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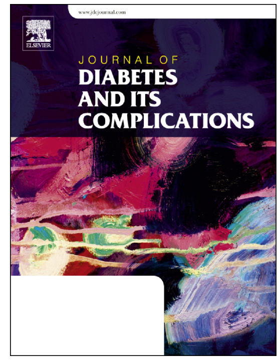

Mannose-binding Lectin and Risk of Infections in Type 2 Diabetes: A Danish cohort study

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Title page

ORIGINAL RESEARCH ARTICLE

Mannose-binding Lectin and Risk of Infections in Type 2 Diabetes: A Danish cohort study

Running head: MBL and Infections in Type 2 Diabetes

Authors and affiliations:

Anne Gedebjerg, PhD[1,2], Reimar Wernich Thomsen, PhD[1], Alisa Devedzic Kjaergaard, PhD[1], Rudi Steffensen, PhD[3], Jens Steen Nielsen, PhD[4,5], Jørgen Rungby, DMSc[6,7], Søren Gunnar Friborg, MD[8], Ivan Brandslund, DMSc[9], Steffen Thiel, PhD[10], Henning Beck-Nielsen, DMSc[4,8], Henrik Toft Sørensen, DMSc[1], Troels Krarup Hansen, DMSc[11], Mette Bjerre, PhD[12]

[1]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

[2]Danish Diabetes Academy, Odense University Hospital, Odense, Denmark

[3]Department of Immunology, Aalborg University Hospital, Aalborg, Denmark

[4]DD2, Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark

[5]Department of Clinical Research, University of Southern Denmark, Odense, Denmark

[6]Department of Endocrinology IC, Bispebjerg University Hospital, Copenhagen, Denmark

[7]Copenhagen Center for Translational Research, Bispebjerg University Hospital, Copenhagen

[8]Diabetes Research Centre, Department of Endocrinology, Odense University Hospital, Odense, Denmark

[9]Department of Biochemistry, Lillebaelt Hospital, Vejle, Denmark

[10]Department of Biomedicine, Aarhus University, Aarhus, Denmark

[11]Steno Diabetes Center Aarhus, Aarhus University Hospital, Aarhus, Denmark

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

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Corresponding author:

Anne Gedebjerg, Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Alle 43-45, DK-8200 Aarhus N, Denmark; Tel: +45 87168253; Fax: +45 87167215

Email: aged@clin.au.dk

ABSTRACT (words 198)

Aims: In individuals at increased risk of infections, e.g., patients with type 2 diabetes, low MBL may have detrimental effects. We used the Mendelian randomization principle to examine whether genetically low MBL is a risk factor for developing infections in patients with type 2 diabetes.

Methods: Serum MBL (n=7305) and MBL genotype (n=3043) were determined in a nationwide cohort of patients with new type 2 diabetes and up to 8 years follow-up for hospital-treated infections and community-based antimicrobial prescriptions. The associations were examined in spline and Cox regression analyses.

Results: 1140 patients (16%) were hospitalized with an infection and 5077 patients (70%) redeemed an antimicrobial prescription. For low (≤100 µg/L) versus intermediate (101–1000 µg/L) serum MBL concentration, the adjusted hazard ratios (aHRs) were 1.13(95% confidence interval, 0.96–1.33) for any hospital-treated infections and 1.19(1.01–1.41) for bacterial infections. Low MBL expression genotype was not associated with risk of any hospital-treated infections except for diarrheal diseases (aHR 2.23[1.04–4.80]). Low MBL expression genotype, but not low serum MBL, was associated with increased risk for antimicrobial prescriptions (aHR 1.18[1.04–2.34] and antibacterial prescriptions 1.20[1.05–1.36]).

Conclusions: Low MBL is a weak causal risk factor for developing infections in patients with type 2 diabetes.

Keywords: Association; cohort study; epidemiology; infection; mannose-binding lectin; type 2 diabetes.

1. Introduction

Mannose-binding lectin (MBL) is a plasma protein that plays an important role in innate immunity[1], as it recognizes and binds to carbohydrate structures of a variety of pathogens, e.g., bacteria, viruses, and fungi.[2] Around 5% of Caucasians have an inherited MBL deficiency leading to reduced plasma levels of MBL protein.[3] A number of clinical studies have reported an association between serum MBL deficiency and elevated risk of lower respiratory tract infections.[4-10] In contrast, a large cohort study found only a weak association between low MBL genotypes and risk of hospitalization with infection among adults, arguing against a strong causal role of MBL for severe infections in the general population.[3] In individuals already at increased risk of infections due to an impaired immune system, e.g., patients with cancer or autoimmune diseases including diabetes,[11-15] low MBL may be clinically important. Patients with type 2 diabetes have rates of antibiotic prescriptions and hospital-treated infections that are approximately 30% higher than for the general population,[16] and more severe infection outcomes including during the current COVID-19 pandemic.[17]

We therefore examined the hypothesis that low MBL is associated with increased risk of infection in 7588 Danish patients with early type 2 diabetes followed for up to 8 years, investigating both serum MBL levels and MBL expression genotypes. According to the Mendelian randomization principle, if both low serum MBL and low MBL genotype are associated with risk of infection, this would suggest a causal role for MBL in the development of infection in type 2 diabetes.

2. Materials and methods

2.1. Study population

The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort has been described in detail previously.[18] Briefly, the DD2 cohort was initiated in November 2010 and is an ongoing cohort of patients recently diagnosed with type 2 diabetes.[18] Enrollment has been continuous from hospital specialist outpatient clinics and general practitioners' offices. Hospital physicians and general practitioners identify newly/recently diagnosed patients with type 2 diabetes and complete an online questionnaire[19] with health-related items (e.g., physical activity, alcohol consumption, and anthropometric measurements) and physical examination items (e.g., waist–hip ratio) for each participant at the time of DD2 enrollment. Fasting blood and urine samples are collected from each participant at enrollment and stored at -80C in the DD2 biobank.[20]

2.2. Cohort definition

This observational, prospective cohort study comprised all DD2 participants, newly diagnosed with T2D, enrolled between November 2010 and December 2016 who gave their consent and had serum MBL levels and/or MBL expression genotypes available. A total of 7588 participants were enrolled in the study, but due to withdrawal of consent or lack of blood sample only 7305 participants were available for MBL serum measurement. DNA material was available for genetic analyses in a total of 3116 participant, but due to failed genotyping for one or more of the six SNPs in the *MBL2* gene only 3043 participants were genotyped for the six SNPs in the *MBL2* gene. A total of 2990 participants had both serum MBL and MBL genotypes (Supplementary Fig. 1).

2.3. Ethics

This study was approved by the Regional Committee on Health Research Ethics for Southern Denmark (record number S-20100082) and by the Danish Data Protection Agency (record number 2008-58-0035). All DD2 participants provided written informed consent.

2.4. Identification of hospital-treated infections

We identified hospital-treated infections occurring after the DD2 enrollment date based on all diagnoses (primary and secondary discharge diagnoses) recorded in the Danish National Registry of Patients. This Registry contains discharge diagnoses from all inpatient hospitalizations in Denmark since 1977 and from all emergency department visits and hospital outpatient clinic contacts since 1995.[21] Discharge diagnoses are coded according to the International Classification of Diseases, Tenth Revision (Supplementary Table 1). In the current study, hospital-treated infections were classified into subtypes consistent with previous studies,[22, 23] i.e., bacterial infections, viral infections, and fungal infections. Bacterial diseases included pneumonia, urinary tract infections, skin infections, sepsis, abscesses, intra-abdominal infections, diarrheal diseases, and other bacterial infections. Viral diseases included influenza and other viral infections.

2.5. Identification of community-based antimicrobial prescriptions

We used the Danish Health Service Prescription Database to identify community-based antimicrobial prescriptions redeemed after the DD2 enrollment date. The Database contains information on all drugs prescribed to patients by either hospital or primary care based physicians and dispensed from any Danish pharmacy.[24] Antimicrobial prescriptions were classified according to the Anatomical Therapeutic Chemical system (Supplementary Table 2). The prescriptions were classified into subtypes consistent with previous studies,[22] i.e., dispensed prescriptions for all antimicrobial agents prescribed for oral treatment of bacterial, viral, and fungal infections. As a proxy for respiratory tract infections, we identified the number of prescriptions dispensed for oral treatment with phenoxymethylpenicillin (the recommended first-line treatment for respiratory tract infections in Denmark) and specific macrolides (erythromycin, roxithromycin, and clarithromycin).[22] As a proxy for skin infections, we identified dispensed prescriptions for dicloxacillin and flucloxacillin.[22] As a proxy for urinary tract infections, we used dispensed prescriptions for pivmecillinam, sulfamethizole, nitrofurantoin, and trimethoprim. Furthermore, we evaluated dispensed prescriptions for commonly prescribed broad-spectrum penicillin in Denmark (amoxicillin and amoxicillin with an enzyme inhibitor), which may be used for several infection types.[22]

2.6. Serum MBL levels

Serum MBL levels are largely genetically determined, and therefore relatively stable over time.[25] Median serum MBL concentration in healthy Caucasians is 800–1000 µg/L.[26] The DD2 biobank measured functional serum MBL levels at the time of enrolment using an in-house time-resolved immuno-fluorometric assay, as described in detail elsewhere.[27] In brief, mannan-coated microtiter wells were incubated with serum samples, and bound MBL was detected with biotin-labeled monoclonal anti-MBL antibodies followed by europium-labeled streptavidin and detection using time-resolved fluorometry. The limit of quantification was 10 µg/L and the intra-assay and inter-assay coefficients of variation were <10%.

