Received: 19 February 2019 | Revised: 22 May 2019 | Accepted: 6 June 2019

DOI: 10.1111/jth.14539

ORIGINAL ARTICLE

Plasma levels of mannose-binding lectin and future risk of venous thromboembolism

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Funding information
Stiftelsen Kristian Gerhard Jebsen; The Danish Research Foundation for Independent Research , Grant/Award Number: DFF-6110-00489; Novo Foundation; The Simon Fougner Hartmann Family Fund; The Odd Fellow Foundation; The Norwegian Council on Cardiovascular Disease

Abstract

Background: Animal and observational studies have suggested a pathophysiological role for complement in venous thromboembolism (VTE), but the initiating mechanisms are unknown. Mannose-binding lectin (MBL) bound to altered host cells leads to activation of the lectin complement pathway, and both high and low MBL levels have been implicated in the pathophysiology of cardiovascular disease.

Objectives: To investigate the association between plasma MBL levels and future risk of incident VTE.

Methods: We conducted a nested case-control study in 417 VTE patients and 849 age-matched and sex-matched controls derived from the general population (Tromsø Study). Plasma MBL levels were measured using enzyme-linked immunosorbent assay. Logistic regression models were used to estimate odds ratio (OR) for VTE across quartiles of plasma MBL levels.

Results: Subjects with plasma MBL levels in the lowest quartile (<435 ng/mL) had a reduced OR for overall VTE (OR 0.79, 95% confidence interval [CI]: 0.56-1.10) and for DVT (OR 0.70, 95% CI: 0.47-1.04) compared to those with MBL in the highest

Manuscript handled by: Flora Peyvandi

Final decision: Flora Peyvandi, 6 June 2019

quartile (≥2423 ng/mL) after multivariable adjustments. For VTE, DVT, and pulmonary embolism (PE) the ORs decreased substantially with decreasing time between blood sampling and VTE event.

Conclusions: Our findings suggest that low plasma MBL levels are associated with reduced risk of VTE, and DVT in particular.

KEYWORDS

complement, deep vein thrombosis, mannose-binding lectin, pulmonary embolism, venous thromboembolism

1 | INTRODUCTION

Venous thromboembolism (VTE), including DVT and pulmonary embolism, affects 1 to 2 per 1000 individuals each year. It is a major public health challenge because of short-term and long-term complications, such as frequent recurrence and potentially death.[1–4] Inherited and environmental risk factors along with changes in blood flow, hypercoagulability, or dysfunction of the vessel wall affect individual thrombosis potential.[5,6] Despite improved awareness and prevention, the incidence of VTE has remained unchanged or even increased marginally over the past decades.[2,7] In order to diminish the health burden of VTE, it is imperative to identify novel biomarkers and unravel underlying disease mechanisms in order to improve risk prediction and provide targeted prevention and treatment.

Recent studies have implicated a role for the complement system in the pathogenesis of VTE due to an extensive cross-talk between the complement and hemostatic systems.[8–10] Complement factor C3 is an acute-phase reactant and a central component in the activation of the complement system.[11] Results from a large population-based cohort in Copenhagen showed that participants with plasma complement C3 levels in the highest tertile had a 58% higher risk of VTE compared to those in the lowest tertile. The risk estimate declined to 31% but was still significant after further adjustment for C-reactive protein (CRP) and body mass index (BMI).[12] In an inferior vena cava stenosis model, C3-deficient mice had a lower incidence of venous thrombosis and developed thrombi that were smaller in weight and size compared to those of wild-type controls.[13] The latter findings may suggest that complement C3 is a mediator rather than only a marker of VTE risk.

