Increased All-Cause Mortality in Patients With Type 1 Diabetes and High-Expression Mannan-Binding Lectin Genotypes: A 12-Year Follow-up Study

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OBJECTIVE

Mannan-binding lectin (MBL) is a complement-activating carbohydrate-recognizing molecule associated with diabetic nephropathy. MBL is associated with all-cause mortality in type 2 diabetes, but whether MBL is associated with mortality in type 1 diabetes remains unknown. We therefore aimed to investigate this.

RESEARCH DESIGN AND METHODS

We studied an existing 12-year prospective cohort with type 1 diabetes with 198 patients with diabetic nephropathy (121 men, age 41 years [95% CI 40–42], estimated glomerular filtration rate [eGFR] 67 mL/min/1.73 m^2 [95% CI 63–70]) and 174 normoalbuminuric patients (103 men, age 43 years [95% CI 41–44], eGFR 93 mL/min/1.73 m^2 [95% CI 91–95]). Mortality rates were compared according to the concentration-determining *MBL2* genotype or the MBL concentration. Patients were classified as having high or low MBL expression genotypes. The effect of MBL concentration was estimated by comparing patients with MBL concentrations above or below the median.

RESULTS

Ninety-eight patients died during follow-up. The unadjusted hazard ratio (HR) for all-cause mortality was 1.61 (95% CI 1.07–2.43) for patients with high MBL expression genotypes versus patients with low MBL expression genotypes (P = 0.023). All-cause mortality was higher in patients with MBL concentrations above the median than in patients with MBL concentrations below the median (unadjusted HR 1.90 [95% CI 1.26–2.87], P = 0.002).

CONCLUSIONS

High MBL expression genotypes and high MBL concentrations are both associated with increased mortality rates in type 1 diabetes compared with low MBL expression genotypes and low MBL concentrations.

Late diabetes complications contribute to excess morbidity and mortality in diabetes. The complement cascade of the immune system is most likely implicated in the development of these complications (1). Diabetes may induce the formation of complement-activating ligands by two mechanisms: altered enzymatic protein glycosylation and increased nonenzymatic advanced glycation end product formation. Both products are hypothesized to be adversely recognized by complement-activating

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pattern-recognition molecules and induce complement auto-attack. Several studies have demonstrated the close association between the lectin pathway of complement activation and diabetic nephropathy. In particular, high concentrations of the carbohydrate pattern–recognition molecule mannan-binding lectin (MBL), which initiates complement activation, are linked to diabetic nephropathy defined by urinary albumin excretion rate (2–8). In the present cohort, we previously showed the cross-sectional association between *MBL2* genotype and diabetic nephropathy (2). We have further substantiated the possible cause-and-effect relationship, as MBL aggravates diabetic kidney changes in mice (9,10).

The circulating MBL concentration is predominantly determined by polymorphisms in the *MBL2* gene (11). However, MBL concentrations are higher in patients with diabetes than in healthy subjects, despite similar *MBL2* genotype distribution (2,6,12,13). Animal studies suggest this to be explained by a secondary increase in MBL concentration after diabetes induction (14). Finally, we recently showed that H-ficolin, another complement-activating pattern-recognition molecule, is also associated with development of diabetic kidney changes (15). This further supports the hypothesis of diabetes-induced complement auto-attack as a mechanism leading to diabetes complications.

High MBL concentrations are associated with increased all-cause mortality in patients with type 2 diabetes (5). Whether a similar relationship exists in type 1 diabetes has not been investigated. We therefore aimed to examine the effect of the concentration-determining *MBL2* genotype and MBL concentration on all-cause mortality in a previously established cohort of patients with type 1 diabetes with 12 years of follow-up.

RESEARCH DESIGN AND METHODS

Subjects

The original cohort of patients with type 1 diabetes, with or without diabetic nephropathy, attending the Steno Diabetes Center in 1993 and the subsequent follow-up has previously been described (16,17). The study prospectively monitored the cohort consisting of patients from our outpatient clinic with persistent normoalbuminuria and patients with diabetic nephropathy matched on sex, age, and duration of diabetes. Mortality data and *MBL2* genotype were obtainable for analysis from 372 patients from this cohort. Follow-up data were obtained as previously described (18). In brief, time and cause of death were identified from the Danish National Death Registry and autopsy reports by two independent observers. Cardiovascular disease (CVD) was assumed as the cause of death unless an alternative cause was unequivocally documented (19). The study complied with the local ethics committee and the Helsinki Declaration. All patients gave their informed consent.

