Serum Levels of Mannose-Binding Lectin and the Risk of Future Coronary Artery Disease in Apparently Healthy Men and Women


Objective—To determine the association between serum levels of mannose-binding lectin (MBL) and the risk of future coronary artery disease (CAD) in apparently healthy men and women.

Methods and Results—We performed a prospective case-control study among apparently healthy men and women nested in the EPIC-Norfolk cohort. Baseline concentrations of MBL were measured in serum samples of 946 patients who experienced a myocardial infarction or died of CAD during follow-up, and 1799 matched controls who remained free of CAD. Among men, median MBL levels were 1.63 ng/mL (interquartile range [IQR]: 0.59 to 3.80) in cases and 1.20 ng/mL (IQR: 0.48 to 3.37) in controls. Among women, median MBL levels were 1.02 ng/mL (IQR: 0.43 to 2.95) in cases and 1.01 ng/mL (IQR: 0.43 to 2.94) in controls. After adjustment, the odds ratio in men for future CAD was 1.59 (95% confidence interval [CI]: 1.09 to 2.32; \( P \) for linearity = 0.01) for those in the highest quartile compared with those in the lowest quartile. In women no such relation was observed.

Conclusions—Elevated levels of MBL are associated with an increased risk of future CAD in apparently healthy men but not in women. The sex difference merits further exploration. (Arterioscler Thromb Vasc Biol. 2006;26:2345-2350.)

Key Words: atherosclerosis ■ coronary artery disease ■ inflammation ■ mannose-binding lectin

Inflammation plays a major role in all phases of atherogenesis from plaque initiation to plaque rupture. Several inflammatory markers have been associated with an increased risk of atherosclerotic vascular disease, including C-reactive protein (CRP),1 SPLA2,2 and interleukin (IL)-6.3 In addition, markers of innate immunity have been shown to predict the development of coronary artery disease (CAD).4–7 However, prospective evidence for a causative role of inflammatory factors is still limited.8 Recently, evidence has emerged concerning the role of mannose-binding lectin (MBL) in the development of atherosclerosis. MBL is a part of the complement cascade and plays an important role in the first line of defense of the innate immune system against pathogenic microorganisms.9,10 MBL recognizes sugar patterns on the surface of many pathogens,11 phospholipids,12 immune complexes,13 and apoptotic cells.14 In the circulation, MBL forms a complex with MBL-associated serine proteases (MASPs). This complex becomes enzymatically active and activates the classical complement route. This facilitates complement-dependent opsonization and subsequent uptake and clearance by phagocytes.11 In addition, there are indications that MBL binds directly to granulocytes, monocytes, and macrophages, which may stimulate the production of pro-inflammatory cytokines.15 Since innate immunity has been implicated in atherogenesis, MBL has been suggested to play a role in the formation of atherosclerotic plaque. However, studies examining the relations between either serum levels of MBL or MBL genetic variants associated with low serum levels of MBL and CAD risk have reported equivocal results.7,16–21,22–24 At present, no conclusive data are available about the relationship between serum MBL levels and future CAD risk in healthy individuals.

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We hypothesized that MBL levels are associated with an increased risk of future CAD in healthy individuals. We tested this hypothesis in a large prospective case-control study nested in the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) prospective population study. We measured serum levels of MBL in apparently healthy men and women at baseline and assessed the development of CAD during a follow-up period of 6 years.
Methods

