

# The Lectin Pathway in Thrombotic Conditions—A Systematic Review

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## Abstract

The lectin pathway of the complement system can activate the coagulation system in vitro, but the role of the lectin pathway in haemostatic activation and thrombosis in vivo is not clear. We performed a systematic review of the existing literature on associations between the lectin pathway and arterial and venous thrombosis, in accordance with the Assessing the Methodological Quality of Systematic Reviews guidelines. PubMed and Embase were searched from January 1990 to March 2017. We included original studies on human study populations investigating associations between the lectin pathway (protein serum levels, genotype or gene expression) and thrombotic conditions or laboratory coagulation markers. Exclusion criteria were case studies including fewer than five cases, conference abstracts or any other language than English. In total, 43 studies were included which investigated associations between the lectin pathway and cardiovascular thrombotic events (CVEs) ( $n = 22$ ), ischaemic stroke ( $n = 9$ ), CVE and stroke ( $n = 1$ ) and other conditions (systemic lupus erythematosus [ $n = 6$ ], sepsis-related coagulopathy [ $n = 3$ ], pulmonary embolism [ $n = 1$ ], asparaginase treatment [ $n = 1$ ]). Studies on the lectin pathway and CVE risk reported discrepant results, as both high and low mannose-binding lectin (MBL) serum levels were found to correlate with increased CVE risk. In ischaemic stroke patients, occurrence of stroke as well as increased stroke severity and poor outcome were consistently associated with high serum MBL. For other thromboembolic conditions, only few studies were identified. In conclusion, lectin pathway activation may negatively influence outcome after ischaemic stroke and possibly contribute to CVE risk. Further research is warranted to elucidate the role of the lectin pathway in other thrombotic conditions.

## Keywords

- ▶ lectin pathway
- ▶ complement proteins
- ▶ mannose-binding lectin
- ▶ thromboembolism
- ▶ blood coagulation

## Introduction

Interplay between the immune system and haemostasis has long been recognized,<sup>1,2</sup> and in recent years, interactions between the complement and coagulation systems have arisen as a field of considerable interest.<sup>3</sup> The lectin pathway

of the complement system is a relatively new player in this field, but increasing amounts of evidence support a crosstalk between lectin pathway proteins and coagulation, at least in vitro.<sup>4</sup> Investigating these interactions more closely may improve our understanding of the pathogenesis of thrombosis in conditions with increased immunologic activation.

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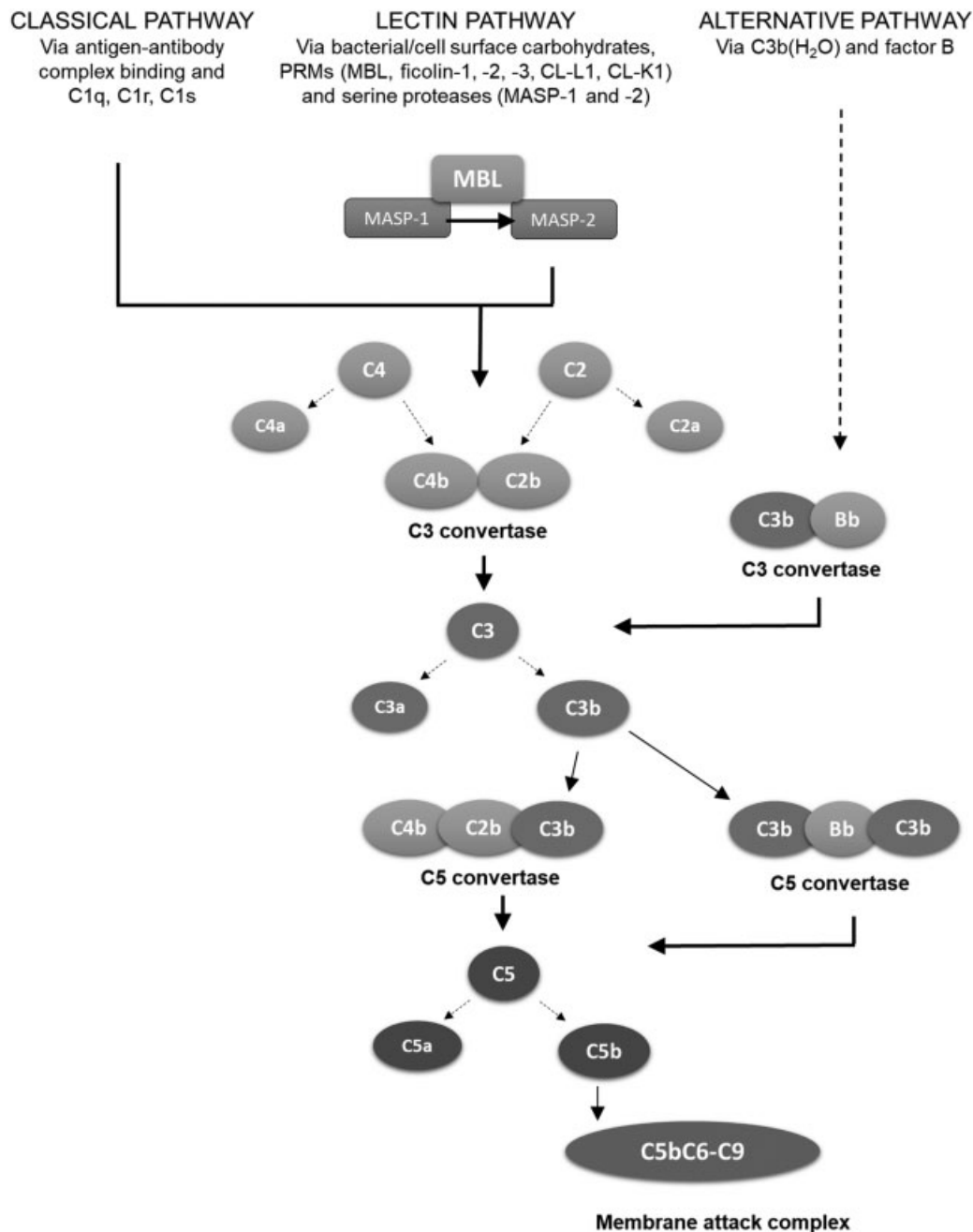
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### The Lectin Pathway

The lectin pathway consists of the pattern recognition molecules mannose-binding lectin (MBL), ficolin-1, -2 and -3 (also named M-ficolin, L-ficolin and H-ficolin) and collectins liver-1 and kidney-1 (CL-L1 and CL-K1), which circulate in complex with the MBL-associated serine proteases (MASP)-1, -2 and -3. The activation of this pathway has been described in detail elsewhere.<sup>5,6</sup> Briefly, the pattern recognition molecules bind to specific carbohydrate structures on microbial and cell

surfaces, and this leads to MASP-1 and -2 activation and subsequent cleavage of C4 and C2, assembly of a C3 convertase and activation of the common complement pathway (► Fig. 1). Besides, two other proteins, MAp44 (or MAP1) and MAp19 (or sMAP), bind to the pattern recognition molecules and are thought to exert a regulatory role in the lectin pathway.<sup>7</sup> Activation of the lectin pathway contributes to the inflammatory response through activation of the common complement pathway and C5a generation.<sup>8</sup> MBL and ficolins also directly



**Fig. 1** Lectin pathway activation. Lectin pathway activation is initiated by recognition of carbohydrate structures by the pattern-recognition molecules like (PRMs) mannose-binding lectin (MBL), ficolins 1–3 and collectin liver-1 (CL-L1) and kidney-1 (CL-K1), which circulate in complex with the MBL-associated serine proteases (MASPs). The binding activates MASPs and causes the subsequent cleaving of complement factors (C) 2 and 4. This generates the split products C2b and C4b, which aggregate to form the C3 convertase complex. Cleavage of C3 further leads to the generation of C5 convertase (C4bC2bC3b complex), C5b production and formation of the terminal membrane attack complex on the surface of the microbe or cell. Dark arrows denote enzymatic activity; dashed arrows denote assembly of split products into active complexes.

interact with C-reactive protein and pentraxin-3,<sup>9–12</sup> contribute to pathogen opsonization<sup>13,14</sup> and stimulate a pro-inflammatory cytokine response.<sup>15</sup> Moderate increases in lectin pathway protein serum concentrations have been described during the acute phase response, although these findings contrast to some degree.<sup>16–20</sup>

### Associations between Lectin Pathway Protein Levels and Disease

Serum levels of lectin pathway proteins show considerable inter-individual variation, particularly the MBL.<sup>21</sup> The genetic background for this variation, as well as possible associations between low serum levels and increased sus-

ceptibility to disease, have been investigated during the years. Some polymorphisms are consistently associated with either higher or lower serum protein levels and with increased disease risk, while such associations are less clear for other polymorphisms. ► **Table 1** provides an overview of the most commonly described lectin pathway gene polymorphisms and of some clinical conditions associated with lower or deficient lectin pathway protein levels.

Around 10% of the general population have low serum MBL levels.<sup>22</sup> The clinical importance of this is still discussed; but it is well established that MBL deficiency is a risk factor for infection in susceptible populations.<sup>23–26</sup> However, the exact role of serum MBL levels in infection and the

**Table 1** Associations between lectin pathway gene polymorphisms, serum concentrations and disease risk

Protein Corresponding gene	Known mutations or polymorphisms leading to altered serum concentration or function	Diseases or increased risk of disease associated with lower or deficient serum concentration or function
MBL <i>MBL2</i>	Lower serum levels: Promoter -550; "H" (wild-type) or "L" (G → C) allele Promoter -221; "Y" (wild-type) or "X" (G → C) allele 5'-untranslated region +4; "P" or "Q" allele (C → T) Exon 1 (wild-type allele denoted "A"); p.Arg52Cys ("D" allele), p.Gly54Asp ("B" allele), p.Gly57Glu ("C" allele)	Deficiency: Bronchiectasia <sup>129</sup> and increased infection risk in vulnerable populations, e.g. neonates <sup>130</sup> and chemotherapy-treated patients <sup>131,132</sup> Worse outcome in pneumonia <sup>133</sup> Increased risk of spontaneous recurrent abortion <sup>134–136</sup>
Ficolin-1 <i>FCN1</i>	Higher serum levels: Promoter -542G > A and -144C > A variants <sup>137</sup> Lower serum levels: p.Ala218Thr, p.Asn289Ser, p.Ser268Pro <sup>138</sup>	Promoter -542 and -144 variants associated with worse outcome in systemic inflammation and leprosy <sup>137,139</sup>
Ficolin-2 <i>FCN2</i>	Higher levels: Promoter -602G > A, promoter 4A > G <sup>140</sup> Lower levels: Promoter -986A > G, p.Ala258Ser <sup>140</sup>	Common variable immunodeficiency <sup>29</sup>
Ficolin-3 <i>FCN3</i>	Deficiency: p.Leu117-frameshift (+1637DelC) <sup>28</sup>	Pneumonia, bronchiectasia, <sup>28,29</sup> and necrotising enterocolitis <sup>30</sup>
CL-L1 <i>COLEC10</i>	Higher serum levels: p.Arg125Trp <sup>141</sup> Deficiency: p.Arg9Ter, p.Cys176Trp, p.Gly77Glu-frameshift <sup>33</sup>	Deficiency: 3MC syndrome <sup>33</sup>
CL-K1 <i>COLEC11</i>	Lower serum levels: p.Arg216His exon 8 variant: G/G and G/A variants associated with lower CL-K1 levels than A/A variant, <sup>142</sup> promoter -9570C > T <sup>141</sup> Deficiency: p.Ser169Pro, p.Gly204Ser, p.Ser217del, p.Phe16-Ser-frameshift, p.Gly101Val-frameshift, exon 1–3 deletion, <sup>34</sup> p.Asp30Ala-frameshift, p.Gly104Val-frameshift, p.Ala126Thr <sup>33</sup>	Tuberculosis <sup>143</sup> Schistosomiasis infection <sup>142</sup> Deficiency: 3MC syndrome <sup>33,34</sup>
MASP-1, MASP-3 and MAp44 <i>MASP1</i>	Deficiency: p.His497Tyr, p.Cys630Arg, p.Gly666Glu, <sup>34</sup> p.Trp3Ter, <sup>33</sup> p.Gly687Arg, p.Trp290Ter <sup>35</sup>	Deficiency: 3MC syndrome <sup>33–35</sup>
MASP-2 and MAp19 <i>MASP2</i>	Lower levels: Asp120Gly, Pro126Leu, His155Arg, Val377Ala, Arg439His, dupCys-His-Asn-His in c.466 (reviewed by Beltrame et al <sup>144</sup> ) p.Asp120Gly associated with impaired binding to MBL/ficolins <sup>145,146</sup>	Lower levels: Symptomatic Chagas' disease, <sup>31</sup> lepromatous leprosy <sup>32</sup> p.Asp120Gly: Association with disease uncertain <sup>147</sup>

Abbreviations: MAp19, MBL-associated protein of 19 kD; MAp44, MBL-associated protein of 44 kD; MASP, MBL-associated protease; MBL, mannose-binding lectin.

Note: 3MC syndrome: Mingarelli, Malpuech, Michels and Carnevale syndrome, characterized by craniofacial abnormalities, developmental delay and intellectual disability.

development of sepsis is not completely understood, as reviewed recently by Charchafieh et al.<sup>27</sup> Only few cases of ficolin<sup>28–30</sup> and MASP-2/Map19 deficiency<sup>31,32</sup> have been described, while CL-L1 and CL-K1 and MASP-1/-3 deficiency is consistently associated with a developmental syndrome (the 3MC syndrome),<sup>33–35</sup> indicating a role for lectin pathway proteins in embryonal development. Besides decreased resistance towards infection in patients with low lectin pathway protein serum levels, there is also a growing interest for the contribution of the lectin pathway to other inflammatory conditions, and for associations between higher serum levels and disease risk. This includes autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus (SLE). Associations between MBL and disease severity<sup>36</sup> and mortality<sup>37</sup> in rheumatoid arthritis are described, and Ammitzboll et al found increased levels of M-ficolin in synovial fluid in rheumatoid arthritis.<sup>38</sup> However, a recent meta-analysis found no association between *MBL2* genotype and occurrence of rheumatoid arthritis.<sup>39</sup> Higher levels of lectin pathway proteins were recently demonstrated in SLE patients,<sup>40</sup> and ficolin-1 was associated with disease severity in SLE.<sup>41</sup> More recently, the lectin pathway has also been associated with metabolic diseases with inflammatory components, such as diabetes<sup>42,43</sup> and atherosclerosis,<sup>44,45</sup> and some studies also reported increased levels of serum MBL in cancer.<sup>46–48</sup> Thus, there is evidence that the lectin pathway may be involved not only in defence against pathogens but in a broad range of clinical conditions.

### Associations between Lectin Pathway Proteins and Coagulation

Several studies have investigated associations between the lectin pathway and coagulation in vitro. An overview is presented in ▶Table 2 and ▶Fig. 2. MASP-1 and -2 show affinity for a range of substrates, and MASP-1 shares similarities with prothrombin and MASP-2 with coagulation factor (F)Xa.<sup>49–51</sup> Recombinant (r) active MASP-1 can cleave fibrinogen, demonstrated by the generation of fibrinopeptides A and B and fibrin  $\gamma_2$ - and  $\alpha_n$  chains,<sup>52,53</sup> and activate FXIII.<sup>52–54</sup> Thus, MASP-1 induces fibrin formation and cross-linking in vitro. Furthermore, Hess et al also found that rMASP-1 generated active thrombin-activatable fibrinolysis inhibitor and prolonged clot lysis time in a plasma-based assay.<sup>53</sup> This indicates that MASP-1 also attenuates fibrinolysis. Also, serum-derived MBL-MASP complexes were found to cleave fibrinogen and activate FXIII.<sup>55</sup> Recently, it was demonstrated that MASP-1 also cleaves prothrombin and gives rise to what appeared to be active thrombin.<sup>56</sup> MASP-1 shortened thromboelastography clotting time and clot formation time in healthy whole blood and plasma<sup>57</sup> as well as clot formation time in a micro-vascular flow model under shear stress,<sup>58</sup> while MASP-1 inhibition in the same flow model delayed the time to clot formation.<sup>58</sup> The ability to cleave prothrombin and generate active thrombin has also been demonstrated for rMASP-2 and MBL-MASP-2 complexes.<sup>59</sup> Notably, for both MASP-1 and MASP-2, cleavage of coagulation proteins and clot formation happened at a

lower rate than FXa- and/or thrombin-induced cleavage,<sup>52,59</sup> and MASP-1- and thrombin-induced clots were also found to differ in density and structure.<sup>53</sup>

There is evidence that the coagulation system can activate the lectin pathway in turn. Endo et al demonstrated that murine ficolin and MBLs bind to fibrinogen and fibrin, and that fibrinogen induces lectin pathway activation in mouse serum.<sup>60</sup> Kozarcanin et al showed that ficolins are capable of binding to the surface of activated platelets, and that fibrin binds MASPs and induce MASP-C1-inhibitor or MASP-anti-thrombin complex formation, indicating MASP activation.<sup>61</sup> Finally, Keizer et al found that tissue factor pathway inhibitor, an endogenous inhibitor of coagulation, can bind and inhibit MASP-2 activation.<sup>62</sup> Thus, crosstalk between the lectin pathway and coagulation occurs at several points, and interactions between lectin pathway proteins and coagulation could contribute to hypercoagulability and increased thrombosis risk in a range of clinical conditions. Our aim was to systematically review the existing literature on associations between the lectin pathway and thrombotic conditions in humans.

