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## **Lectin pathway proteins of the complement system in normotensive pregnancy and preeclampsia**

Running title: The lectin pathway in pregnancy and preeclampsia

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**Abbreviations** CL-L1, collectin liver 1; GA, gestational age (weeks); IUGR, intrauterine growth restriction; IQR, interquartile range; MAP19/MAP44, MBL-associated protein of 19/44 kDa; MASP, MBL-associated serine protease; MBL, mannose-binding lectin.

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## **Abstract**

**Problem:** The lectin pathway of the complement system may be involved in the pathogenesis of preeclampsia. We aimed to investigate changes in serum concentrations of a broad range of lectin pathway proteins during normal pregnancy and their association with preeclampsia, placental infarctions and intrauterine growth restriction (IUGR).

**Method of study:** We included 51 women with normotensive pregnancies and 54 women with pregnancies complicated by preeclampsia. Blood samples were obtained at gestational weeks 16, 33, 37, and after delivery for the normotensive pregnant women and before and after delivery for women with preeclampsia. Mannose-binding lectin (MBL), H- and M-ficolin, collectin liver-1 (CL-L1), MBL-associated serine proteases (MASPs)-1, -2 and -3 and MBL-associated proteins of 19 (MAP19) and 44 (MAP44) kDa were analysed. Clinical information was obtained from medical records. The placentae were examined by two experienced perinatal pathologists.

**Results:** Lectin pathway protein concentrations generally increased during normal pregnancy and decreased after delivery in both normotensive pregnant women and women with preeclampsia. Exceptions were MASP-3 which increased after delivery in both groups ( $p<0.0001$ ) and H-ficolin which increased after delivery in preeclampsia ( $p<0.0001$ ). H-ficolin ( $p<0.0001$ ), M-ficolin ( $p=0.005$ ), and MASP-3 ( $p=0.03$ ) concentrations were lower in women with preeclampsia than in normotensive pregnant women. Low MASP-3 concentrations were associated with placental infarction ( $p=0.03$ ) and IUGR ( $p=0.04$ ). Low H-ficolin concentrations were associated with IUGR ( $p<0.01$ ).

**Conclusions:** In general, lectin pathway protein serum concentrations increased during normal pregnancy. H-ficolin and MASP-3 may be involved in the pathophysiology of preeclampsia and IUGR and could be potential future preeclampsia biomarkers.

**Keywords (MeSH):** “Complement Pathway, Mannose-Binding Lectin”; “Ficolin”; “Mannose-Binding Protein-Associated Serine Proteases”; “Pre-Eclampsia”; “Intrauterine Growth Restriction”

## Introduction

Preeclampsia affects approximately 5% of all pregnancies worldwide<sup>1</sup> and is a major cause of maternal and perinatal morbidity and mortality.<sup>2</sup> The condition is characterized by new-onset hypertension after the 20<sup>th</sup> gestational week and proteinuria and is associated with potentially life-threatening maternal organ dysfunction as well as intrauterine growth restriction (IUGR) and preterm birth.<sup>3,4</sup> The cause of preeclampsia is debated, but an abnormal trophoblast invasion and impaired remodelling of uterine spiral arteries are considered the main pathogenetic factors.<sup>5</sup> Furthermore, preeclampsia is characterised by enhanced apoptotic<sup>6</sup> and inflammatory changes<sup>7</sup> in the placenta as well as excess placental infarction.<sup>8</sup>