2.7. MBL expression genotypes

Serum MBL levels vary widely between individuals mainly due to six common single nucleotide polymorphisms (SNPs) in the *MBL2* gene.[28] Three SNPs at codons 52, 54, and 57 of exon 1 are frequently referred to as variant D, B, and C, respectively, while the wild-type allele is referred to as allele A. Important SNPs in the promotor region are H/L and X/Y. Because of linkage disequilibrium, the six SNPs give rise to seven major haplotypes: HYPA, LYQA, LYPA, LXPA, LYPB, LYQC, and HYPD. These can be further combined into three MBL expression genotypes – low, intermediate, and high[4, 29] – corresponding to serum MBL levels.[28] TaqMan genotyping assays were used to genotype the six SNPs in the *MBL2* gene (rs11003125, rs7096206, rs7095891, rs5030737, rs1800451, and rs1800450) in the first 3043 consecutive patients in the DD2 cohort,[28] as described in detail elsewhere.[30]

2.8. Covariates

From the online DD2 questionnaire[19] and linked administrative and medical registries, we extracted information on covariates at the time of DD2 enrollment. Selection of covariates was based on their known association with serum MBL levels and/or risk of infections (Supplementary Table 3).

2.9. Biochemical analysis

High-sensitivity C-reactive protein (hs-CRP, mg/L) was determined by an in-house Time Resolved Immuno-fluorometric Assay, as previously described.[31] Samples were diluted 1000-fold and measured in duplicate. Intra-assay and inter-assay coefficients of variation were <5% and <6%, respectively. HbA1c, fasting blood glucose, and lipids were measured using hospitals' routine analysis procedures.

2.10. Statistical analysis

We examined the association between serum MBL levels, as a continuous variable, and risk of outcomes using restricted cubic spline models with five degrees of freedom. In addition, serum MBL levels were categorized as low (≤100 µg/L), intermediate (101–1000 µg/L), or high (>1000 µg/L).[30] Consistent with previous findings suggesting an U-shaped association of MBL with mortality and vascular outcomes (i.e., that intermediate MBL levels may be the most advantageous[30, 32, 33]), the intermediate serum MBL category (101–1000 µg/L) was used as reference.[30] The MBL expression genotypes were categorized into low, intermediate, or high, shown previously to correlate with serum MBL levels.[30, 34]

The cumulative incidence of hospital-treated infections and community-based antimicrobial prescriptions, with death as a competing risk, was plotted using STATA's stcompet command, and incidence rates were calculated using STATA's stptime command. Cox regression analysis, with time-on-study as time scale, was used to compute hazard ratios (HRs) and 95% confidence intervals (CIs) for outcomes. We detected no violations of the proportional hazards assumption.

We used three different adjustment models. In Model 1, HRs were adjusted for sex and age. In Model 2, HRs were adjusted for sex, age, diabetes duration, and levels of hs-CRP. In the fully adjusted Model 3, HRs were additionally adjusted for waist circumference, waist–hip ratio, body mass index, physical activity, smoking, alcohol consumption, comorbidities, fasting blood glucose level, HbA1c, total cholesterol level, low-density lipoprotein level, high-density cholesterol level, triglyceride level, and treatment with anti-diabetes and lipid-lowering agents. This extensive adjustment was to ensure robustness of potential associations between MBL levels and infections. Missing values for each covariate (n=5–3966; 0.1%–54%; Table 1) were imputed, in order to create a complete dataset.[30] We did not impute MBL expression genotype when this information was missing (n=4262; 58%).

In all analyses, since infections and antimicrobial prescriptions are generally common and repetitive events, we did not exclude individuals with a history of previous events prior to blood sampling (i.e., the DD2 enrollment date), assuming that most patients with an infectious disease history would have fully recovered if they were able to attend the DD2 enrollment examination. We followed the patients from index date (i.e., DD2 enrollment date) until a first infection occurred (separately for hospital-diagnosed infections and antimicrobial prescriptions), emigration, death, or end of follow-up, whichever came first. We did not consider recurrent infectious events during follow-up in the analyses, due to relative short follow-up time and few events in the exposure groups (three categories). Instead, we display descriptive characteristics of recurrent event infections. For hospital-treated infections, end of follow-up was August 10, 2018. For community-based antimicrobial prescriptions, due to data availability the end of follow-up was December 31, 2017. Vital and emigration status, as well as exact dates of death, were obtained from the Danish Civil Registration System.[35]

Hardy–Weinberg equilibrium was evaluated by a χ^2 test to assess risk of genotype misclassification. To evaluate the association between MBL expression genotype and serum MBL levels, we performed a Cuzick non-parametric test for trend and calculated R^2 using a simple linear regression.

2.11. Sensitivity analysis

We performed a sensitivity analysis excluding patients with serum CRP levels above 10 mg/L (n=641; 9%) at time of enrollment, to eliminate those with possible ongoing infections and/or underlying autoimmune disease at the time of MBL testing.[23] Analyses were performed using STATA version 14.2.

3. Results

3.1. Baseline characteristics

The study included 7588 patients with type 2 diabetes, among whom 7305 (96%) had a serum MBL measurement available and 3043 (42%) were genotyped for the six SNPs in the *MBL2* gene (Supplementary Fig. 1). The cohort was followed for up to 8 years, with a median follow-up of 4.5 years (inter-quartile range [IQR]: 3.0–5.5 years) for any hospital-treated infection and 1.5 years (IQR: 0.5–3.1 years) for the much more common event of any community-based antimicrobial prescription. 1140 patients (16%) were hospitalized with an infection and a total 5077 patients

(70%) redeemed a community-based antimicrobial prescription. Supplementary Table 4 presents these events by patient subtype. During follow-up, a total of 14% of individuals experienced a bacterial infection-related hospitalization (relatively more males than females, Supplementary Table 5). Of these, 67% had only one, 20% had two, and 12% had three or more bacterial infection-related hospitalizations (Supplementary Table 5). Increasing number of hospitalizations was not associated with increased serum MBL levels, MBL expression genotype, or increased follow-up time (Supplementary Table 5). There was a step-wise increase in number of hospitalizations with increasing serum CRP levels and CCI (Supplementary Table 5).

There were no clear differences in baseline characteristics across different serum MBL and MBL expression genotype categories (Table 2, Supplementary Table 6).

The MBL genotype frequencies in type 2 diabetes patients were 15% for the low MBL expression genotype, 31% for the intermediate, and 55% for the high. Serum MBL levels were strongly associated with MBL expression genotypes (Supplementary Table 7, Supplementary Fig. 2; R^2=0.31, P for trend $<1\times10^{-300}$). Median serum MBL levels for patients with low, intermediate, and high MBL expression genotypes were 10 μg/L (IQR: 10–26 μg/L), 321 μg/L (IQR: 199–545 μg/L), and 1527 μg/L (IQR: 974–2394 μg/L). We detected no major deviations in Hardy-Weinberg equilibrium (Supplementary Table 8).

3.2. Serum MBL and hospital-treated infections

Cumulative incidence curves and spline models showed an association of low serum MBL with increased risk of any hospital-treated infection (Figs. 1A & 2A). The L-shape of the spline model was preserved for hospital-treated bacterial infection (Fig. 2C) and most infection subtypes (pneumonia, urinary tract infections, diarrheal diseases, and other bacterial infections) but not for all (skin infections, intra-abdominal, viral, and fungal infections) (Supplementary Fig. 3). Incidence rates are shown in Supplementary Table 9.

