Mannose-Binding Lectin and Mortality in Type 2 Diabetes

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Background: Inflammation and complement activation initiated by mannose-binding lectin (MBL) may be implicated in the pathogenesis of diabetic vascular complications. We evaluated the relationship between serum MBL and mortality and development of albuminuria in type 2 diabetes.

Methods: Levels of MBL and C-reactive protein (CRP) were measured at baseline in 326 patients with type 2 diabetes who attended the Steno Diabetes Center, Gentofte, Denmark, for control. Urinary albumin excretion was determined annually, and the vital status of all patients was traced after more than 15 years of follow-up.

Results: During follow-up, 169 patients died. The risk of dying was significantly higher among patients with MBL levels greater than or equal to 1000 µg/L than among patients with levels less than 1000 µg/L (hazard ratio, 1.5; 95% confidence interval, 1.1-2.1; \( P = .005 \)). After adjustment for known confounders, MBL remained a significant risk factor for death from any cause. It added to the predictive power of CRP, and mortality was significantly higher among patients with both high MBL (\( \geq 1000 \) µg/L) and high CRP (above the median, 3.6 mg/L) levels than among patients with both low MBL and low CRP levels (hazard ratio, 2.7; 95% confidence interval, 1.7-4.3; \( P < .001 \)). Normoalbuminuric patients with both high MBL and high CRP levels at baseline had a significantly increased risk of developing microalbuminuria or macroalbuminuria compared with patients with both low MBL and low CRP levels (hazard ratio, 2.6; 95% confidence interval, 1.5-4.4; \( P < .001 \)).

Conclusion: In patients with type 2 diabetes, measurements of MBL alone or in combination with CRP can provide prognostic information on mortality and the development of albuminuria.

Arch Intern Med. 2006;166:2007-2013

Type 2 diabetes is associated with significantly increased morbidity and mortality, particularly from cardiovascular and microvascular disease. Outcome can be greatly improved with new targeted intensive treatment algorithms, but tools to better predict the course of disease in individual patients are still needed to provide optimal use of available resources.

The complement system has evolved as a central part of the innate immune defense against invading microorganisms. On activation, however, complement may cause damage to innocent bystander cells through deposition of the membrane attack complex and initiate inflammation via release of the anaphylatoxins C5a and C3a. Mannose-binding lectin (MBL) is a liver-derived serum protein that activates complement by means of MBL-associated serine proteases. Levels of MBL are under genetic control and vary significantly from person to person primarily owing to frequently occurring genetic polymorphisms. Functional MBL deficiency occurs in as many as 10% of the normal population, and these individuals may be at increased risk of infections. It can also increase the risk of detrimental disease activity and thrombogenesis in patients with autoimmune diseases such as systemic lupus erythematosus.

Chronic low-grade inflammation, as indicated by increased levels of the inflammatory marker C-reactive protein (CRP), is believed to play a central role in the development of cardiovascular disease. C-reactive protein is an established marker of cardiovascular and all-cause mortality in patients with type 2 diabetes and is an independent determinant of changes in urinary albumin excretion over time. Mannose-binding lectin may aggravate local and systemic inflammation through...
complement activation,8 and it has been documented that inhibition of the complement cascade both at the level of MBL and further downstream improves outcome in patients with acute myocardial infarction.9,10 In patients with type 1 diabetes, high levels of circulating MBL and genotypes related to high MBL levels have been associated with the development of diabetic nephropathy and the presence of cardiovascular disease.11,12

The relationship between MBL levels and mortality and development of albuminuria in patients with type 2 diabetes remains unknown. To address this issue, we measured MBL levels at baseline in a well-characterized cohort of patients with type 2 diabetes who received more than 15 years of follow-up.

