

# Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk

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**Inflammation can predispose to myocardial infarction (MI), and mannan binding lectin (MBL) promotes phagocytic clearance of inflammatory agents, but the predictive value of MBL levels for MI is not known. MBL was analyzed in subgroups of the population-based Reykjavik study, a cohort of 19,381 participants recruited from 1967. MBL levels were very stable over time (self correlation: 0.86). In a cross-sectional group from the original cohort ( $n = 987$ ), high MBL ( $>1,000 \mu\text{g/L}$ ) was associated with a greatly lowered odds ratio for MI (0.64,  $P < 0.001$ ). To verify this finding, a nested case control sample ( $n = 1,309$ ) was randomly selected from the cohort. High MBL at recruitment was also associated with decreased MI risk in this follow-up group, but to a lesser extent and not significant for the whole group, smokers, or hypertensive individuals. However, high MBL was as in the cross-sectional group, associated with greatly decreased MI risk in diabetic ( $P = 0.02$ ) or hypercholesterolemic individuals ( $P = 0.004$ ). This also applied to raised erythrocyte sedimentation rate ( $P = 0.007$ ). Diabetic patients with high MBL did not have a higher MI risk than nondiabetic individuals. Our findings indicate that high MBL may predict decreased likelihood of MI, particularly in diabetics, and are consistent with the possibility that MBL may promote clearance of atherogenic agents.**

## CORRESPONDENCE

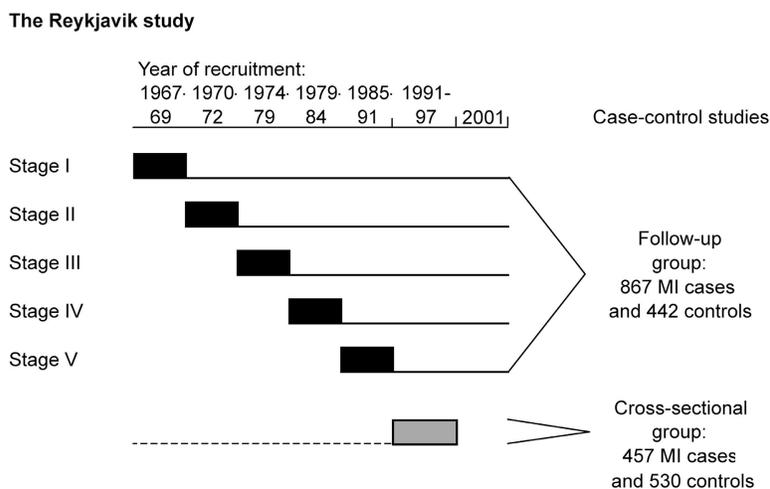
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Abbreviations used: ESR, erythrocyte sedimentation rate; G1cNAc, *N*-acetylglucosamine; LDL, low density lipoprotein; MBL, mannan binding lectin; MI, myocardial infarction; ROC, receiver operating characteristic; SLE, systemic lupus erythematosus.

Several inflammatory markers have been associated with coronary heart disease (1–4), and this was recently demonstrated for erythrocyte sedimentation rate (ESR; reference 3) and for C-reactive protein in the Reykjavik study (4). Complement-dependent opsonization can facilitate noninflammatory clearance of immune complexes, microorganisms, and apoptotic cells within the liver and spleen (5–9), and mannan binding lectin (MBL) is a serum protein that can activate the complement system and thereby promote phagocytic clearance of various inflammatory agents (10, 11). MBL is an oligomeric C-type lectin that recognizes certain sugar patterns on the surface of many microorganisms (10, 12) and self-components including apoptotic cells (8, 13), phospholipids (14), and immune complexes (15, 16). The serum concentration of MBL varies from undetectable up to 10,000  $\mu\text{g/L}$  with a median around 1,000  $\mu\text{g/L}$  in Caucasians (17, 18). Low levels of MBL ( $<1,000 \mu\text{g/L}$ ) are mostly caused by three point

mutations (O alleles) in exon 1 of the MBL gene (in codons 52, 54, and 57) that disrupt the assembly of the oligomers and also by a promoter polymorphism that is associated with low MBL production (LX). MBL level of 1,000  $\mu\text{g/L}$  discriminates fairly well between individuals with and without these variant MBL genotypes (12, 18–20). MBL levels can, however, vary markedly between individuals carrying the same genotype (18, 19). However, within-person variation of MBL concentration is fairly small also in patients with chronic inflammatory diseases (17, 21), but can temporarily increase up to threefold during acute phase responses (10, 22). It is not known how much MBL is required for optimal function in vivo, but low serum levels or variant MBL genotypes have been associated with certain infections and inflammatory diseases including systemic lupus erythematosus (SLE; references 12 and 20–22).