Baseline Characterization

Blood pressure was recorded as the average of two measurements after supine rest for 10 min. HbA_{1c} was measured by high-performance liquid chromatography (Bio-Rad, Hercules, CA, USA), and hs-CRP was quantified by an in-house ELISA, as previously described (2). Serum creatinine was measured by the Jaffé kinetic method.

Urinary albumin excretion (UAE) in 24 h collections was measured by enzyme immunoassay (20). UAE $\leq$30 mg/24 h was classified as normoalbuminuria. Diabetic nephropathy was defined as UAE $\geq$300 mg/24 h in two or more of three consecutive urine collections and concomitant retinopathy in the absence of nondiabetic explanations.

MBL2 Genotype and Serum MBL Concentration

MBL2 genotype and serum MBL concentration were obtained from blood samples drawn in the morning after an overnight fast, as previously described (21,22). In brief, common *MBL2* polymorphisms were identified in the promoter (H/L at −550 and X/Y at −221), in an untranslated sequence (P/Q at +4), and in the wild-type A allele on exon 1, (D, B, and C in codon 52, 54, 57, respectively) using real-time PCR with TaqMan single-nucleotide genotyping (Applied Biosystems, Foster City, CA) (22). As previously described, only the X/Y polymorphism of the promoter and the polymorphisms in exon 1 had a significant effect on the MBL concentration (2). The *MBL2* genotypes were divided into two groups, based on the resulting MBL concentration (2), designated as high MBL expression genotypes and low MBL expression genotypes (2). As described previously, YA/YA and XA/YA lead to the highest circulating MBL concentrations, and these are therefore designated as high MBL expression genotypes (11). YO/YO, XA/YO, YA/YO, and XA/XA (with "O" indicating mutations in exon 1) lead to lower MBL concentrations and are designated as low MBL expression genotypes (11). The haplotype distribution of the full cohort has been published previously (2).

The quantification of the circulating MBL concentration was performed by an in-house time-resolved immunofluorometric assay, as previously described (21).

Statistics

Baseline data were compared by χ^2 test, unpaired Student *t* test, and rank sum test, as appropriate. The MBL or hs-CRP concentration was missing for two patients. The Spearman correlation was used to estimate the strength and significance of the association between MBL and HbA_{1c} concentrations. Survival time was compared by the log-rank test and Cox proportional hazards analysis in complete case analyses. Traditional risk factors of mortality were investigated as potential confounders of the association between MBL and mortality. We thus investigated the possible confounding role of sex, diabetes duration, HbA_{1c}, systolic blood pressure, diabetic nephropathy, UAE, cholesterol, and smoking. Except diabetic nephropathy, none of these were associated with the exposure (*MBL2* genotype), as reported in Table 1. In addition, a collapsibility-based definition of confounders was applied, meaning that a potential confounder should change the estimated measure of association between *MBL2* genotype and mortality by more than 10% (23). From this approach, none of the traditional risk factors listed above confounded the association. For these reasons, none of the traditional risk factors were adjusted for as confounders. However, because the literature suggests that *MBL2* genotype and diabetic nephropathy are associated, we did wish to investigate the effect of diabetic nephropathy in an exploratory analysis assuming it to be on the causal pathway between MBL and death. Evaluation of time to death assumed attributable to CVD was adjusted for death of other causes as a competing risk.

Estimates are given as mean (95% CI), median (interquartile range [IQR]), or

Table 1—Baseline characteristics among 201 patients with high MBL expression genotypes and 171 patients with low MBL expression genotypes

	High MBL expression genotypes (*n* = 201)	Low MBL expression genotypes (*n* = 171)	*P* value
Age (years)	42 (41–43)	41 (40–43)	0.28
Males, *n* (%)	119 (59)	105 (61)	0.67
Diabetes duration (years)	26 [22–32]	27 [22–33]	0.40
Current smokers, *n* (%)	101 (50)	73 (43)	0.15
Systolic blood pressure (mmHg)	144 (141–147)	140 (137–144)	0.16
Diastolic blood pressure (mmHg)	82 (80–83)	81 (79–82)	0.37
HbA_{1c} (%)	9.1 (8.9–9.3)	9.0 (8.8–9.2)	0.43
HbA_{1c} (mmol/mol)	76 (74–78)	75 (73–77)	0.43
Cholesterol (mmol/L)	5.3 (5.1–5.5)	5.2 (5.0–5.3)	0.36
hs-CRP (mg/L)	1.09 [0.44–2.27]	1.09 [0.56–2.68]	0.62
Diabetic nephropathy, *n* (%)	115 (57)	83 (49)	0.10
Urinary albumin excretion (mg/24 h)	143 [10–1,030]	23 [7–761]	0.15
eGFR (mL/min/1.73 m^2)	77 (73–81)	81 (77–85)	0.15
Serum MBL (μg/L)	3,390 [2,371–5,341]	619 [244–1,044]	<0.001

Data are expressed as mean (95% CI) or median [IQR] or as indicated. *P* values are from the *t* test or rank sum test, as appropriate. MBL or hs-CRP concentration was missing for two patients.

number of persons (%), unless explained otherwise. *P* values <0.05 were considered as statistically significant. STATA 13 software (StataCorp LP) was used for all analyses.