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A dysregulated maternal immune response is regarded a key factor in the abnormal placental establishment in early pregnancy,<sup>9</sup> and the complement system has been suggested as an important contributor,<sup>10</sup> as signs of systemic increased complement activation in preeclampsia with increased classical and terminal pathway activity have been described.<sup>11</sup> The complement system is part of the innate immune system, providing a first line defence against infection. The system is also an intimate part of the recognition and clearing of altered-self structures, e.g. as may occur through pregnancies.<sup>12,13</sup> Complement activation generates inflammatory mediators which facilitate a crucial danger signal to surrounding cells and migrating leucocytes.<sup>14</sup> The lectin pathway, which is the most recently described complement activation pathway, has gained much interest in the development of preeclampsia and IUGR.<sup>15-21</sup> The lectin pathway consists of the pattern recognition molecules mannose-binding lectin (MBL), ficolins (H-, L- and M-) and collectins liver-1 (CL-L1) and kidney-1 (CL-K1), together with the MBL-associated serine proteases (MASPs)-1, -2 and -3 and the regulatory MBL-associated proteins of 19 kDa (MAp19) and 44 kDa (MAp44).<sup>22</sup> MBL may be directly involved in the impairment of spiral artery remodelling and trophoblast invasion in early pregnancy.<sup>15,16</sup> Furthermore, the pattern recognition molecules recognise not only pathogens but also necrotic or apoptotic cells.<sup>12,13</sup> Thus, pattern recognition and subsequent complement activation in the preeclampsia-affected placenta may contribute to inflammation and placental insufficiency later in pregnancy. Finally, MASP-1 and -2 enhance prothrombin activation, fibrin formation and clot stabilisation *in vitro*.<sup>23-25</sup> This may aggravate placental infarction and further impair placental function.

Increased circulating MBL concentrations have been reported for pregnant women with preeclampsia compared with normotensive pregnant women.<sup>17,18</sup> One study found an association between high MBL serum concentration and impaired placental perfusion.<sup>18</sup> Others found that *MBL2* genotypes normally causing high MBL serum concentrations were more prevalent in women with preeclampsia than in healthy pregnant women.<sup>19,20</sup> These findings indicate a protective effect of low serum-MBL on preeclampsia risk. However, this was contradicted by a study which found a higher prevalence of *MBL2* genotypes associated

with low MBL serum concentrations in preeclampsia than in normotensive pregnancy.<sup>26</sup>

Other studies reported no differences in MBL serum concentrations<sup>27-29</sup> or *MBL2* genotype distribution<sup>30</sup> between women with preeclampsia and normotensive pregnant women.

Concerning other lectin pathway proteins, two studies reported lower serum concentrations of H- and L-ficolin in preeclampsia than in normotensive pregnancy.<sup>21,27</sup> Finally, a recent study reported that several *MASP1* polymorphisms which may impair MASP-1 synthesis were associated with a higher risk of preeclampsia.<sup>31</sup> Serum concentrations of MASP-1, -2 or -3, M-ficolin or CL-L1/CL-K1 have not been investigated previously in women with preeclampsia. Thus, the association between MBL and preeclampsia is still not fully elucidated, while the role of other lectin pathway proteins in preeclampsia is not well investigated.

The present study aimed to investigate changes in serum concentrations of lectin pathway proteins during normal pregnancy and their possible association with the development of preeclampsia, placental infarctions and IUGR.

## **Materials and methods**

### **Design and study population**

The present study included two groups of pregnant women; a) normotensive pregnant women and b) pregnant women with preeclampsia. The women were prospectively enrolled at the Departments of Gynaecology and Obstetrics, Hillerød Hospital and Glostrup Hospital, Denmark, from January 2004 to August 2006 as part of a previous study.<sup>32</sup> Normotensive pregnant women were enrolled at their routine antenatal care visit at gestational age (GA) 16-17 weeks and followed during pregnancy and after delivery. Consecutive blood samples were obtained at GA 16, 33 and 37 ( $\pm 1$  week) and 1 month after delivery. A total of 580 women were enrolled in the original cohort, with serum available from 51 normotensive, healthy pregnant women for the present study. Pregnant women with suspected preeclampsia were enrolled from the obstetric department when referred for acute or

subacute assessment. Only women with a confirmed diagnosis of preeclampsia were included in the final cohort. Blood samples were obtained at inclusion (median GA 35+4) and after delivery (see Table 1). Sixty-one women were included, with serum available from 54 women for the present study.

All participants gave written informed consent to be included in the original study, which was carried out in accordance with the Helsinki Declaration and approved by the Committee on Biomedical Research Ethics of the Capital Region of Denmark (record no: H-Ø-2005-2-04) and the Danish Data Protection Agency (record no: 2010-41-5119). All health care data were fully anonymised for the present study.