Several studies have analyzed MBL in relation to atherosclerotic outcomes. Low MBL



**Figure 1. The design of the Reykjavik study.** The cohort consisted of all men born between 1907 and 1934 and all women born between 1908 and 1935 that lived in the greater Reykjavik area. Approximately 70% participated, with 9,323 men and 10,058 women. The follow-up group was

levels have been reported to increase the risk of graft occlusion after bypass surgery (23), and variant MBL genotypes have been associated with carotid artery plaques (24) and coronary heart disease as evident by coronary artery surgery (25, 26), coronary events (27), and myocardial infarction (MI) in patients with SLE (28). However, there was no correlation between MBL levels and peripheral arterial disease in another study (29), and recent case control studies did not find significant association between variant MBL genotypes and hospitalization due to cardiovascular disorders (30) or vascular complications in type 1 diabetes (31).

The predictive value of MBL levels for MI has not been analyzed before. We postulated that high MBL might predict decreased likelihood of MI either independently or in the context of risk factors that may give rise to MI through diverse pathogenic mechanisms. To evaluate these possibilities, we first studied cross-sectional and then follow-up case control groups from the Reykjavik study (32).

randomly selected from stages one to five of the study. The cross-sectional group was recruited from the original cohort and consisted of cases with a history of MI at the age of 70 yr and controls, 70 yr and older, without evidence of MI.

## RESULTS

The study groups were, as shown in Fig. 1, subsamples from the Reykjavik study, a prospective study initiated in 1967. In brief, the first, referred to as the cross-sectional group, was a sample from the original cohort of cases with a history of MI at the age of 70 yr and controls, 70 yr and older, without evidence of MI. The second, referred to as the follow-up group, was a randomly selected nested sample of middle-aged individuals from stages one to five of the Reykjavik study with an average follow-up time of 27 yr.

### Baseline associations and long-term stability of MBL

Baseline characteristics of the study participants are shown in Table I. The within-self correlation coefficient for MBL levels was 0.86 (95% confidence interval: 0.80–0.91) among 96 participants who provided paired serum samples at an average interval of 20 yr (7–29), which is above the long-term consistency for total serum cholesterol (0.60), ESR (0.67),

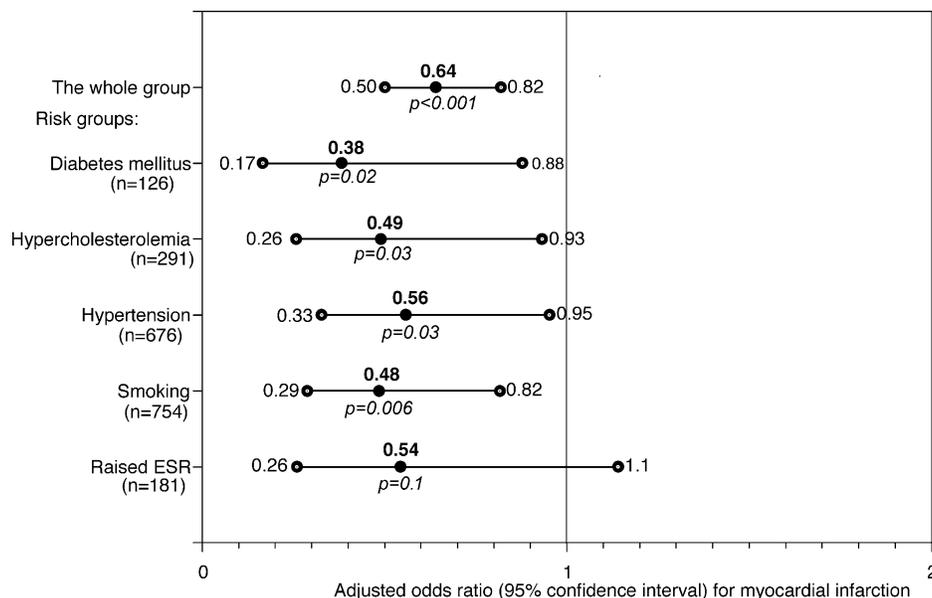
**Table I.** Baseline characteristics of study participants

	Cross-sectional group		Follow-up group	
	MI cases	Controls	MI cases	Controls
Number in group	457	530	867	442
Gender (male/female)	356/101	410/120	500/367	221/221
Age at examination (yr)	70 (66–73)	75 (72–78)	56 (51–62)	55 (50–61)
Diabetes mellitus (%) <sup>a</sup>	15.3	10.6	5.2	2.5
Total cholesterol (mmol/L)	5.9 (5.2–6.7)	6.0 (5.3–6.9)	6.8 (6.1–7.6)	6.5 (5.7–7.2)
Total triglyceride (mmol/L)	1.3 (1.0–1.9)	1.1 (0.85–1.5)	1.1 (0.85–1.5)	1.0 (0.76–1.4)
Hypertension (%) <sup>b</sup>	67.6	63.8	67.6	53.6
Current or former smoker (%)	80.5	68.1	76.9	67.9
Body mass index (kg/m <sup>2</sup> )	26.7 (24.5–30.0)	26.1 (23.6–28.4)	25.6 (23.3–28.3)	25.1 (22.7–27.4)
ESR (mm/h)	7 (3–13)	6 (3–12)	9 (4–17)	8 (3–14)

Data are median values (lower and upper quartile) or percentages.

<sup>a</sup>Diabetes mellitus diagnosis was based on a questionnaire, fasting plasma glucose >7 mmol/L, and a 2-h glucose tolerance test >11.1 mmol/L.