Compared to the intermediate serum MBL category, the adjusted HR (Model 3) for the low serum MBL level was 1.13 (95% CI 0.96–1.33) for any infections, 1.19 (95% CI 1.01–1.41) for bacterial infections, 0.71 (95% CI 0.35–1.46) for viral infections, and 0.44 (95% CI 0.09–2.06) for fungal infections (Fig. 3). The association between low serum MBL levels and bacterial infections was primarily driven by pneumonia (aHR 1.30, 95% CI 0.98–1.70), diarrheal diseases (aHR 1.77, 95% CI 0.97–3.23), and other bacterial infections (aHR 1.50, 95% CI 1.00–2.24) (Fig. 4). Compared to the intermediate serum MBL category, high serum MBL was not associated with increased risk of any infection or subtypes. However, the aHR for high serum MBL was particularly high 1.91 (95% CI 0.89–4.11) for fungal infections (Fig. 3).

3.3. MBL expression genotype and hospital-treated infections

Cumulative incidence curves showed that the low MBL expression genotype was associated with increased risk of any hospital-treated infection (Fig. 1B). Incidence rates are shown in Supplementary Table 9.

Compared to the intermediate MBL expression genotype, the adjusted HR (Model 3) for the low MBL expression genotype was 1.08 (95% CI 0.84–1.38) for any infections, 1.13 (95% CI 0.88–1.46) for bacterial infections, and 1.02 (95% CI 0.38–2.71) for viral infections (Fig. 3). Infection subtype analyses showed a clear association between low MBL expression genotype and diarrheal diseases (aHR 2.23, 95% CI 1.04–4.80) (Fig. 4). Compared to the intermediate MBL genotype expression category, high genotype expression was not associated with increased risk of any infections or subtypes. However, the aHR for high MBL expression genotype was particularly high 1.96 (95% CI 0.75–5.10) for fungal infections (Fig. 3).

3.4. Serum MBL and antimicrobial prescriptions

Cumulative incidence curves and spline models showed no clear association of low serum MBL with increased risk of any antimicrobial prescriptions (Figs. 1C & 2B), including antibacterial, antiviral, and antifungal prescriptions (Fig. 1, Supplementary Fig. 4). Incidence rates are shown in Supplementary Table 9.

Compared to the intermediate serum MBL category, the adjusted HR (Model 3) for low serum MBL was 1.06 (95% CI 0.98–1.15) for any antimicrobial prescriptions, 1.07 (95% CI 0.99–1.16) for antibacterial prescriptions, 0.73 (95% CI 0.54–0.99) for antiviral prescriptions, and 1.07 (95% CI 0.88–1.30) for antifungal prescriptions (Fig. 5). Results for individual subtypes of antibacterial prescriptions were similar (Fig. 6). Compared to intermediate MBL levels, the adjusted HRs (Model 3) for antiviral prescriptions were 0.73 (95% CI 0.54–0.99) for the low serum MBL category and 0.63 (95% CI 0.50–0.81) for the high serum MBL category. Additionally, high serum MBL was associated with increased risk of antifungal prescriptions with an aHR of 1.17 (1.01–1.36) (Fig. 5).

3.5. MBL expression genotype and antimicrobial prescriptions

Cumulative incidence curves showed that the low MBL expression genotype patients tended to have the highest risk of any antimicrobial prescriptions (Fig. 1D). Incidence rates are shown in Supplementary Table 9.

Compared to the intermediate MBL expression genotype, the aHR (Model 3) for the low MBL expression genotype was 1.18 (95% CI 1.04–1.34) for any antimicrobial prescriptions, 1.20 (95% CI 1.05–1.36) for antibacterial, 1.10 (95% CI 0.70–1.72) for antiviral, and 0.90 (95% CI 0.66–1.21) for antifungal prescriptions (Fig. 5). The risk estimate was highest for prescriptions for skin infections (aHR 1.22, 95% CI 0.97–1.53) (Fig. 6). An additional finding was that the high MBL expression genotype was also associated with a slightly increased risk of any antimicrobial prescriptions (aHR 1.10, 95% CI 1.00–1.20) and antibacterial prescriptions (aHR 1.11, 95% CI 1.02–1.22) (Fig. 5).

3.6. Sensitivity analyses

Overall, the sensitivity analyses restricted to patients with a CRP level below 10 mg/L (Supplementary Figs. 5–16) yielded results similar to those in the main analyses.

4. Discussion

In this cohort study of 7305 patients recently diagnosed with type 2 diabetes, we found evidence that genetically low MBL is a weak causal risk factor for developing infectious disease.

Our findings corroborate and extend findings from a previous Danish cohort study of 9245 individuals from the general population followed for up to 24 years.[3] This study found relative risks in the range of 1.1 to 1.7 for a number of infectious diseases (e.g., pneumonia and diarrheal diseases) associated with low versus high MBL expression genotype.[3] Even though MBL is a pivotal factor in the innate immune system, initiating the complement cascade and promoting pathogen clearance,[26, 27] the many redundant effector functions of the immune systems, e.g., other soluble pattern recognition molecules such as ficolins, may be able to compensate for the function of MBL in adults with MBL deficiency.[3] Accordingly, it has been suggested that MBL deficiency may only increase risk of infections when other parts of the immune system are compromised,[8] e.g., by chemotherapy,[13] autoimmune and inflammatory diseases such as diabetes, or cancer.[12] MBL deficiency in combination with type 2 diabetes may act as a dual hit to the immune system and thus increase the susceptibility of infections. We found that low MBL was a relatively weak risk factor for developing infections in type 2 diabetes, mostly driven by a slightly increased risk for bacterial infections, with a stronger signal for diarrheal diseases in particular. However, our findings do not suggest that MBL deficiency is of substantial clinical relevance in relation to vulnerability to infections. Recurrent infections, with chronic diarrhea as the most common complaint, has been associated with MBL deficiency in a small study of 104 patients.[36] We found no indication of an association between MBL deficiency and recurrent hospital-treated bacterial infections. This accords with a case-control study of 120 MBL-deficient adults showing that individuals with the low MBL expression genotype were more likely to suffer from gastrointestinal disease than individuals with the intermediate or high MBL expression genotype.[37] The participants in the DD2 cohort are newly diagnosed with type 2 diabetes with a median diabetes duration 1.3 years, one could speculate that risk of infection may increase with diabetes duration or concomitant complications.

As an additional finding, we found that high serum MBL and corresponding high MBL expression genotype were compatible with a two-fold increased risk of hospital-treated fungal infections. MBL binds to different fungal pathogens through carbohydrate ligands such as mannan.[2, 38] In patients in a high-risk intensive-care unit no association between low or high MBL levels and Candida colonization and intra-abdominal candidiasis were found.[39] Thus, further studies are needed to address a possible causal association between MBL and fungal infections.

Main strengths of this large nationwide cohort study include the assessment of detailed covariates and outcomes available in the DD2 database, DD2 biobank, and by linkage with high-quality population-based health registries.[40] These resources provide nearly 100% completeness for serum MBL and complete follow-up for outcome events, both when defining an infectious disease event as a hospital contact due to infection and as a redeemed prescription for an antimicrobial agent.

Limitations of this study include the possibility of survival bias and selection bias for patients with certain MBL levels and outcome risks before entering the DD2 cohort. Such bias would likely lead to decreased participation of patients with a severe type 2 diabetes phenotype or high infectious disease risk and likely bias results towards the null hypothesis. Of note, age, demographic characteristics, diabetes characteristics and comorbidities of early type 2 diabetes patients in the DD2 cohort are very similar to those of patients with early treated type 2 diabetes in everyday clinical practice in Denmark.[18, 41] Misclassification of diagnoses, prescriptions, or genotypes in our cohort may have happened. However, the validity of major diagnoses in our registries, including for infectious disease diagnoses is high[42], and the MBL genotype frequencies were similar to frequencies in the general population[3] and in patients with type 1 diabetes.[26] Another potential limitation is that prescribed antimicrobial drugs are only a proxy for true presence of infection. In the community setting, antimicrobial agents might be prescribed outside their proper indication, and general practitioners' approach to treating infectious diseases may vary,[22] which may have resulted in some outcome misclassification. However, we expect such misclassification to be non-differential as regards the patients' MBL level, likely biasing results towards the null hypothesis. Yet another potential limitation is that we did not account for autoimmune diseases, which are associated with increased risk of infection. However, excluding patients with serum CRP levels above 10 mg/L, to eliminate those with possible ongoing infections and/or autoimmune disease at time of blood sampling did not alter the results, and thus cannot explain our findings. Finally, due to our relatively low sample size and thus low precision (i.e., wide 95% CI), we acknowledge that we lack power to show robust results. However, as several point estimates were compatible with an association and may be biologically plausible, our findings justify and encourage future efforts in investigating the role of MBL in the development of and/or predisposition to infections. Indeed, we have previously shown that MBL may be clinically important in patients with T2D, since patients with low as well as high serum MBL have an increased risk of cardiovascular events, and thus may benefit from a more aggressive preventative treatment.[30]

In conclusion, this cohort study supports that genetically low MBL is a weak causal risk factor for infections in patients with type 2 diabetes. A possible role of low MBL in causing bacterial infections and diarrheal diseases in particular in patients with type 2 diabetes merits further studies.

