

Mannose-Binding Lectin Genotype and Phenotype in Patients With Type 2 Diabetes and Myocardial Infarction

A report from the DIGAMI 2 trial

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OBJECTIVE — The present study characterizes mannose-binding lectin (MBL), an activator of the complement system and thereby important for inflammatory activation, in patients with diabetes and myocardial infarction.

RESEARCH DESIGN AND METHODS — Serum (S)-MBL was determined at hospital admission in 387 patients with type 2 diabetes (median age 70 years; 68% male) with myocardial infarction, and genotyping was performed in 287 patients. Cardiovascular events (cardiovascular mortality and nonfatal myocardial infarction or stroke) were recorded during 2.5 years.

RESULTS — Median S-MBL was 1,212 $\mu\text{g/l}$ (interquartile range [IQR] 346–2,681 $\mu\text{g/l}$). Of the subjects, 54% in the geno- and phenotype subgroup had a high-coding MBL genotype (median S-MBL = 2,658 $\mu\text{g/l}$ [IQR 1,715–3,829]) and 46% a low-coding MBL genotype (373 $\mu\text{g/l}$ [100–765]). S-MBL did not correlate with age, BMI, creatinine clearance, glucose, or A1C. Cardiovascular events occurred in 136 (35%) patients. S-MBL did not predict events in univariable analyses (hazard ratio 0.93 [95% CI 0.85–1.01]; $P = 0.09$). In unadjusted analyses, the risk of events was lower in patients with a high genotype and S-MBL above the median for their genotype (0.49 [0.26–0.92]; $P = 0.026$) than for patients with a low genotype and S-MBL below the median for their genotype. The prediction capacity of the geno- and phenotype model was of borderline significance in adjusted Cox regression.

CONCLUSIONS — Patients with type 2 diabetes and myocardial infarction have MBL genotypes that are similar to those known in the general population. The combination of a low-coding MBL genotype with a low S-MBL appears to be prognostically unfavorable, but the association is blunted by traditional risk markers.

Diabetes Care 33:2451–2456, 2010

Macrovacular complications, not the least is myocardial infarction, are leading causes of death in patients with type 2 diabetes. An improved understanding of pathophysiological mechanisms is a prerequisite for the development of new treatment targets. Chronic, low-grade inflammation is a component in the development of atherosclerosis and coronary artery disease (1).

There is evidence of increased inflammatory activation and downstream markers of inflammation, e.g., C-reactive protein, predict an increased risk of cardiovascular mortality and morbidity (1). To enable a successful modulation of disease progression, it is, however, likely that the earlier stages of the inflammatory process should be targeted.

Mannose-binding lectin (MBL) is a

pattern recognition molecule of the innate immune system that has attracted interest in the context of cardiovascular disease (2). MBL activates the lectin pathway, one of three (classic, lectin, or alternative) arms, which initiates the complement cascade (3). Although MBL acts as an acute-phase reactant, circulating levels remain stable over time, only increasing two- to threefold during stress (3). However, genetic regulation accounts for a considerable variation in MBL concentration among individuals. MBL deficiency is associated with autoimmune, inflammatory, and vascular diseases (4). Information on a potential link between MBL and cardiovascular complications is contradictory. MBL levels were higher among patients with acute coronary syndromes than among control subjects in a recent study (5) and in another study comprising 890 patients with ST-segment myocardial infarction undergoing percutaneous coronary intervention, functional deficiency of complement MBL, defined as ≤ 100 ng/ml, was associated with reduced mortality but not with a decreased risk of the combined end points of death and shock or death, shock, and congestive heart failure (6). In contrast, low MBL levels and *MBL2* genotypes have been linked to atherosclerosis (7,8), and individuals with MBL deficiency have a higher risk of coronary artery disease (9,10).

The aim of the present report was to characterize MBL geno- and phenotypes in patients with type 2 diabetes and acute myocardial infarction (AMI). A secondary aim was to test the hypothesis that this information may be useful as a prognostic marker in such patients.

RESEARCH DESIGN AND METHODS

The Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI 2) trial compared three different management strategies in patients with type 2 diabetes and suspected AMI. An extensive description of the study design has been presented elsewhere (11). In short, 1,253

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Received 18 May 2010 and accepted 24 July 2010. Published ahead of print at <http://care.diabetesjournals.org> on 6 August 2010. DOI: 10.2337/dc10-0903.