<sup>b</sup>Hypertension was defined as blood pressure >140/90 mm Hg or the use of antihypertensive medications.



**Figure 2. The cross-sectional group.** Modifying effect associated with high MBL on the risk of MI in individuals with different risk factors. Adjusted odds ratios for individuals with MBL levels  $> 1,000 \mu\text{g/L}$  compared with those with MBL  $< 1,000 \mu\text{g/L}$  with 95% confidence intervals. An odds ratio of one (the vertical line) is the MI risk associated with each risk factor in-

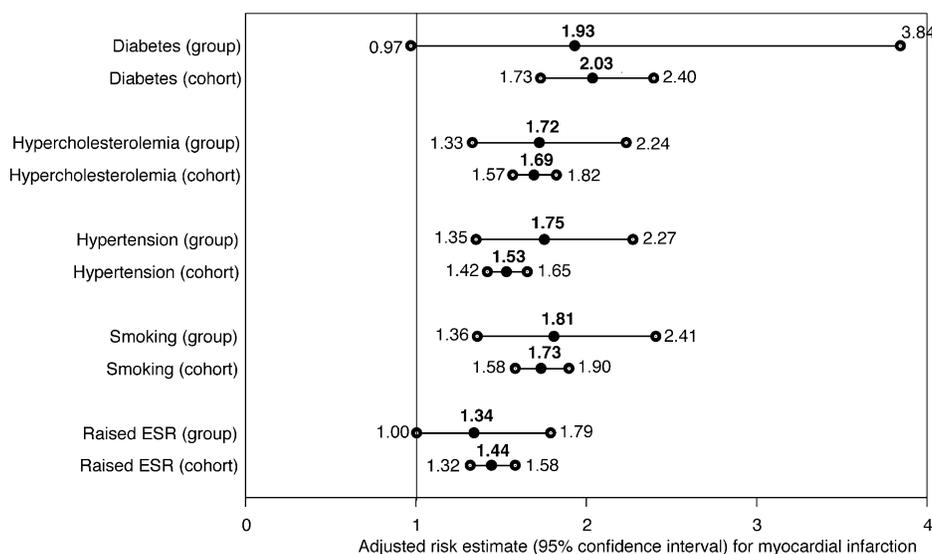
dependent of MBL levels. The multiple logistic regression model was adjusted simultaneously for gender, age, body mass index, diabetes mellitus, hypercholesterolemia, hypertension, smoking, and raised ESR with the exception of the analyzed covariate. The definition of risk factors is described in Materials and Methods.

and systolic (0.66) and diastolic (0.53) blood pressure, but similar to that for body mass index in the Reykjavik study cohort (4). MBL levels did not correlate with the age of the participants, diabetes mellitus, total serum cholesterol, systolic or diastolic blood pressure, body mass index, or ESR. In the follow-up group, smokers had higher MBL than those who had never smoked with median levels of 1,265 and 1,046  $\mu\text{g/L}$ , respectively ( $P < 0.001$ ), and men had higher MBL than women with median levels of 1,339 and 1,034

$\mu\text{g/L}$ , respectively ( $P < 0.001$ ), but these differences were not observed in the cross-sectional group.

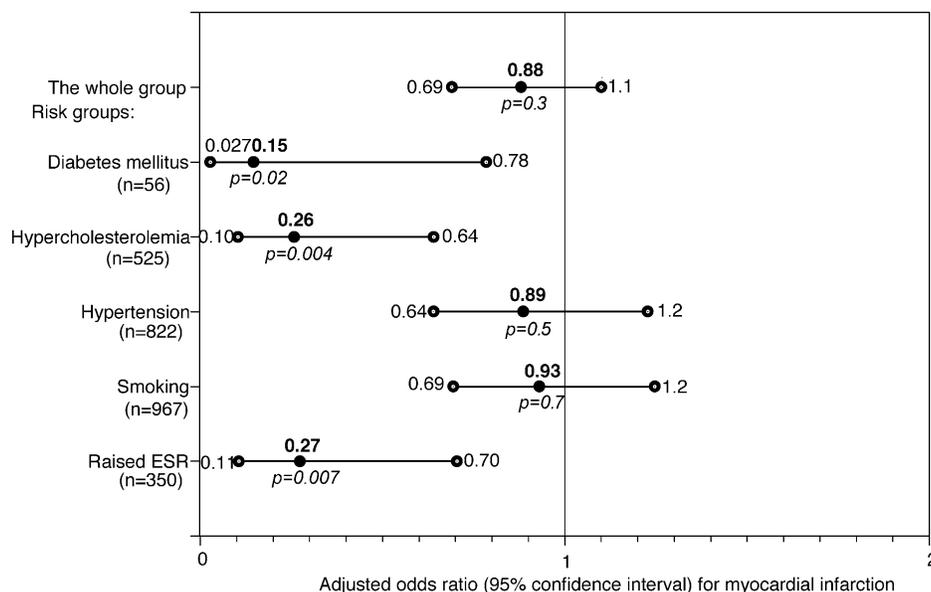
### MBL and the risk of MI

A cut-off level of 1,000  $\mu\text{g/L}$  was used to assess whether a relatively high level of MBL is associated with decreased risk of MI, but most individuals without variant MBL genotypes have levels above this (18–20). This is approximately the median MBL concentration in Caucasians (18) including