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Conflicts of Interest

None.

Author Contributions

H.B.N., H.T.S., and J.R. participated in planning and designing the parent DD2 project cohort study. R.W.T, A.D.K., J.S.N., J.R., S.F., I.B., H.B.N., H.T.S., T.K.H., and M.B. designed the current study. M.B. was responsible for serum MBL and hs-CRP measurements, and R.S. was responsible for MBL genotyping. I.B. was responsible for obtaining data from the biobank and overseeing the other biochemical analyses. A.G., A.D.K., and R.W.T. participated in the design of the current study, and A.G. performed the statistical analyses. A.G. drafted the article, with help from A.D.K., R.W.T., and M.B. All other authors have critically reviewed the manuscript. All authors contributed substantially to the study, revised the manuscript for intellectual content, and approved the final version to be submitted. A.G., R.W.T., and M.B. are the guarantors of this work and, as such, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses.

Prior presentation

This study was presented as a poster presentation at the annual virtual meeting of the European Association for the Study of Diabetes (EASD), 21-25 September, 2020.

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Table 1. Missing covariates for the serum MBL and MBL expression genotype cohorts within the DD2 cohort.

	Serum MBL cohort		MBL expression genotype cohort	
	Missing, n (%)	Total	Missing, n (%)	Total
Sex	0 (0.0)	7305	0 (0.0)	3043
Age	0 (0.0)	7305	0 (0.0)	3043
Diabetes duration	0 (0.0)	7305	0 (0.0)	3043
Waist circumference	13 (0.18)	7305	<5[b] (0.2)	3043
Waist–hip ratio	11 (0.15)	7305	<5 [b] (0.2)	3043
BMI[a]	569 (7.79)	7305	245 (8.05)	3043
Alcohol intake	0 (0.0)	7305	0 (0.0)	3043
Physical activity	<5[b] (0.0)	7305	0 (0.0)	3043
Smoking[a]	1900 (26.01)	7305	612 (20.11)	3043
CCI score	0 (0.0)	7305	0 (0.0)	3043
Anti-diabetes drug use	0 (0.0)	7305	0 (0.0)	3043
Lipid-lowering drug use	0 (0.0)	7305	0 (0.0)	3043
Fasting blood glucose	2028 (27.76)	7305	368 (12.09)	3043
HbA1c[a]	1548 (21.19)	7305	486 (15.97)	3043
Total cholesterol[a]	3966 (54.29)	7305	1244 (40.88)	3043
LDL cholesterol[a]	1763 (24.13)	7305	550 (18.07)	3043
HDL cholestero[a]	3951 (54.09)	7305	1245 (40.91)	3043
Triglycerides[a]	1849 (25.31)	7305	572 (18.80)	3043
hs-CRP	5 (0.07)	7305	56 (1.84)	3043

[a]By August 2018, a total of 5847 DD2 patients (80%) in the serum MBL cohort and 2597 DD2 patients (85%) in the MBL genotype cohort had been linked to the Danish Diabetes Database for Adults.
[b] Exact number of missing too low to be displayed according to Danish data protection regulations.

Table 2. Characteristics of DD2 Cohort Participants at Baseline by Serum MBL Category.

	Low serum MBL (≤100 μg/L)	Intermediate serum MBL (101–1000 μg/L)	High serum MBL (>1000 μg/L)
Total, N (%)	1295 (17.7)	2975 (40.7)	3035 (41.6)
Male sex, n (%)	727 (56.1)	1612 (54.2)	1939 (63.9)
Median age (IQR), years	61.6 (52.7–69.0)	61.9 (53.1–68.7)	62.3 (53.0–68.8)
Median diabetes duration (IQR), years	1.3 (0.3–2.9)	1.4 (0.4–2.9)	1.2 (0.3–2.9)
Median waist circumference (IQR), cm	106 (97–117)	107 (97–117)	105 (96–115)
Median waist–hip ratio (IQR)	0.98 (0.92–1.04)	0.98 (0.92–1.04)	0.98 (0.93–1.04)
Median body mass index (IQR), kg/m^2	30.5 (27.1–34.5)	30.7 (27.4–34.7)	29.7 (26.4–33.7)
High alcohol intake[a], n (%)	74 (5.7)	211 (7.1)	190 (6.3)
Physical activity[b] (IQR), days/week	3 (2–7)	3 (2–7)	4 (2–7)
Smoking, n (%)			
Never	434 (45.5)	1039 (47.7)	1052 (46.3)
Former	351 (36.8)	749 (34.4)	750 (33.0)
Current	170 (17.8)	389 (17.9)	471 (20.7)
CCI score[c], n (%)			
0	882 (68.1)	2034 (68.4)	2109 (69.5)
1-2	339 (26.2)	783 (26.3)	763 (25.1)
3	74 (5.7)	158 (5.3)	163 (5.4)
Anti-diabetes drug use, n (%)	1080 (83.4)	2547 (85.6)	2582 (85.1)
Lipid-lowering drug use, n (%)	932 (72.0)	2176 (73.1)	2037 (67.1)
Median fasting blood glucose level (IQR), mmol/L	7.1 (6.3–8.1)	7.1 (6.3–8.2)	7.2 (6.4–8.3)
Median HbA1c (IQR), mmol/mol	49 (44–55)	49 (43–55)	49 (44–56)
Median HbA1c (IQR), %	6.6 (6.2–7.2)	6.6 (6.1–7.2)	6.6 (6.2–7.3)
Median total cholesterol level (IQR), mmol/L	4.4 (3.8–5.2)	4.3 (3.7–5.1)	4.3 (3.7–5.1)
Median LDL cholesterol level (IQR), mmol/L	2.1 (1.7–2.7)	2.2 (1.7–2.8)	2.2 (1.7–2.9)
Median HDL cholesterol level (IQR), mmol/L	1.2 (1.0–1.4)	1.2 (1.0–1.4)	1.2 (1.0–1.5)
Median triglyceride level (IQR), mmol/L	1.7 (1.2–2.5)	1.7 (1.2–2.4)	1.6 (1.1–2.3)
Median hs-CRP level (IQR), mg/L	2.0 (0.8–4.7)	2.0 (0.9–4.5)	1.9 (0.8–4.3)

Abbreviations: MBL, mannose-binding lectin; IQR, interquartile range; CCI, Charlson Comorbidity Index; hs-CRP, high-sensitivity C-reactive protein
[a]High alcohol intake was defined as >14/21 alcoholic drinks/week for women/men.
[b]Days per week with a minimum of 30 minutes of physical activity.
[c]CCI (Charlson Comorbidity Index) score excluding diabetes.
Numbers of participants varied because of availability of data (see Table 1 for missing covariates).

Figure Legends

Fig. 1. Time-to-event curves of any hospital-treated infections and any community-based antimicrobial prescriptions by serum MBL levels and MBL expression genotype categories. Cumulative incidence plots of any hospital-treated infections (A and B) and any community-based antimicrobial prescriptions (C and D) by serum MBL (A and C) and MBL expression genotype (B and D) categories. Cumulative incidence estimates are based on time from the DD2 enrollment date to the first event, with risk of death representing a competing risk.

Fig. 2. Risk of hospital-treated infections and community-based antimicrobial prescriptions by serum MBL levels. Any infections (A), bacterial infections (C), viral infections (E), fungal infections (G), any prescriptions (B), antibacterial prescriptions (D), antiviral prescriptions (F), and antifungal prescriptions (H). The solid lines indicate the hazard ratios and the dotted lines indicate 95% confidence intervals. Serum MBL as a continuous variable was modeled with five restricted cubic splines.

Fig. 3. Hazard ratios of any hospital-treated infections, and bacterial, viral, and fungal infections by serum MBL levels and MBL expression genotype categories.
Model 3 was adjusted for sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, alcohol consumption, comorbidities, fasting blood glucose, HbA1c, total cholesterol, LDL, HDL, triglycerides, and use of anti-diabetes, and lipid-lowering drugs. Values for missing covariates were estimated using multiple imputation (Table 1).

Fig. 4. Hazard ratios of hospital-treated bacterial infection subtypes by serum MBL levels and MBL expression genotype categories.

