance of the sampling time for the interpretation of MBL levels in these patients. In our cohort candidemia was only detected in two patients, which might be related to early diagnosis and treatment or the more frequent use of pre-emptive antifungal treatment (18/89). Lastly, *MBL2* variant alleles were similarly distributed in adult leukemia patients with and without chronic disseminated candidiasis in a third study(35).

Given conflicting evidence from previous studies and negative results from our comprehensive assessment of both MBL geno- and phenotype, MBL probably has a very limited effect in the context of invasive *Candida* infection in adults but further studies with a larger patient population are desirable. This is in contrast to vulvovaginal candidiasis, where a recent meta-analysis demonstrated an increased risk in women harboring the *MBL2* exon 1 codon 54 (A/B) variant(36). We can only speculate that other PRR (e.g. toll-like receptor 4(6)) including other lectin PRR (e.g. ficolin-2(37) or collectin-11(24)) might partially compensate for MBL deficiency in the setting of invasive *Candida* infection. Supporting our hypothesis levels of at least one ficolin and/or MASP-2 were above or close to the median in every single patient with severe MBL deficiency in our study (data not shown).

Ficolins have been implicated in fungal infections recently(20, 21, 38), and ficolin-2 was shown to recognize 1-3- $\beta$ -D-glucan (the major components of *Candida* cell walls) leading to complement activation(37) more than ten years ago. To our knowledge, data on other ficolins and human studies investigating their role in *Candida* infections are lacking. Similar to MBL we were not able to demonstrate a role of ficolin-1, -2, -3, CL-L1 or MASP-2 levels in the predisposition to heavy *Candida* colonization or IAC in our cohort. In line, a recent genome-wide association study failed to identify polymorphisms in lectin pathway PRR as risk factor for candidemia(19). Larger cohorts are required to

confirm our phenotypic findings including data on lectin PRR variant alleles not investigated in the current study.

Our study has limitations including the lack of data on ficolin, CL-L1 and MASP-2 variant alleles in the setting of invasive *Candida* infection. In addition, the use of pre-emptive antifungal treatment in 20% of patients in our study reduced the power for the analysis of IAC and might have influenced our results if those patients had been included in the IAC analysis. The significance of our observation is limited by the small sample size of the analyzed cohort and the multiple comparisons investigated. Significant differences as described might be a chance result in the setting of multiple statistical analyses. Vice versa, it is possible that small differences in the presence of IAC or heavy *Candida* colonization according to lectin pathway protein levels or *MBL2* SNPs may only be detectable in a larger cohort of patients. In addition, our results do not necessarily exclude a potential association of MBL and the lectin pathway with invasive candidiasis not caused by intraabdominal infection (e.g. candidemia associated with the presence of intravascular catheters or in the setting of hematologic malignancies).

In conclusion, this study does not support an important role for MBL or other lectin pathway proteins in the predisposition to *Candida* colonization or intra-abdominal infection in high-risk surgical ICU patients. Our present state of knowledge indicates that possible effects of lectin pathway protein abnormalities are likely overwhelmed by conventional risks factors and/or more powerful SNPs in other key immune genes or that a single lectin pathway protein deficiency (such as MBL deficiency) is compensated by other PRRs including lectin pathway PRR in the setting of invasive *Candida* infection.

**Conflict of interest:**

No conflict of interest.