Mannose-binding lectin (MBL) is a pattern recognition molecule that binds to carbohydrates such as mannose on pathogens or damaged host cells and thereby activates the lectin pathway of the complement system.[14,15] The MBL circulates in molecular complexes with serine proteases called MBL-associated serine protease-1, MBL-associated serine protease-2, and MBL-associated serine protease-3 (MASPs-1,-2,-3).[14,15] The MASP-1 and MASP-2 are activated when MBL binds to specific carbohydrate structures on microbial and cell surfaces. This leads to cleavage of complement factors C4 and C2 and the formation of C4b2b convertase, with subsequent activation of C3 and the common complement pathway.[16] *In vitro* studies have shown that MASP-1 has thrombinlike activity and can cleave factor XIII (FXIII), fibrinogen, high-molecular-weight kininogen, and thrombin-activatable fibrinolysis inhibitor, while MASP-2 can cleave prothrombin to thrombin.[15,17] The MASPs can activate and stabilize clot formation,[15] and *in vivo* animal studies show that MASPs likely have a role in thrombogenesis.[18,19]

Essentials

- The initiating mechanisms for the role of complement in VTE is unknown.
- Mannose-binding lectin (MBL) leads to activation of the lectin complement pathway.
- Low plasma MBL was associated with a reduced risk of VTE, especially DVT.
- The OR for VTE decreased with decreasing time between blood sampling and event.

Plasma levels of MBL are largely determined by genotypes of the *MBL2* gene[20] and remain rather stable within individuals despite a moderate increase during an acute-phase response.[21,22] The MBL levels vary markedly between individuals because of the variation in the *MBL2* gene,[23] and approximately 5% to 20% of the population is MBL-deficient with functional levels below 100 ng/mL.[24–27] Thus, low levels of MBL have been suggested as a reliable surrogate marker of variation in the *MBL2* gene. The association between plasma levels of MBL and risk of VTE has not been thoroughly investigated. Given the procoagulant effects of MASPs *in vitro* and in animal models, it is likely that low levels of MBL would protect against development of VTE. However, in patients with systemic lupus erythematosus, *MBL2*-deficient genotypes were associated with increased[28] or unchanged[29] risk of VTE, whereas low plasma levels of MBL (<100 ng/mL) were associated with increased VTE risk in a small case-control study recruited from the general population.[30] The conflicting results may partly be explained by chance because of the low number of participants included in these studies, inconsistent patient selections, or the retrospective nature of the case-control study with the potential risk of reverse causation. The aim of the present study was therefore to investigate the association between plasma levels of MBL and risk of VTE in a nested case-control study derived from the general population.

2 | METHODS

2.1 | Study population

The Tromsø Study is a single-center, population-based cohort, with repeated health surveys of inhabitants of Tromsø, Norway. Members of the population aged ≥25 years living in the municipality of Tromsø were invited to participate in the fourth survey, conducted in 1994-1995. A total of 27 158 subjects participated (77% of those invited) and were followed from the date of inclusion until an adjudicated incident VTE event, migration, death, or end of follow-up (1 September 2007). All first lifetime events of VTE occurring among the participants in this period were identified using the hospital discharge diagnosis registry, the autopsy registry, and the radiology procedure registry from University Hospital of North Norway (UNN), which is the sole provider of diagnostic radiology and treatment of VTE in the Tromsø area. Participants with a history of VTE before baseline were excluded. Trained personnel adjudicated and recorded each VTE by extensively reviewing medical records. The identification and adjudication process of VTEs has previously been described in detail.[31] In short, the adjudication criteria for VTE were presence of signs and symptoms of DVT or PE combined with objective confirmation by radiological procedures, which resulted in initiation of treatment (unless contraindications were specified). A VTE occurring in the presence of one or more provoking factors was classified as provoked. Provoking factors were surgery or trauma (within 8 weeks before the event), acute medical condition (acute myocardial infarction, acute ischemic stroke, acute infections), immobilization (bed rest >3 days or confinement to wheelchair within the last 8 weeks, or long-distance travel ≥4 h within the last 14 days), or other factors specifically described as provoking by a physician in the medical record (e.g., intravascular catheter).

There were 462 individuals who experienced a VTE event during the follow-up period (1994-2007). For each case, two age-matched and sex-matched controls, who were alive at the index date of the VTE event, were randomly sampled from the source cohort ($n = 924$). In total, 45 cases and 75 controls did not have plasma samples of sufficient quality available for the analyses. Thus, our final nested case-control study consisted of 417 cases and 849 controls. The regional committee for medical and health research ethics approved the study, and all participants provided written consent.