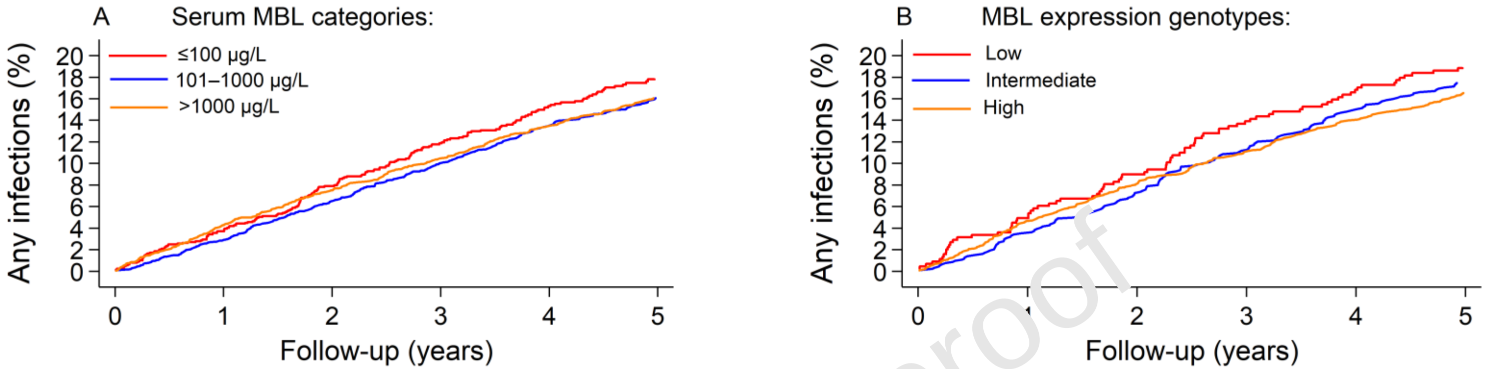
Model 3 was adjusted for sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, alcohol consumption, comorbidities, fasting blood glucose, HbA1c, total cholesterol, LDL, HDL, triglycerides, and use of anti-diabetes, and lipid-lowering drugs. Values for missing covariates were estimated using multiple imputation (Table 1).

Fig. 5. Hazard ratios of any community-based antimicrobial prescriptions, and antibacterial, antiviral, and antifungal prescriptions by serum MBL levels and MBL expression genotype categories.

Model 3 was adjusted for sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, alcohol consumption, comorbidities, fasting blood glucose, HbA1c, total cholesterol, LDL, HDL, triglycerides, and use of anti-diabetes, and lipid-lowering drugs. Values for missing covariates were estimated using multiple imputation (Table 1).

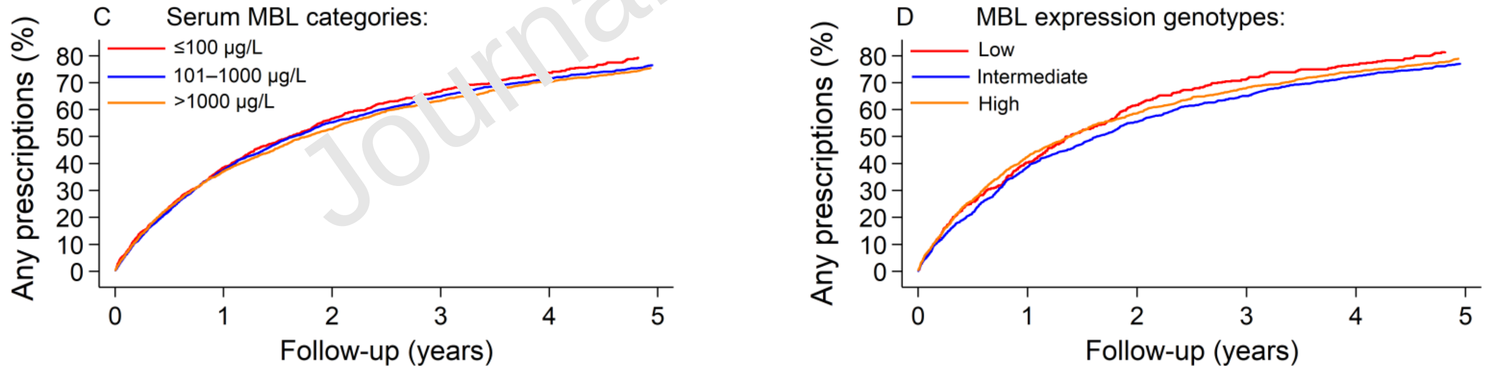
Fig. 6. Hazard ratios of community-treated prescription subtypes by serum MBL levels and MBL expression genotype categories.

Model 3 was adjusted for sex, age, diabetes duration, hs-CRP, waist circumference, waist–hip ratio, body mass index, physical activity, smoking, alcohol consumption, comorbidities, fasting blood glucose, HbA1c, total cholesterol, LDL, HDL, triglycerides, and use of anti-diabetes, and lipid-lowering drugs. Values for missing covariates were estimated using multiple imputation (Table 1).



Number at risk by serum MBL						
≤100 µg/L	1295	1239	1141	950	727	449
101–1000 µg/L	2975	2879	2642	2224	1699	1085
>1000 µg/L	3035	2882	2673	2276	1767	1136

Number at risk MBL genotype						
Low	446	423	403	379	361	350
Intermediate	939	904	861	819	781	752
High	1658	1569	1499	1435	1377	1319



Number at risk by serum MBL						
≤100 µg/L	1295	792	502	308	177	68
101–1000 µg/L	2975	1850	1177	760	427	171
>1000 µg/L	3035	1893	1271	819	486	191

Number at risk MBL genotype						
Low	446	265	169	126	101	55
Intermediate	939	573	412	321	253	140
High	1658	946	673	513	407	224

Figure 1.