METHODS

STUDY DESIGN

We conducted a prospective observational study in all white patients with type 2 diabetes who attended the Steno Diabetes Center, Gentofte, Denmark, for control between January and December 1987. We excluded 6 patients in whom baseline samples were not available, leaving 326 patients for follow-up. The study was approved by the ethics committee, and all subjects gave written informed consent before entering the study. The diagnosis of type 2 diabetes was defined as (1) diabetes treated by diet and/or oral antidiabetic drugs, (2) insulin-treated diabetes with normal body mass index (weight in kilograms divided by the height in meters squared) above normal (≥27 in men and ≥25 in women), or (3) insulin-treated diabetes with a normal body mass index and a glucagon-stimulated C peptide value greater than or equal to 1.8 ng/mL (≥0.60 nmol/mL).

At the baseline examination, a random-zero sphygmonanometer (Hawksley & Sons, Lancing, England) was used to measure arterial blood pressure after 10 minutes of rest with the patients in the supine position. Prior cardiovascular disease was defined as a history of myocardial infarction or stroke from the World Health Organization cardiovascular questionnaire.9 Current smokers (>1 cigarette, cigar, or pipe per day) and former smokers were recorded as ever smokers, while never smokers were defined as persons who stated that they had never smoked. Urinary albumin excretion was determined at baseline and at subsequent annual follow-up visits from 1990 through 1997. Patients were classified as having normoalbuminuria (urinary albumin excretion <30 mg/d), microalbuminuria (30-299 mg/d), or macroalbuminuria (≥300 mg/d) and remained in a given category until progression to a higher excretion rate.

The vital status of all study patients was traced through linkage to the Danish Civil Registration System at the beginning of 2004, with the date of death (n=169) or January 1, 2004, as the last day of observation. The cause of death was obtained from death certificates, including available information from necropsy reports by 2 independent observers. An age- and sex-matched control group composed of healthy subjects (n=80) recruited among blood donors was included for comparison of MBL levels in patients with type 2 diabetes with those in healthy subjects.

LABORATORY ANALYSES

Blood samples were collected at baseline in the nonfasting state and stored at −70°C. Hemoglobin A1c (reference range, 4.1%-6.1%), serum total cholesterol, and serum creatinine levels were measured using standard methods. The urinary albumin concentration was determined in 24-hour urine collections by radioimmunoassay (sensitivity, 0.5 mg/L; coefficient of variation [CV], 9%) until 1992 and by enzyme-linked immunosorbent assay thereafter (sensitivity, 0.001 mg/L; CV, 8%).13 Serum MBL levels were measured using an in-house time-resolved immunofluorometric assay.14 The lower detection level was 5 µg/L, and the intra-assay and interassay CVs were below 10%. In healthy subjects, the median day-to-day variability in MBL concentrations expressed as CV was 6%.13 A highly sensitive in-house enzyme-linked immunosorbent assay was used to measure CRP with an interassay CV of 14%.7

STATISTICAL ANALYSES

For nonnormally distributed variables, values are given as medians with interquartile ranges (IQRs). All other values are given as means ± SDs. Comparisons between groups were performed by means of unpaired t test, Mann-Whitney U test, or Kruskall-Wallis test as appropriate. Spearman correlation with 2-tailed probability values was used to estimate the strength of association between variables. The cumulative risk of death was calculated based on the entire follow-up period ending in 2004 with a life table method, taking into account differences in the interval of follow-up. The method makes proper allowances for the observations that areensored and makes use of information from all subjects during follow-up to the time of event or censoring. Groups were compared using the log-rank test and displayed on Kaplan-Meier plots. To evaluate the effect of baseline MBL levels on mortality and on the development of persistent microalbuminuria, Cox proportional hazards regression analyses were performed. Multivariate Cox analyses were used to evaluate the relative contributions of available clinical covariates, such as sex, age, known diabetes duration, systolic and diastolic blood pressure, body mass index, serum total cholesterol, hemoglobin A1c, urinary albumin excretion (normoalbuminuria, microalbuminuria, or macroalbuminuria), and smoking, with backward elimination of nonsignificant variables. The best discriminative cutoff value for MBL as predictor of mortality was established from receiver operating characteristic curve (ROC) analysis. Statistical significance was assumed for P<.05. All statistical calculations were performed by one of us (T.K.H.) using SPSS for Windows (Version 13.0; SPSS Inc, Chicago, Ill).