2.2 | Baseline measurements

Height (to the nearest centimeter) and weight (to the nearest 0.5 kg) were measured in participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight divided by the square of height in meters (kg/m^2). A self-administered questionnaire was used to collect a detailed history of previous cardiovascular disease (CVD) events (stroke, angina pectoris, transient ischemic attack, and myocardial infarction), recurrent VTE, diabetes mellitus, and other concurrent diseases. The questionnaire also included questions about dietary habits, physical exercise, smoking, and alcohol consumption.

2.3 | Blood sample collection and storage of blood products

At inclusion in Tromsø 4 (1994-1995), non-fasting blood was collected from an antecubital vein into 5-mL vacutainers (Becton Dickinson, Le Pont de Claix, France) containing EDTA (K_3-EDTA 40 μL, 0.37 mol/L per tube) as an anticoagulant. Platelet poor plasma was prepared by centrifugation at 3000 *g* for 10 min at room temperature, after which the supernatant was transferred into cryovials (Greiner Labortechnik, Nürtingen, Germany) in 1-mL aliquots and stored at −80°C.

For biomarker measurements in plasma, samples were thawed in a water bath at 37°C for 5 min, followed by centrifugation for 2 min at 13 000 *g* to obtain platelet-freeplasma.

2.4 | Measurements of plasma levels of CRP and MBL

Plasma levels of high-sensitivity C-reactive protein were measured in duplicates using commercially available reagents by enzyme immunoassay (R&D Systems, Minneapolis, MN) in a 384 format using the combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT) dispenser/washer (EL406). Absorption was read at 450 nm with a wavelength correction set to 540 nm using an EIA plate reader (Synergy H1 Hybrid, BioTek, Vinooski, VT). The intraindividual and interindividual coefficients of variation were 2.6% and 9.1%, respectively. Oligomerized MBL was measured using enzyme-linked immunosorbent assay (Bioporto Diagnostics A/S, Hellerup, Denmark) according to the manufacturer's instructions. The coefficient of variation was in the range of 3.8% to 5.5%.

2.5 | Statistical analysis

Statistical analyses were carried out using Stata version 15 (StataCorp LLC, College Station, TX, USA) andR version 3.5.2 (The R Foundation for Statistical Computing, Vienna, Austria). The MBL was categorized according to quartile cutoffs in the control population (<435, 435-1367, 1368-2422, ≥2423 ng/mL). Means and proportions of baseline characteristics across quartiles of MBL were calculated using descriptive statistics. Logistic regression models were used to calculate OR of VTE with 95% CI according to quartiles of MBL. The highest MBL quartile was used as the reference group. We also calculated the *P* value for linear trend across decreasing quartiles of MBL. Separate analyses were also conducted with unprovoked VTE, DVT, and PE as the outcomes.

The results were based on a single baseline measurement with long follow-up (>12 years for many individuals) and could be

TABLE 1 Distribution of baseline characteristics according to quartiles of plasma levels of MBL

	Quartiles MBL			
	Q1 (<435 ng/mL)	Q2 (435-1367 ng/mL)	Q3 (1368-2422 ng/mL)	Q4 (≥2423 ng/mL)
n	310	320	311	325
Age (years)	62 ± 13	60 ± 13	61 ± 14	59 ± 15
Sex, % men (*n*)	44.5 (138)	45.6 (146)	43.7 (136)	54.7 (178)
BMI, kg/m^2	26.8 ± 4.3	26.7 ± 4.4	26.7 ± 4.3	25.4 ± 3.8
Smoking, % (*n*)	28.7 (89)	28.4 (91)	28.3 (88)	38.8 (126)
hsCRP, mg/L	1.71 ± 1.5	1.67 ± 1.4	1.50 ± 1.2	1.63 ± 1.4
CVD[a], % (*n*)	16.1 (50)	13.1 (42)	18.0 (56)	16.3 (53)
Cancer[b], % (*n*)	3.1 (10)	6.8 (21)	4.4 (14)	3.9 (12)
Diabetes[c], % (*n*)	2.60 (8)	5.31 (17)	3.87 (12)	4.01 (13)

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein; MBL, mannose-binding lectin.