**Figure 3. Comparison of the risk of MI in individuals with each risk factor in the follow-up group ( $n = 1,309$ ) and the whole Reykjavik study cohort ( $n = 19,381$ ).** Odds ratio estimates from a multiple logistic regression model for the follow-up group and hazard ratio estimates from

a Cox regression for the whole Reykjavik study cohort are shown with 95% confidence intervals adjusted for the same variables as in Fig. 2. The definition of risk factors is described in Materials and Methods.



**Figure 4. The follow-up group.** Modifying effect associated with high MBL on the risk of MI in individuals with different risk factors. Adjusted odds ratios for individuals with MBL levels  $>1,000 \mu\text{g/L}$  compared with those with MBL  $<1,000 \mu\text{g/L}$  with 95% confidence intervals. An odds ratio

of one (the vertical line) is the MI risk associated with each risk factor independent of MBL levels. The multiple logistic regression model shown is adjusted for the same variables as in Fig. 2. The definition of risk factors is described in Materials and Methods.

Icelanders (17), and we hereafter refer to MBL levels  $>1,000 \mu\text{g/L}$  as high and MBL levels  $<1,000 \mu\text{g/L}$  as low.

In the cross-sectional group of individuals with a history of MI at the age of 70 and event-free controls above that age, the odds ratio for MI was 0.64 (95% confidence interval: 0.50–0.82;  $P < 0.001$ ) among individuals with high MBL ( $>1,000 \mu\text{g/L}$ ) compared with individuals with low MBL (Fig. 2). The group was then stratified according to conventional risk factors to assess the effect of high MBL in relation to different risk factors. It should be noted that most individuals had more than one risk factor and, with the exception of the analyzed covariate, the multiple logistic regression model adjusted simultaneously for gender, age, body mass index, diabetes mellitus, hypercholesterolemia, hypertension, smoking, and raised ESR. High MBL was associated with a lowered odds ratio for MI in the context of all these risk factors. The odds ratio estimate was lowest for diabetic patients, but only a trend was observed for participants with raised ESR.

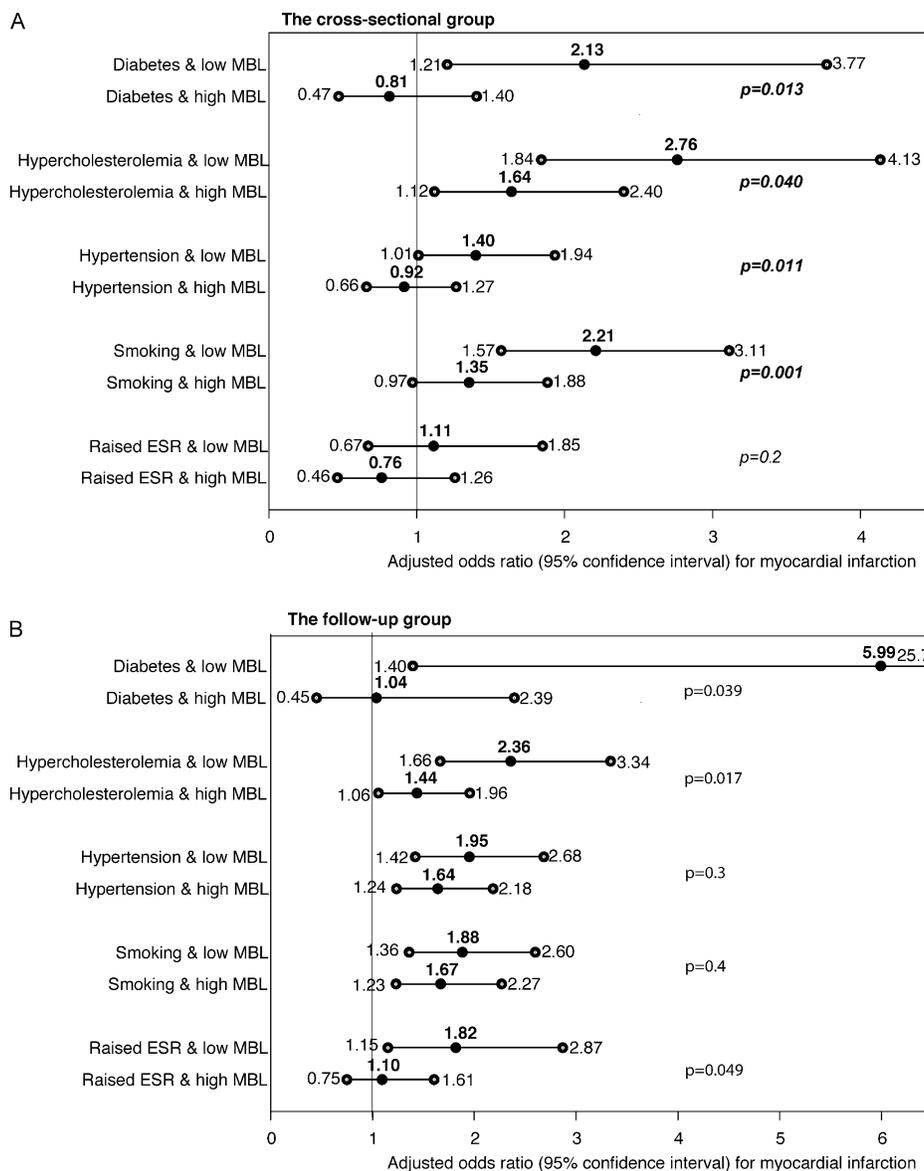
The predictive value of these findings was then evaluated in the prospectively assembled case control group, referred to as the follow-up group, which was randomly selected from the original Reykjavik study. As shown in Fig. 3, the risk profile in this study group largely reflected the whole Reykjavik study cohort ( $n = 19,381$ ). High MBL was also associated with a decreased MI risk in this group (Fig. 4), but to a lesser extent and not significantly in the group as a whole (Fig. 4). However, high MBL was, as in the cross-sectional group, associated with greatly decreased risk of MI in patients with diabetes or hypercholesterolemia with adjusted odds ratios of 0.15 (0.027–0.78) and 0.26 (0.10–0.64), respectively. This also applied for participants with raised ESR with an ad-