### Materials and Methods

The present systematic review was conducted in accordance with the Assessing the Methodological Quality of Systematic Reviews guidelines.<sup>63</sup> The protocol was published in the PROSPERO database (reg. no. CRD42017070207, www.crd.york.ac.uk/prospero).

### Literature Search

PubMed and Embase were searched from 1 January 1990 to 29 March 2017. No additional filters were set. Free-text as well as MeSH terms/Emtree-preferred terms were used. Keywords and search combinations were as follows:

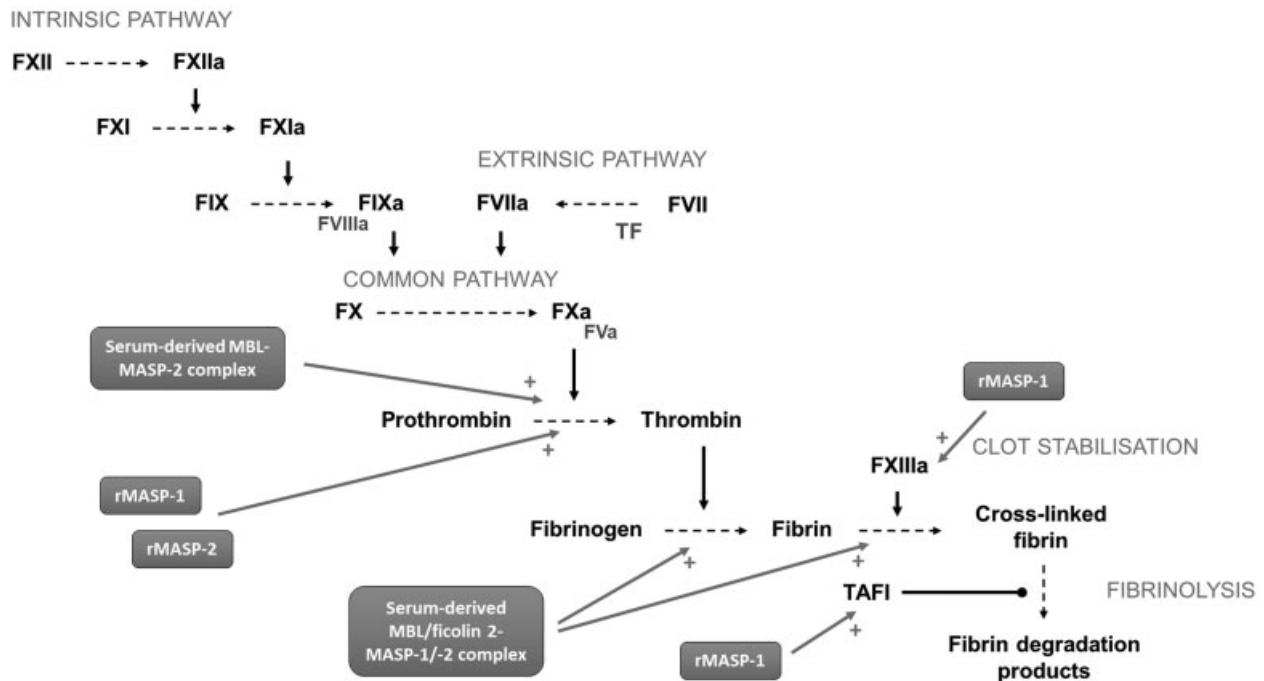
### PubMed

((((((((((((((((((((((((("Complement Pathway, Mannose-Binding Lectin"[Mesh]) OR "Mannose-Binding Lectin"[Mesh]) OR "Mannose-Binding Protein-Associated Serine Proteases"[Mesh]) OR "ficolin" [Supplementary Concept]) OR "lectin pathway") OR "lectin pathway activation") OR "mannose-binding lectin") OR "mannan-binding lectin") OR "mannose-binding lectin associated serine protease") OR "mannose-binding lectin associated serine proteases") OR "map19") OR "map44") OR "collectin I1") OR "collectin 10") OR ficolin\*)) AND (((((((((((((((((((((((("Embolism and Thrombosis" [Mesh])) OR "Myocardial Ischemia"[Mesh]) OR "Stroke" [Mesh]) OR "Mesenteric Vascular Occlusion"[Mesh]) OR "Renal Artery Obstruction"[Mesh]) OR "Peripheral Arterial Disease"[Mesh]) OR "Disseminated Intravascular Coagulation"[Mesh]) OR ("Blood Coagulation"[Mesh]) OR "Platelet Activation"[Mesh]) OR "blood coagulation") OR "platelet activation") OR "arterial thrombosis") OR "venous thrombosis") OR "venous thromboembolism") OR "disseminated intravascular coagulation") OR "myocardial infarction") OR "cardiovascular disease") OR "ischemic stroke") OR "hemorrhagic stroke") OR "renal artery thrombosis") OR

**Table 2** Overview of studies investigating associations between the lectin pathway and coagulation in vitro

Author, year	LP protein	Research question addressed Coagulation protein or activation assay	Findings
Jenny et al <sup>58</sup> 2018	rMASP-1	Influence of MASP-1 and inhibition of MASP-1 on blood clot formation Whole blood, micro-vascular flow model	MASP-1 addition accelerates clot formation while inhibition of MASP-1 prolongs time to clot formation
Kozarcanin et al <sup>61</sup> 2016	MASP-1 and -2, ficolins, MBL	Ability of coagulation factors and platelets to activate MASPs Platelets, fibrin LP-platelet binding assessed with flow cytometry	Activated platelets bind ficolin-1, -2 and -3 on surface Fibrin and fibrin d-dimer bind MASP-1 and -2 and induce MASP-C1inh/-anti-thrombin complex formation
Jenny et al <sup>57</sup> 2015	rMASP-1	Influence of MASP-1 addition on blood clotting Thromboelastography; whole blood, platelet-poor plasma, purified fibrinogen solution Prothrombin	MASP-1 addition shortens thromboelastography clotting time and clot formation time in the presence of prothrombin
Jenny et al <sup>56</sup> 2015	rMASP-1	Ability of MASP-1 to cleave wild-type or mutant prothrombin; characterization of cleavage sites and products Prothrombin (wild-type or mutant at cleaving sites R271, R320, R393)	MASP-1 cleaves prothrombin at R271 and R393 and gives rise to active thrombin species
Keizer et al <sup>62</sup> 2015	rMASP-2; LP activation (C4 deposition)	Ability of TFPI to inhibit MASP-2; characterization of inhibitory domain Tissue factor pathway inhibitor (TFPI)	TFPI (Kunitz-2 domain) inhibits rMASP-2 activity (chromogenic assay) and human serum LP activation
Hess et al <sup>53</sup> 2012	rMASP-1	Ability of MASP-1 to activate coagulation and inhibit fibrinolysis Clot formation and lysis assay, prothrombin fragment 1 + 2, fibrinopeptide A, thrombin-activatable fibrinolysis inhibitor, FXIII activation Citrat plasma and purified fibrinogen and FXIII (human)	MASP-1 activates FXIII, induces generation of prothrombin fragment 1 + 2 and fibrinopeptide A, activates thrombin-activatable fibrinolysis inhibitor and prolongs clot lysis
Endo et al <sup>60</sup> 2010	rFicolin A, rMBL-A, rMBL-C (mouse) LP activation (C4 deposition)	Ability of fibrinogen/fibrin to bind ficolin and MBL and to activate LP Fibrinogen and fibrin (human)	Murine ficolin and MBL binds to fibrinogen (A $\alpha$ and B $\beta$ chains) and fibrin ( $\alpha$ and $\beta$ chains) in dose-dependent manner Fibrinogen induces LP activation in mouse serum
Skjoedt et al <sup>149</sup> (conference abstract)	MAP44	Ability of MAP44 to inhibit platelet function and coagulation Platelet activation and aggregation, whole blood clotting, fibrin clot formation and lysis in plasma (human)	MAP44 attenuates platelet activation and aggregation, clot amplitude in whole blood (intrinsic pathway) and fibrin clot formation
Gulla et al <sup>55</sup> 2010	Ficolin- or MBL-MASP-1/-2 complexes (serum-derived)	Ability of ficolin-/MBL-MASP complexes to activate coagulation Fibrin clot formation in citrated plasma and purified fibrinogen/FXIII solution (human)	Ficolin-/MBL-MASP complexes induces FXIII activation and fibrinogen cleavage in purified systems and clot formation in plasma
Kraruup et al <sup>52</sup> 2008	rMASP-1	Ability of MASP-1 to cleave coagulation proteins Fibrinogen, FXIII (human)	MASP-1 cleaves FXIII (presence of activated A-chain) and fibrinogen (presence of $\gamma$ -chains and fibrinopeptide B) but at a significantly lower rate than thrombin
Kraruup et al <sup>59</sup> 2007	rMASP-2, rMBL-A-MASP-2 complexes (rat)	Ability of MASP-2 to cleave and activate prothrombin Prothrombin, thrombin activity (VPR-AMC turnover), fibrin formation (turbidimetry, <sup>125</sup> I-labeled fibrin deposition) in purified solutions (human)	rMASP-2 cleaves prothrombin (presence of pre-thrombin and fragment 1) and generates active thrombin, but at a lower rate than FXa MBL-MASP-2 complexes induce fibrin formation on mannan-coated plates and <i>S. aureus</i> -coated beads in the presence of prothrombin
Hajela et al <sup>54</sup> 2002	rMASP-1	Ability of MASP-1 to cleave fibrinogen Purified fibrinogen/FXIII (human)	MASP-1 cleaves fibrinogen and induces crosslinking (generation of $\gamma_2$ - and $\alpha_n$ -chains)

Abbreviations: C, complement factor; F, coagulation factor; MAP44, MBL-associated protein of 44 kD; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; r, recombinant.



**Fig. 2** Proteases of the lectin pathway induce thrombin and fibrin generation, stabilize the fibrin clot and impair fibrinolysis. The figure displays our current knowledge of the interactions between MASP-1 and -2 and the coagulation and fibrinolytic system.<sup>52-62</sup> Abbreviations: MASP, MBL-associated serine protease; MBL, mannose-binding lectin; rMASP, recombinant MASP; TF, tissue factor; TAFI, thrombin-activated fibrinolysis inhibitor. Roman numerals denote coagulation factors and the suffix 'a' denotes an activated factor. Black arrows denote enzymatic activity of coagulation factors on the next step in the cascade. Grey arrows with '+' denotes activation by lectin pathway proteases.

“mesenteric thrombosis”) OR “hepatic thrombosis”) OR “peripheral artery occlusion”).

### Embase

('Mannose binding lectin'/exp OR 'Complement lectin pathway'/exp OR 'Complement activation'/exp OR 'Mannan binding lectin associated serine proteinase'/exp OR 'Ficolin'/exp OR 'Map19' OR 'Map44' OR 'Collectin liver 1') AND ('Thromboembolism'/exp OR 'Heart muscle ischemia'/exp OR 'Heart infarction'/exp OR 'Brain ischemia'/exp OR 'Cerebrovascular accident'/exp OR 'Disseminated intravascular clotting'/exp OR 'Blood clotting').

Other relevant literature was identified by checking the reference lists of recent original studies and reviews and employing the 'cited-by' function in Scopus.

### Inclusion and Exclusion

The inclusion criteria were: (1) original work, (2) human study population and (3) investigating associations between the lectin pathway and thromboembolic disease or laboratory coagulation parameters (surrogate endpoints for increased thrombosis risk). Both interventional and observational studies, including prospective cohort studies as well as case-control studies, were eligible for inclusion. Accepted endpoints for the lectin pathway were plasma or serum protein levels, genetic polymorphisms, gene expression and activation assays for one or more of the following proteins: MBL, ficolin-1, -2, -3, CL-L1 or -K1, MASP-1, -2, -3, MAp44 and MAp19. Accepted endpoints for thromboem-

bolic disease were both microscopic thrombosis (e.g. disseminated intra-vascular coagulation) and large vessel occlusion, and both arterial and venous thrombosis. We included acute ischaemic stroke and cardiovascular thrombotic events (CVEs) defined as acute coronary syndrome (myocardial infarction [MI] and unstable angina pectoris) but not transient cerebral ischaemia, stable angina pectoris or atherosclerotic lesions without sign of thrombosis. Exclusion criteria were (1) case studies including fewer than five cases, (2) other language than English and (3) conference abstracts.

Fifty abstracts were randomly selected and screened for either exclusion or full-text reading by all three authors, and any disagreement was solved by consensus. The remaining abstracts were screened by J.B.L. In a similar fashion, 20 papers proceeding to full-text reading were randomly selected and read in full by all authors, and any disagreement was again solved by consensus. Inclusion or exclusion of the remaining papers were performed by J.B.L., and in case of doubt, all three authors discussed the study in question.

### Data Extraction and Quality Assessment

Data extraction from the included articles was performed by J.B.L. and checked by C.L.H. and A.M.H. Study quality was assessed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies<sup>64</sup> and the Quality Assessment Tool for Case-Control Studies,<sup>65</sup> National Institutes of Health, USA. The quality assessment tools provide a guide for

systematically assessing the risk of different types of bias in the study and thus the internal validity of the results. The tools comprise a list of items concerning key methodological points and specific criteria to be fulfilled for each item. A numeric rating scale is not employed; instead, each study is rated 'good', 'fair' or 'poor' according to the estimated risk of serious bias (low, intermediate or high), which is based on the number and types of criteria not fulfilled. Quality assessment was performed by all the three authors for all included studies, and disagreement was solved by consensus.

**Data Synthesis**

The included studies were heterogeneous regarding the design, study populations and endpoints, thus a complete

meta-analysis of the data was not performed. Forest plots were made for the CVE sub-group to visualize results and support our qualitative data synthesis using Stata 14 (Stata-Corp, Texas, United States). If data for forest plots could not be extracted from the included article, an attempt to obtain data was made via emails to the corresponding authors.

**Results**

In total, 43 original articles were included in the present review and are presented in ►Tables 3–6. The screening and inclusion/exclusion process is displayed in detail in ►Fig. 3.