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## Tables

**Table 1: Patient's demographic and clinical characteristics**

<b>Characteristics</b>	<b>(n=89)</b>
Age in years, median (range)	61 (22-86)
Female sex, n (%)	30 (34)
<b>Inclusion criteria, n (%)</b>	
Recurrent gastrointestinal perforation	68 (76)
Acute necrotizing pancreatitis	21 (24)
<b>Primary diagnosis at ICU admission, n (%)</b>	
Intra-abdominal tumor	23 (26)
Intestinal ischemia	20 (22)
Acute necrotizing pancreatitis	20 (22)
Gastro-intestinal perforation	10 (11)
Gastro-intestinal bleeding	5 (6)
Ruptured aneurysm of abdominal aorta	4 (4)
Others	7 (8)
<b>Clinical severity at inclusion</b>	
SAPS II score, median (range)	51 (13-87)
APACHE II score, median (range)	23 (5-37)
Severe sepsis or septic shock, n (%)	50 (56)
Mortality, n (%) <sup>1</sup>	15 (17)
Duration of hospital stay in days, median (range)	44.5 (9-176)
Duration of ICU stay in days, median (range)	13 (3-74)
<b>Risk factors for <i>Candida</i> infection at inclusion</b>	

Central venous catheter	87 (98)
Proton pump inhibitors	86 (97)
Urinary catheter	86 (97)
Total parenteral nutrition	84 (94)
Antibacterial therapy	77 (87)
Invasive mechanical ventilation > 24h	61 (69)

#### **Antifungal therapy**

None	45 (51)
Pre-emptive therapy for suspected IAC	18 (20)
Therapy for documented infection	26 (29)

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Abbreviations: IAC, intra-abdominal candidiasis; ICU, intensive-care unit; SAPS, Simplified Acute Physiology Score; APACHE score, Acute Physiology and Chronic Health Evaluation score;

<sup>1</sup>Mortality was analysed during the high-risk period until discharge from ICU

**Table 2:** Time-dependent analysis of lectin pathway protein levels according to the presence of heavy *Candida* colonization or IAC

Variables	Heavy <i>Candida</i> colonization (CCI $\geq$ 0.4, n=42/89)		IAC (n=27/71) <sup>a</sup>	
	HR (95% CI)	P value	HR (95% CI)	P value
MBL serum levels <sup>b</sup>	0.84 (0.69-1.04)	0.1	0.74 (0.57-0.96)	<b>0.02</b>
MBL < 0.5 $\mu$ g/mL	1.18 (0.63-2.21)	0.6	0.89 (0.40-1.98)	0.7
MBL < 0.1 $\mu$ g/ml	1.15 (0.51-2.60)	0.7	0.71 (0.21-2.35)	0.6
MASP-2 serum levels <sup>b</sup>	1.33 (0.89-1.99)	0.2	0.73 (0.41-1.32)	0.3
FCN-1 serum levels <sup>b</sup>	0.64 (0.38-1.07)	0.1	0.75 (0.42-1.36)	0.3
FCN-2 serum levels <sup>b</sup>	1.03 (0.81-1.30)	0.8	0.81 (0.60-1.09)	0.2
FCN-3 serum levels <sup>b</sup>	1.04 (0.95-1.13)	0.4	0.97 (0.87-1.07)	0.6
CL-L1 serum levels <sup>c</sup>	0.95 (0.71-1.27)	0.7	0.78 (0.56-1.09)	0.2

Time-dependent analysis (Cox regression) of clinical endpoints and lectin pathway proteins during the 4-week high-risk period considering the diagnosis of recurrent gastrointestinal perforation or acute necrotizing pancreatitis as the starting date, with censoring at death or ICU discharge. HR>1 corresponds to an increased risk.

Abbreviations: CI, confidence interval; CL-L1, collectin liver 1, FCN, ficolin; HR, hazard ratio; MASP, mannose-binding lectin-associated serine protease; MBL, mannose-binding lectin; IAC, intra-abdominal candidiasis.

<sup>a</sup> Patients with pre-emptive antifungal therapy were excluded from the analysis of IAC (n=18).

<sup>b</sup> per 1  $\mu$ g/ml increase in lectin pathway protein serum levels.

<sup>c</sup> per 100 ng/ml increase in CL-L1 serum levels.