Lectin pathway protein concentrations in healthy, non-pregnant female blood donors (n=149), enrolled from the blood bank at the Department of Clinical Immunology, Aarhus University Hospital, Denmark, have previously been described by our group.<sup>33</sup>

### **Clinical outcomes**

Danish preeclampsia patients are diagnosed according to the criteria stated in the American College of Obstetricians and Gynecologists' task force report on hypertension in pregnancy.<sup>3</sup>

1) blood pressure  $\geq 140$  mmHg systolic and/or  $\geq 90$  mmHg diastolic measured on at least two separate occasions after GA 20 and 2) proteinuria  $>300$  mg/24 h or protein/creatinine ratio  $>0.3$ . Severe preeclampsia was defined as a blood pressure  $\geq 160$  mmHg systolic and/or  $\geq 110$  mmHg diastolic on two separate occasions or one or more symptoms or laboratory findings related to renal, hepatic, pulmonary or cerebral dysfunction or the presence of HELLP (haemolysis, elevated liver enzymes and low platelet count).<sup>3</sup> IUGR was defined as foetal weight  $>2$  standard deviations (equal to -22%) below mean weight for GA using Marsal's growth charts as reference.<sup>34</sup>



## **Placental pathology**

Placentae obtained from study participants were fixated in 10% formalin immediately after delivery and transported to the Department of Pathology, Hillerød Hospital, Denmark.

Placental examination was performed by one of two experienced perinatal pathologists affiliated to the project. Both were blinded to the clinical outcomes. Infarction of up to 5% of the placental parenchyma is considered a normal finding in full-term healthy pregnancies;<sup>4</sup> therefore we defined  $\geq 10\%$  infarction of the total placental parenchyma as clinically significant.

Data on placental fibrin deposition and inflammation were available, but since the number of events were low (fibrin deposition,  $n=3$ ; inflammation,  $n=4$ ), statistical analysis was not performed for these variables.

## **Lectin pathway protein analysis**

Blood samples were drawn in 5 ml serum tubes (Venosafe Z Serum Clot Activator, Terumo Europe, Belgium) and centrifuged at 2,773 g for 20 minutes at 4°C. Serum was stored at -80°C within two hours of sampling. Lectin pathway protein concentrations were analysed with time-resolved immunofluorometric assays at the Department of Biomedicine, Aarhus University, Denmark between November 2017 and May 2018. The analyses were performed as previously described<sup>35-40</sup> except for a few changes in the H-ficolin and MASP-1 assays. Briefly, serum was thawed, diluted in assay buffers and added to microtiter wells (FluoroNunc, Thermo Scientific<sup>TM</sup>, Hvidovre, Denmark) coated with relevant capture antibodies, mannan (for MBL assay) or acetylated bovine serum albumin (for H-ficolin assay). Standards, samples and controls were added automatically to plates using a Janus® Varispan automated work station (PerkinElmer, Waltham, MA, USA). In-house biotinylated antibodies, europium-labelled streptavidin (PerkinElmer, Waltham, MA, USA) and enhancement solution (Ampliqon, Odense, Denmark) were added in successive steps with triple washing in between. The europium fluorescence intensity was detected with a Victor X5® fluorometer (PerkinElmer, Waltham, MA, USA).

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MASP-1 was analysed as previously described by our group,<sup>33</sup> except that plates were coated with murine anti-MASP-1 antibodies (clone 1H1G6, Genscript, lot no A217100014) instead of the previously used antibody. For H-ficolin, acetylated bovine serum albumin was used as the capturing agent as described by Hein et al.<sup>41</sup> The wells were coated with 10 µg/ml acetylated bovine serum albumin (Sigma® B2518) diluted in coating buffer (0.1 M sodium bicarbonate, pH 9.6 with 0.09% (w/v) sodium azide). Standards, controls and samples were diluted in tris-buffered saline with 0.05% Tween-20, 5 mM CaCl<sub>2</sub> and 1 mg/ml human serum albumin, and in-house biotinylated mouse anti-human H-ficolin (clone 4H5, HyCult® Biotechnology cat# HM2089b) was used as detecting antibody as previously described by our group.<sup>42</sup>

Each microtiter plate contained three quality controls. All standards, controls and samples were added in duplicate, and a coefficient of variation of 15% was accepted. The person performing the analysis (JBL) was blinded to placental pathology and IUGR status at the time of analysis.