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patients were randomly assigned to one of three study arms receiving 1) a 24-h insulin-glucose infusion, followed by subcutaneous insulin-based long-term glucose control (group 1, $n = 474$); 2) the same initial treatment, followed by glucose-lowering treatment according to local practice (group 2, $n = 473$); or 3) glucose-lowering treatment according to local practice (group 3, $n = 306$). There was no significant difference in total mortality or nonfatal myocardial infarction and stroke during the median follow-up period of 2.1 years. This allowed the possibility of combining all DIGAMI 2 patients into one cohort suitable for post hoc epidemiological analyses disregarding randomized group belonging. The present study consists of 387 participants in a biochemistry substudy, in which serum (S) MBL was determined at hospital admission. In 287 of them, samples were available for both S-MBL and *MBL2* genotyping.

Investigations

Samples for assessments of the following variables were obtained as soon as possible after hospital admission: electrolytes, serum creatinine, blood lipids, glucose (millimoles per liter in whole blood), A1C, and S-MBL. Samples for genetic analyses were obtained at the time of hospital discharge. A1C was analyzed by high-performance liquid chromatography with an upper normal limit of 5.3%. Creatinine clearance (milliliters per min) was calculated using the Cockcroft-Gault method. S-MBL levels were measured using an in-house, time-resolved immunofluorometric assay (12). The lower detection level was 10 $\mu\text{g/l}$, and the intra-assay and inter-assay coefficients of variation were $<10\%$.

A full description of the genotyping of the patients is found in the supplementary data (available in an online appendix at <http://care.diabetesjournals.org/cgi/content/full/dc10-0903/DC1>). In short, DNA was extracted from whole blood and analyzed using real-time PCR with TaqMan single nucleotide genotyping assays (Applied Biosystems, Foster City, CA) (13–15).

The presence of one of the three structural mutations in the *MBL2* gene, located at 10q21.1, the D, B, and C variants (designated as “O”), significantly reduces circulating MBL. Of the three known promoter polymorphisms, only the X/Y polymorphism influences S-MBL, causing reduced MBL levels (5). In the present report, genotypes were categorized according to polymorphisms in the

MBL2 gene as AA (wild-type), AO (heterozygote), or OO (homozygote). After the addition of information on the promoter region, genotypes were classified as high (including all AA with the exclusion of homozygotes for the X promoter) or low, as determined previously by Hansen et al. (16).

A composite of cardiovascular death, reinfarction, or stroke, adjudicated by an independent committee composed of experienced cardiologists, served as the primary end point.

Statistical methods

Continuous variables are presented as median and interquartile range (IQR), unless otherwise stated. The Wilcoxon Mann-Whitney test was used to study differences between groups of patients stratified by dichotomous variables, and the association between continuous variables was assessed by means of Spearman rank correlation. The Jonckheere-Terpstra test was used to compare differences between genotypes and combinations of geno- and phenotypes. The relationships between S-MBL and age, sex, BMI, previous myocardial infarction, heart failure, hypertension, creatinine clearance, B-glucose, and A1C at admission were studied by regression analyses.

The relationship between S-MBL and the primary end point was assessed using Cox proportional hazards regression and presented as hazard ratios (HR) and 95% CIs. Because of skewed distribution, S-MBL was log-transformed before analysis. No model building was performed in the principal analysis. For the outcome analysis, S-MBL was dichotomized below or above a level of 1,000 $\mu\text{g/l}$ according to previous findings, demonstrating that this cutoff level has high sensitivity and specificity for risk prediction (10,17). Patients were also classified according to their genotypes in relation to the median S-MBL concentration for the respective genotype: high/above indicates high genotype with S-MBL above the median for this genotype; high/below indicates high genotype with S-MBL below the median for this genotype; low/above indicates low genotype with S-MBL above the median for this genotype; and low/below indicates low genotype with S-MBL below the median for this genotype.

To illustrate time trends for cardiovascular outcome, Kaplan-Meier curves were drawn using S-MBL less than or greater than 1,000 $\mu\text{g/l}$ and the combination of geno- and phenotypes (high/

above, high/below, low/above, and low/below) as strata. A log-rank test for trend was applied to assess differences in event patterns.