[a] Self-reported history of cardiovascular disease (myocardial infarction, angina, stroke).

[b] History of cancer before baseline.

[c] Information on diabetes status was missing in four persons.

TABLE 2 Characteristics of the VTE events (*n* = 417)

	% (n)
Age at VTE (years)	67.3 ± 13.7
Sex (males)	48.2 (201)
Deep vein thrombosis	62.4 (260)
Pulmonary embolism	37.6 (157)
Unprovoked VTE	42.2 (176)
Provoked VTE	57.8 (241)
Surgery/trauma	22.3 (93)
Acute medical condition	15.6 (65)
Cancer	21.3 (89)
Immobilization	18.0 (75)
Other factors	4.1 (17)

Abbreviation: VTE, venous thromboembolism.

influenced by regression dilution bias. To address this, we performed analyses that restricted maximum time from blood sampling in Survey 4 of the Tromsø Study (Tromsø 4) to the VTE events, while keeping all controls in the analyses. The logistic regression analyses on time restrictions were set to require at least 10 VTE events, and ORs were generated at every 0.1-year increase in time since blood sampling and plotted as a function of the maximum time.

3 | RESULTS

The distribution of baseline characteristics of study participants according to quartiles of MBL is shown in Table 1. The mean age (ranging from 59 to 62 years) was similar across quartiles. The mean BMI was lowest (25.4 kg/m^2) in the highest quartile of MBL. The proportions of males and smokers were highest in the highest quartile (54.7% and 38.8%, respectively). The proportion of participants with cancer was highest (6.8%) in the second lowest quartile. There was no obvious trend in the mean high-sensitivity C-reactive protein measurements and the proportion of participants with a history of CVD across quartiles.

The characteristics of the VTE patients are shown in Table 2. The mean age at the time of VTE was 67.3 years, and 48.2% were men. In total, 62.4% of the events were DVTs and 37.6% of the events were PEs, and 42.2% of the events were unprovoked. Surgery/trauma was the most common provoking factor (22.3%), followed by cancer (21.3%), immobilization (18.0%), and acute medical conditions (15.6%).

The risk of VTE, DVT, and PE across quartiles of plasma levels of MBL is shown in Table 3. Subjects with plasma MBL levels in the lowest quartile (<435 ng/mL) had a lower OR for VTE (OR 0.87, 95% CI: 0.62-1.21) compared to those with MBL in the highest quartile (≥2423 ng/mL) in a model adjusted for age and sex. The OR for VTE was slightly lower with further adjustment for BMI and CRP (OR 0.79, 95% CI: 0.56-1.10). The association was stronger for DVT than PE. Subjects with plasma MBL levels in the lowest quartile (<435 ng/mL) had a lower OR for DVT (OR 0.76, 95% CI: 0.51-1.13) compared to those with MBL in the highest quartile (≥2423 ng/mL) in a model adjusted for age and sex, and the OR decreased further (OR 0.70, 95% CI: 0.47-1.04) after additional adjustment for BMI and C-reactive protein. There was no clear association between plasma levels of MBL and risk of PE. The ORs for unprovoked events were essentially similar to the ORs of all (provoked and unprovoked) events (Table 4).

To consider the possibility of underestimating ORs because of regression dilution bias, we estimated ORs for VTE and subgroups (DVT and PE) among subjects with lowest (lowest quartile) versus highest (highest quartile) plasma MBL as a function of time between blood sampling and the VTE events (Figure 1). The OR by low plasma MBL was substantially lower with shortened time between the blood sampling and the VTE events. The ORs for DVT

TABLE 3 Odds ratios with 95% confidence intervals for venous thromboembolism and VTE subgroups (DVT and PE) according to quartiles of plasma levels of mannose-binding lectin