The EPIC study (EPIC: European Prospective Investigation into Cancer and Nutrition), a collaborative study of 9 countries in Europe, was designed to assess the determinants of cancer and other diseases. The EPIC-Norfolk cohort which is a part of the EPIC study has been described in detail previously. In brief, investigators recruited 25,663 men and women, all residents in Norfolk (United Kingdom), from general practices and performed a baseline survey between 1993 and 1997. Trained nosologists obtained vital status of the entire cohort based on death certificates of the United Kingdom Office of National Statistics and linkage of the unique National Health Service number linkage with the East Norfolk Health Authority (ENCORE) database, which identifies all hospital contacts throughout England and Wales for Norfolk residents. Follow-up information was obtained up to January 2003, an average of ~6 years. The study was approved by the Norwich District Health Authority Ethics Committee and all participants gave informed consent. We performed a nested case-control study of coronary artery disease in the EPIC-Norfolk cohort. The design, methods and validation of this CAD case control study have been described in detail elsewhere. In brief, we identified apparently healthy participants from the cohort who developed a myocardial infarction or died of coronary heart disease. Two controls were matched to these cases based on sex, age (within 5 years), data of visit (within 3 months), and duration of follow-up time in the study. Controls were those who remained alive and did not have a myocardial infarction during follow-up. For this nested study we excluded participants from the EPIC-Norfolk cohort who reported a history of myocardial infarction or stroke at the baseline visit.

Biochemical Analyses

Qualified staff collected blood from all participants at the baseline visit. The Department of Clinical Biochemistry, University of Cambridge processed the samples for assay. Serum levels of total cholesterol, high-density lipoprotein cholesterol (HDL-c), and triglycerides were measured on fresh samples with the RA 1000 (Bayer Diagnostics, Basingstoke, UK), and low-density lipoprotein cholesterol (LDL-c) levels were calculated with the Friedewald formula. In addition, serum samples were stored at −80°C for future analyses. We measured serum concentrations of CRP with a sandwich-type enzyme linked immunosorbent assay (ELISA) as previously described, and related the results of this ELISA to a standard consisting of commercially available CRP (Behringwerke AG, Marburg, Germany). We measured serum MBL levels with a commercially available ELISA kit from Sanquin Research (Amsterdam, The Netherlands). We incubated the samples in mannan-coated plates. After washing, we visualized the binding of MBL by incubation with biotinylated monoclonal antibody against MBL (CLB anti-MBL-1). Samples were analyzed in random order and researchers and laboratory personnel were blinded as to case status of the samples.

Statistical Analysis

MBL levels had a skewed distribution and were therefore log-transformed before being used in statistical analyses, but in Tables we present untransformed medians and corresponding interquartile ranges (IQR). MBL quartiles were based on the sex-specific distribution among the controls. Sex-specific analyses were performed using sex-specific quartile cut-offs. To estimate the relative risk of future CAD, we calculated odds ratios (OR) and corresponding 95% confidence intervals (95% CI) per MBL quartile and a probability value for linearity across quartiles. ORs were calculated using conditional logistic regression, taking into account the matching for sex and age and enrollment time. ORs were adjusted for cardiovascular risk factors which were significantly related to MBL levels. In a second regression model, ORs were additionally adjusted for serum levels of CRP. The lowest quartile was used as the reference category.

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<th>TABLE 1. Study Population</th>
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<td>C-reactive protein, mg/L</td>
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<td>MBL, ng/mL</td>
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Women

|                           | (n=547)  | (n=294) |
| Age, year                 | 67±7     | 67±7    |
| Smoking                   |          |         |
| Current                   | 7.0 (38) | 15.1 (44) |
| Former                    | 36.3 (197) | 37.1 (108) |
| Never                     | 56.6 (307) | 47.8 (139) |
| Body mass index, kg/m²    | 26.3±4.1 | 27.3±4.5 |
| Diabetes                  | 1.3 (7)  | 5.4 (16) |
| Systolic blood pressure, mm Hg | 139±19 | 144±20 |
| Diastolic blood pressure, mm Hg | 82±11 | 85±12 |
| Total cholesterol, mmol/L | 6.7±1.2 | 6.9±1.3 |
| LDL cholesterol, mmol/L   | 4.3±1.1 | 4.5±1.1 |
| HDL cholesterol, mmol/L   | 1.58±0.41 | 1.44±0.39 |
| Triglycerides, mmol/L     | 1.5 (1.1–2.2) | 1.9 (1.5–2.6) |
| C-reactive protein, mg/L  | 1.6 (0.7–3.7) | 2.6 (1.1–5.1) |
| MBL, ng/mL                | 1.01 (0.43–2.94) | 1.02 (0.43–2.95) |

| Data are presented as mean±SD, % (n), or median (interquartile range). |
| HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; MBL, mannose binding lectin. Means, percentages, and medians may be based on fewer observations than the indicated number of subjects. |