Cox proportional hazards regression was used to test the prediction capacity of dichotomized S-MBL (less than or greater than 1,000 $\mu\text{g/l}$), as well as of the combination of geno- and phenotypes. Cox proportional hazards regression models were adjusted with the same covariates as those used in the regression analyses.

Two-tailed statistical tests were used at a 5% significance level. SAS (version 9.2) was used for all statistical analyses.

Ethical considerations

The study followed the recommendations of the Declaration of Helsinki, and local ethics review boards approved the protocol. Written informed consent was obtained from all patients before enrollment.

RESULTS

The baseline characteristics of patients with S-MBL measured at admission are presented in Table 1. S-MBL varied between 10 and 6,382 $\mu\text{g/l}$ (median 1,212 $\mu\text{g/l}$; mean 1,666 $\mu\text{g/l}$; IQR 346–2,681 $\mu\text{g/l}$). Patients ($n = 287$) with both an S-MBL and *MBL2* genotype had baseline characteristics and S-MBL levels similar to those of the total cohort of patients with S-MBL available ($n = 387$). There were no significant differences in baseline characteristics between patients with high ($n = 156$) or low genotypes ($n = 131$) (Table 1). The median S-MBL in patients with a high-coding genotype was 2,658 $\mu\text{g/l}$ (IQR 1,715–3,829) compared with 373 $\mu\text{g/l}$ (100–765) in those with a low-coding genotype.

The genetic analyses revealed that 173 patients belonged to genotype AA (60.3%), 104 to AO (36.2%), and 10 to OO (3.5%). S-MBL differed significantly among patients with different genotypes (Jonckheere-Terpstra test; $P < 0.001$). The respective medians and IQRs for the three groups were AA 2,538 $\mu\text{g/l}$ (IQR 1,498–3,772), AO 384 $\mu\text{g/l}$ (130–702), and OO 12 $\mu\text{g/l}$ (10–65).

There were no significant differences in S-MBL between men and women or between patients with or without a history of myocardial infarction, heart failure, or hypertension (Table 2). Moreover, there were no significant correlations between S-MBL and age, BMI, creatinine clearance, glucose, and A1C at admission (Spearman correlation).

Table 1—Baseline characteristics of all patients with S-MBL, of patients with both S-MBL and genotype, and of patients grouped according to genotype (encoding high or low S-MBL concentration)

Variable	All patients	Geno- and phenotyped patients	High genotype	Low genotype
<i>n</i>	387	287	156	131
Age (years)	70.0 (61.1–77.1)	69.9 (60.7–76.7)	67.9 (60.4–75.7)	70.7 (61.1–77.1)
Male sex	264 (68)	194 (68)	107 (69)	87 (66)
BMI (kg/m ²)	28 (26–31)	28 (26–31)	28 (26–31)	28 (25–31)
Diabetes duration (years)	6.0 (2.0–14.0)	6.0 (1.0–14.0)	5.5 (0–13.0)	8.0 (2.0–14.5)
Blood pressure (mmHg)				
Systolic	130 (120–150)	130 (120–150)	135 (120–150)	130 (115–145)
Diastolic	74 (65–85)	74 (63–85)	75 (64–85)	70 (60–80)
Previous medical history				
Myocardial infarction	149 (39)	109 (38)	59 (38)	50 (38)
Angina pectoris	202 (52)	145 (51)	79 (51)	66 (50)
Heart failure	77 (20)	53 (19)	25 (16)	28 (21)
Hypertension	214 (55)	160 (56)	92 (59)	68 (52)
Hyperlipidemia	134 (35)	101 (35)	51 (33)	50 (38)
Current smoker	84 (22)	61 (21)	32 (21)	29 (22)
Medication before admission				
Insulin	141 (36)	104 (36)	51 (33)	53 (40)
Metformin	102 (26)	73 (25)	35 (22)	38 (29)
Sulfonylurea	150 (39)	108 (38)	50 (32)	58 (44)
β-Blocker	176 (46)	123 (43)	69 (45)	54 (41)
Aspirin	204 (53)	148 (52)	87 (56)	61 (47)
ACE inhibitor	122 (32)	94 (33)	53 (34)	41 (31)
Lipid-lowering	119 (31)	90 (31)	46 (30)	44 (34)
Biochemical analysis at admission				
Blood glucose (mmol/l)	11.8 (9.2–14.9)	11.9 (9.5–14.9)	12.0 (9.3–15.1)	11.9 (9.6–14.9)
A1C (%)	7.1 (6.2–8.5)	7.1 (6.3–8.6)	7.0 (6.3–8.7)	7.3 (6.3–8.5)
Serum creatinine (μmol/l)	91 (78–112)	89 (75–107)	88 (75–108)	90 (76–106)
Creatinine clearance (ml/min)	72 (49–92)	74 (51–96)	78 (52–97)	70 (49–95)
Serum cholesterol (mmol/l)	5.0 (4.2–5.8)	5.1 (4.4–5.9)	5.1 (4.6–5.9)	4.9 (4.2–6.0)
Serum triglycerides (mmol/l)	1.7 (1.2–2.6)	1.8 (1.2–2.7)	1.9 (1.2–2.7)	1.7 (1.1–2.7)