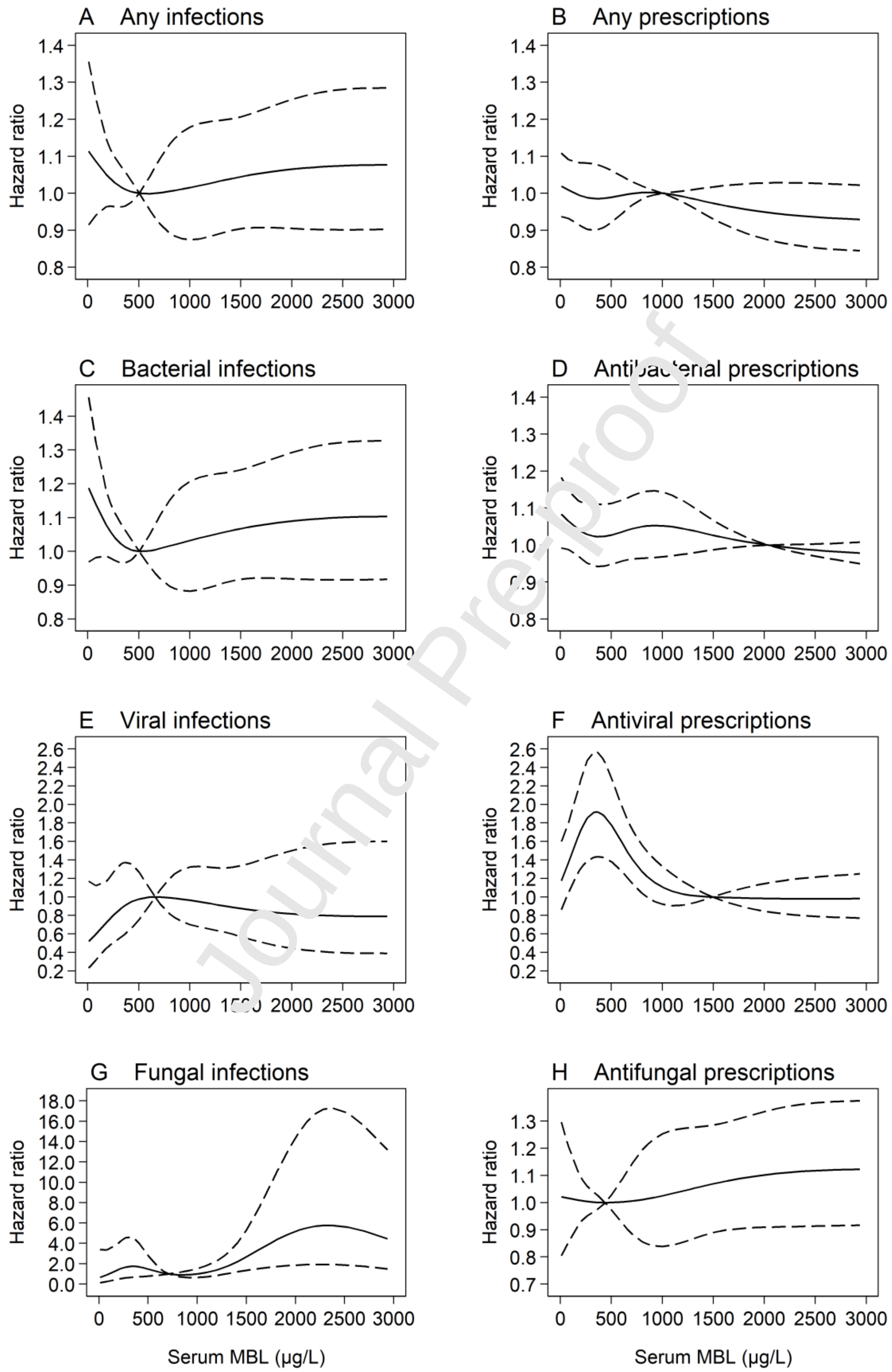
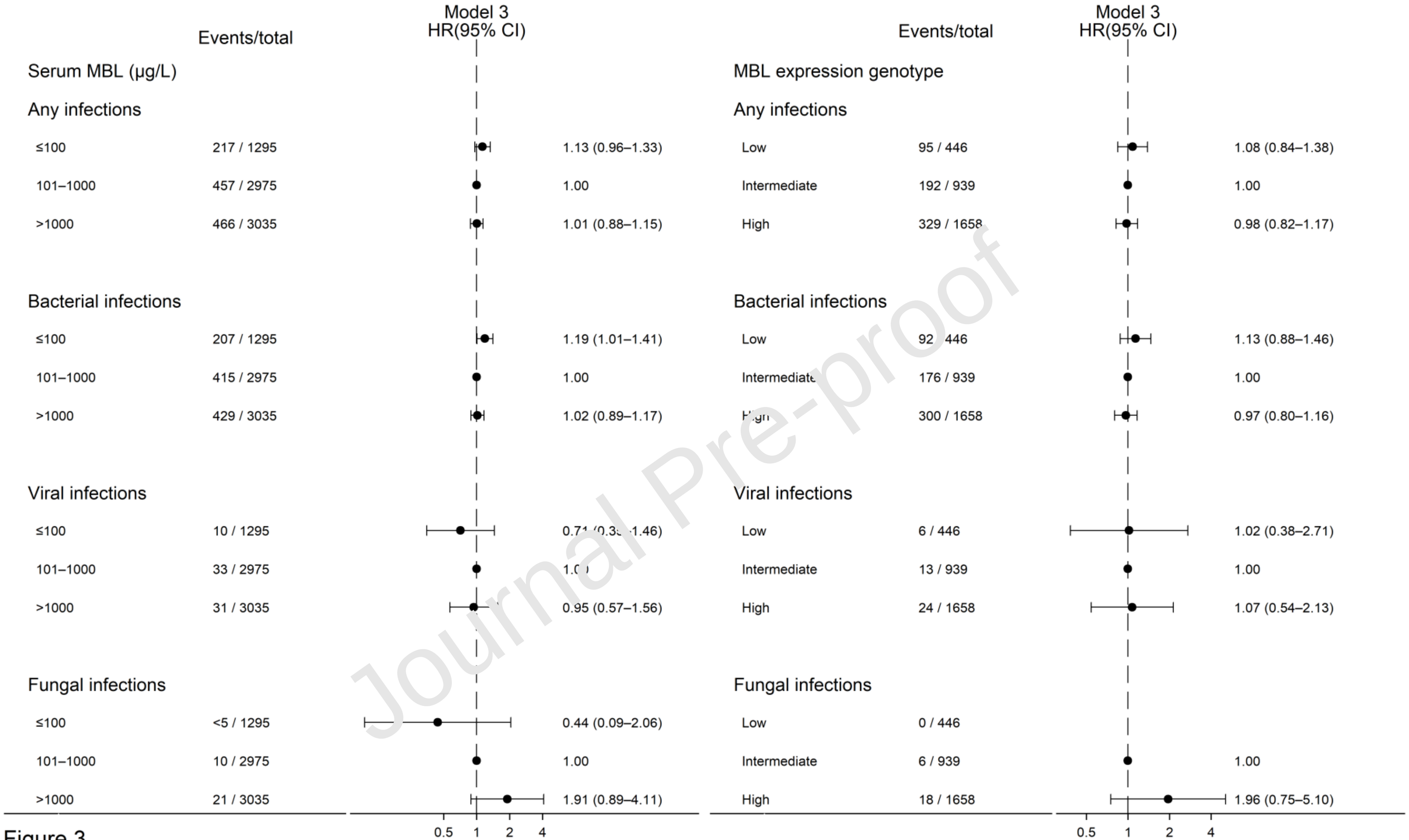

A Any infections
B Any prescriptions
C Bacterial infections
D Antibacterial prescriptions
E Viral infections
F Antiviral prescriptions
G Fungal infections
H Antifungal prescriptions
Hazard ratio
Serum MBL (µg/L)

Figure 2.


Events/total
Model 3
HR(95% CI)
Serum MBL (µg/L)
Any infections
≤100 217 / 1295 1.13 (0.96–1.33)
101–1000 457 / 2975 1.00
>1000 466 / 3035 1.01 (0.88–1.15)
Bacterial infections
≤100 207 / 1295 1.19 (1.01–1.41)
101–1000 415 / 2975 1.00
>1000 429 / 3035 1.02 (0.89–1.17)
Viral infections
≤100 10 / 1295
101–1000 33 / 2975
>1000 31 / 3035 0.95 (0.57–1.56)
Fungal infections
≤100 <5 / 1295 0.44 (0.09–2.06)
101–1000 10 / 2975 1.00
>1000 21 / 3035 1.91 (0.89–4.11)
0.5 1 2 4
Events/total
Model 3
HR(95% CI)
MBL expression genotype
Any infections
Low 95 / 446 1.08 (0.84–1.38)
Intermediate 192 / 939 1.00
High 329 / 1658 0.98 (0.82–1.17)
Bacterial infections
Low 1.13 (0.88–1.46)
176 / 939 1.00
300 / 1658 0.97 (0.80–1.16)
Viral infections
Low 6 / 446 1.02 (0.38–2.71)
Intermediate 13 / 939 1.00
High 24 / 1658 1.07 (0.54–2.13)
Fungal infections
Low 0 / 446
Intermediate 6 / 939 1.00
High 18 / 1658 1.96 (0.75–5.10)
0.5 1 2 4
Figure 3.