## Statistics

Data were visually assessed for Gaussian distribution using quantile-quantile plots, and logarithmic transformation was used if the original data did not follow a Gaussian distribution. Since a Gaussian distribution could not be achieved for all lectin pathway proteins, protein concentrations were generally displayed as medians with interquartile ranges (IQR). Differences in lectin pathway protein concentrations between groups were analysed with unpaired t-test if data followed a Gaussian distribution, otherwise with Mann-Whitney test. Changes observed during pregnancy within the normotensive pregnancy group were analysed using repeated measures analysis of variance or Friedman's test. Differences in protein concentrations before and after delivery within groups were analysed with paired t-test if data followed a Gaussian distribution, otherwise with Wilcoxon's matched pairs signed rank test. Since this was an exploratory study analysing multiple lectin pathway proteins, and

since the size of the cohort was determined in beforehand, a sample size calculation was not performed.

All statistical analyses were performed with Stata® 14 (StataCorp, Texas, USA), and graphs were prepared using Prism 7.0 (GraphPad, La Jolla, CA, USA).

## Results

### Study population

In total, serum samples were available from 51 normotensive pregnant women, of which 37 had a post-delivery sample. For women with preeclampsia, serum samples were available for 54 women, of which 51 had a post-delivery sample. Demographic and clinical characteristics are shown in Table 1.

### Lectin pathway protein serum concentrations

Lectin pathway protein concentrations in normotensive pregnant women and women with preeclampsia at all sampling times are displayed in Supplementary Table 1. Lectin pathway protein concentrations (IQR) in healthy non-pregnant women are displayed as dotted lines in Figures 1 and 2.

### *Changes during and after pregnancy*

#### *Normotensive pregnant women*

All lectin pathway proteins except M-ficolin and MASP-3 increased significantly during pregnancy in the normotensive group (Figure 1). The median increase from GA 16 to GA 37 ranged from 11% (IQR: 0-23%) for CL-L1 to 39% (IQR: 27-51%) for MAp19 (all  $p < 0.0001$ ). For H-ficolin, the increase occurred primarily from GA 16 to GA 33 (median increase 19% (IQR: 11-25%),  $p < 0.0001$ ) with a drop from GA 33 to GA 37. A decrease from GA 37 to after delivery was observed for almost all proteins, ranging from 15% (IQR: 1-24%) for H-ficolin to

54% (IQR: 48%-76%) for MBL (all  $p<0.001$ ). An exception was MASP-3, which displayed a 30% (IQR: 8-44%) increase from GA 37 to after delivery ( $p<0.001$ ).

#### *Women with preeclampsia*

In the preeclampsia group (Figure 2), a similar pattern was observed as for the normotensive group with significant decreases in most protein concentrations after delivery, ranging from 12% (IQR: -21-31%) for M-ficolin to 58% (IQR: 48-62%) for MASP-1. Exceptions were H-ficolin and MASP-3 which showed a median increase after delivery of 30% (IQR: 15-65%) for H-ficolin and 53% (IQR: 34-81%) for MASP-3, both  $p<0.0001$ . MASP-2 did not change significantly ( $p=0.22$ ).

We also examined the influence of preeclampsia severity on the lectin pathway and observed no difference in lectin pathway protein concentrations between women with mild or moderate preeclampsia ( $n=11$ ) and women with severe preeclampsia or HELLP ( $n=43$ ) ( $p>0.16$  for all proteins).

A proportion of the women with preeclampsia received betamethasone (Celeston®) for foetal lung maturation (43%). We observed no difference in lectin pathway protein levels between women who received betamethasone ( $n=23$ ) and women who did not ( $n=31$ ) (all  $p>0.15$ ), except for M-ficolin which was numerically but not significantly lower in women who received betamethasone than women who did not (median 3,373 (IQR: 2,659-5,014) ng/ml vs 4,116 (3,372-4,978) ng/ml,  $p=0.10$ ), and MASP-3 which was lower in women who received betamethasone than in women who did not (4,349 (3,803-5,120) vs 5,252 (4,467-6,206),  $p=0.03$ ).