RESULTS

BASELINE CHARACTERISTICS

Table 1 presents baseline characteristics of the cohort divided according to survival and baseline MBL status. The MBL levels were not significantly different in patients with type 2 diabetes compared with healthy subjects (666 µg/L [IQR, 176-1778 µg/L] vs 728 µg/L [IQR, 252-1698 µg/L]; P=.77). The mean age (50.4±6.3 years) and sex distribution (male/female, 59%/41%) of the control subjects were comparable to those of the patients. Among diabetic patients, the MBL concentrations were significantly higher in men than in women (905 µg/L [IQR, 289-2075 µg/L] vs 829 µg/L [IQR, 232-1944 µg/L]; P<.001) and in ever smokers than in never smokers (829 µg/L [IQR, 232-1944 µg/L] vs 461 µg/L [IQR, 62-1352 µg/L]; P=.005). The MBL concentrations were not correlated with age, diabetes duration, blood pressure, body mass index, serum total cholesterol levels, hemoglobin
A1c levels, serum creatinine levels, urinary albumin excretion, diabetes treatment, or antihypertensive treatment.

The MBL and CRP levels were not correlated in the entire cohort (r = 0.013; P = .81) or in subgroups of patients who were classified according to albuminuria status, sex, or smoking status. In contrast to MBL concentrations, CRP concentrations did not differ between men and women (3.3 mg/L [IQR, 1.3-7.1 mg/L] vs 4.0 mg/L [IQR, 1.6-9.3 mg/L%; P = .15] or between ever smokers and never smokers (3.8 mg/L [IQR, 1.7-7.6 mg/L] vs 2.9 mg/L [IQR, 0.9-7.8 mg/L]; P = .27).

MORTALITY

The median duration of follow-up was 15.6 years (IQR, 7.8-16.8 years), and during this observation time, 169 patients (52%) died. In 87 (51%) of these patients, the primary cause of death was classified as cardiovascular disease, while 10 patients (6%) died of end-stage renal failure, and 72 patients (42%) died of other causes, including cancer, infection, and unknown causes.

Median serum MBL concentrations were almost twice as high at baseline among patients who later died than among survivors (Table 1). A similar difference was seen when patients with normoalbuminuria at baseline were analyzed separately: 905 µg/L (IQR, 266-2047 µg/L) vs 497 µg/L (IQR, 124-1501 µg/L) in nonsurvivors and survivors, respectively (P = .05). To establish the best discriminative cutoff value for MBL as a predictor of survival, a ROC curve was constructed using increments of 200 µg/L of MBL. From this analysis, the MBL cutoff with the maximal sum of sensitivity and specificity was identified as 1000 µg/L (sensitivity, 0.54; specificity, 0.60; and area under the ROC curve, 0.58; P = .01). A similar ROC curve analysis in patients with normoalbuminuria at baseline revealed the same optimal cutoff level of 1000 µg/L. When the 326 patients were divided according to this cutoff level, the mortality rate during follow-up was 61% in patients with MBL levels above 1000 µg/L, compared with 46% in patients with MBL levels below 1000 µg/L (hazard ratio, 1.5; 95% confidence interval [CI], 1.1-2.1; P = .005) (Figure 1). Among the 191 patients with normoalbuminuria at baseline, those with a baseline MBL level above 1000 µg/L had a mortality rate of 49% during follow-up compared with 35% in normoalbuminuric patients with a MBL level below 1000 µg/L (hazard ratio, 1.7; 95% CI, 1.1-2.6; P = .03) (Figure 1).