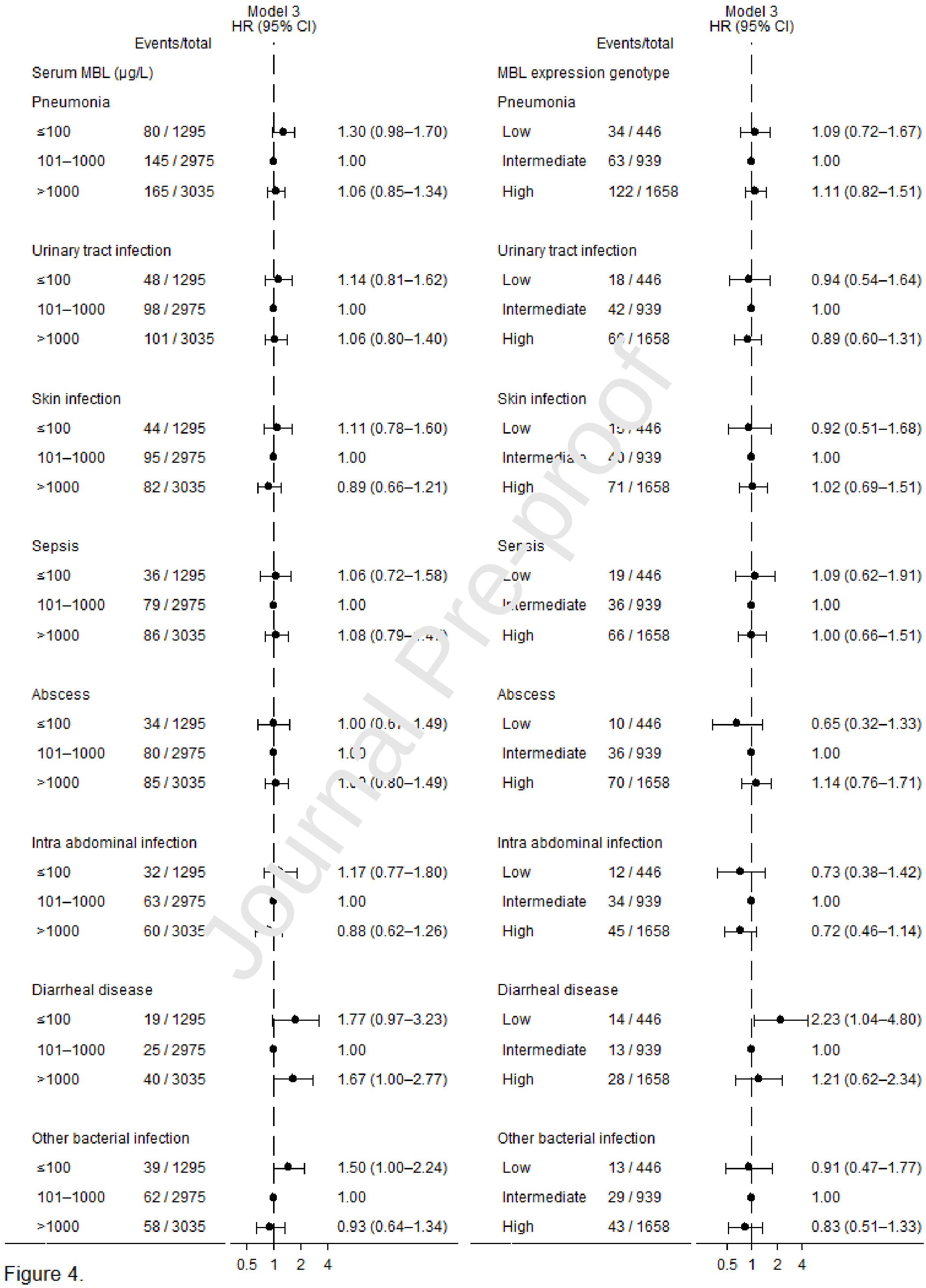

Model 3
HR (95% CI)
Events/total
Serum MBL (μg/L)
Pneumonia
≤100 80 / 1295 1.30 (0.98–1.70)
101–1000 145 / 2975 1.00
>1000 165 / 3035 1.06 (0.85–1.34)
Urinary tract infection
≤100 48 / 1295 1.14 (0.81–1.62)
101–1000 98 / 2975 1.00
>1000 101 / 3035 1.06 (0.80–1.40)
Skin infection
≤100 44 / 1295 1.11 (0.78–1.60)
101–1000 95 / 2975 1.00
>1000 82 / 3035 0.89 (0.66–1.21)
Sepsis
≤100 36 / 1295 1.06 (0.72–1.58)
101–1000 79 / 2975 1.00
>1000 86 / 3035 1.08 (0.79–
Abscess
≤100 34 / 1295 1.00 (0.6 1.49)
101–1000 80 / 2975 1.00
>1000 85 / 3035 (0.80–1.49)
Intra abdominal infection
≤100 32 / 1295 1.17 (0.77–1.80)
101–1000 63 / 2975 1.00
>1000 60 / 3035 0.88 (0.62–1.26)
Diarrheal disease
≤100 19 / 1295 1.77 (0.97–3.23)
101–1000 25 / 2975 1.00
>1000 40 / 3035 1.67 (1.00–2.77)
Other bacterial infection
≤100 39 / 1295 1.50 (1.00–2.24)
101–1000 62 / 2975 1.00
>1000 58 / 3035 0.93 (0.64–1.34)
0.5 1 2 4
Model 3
HR (95% CI)
Events/total
MBL expression genotype
Pneumonia
Low 34 / 446 1.09 (0.72–1.67)
Intermediate 63 / 939 1.00
High 122 / 1658 1.11 (0.82–1.51)
Urinary tract infection
Low 18 / 446 0.94 (0.54–1.64)
Intermediate 42 / 939 1.00
High / 1658 0.89 (0.60–1.31)
Skin infection
Low 0.92 (0.51–1.68)
Intermediate 1.00
High 71 / 1658 1.02 (0.69–1.51)
Sepsis
Low 19 / 446 1.09 (0.62–1.91)
Intermediate 36 / 939 1.00
High 66 / 1658 1.00 (0.66–1.51)
Abscess
Low 10 / 446 0.65 (0.32–1.33)
Intermediate 36 / 939 1.00
High 70 / 1658 1.14 (0.76–1.71)
Intra abdominal infection
Low 12 / 446 0.73 (0.38–1.42)
Intermediate 34 / 939 1.00
High 45 / 1658 0.72 (0.46–1.14)
Diarrheal disease
Low 14 / 446 2.23 (1.04–4.80)
Intermediate 13 / 939 1.00
High 28 / 1658 1.21 (0.62–2.34)
Other bacterial infection
Low 13 / 446 0.91 (0.47–1.77)
Intermediate 29 / 939 1.00
High 43 / 1658 0.83 (0.51–1.33)
0.5 1 2 4

Figure 4.