justed odds ratio of 0.27 (0.11–0.70), but in hypertensive individuals and smokers, high MBL was associated with only marginally decreased MI risk that was not significant.

To evaluate further the association between MBL and MI in individuals with a particular risk factor, these subgroups were categorized by their MBL level and compared with individuals without that risk factor. The baseline MI risk in individuals with each risk factor independent of MBL is shown in Fig. 3 for the follow-up group. High MBL was associated with decreased risk of MI in all risk groups of both study groups, but a marked variation was observed in this respect for the different risk factors. Thus, diabetic patients with high MBL were not at more risk of MI than nondiabetic individuals, and this applied both to the cross-sectional and follow-up groups (Fig. 5, A and B). In the cross-sectional group, hypertensive individuals and smokers with high MBL were also not at enhanced risk of MI (Fig. 5 A), and this also applied for individuals with raised ESR in the follow-up group (Fig. 5 B). However, hypercholesterolemic individuals with high MBL had enhanced risk of MI compared with participants with normal cholesterol levels, but the risk was much lower than that of hypercholesterolemic individuals with low MBL in both study groups. Thus, if MBL was high, the risk of MI associated with certain risk factors was not significantly different from that observed for the study groups as a whole.

#### Distribution of MBL levels in MI cases and controls

The controls had higher MBL levels than the MI patients in the cross-sectional group with median levels of 1,195 and 938  $\mu\text{g/L}$ , respectively ( $P = 0.002$ ), whereas the distribution of the MBL levels was similar in MI cases and controls in the follow-up group as a whole (Fig. 6 A). However, when



**Figure 5.** Risk of MI in individuals with different risk factors categorized into subgroups according to their MBL levels (above or below 1,000  $\mu\text{g/L}$ ) and compared with all the individuals without that risk factor, independent of their MBL level. (A) The cross-sectional group and (B) the follow-up group. Odds ratio estimates from a multiple logistic

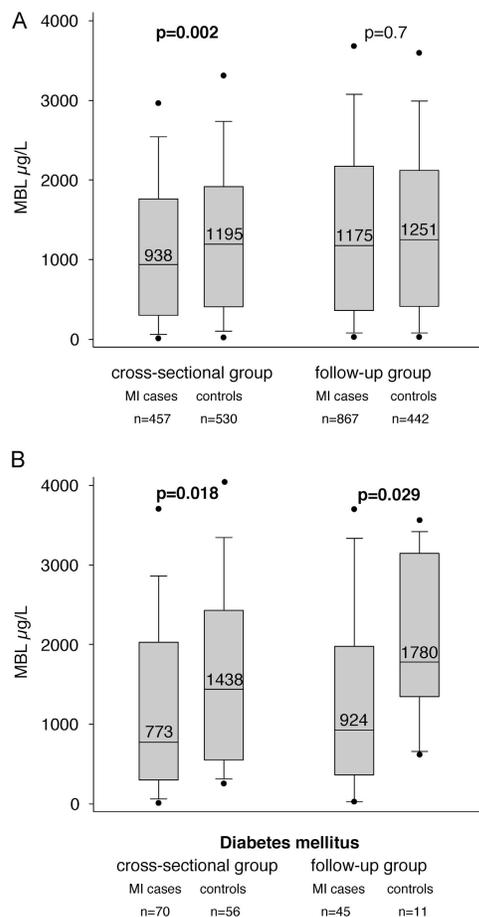
regression model adjusted for the same variables as in Fig. 2. The p-values indicate the significance of the difference between the subgroups. The definition of risk factors is described in Materials and Methods, and the number of participants with each risk factor is shown in Figs. 2 and 4.