Data are *n* (%) or median (quartile 1–quartile 3). All variables refer to the time of hospital admission, unless otherwise stated.

A univariable regression analysis of S-MBL and age, sex, BMI, creatinine clearance, glucose, A1C, a history of myocardial infarction, heart failure, and hypertension was performed separately in the dataset with all patients (*n* = 387) and in the geno- and phenotyped patients (*n* = 287). Only hypertension showed a weak relationship (unadjusted *P* = 0.05) in patients with both geno- and phenotype available (data not shown).

Mortality and morbidity

During the follow-up period of 2.5 (range 1.04–3.00) years, 35% of the total cohort (*n* = 387) had a cardiovascular event (Table 3). The corresponding number in the subgroup with both S-MBL and genotype available (*n* = 287) was 30.0%.

The predictive value of S-MBL for the composite end point, its separate components, and total mortality are shown in Table 3. No model building was per-

formed, because S-MBL did not predict the primary end point or any components in the univariable analysis.

Patients with S-MBL >1,000 μg/l had lower event rates than those below that level in a univariable Cox regression analysis (HR 0.68 [95% CI 0.48–0.95]; *P* = 0.02). This pattern remained after univariable adjustments for age, admission glucose, BMI, sex, previous myocardial infarction, and hypertension, but not with adjustment for creatinine clearance, A1C, or previous heart failure. However, the dichotomized S-MBL did not predict events (*P* = 0.09) in a multiple Cox regression model adjusting for significant confounders using a best subset selection criterion (age, BMI, admission glucose, creatinine clearance, and myocardial infarction).

When we grouped the patients according to their genotype and S-MBL above or below the median for their geno-

type (high/above [*n* = 78], high/below [*n* = 78], low/above [*n* = 65], and low/below [*n* = 66]), there was a significant trend among the four groups to predict survival free from cardiovascular event (log-rank test *P* = 0.01) (Fig. 1). Using the low/below group as a reference in Cox regression analyses, patients with high/above were the only group with a significantly lower event rate (HR = 0.49 [95% CI 0.26–0.92]; *P* = 0.03). For patients in the high/below and low/above groups compared with low/below, the HRs were 0.66 ([0.37–1.17], *P* = 0.15) and 0.89 ([0.51–1.57], *P* = 0.69), respectively. These results remained after univariable adjustments for admission glucose, A1C, BMI, sex, previous myocardial infarction, and hypertension, but not for age, creatinine clearance, or previous heart failure. In a multiple Cox regression model adjusting for significant confounders using a best subset selection criterion (age, BMI,

Table 2—Differences between groups of patients stratified by dichotomous variables (Wilcoxon Mann-Whitney test) and associations between continuous variables (Spearman rank correlation) in all patients with S-MBL and in patients with both S-MBL and genotype available