We grouped the studies first according to their major clinical focus and second according to their design. Among

**Table 3** Studies investigating associations between the lectin pathway and occurrence of cardiovascular events

Author, year, rating	Design Lectin pathway parameters Laboratory methods	Study population	Endpoint(s) for cardiovascular events (CVE)	Results
<b>Prospective studies including nested case-control studies (n = 10)</b>				
Poppelaars et al <sup>66</sup> 2016 Rating: Good	Longitudinal Plasma MBL (EDTA), ELISA	Patients: end-stage kidney disease on haemodialysis (n = 107) Mean follow-up: 27 months Censored if received transplantation Age: 63 ± 16 years Gender (M/F, %): 66/34	(1) Cardiac events (CE) alone: MI, UAP, CABG or PCI (2) CVE: MI, UAP, CABG, PCI, ischaemic stroke or claudication (3) All-cause mortality	↑ CE and ↑ CVE asso- ciated with ↓ MBL (adjusted HR = 3.96 [1.49–10.54] for CE and 3.98 [1.88–8.42] for CVE) Mortality not asso- ciated with MBL (HR not reported) MBL improved predic- tion of CVE (Harrell's C = 0.76 vs. 0.73, p = 0.01 in a model with vs. without MBL)
Siezenga et al <sup>67</sup> 2011 Rating: Good	Longitudinal MBL2 genotype Codon 52, 54, 57 Serum MBL, ELISA	Patients: type 2 dia- betes (n = 134) Mean follow-up: 7.7 years Age: 51 ± 11 years Gender (M/F, %): 46/54 Ethnicity: South Asian	MI, PCI, CABG or sud- den cardiac death	↑ CVE in low versus intermediate/high genotype (HR = 3.43 [1.24– 9.49]) CVE not associated with serum MBL (HR = 0.93 [0.50–1.73])
Troelsen et al <sup>37</sup> 2010 Rating: Fair	Longitudinal MBL2 genotype Promoter -221, codon 52, 54, 57 Serum MBL, ELISA	Patients: rheumatoid arthritis (n = 229) Mean follow-up: 10.3 years Gender (M/F, %): 18/82	(1) CVE-related mor- tality (2) All-cause mortality	↑ CVE-related and overall mortality asso- ciated with high versus intermediate/low gen- otype (adjusted HR = 2.0 [1.0–4.1] for CVE-related and 1.7 [1.1–2.8] for overall) ↑ CVE and overall mortality associated with ↑ serum MBL (adjusted HR = 1.3 [1.0–1.5] for CVE and 1.2 [1.0–1.3] for overall)

(Continued)

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**Table 3** (Continued)

Author, year, rating	Design Lectin pathway parameters Laboratory methods	Study population	Endpoint(s) for cardiovascular events (CVE)	Results
<b>Prospective studies including nested case-control studies (n = 10)</b>				
Troelsen et al <sup>68</sup> 2007 <sup>a</sup> Rating: Good	Longitudinal <i>MBL2</i> genotype Promoter -221, codon 52, 54, 57 Serum MBL, ELISA	Patients: rheumatoid arthritis (n = 229) Mean follow-up: 9.5 years Gender (M/F, %): 18/82	(1) MI (2) CVE-related mortality	↑ CVE in high versus intermediate/low genotype (adjusted HR = 5.0 [1.4–17.5] for MI and 4.1 [1.2–14.3] for mortality) ↑ CVE associated with ↑ serum MBL (adjusted HR = 6.3 [2.0–20.1] for MI and 10.5 [2.7–41.3] for mortality)
Berger et al <sup>69</sup> 2007 Rating: Fair	Longitudinal <i>MBL2</i> genotype Codon 52, 54, 57 Serum MBL, ELISA	Patients: pancreas-kid- ney transplant recipi- ents (genotype, n = 97; serum MBL, n = 99) Follow-up: 12 years High/low MBL: Age: 40 ± 8/41 ± 7 years Gender (M/F, %): 68/32 and 61/39	(1) CVE-related mor- tality (2) Overall mortality	↑ CVE mortality asso- ciated with ↑ serum MBL (0/34 deaths vs. 11/65 deaths, p = 0.01) ↑ overall mortality associated with ↑ serum MBL (adjusted HR = 4.4 [1.30–15.10]) Mortality not asso- ciated with genotype in multivariate analysis (HR not reported)
Keller et al <sup>70</sup> 2006 Rating: Good	Nested case-control Serum MBL, ELISA	Patients: CVE during follow-up (n = 781) Controls matched for age, gender, date of visit (± 3 months) and duration of follow- up (n = 1,505) Mean follow-up: 6 years Cases/controls: Age: Men 65 ± 8/ 64 ± 8, years; women 67 ± 7/67 ± 7 years Gender (M/F, %): 62/38 and 64/36	MI or CVE-related mortality	In men: ↑ CVE asso- ciated with ↑ MBL (adjusted OR = 1.55 [1.08–2.22] in highest vs. lowest MBL quar- tile) In women: no associa- tion between MBL and CVE (adjusted OR = 0.98 [0.61–1.56])
Saevarsdottir et al <sup>71</sup> 2005 Rating: Good	Nested case-control Serum MBL, ELISA	Patients: CVE during follow-up (n = 867) Controls (n = 442), matching not reported Mean follow-up: 27 years Cases/controls: Age: 56 (51–62) years /55 (50–61) years Gender (M/F, %): 58/42 and 50/50	MI or CVE-related mortality	Overall: No association between CVE and MBL (OR = 0.88 [0.69–1.1]) ↓ CVE associated with ↑ MBL in patients with diabetes (OR = 0.15 [0.03–0.78]) and hypercholesterolaemia (OR = 0.26 [0.10–0.64])
Hertle et al <sup>45</sup> 2016 Rating: Fair	Longitudinal <i>MBL2</i> genotype Codon 52, 57 Plasma MBL (EDTA),	Healthy or diabetic subjects (n = 495); Type 2 diabetes or impaired glucose	MI, ischaemic stroke, CABG or PCI during follow-up	No associations between LP and CVE <i>MBL2</i> genotype: OR = 1.60 [0.73–3.51])



Table 3 (Continued)

Author, year, rating	Design Lectin pathway parameters Laboratory methods	Study population	Endpoint(s) for cardiovascular events (CVE)	Results
<b>Prospective studies including nested case-control studies (n = 10)</b>				
	MASP-1, -2, -3, MAp44 (citrate), ELISA	tolerance: 48% Follow-up: 7 years Age: 60 ± 7 years Gender (M/F, %): 61/39		MASPs, MAp44: ORs not reported
Vengen et al <sup>72</sup> 2012 Rating: Good	Nested case-control <i>MBL2</i> genotype Promoter -221, codon 52, 54, 57 <i>FCN1</i> promoter -542 SNP; <i>FCN2</i> exon 8 +6359 and +6424; <i>FCN3</i> +1637 SNP	Patients: MI during follow-up (n = 370) Controls matched for age and gender (n = 370) Mean follow-up: 12 years Cases: Age: 48 (range: 29–62) years Gender (M/F, %): 76/24	MI	↑ MI in low versus intermediate/high <i>MBL2</i> genotype (adjusted OR = 2.02 [1.17–3.47]) No association between <i>FCN</i> polymorphisms and MI (all p > 0.19, OR not reported)
Vengen et al <sup>73</sup> 2017 <sup>b</sup> Rating: Good	Nested case-control Serum ficolin-1, -2, -3, MASP-3, MAp44, ELISA	Patients: MI during follow-up (n = 370) Controls matched for age and gender (n = 370) Mean follow-up: 12 years Cases: Age: 48 (range: 29–62) years Gender (M/F, %): 76/24	MI	↑ MI in highest tertile of ficolin-2 (adjusted OR = 1.55 [1.04–2.30]) ↓ MI in highest tertile of MASP-3 (adjusted OR = 0.63 [0.04–0.94]) No association with MI for ficolin-1 (OR = 0.77 [0.52–1.13]), ficolin-3 (OR = 1.17 [0.79–1.73]) or MAp44 (OR = 0.86 [0.59–1.27])
<b>Cross-sectional and case-control studies (n = 4)</b>				
Pesonen et al <sup>74</sup> 2009 Rating: Fair	Case-control <i>MBL2</i> genotype Promoter -550 and 221, codon 52, 54, 57 Serum MBL, ELISA	Patients: UAP (n = 113) or MI (n = 241) Controls: healthy volunteers matched for age, gender and parish (n = 334) Cases: Age: 63 years Gender (M/F, %): 78/22	(1) UAP (2) MI	↑ serum MBL and high genotype associated with ↑ UAP (adjusted OR = 1.25 [p = 0.006] for serum MBL and 1.16 [p = 0.01] for genotype) and with ↑ MI (adjusted OR = 1.28 [p < 0.001] for serum MBL and 1.12 [p = 0.007] for genotype)
Hansen et al <sup>75</sup> 2004 Rating: Fair	Case-control <i>MBL2</i> genotype Promoter -550 and 221, UTR +4, codon 52, 54, 57 Serum MBL, TRIFMA	Patients: type 1 diabetes and nephropathy (n = 199) or normoalbuminuria (n = 192) matched for age, gender and duration of diabetes Controls: healthy volunteers matched for age (n = 100) Cases: Age: 41 ± 10 years Gender (M/F, %): 61/39 Ethnicity: Caucasian	MI, stroke or claudication	↑ serum MBL in patients with previous CVE (mean difference 880 µg/L, p = 0.02) This difference persisted in patients with high genotype (p < 0.001) but not low genotype (p = 0.31)

(Continued)

**Table 3** (Continued)

Author, year, rating	Design Lectin pathway parameters Laboratory methods	Study population	Endpoint(s) for cardiovascular events (CVE)	Results
<b>Cross-sectional and case-control studies (n = 4)</b>				
Frauenknecht et al <sup>95</sup> 2013 Rating: Fair	Case-control Plasma MASP-1, -2, -3 or MAp44 (citrate), TRIFMA	Patients: MI (n = 49) Controls: healthy volunteers (n = 50) and patients with stable CAD (n = 104) Patients: Age: 57 ± 9 years Gender (M/F, %): 80/20	MI	↑ MASP-1 and ↓ MASP-2 in MI compared with controls (MASP-1: 11.93 vs. 9.44 µg/mL, p < 0.001; MASP-2: 369.8 vs. 442.9 ng/mL, p = 0.005) ↔ MASP-3 or MAp44 between groups (p > 0.05)
Zhang et al <sup>76</sup> 2013 Rating: Fair	Case-control Plasma MASP-2 (anti-coagulant not reported), ELISA	Patients: MI (n = 29) Controls: healthy volunteers (n = 50); patients with stable CAD (n = 27) Patients: Age: 61 (32–72) years Gender (M/F, %): 69/31 Ethnicity: African American 59%, Caucasian 10%, Hispa- nic 31%	MI	↓ MASP-2 in MI versus healthy or CAD controls (235 vs. 460 [healthy] and 471 ng/mL [CAD], p < 0.01)

Abbreviations: CABG, coronary artery bypass grafting; CAD, coronary artery disease; CPB, cardiopulmonary bypass; CVE, cardiovascular event; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FCN, gene encoding ficolin; HR, hazard ratio; LP, lectin pathway; MAp44, MBL-associated protein of 44 kDa (also known as MAP1); MASP, MBL-associated protease; MBL, mannose-binding lectin; MBL2, gene encoding MBL; MI, myocardial infarction; OR, odds ratio; PCI, percutaneous coronary intervention; SNP, single-nucleotide polymorphism; STEMI, ST-segment elevation myocardial infarction; TRIFMA, time-resolved immunofluorometric assay; UAP, unstable angina pectoris.

Notes: Study population: Age, gender and ethnicity are stated if reported in the study. Age is rounded to whole years and indicated by mean ± standard deviation or median (interquartiles) unless otherwise specified. Results: Numbers in parentheses after OR/HR are 95% confidence intervals unless otherwise specified.

<sup>a</sup>Same study population as Troelsen et al 2010.<sup>37</sup>

<sup>b</sup>Same study population as Vengen et al 2012.<sup>72</sup>

the included studies, 22 focused on CVE, predominantly MI,<sup>37,45,66–85</sup> 9 on ischaemic stroke,<sup>86–94</sup> 1 on both CVE and stroke<sup>95</sup> and 11 on other conditions (SLE, n = 6,<sup>41,61,96–99</sup> disseminated intravascular coagulation [DIC], n = 3,<sup>102,103,105</sup> pulmonary embolism, n = 1<sup>100</sup> and L-asparaginase-treated pediatric patients, n = 1<sup>101</sup>).

Out of 23 CVE studies, 16 investigated associations between the lectin pathway and occurrence of CVE,<sup>37,45,66–76,83,84,95</sup> while 9 of the 23 prospectively investigated associations between the lectin pathway and outcome (e.g. mortality or recurrent MI) in patients with existing CVE.<sup>77–85</sup> Among the 10 studies on ischaemic stroke, 8 investigated associations between lectin pathway proteins and occurrence of stroke (case-control design),<sup>86–92,95</sup> and 6 of the 10 studies investigated associations between lectin pathway proteins and stroke severity and/or functional outcome in existing stroke (prospective or cross-sectional design).<sup>88–90,92–94</sup>

The majority of studies (n = 33) included MBL serum/plasma levels and/or MBL2 genotype, while nine studies investigated one or more MASPs,<sup>45,61,73,76,84,85,94,95,100</sup>

seven studies one or more ficolins,<sup>41,72,73,83,86,88,92</sup> five studies MAp44<sup>45,73,83,84,95</sup> and one investigated CL-K1.<sup>102</sup> Studies including MBL2 genotype all investigated polymorphisms in codon 52, 54 and 57 in exon 1.<sup>37,45,67–69,72,74,75,77,80,94,96–99,103</sup> Additionally, some included one or more of three polymorphisms in the promoter or 5' untranslated region<sup>37,68,72,74,75,77,80,94,97,99</sup> (please refer to ►Table 1). All studies classified the patients' genotypes or haplotypes after their known association with MBL expression and serum levels. Thus, genotype was classified overall as 'high' (wild-type), 'intermediate' or 'low' in these studies, and this classification is used throughout the text and tables of the present review.

The overall study quality was moderate to high, with 22 (51%) rating 'good', 17 (40%) rating 'fair' and 4 (9%) rating 'poor'. The individual ratings are provided in ►Tables 3–6.

Below, we summarize the findings of the included studies on associations between the lectin pathway and thrombotic events.

**Table 4** Studies investigating associations between the lectin pathway and disease severity or outcome in existing cardiovascular disease

Author, year, rating	Design Lectin pathway parameters	Study population	Endpoints for disease severity or outcome	Results
Mellbin et al <sup>77</sup> 2010 Rating: Good	Longitudinal, baseline blood sampling shortly after MI diagnosis MBL2 genotype Promoter -221, codon 52, 54, 57 Serum MBL, TRIFMA	Patients: type 2 diabetes and MI ( $n = 387$ serum only and $n = 287$ serum and genotype) Mean follow-up: 2.5 years Age: 70 (61–77) years Gender (M/F, %): 68/32	Recurrent MI, ischaemic stroke or CVE-related mortality	No association between CVE and serum MBL or genotype (HR = 0.93 [0.85–1.01])
Trendelenburg et al <sup>78</sup> 2010 Rating: Good	Longitudinal, baseline blood sampling before PCI Serum MBL, ELISA	Patients: STEMI, undergoing primary PCI ( $n = 890$ ) Follow-up: 90 days Low/high MBL Age: 60 (53–73) years /60 (51–70) years Gender (M/F, %): 76/42 and 79/21	(1) All-cause mortality (2) Cardiac dysfunction: Composite endpoint of death, cardiogenic shock or heart failure	↓ MBL associated with ↓ all-cause mortality (HR = 0.14 [0.02–1.02], $p = 0.02$ ) but not with cardiac dysfunction (HR = 0.89 [0.52–1.53])
Haahr-Pedersen et al <sup>79</sup> 2009 Rating: Good	Longitudinal, baseline blood sampling before PCI Plasma MBL (EDTA), TRIFMA	Patients: STEMI, undergoing primary PCI ( $n = 74$ ) Follow-up: 48 hours Ejection fraction > 35%/< 35% Age: 62 years <sup>58–65</sup> /63 (59–68) years Gender (M/F, %): 79/21 and 81/19	Cardiac dysfunction: left ventricle ejection fraction < 35%	Cardiac dysfunction associated with ↑ MBL (adjusted OR = 5.5 [1.5–19.3])
Collard et al <sup>80</sup> 2007 Rating: Good	Longitudinal MBL2 genotype Promoter -550 and 221, UTR +4, codon 52, 54, 57	Patients undergoing first-time isolated CABG ( $n = 843$ ) Follow-up: 3 days post-operation MI/no MI Age: $64 \pm 11$ years / $65 \pm 10$ years Gender (M/F, %): 74/26 and 79/21 Ethnicity: Caucasian	Post-operative MI	The combination of a 5' and a 3' haplotype encoding ↑serum MBL was associated with ↑ MI (adjusted OR = 4.72 [1.41–15.85]) No association found between any haplotype alone and MI (ORs not reported)
Ueland et al <sup>81</sup> 2006 Rating: Fair	Longitudinal, blood sampling at study enrolment and after 1 month Plasma MBL (EDTA), EIA	Patients: MI and ↓ left ventricle ejection fraction ( $n = 234$ ) Mean follow-up: 2.7 years Low/high MBL: Age: $68 \pm 10$ years / $67 \pm 10$ years Gender (M/F, %): 63/37 and 74/26	(1) Recurrent MI (2) Overall mortality	↑ recurrent MI at 1 mo associated with ↓ MBL (adjusted HR = 2.3 [1.2–4.5] to 2.7 [1.4–5.2] depending on the model used) No association between MBL and overall mortality (OR not reported)
Limnell et al <sup>82</sup> 2002 Rating: Poor	Retrospective, blood sampling at the end of follow-up Serum MBL, EIA	Patients undergoing CABG with one or more venous graft ( $n = 62$ ) Median follow-up: 1.3 years Age: 56 (43–67) years Gender: all male	Graft occlusion	↑ graft occlusion associated with ↓ MBL (adjusted OR = 11.5 [1.24–106])