In a multivariate Cox proportional hazards model with adjustment for known confounders, MBL remained an independent risk factor for death after 10 and 15 years of follow-up (Table 2). When the smaller group of patients with normoalbuminuria at baseline was considered separately, MBL was a significant and independent predictor of mortality after 10 years of follow-up (hazard ratio, 2.5; 95% CI, 1.3-4.8; P = .006), but not significantly so after 15 years of follow up (hazard ratio, 1.4; 95% CI, 0.9-2.2; P = .17). When patients were divided according to the overall median MBL level of 666 µg/L instead of the 1000-µg/L cutoff, similar results were obtained in the univariate and multivariate analyses (data not shown).

Prior cardiovascular disease is a known risk factor for all-cause mortality in type 2 diabetes. In our cohort, 32 patients had a history of cardiovascular disease at baseline, and these patients had significantly higher MBL levels than patients without prior cardiovascular disease: 1436 µg/L (IQR, 353-2309 µg/L) vs 655 µg/L (IQR, 162-1722 µg/L); P = .03. When the group of 296 patients without prior cardiovascular disease was analyzed separately, MBL levels above 1000 µg/L were still associated with a significant higher risk of death (hazard ratio, 1.5; 95% CI, 1.2-2.1; P = .001).

### Table 1. Baseline Characteristics According to Survival Status and Baseline Mannose-Binding Lectin (MBL) Level

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Survived (n = 157)</th>
<th>Died (n = 169)</th>
<th>P Value</th>
<th>MBL&lt;1000 µg/L (n = 189)</th>
<th>MBL&lt;1000 µg/L (n = 137)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, % men</td>
<td>55</td>
<td>69</td>
<td>.01</td>
<td>53 ± 9</td>
<td>72 ± 8</td>
<td>.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>50 ± 9</td>
<td>57 ± 6</td>
<td>&lt;.001</td>
<td>53 ± 9</td>
<td>54 ± 8</td>
<td>.32</td>
</tr>
<tr>
<td>Diabetes duration, y</td>
<td>5 ± (2-10)</td>
<td>9 ± (3-12)</td>
<td>.004</td>
<td>6 ± (2-10)</td>
<td>7 ± (3-13)</td>
<td>.04</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>144 ± 20</td>
<td>157 ± 23</td>
<td>&lt;.001</td>
<td>151 ± 23</td>
<td>150 ± 23</td>
<td>.76</td>
</tr>
<tr>
<td>Systolic</td>
<td>85 ± 11</td>
<td>87 ± 13</td>
<td>.09</td>
<td>86 ± 11</td>
<td>86 ± 11</td>
<td>.92</td>
</tr>
<tr>
<td>Diastolic</td>
<td>28 ± 5</td>
<td>29 ± 5</td>
<td>.45</td>
<td>29 ± 5</td>
<td>28 ± 5</td>
<td>.35</td>
</tr>
<tr>
<td>BMI</td>
<td>Serum total cholesterol, mg/dL</td>
<td>234 (207-269)</td>
<td>242 (203-294)</td>
<td>.15</td>
<td>242 (203-286)</td>
<td>234 (207-277)</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.6 (6.4-8.9)</td>
<td>8.2 (6.7-9.7)</td>
<td>.01</td>
<td>7.8 (6.3-9.3)</td>
<td>8.1 (6.8-9.6)</td>
<td>.10</td>
</tr>
<tr>
<td>Serum creatinine, µmol/L</td>
<td>71 (59-80)</td>
<td>80 (67-96)</td>
<td>&lt;.001</td>
<td>74 (64-89)</td>
<td>76 (65-91)</td>
<td>.33</td>
</tr>
<tr>
<td>Urinary albumin excretion, %</td>
<td>73/20/7</td>
<td>46/32/22</td>
<td>&lt;.001</td>
<td>61/24/15</td>
<td>55/30/15</td>
<td>.46</td>
</tr>
<tr>
<td>ever smoker, %</td>
<td>65</td>
<td>78</td>
<td>.01</td>
<td>66</td>
<td>80</td>
<td>.004</td>
</tr>
<tr>
<td>MBL, µg/L</td>
<td>510 (128-1556)</td>
<td>920 (266-1945)</td>
<td>.02</td>
<td>237 (52-528)</td>
<td>2018 (1461-2680)</td>
<td>NA</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>24 (0.8-5.3)</td>
<td>4.7 (1.9-8.8)</td>
<td>&lt;.001</td>
<td>3.6 (1.3-7.0)</td>
<td>3.7 (1.4-8.0)</td>
<td>.59</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the height in meters squared); HbA1c, hemoglobin A1c.; NA, not applicable.