**Table 3:** Time-dependent analysis of *MBL2* SNPs according to the presence of heavy *Candida* colonization or IAC

Variables	Heavy <i>Candida</i> colonization (CCI $\geq$ 0.4, n=42/89)			IAC (n=27/71) <sup>a</sup>		
	MA/HF	HR (95% CI)	P value	MA/HF	HR (95% CI)	P value
<i>MBL2</i> exon variants						
<i>rs1800451</i> (A/C)	0.02	3.85 (1.18-12.58)	<b>0.03</b>	0.02	2.71 (0.64-11.59)	0.2
<i>rs1800450</i> (A/B)	0.12	0.71 (0.33-1.53)	0.4	0.12	0.59 (0.20-1.70)	0.3
<i>rs5030737</i> (A/D)	0.08	1.03 (0.43-2.45)	1	0.08	1.41 (0.49-4.10)	0.5
<i>MBL2</i> promoter variants						
<i>rs11003125</i> (H/L)	0.37	0.70 (0.38-1.31)	0.3	0.37	0.54 (0.25-1.17)	0.1
<i>rs7096206</i> (Y/X)	0.22	1.58 (0.86-2.90)	0.1	0.23	1.43 (0.67-3.04)	0.4
<i>rs7095891</i> (P/Q)	0.21	1.54 (0.84-2.82)	0.2	0.21	2.25 (1.05-4.80)	<b>0.04</b>
<i>MBL2</i> genotypes						
High producing		Reference			Reference	
Intermediate producing		1.08 (0.53-2.21)	0.8		1.20 (0.50-2.91)	0.8
Low producing		1.56 (0.69-3.55)	0.3		0.98 (0.32-3.01)	1.0
<i>MBL2</i> haplotypes						
HYPA	0.32	0.67 (0.35-1.27)	0.2	0.31	0.59 (0.26-1.36)	0.2
LXPA	0.22	1.60 (0.90-2.84)	0.1	0.23	1.46 (0.73-2.95)	0.3



LYQA	0.20	1.25 (0.69-2.27)	0.5	0.19	1.92 (0.93-3.95)	0.1
LYPB	0.12	0.64 (0.29-1.44)	0.3	0.13	0.52 (0.16-1.68)	0.3
HYPD	0.05	1.16 (0.50-2.71)	0.7	0.05	1.86 (0.67-5.17)	0.2
LYPA	0.05	0.89 (0.25-3.17)	0.9	0.05	1.07 (0.29-3.93)	0.9

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Time-dependent analysis (Cox regression, dominant model of inheritance (wildtype vs. hetero- and homozygous)) of clinical endpoints and *MBL2* SNPs, haplo- and genotypes proteins during the 4-week high-risk period considering the diagnosis of recurrent gastrointestinal perforation or acute necrotizing pancreatitis as the starting date, with censoring at death or ICU discharge. *MBL2* genotypes were classified as low (XA/YO, YO/YO), intermediate (XA/XA, YA/YO) or high (YA/YA, XA/YA) with exon variant alleles collectively designated as O and the wild-type gene as A, and the promoter variant allele (rs7096206) and the wild-type gene designated as X and Y, respectively. HR>1 corresponds to an increased risk.

Abbreviations: CI, confidence interval; HR, hazard ratio; MA/HF, minor allele and haplotype frequencies, respectively; MASP, mannose-binding lectin-associated serine protease; MBL, mannose-binding lectin; IAC, intra-abdominal candidiasis.

<sup>a</sup> Patients with pre-emptive antifungal therapy were excluded from the analysis of IAC (n=18).

**Figure legends:**

**Figure 1:** Serum levels of lectin pathway proteins MBL (A), MASP-2 (B), ficolin-1 (C), ficolin-2 (D), ficolin-3 (E) and collectin liver 1 (F) in patients who developed IAC (n=27) during a 4-week high-risk period compared to patients without IAC (n=44, controls). Data are reported as scatter-dot plots and medians. Mann-Whitney-U- (MBL) or Student's t-test (MASP-2, ficolin-1, -2, -3, collectin liver 1) p values are indicated. Abbreviations: CL-L1, collectin liver 1; MASP, mannose-binding lectin-associated serine protease; MBL, mannose-binding lectin; IAC, intra-abdominal candidiasis