(Continued)

**Table 4** (Continued)

Author, year, rating	Design Lectin pathway parameters	Study population	Endpoints for disease severity or outcome	Results
Schoos et al <sup>83</sup> 2013 Rating: Fair	(1) Case-control: MI occurrence (2) Longitudinal: Cardiac function after MI Blood sampling at day 1, 4, 7 and 31 after MI Plasma MBL, ficolin-2, -3 or MAp44 (EDTA), ELISA	Patients: MI undergoing primary PCI ( <i>n</i> = 55) Controls: reference intervals in healthy individuals Follow-up: 6 months Low/high ficolin-2: Age: 59 (51–63) years /54 (50–62) years Gender (M/F, %): 76/24 and 82/18	(1) MI (2) Cardiac function: Infarct size, end-diastolic volume/body surface area, end-systolic volume/body surface area) and left ventricle ejection fraction	↓ ficolin-2 in MI patients versus controls (day 1: 870 vs. 3,370 ng/mL, <i>p</i> not reported) ↔ in MBL, ficolin-3 or MAp44 in MI versus controls, <i>p</i> not reported ↑ ficolin-2 alone associated with ↑ end-diastolic volume and ↑ end-systolic volume at baseline but not 6 mo ( <i>p</i> = 0.004 and < 0.001) A combination of ↑ ficolin-2 AND ↑ MBL or ↓ MAp44 was associated with ↑ EDV and ESV from baseline to 6 mo ( <i>p</i> = 0.006 and <i>p</i> = 0.025)
Holt et al <sup>84</sup> 2014 Rating: Fair	(1) Case-control: MI occurrence (2) Longitudinal: Salvaged myocardium Blood sample before PCI Plasma MASP-1, -3 or MAp44 (EDTA), TRIFMA	Patients: MI ( <i>n</i> = 122) undergoing PCI Controls: Healthy blood donors ( <i>n</i> = 140) Follow-up: 30 days Age: 63 (56–72) years Gender (M/F, %): 77/23	Salvage index calculated from myocardial area at risk estimated before PCI and final infarct size	(1) ↑ MASP-1, MASP-3 and MAp44 in MI compared with controls ( <i>p</i> < 0.001–0.002) (2) No correlation between LP proteins and salvage index or area at risk (Spearman's <i>r</i> : -0.12 to 0.02, all <i>p</i> > 0.12)
Mellbin et al <sup>85</sup> 2012 <sup>a</sup> Rating: Good	Longitudinal, blood sample at study enrolment and 3 months Plasma MASP-2 (anticoagulant not reported), TRIFMA	Patients: type 2 diabetes and MI ( <i>n</i> = 397) Mean follow-up: 2.4 years Age: 70 (61–77) years Gender (M/F, %): 68/32	CVE: recurrent MI, ischaemic stroke or CVE-related death	No association between MASP-2 at admission or 3 mo and CVE (HR = 0.86 [0.62–1.20])

Abbreviations: CABG, coronary artery bypass grafting; CAD, coronary artery disease; CPB, cardiopulmonary bypass; CVE, cardiovascular event; EDTA, ethylenediaminetetraacetic acid; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; FCN, gene encoding ficolin; HR, hazard ratio; LP, lectin pathway; MAp44, MBL-associated protein of 44 kDa (also known as MAP1); MASP, MBL-associated protease; MBL, mannose-binding lectin; MBL2, gene encoding MBL; MI, myocardial infarction; OR, odds ratio; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty; STEMI, ST-segment elevation myocardial infarction; TRIFMA, time-resolved immunofluorometric assay; UAP, unstable angina pectoris.

Note: Study population: Age, gender and ethnicity are stated if reported in the study. Age is rounded to whole years and indicated by mean ± standard deviation or median (interquartiles) unless otherwise specified. Results: Numbers in parentheses after OR/HR are 95% confidence intervals unless otherwise specified.

<sup>a</sup>Same study population as Mellbin et al 2010.<sup>77</sup>

## Cardiovascular Events

### The Lectin Pathway and Occurrence of Cardiovascular Events

Studies investigating the lectin pathway and CVE occurrence are presented in ► **Table 3**. The following endpoints for CVE were investigated: MI,<sup>72–74,76,95</sup> unstable angina pectoris,<sup>74</sup>

CVE-related mortality,<sup>37,69</sup> all-cause mortality,<sup>37,66</sup> myocardial revascularization procedure (coronary artery bypass grafting [CABG] or percutaneous coronary intervention [PCI]), or a composite endpoint consisting of two or more of these.<sup>45,66–68,70,71,75</sup> Some studies investigated the association between the lectin pathway and CVE in patients with other clinical conditions such as diabetes mellitus,<sup>45,67,71,75</sup>

**Table 5** The lectin pathway in ischaemic stroke

Author, year, rating	Design Lectin pathway parameters	Study population	Endpoints for stroke severity or outcome	Results
Kouchaki et al <sup>86</sup> 2017 Rating: Good	Cross-sectional, blood sampling at admission Serum ficolin-1, ELISA	Patients ( $n = 82$ ) Age: $70 \pm 14$ years Gender (M/F, %): 51/49	NIHSS at admission	$\uparrow$ ficolin-1 correlated moderately with $\uparrow$ NIHSS (multivariate analysis, $\beta = 0.73$ , $p < 0.001$ )
Huang et al <sup>87</sup> 2016 Rating: Good	Case-control, blood sampling on the morning after admission Serum MBL, TRIFMA	Patients ( $n = 175$ ) Controls: healthy volunteers matched for age and gender ( $n = 175$ ) Age: 68 (57–75) years Gender (M/F, %): 56/44	(1) Stroke occurrence (2) NIHSS at admission (all) (3) Infarct size ( $n = 134$ )	$\uparrow$ MBL associated with stroke occurrence: Area under ROC curve = 0.76 (0.71–0.83) MBL correlated moderately with NIHSS score ( $r = 0.70$ , $p < 0.0001$ ) and weakly with infarct size <sup>1</sup> ( $r = 0.49$ , $p < 0.001$ )
Zangari et al <sup>88</sup> 2016 Rating: Fair	(1) Case-control: Stroke occurrence (2) Longitudinal: Severity and outcome after stroke Blood sampling at 6 h after admission (cohort 1) or 48 h, 4 d and 1 mo after admission (cohort 2) Plasma MBL, ficolin-1, -2, -3 (EDTA), ELISA	Patients ( $n = 165$ ) Two cohorts; different blood sampling times but same inclusion/exclusion criteria (cohort 1, $n = 80$ , cohort 2, $n = 85$ ) Controls: next-of-kin's to cases, matched on age and gender ( $n = 61$ ) Follow-up: 3 months Cohort 1/cohort 2: Age > 50: 94% years /86% years Gender (M/F, %): 41/59 and 58/42 Caucasian: 100% and 96%	(1) Stroke occurrence (2) NIHSS at admission (3) Modified Rankin Scale	$\downarrow$ ficolin-1 and -3 associated with stroke: Area under ROC curve = 0.91 for ficolin-1 and 0.68 for ficolin-3 at 6 hour (95% CIs not reported) Ficolin-1 at 6 h improved prediction of outcome (area under ROC = 0.93 [0.87–0.98] vs. 0.87 [0.77–0.96] for NIHSS + age alone) Unfavourable outcome associated with $\downarrow$ ficolin-1 at 6 h (adjusted OR = 2.21 [1.11–4.39]) but not ficolin-3 (OR = 0.99 [0.93–1.05]) or other LP proteins (all $p > 0.05$ ) No association between LP and initial stroke severity (data not shown)
Song et al <sup>89</sup> 2015 Rating: Good	(1) Case-control: Stroke occurrence (2) Longitudinal: Outcome after stroke Blood sampling at first morning after admission Serum MBL, TRIFMA	Patients with type 2 diabetes ( $n = 188$ ) Controls: healthy volunteers, matched for age and gender ( $n = 100$ ) Follow-up: 1 year Age: 68 (57–79) years Gender (M/F, %): 56/44 Ethnicity: all Chinese	(1) Stroke occurrence (2) NIHSS at admission (3) Modified Rankin Scale (4) All-cause mortality	Unfavourable outcome associated with $\uparrow$ MBL (adjusted OR = 8.99 [2.21–30.12], area under ROC curve = 0.75 [0.68–0.83]) $\uparrow$ mortality associated with $\uparrow$ MBL (adjusted OR = 13.22 [2.05–41.21], area under ROC curve = 0.85 [0.80–0.90]) MBL correlated moderately with NIHSS score (Spearman, $r = 0.71$ , $p = 0.009$ )

(Continued)

**Table 5** (Continued)

Author, year, rating	Design Lectin pathway parameters	Study population	Endpoints for stroke severity or outcome	Results
Zhang et al <sup>90</sup> 2015 Rating: Good	(1) Case-control: Stroke occurrence (2) Longitudinal: Outcome after stroke Blood sampling at admission Serum MBL, ELISA	Patients ( <i>n</i> = 231) Controls: healthy volunteers matched for age and gender ( <i>n</i> = 100) Follow-up: 90 days ( <i>n</i> = 222 completed follow-up) Good/poor outcome: Age: 61 (55–73) years /69 (61–76) years Gender (M/F, %): 62/38 and 60/40 Ethnicity: all Chinese	(1) Stroke occurrence (2) NIHSS at admission (3) Modified Rankin Scale (4) All-cause mortality	Unfavourable outcome associated with ↑ MBL (adjusted OR = 5.28 [2.88–10.67]) ↑ mortality associated with ↑ MBL (adjusted OR = 6.99 [3.55–13.97]) MBL correlated weakly with NIHSS score ( <i>r</i> = 0.33, <i>p</i> < 0.001)
Wang et al <sup>91</sup> 2014 Rating: Fair	Case-control, blood sampling at first morning after admission Serum MBL, TRIFMA	Patients ( <i>n</i> = 148) Controls: healthy volunteers, matched for age and gender ( <i>n</i> = 100) Cases: Age: 68 (59–75) years Gender (M/F, %): 54/46 Ethnicity: all Chinese	(1) NIHSS at admission (all) (2) Infarct size ( <i>n</i> = 112)	↑ MBL in stroke versus controls (1,332 vs. 678 μg/L, multiple logistic regression OR = 1.002 [1.001–1.008]) ↑ MBL correlated weakly with stroke severity (Spearman, <i>r</i> = 0.61, <i>p</i> = 0.004) ↑ MBL with ↑ infarct size (ANOVA, <i>p</i> < 0.0001) Area under ROC curve = 0.76 (0.70–0.82)
Frauenknecht et al <sup>95</sup> 2013 Rating: Fair	Case-control Plasma- MASP-1, -2, -3, MAp44 (citrate), TRIFMA	Patients ( <i>n</i> = 66) Controls: healthy volunteers ( <i>n</i> = 50) and patients with stable coronary artery disease ( <i>n</i> = 104) Stroke patients: Age: 65 ± 16 years Gender (M/F, %): 62/38	Stroke occurrence	↓ MASP-2 in stroke compared with controls. ↔ MASP-1, MASP-3 or MAp44 between groups ( <i>p</i> > 0.05)
Füst et al <sup>92</sup> 2011 Rating: Poor	(1) Case-control: Stroke occurrence (2) Longitudinal: Outcome after stroke Blood sampling at 12 h and at 3–4 d after admission Serum ficolin-2 and -3, ELISA	Patients ( <i>n</i> = 65) Controls: patients with stable carotid artery stenosis ( <i>n</i> = 135) and healthy volunteers ( <i>n</i> = 100) Follow-up: until discharge Age: 70 ± 10 years Gender (M/F, %): 51/49	(1) NIHSS at admission, good (< 16) versus poor (≥ 16) (2) Modified Rankin Scale at discharge	Unfavourable outcome associated with ↓ ficolin-3 at day 3 (OR = 5.63 [1.50–21.15]) No difference in mean ficolin-2 between outcome groups, <i>p</i> = 0.30, OR not computed Poor NIHSS associated with ↑ ficolin-3 at day 3 ( <i>p</i> = 0.02), but not at admission No association between NIHSS and ficolin-2 ( <i>p</i> = 0.30)
Osthoff et al <sup>93</sup> 2011 Rating: Good	Longitudinal, blood sampling at admission Serum MBL, ELISA	Patients receiving either conservative treatment ( <i>n</i> = 287) or thrombolysis ( <i>n</i> = 66)	(1) Stroke severity: NIHSS at admission (all), infarct size (in 199 patients) (2) Modified Rankin	Conservative treatment group: Unfavourable outcome associated with ↑ MBL:

Table 5 (Continued)

Author, year, rating	Design Lectin pathway parameters	Study population	Endpoints for stroke severity or outcome	Results
		Follow-up: 90 days Age: 75 (63–82) years Gender (M/F, %): 59/41	Scale (3) Barthel Index	-Rankin Scale (adjusted OR = 1.23 [1.02–1.48] for continuous serum MBL and 0.38 [0.14–0.98] in low vs. high MBL) -Barthel Index (adjusted OR = 1.26 (1.04–1.54) for continuous data) ↑ severity and ↑ infarct size associated with ↑ MBL ( $p = 0.025$ and $0.003$ ) No associations in thrombolysis group (no difference in median MBL between outcome or severity groups, all $p > 0.50$ )
Cervera et al <sup>94</sup> 2010 Rating: Good	Longitudinal MBL2 genotype Promoter -221, codon 52, 54, 57 MASP2 SNP D120G Serum MBL, MASP-2, ELISA	Patients with ischaemic ( $n = 109$ ) or haemorrhagic ( $n = 26$ ) stroke Follow-up: 90 days Low/high MBL: Age: $74 \pm 13$ years / $73 \pm 12$ years Gender (M/F, %): 38/62 and 53/47	Favourable versus unfavourable outcome, 'favourable' defined as modified Rankin Scale $< 2$ , NIHSS score $< 2$ and Barthel Index $\geq 95\%$	Unfavourable outcome associated with ↑ serum MBL (adjusted OR = 1.29 [1.02–1.69]) and high genotype (adjusted OR = 10.85 [1.87–62.94]) No association between MASP2 polymorphism and outcome (OR = 0.92 [0.23–3.73])

Abbreviations: CI, confidence interval; CT, computed tomography; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; LP, lectin pathway; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; MBL2, gene encoding MBL; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; ROC, receiver operator characteristics; TRIFMA, time-resolved immunofluorometric assay; WHO, World Health Organization.

Note: Study population: 'Patients' refer to patients with ischaemic stroke (cases) unless otherwise specified. Stroke was verified with CT/MRI or according to the WHO criteria for all studies. Age, gender and ethnicity are stated if reported in the study. Age is rounded to whole years and indicated by mean  $\pm$  standard deviation or median (interquartiles) unless otherwise specified. Endpoints: All studies defined 'favourable' outcome as Rankin Scale  $< 2$  points or Barthel Index  $\geq 95\%$ . Results: Numbers in parentheses after OR/HR/area under ROC curve are 95% confidence intervals unless otherwise specified.

hypercholesterolaemia,<sup>71</sup> rheumatoid arthritis,<sup>37,68</sup> end-stage kidney disease<sup>66</sup> or pancreas-kidney transplantation.<sup>69</sup> Thus, the study populations were heterogeneous, and we summarize their main findings here with this limitation in mind.

Overall, the reported associations between MBL and CVE occurrence varied considerably between studies with regards to effect size as well as direction. Two case-control studies found that CVE occurrence was associated with increased MBL serum levels<sup>74,75</sup> and high genotype.<sup>74</sup> This was supported by prospective studies, which all reported increased CVE occurrence in patients with high serum MBL levels<sup>69,70</sup> or high genotype,<sup>37,68</sup> however, Keller et al only found this association in a sub-population of men.<sup>70</sup> In contrast to this increased CVE risk related to high MBL, three studies reported that CVE was associated with low

MBL,<sup>66,67,71</sup> although Saevarsdottir et al only found this association in sub-groups with hypercholesterolaemia and diabetes.<sup>71</sup> It was possible to extract/obtain data from six studies investigating serum MBL and five studies investigating MBL2 genotype to perform forest plots of crude or adjusted odds ratios for CVE with high serum MBL or high genotype (► Fig. 4). The forest plots support that high serum MBL or genotype was not consistently associated with a higher CVE risk in the included studies.