Quartiles of MBL (ng/mL)	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Overall VTE				
≥2423	212	113	Reference	Reference
1368-2422	213	98	0.87 (0.62-1.21)	0.81 (0.58-1.13)
435-1367	212	108	0.96 (0.69-1.33)	0.88 (0.63-1.22)
<435	212	98	0.87 (0.62-1.21)	0.79 (0.56-1.10)
P for trend			0.6	0.2
DVT				
≥2423	212	75	Reference	Reference
1368-2422	213	63	0.84 (0.57-1.23)	0.79 (0.53-1.17)
435-1367	212	65	0.87 (0.59-1.27)	0.80 (0.54-1.18)
<435	212	57	0.76 (0.51-1.13)	0.70 (0.47-1.04)
P for trend			0.2	0.1
PE				
≥2423	212	38	Reference	Reference
1368-2422	213	35	0.92 (0.56-1.52)	0.85 (0.51-1.41)
435-1367	212	43	1.14 (0.71-1.84)	1.04 (0.64-1.69)
<435	212	41	1.09 (0.67-1.76)	0.96 (0.59-1.57)
P for trend			0.6	0.9

Note: Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, body mass index, and C-reactive protein.

Abbreviations: CI, confidence interval; DVT, deep vein thrombosis; OR, odds ratio; PE, pulmonary embolism; VTE, venous thromboembolism.

and PE showed essentially similar patterns to the ORs for overall VTE (Figure 1) and decreased substantially, particularly for PE, with shortened time between blood sampling and the respective events.

In the sensitivity analyses, we tested whether the association between low plasma MBL levels and low OR for VTE was influenced by comorbidities that could occur as a consequence of low MBL levels and were established triggers for VTE (Tables S1 and S2). The ORs are shown for VTE and subgroups (DVT and PE) in quartiles of MBL in participants without cancer (Table S1) and without those who developed myocardial infarction or stroke or had acute infections that required hospitalization during the last 3 months before the VTE event (Table S2). The results were essentially similar to those of the total study population, indicating that the association between plasma MBL and VTE risk was not influenced by other comorbidities such as cancer, arterial CVD, and acute infection.

4 | DISCUSSION

In the present study, we investigated the association between plasma MBL levels and future risk of VTE in a large nested case-control study derived from the general population. We found that approximately 13% of the participants had low levels of plasma MBL (100-499 ng/mL) and that 12% of participants were MBL-deficient (<100 ng/mL), results that are similar to findings from previous studies of Scandinavian populations.[32,33] The risk of VTE, and DVT in particular, was lower in subjects with low plasma levels of MBL. Subjects with plasma MBL levels in the lowest quartile had a 30% lower OR for DVT (OR: 0.70; 95% CI: 0.47-1.04) compared to those with plasma MBL in the highest quartile. The ORs for VTE, and PE in particular, by plasma MBL decreased substantially with shortened time between blood sampling and the VTE events and were not influenced by other comorbidities such as cancer, arterial CVD, or acute infection. Our findings support the hypothesis that low plasma levels of MBL protect against VTE.

Our study is, to the best of our knowledge, the first to investigate the association between plasma levels of MBL and future risk of VTE in the general population. Subjects with MBL levels in the lowest quartile had a 21% and 30% lower OR of VTE and DVT, respectively, compared to those in the highest quartile. Even though plasma levels of MBL are mainly determined by the *MBL2* genotype,[20,34] they are also influenced by age, sex, and hormonal status and may increase 2-fold to 3-fold upon inflammatory responses.[21,35] Plasma levels of modifiable biomarkers are expected to change over time. Fluctuations in exposure during follow-up will lead to a phenomenon called regression dilution bias,[36] which usually results in an underestimation of the true association between exposure and outcome. Accordingly, we found that the risk of VTE by plasma levels of MBL declined substantially with shortened time between blood sampling and VTE (Figure 1).