MBL levels were compared between MI cases and controls with individual risk factors, the difference was, again, most marked in the diabetic patients (Fig. 6 B). Thus, the diabetic controls had nearly two times higher median MBL levels than the diabetic MI cases, and this applied both to the cross-sectional ( $P = 0.018$ ) and the follow-up ( $P = 0.029$ ) groups.

The modifying effect associated with MBL on the risk of MI was observed in both genders. The findings were unaffected by changes in cutoff values, including analysis of tertiles. Furthermore, the receiver operating characteristic (ROC) analysis revealed that the 1,000- $\mu\text{g/L}$  cutoff for MBL discriminated best between MI cases and controls with diabetes in the follow-up group (Fig. 7).

## DISCUSSION

Previous studies of MBL genotypes and various atherosclerotic outcomes have not been conclusive (24–27, 30), and the predictive power of serum MBL levels in relation to MI has not been analyzed before. Our findings are in accordance with a recent prospective cohort study, in which variant MBL genotypes were only marginally associated with increased risk of cardiovascular hospitalization or death in general, and this was not significant when retested in two case control studies (30). However, the findings in both of our study groups showed a similar trend when MBL was analyzed in the context of conventional risk factors for MI. Thus, high MBL was associated with greatly decreased risk

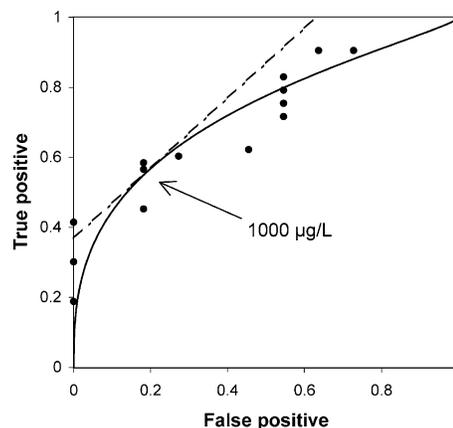


**Figure 6.** Distribution of MBL levels in MI cases and controls of both study groups (A) as a whole and (B) in diabetic individuals. The boxes show the median values and interquartiles, and the fifth percentiles of MBL concentrations are shown by the dots.

of MI in patients with diabetes or hypercholesterolemia, and diabetic patients were not at enhanced risk of MI if they had MBL above the median population level.

Chronic inflammation is now generally accepted to be a risk factor for MI and this applies for patients with chronic inflammatory diseases such as SLE and rheumatoid arthritis (1). A recent study found a strong association between variant MBL genotypes and the risk of MI in SLE (28), and it is interesting in this context that we also observed that high MBL was associated with reduced MI risk in individuals with raised ESR in the follow-up group, as ESR levels have previously been shown to predict future risk of MI in the Reykjavik study cohort (3). The cutoff for raised ESR was set at 15 mm/h (2), but the effect was also observed, although not as marked, at lower cutoff levels. C-reactive protein is another inflammatory marker predictive of coronary heart disease in the Reykjavik cohort (4), but these measurements were not available in this study.

Two reports have shown an association between variant MBL genotypes and coronary artery surgery (25, 26), and the latter also indicated that the risk of new coronary events in these patients was restricted to individuals with raised an-



**Figure 7.** ROC analysis of MBL levels for predicting MI in diabetic patients of the follow-up group. The dots represent empirical values of true/false positive fractions for MBL levels of 200–2,800 in increments of 200. The slope of the curve was unity for the 1,000- $\mu\text{g/L}$  cutoff, so MBL levels  $>1,000 \mu\text{g/L}$  are more representative of a control than a case and vice versa.

tibodies against *Chlamydia pneumoniae* (26). MBL binds this (33) and various other microorganisms (12), and chronic infections may aggravate the atherosclerotic process, possibly by promoting inflammatory state (1) and autoimmune processes (34).

Although high MBL was associated with a greatly reduced odds ratio for MI in the cross-sectional group, this did not reach significance for the follow-up group as a whole. A recent report from the Strong Heart study showed that variant MBL genotypes were associated with about threefold increased risk of coronary heart disease in American Indians (27). In view of our finding that the strongest association between high MBL and reduced risk of MI was observed in diabetic patients, it is of special interest that two thirds of the cases and one third of the controls in the Strong Heart study had diabetes. This might explain the strong association between variant MBL genotypes and MI risk observed in the group as a whole, but the participants were not stratified according to risk factors in this study. Another cross-sectional study focusing on the association between MBL and nephropathy in type 1 diabetes showed higher MBL levels in diabetic patients with macrovascular disease, including ischemic heart disease, stroke, and peripheral vascular disease compared with diabetic patients without history or signs of such complications. However, the frequency of variant MBL genotypes was not significantly different between the groups, and the difference in MBL concentration between the groups was primarily driven by individuals without variant MBL genotypes, suggesting that the higher MBL levels in the patients with vascular complications may reflect an ongoing acute phase response (31). It should be noted that very few diabetic patients in our study groups were receiving insulin, and the pathogenic mechanisms of vascular complications in type 1 and 2 diabetes might be different.