Regarding other lectin pathway proteins, no clear patterns emerged. Two studies reported higher MASP-1 levels in patients with MI compared with healthy controls;<sup>84,95</sup> two studies found lower MASP-2 levels in patients with MI versus healthy controls;<sup>76,95</sup> and finally, two studies reported associations between MI and higher MASP-3 levels.<sup>73,84</sup> Vengen et al also found MI to be associated with higher ficolin-2

**Table 6** The lectin pathway in other pro-thrombotic conditions

Author, year, rating	Design Lectin pathway parameters	Study population	Endpoints for thromboembolic disease or coagulation activity	Results
<b>Systemic lupus erythematosus (SLE)</b>				
Kozarcanin et al <sup>61</sup> 2016 Rating: Fair	Cross-sectional MASP-1/-2 activation (MASP-1/-2 in complex with C1-inhibitor or anti-thrombin)	Patients: SLE ( $n = 69$ ), multi-trauma ( $n = 10$ ) Trauma: Age: 23–80 years Gender (M/F, %): 80/20	SLE: Platelet activation markers, platelet count, FXIa, FXIIa Trauma: Platelet count, PT, aPTT, TAT- complex and fibrinogen	SLE: Positive correlation between MASP complexes and platelet activation, FXIa and FXIIa (rho values, 0.31–0.74, all $p < 0.001$ ) Trauma: Positive correlation between MASP complexes and PT, TAT-complex and fibrinogen, but not aPTT or platelet count
Hein et al <sup>41</sup> 2015 Rating: Poor	Cross-sectional Plasma ficolin-1, -2, -3 (EDTA), ELISA	Patients: SLE ( $n = 68$ ) Age: 40 (range: 21– 76) years Gender (M/F,%): 9/91	History of arterial or venous thrombosis	↑ ficolin-1 in patients with a history of arterial thrombosis ( $p = 0.005$ ) No difference in ficolin- 2 or -3 for arterial thrombosis and no difference for venous thrombosis (all $p > 0.05$ )
Jönsen et al <sup>96</sup> 2007 Rating: Good	Longitudinal <i>MBL2</i> genotype Promoter -550, -221, codon 52, 54, 57	Patients: SLE ( $n = 143$ ) Mean follow-up: 15 years Age at enrolment not reported Gender (M/F, %): 12/88	Cerebrovascular, coronary or peripheral vascular event	No association between <i>MBL2</i> genotype and thrombotic events (adjusted OR = 3.3 [0.80–14])
Font et al <sup>97</sup> 2007 Rating: Fair	Cross-sectional <i>MBL2</i> genotype Promoter -550, -221, UTR +4, codon 52, 54, 57	Patients: SLE ( $n = 114$ ) Age: 41 ± 1 years Gender (M/F, %): 7/93	Previous cerebrovascular thrombosis (arterial or venous), MI, intra-abdominal artery thrombosis, DVT or PE	No association between <i>MBL2</i> genotype and thrombotic events in multivariate analysis (OR not reported)
Calvo-Alén et al <sup>98</sup> 2006 Rating: Fair	Longitudinal <i>MBL2</i> genotype Codon 52, 54, 57	Patients: SLE ( $n = 415$ ) Mean follow-up: 3.9 years Thrombosis: yes/no Age: 44 ± 15 years /36 ± 12 years Gender (M/F, %): 17/83 and 9/91 Ethnicity: Hispanic: 23% and 30% African: 40% and 40% Caucasian: 37% / 30%	MI, angina pectoris, CABG, ischaemic stroke, transient ischaemic attack or peripheral vascular disease	No significant association between <i>MBL2</i> genotype and thrombotic events, except cerebrovascular events alone: ↑ events in low versus intermediate/high genotype ( $p = 0.049$ )
Øhlschlaeger et al <sup>99</sup> 2004 Rating: Good	Longitudinal <i>MBL2</i> genotype Promoter -550, -221, codon 52, 54, 57	Patients: SLE ( $n = 91$ ) Mean follow-up: 11 years Gender (M/F, %): 10/90 Ethnicity: Caucasian	MI, cerebrovascular event, peripheral vascular disease, DVT or PE	↑ arterial events in patients with low versus intermediate/high genotype (adjusted HR = 7.0 [1.9–25.4]) No association between genotype and venous thrombosis (data not shown)

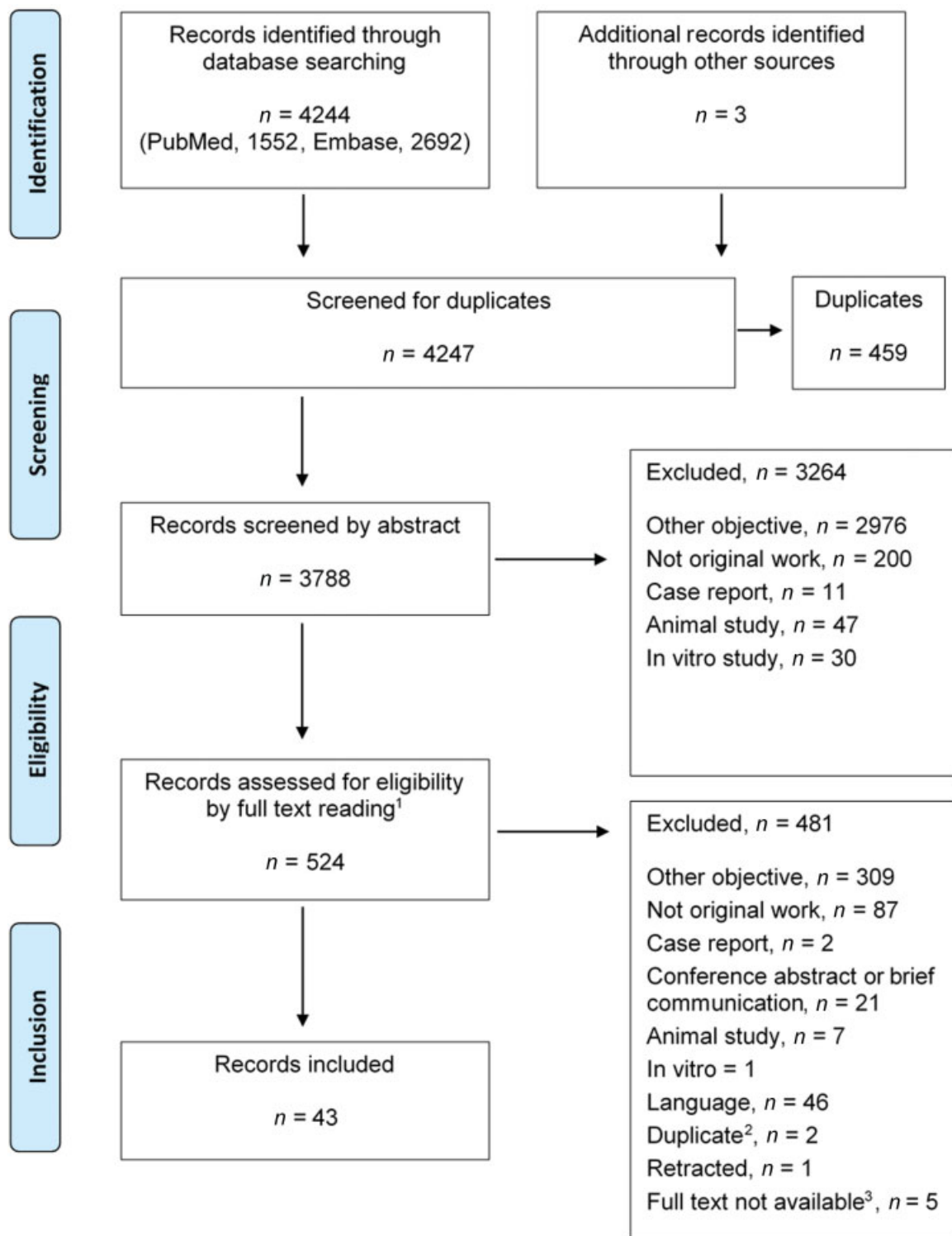


Table 6 (Continued)

Author, year, rating	Design Lectin pathway parameters	Study population	Endpoints for thromboembolic disease or coagulation activity	Results
<b>Disseminated intravascular coagulation (DIC)</b>				
Sprong et al <sup>103</sup> 2009 Rating: Fair	Cross-sectional MBL2 genotype Codon 52, 54, 57	Patients: septic shock ( <i>n</i> = 18) Age: 6 months–7 years Gender (M/F, %): 50/50	Platelet count, fibrinogen, ISTH DIC score	↑ platelet count and fibrinogen and ↓ DIC score in children with low genotype ( <i>n</i> = 2)
Zhao et al <sup>104</sup> 2015 Rating: Good	(1) Cross-sectional: DIC at admission (2) Longitudinal: DIC development after admission Blood sampling at admission Serum MBL, ELISA	Patients: sepsis with/without shock ( <i>n</i> = 267) Follow-up: until discharge DIC/no DIC at admission: Age: 67 ± 15 years / 68 ± 14 years Gender (M/F, %): 61/39 and 60/40	Overt DIC (ISTH DIC score ≥ 5) at admission or developed during hospitalization, assessed once daily	MBL did not predict DIC independently (OR or area under ROC curve not reported) ↑ MBL in DIC versus no DIC (125 vs. 116 ng/L, <i>p</i> = 0.007)
Takahashi et al <sup>102</sup> 2014 Rating: Good	Case-control Plasma CL-K1 (citrate), ELISA	Patients: DIC of different aetiology ( <i>n</i> = 549) Controls: Patients without DIC ( <i>n</i> = 82) and healthy controls ( <i>n</i> = 27) DIC/no DIC: Age: 67 (53–78) years / 64 (51–73) years Gender (M/F, %): 56/44 and 60/40 Ethnicity: Caucasian	DIC: biphasic aPTT waveform present	DIC associated with ↑ CL-K1 (OR = 1.93 [1.04–3.87])
<b>Others</b>				
Lv et al <sup>100</sup> 2013 Rating: Poor	Case-control LP mRNA expression (whole genome expression)	Patients: PE ( <i>n</i> = 20) Controls: patients without PE matched for age and gender ( <i>n</i> = 20) Cases: Age: 70 ± 14 y Gender (M/F, %): 55/45	PE	↓ mRNA expression for MBL and MASP-1 in PE versus controls ( <i>p</i> < 0.05); no difference for MASP-2 ( <i>p</i> -value not reported)
Merlen et al <sup>101</sup> 2015 Rating: Fair	Longitudinal, blood sampling before therapy and every 3 wk until end of therapy Serum MBL, EIA	Patients: acute lymphoblastic leukaemia receiving L-asparaginase ( <i>n</i> = 97) Follow-up: 25 wk Age: 6.4 (range: 1–17) years Gender (M/F, %): 56/44	Plasma-fibrinogen and -anti-thrombin	↓ MBL, fibrinogen and anti-thrombin during therapy versus baseline MBL levels correlated moderately with fibrinogen (Spearman, <i>r</i> = 0.58, <i>p</i> < 0.0001) and anti-thrombin ( <i>r</i> = 0.40, <i>p</i> < 0.0001)

Abbreviations: aPTT, activated partial thromboplastin time; CABG, coronary artery bypass grafting; CL-K1, collectin kidney 1; DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; F, coagulation factor; HR, hazard ratio; ISTH, International Society on Thrombosis and Haemostasis; LP, lectin pathway; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; MBL2, gene encoding MBL; MI, myocardial infarction; mRNA, messenger ribonucleic acid; OR, odds ratio; PE, pulmonary embolism; PT, prothrombin time; SLE, systemic lupus erythematosus; ROC, receiver operator characteristics; TAT, thrombin-anti-thrombin.

Note: Study population: Age, gender and ethnicity are stated if reported in the study. Age is rounded to whole years and indicated by mean ± standard deviation or median (interquartiles) unless otherwise specified. Results: Numbers in parentheses after OR/HR/area under ROC curve are 95% confidence intervals unless otherwise specified.



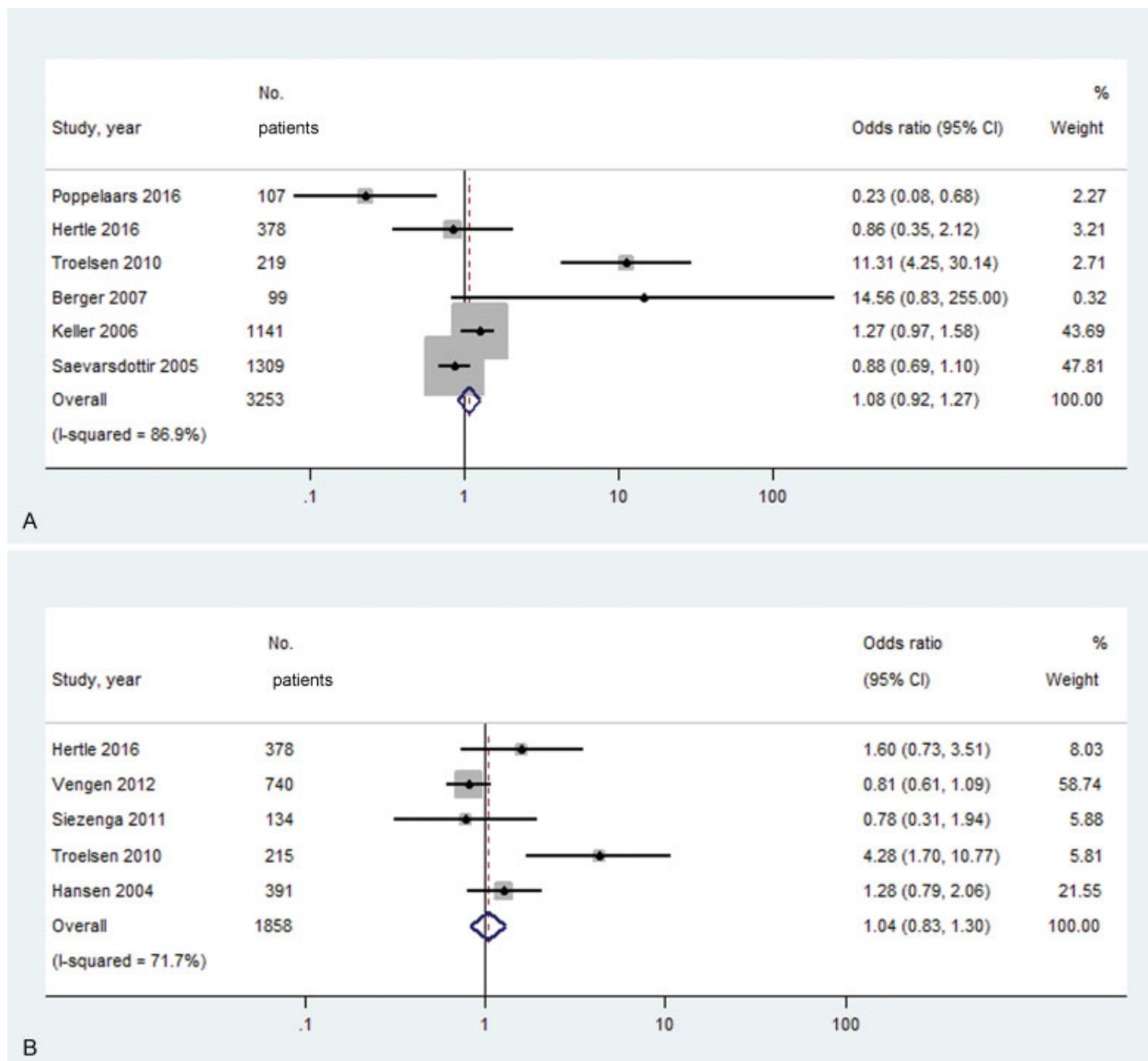
**Fig. 3** Inclusion and exclusion process. Notes: <sup>1</sup>Including 378 with no available abstract; <sup>2</sup>Duplicate not caught in initial duplicate screening; <sup>3</sup>Could not be obtained via the Aarhus University Library/Royal Danish Library.

levels,<sup>73</sup> while Schoos et al reported the opposite.<sup>83</sup> No associations between ficolin-1 or -3 and CVE were observed.<sup>72,73,83</sup>