RESULTS

Baseline

Follow-up data were obtainable on 198 patients with diabetic nephropathy (121 men, age 41 years [95% CI 40–42], eGFR 67 mL/min/1.73 m^2 [95% CI 63–70]) and 174 patients with persistent normoalbuminuria (103 men, age 43 years [95% CI 41–44], eGFR 93 mL/min/1.73 m^2 [95% CI 91–95]). None of the patients entering the cohort with normoalbuminuria had developed macroalbuminuria at follow-up.

Baseline characteristics of patients grouped by *MBL2* genotype are compared in Table 1. Patients in the two groups had similar distributions of age, sex, diabetes duration, smoking status, blood pressure, HbA_{1c}, cholesterol, hs-CRP, UAE, and eGFR. As expected, the serum MBL concentration was significantly higher among patients preclassified as having high MBL expression genotypes than among those preclassified as having low MBL expression genotypes. The distribution of diabetic nephropathy in the original cohort has previously been described (2). In the current study, including only patients with available survival data, 115 patients (57%) with high MBL expression genotypes had diabetic nephropathy compared with 83 patients (49%) with low MBL expression genotypes (*P* = 0.10).

In accordance with the findings in the original cross-sectional study on the full cohort, MBL concentration correlated weakly to HbA_{1c} in the current study among all patients (correlation coefficient = 0.16, *P* = 0.002) (2). This correlation was stronger within patients with high MBL expression genotypes (correlation coefficient = 0.35, $P < 0.001$), whereas no correlation was found among patients with low MBL expression genotypes.

Mortality by *MBL2* Genotype

Ninety-eight patients died through a median follow-up time of 12.6 years (IQR 11.9–12.7). Among patients who died during follow-up, 63% had high MBL expression genotypes compared with only 51% among survivors (*P* = 0.033, Fig. 1*A*). The all-cause mortality rate was significantly higher among patients with high MBL expression genotypes compared with those with low MBL expression genotypes (31% vs. 21%, log-rank *P* = 0.022; Fig. 1*B*).

The HR for all-cause mortality of patients with high MBL expression genotypes relative to patients with low MBL expression genotypes was estimated by Cox proportional hazards regression analyses (Table 2). In the unadjusted model, the HR for all-cause mortality

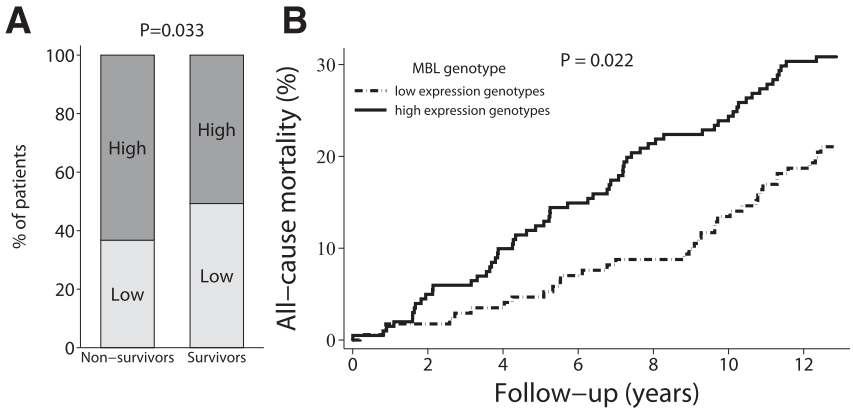

Figure 1—Association of MBL genotypes and mortality in type 1 diabetes. *A*: Distribution of high vs. low MBL expression genotypes in patients who died of any cause vs. all survivors. *B*: All-cause mortality rates in patients with high and low MBL expression genotypes. The *P* value refers to the log-rank test.

was 1.61 (95% CI 1.07–2.43) for patients with the high MBL expression genotypes compared with those with low MBL expression genotypes.