The strength of our study is large groups of both genders from a population-based cohort, which is typical for West-

ern societies in terms of cardiovascular risk. The average follow-up time was 27 yr in the follow-up group, the rates of participation and follow-up were high (32), and the MBL levels were found to be very stable when measured in the same individuals on average 20 yr apart. This means that adults or middle-aged individuals with a high or low level of MBL are likely to have very similar MBL levels until the age of 70 yr at least and after the occurrence of MI. The diabetic subgroups were, as expected, small compared with the whole study groups, and this limits further analysis. The reduced risk associated with MBL was nevertheless strong and notably, the diabetic patients were not at more risk of MI than nondiabetic individuals if they had high MBL.

Various arbitrary cutoff levels have previously been used to define insufficient MBL levels (19, 21), but we argued that it might be more appropriate to ask whether high MBL is associated with decreased risk. Several previous studies are based on MBL genotyping (24–27), but DNA samples were not available in this study, which was initiated in 1967. The monoclonal antibody used in this study for measuring MBL levels (clone 131–01; Statens Serum Institute) preferentially detects functional oligomers of MBL (19). The 1,000- $\mu\text{g/L}$  cutoff, which approximates the median level of the Icelandic population (17), was chosen as it discriminates fairly well between individuals with and without variant MBL genotypes (12, 18–20), and levels below this have been reported to be insufficient for clearing infections (21, 22, 33). Analysis of continuous MBL values and tertiles gave similar results, and evaluation of the 1,000- $\mu\text{g/L}$  cutoff for predicting MI in diabetic individuals showed this level to discriminate best between MI cases and controls.

Our data do not exclude the possibility that MBL could be a surrogate marker for risk of MI. However, the MBL levels did not correlate with any of the measured variables, and there was no correlation between MBL concentrations and the risk factors except that men had higher MBL levels than women and smokers had higher MBL levels than nonsmokers, but this only applied to the follow-up group. More frequent respiratory infections in smokers might explain this as MBL concentration can increase up to threefold during acute phase responses (22). Our findings are unlikely to be confounded by this, and it would, in any case, be more likely to diminish than augment the observed differences. The difference between genders has not been reported before and it should be noted that the modifying effects associated with MBL on MI risk were observed in both men and women. As the gender difference was only observed in the middle-aged follow-up group and not in the elderly cross-sectional group, this difference may perhaps be due to some hormonal influences on MBL production, as has for instance been shown for growth hormone (35, 36).

This study was not designed to elucidate causal mechanisms. However, as high MBL levels were associated with decreased likelihood of MI, particularly in diabetic individuals and also in hypercholesterolemia, it is interesting to note that both hyperglycemia and hyperlipidemia increase the flux of glucose through the hexosamine biosynthetic pathway,

leading to accumulation of *N*-acetylglucosamine (GlcNAc). This sugar is the principal substrate for O-linked and N-linked glycosylation of various proteins (37). MBL has high affinity for GlcNAc (10) and has been shown to bind glycoproteins with terminal GlcNAc residues (38, 39). Furthermore, GlcNAc and mannose residues, which MBL also binds strongly, are exposed on the apolipoprotein B-100 component of oxidized low density lipoprotein (LDL; references 40–42). Oxidation of LDL promotes the formation of immune complexes, and this has been associated with an increased risk of MI (42–44), especially in diabetic patients (45). Glycation of the apolipoprotein B-100 of LDL is increased in diabetic patients and glycated LDL is vulnerable for oxidation (46). MBL has been shown to bind immune complexes (11, 15, 16), and we have already demonstrated that MBL can bind oxLDL in vitro (unpublished data), as has been demonstrated for the membrane-bound lectin-like oxLDL receptor 1, another C-type lectin (47). Thus, the findings are consistent with the possibility that MBL may facilitate noninflammatory phagocytic clearance of atherogenic agents by the liver and spleen and thereby reduce their arterial deposition. Inflammation and apoptosis probably render atherosclerotic plaques more vulnerable to rupture that may cause coronary occlusion (48). MBL has also been shown to decrease the release of proinflammatory cytokines from monocytes (12), greatly decrease the risk of a systemic inflammatory response (20), and mediate noninflammatory clearance of apoptotic cells (8, 13). Our findings suggest that MBL may predict lower MI risk in individuals with chronic inflammatory disorders. MBL replacement therapy has already finished a phase I clinical study (49) and might have a therapeutic potential in the appropriate clinical settings.

We conclude that a relatively high level of MBL may predict decreased likelihood of MI, particularly in diabetic patients. As diabetic patients with high MBL had a similar risk estimate for MI as nondiabetic individuals, MBL measurement has a potential usefulness as an adjunct to cardiac risk assessment and might actually aid in the evaluation of the need for preventive treatment. Our observations are in accordance with and extend previous findings and are suggestive of a biological relevance of MBL in the pathogenic process leading to coronary occlusion, perhaps by promoting clearance of atherogenic agents.

## MATERIALS AND METHODS

**Subjects and study design.** The study was performed in accordance with the Helsinki Declaration and was approved by the National Bioethics Committee and the Data Protection Authority in Iceland. All participants gave informed consent.