Four studies analysed MAp44, of which three found no association between this protein and CVE,<sup>45,73,95</sup> while one study reported increased MAp44 levels in MI patients versus healthy controls.<sup>84</sup>

### The Lectin Pathway and Outcome in Existing Cardiovascular Disease

The nine studies investigating the association between lectin pathway proteins and outcome in patients with pre-existing CVE are presented in ►Table 4. All studies included study populations with recent MI; most having undergone either CABG or PCI in the early phase of the study. The following



**Fig. 4** Forest plots of odds ratios for cardiovascular events with (A) high versus low serum levels of mannose-binding lectin (MBL) and (B) MBL2 genotype associated with high (wild-type) versus intermediate or MBL serum level. Dotted vertical lines represent pooled or overall odds ratio.

endpoints for outcome or severity of CVE were investigated: recurrent MI,<sup>80,81</sup> reduced left ventricle ejection fraction (heart failure),<sup>79,83</sup> change in end-systolic/-diastolic volume,<sup>83</sup> infarct size,<sup>83</sup> myocardial salvage index,<sup>84</sup> early CABG venous graft occlusion,<sup>82</sup> CVE-related mortality, all-cause mortality<sup>81</sup> or a composite endpoint consisting of two or more of these.<sup>77,78,85</sup>

Three studies reported that low serum MBL or low genotype was associated with a better outcome after MI.<sup>78–80</sup> In contrast, two studies found an association between low MBL and early recurrent MI<sup>81</sup> or graft occlusion after CABG,<sup>82</sup> while Mellbin et al found no association between MBL or MASP-2 and CVE.<sup>77,85</sup> Holt et al investigated MASP-1, MASP-3 and MAp44 and found no association between either of these proteins and myocardial salvage after PCI.<sup>84</sup> Schoos et al demonstrated no association between MAp44 alone and cardiac function either, but reported that both high ficolin-2 alone and a combination of

high ficolin and low MAp44 were associated with impaired cardiac function (higher end-systolic and end-diastolic volumes).<sup>83</sup>

### Ischaemic Stroke

#### *The Lectin Pathway and Occurrence of Ischaemic Stroke*

Eight case-control studies investigated lectin pathway patients with ischaemic stroke versus healthy controls.<sup>86–92,95</sup> In general, stroke was diagnosed according to valid diagnostic criteria and/or verified with radiological imaging. Among the five studies analysing MBL, four studies reported higher serum MBL levels in stroke patients compared with healthy controls.<sup>87,89–91</sup> High MBL was moderately associated with the occurrence of stroke with areas under the receiver operator characteristics (ROC) curves = 0.76.<sup>87,91</sup> In contrast, one study found no association between plasma MBL and ischaemic stroke.<sup>88</sup>

Zangari et al found lower levels of ficolin-1, -2 and -3 during the early phase of stroke and a strong association between low ficolin-1 at 6 hours and stroke occurrence (area under the ROC curve = 0.91).<sup>88</sup> This was in accordance with Füst et al who also reported low ficolin-2 and -3 levels at admission in stroke patients.<sup>92</sup> Frauenknecht et al found lower levels of MASP-2 in stroke patients compared with controls, but demonstrated no difference for MASP-1, -3 or MASP44.<sup>95</sup>

#### *The Lectin Pathway and Functional Outcome or Severity of Stroke*

The following endpoints were used for stroke severity and functional outcome after stroke: initial stroke severity assessed with National Institute of Health Stroke Scale (NIHSS)<sup>86-93</sup> where increasing score indicates increasing stroke severity, infarction volume estimated with magnetic resonance imaging,<sup>87,91,93</sup> functional outcome assessed with modified Rankin Scale<sup>88-90,92,93</sup> or Barthel Index<sup>93</sup> or a composite endpoint of NIHSS, modified Rankin Scale and Barthel Index.<sup>94</sup> A favourable outcome on the Rankin Scale was typically defined as < 2 points and on the Barthel Index as > 95%.

In general, the studies demonstrated an association between high MBL and increased stroke severity and unfavourable outcome. MBL was reported to correlate positively with NIHSS (though only weakly to moderately)<sup>87,89,90</sup> and with infarct size.<sup>91</sup> Several studies found that high MBL was independently associated with both higher mortality and unfavourable outcome on Rankin Scale<sup>89,90,93</sup> and Barthel Index.<sup>93</sup> The only study investigating *MBL2* genotype supported these findings, reporting that both high serum MBL and high genotype was associated with an unfavourable outcome.<sup>94</sup> Song et al calculated the ability of MBL to predict mortality and functional outcome, finding moderate areas under the ROC curves (0.85 for mortality and 0.75 for Rankin Scale).<sup>89</sup> Only one study did not find an association between MBL and stroke severity or functional outcome.<sup>88</sup>

With regards to other lectin pathway proteins, Kouchaki et al found a significant positive correlation between ficolin-1 and NIHSS score,<sup>86</sup> indicating that high ficolin-1 levels are associated with more severe stroke. This contrasted with Zangari et al who found no association between ficolins and NIHSS score, but reported an association between low ficolin-1 and unfavourable outcome on modified Rankin Scale.<sup>88</sup> Füst et al found that low ficolin-3 at day 3 after stroke was associated with unfavourable outcome, but also with less initial stroke severity.<sup>92</sup>

### **Other Conditions**

#### **Systemic Lupus Erythematosus**

Four studies examined associations between *MBL2* genotypes and thrombotic events in SLE patients.<sup>96-99</sup> Øhlenschlaeger et al reported that low genotype was associated with increased risk of arterial thrombosis, defined as cardiovascular, cerebrovascular or peripheral vascular events, but not with venous thrombosis.<sup>99</sup> The three remain-

ing studies found no independent association<sup>96,97</sup> or only a borderline significant association<sup>98</sup> between *MBL2* genotype and thrombotic events.

Hein et al reported that SLE patients with a history of arterial but not venous thrombosis had significantly higher circulating ficolin-1 levels.<sup>41</sup> The method for verifying a previous thrombotic event was not reported, nor could it be found in the original source cited. Kozarcanin et al did not include clinical endpoints, but investigated correlations between MASP-1 and -2 activation and platelet and coagulation parameters in SLE patients and multi-trauma patients.<sup>61</sup> The authors reported that MASP activation correlated significantly with activation of both primary and secondary haemostasis in both patient groups.

#### **Disseminated Intravascular Coagulation**

Sprong et al investigated the association between *MBL2* genotype and coagulation parameters and clinical manifestations indicating DIC in children with meningococcal disease and septic shock.<sup>103</sup> Children with low genotype ( $n = 2$  of 18) had a lower DIC score, and their platelet counts and fibrinogen levels were markedly closer to the normal range compared with children with intermediate or high genotype. Due to the small number of patients, statistical significance was not calculated. In a study on adult sepsis patients, Zhao et al reported higher MBL levels in patients with overt DIC at admission and in patients who developed DIC later compared with patients without DIC, but MBL was not found to be an independent predictor for the development of DIC.<sup>104</sup> In both the DIC and non-DIC group, the mean MBL serum level was well below the range reported by other authors in healthy individuals,<sup>21</sup> and the difference between the DIC and non-DIC group, although statistically significant, was subtle. Finally, Takahashi et al investigated CL-K1 in 549 patients with DIC of various aetiologies and found that DIC was associated with higher CL-K1 levels.<sup>102</sup>

#### **Other Studies**

Lv et al conducted a case-control study in patients with pulmonary embolism and healthy controls and extensively investigated complement-related messenger ribonucleic acid (mRNA) expression.<sup>100</sup> They found lower expression of *MBL2* and *MASP2* mRNA in patients with pulmonary embolism compared with healthy controls.

Finally, Merlen et al investigated serum-MBL, fibrinogen, anti-thrombin and thrombotic events in paediatric patients who were treated with L-asparaginase,<sup>101</sup> a chemotherapeutic drug with a known pro-thrombotic effect. The main finding related to the scope of the present review was that MBL correlated positively, although only weakly, with both fibrinogen and anti-thrombin.

### **Discussion**

The present systematic review did not reveal a consistent association between the lectin pathway and thrombotic conditions. MBL serum levels and/or genotype were the most commonly investigated lectin pathway parameters.

An association between MBL and CVE occurrence or outcome was observed in several studies but differed in both effect size and direction. In ischaemic stroke patients, high serum MBL was consistently associated with occurrence of stroke as well as increased stroke severity and unfavourable outcome. Only few studies on other thrombotic conditions were identified.

In general, the included studies were of high quality, with 90% rating 'good' or 'fair' using the National Institute of Health quality assessment tools. This indicates a low risk of bias and overall high validity of the results. Among the 43 studies, 29 (67%) were prospectively designed, while the rest was cross-sectional or case-control studies. CVE ( $n = 23$ ), ischaemic stroke ( $n = 10$ ), SLE-associated thrombosis ( $n = 6$ ) and sepsis-associated DIC ( $n = 3$ ) were the most commonly investigated thrombotic conditions. Almost all studies had clinical thromboembolic disease as their main outcome, and only five studies used laboratory coagulation parameters as endpoints.<sup>61,101-104</sup> A variety of materials (serum, ethylenediaminetetraacetic acid plasma, citrated plasma) were used for lectin pathway protein analysis, as well as different assays (enzyme-linked immunosorbent assay, time-resolved immunofluorometric assay); however, there did not seem to be an association between the materials or assays used and the reported results.

As summarized in the introduction, the available *in vitro* evidence consistently reports that MBL and MASP-1 and -2 can activate the coagulation system. This is supported by animal studies. Studies on induced carotid artery and cerebral thrombosis in murine models found that MBL-deficient mice had preserved carotid blood flow<sup>105,106</sup> and less intravascular fibrin deposition<sup>107</sup> compared with wild-type mice. Takahashi et al demonstrated a prolonged tail bleeding time in MBL-deficient mice which could be restored with recombinant MBL. Taken together, the available evidence from *in vitro* and animal studies thus suggests a pro-coagulant effect of lectin pathway activation and a protective role of MBL deficiency on thrombus development.

The results of the present systematic review do not unequivocally support these findings of *in vitro* and animal studies. The included studies reported conflicting results, especially regarding the role of the lectin pathway in cardiovascular thrombosis. Both high<sup>37,69</sup> and low<sup>66,67</sup> MBL levels were reported to be risk factors for CVE, while two large cohort studies found no overall influence of serum MBL on future CVE risk.<sup>70,71</sup> A probable explanation of these conflicting results could be the diversity in study populations; they all carried an increased CVE risk but differed with regards to the underlying pathophysiology and baseline risk of thrombotic events. Furthermore, follow-up times varied, and a variety of endpoints or combinations of endpoints were employed.

The studies on ischaemic stroke more consistently reported associations between high MBL levels and stroke occurrence as well as severity<sup>87,89-91,93</sup> with a single exception.<sup>88</sup> The ficolins were investigated in three studies only.<sup>86,88,92</sup> Associations between both ficolin-1 and ficolin-3 and stroke severity and long-term outcome were

described; however, the findings were contrasting, and the scarcity of studies make it difficult to conclude on the role of ficolins in ischaemic stroke. An association between high MBL and unfavourable outcome was also consistently reported after stroke,<sup>89,90,93,94</sup> again with one exception,<sup>88</sup> as recently reviewed by Fumagalli and De Simoni,<sup>108</sup> but this association was not consistently reported after MI.<sup>77-83</sup> An important point is that the functional outcome after an ischaemic event is not only related to the thrombus itself but also to other factors, among which is ischaemia-reperfusion injury. Interestingly, increasing evidence points to the lectin pathway as a possible player in ischaemia-reperfusion injury, showing that the lectin pathway contributes to the increased inflammation, tissue damage and necrosis in ischaemia-reperfusion injury, together with other mechanisms.<sup>109-111</sup> This is supported by several animal studies which report smaller infarct size and improved functional outcome in MBL- or MASP-2-deficient mice after cerebral<sup>107,112-114</sup> or myocardial<sup>105,109,115-117</sup> ischaemia-reperfusion injury. This association could be a possible explanation for the association between MBL and unfavourable outcome in stroke observed in the present review. However, this does not explain why a similar association with unfavourable outcome after MI was not consistently observed.

In SLE patients, one well-conducted follow-up study demonstrated that low MBL genotype was associated with higher risk of arterial thrombotic events,<sup>99</sup> but three other studies on SLE patients could not reproduce these findings.<sup>96-98</sup> Two studies investigated associations between MBL and sepsis-related DIC<sup>103,104</sup> and both reported higher MBL levels to be associated with more severe coagulation disturbances. However, one study was very small ( $n = 18$ ),<sup>103</sup> while in the second study, only a discrete difference in MBL levels was observed between the DIC and non-DIC group, and MBL was not found to be an independent predictor of DIC in sepsis.<sup>104</sup> Thus, the influence of the lectin pathway on coagulation and thrombosis risk in both SLE and sepsis remains uncertain.

We included both arterial and venous thrombosis in the present review, with the majority of included studies focusing on arterial thrombotic events. Although arterial and venous thrombosis include several common factors, the mechanisms behind them differ considerably. Coronary and cerebral artery thrombosis usually includes a component of atherosclerosis. This is a complex condition characterized by chronic low-grade inflammation with activation of several immune system components,<sup>118,119</sup> which may have been on-going for a long period of time before the major thrombotic event takes place. Activated platelets are also a key component of atherosclerosis, as they are activated by oxidized LDL and activated endothelial cells in the atherosclerotic plaque. In turn, they activate and uphold inflammation by facilitating monocyte migration, activating neutrophils and secreting pro-inflammatory factors.<sup>120,121</sup> Interestingly, cholesterol crystals in the atherosclerotic plaque may play a role in activating the lectin pathway directly,<sup>122</sup> and in diabetic patients, altered endothelial

glycosylation patterns may further cause MBL binding and lectin pathway activation.<sup>42</sup> Venous thrombosis, on the other hand, is driven largely (though not exclusively) by activation of secondary haemostasis, and traditional risk factors are hypercoagulability, loss of endothelial integrity with tissue factor exposure and decreased blood flow or stasis.<sup>123</sup> Hypercoagulability can be inherited, but one of the major risk factors for venous thrombosis is inflammation, potentially induced by bacterial infection, autoimmune disease and traumatic or surgical acute phase response. The inflammation-induced hypercoagulability is driven by pro-inflammatory cytokines such as interleukin-6 and caused by an increased production of pro-coagulant factors such as tissue factor, fibrinogen, FVIII, von Willebrand factor and plasminogen activator inhibitor-1, as well as decreased production of the endogenous anti-coagulants, anti-thrombin protein C and S.<sup>123,124</sup> The venous thrombosis is often an acute event in itself, but local inflammatory changes in the vessel can persist and lead to chronic venous disease.<sup>125,126</sup>

The lectin pathway is an interesting potential contributor to inflammation-driven hypercoagulability and thrombosis through direct activation by MASPs, as described in the introduction. Since there is considerable crosstalk and mutual activation between the coagulation system and the lectin pathway, they may well activate each other in thrombotic and inflammatory conditions and thereby uphold the inflammatory and hyper-coagulable response in a vicious cycle. This emerging concept of immunothrombosis—or thromboinflammation—has recently been discussed.<sup>123,127</sup> However, it should be noted that since changes in both lectin pathway proteins and coagulation factors occur during the acute phase response, any observed correlation between plasma levels does not equal causation, but may simply indicate that an acute phase reaction is on-going. It should also be considered that although there is solid evidence that MASPs are able to induce clot formation *in vitro*, this occurs at a lower rate than thrombin-induced clot formation.<sup>52</sup> Furthermore, MASP-1-induced fibrin clots were found to be less dense and with thicker fibres than thrombin-induced clots.<sup>53</sup> This type of clots has been found more susceptible to lysis.<sup>128</sup> Thus, the lectin pathway may have not a merely pro-coagulant but also a modulatory effect on coagulation, which could be clinically important.

The strengths of the present review were that we assessed the literature systematically, searching both PubMed and Embase and using broad search terms to minimize the risk of overlooking relevant studies. We performed a formal quality assessment and found the vast majority of studies to be of good or fair quality, which strengthens the validity of their results and our conclusions. The majority of studies included clinical endpoints for thromboembolic disease. However, some limitations should be mentioned. The study populations and endpoints for thrombosis were heterogeneous, and only few studies on thromboembolic conditions other than CVE and stroke were identified. This limits the value of comparison across all the identified studies and makes it impossible to conclude on the significance of the lectin pathway in the development of thrombotic conditions in a broader clinical setting.