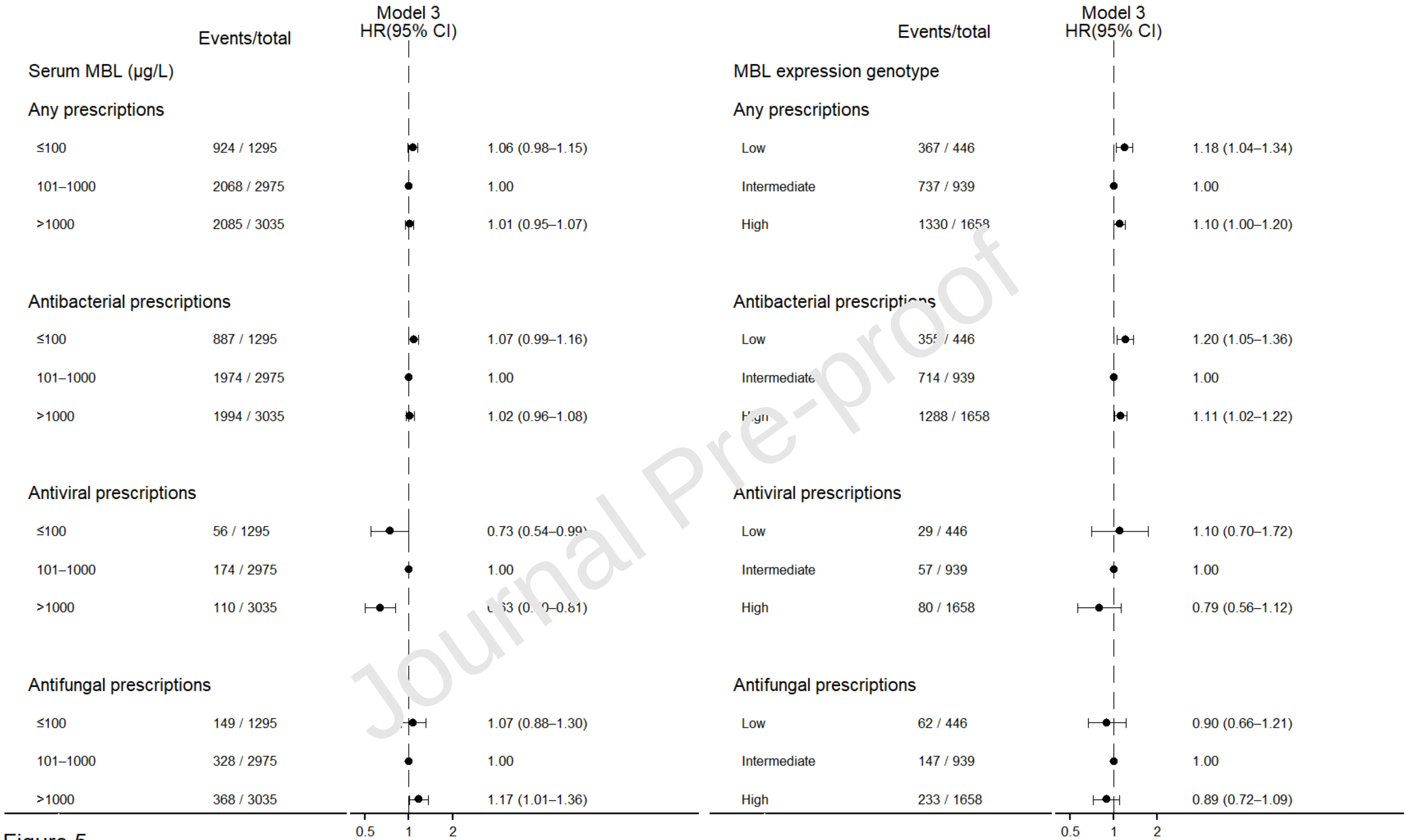

Events/total
Model 3
HR(95% CI)
Serum MBL (µg/L)
Any prescriptions
≤100 924 / 1295 1.06 (0.98–1.15)
101–1000 2068 / 2975 1.00
>1000 2085 / 3035 1.01 (0.95–1.07)
Antibacterial prescriptions
≤100 887 / 1295 1.07 (0.99–1.16)
101–1000 1974 / 2975 1.00
>1000 1994 / 3035 1.02 (0.96–1.08)
Antiviral prescriptions
≤100 56 / 1295 0.73 (0.54–0.99)
101–1000 174 / 2975 1.00
>1000 110 / 3035 63 (0. 0–0.81)
Antifungal prescriptions
≤100 149 / 1295 1.07 (0.88–1.30)
101–1000 328 / 2975 1.00
>1000 368 / 3035 1.17 (1.01–1.36)
0.5
1
2
Events/total
Model 3
HR(95% CI)
MBL expression genotype
Any prescriptions
Low 367 / 446 1.18 (1.04–1.34)
Intermediate 737 / 939 1.00
High 1330 / 1658 1.10 (1.00–1.20)
Antibacterial prescriptions
Low 355 / 446 1.20 (1.05–1.36)
Intermediate 714 / 939 1.00
High 1288 / 1658 1.11 (1.02–1.22)
Antiviral prescriptions
Low 29 / 446 1.10 (0.70–1.72)
Intermediate 57 / 939 1.00
High 80 / 1658 0.79 (0.56–1.12)
Antifungal prescriptions
Low 62 / 446 0.90 (0.66–1.21)
Intermediate 147 / 939 1.00
High 233 / 1658 0.89 (0.72–1.09)
0.5
1
2

Figure 5.

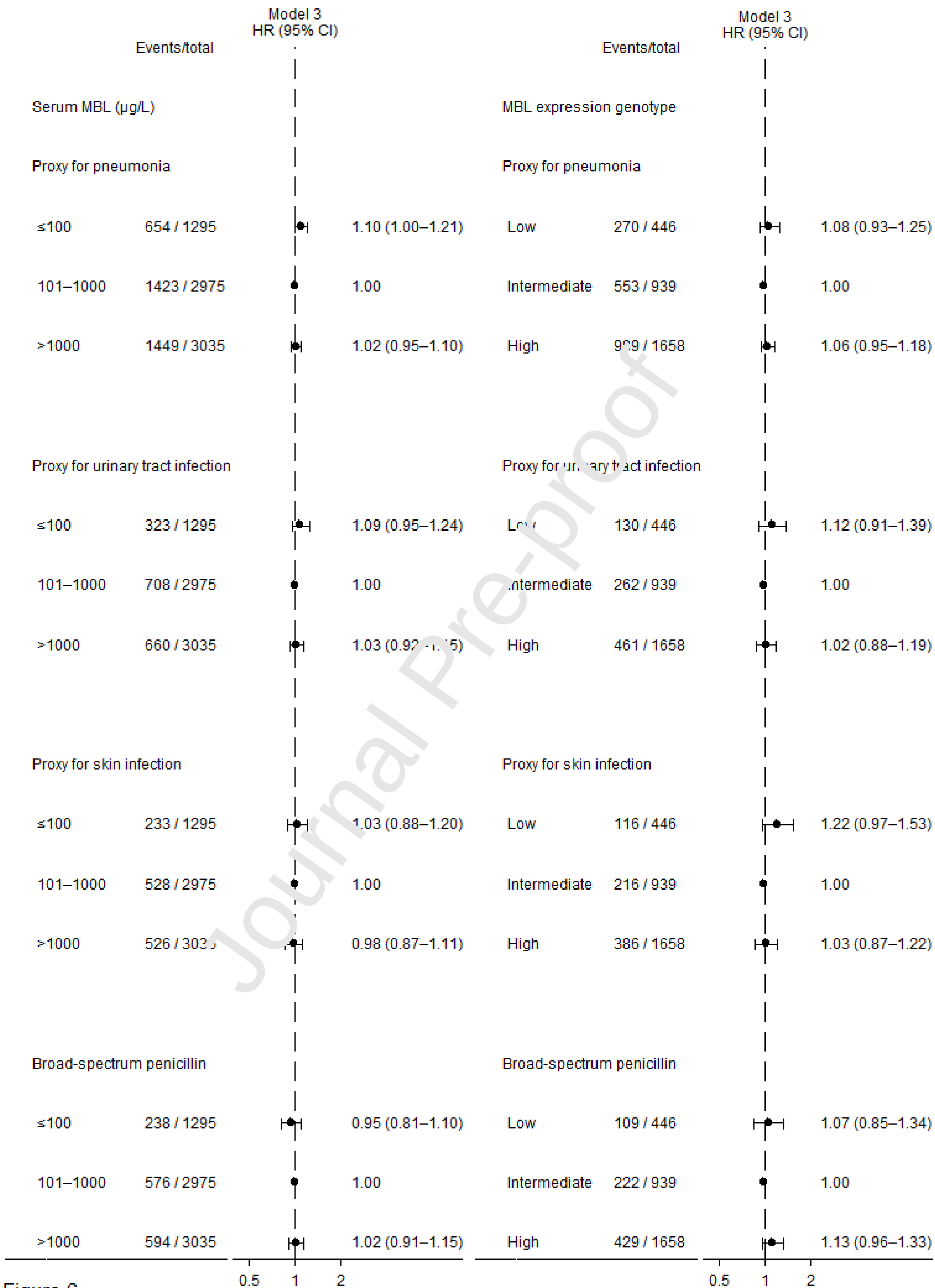

Model 3
HR (95% CI)
Events/total
Serum MBL (µg/L)
Proxy for pneumonia
≤100 654 / 1295 1.10 (1.00–1.21)
101–1000 1423 / 2975 1.00
>1000 1449 / 3035 1.02 (0.95–1.10)
Proxy for urinary tract infection
≤100 323 / 1295 1.09 (0.95–1.24)
101–1000 708 / 2975 1.00
>1000 660 / 3035
Proxy for skin infection
≤100 233 / 1295 1.03 (0.88–1.20)
101–1000 528 / 2975 1.00
>1000 0.98 (0.87–1.11)
Broad-spectrum penicillin
≤100 238 / 1295 0.95 (0.81–1.10)
101–1000 576 / 2975 1.00
>1000 594 / 3035 1.02 (0.91–1.15)
0.5 1 2
Model 3
HR (95% CI)
Events/total
MBL expression genotype
Proxy for pneumonia
Low 270 / 446 1.08 (0.93–1.25)
Intermediate 553 / 939 1.00
High / 1658 1.06 (0.95–1.18)
130 / 446 1.12 (0.91–1.39)
262 / 939 1.00
High 461 / 1658 1.02 (0.88–1.19)
Proxy for skin infection
Low 116 / 446 1.22 (0.97–1.53)
Intermediate 216 / 939 1.00
High 386 / 1658 1.03 (0.87–1.22)
Broad-spectrum penicillin
Low 109 / 446 1.07 (0.85–1.34)
Intermediate 222 / 939 1.00
High 429 / 1658 1.13 (0.96–1.33)
0.5 1 2

Figure 6.

Author Statement

All authors have seen and approved the content, have contributed significantly to the work, and fulfill the criteria given in the Authorship paragraph.
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Author Contributions

H.B.N., H.T.S., and J.R. participated in planning and designing the parent DD2 project cohort study. R.W.T, A.D.K., J.S.N., J.R., S.F., I.B., H.B.N., H.T.S., T.K.H., and M.B. designed the current study. M.B. was responsible for serum MBL and hs-CRP measurements, and R.S. was responsible for MBL genotyping. I.B. was responsible for obtaining data from the biobank and overseeing the other biochemical analyses. A.G., A.D.K., and R.W.T. participated in the design of the current study, and A.G. performed the statistical analyses. A.G. drafted the article, with help from A.D.K., R.W.T., and M.B. All other authors have critically reviewed the manuscript. All authors contributed substantially to the study, revised the manuscript for intellectual content, and approved the final version to be submitted. A.G., R.W.T., and M.B. are the guarantors of this work and, as such, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- In persons at increased baseline risk of infections such as patients with type 2 diabetes, low MBL may have detrimental effects but studies are nonexistent.
- Genetically low serum MBL was weakly associated with increased risk of developing infections in patients with type 2 diabetes.
- This association was mainly driven by an association with bacterial infections.
- Low MBL is only a weak causal risk factor for developing infections in patients with type 2 diabetes.
- A possible role of low MBL in causing bacterial infections in patients with type 2 diabetes merits further studies.