Variable	All patients	P value	Geno- and phenotyped patients	P value
n	387		287	
Wilcoxon Mann-Whitney test	S-MBL (μg/l)		S-MBL (μg/l)	
Sex				
Male	1,357 (337–2,835)	0.44	1,555 (301–3,287)	0.19
Female	1,046 (347–2,394)		1,071 (371–2,336)	
Previous myocardial infarction				
Yes	1,354 (346–2,568)	0.71	1,475 (303–2,878)	0.96
No	1,158 (347–2,836)		1,297 (346–2,930)	
Previous heart failure				
Yes	943 (241–2,100)	0.13	943 (239–2,392)	0.23
No	1,270 (373–2,860)		1,446 (386–2,930)	
Previous hypertension				
Yes	1,337 (386–2,836)	0.18	1,542 (407–2,941)	0.09
No	1,041 (234–2,605)		1,026 (177–2,778)	
Spearman rank correlation	Correlation coefficient		Correlation coefficient	
Age	−0.08	0.13	−0.08	0.21
BMI	−0.03	0.52	−0.03	0.65
Biochemical analysis at admission				
Creatinine clearance	0.08	0.15	0.04	0.49
Glucose	0.06	0.22	0.05	0.43
A1C	−0.01	0.84	−0.04	0.48

Data are median (quartile 1–quartile 3) or correlation coefficient.

creatinine clearance, and previous myocardial infarction), the predictive capacity of the geno- and phenotype model was of borderline significance ($P = 0.07$).

CONCLUSIONS— This study is the first to examine the distribution of MBL geno- and phenotypes and the impact of MBL on prognosis in patients with type 2 diabetes and myocardial infarction. The patients had a *MBL2* genotype distribution similar to that of the general population and S-MBL did not contribute much

information on cardiovascular prognosis beyond traditional risk markers. The results suggest that the combination of a low geno- and phenotype is associated with a poorer outcome, but they does not explain whether a low or a high MBL level is harmful in this patient group.

In the present cohort, the distribution of MBL genotypes was similar to that in a general European population (18,19), with the majority of patients, 60%, belonging to the wild-type genotype (AA), whereas the remainder had one or two

alleles with mutations (AO or OO). As expected, the phenotypes were distributed with the highest S-MBL levels in patients belonging to the AA group and in those classified as having a high genotype, i.e., also including information on polymorphisms in the promoter region (19).

The present S-MBL median of 1,212 μg/l, with no sex difference, was higher than that detected in outpatients with type 2 diabetes and healthy control subjects (median 666 and 728 μg/l, respectively) (17). One explanation for the higher S-MBL in this cohort is that MBL was measured at admission for AMI and was thereby possibly influenced by stress. In a study of patients with acute coronary syndrome and matched control subjects, patients had higher MBL than control subjects (median 855 and 441 μg/l; $P < 0.0001$) (5).

The present study does not resolve the issues of whether a low or a high S-MBL and which genotypes are harmful in patients with type 2 diabetes and myocardial infarction. In the unadjusted analysis, S-MBL $< 1,000$ μg/l and the combination of a low genotype with S-MBL below the median for this genotype was associated with a poorer outcome, but this finding did not remain after adjustments.

One limitation of the present report is that patients with both geno- and phenotypes available had a lower event rate than that for the total cohort (Table 3). One possible explanation is that genotypes were measured at the time of hospital discharge, thereby eliminating patients who died in the hospital from the genotyped subgroup. The results of the correlation and regression analyses for continuous S-MBL were, however, similar in the two groups, suggesting that findings in the genotyped group are representative. Only a larger study will be able to elucidate the prognostic significance of determining MBL geno- and phenotypes.

Table 3—Number and proportion of events and the predictive value of S-MBL (Cox proportional hazards regression models) for respective events in all patients with S-MBL and in patients with both S-MBL and genotype available

	All patients			Geno- and phenotyped patients		
	n (%)	HR (95% CI)	P value	n (%)	HR (95% CI)	P value
n		387			287	
Death	95 (24.6)	0.93 (0.84–1.04)	0.20	56 (19.5)	0.97 (0.85–1.12)	0.71
CV death	77 (19.9)	0.95 (0.85–1.08)	0.43	43 (15.0)	0.99 (0.84–1.16)	0.89
Reinfarction	58 (15.0)	0.95 (0.83–1.09)	0.45	41 (14.3)	0.93 (0.79–1.09)	0.35
Stroke	25 (6.5)	0.87 (0.72–1.06)	0.17	20 (7.0)	0.89 (0.71–1.10)	0.27
CV event	136 (35.1)	0.93 (0.85–1.01)	0.09	86 (30.0)	0.92 (0.82–1.02)	0.12

CV, cardiovascular.