Previously, few studies have investigated the association between MBL and VTE risk. In a cohort of 91 Danish patients with systemic lupus erythematosus followed for 9 years, 14 developed VTE and the *MBL2* genotype was not associated with risk of VTE.[29] In a cross-sectional

TABLE 4 Odds ratios with 95% confidence intervals for unprovoked venous thromboembolic events and unprovoked events in venous thromboembolic event subgroups (DVT and PE) according to quartiles of plasma levels of mannose-binding lectin

Quartiles of MBL (ng/mL)	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Unprovoked VTE				
≥2423	212	46	Reference	Reference
1368-2422	213	42	0.93 (0.59-1.47)	0.85 (0.53-1.36)
435-1367	212	47	1.04 (0.66-1.63)	0.94 (0.60-1.49)
<435	212	41	0.92 (0.58-1.46)	0.80 (0.50-1.29)
P for trend			0.9	0.5
Unprovoked DVT				
≥2423	212	28	Ref	Ref
1368-2422	213	29	1.05 (0.60-1.84)	0.98 (0.55-1.72)
435-1367	212	26	0.95 (0.53-1.67)	0.86 (0.48-1.53)
<435	212	21	0.78 (0.43-1.42)	0.67 (0.36-1.24)
P for trend			0.4	0.2
Unprovoked PE				
≥2423	212	18	Ref	Ref
1368-2422	213	13	0.74 (0.35-1.54)	0.68 (0.32-1.43)
435-1367	212	21	1.19 (0.62-2.30)	1.09 (0.56-2.12)
<435	212	20	1.15 (0.59-2.24)	1.01 (0.52-2.00)
P for trend			0.4	0.6

Note: Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, body mass index, and C-reactive protein.

Abbreviations: CI, confidence interval; DVT, deep vein thrombosis; MBL, mannose-binding lectin; PE, pulmonary embolism; VTE, venous thromboembolism.

study of 114 Spanish SLE patients, the patients with *MBL2*-low genotypes had a higher prevalence of VTE than those with normal MBL genotypes (22% vs. 4%, respectively, $P = 0.016$).[28] However, the increased VTE risk was, according to the authors, at least in part attributed to the coexistence of antiphospholipid syndrome. There are several possible explanations for the apparent conflict with our results showing a protective effect of low plasma MBL levels on future risk of VTE. First, MBL deficiency is a predisposing factor for the incidence[37,38] and severity of systemic lupus erythematosus,[39] as well as the frequency of infectious complications. Systemic lupus erythematosus[40] and acute infectious diseases[41] are associated with increased risk of VTE and may therefore counterbalance the beneficial effect of low MBL levels. Second, although mainly determined by the *MBL2* genotype, there is no stringent relationship between *MBL2* genotypes tested in previous studies and plasma MBL levels. In a merged population consisting of 1642 healthy individuals, *MBL2*-deficient genotypes had sensitivity of 82%, specificity of 82%, and negative predictive value of 98% to predict serum levels of MBL <500 ng/mL.[42] The established *MBL2*-deficient genotypes will therefore lead to non-differential misclassification of plasma MBL levels that could lead to an underestimation of the true association between low MBL and VTE risk.[36,43] In a small retrospective case-control study including 24 patients with unprovoked VTE without comorbidities and 24 age-matched and sex matched controls,[30] we found that the prevalence of MBL-deficiency (MBL <100 ng/mL) was higher in VTE patients (33.3%) than in age-matched and sex-matched controls (12.5%). The higher prevalence of MBL deficiency in VTE patients in the case-control study was surprising and unexpected and encouraged us to perform a larger prospective study with sufficient power and to avoid the possibility of reverse causation.

We hypothesized that low MBL levels would protect against development of VTE. The procoagulant effects of MASPs on coagulation factors, endothelial cells, and platelets link MBL and the lectin pathway to thrombogenesis.[15,17–19,44] Both MASP-1 and MASP-2 have been shown to cleave prothrombin to thrombin.[17,45] The MASP-1 has a thrombinlike substrate specificity and cleaves fibrinogen, FXIII, high-molecular-weight kininogen, and thrombin-activatable fibrinolysis inhibitor, thereby contributing to both clot formation and stabilization.[15,17,18,46] Like thrombin, MASP-1 can activate PAR4, a receptor responsible for the activation and aggregation of platelets as well as proinflammatory processes such as leukocyte recruitment to endothelial cells.[44] Human umbilical vein endothelial cells exposed to oxidative stress, such as hypoxia-reperfusion, are able to bind MBL and thereby activate the complement system through the lectin pathway.[47–49] *In vivo* animal models have shown that the lectin pathway is indeed activated in ischemia-reperfusion and furthermore in thrombus formation. In a model where knock-in mice expressed human MBL, the monoclonal antibody 3F8 inhibiting MBL prevented arterial thrombosis and limited the injury in infarction.[50] A rat model of ischemia-reperfusion injury showed that anti-MBL antibodies protected the myocardium against tissue injury.[51] The MBL-MASP complexes, particularly with MASP-1, were found to play a role in arterial thrombus formation both *in vitro* and *in vivo* in a mouse thrombosis