Results

MBL Levels in the Study Population

Table 1 shows the sex-specific distribution of risk factors at baseline for cases and controls. As expected, levels of risk factors including blood pressure, total cholesterol, LDL-c, triglycerides, CRP, diabetes, cigarette smoking habit, and body mass index were higher and HDL-c lower in those in whom CAD developed during follow-up compared with those who remained free from CAD. Among men, median MBL levels were higher in cases when compared with controls. Among women, median MBL levels were similar in cases and controls.

MBL Levels and Other Risk Factors

Table 2 shows the relationship between cardiovascular risk factors and MBL quartiles.
MBL levels were correlated with sex, HDL-c, and CRP levels. These correlations were consistent over the MBL quartile. Among men, MBL levels were correlated with CRP levels. Among women, no such correlation with CRP was noted, but a significant correlation was seen with body mass index. There was no significant relationship between MBL levels and other cardiovascular risk factors, including age, history of diabetes, smoking, blood pressure, LDL-c, and triglycerides.

**MBL Levels and the Risk of Future CAD**

Table 3 presents the ORs for future CAD according to quartile of MBL among men and women (Table 3). In men, after adjustment for determinants of cardiovascular risk and MBL levels, the risk of future CAD was 1.55 (95% CI, 1.08 to 2.22) for men in the highest MBL quartile compared with those in the lowest (P for linearity=0.01). This remained significant after additional adjustment for CRP. The Figure shows ORs for future CAD in men stratified by quartiles of CRP and MBL. The relationship between MBL levels and future CAD was solely accounted for by men, whereas in women no such a relation was found (P for linearity=0.2).

**Discussion**

In this large prospective study among apparently healthy people, we showed that in men high serum levels of MBL are associated with an increased risk of future coronary artery disease. This relationship was independent of established risk factors.
cardiovascular risk factors. This observation suggests that MBL levels may reflect or contribute to a pathophysiological process relevant in the development of atherosclerosis.

Recently, evidence emerged about a role of MBL in CAD. In experimental models it was shown that the MBL pathway is involved in ischemia-induced complement activation in mice. Consequently, the administration of anti-MBL antibodies protects the heart from ischemia-reperfusion injury by reducing neutrophil infiltration and attenuating pro-inflammatory gene expression. In humans, it was recently shown that high MBL levels are associated with a high incidence of re-stenosis in patients with atherosclerotic disease of the carotid artery. Moreover, type I diabetic patients with a history of cardiovascular disease had significantly elevated MBL levels when compared with type I diabetic patients without vascular complication. In our study, we were able to show an increased risk for CAD in apparently healthy men with high MBL levels. In women, however, no such an association was seen. Sex differences with regard to MBL levels and cardiovascular risk have been reported before but the causes are presently unknown. Endocrine status plays an important role in the regulation of MBL levels. Hormonal differences and cardiovascular disease are an ongoing topic of research. Anti-inflammatory properties of estrogen have sparked much interest and may comprise complement activation. Interestingly, endothelial tissue from males may be more susceptible than that from females to the acute effects of complement activation. Differences in the expression of complement components in adipose tissue of men and women have also been observed. Furthermore, we observed significant differences in CRP levels among the MBL quartiles with highest CRP levels in the highest MBL quartile. High CRP and MBL levels could both be a sign of an inflammatory state. MBL concentrations are normally stable within one person, but can rise 3-fold during the acute phase response. Therefore, the differences in MBL levels between male cases and controls could be partially attributable to the differences in the inflammatory state of these individuals. However, the relationship between MBL levels and future CAD risk persisted on additional adjustment for CRP.