*Data, other than percentages, are expressed as mean ± SD or median (25th-75th percentile).
†Includes current smokers (>1 cigarette, cigar, or pipe per day) and former smokers.
In the high MBL/high CRP group compared with the low MBL/low CRP group (hazard ratio, 3.5; 95% CI, 1.8-6.7; P<.001). In a multivariate Cox proportional hazards model that included all patients, the adjusted hazard ratio was 2.2 (95% CI, 1.4-3.5; P=.001) in the high MBL/high CRP group compared with the low MBL/low CRP group. When only patients with normoalbuminuria at baseline were included in the same model, the hazard ratio was 2.7 (95% CI, 1.4-5.4; P=.005) in the high MBL/high CRP group compared with the low MBL/low CRP group.

**PROGRESSION TO MICROALBUMINURIA OR MACROALBUMINURIA**

Of 191 patients with normoalbuminuria at baseline, 99 progressed to microalbuminuria or macroalbuminuria during the first 10 years of follow-up (68 to microalbuminuria, 31 to macroalbuminuria). Overall mortality rates were not significantly different among progressors and nonprogressors (44% vs 36%; P=.24). Progressors had higher baseline levels of MBL and CRP than non-progressors (MBL, 782 µg/L [IQR, 236-2036 µg/L] vs 581 µg/L [IQR, 119-1517 µg/L] [P=.06]; and CRP, 3.8 mg/L [IQR 1.4-7.9 mg/L] vs 1.9 mg/L [IQR, 0.7-4.4 mg/L] [P=.005]), but in a multivariate Cox proportional hazards model, neither MBL levels nor CRP levels were independent predictors of later development of albuminuria. However, when the predictive values of the 2 markers were combined, it appeared that patients with both high MBL levels and high CRP levels at baseline had a significantly increased risk of developing microalbuminuria or macroalbuminuria compared with patients with both low MBL levels and low CRP levels (hazard ratio, 2.6; 95% CI, 1.5-4.4; P=.001) (Figure 3). The combined variable remained an independent predictor of progression to microalbuminuria or macroalbuminuria in a Cox proportional hazards model (adjusted hazard ratio, 2.4; 95% CI, 1.4-4.1; P<.001), when patients with high MBL and CRP levels at baseline were compared with patients with low MBL and CRP levels.

**COMMENT**

The incidence of type 2 diabetes mellitus is rapidly increasing, and according to the World Health Organization, more than 170 million persons are currently affected worldwide. Improved risk stratification to allow optimal targeted use of available resources is thus essential. Our study demonstrates that measurement of MBL in white patients with type 2 diabetes—alone or in combination with CRP—provides prognostic information on all-cause mortality and development of albuminuria that is independent of previously known risk factors. Limitations of our analyses are the relatively small study size and the modest size of the observed effects as well as the unavailability of DNA samples for the analysis of MBL genotypes.

The current findings may have implications for future preventive therapies. Long-term intensified intervention aimed at multiple risk factors was recently shown...
to reduce the risk of cardiovascular and microvascular events by about 50% in patients with type 2 diabetes and microalbuminuria. The same approach may well prove beneficial in high-risk normoalbuminuric patients, and biologic markers to better identify such patients are therefore needed. Measurement of MBL added to the predictive power of the well-established prognostic marker CRP, and the 15-year mortality rate among normoalbuminuric patients in the high MBL/high CRP group (approximately 61%) came close in magnitude to the mortality rate in patients with microalbuminuria at baseline (approximately 63%). Moreover, the combination of MBL and CRP was an independent predictor of future development of microalbuminuria or macroalbuminuria and added information to previously described risk factors in the identification of high-risk patients.