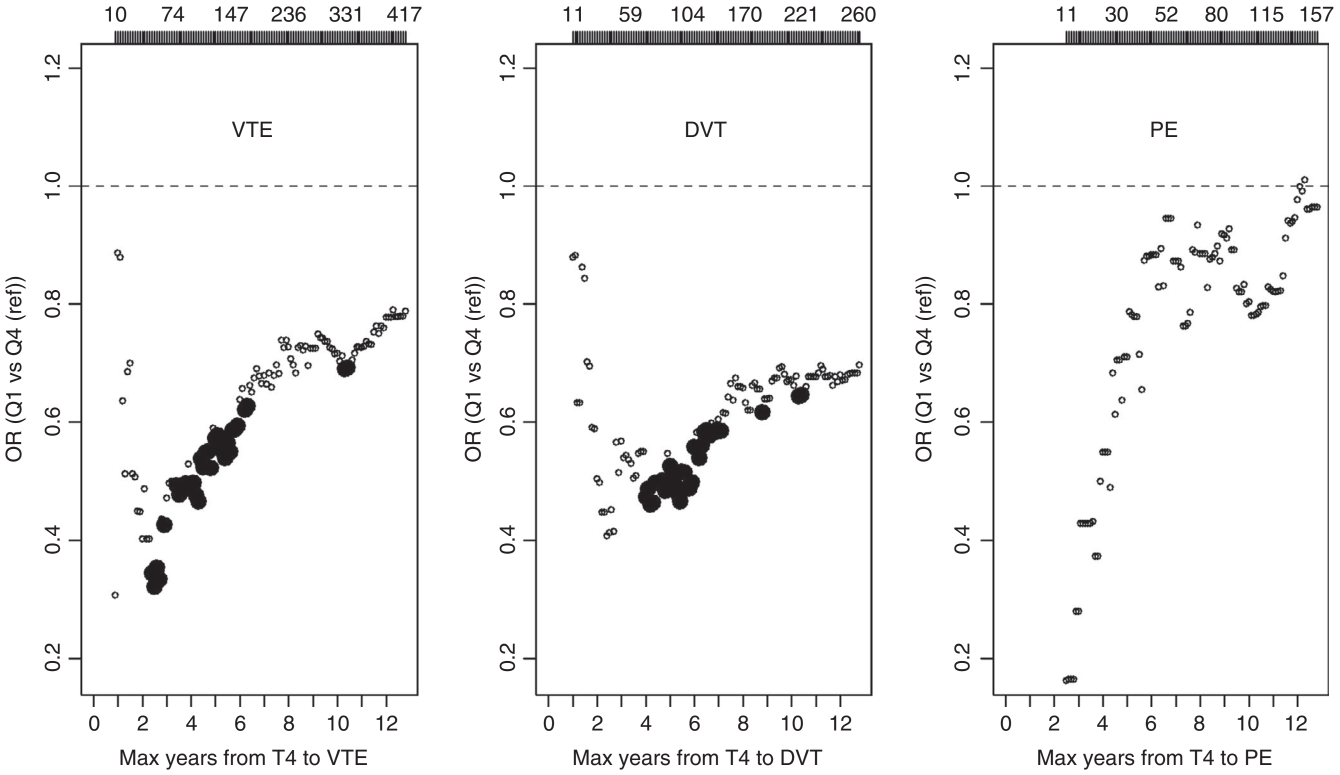

FIGURE 1 Plots of estimated ORs for overall VTE, DVT, and PE as a function of time from blood sampling in the fourth survey of the Tromsø Study (1994-1995) and event in analyses adjusted for age, sex, BMI, and hsCRP. Large, solid circles indicate ORs with *P* values < 0.05. CI, confidence interval; DVT, deep vein thrombosis; OR, odds ratio; PE, pulmonary embolism; VTE, venous thromboembolism