Previous studies have found MBL is associated with diabetic nephropathy; thus, we investigated the effect of diabetic nephropathy in the Cox proportional hazards regression. Because diabetic nephropathy is indicated to be on the causal pathway between MBL and mortality, we expected the measure of association to attenuate in the adjusted model. This association between *MBL2* genotype and mortality did appear to be partly driven through diabetic nephropathy as the HR declined to 1.47 (95% CI 0.97–2.21) and lost statistical significance after adjustment for diabetic nephropathy (Table 2). No further model adjustments were made, as described in the STATISTICS section above.

Sixty patients died without confirmation of a non-CVD cause. These patients were therefore assumed to have died of CVD, as described in the RESEARCH DESIGN AND METHODS. When the *MBL2* genotype distribution within these patients was compared with survivors, a tendency for the same pattern as for all-cause mortality was seen, although not reaching statistical significance ($P = 0.08$). The difference in the assumed CVD mortality rate between the *MBL2* genotypes was not statistically significant, but the same direction of association was observed as observed for all-cause mortality (data not shown, $P = 0.11$).

Mortality by Circulating MBL Concentration

The association between the circulating MBL concentration and the mortality rate was subsequently examined. Among all patients, the MBL concentrations in patients who died of all causes were significantly higher than in survivors (3,177 μg/L [IQR 726–5,053] vs. 1,624 μg/L [IQR 644–3,060], $P < 0.001$). Among patients with high MBL expression genotypes, patients who died of any cause had higher MBL concentration (4,425 μg/L [IQR 3,179–6,225]) than survivors (3,040 μg/L [IQR 2,086–4,759], $P < 0.001$). No difference was found in the MBL concentration among patients with low MBL expression genotypes who died (597 μg/L [IQR 131–758]) compared with patients who survived (629 μg/L [IQR 282–1,052], $P = 0.17$).

The association between the circulating MBL concentration and the mortality rate was investigated further in survival time analyses. We divided the patients into two groups having an MBL concentration above or below the median MBL concentration (1,895 μg/L) of the total cohort as illustrated in Fig. 2. The all-cause mortality rate was significantly higher in patients with an MBL concentration above the median compared with patients with an MBL concentration below the median (Fig. 2). Patients with an MBL concentration above the median were estimated to have an unadjusted HR of 1.90 (95% CI 1.26–2.87) for all-cause mortality compared with patients with an MBL concentration below the median (Table 2). In an exploratory analysis adding diabetic nephropathy to the model, the HR was 1.70 (95% CI 1.19–2.71), remaining statistically significant (Table 2).

Furthermore, patients assumed to have died of CVD had a higher MBL concentration at baseline compared with survivors (3,378 μg/L [IQR 726–5,822] vs. 1,624 μg/L [IQR 644–3,060], respectively, $P = 0.002$). This relation was also seen among patients with high MBL expression genotypes (5,286 μg/L [IQR 3,378–7,282] vs. 3,040 μg/L [IQR 2,086–4,759], respectively, $P < 0.001$). No difference was found among patients with low MBL expression genotypes (617 μg/L [IQR 163–758] vs. 629 μg/L [IQR 282–1,052], respectively, $P = 0.33$).

The assumed CVD mortality rate was significantly increased for patients with MBL concentrations above the median compared with those with MBL concentrations below (data not shown, $P = 0.032$).

CONCLUSIONS

The current study identifies a close relationship between MBL and mortality in patients with type 1 diabetes. In the study cohort, the mortality risk during 12 years of follow-up was significantly higher among patients carrying the *MBL2* genotypes that produce high concentrations of MBL compared with patients with low MBL–producing genotypes. These findings add to the previously described association between diabetic nephropathy and MBL and point to a potential role of *MBL2* genotype and MBL concentration as risk factors for diabetes complications and mortality.

The circulating MBL concentration varies considerably between subjects because of polymorphisms in the encoding *MBL2* gene, whereas within-subject variations are much smaller (11,24). In the present cohort, we found that high MBL expression genotypes were associated with an increased mortality rate. Furthermore, MBL concentration was also strongly associated with mortality, as expected from the close relationship between the *MBL2* genotype and the circulating MBL concentration. These findings suggest that the risk of death among patients with type 1 diabetes might be predicted from the *MBL2* genotype and the MBL concentration. In these patients, improved treatment of classical risk factors (e.g., glycemic control and treatment of hypertension) might be particularly rewarding.

The positive correlation between HbA_{1c} and MBL concentrations in the current study supports that the MBL concentration in humans increases within a given genotype as a consequence of diabetes in agreement with animal studies (14).