Earlier studies regarding the role of MBL in atherogenesis in humans are not conclusive. In one population-based study, DNA analysis in 434 apparently healthy Native Americans revealed that genotypes coding for diminished levels of MBL are predictive of CAD. However, measuring MBL protein levels may be more reliable than genotyping. MBL protein levels vary widely between individuals. This is partly influenced by variant MBL genotypes, but serum levels vary markedly between persons with an identical genotype. Depending on the antibody used, however, different findings can be seen that may also contribute to the lack of consensus regarding the role of MBL in atherosclerotic vascular disease. In a nested case-control study in the Reykjavik cohort,
Saevarsdotir showed that MBL levels are not associated with an increased risk of CAD, but in a subanalysis high MBL levels were associated with a lower risk of CAD in diabetic subjects.24 We observed several differences with our own study. First, there is a difference in power, because we included twice as many patients in the study. Second, contrary to the former study we matched all cases for both age and sex and enrollment-time and subsequently we performed sex-specific analysis. Finally, we measured also CRP levels, which made it possible to correct for CRP levels during the statistical analysis.

There are several mechanisms by which MBL can function atherogenic. MBL activates the complement system which has been implicated in atherogenesis and was recently shown to be associated with increased cardiovascular risk in patients with advanced atherosclerosis.4 Indeed, increased deposition of complement iC3b in ruptured and vulnerable plaques suggests a role for complement activation in acute coronary syndromes.24 Furthermore, endothelial oxidative stress, which plays a major role in atherogenesis, activates complement via the lectin complement pathway in human cell cultures. In addition, in this experimental model, anti-MBL monoclonal antibodies inhibited MBL and C3 deposition after endothelial oxidative stress.35 MBL, however, not only is involved in complement activation but also is a potent regulator of inflammatory pathways.36 Indeed, MBL was shown to enhance the production of chemokines by macrophages.37 and may thereby enhance phagocyte recruitment to the subendothelium. Moreover, it was recently shown that MBL binds to leukocytes and can thereby modulate inflammation. Of note, this study suggested that such binding only occurred at extravascular sites in individuals possessing MBL genotypes conferring MBL sufficiency.38 This may help explain why high MBL levels can influence the atherosclerotic process in the subendothelium. These data support a role for MBL in the atherogenesis and possibly in plaque destabilization and the development of coronary events.

A number of issues have to be taken into account when interpreting the results of the present study. Serum levels of MBL were determined in a single sample that was obtained at a nonuniform time of the day. Diurnal variation and variation over time could have affected the MBL concentrations, although such variation has been shown to be limited compared with other cardiovascular risk factors. In addition, we cannot rule out that sample storage at −80°C for 6 to 10 years may have affected the detection of MBL. However, both these limitations would lead to increased random measurement error, which leads to an underestimation of any relationship, and therefore do not negate our findings. Although the current study was not designed to establish whether the relationship between MBL and CAD is causal, it is unlikely that differences in MBL serum levels occurred as a consequence of cardiovascular events because individuals with symptomatic cardiovascular disease were excluded from our analysis. However, we cannot exclude the possibility that MBL concentration is a marker of advanced subclinical atherosclerosis.

We conclude that in this nested case-control study elevated serum concentrations of MBL are associated with an increased risk of future CAD in apparently healthy men. This relationship was independent of other cardiovascular risk factors. These prospective data support the hypothesis that the immune system plays an important role in the development of atherosclerosis and its prominent clinical manifestation, CAD. The lack of association in women warrants further investigation and may provide insights into possible explanations for the sex difference in coronary heart disease susceptibility.

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Disclosures
None.

References
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