Two separate case control groups, recruited by the Icelandic Heart Association, were analyzed (Table I). As shown in Fig. 1, these groups were subsamples from the Reykjavik study, a prospective study initiated in 1967 that has been described in detail (32). In brief, all men born between 1907 and 1934 and all women born between 1908 and 1935 and living in the greater Reykjavik area, where 52% of the nation lived, were invited and ~70% participated, a total of 9,323 men and 10,058 women.

The first, referred to as the cross-sectional group, was a case control sample from the original Reykjavik study cohort of cases with a history of

MI at the age of 70 yr and controls, 70 yr and older, without evidence of MI. The participants were recruited again between 1991 and 1997 for clinical assessment and sampling of blood. Serum was available for 457 cases and 530 gender-matched controls who had not been diagnosed with MI.

The second, referred to as the follow-up group, was a randomly selected nested sample of middle-aged individuals from stages one to five of the Reykjavik study that were recruited between 1967 and 1991. Individuals with a history of MI at recruitment were excluded. Baseline serum samples were available from 1,309 individuals, including 867 participants who had suffered MI during the follow-up time and 442 event-free controls. The average follow-up time was 27 yr (6–34) in December 2001.

96 individuals ended up in both groups and were used as a basis for within-self correlation measures of MBL levels.

**Evaluation and ascertainment of events.** The evaluation of both groups included a structured interview, physical examination, collection of serum samples, and various laboratory tests. Endpoints were nonfatal MI according to the MONICA criteria (50) or death from coronary heart disease, verified from death certificates, hospital, and necropsy records with >99% follow-up yield. Detailed account of the recruitment process and ascertainment of events has been published elsewhere (3, 32). Biochemical and hematological measurements were made at recruitment by standard methods as described previously, and the same methodology was used for both study groups (32). For this study, risk factors for coronary heart disease were defined as follows. Hypercholesterolemia was defined as total cholesterol >7 mmol/L or the use of lipid-lowering medications (9% of the cross-sectional group but not recorded in the follow-up study as their use was very uncommon in Iceland during the study period). The lipid analysis was based on total cholesterol as measurement of high density lipoprotein was only available at a late stage of the study. Smoking was recorded as a risk factor in participants who smoked at study entry or had a history of former smoking. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or the use of antihypertensive medications (20% in both the cross-sectional and follow-up groups). The diagnosis of diabetes mellitus was based on a questionnaire and fasting plasma glucose >7 mmol/L or an oral glucose tolerance test with plasma glucose >11.1 mmol/L 2 h after a 75-g glucose load, according to the American Diabetes Association's criteria. It was not possible to distinguish type 1 and 2 diabetes, but very few of the diabetic individuals were treated with insulin. In addition to participants recorded with diabetes mellitus at entry to the study, those who were subsequently diagnosed with diabetes, but >5 yr before the occurrence of MI, were included as diabetics in the analysis. Inflammation was considered present if ESR was >15 mm/h (2).

**Measurement of MBL.** MBL was measured blindly in serum samples taken at entry when the risk factors were first recorded. A sandwich ELISA system was used that has been described in detail (17). The monoclonal antibody used in this assay (HYB 131–1; Statens Serum Institute) predominantly binds to the functional oligomers of MBL (19). In brief, two dilutions of the test sera ( $1/7$  and  $1/63$ ) and three control sera with low, medium, and high MBL concentration were included in each test run and calibrated according to a standard. The detection limit of the assay was set at 50  $\mu\text{g/L}$  and the intra- and inter-assay variabilities were 3.7 and 11.7%, respectively. The sera were kept at  $-20^\circ\text{C}$  and had never been thawed. The distribution of the MBL concentrations was independent of the age of the serum samples.

**Statistical analysis.** Mann-Whitney Rank Sum test was used to compare continuous variables between groups. The association between events of MI and each risk factor was estimated as an odds ratios with a 95% confidence interval (OR [95% CI]) from a multiple logistic regression model adjusted simultaneously for gender, age, body mass index, diabetes mellitus, hypercholesterolemia, hypertension, smoking, and raised ESR, with the exception of the analyzed covariate. The logistic regression model was defined by using a backward selection method beginning with all two-way interactions. Interactions with  $p$ -values <0.2 stayed in the model. The effect of

high MBL on the risk of MI in groups with enhanced risk was assessed in two ways: (a) by the change in odds ratio estimates from a baseline risk of one for each risk group and (b) by comparing the risk of MI in each group, categorized into those with high and low MBL, to the risk of MI in individuals without that risk factor. Hazard ratio estimates from a Cox regression adjusted for the variables described above were calculated for the whole Reykjavik study cohort to assess whether the follow-up group was a reflective sample of the whole cohort. Within-self correlation of MBL in paired serum samples was estimated with a repeated measures model with sex and age as covariates. An ROC analysis was used to evaluate the sensitivity and specificity of the 1,000- $\mu\text{g/L}$  cutoff for predicting MI in diabetic individuals. Statistical analysis was performed using SAS (SAS/STAT Version 9.1; SAS Institute Inc.). All tests were two-sided and the level of significance was set at 0.05.

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