The association between MBL and diabetic nephropathy has been observed

Table 2—HR for all-cause mortality by MBL genotype and MBL concentration

	HR (95% CI)		HR (95% CI)	
Model	High vs. low MBL expression genotype	*P* value	High vs. low MBL concentration	*P* value
Unadjusted	1.61 (1.07–2.43)	0.023	1.90 (1.26–2.87)	0.002
Adjusted for diabetic nephropathy	1.47 (0.97–2.21)	0.068	1.79 (1.19–2.71)	0.006

HR (95% CI) of all-cause mortality comparing patients with high MBL expression genotypes relative to patients with low MBL expression genotypes and also the HR (95% CI) of all-cause mortality by MBL concentration in patients with an MBL concentration above the median concentration of 1,895 μg/L compared with patients with an MBL concentration below the median.

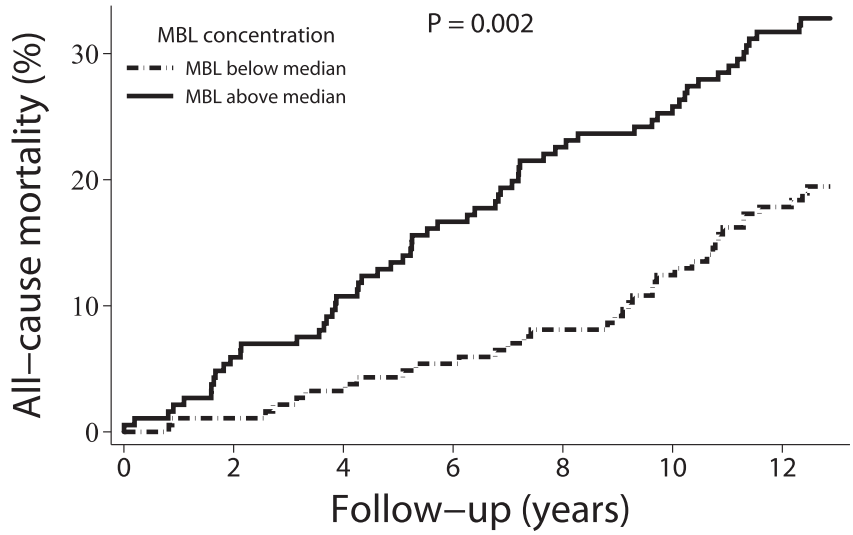

Figure 2—Association of circulating MBL level and mortality in type 1 diabetes. All-cause mortality rates in patients with MBL concentration above or low below the median in the cohort (1,895 μg/L). The *P* value refers to the log-rank test.

in several clinical studies (2–8). Also, the *MBL2* genotype in the present cohort was marginally unequally distributed between patients with or without diabetic nephropathy. Animal studies using MBL knockout mice indicate a causal role of MBL in diabetes-induced kidney changes (9,10). From these findings and the effect of diabetic nephropathy on mortality, we expected that diabetic nephropathy would explain most of the association between the *MBL2* genotype and the mortality rate (25). In exploratory analyses, we investigated whether this association could be mediated through diabetic nephropathy as an intermediate factor on the causal pathway between MBL and mortality. Both HR point estimates for the association between MBL2 genotype/MBL concentration and mortality risk were attenuated by the addition of diabetic nephropathy. This suggests that the effect of MBL is partly mediated through diabetic nephropathy.

On the basis of the present and previous results, we hypothesize that diabetes-induced alternations in enzymatic glycosylations and/or increased formation of advanced glycation end products lead to formation of patterns recognized by MBL. As shown in vitro, complement activation and sublytic formation of the terminal complement complex on mammalian cells may induce release of growth factors, leading to vascular alternations (26–31). In addition, glycation of complement regulatory proteins has been shown to decrease their regulatory capacity and increase complement activation in diabetes (32,33). These mechanisms might contribute to development of late diabetes complications and to the excess mortality in diabetes.

In conclusion, the current study suggests that *MBL2* genotype and MBL concentration may serve as important prognostic indicators for mortality in patients with type 1 diabetes. Intensified diabetes control might prove particularly beneficial in patients with *MBL2* genotypes that produce high concentrations of MBL. Future preclinical studies should aim to determine the possible beneficial effects of selective MBL or complement inhibition on diabetes-associated complications and mortality.

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Author Contributions. H.-H.P., P.R., and L.T. designed and performed the study. J.A.Ø., S.T., M.L., R.S., A.F., and T.K.H. researched data for the manuscript. J.A.Ø. wrote the manuscript draft. All authors reviewed and edited the manuscript and contributed to the discussion. J.A.Ø. and T.K.H. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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