To conclude, the existing evidence indicates that lectin pathway activation is associated with poor outcome after ischaemic stroke. Furthermore, there is some evidence for involvement of the lectin pathway in CVEs, although the exact role of the lectin pathway in this setting is not yet fully elucidated. With regards to other thrombotic conditions, only sparse clinical evidence exists, and future studies in this field are warranted.

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#### Conflict of Interest

None.

#### References

- 1 Esmon CT, Xu J, Lupu F. Innate immunity and coagulation. *J Thromb Haemost* 2011;9(Suppl 1):182–188
- 2 van der Poll T, Herwald H. The coagulation system and its function in early immune defense. *Thromb Haemost* 2014;112(04):640–648
- 3 Oikonomopoulou K, Ricklin D, Ward PA, Lambris JD. Interactions between coagulation and complement—their role in inflammation. *Semin Immunopathol* 2012;34(01):151–165
- 4 Degn SE, Jensenius JC, Bjerre M. The lectin pathway and its implications in coagulation, infections and auto-immunity. *Curr Opin Organ Transplant* 2011;16(01):21–27
- 5 Degn SE, Thiel S, Jensenius JC. New perspectives on mannan-binding lectin-mediated complement activation. *Immunobiology* 2007;212(4-5):301–311
- 6 Garred P, Genster N, Pilely K, et al. A journey through the lectin pathway of complement-MBL and beyond. *Immunol Rev* 2016; 274(01):74–97
- 7 Matsushita M, Endo Y, Fujita T. Structural and functional overview of the lectin complement pathway: its molecular basis and physiological implication. *Arch Immunol Ther Exp (Warsz)* 2013;61(04):273–283
- 8 Dunkelberger JR, Song WC. Complement and its role in innate and adaptive immune responses. *Cell Res* 2010;20(01): 34–50
- 9 Ma YJ, Doni A, Hummelshøj T, et al. Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition. *J Biol Chem* 2009;284(41):28263–28275
- 10 Ma YJ, Doni A, Skjoed MO, et al. Heterocomplexes of mannan-binding lectin and the pentraxins PTX3 or serum amyloid P component trigger cross-activation of the complement system. *J Biol Chem* 2011;286(05):3405–3417
- 11 Ng PM, Le Saux A, Lee CM, et al. C-reactive protein collaborates with plasma lectins to boost immune response against bacteria. *EMBO J* 2007;26(14):3431–3440
- 12 Zhang J, Yang L, Ang Z, et al. Secreted M-ficolin anchors onto monocyte transmembrane G protein-coupled receptor 43 and cross talks with plasma C-reactive protein to mediate immune signaling and regulate host defense. *J Immunol* 2010;185(11): 6899–6910
- 13 Frederiksen PD, Thiel S, Larsen CB, Jensenius JC. M-ficolin, an innate immune defence molecule, binds patterns of acetyl groups and activates complement. *Scand J Immunol* 2005;62(05):462–473
- 14 Matsushita M, Endo Y, Fujita T. Cutting edge: complement-activating complex of ficolin and mannan-binding lectin-associated serine protease. *J Immunol* 2000;164(05):2281–2284

- 15 Luo F, Sun X, Wang Y, et al. Ficolin-2 defends against virulent mycobacteria tuberculosis infection in vivo, and its insufficiency is associated with infection in humans. *PLoS One* 2013;8(09): e73859
- 16 Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. *Clin Exp Immunol* 1992;90(01):31–35
- 17 Dean MM, Minchinton RM, Heatley S, Eisen DP. Mannose binding lectin acute phase activity in patients with severe infection. *J Clin Immunol* 2005;25(04):346–352
- 18 Plovsing RR, Berg RM, Munthe-Fog L, et al. Alveolar recruitment of ficolin-3 in response to acute pulmonary inflammation in humans. *Immunobiology* 2016;221(05):690–697
- 19 Van Till JW, Boermeester MA, Modderman PW, et al. Variable mannose-binding lectin expression during postoperative acute-phase response. *Surg Infect (Larchmt)* 2006;7(05):443–452
- 20 Ytting H, Christensen IJ, Basse L, et al. Influence of major surgery on the mannan-binding lectin pathway of innate immunity. *Clin Exp Immunol* 2006;144(02):239–246
- 21 Trolldborg A, Hansen A, Hansen SW, Jensenius JC, Stengaard-Pedersen K, Thiel S. Lectin complement pathway proteins in healthy individuals. *Clin Exp Immunol* 2017;188(01):138–147
- 22 Thiel S. Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins and associated proteins. *Mol Immunol* 2007;44(16):3875–3888
- 23 Bronkhorst MW, Lomax MA, Vossen RH, Bakker J, Patka P, van Lieshout EM. Risk of infection and sepsis in severely injured patients related to single nucleotide polymorphisms in the lectin pathway. *Br J Surg* 2013;100(13):1818–1826
- 24 De Pascale G, Cutuli SL, Pennisi MA, Antonelli M. The role of mannose-binding lectin in severe sepsis and septic shock. *Mediators Inflamm* 2013;2013:625803
- 25 Eisen DP, Dean MM, Thomas P, et al. Low mannose-binding lectin function is associated with sepsis in adult patients. *FEMS Immunol Med Microbiol* 2006;48(02):274–282
- 26 Schlapbach LJ, Mattmann M, Thiel S, et al. Differential role of the lectin pathway of complement activation in susceptibility to neonatal sepsis. *Clin Infect Dis* 2010;51(02):153–162
- 27 Charchafieh J, Rushbrook J, Worah S, Zhang M. Activated complement factors as disease markers for sepsis. *Dis Markers* 2015; 2015:382463
- 28 Munthe-Fog L, Hummelshøj T, Honoré C, Madsen HO, Permin H, Garred P. Immunodeficiency associated with FCN3 mutation and ficolin-3 deficiency. *N Engl J Med* 2009;360(25):2637–2644
- 29 Metzger ML, Michelfelder I, Goldacker S, et al. Low ficolin-2 levels in common variable immunodeficiency patients with bronchiectasis. *Clin Exp Immunol* 2015;179(02):256–264
- 30 Schlapbach LJ, Thiel S, Kessler U, Ammann RA, Aebi C, Jensenius JC. Congenital H-ficolin deficiency in premature infants with severe necrotising enterocolitis. *Gut* 2011;60(10):1438–1439
- 31 Boldt AB, Luz PR, Messias-Reason IJ. MASP2 haplotypes are associated with high risk of cardiomyopathy in chronic Chagas disease. *Clin Immunol* 2011;140(01):63–70
- 32 Boldt AB, Goeldner I, Stahlke ER, Thiel S, Jensenius JC, de Messias-Reason IJ. Leprosy association with low MASP-2 levels generated by MASP2 haplotypes and polymorphisms flanking MAP19 exon 5. *PLoS One* 2013;8(07):e69054
- 33 Munye MM, Diaz-Font A, Ocaka L, et al. COLEC10 is mutated in 3MC patients and regulates early craniofacial development. *PLoS Genet* 2017;13(03):e1006679
- 34 Rooryck C, Diaz-Font A, Osborn DP, et al. Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. *Nat Genet* 2011;43(03):197–203
- 35 Sirmaci A, Walsh T, Akay H, et al. MASP1 mutations in patients with facial, umbilical, coccygeal, and auditory findings of Carnevale, Malpuech, OSA, and Michels syndromes. *Am J Hum Genet* 2010;87(05):679–686
- 36 Jacobsen S, Garred P, Madsen HO, et al. Mannose-binding lectin gene polymorphisms are associated with disease activity and physical disability in untreated, anti-cyclic citrullinated peptide-positive patients with early rheumatoid arthritis. *J Rheumatol* 2009;36(04):731–735
- 37 Troelsen LN, Garred P, Jacobsen S. Mortality and predictors of mortality in rheumatoid arthritis—a role for mannose-binding lectin? *J Rheumatol* 2010;37(03):536–543
- 38 Ammitzboll CG, Thiel S, Ellingsen T, et al. Levels of lectin pathway proteins in plasma and synovial fluid of rheumatoid arthritis and osteoarthritis. *Rheumatol Int* 2012;32(05):1457–1463
- 39 Epp Boschmann S, Goeldner I, Tuon FF, Schiel W, Aoyama F, de Messias-Reason IJ. Mannose-binding lectin polymorphisms and rheumatoid arthritis: a short review and meta-analysis. *Mol Immunol* 2016;69:77–85
- 40 Trolldborg A, Thiel S, Laska MJ, Deleuran B, Jensenius JC, Stengaard-Pedersen K. Levels in plasma of the serine proteases and associated proteins of the lectin pathway are altered in patients with systemic lupus erythematosus. *J Rheumatol* 2015;42(06): 948–951
- 41 Hein E, Nielsen LA, Nielsen CT, et al. Ficolins and the lectin pathway of complement in patients with systemic lupus erythematosus. *Mol Immunol* 2015;63(02):209–214
- 42 Jenny L, Ajjan R, King R, Thiel S, Schroeder V. Plasma levels of mannan-binding lectin-associated serine proteases MASP-1 and MASP-2 are elevated in type 1 diabetes and correlate with glycaemic control. *Clin Exp Immunol* 2015;180(02):227–232
- 43 Axelgaard E, Østergaard JA, Thiel S, Hansen TK. Diabetes is associated with increased autoreactivity of mannan-binding lectin. *J Diabetes Res* 2017;2017:6368780
- 44 Best LG, Davidson M, North KE, et al. Prospective analysis of mannose-binding lectin genotypes and coronary artery disease in American Indians: the Strong Heart Study. *Circulation* 2004; 109(04):471–475
- 45 Hertle E, Arts IC, van der Kallen CJ, et al. Distinct longitudinal associations of MBL, MASP-1, MASP-2, MASP-3, and MASP4 with endothelial dysfunction and intima-media thickness: the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) Study. *Arterioscler Thromb Vasc Biol* 2016;36(06):1278–1285
- 46 Ytting H, Christensen IJ, Thiel S, Jensenius JC, Nielsen HJ. Pre- and postoperative levels in serum of mannan-binding lectin associated serine protease-2 -a prognostic marker in colorectal cancer. *Hum Immunol* 2008;69(07):414–420
- 47 Storm L, Christensen IJ, Jensenius JC, Nielsen HJ, Thiel S; Danish Study Group on Early Detection of Colorectal Cancer. Evaluation of complement proteins as screening markers for colorectal cancer. *Cancer Immunol Immunother* 2015;64(01):41–50
- 48 Swierzko AS, Szala A, Sawicki S, et al. Mannose-binding lectin (MBL) and MBL-associated serine protease-2 (MASP-2) in women with malignant and benign ovarian tumours. *Cancer Immunol Immunother* 2014;63(11):1129–1140
- 49 Ambrus G, Gál P, Kojima M, et al. Natural substrates and inhibitors of mannan-binding lectin-associated serine protease-1 and -2: a study on recombinant catalytic fragments. *J Immunol* 2003;170(03):1374–1382
- 50 Presanis JS, Hajela K, Ambrus G, Gál P, Sim RB. Differential substrate and inhibitor profiles for human MASP-1 and MASP-2. *Mol Immunol* 2004;40(13):921–929
- 51 Dobó J, Harmat V, Beinrohr L, Sebestyén E, Závodszy P, Gál P. MASP-1, a promiscuous complement protease: structure of its catalytic region reveals the basis of its broad specificity. *J Immunol* 2009;183(02):1207–1214
- 52 Krarup A, Gulla KC, Gál P, Hajela K, Sim RB. The action of MBL-associated serine protease 1 (MASP1) on factor XIII and fibrinogen. *Biochim Biophys Acta* 2008;1784(09):1294–1300
- 53 Hess K, Ajjan R, Phoenix F, Dobó J, Gál P, Schroeder V. Effects of MASP-1 of the complement system on activation of coagulation factors and plasma clot formation. *PLoS One* 2012;7(04):e35690

- 54 Hajela K, Kojima M, Ambrus G, et al. The biological functions of MBL-associated serine proteases (MASPs). *Immunobiology* 2002;205(4-5):467-475
- 55 Gulla KC, Gupta K, Krarup A, et al. Activation of mannan-binding lectin-associated serine proteases leads to generation of a fibrin clot. *Immunology* 2010;129(04):482-495
- 56 Jenny L, Dobó J, Gál P, Schroeder V. MASP-1 induced clotting—the first model of prothrombin activation by MASP-1. *PLoS One* 2015;10(12):e0144633
- 57 Jenny L, Dobó J, Gál P, Schroeder V. MASP-1 of the complement system promotes clotting via prothrombin activation. *Mol Immunol* 2015;65(02):398-405
- 58 Jenny L, Dobó J, Gál P, Pál G, Lam WA, Schroeder V. MASP-1 of the complement system enhances clot formation in a microvascular whole blood flow model. *PLoS One* 2018;13(01):e0191292
- 59 Krarup A, Wallis R, Presanis JS, Gál P, Sim RB. Simultaneous activation of complement and coagulation by MBL-associated serine protease 2. *PLoS One* 2007;2(07):e623
- 60 Endo Y, Nakazawa N, Iwaki D, Takahashi M, Matsushita M, Fujita T. Interactions of ficolin and mannose-binding lectin with fibrinogen/fibrin augment the lectin complement pathway. *J Innate Immun* 2010;2(01):33-42
- 61 Kozarcanin H, Lood C, Munthe-Fog L, et al. The lectin complement pathway serine proteases (MASPs) represent a possible crossroad between the coagulation and complement systems in thromboinflammation. *J Thromb Haemost* 2016;14(03):531-545
- 62 Keizer MP, Pouw RB, Kamp AM, et al. TFPI inhibits lectin pathway of complement activation by direct interaction with MASP-2. *Eur J Immunol* 2015;45(02):544-550
- 63 AMSTAR-Assessing the Methodological Quality of Systematic Reviews; 2015. Available at: <https://amstar.ca/>. Accessed August 29, 2017
- 64 Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies; 2014. Available at: <https://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cardiovascular-risk-reduction/tools/cohort>. Accessed May 1, 2017
- 65 Quality Assessment of Case-Control Studies; 2014. Available at: <https://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cardiovascular-risk-reduction/tools/case-control>
- 66 Poppelaars F, Gaya da Costa M, Berger SP, et al. Strong predictive value of mannose-binding lectin levels for cardiovascular risk of hemodialysis patients. *J Transl Med* 2016;14(01):236
- 67 Siezenga MA, Shaw PK, Daha MR, Rabelink TJ, Berger SP. Low mannose-binding lectin (MBL) genotype is associated with future cardiovascular events in type 2 diabetic South Asians. A prospective cohort study. *Cardiovasc Diabetol* 2011;10:60
- 68 Troelsen LN, Garred P, Madsen HO, Jacobsen S. Genetically determined high serum levels of mannose-binding lectin and agalactosyl IgG are associated with ischemic heart disease in rheumatoid arthritis. *Arthritis Rheum* 2007;56(01):21-29
- 69 Berger SP, Roos A, Mallat MJ, et al. Low pretransplantation mannose-binding lectin levels predict superior patient and graft survival after simultaneous pancreas-kidney transplantation. *J Am Soc Nephrol* 2007;18(08):2416-2422
- 70 Keller TT, van Leuven SI, Meuwese MC, et al. Serum levels of mannose-binding lectin and the risk of future coronary artery disease in apparently healthy men and women. *Arterioscler Thromb Vasc Biol* 2006;26(10):2345-2350
- 71 Saevarsdottir S, Oskarsson OO, Aspelund T, et al. Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. *J Exp Med* 2005;201(01):117-125
- 72 Vengen IT, Madsen HO, Garred P, Platou C, Vatten L, Videm V. Mannose-binding lectin deficiency is associated with myocardial infarction: the HUNT2 study in Norway. *PLoS One* 2012;7(07):e42113
- 73 Vengen IT, Enger TB, Videm V, Garred P. Pentraxin 3, ficolin-2 and lectin pathway associated serine protease MASP-3 as early predictors of myocardial infarction - the HUNT2 study. *Sci Rep* 2017;7:43045
- 74 Pesonen E, Hallman M, Sarna S, et al. Mannose-binding lectin as a risk factor for acute coronary syndromes. *Ann Med* 2009;41(08):591-598
- 75 Hansen TK, Tarnow L, Thiel S, et al. Association between mannose-binding lectin and vascular complications in type 1 diabetes. *Diabetes* 2004;53(06):1570-1576
- 76 Zhang M, Hou YJ, Cavusoglu E, et al. MASP-2 activation is involved in ischemia-related necrotic myocardial injury in humans. *Int J Cardiol* 2013;166(02):499-504
- 77 Mellbin LG, Hamsten A, Malmberg K, et al. Mannose-binding lectin genotype and phenotype in patients with type 2 diabetes and myocardial infarction: a report from the DIGAMI 2 trial. *Diabetes Care* 2010;33(11):2451-2456
- 78 Trendelenburg M, Theroux P, Stebbins A, Granger C, Armstrong P, Pfisterer M. Influence of functional deficiency of complement mannose-binding lectin on outcome of patients with acute ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Eur Heart J* 2010;31(10):1181-1187
- 79 Haahr-Pedersen S, Bjerre M, Flyvbjerg A, et al. Level of complement activity predicts cardiac dysfunction after acute myocardial infarction treated with primary percutaneous coronary intervention. *J Invasive Cardiol* 2009;21(01):13-19
- 80 Collard CD, Shernan SK, Fox AA, et al. The MBL2 'LYQA secretor' haplotype is an independent predictor of postoperative myocardial infarction in whites undergoing coronary artery bypass graft surgery. *Circulation* 2007;116(11, Suppl):I106-I112
- 81 Ueland T, Espevik T, Kjekshus J, et al. Mannose binding lectin and soluble Toll-like receptor 2 in heart failure following acute myocardial infarction. *J Card Fail* 2006;12(08):659-663
- 82 Limnell V, Aittoniemi J, Vaarala O, et al. Association of mannan-binding lectin deficiency with venous bypass graft occlusions in patients with coronary heart disease. *Cardiology* 2002;98(03):123-126
- 83 Schoos MM, Munthe-Fog L, Skjoed MO, et al. Association between lectin complement pathway initiators, C-reactive protein and left ventricular remodeling in myocardial infarction—a magnetic resonance study. *Mol Immunol* 2013;54(3-4):408-414
- 84 Holt CB, Thiel S, Munk K, Østergaard JA, Bøtker HE, Hansen TK. Association between endogenous complement inhibitor and myocardial salvage in patients with myocardial infarction. *Eur Heart J Acute Cardiovasc Care* 2014;3(01):3-9
- 85 Mellbin LG, Bjerre M, Thiel S, Hansen TK. Complement activation and prognosis in patients with type 2 diabetes and myocardial infarction: a report from the DIGAMI 2 trial. *Diabetes Care* 2012;35(04):911-917
- 86 Kouchaki E, Babamohammadi A, Nikouejad H, Sehat M. Association of serum levels of pentraxin-3, M-ficolin, and surfactant protein A with the severity of ischemic stroke. *Iran J Allergy Asthma Immunol* 2017;16(02):140-146
- 87 Huang J, Ma X, Li Q, et al. Serum mannose-binding lectin levels in patients with ischemic stroke. *Int J Clin Exp Med* 2016;9(08):16332-16338
- 88 Zangari R, Zanier ER, Torgano G, et al; LEPAS group. Early ficolin-1 is a sensitive prognostic marker for functional outcome in ischemic stroke. *J Neuroinflammation* 2016;13:16
- 89 Song FY, Wu MH, Zhu LH, Zhang ZQ, Qi QD, Lou CL. Elevated serum mannose-binding lectin levels are associated with poor outcome after acute ischemic stroke in patients with type 2 diabetes. *Mol Neurobiol* 2015;52(03):1330-1340
- 90 Zhang ZG, Wang C, Wang J, et al. Prognostic value of mannose-binding lectin: 90-day outcome in patients with acute ischemic stroke. *Mol Neurobiol* 2015;51(01):230-239
- 91 Wang ZY, Sun ZR, Zhang LM. The relationship between serum mannose-binding lectin levels and acute ischemic stroke risk. *Neurochem Res* 2014;39(02):248-253