model.[19] We would expect a similar activation of the lectin pathway in the valvular sinuses of the deep veins, where DVT has been shown to originate,[52] because of the severe local hypoxia.[53] As plasma MBL levels correlate well with lectin pathway activity,[30,54] it is reasonable to presume that low MBL levels to some extent would suppress lectin pathway activity and in this way limit thrombus formation.

Mannose-binding lectin-deficient individuals are susceptible to other diseases, such as various types of infectious disease, autoimmune disorders, and arterial CVD.[35,55] These diseases are known to be associated with VTE risk[40,41,56,57] and could thereby counterbalance the potential beneficial effect of MBL deficiency. Mannose-binding lectin deficiency has been associated with advanced atherosclerosis[58,59] and a higher risk of myocardial infarction, independent of other traditional risk factors.[60,61] In contrast, other studies have reported an association between high levels of MBL and risk of ischemic stroke[62–64] and coronary artery disease.[65,66] In our study, low plasma levels of MBL protected against future risk of VTE, and the risk estimates remained similar in the sensitivity analyses accounting for other diseases (Tables S1 and S2).

The strengths of our study include the recruitment of VTE patients from a population-based cohort with age-matched and sex-matched controls from the same source population. It is a large prospective study where blood samples were collected before VTE, allowing assumptions on the direction of the association between exposure (plasma levels of MBL) and outcome (VTE). The blood samples used for plasma MBL analysis were drawn in 1994-1995 and stored at −80°C for up to 22 years. The long storage time could potentially affect the plasma levels of MBL. However, it is unlikely that it would affect the results, as the potential storage effect would be similar in cases and controls. Plasma samples were thawed and refrozen at least twice in preparation for analysis. Nonetheless, this did not likely affect our results as plasma MBL measurements have been shown to remain stable for at least seven freeze/thaw cycles.[67] Plasma MBL was only measured at baseline, and changes in MBL level during follow-up (up to 12 years) could result in underestimation of the true association.[36] Accordingly, we found that the favorable effect of low plasma MBL levels on VTE risk diminished substantially with prolonged time between blood sampling and the VTE event. Of note, the majority of our results did not reach statistical significance and should therefore be interpreted with caution.

In conclusion, the results from our nested case-controls study indicate that low plasma MBL levels were associated with reduced risk of VTE, and DVT in particular. Our findings should be validated and extended to investigate whether *MBL2*-deficient genotypes are associated with reduced VTE risk in population-based cohorts.

ACKNOWLEDGMENTS

K. G. Jebsen TREC is supported by an independent grant from Stiftelsen Kristian Gerhard Jebsen. This study was also financially supported by the Norwegian Council on Cardiovascular Disease, the Odd Fellow Foundation, and the Simon Fougner Hartmann Family Fund. P. G. was funded by the Novo Foundation, the Danish Research Foundation for Independent Research (DFF-6110-00489), and the Svend Andersen Research Foundation.

CONFLICT OF INTERESTS

The authors state that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Robin A. Liang analyzed data, wrote, and revised the manuscript. Ina I. Høiland wrote and revised the manuscript. Thor Ueland and Pål Aukrust performed the laboratory analysis and revised the manuscript. Kristian Hindberg and Sigrid K. Brækkan analyzed data and participated in the revision of the manuscript. Omri Snir and Peter Garred provided intellectual input and revised the manuscript. John-Bjarne Hansen and Tom Eirik Mollnes designed the study and participated in the writing and revision of the manuscript. All the authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Liang RA, Høiland II, Ueland T, et al. Plasma levels of mannose-binding lectin and future risk of venous thromboembolism. *J Thromb Haemost*. 2019;00:1–9. https://doi.org/10.1111/jth.14539