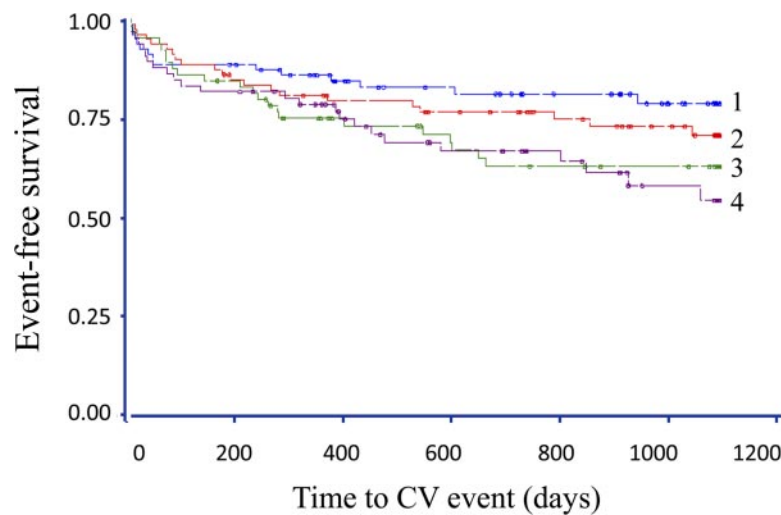


Figure 1—Kaplan-Meier curves for cardiovascular (CV) events in patients grouped according to genotype (encoding high or low S-MBL) and S-MBL level (above or below median S-MBL concentration for respective genotype) (log-rank test for trend $P = 0.01$). 1, high/above; 2, high/below; 3, low/above; 4, low/below. ●, censored event.

Several studies have linked a low S-MBL or genotypes associated with a low S-MBL to cardiovascular disease (7–10,20). There are, however, reports of an association between macrovascular disease and high MBL (5,6,21). These discrepant findings may relate to differences in study design, the composition of patient populations, and the type of events studied. Pesonen et al. (5) noted that patients with acute coronary syndrome had high S-MBL and genotypes, but this was in comparison with healthy control subjects. Furthermore, patients with type 2 diabetes may react differently to MBL than those without type 2 diabetes. In the study by Trendelenburg et al. (6) few patients had diabetes. In studies with a high proportion of patients with diabetes, low S-MBL or low MBL genotypes were related to a poor prognosis (9,10).

The possible relationship between low MBL and an increased risk of atherosclerosis may have several explanations. In a study comprising 2,176 children, homozygosity for the MBL variant alleles was associated with a greater impairment in flow-mediated dilation during infection compared with that in control subjects without infection, suggesting that an MBL gene-environment interaction, possibly occurring early in life, may have an impact on the initiation and progression of atherosclerosis (22).

The idea underlying the use of the combination of genotype and MBL levels below or above the median for the respec-

tive genotype was to combine information on genetic susceptibility and ongoing inflammation. The inability of this combination to predict risk in the present cohort may be due to a lack of power, but it also indicates that easily available factors such as age and renal function are more useful as clinical risk markers than S-MBL.

MBL may play a different role in different settings, which limits its usefulness as an overall risk marker or a treatment target. However, before abandoning the concept of MBL and the notion of the potential importance of the innate immune system for the progression of cardiovascular diseases, it is important to look further into downstream steps of the complement cascade.

Acknowledgments—This study was supported by grants from the Swedish Heart-Lung Foundation, the Swedish Research Council (8691), and AFA Insurance and by unconditional grants from Aventis Sweden and Novo Nordisk Denmark.

No other potential conflicts of interest relevant to this article were reported.

L.G.M., L.R., and T.K.H. researched and prepared data, wrote the manuscript, and revised/edited the manuscript. A.H. and R.S. performed laboratory analyses, contributed to discussion, and revised/edited the manuscript. K.M. researched data and contributed to discussion. J.Ö. assisted in statistical analyses and reviewed/edited the manuscript.

We thank Mattias Molin, BSc, Statistical Consulting Group, Göteborg, Sweden, for valuable support with the database.

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