- 92 Füst G, Munthe-Fog L, Illes Z, et al. Low ficolin-3 levels in early follow-up serum samples are associated with the severity and unfavorable outcome of acute ischemic stroke. *J Neuroinflammation* 2011;8:185
- 93 Osthoff M, Katan M, Fluri F, et al. Mannose-binding lectin deficiency is associated with smaller infarction size and favorable outcome in ischemic stroke patients. *PLoS One* 2011;6(06):e21338
- 94 Cervera A, Planas AM, Justicia C, et al. Genetically-defined deficiency of mannose-binding lectin is associated with protection after experimental stroke in mice and outcome in human stroke. *PLoS One* 2010;5(02):e8433
- 95 Frauenknecht V, Thiel S, Storm L, et al. Plasma levels of mannan-binding lectin (MBL)-associated serine proteases (MASPs) and MBL-associated protein in cardio- and cerebrovascular diseases. *Clin Exp Immunol* 2013;173(01):112–120
- 96 Jönsen A, Gullstrand B, Güner N, et al. Genetically determined mannan-binding lectin deficiency is of minor importance in determining susceptibility to severe infections and vascular organ damage in systemic lupus erythematosus. *Lupus* 2007;16(04):245–253
- 97 Font J, Ramos-Casals M, Brito-Zerón P, et al. Association of mannose-binding lectin gene polymorphisms with antiphospholipid syndrome, cardiovascular disease and chronic damage in patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 2007;46(01):76–80
- 98 Calvo-Alén J, Alarcón GS, Tew MB, et al; LUMINA Study Group. Systemic lupus erythematosus in a multiethnic US cohort: XXXIV. Deficient mannose-binding lectin exon 1 polymorphisms are associated with cerebrovascular but not with other arterial thrombotic events. *Arthritis Rheum* 2006;54(06):1940–1945
- 99 Øhlenschlaeger T, Garred P, Madsen HO, Jacobsen S. Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. *N Engl J Med* 2004;351(03):260–267
- 100 Lv W, Wang L, Duan Q, et al. Characteristics of the complement system gene expression deficiency in patients with symptomatic pulmonary embolism. *Thromb Res* 2013;132(01):e54–e57
- 101 Merlen C, Bonnefoy A, Wagner E, et al. L-Asparaginase lowers plasma antithrombin and mannan-binding-lectin levels: impact on thrombotic and infectious events in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer* 2015;62(08):1381–1387
- 102 Takahashi K, Ohtani K, Larvie M, et al. Elevated plasma CL-K1 level is associated with a risk of developing disseminated intravascular coagulation (DIC). *J Thromb Thrombolysis* 2014;38(03):331–338
- 103 Sprong T, Mollnes TE, Neeleman C, et al. Mannose-binding lectin is a critical factor in systemic complement activation during meningococcal septic shock. *Clin Infect Dis* 2009;49(09):1380–1386
- 104 Zhao X, Chen YX, Li CS. Predictive value of the complement system for sepsis-induced disseminated intravascular coagulation in septic patients in emergency department. *J Crit Care* 2015;30(02):290–295
- 105 Pavlov VI, Skjoedt MO, Siow Tan Y, Rosbjerg A, Garred P, Stahl GL. Endogenous and natural complement inhibitor attenuates myocardial injury and arterial thrombogenesis. *Circulation* 2012;126(18):2227–2235
- 106 Takahashi K, Chang WC, Takahashi M, et al. Mannose-binding lectin and its associated proteases (MASPs) mediate coagulation and its deficiency is a risk factor in developing complications from infection, including disseminated intravascular coagulation. *Immunobiology* 2011;216(1-2):96–102
- 107 de la Rosa X, Cervera A, Kristoffersen AK, et al. Mannose-binding lectin promotes local microvascular thrombosis after transient brain ischemia in mice. *Stroke* 2014;45(05):1453–1459
- 108 Fumagalli S, De Simoni MG. Lectin complement pathway and its bloody interactions in brain ischemia. *Stroke* 2016;47(12):3067–3073
- 109 Jordan JE, Montalto MC, Stahl GL. Inhibition of mannose-binding lectin reduces postischemic myocardial reperfusion injury. *Circulation* 2001;104(12):1413–1418
- 110 Hart ML, Ceonzo KA, Shaffer LA, et al. Gastrointestinal ischemia-reperfusion injury is lectin complement pathway dependent without involving C1q. *J Immunol* 2005;174(10):6373–6380
- 111 Busche MN, Pavlov V, Takahashi K, Stahl GL. Myocardial ischemia and reperfusion injury is dependent on both IgM and mannose-binding lectin. *Am J Physiol Heart Circ Physiol* 2009;297(05):H1853–H1859
- 112 Ducruet AF, Sosunov SA, Zacharia BE, et al. The neuroprotective effect of genetic mannose-binding lectin deficiency is not sustained in the sub-acute phase of stroke. *Transl Stroke Res* 2011;2(04):588–599
- 113 Orsini F, Villa P, Parrella S, et al. Targeting mannose-binding lectin confers long-lasting protection with a surprisingly wide therapeutic window in cerebral ischemia. *Circulation* 2012;126(12):1484–1494
- 114 Orsini F, Chrysanthou E, Dudler T, et al. Mannan binding lectin-associated serine protease-2 (MASP-2) critically contributes to post-ischemic brain injury independent of MASP-1. *J Neuroinflammation* 2016;13(01):213
- 115 Walsh MC, Bourcier T, Takahashi K, et al. Mannose-binding lectin is a regulator of inflammation that accompanies myocardial ischemia and reperfusion injury. *J Immunol* 2005;175(01):541–546
- 116 La Bonte LR, Dokken B, Davis-Gorman G, Stahl GL, McDonagh PF. The mannose-binding lectin pathway is a significant contributor to reperfusion injury in the type 2 diabetic heart. *Diab Vasc Dis Res* 2009;6(03):172–180
- 117 Schwaeble WJ, Lynch NJ, Clark JE, et al. Targeting of mannan-binding lectin-associated serine protease-2 confers protection from myocardial and gastrointestinal ischemia/reperfusion injury. *Proc Natl Acad Sci U S A* 2011;108(18):7523–7528
- 118 Pant S, Deshmukh A, Gurumurthy GS, et al. Inflammation and atherosclerosis—revisited. *J Cardiovasc Pharmacol Ther* 2014;19(02):170–178
- 119 Viola J, Soehnlein O. Atherosclerosis - a matter of unresolved inflammation. *Semin Immunol* 2015;27(03):184–193
- 120 Langer HF, Gawaz M. Platelet-vessel wall interactions in atherosclerotic disease. *Thromb Haemost* 2008;99(03):480–486
- 121 Lisman T. Platelet-neutrophil interactions as drivers of inflammatory and thrombotic disease. *Cell Tissue Res* 2018;371(03):567–576
- 122 Pilely K, Rosbjerg A, Genster N, et al. Cholesterol crystals activate the lectin complement pathway via ficolin-2 and mannose-binding lectin: implications for the progression of atherosclerosis. *J Immunol* 2016;196(12):5064–5074
- 123 Vazquez-Garza E, Jerjes-Sanchez C, Navarrete A, Joya-Harrison J, Rodriguez D. Venous thromboembolism: thrombosis, inflammation, and immunothrombosis for clinicians. *J Thromb Thrombolysis* 2017;44(03):377–385
- 124 Levi M, Schultz M, van der Poll T. Sepsis and thrombosis. *Semin Thromb Hemost* 2013;39(05):559–566
- 125 Takase S, Schmid-Schönbein G, Bergan JJ. Leukocyte activation in patients with venous insufficiency. *J Vasc Surg* 1999;30(01):148–156
- 126 Takase S, Bergan JJ, Schmid-Schönbein G. Expression of adhesion molecules and cytokines on saphenous veins in chronic venous insufficiency. *Ann Vasc Surg* 2000;14(05):427–435
- 127 Ekdahl KN, Teramura Y, Hamad OA, et al. Dangerous liaisons: complement, coagulation, and kallikrein/kinin cross-talk act as a linchpin in the events leading to thromboinflammation. *Immunol Rev* 2016;274(01):245–269
- 128 Gabriel DA, Muga K, Boothroyd EM. The effect of fibrin structure on fibrinolysis. *J Biol Chem* 1992;267(34):24259–24263

- 129 Litzman J, Freiberger T, Grimbacher B, et al. Mannose-binding lectin gene polymorphic variants predispose to the development of bronchopulmonary complications but have no influence on other clinical and laboratory symptoms or signs of common variable immunodeficiency. *Clin Exp Immunol* 2008;153(03):324–330
- 130 Luo J, Xu F, Lu GJ, Lin HC, Feng ZC. Low mannose-binding lectin (MBL) levels and MBL genetic polymorphisms associated with the risk of neonatal sepsis: an updated meta-analysis. *Early Hum Dev* 2014;90(10):557–564
- 131 Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. *Lancet* 2001;358(9282):637–638
- 132 Mullighan CG, Heatley S, Doherty K, et al. Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation. *Blood* 2002;99(10):3524–3529
- 133 Garcia-Laorden MI, Sole-Violan J, Rodriguez de Castro F, et al. Mannose-binding lectin and mannose-binding lectin-associated serine protease 2 in susceptibility, severity, and outcome of pneumonia in adults. *J Allergy Clin Immunol* 2008;122(02):368–374, 374.e1–374.e2
- 134 Kruse C, Rosgaard A, Steffensen R, Varming K, Jensenius JC, Christiansen OB. Low serum level of mannan-binding lectin is a determinant for pregnancy outcome in women with recurrent spontaneous abortion. *Am J Obstet Gynecol* 2002;187(05):1313–1320
- 135 Christiansen OB, Kilpatrick DC, Souter V, Varming K, Thiel S, Jensenius JC. Mannan-binding lectin deficiency is associated with unexplained recurrent miscarriage. *Scand J Immunol* 1999;49(02):193–196
- 136 Kilpatrick DC, Bevan BH, Liston WA. Association between mannan binding protein deficiency and recurrent miscarriage. *Hum Reprod* 1995;10(09):2501–2505
- 137 Munthe-Fog L, Hummelshøj T, Honoré C, et al. Variation in FCN1 affects biosynthesis of ficolin-1 and is associated with outcome of systemic inflammation. *Genes Immun* 2012;13(07):515–522
- 138 Ammitzbøll CG, Kjær TR, Steffensen R, et al. Non-synonymous polymorphisms in the FCN1 gene determine ligand-binding ability and serum levels of M-ficolin. *PLoS One* 2012;7(11):e50585
- 139 Boldt AB, Sanchez MI, Stahlke ER, et al. Susceptibility to leprosy is associated with M-ficolin polymorphisms. *J Clin Immunol* 2013;33(01):210–219
- 140 Munthe-Fog L, Hummelshøj T, Hansen BE, et al. The impact of FCN2 polymorphisms and haplotypes on the Ficolin-2 serum levels. *Scand J Immunol* 2007;65(04):383–392
- 141 Bayarri-Olmos R, Hansen S, Henriksen ML, et al. Genetic variation of COLEC10 and COLEC11 and association with serum levels of collectin liver 1 (CL-L1) and collectin kidney 1 (CL-K1). *PLoS One* 2015;10(02):e0114883
- 142 Antony JS, Ojuronbe O, Krensner PG, Velavan TP. Lectin complement protein Collectin 11 (CL-K1) and susceptibility to urinary schistosomiasis. *PLoS Negl Trop Dis* 2015;9(03):e0003647
- 143 Troegeler A, Lugo-Villarino G, Hansen S, et al. Collectin CL-LK is a novel soluble pattern recognition receptor for mycobacterium tuberculosis. *PLoS One* 2015;10(07):e0132692
- 144 Beltrame MH, Boldt AB, Catarino SJ, et al. MBL-associated serine proteases (MASPs) and infectious diseases. *Mol Immunol* 2015;67(01):85–100
- 145 Stengaard-Pedersen K, Thiel S, Gadjeva M, et al. Inherited deficiency of mannan-binding lectin-associated serine protease 2. *N Engl J Med* 2003;349(06):554–560
- 146 Sokolowska A, Szala A, St Swierzko A, et al. Mannan-binding lectin-associated serine protease-2 (MASP-2) deficiency in two patients with pulmonary tuberculosis and one healthy control. *Cell Mol Immunol* 2015;12(01):119–121
- 147 García-Laorden MI, García-Saavedra A, de Castro FR, et al. Low clinical penetrance of mannose-binding lectin-associated serine protease 2 deficiency. *J Allergy Clin Immunol* 2006;118(06):1383–1386
- 148 Skjødt M-O, Munthe-Fog L, Hansen KM, Garred P. Inhibition of the coagulation system by the complement regulator MBL/Ficolin-Associated Protein-1 (MAP-1). *Molecular Immunology* 2014;61(02):219