Different information is clearly conveyed by measurement of MBL and CRP, as there was no correlation between the 2 proteins in the present study or in previous studies of patients with type 1 diabetes. A strategy that includes measurement of both CRP and MBL may thus improve risk stratification in a clinical setting. Repeated measurements over a time span of 15 to 20 years show a very high correlation of MBL concentrations, exceeding the long-term consistency of known risk markers such as total serum cholesterol and systolic and diastolic blood pressure. Concentrations of MBL show no diurnal variation and are independent of renal function, and the variations in MBL levels during acute phase responses are very small compared with the changes seen with CRP. DNA samples for MBL genotyping were not available in the present study, but circulating MBL levels reflect the genotype, and protein measurements in serum samples are more convenient for the purpose of risk assessment.

While the ability to mount an effective inflammatory response against infections has been one of the most powerful selection pressures through evolution, the very same mechanisms may be detrimentally involved in a variety of modern-day disorders, eg, autoimmune and cardiovascular diseases. Circulating MBL has the ability to effectively initiate inflammation through the enzymatic activation cascades of complement. Under normal circumstances, MBL does not react with the host's own tissues, but changes in cell surface glycations, as those seen after cellular hypoxia, may lead to increased MBL deposition and complement activation. Mannose-binding lectin has been shown to aggravate ischemic injury in acute myocardial infarction, and downstream inhibition of the complement system with a C5 inhibitor significantly reduced mortality after percutaneous coronary intervention in patients with myocardial infarction. Patients with intrinsically higher levels of MBL may thus be at increased risk of inflammatory damage after ischemia and reperfusion, which could explain the association between MBL levels and mortality rates that was observed in the present study.

In contrast to our current findings in patients with type 2 diabetes and previous findings in patients with type 1 diabetes, there was no association between MBL genotypes and morbidity or mortality in a large epidemiological population-based study of 9245 white subjects in the adult Danish population, and among American Indians the presence of coronary disease was actually higher in patients with variant MBL genotypes associated with low levels of MBL. The reason for this difference may be the presence of hyperglycemia in diabetic patients, which is known to cause protein glycation, leading to the formation of advanced glycation end products. Glycosylation changes may cause increased MBL autoreactivity and therefore explain why diabetic subjects are more susceptible to unfavorable effects of high MBL concentrations. Moreover, emerging data indicate that increased glycation inactivates important membrane-bound complement regulatory proteins, such as CD59, that normally serve to prevent self-injury from insertion of the terminal membrane attack complex of complement. Complement activation from any cause may thus have more widespread consequences in diabetic patients and contribute to the ongoing inflammation and
Specific inhibition of complement at different levels of the enzymatic cascade is currently being evaluated for the treatment of ischemia-reperfusion injury after acute myocardial infarction and acute humoral rejection in renal transplantation. It remains to be clarified whether the same strategy may be a therapeutic option in diabetes. Patients with complement deficiencies have an increased susceptibility to infection and immune complex diseases, and long-term inhibition of complement may cause similar adverse effects. However, it is quite plausible that targeted down-regulation rather than complete blockade of complement would be sufficient to evade the detrimental proinflammatory effects of MBL and the complement system in type 2 diabetes.

Accepted for Publication: June 29, 2006.

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Financial Disclosure: None reported.

Funding/Support: This work was supported by research grants from the Danielsen Foundation, the Dagnan Marshall Foundation, the Danish Medical Research Council, and the Danish Diabetes Association.

Role of the Sponsor: The funding sources had no involvement in the study design; collection, analysis, and interpretation of data; writing of the report; or decision to submit the manuscript for publication.

Acknowledgment: We thank Inga Bisgaard for expert technical assistance and Peter Hovind, MD, DMS, for reviewing death certificates.