#### ***Women with preeclampsia versus normotensive pregnant women***

H-ficolin, M-ficolin and MASP-3 concentrations were significantly lower in women with preeclampsia than in normotensive pregnant women in late pregnancy, as displayed in Figure 2. CL-L1 and MASP-1 concentrations were higher in women with preeclampsia than

in normotensive pregnant women. MBL, MAP19, MAP44 and MASP-2 concentrations did not differ between women with preeclampsia and normotensive women (all p-values>0.40).

### **Association between lectin pathway proteins and placental infarction and IUGR**

Lectin pathway protein concentrations in women with and without placental infarction and with and without IUGR are shown in Figures 3 and 4. We observed significantly lower MASP-3 concentrations in women with  $\geq 10\%$  placental infarctions (n=12) than in women with no or  $<10\%$  placental infarctions (n=80) (median 4,447 (IQR: 4103-5392) ng/ml vs 5,116 (IQR: 4,343-5,990) ng/ml, p=0.03). Likewise, MASP-3 concentrations were lower in women with IUGR (n=21) than in women without IUGR (n=84) (median: 4,545 (IQR: 4,110-5,143) ng/ml vs 5,312 (IQR: 4,392-6,244) ng/ml, p=0.04). We also observed lower H-ficolin concentrations in women with IUGR than in women without IUGR (median 19,504 (IQR: 17,968-22,134) ng/ml vs 23,912 (IQR: 20,338-29,440) ng/ml, p=0.009). When examined in women with preeclampsia only, we still found that women with  $\geq 10\%$  placental infarctions (n=11) had lower MASP-3 than women with no or  $<10\%$  infarction (n=42) (median 4,349 (IQR: 4,014-5,143) ng/ml vs 5,006 (IQR: 4,110-5,920) ng/ml, p=0.05). Women with preeclampsia and IUGR (n=17) had numerically but not statistically significantly lower MASP-3 and H-ficolin than women with preeclampsia without IUGR (n=37) (MASP-3, median 4,349 (IQR: 4,014-5,120) ng/ml vs 5,007 (IQR: 4,373-5,846) ng/ml, p=0.11; H-ficolin, 18,821 (IQR: 17,820-22,046) ng/ml vs 20,635 (17,793-22,481) ng/ml, p=0.22). No significant differences were found for the other lectin pathway proteins.

### **Discussion**

We showed that lectin pathway protein concentrations increased during pregnancy and decreased after delivery. Exceptions were MASP-3, which increased after delivery in both normotensive pregnant women and women with preeclampsia, and H-ficolin, which increased after delivery in women with preeclampsia. H-ficolin, M-ficolin and MASP-3

concentrations were lower in women with preeclampsia than in normotensive pregnant women. Low MASP-3 concentrations were associated with excess placental infarction and IUGR, and low H-ficolin concentrations were associated with IUGR.

Our results demonstrate that lectin pathway protein concentrations generally increase during normal pregnancy and decrease after delivery, to a level similar to healthy non-pregnant women.<sup>33</sup> An exception was MASP-3 which increased after delivery. Our results are supported by van de Geijn et al who found an approximate increase of 50% in serum MBL during normal pregnancy and a decline again six weeks after delivery.<sup>43</sup> Consecutive serum concentrations of MASP-1, -2 and -3 and MAp19 and -44 during pregnancy have not been described previously. We were not able to follow women with preeclampsia at several time points during pregnancy, but we found a similar pattern of higher concentrations in late pregnancy and a decline after delivery, except for MASP-3 and H-ficolin, which were low in late pregnancy and increased after delivery. Our findings support an upregulation of innate immunity during pregnancy, with a return towards pre-pregnancy conditions when the maternal circulation is no longer exposed to the placenta.<sup>43</sup>

We observed lower concentrations of H-ficolin, M-ficolin and MASP-3 in women with preeclampsia than in normotensive pregnant women. This supports previous findings of lower H-ficolin levels in pregnancies complicated by preeclampsia as compared with normotensive pregnancies.<sup>21,27</sup> Yet, M-ficolin and MASP-3 have not been investigated previously in pregnancies complicated by preeclampsia, whereas MBL is extensively investigated in preeclampsia.<sup>17-20,28,29</sup> Some previous studies described increased MBL concentrations in preeclampsia compared with normotensive pregnancy<sup>17,18</sup> while others found no difference.<sup>28,29</sup> Our results support the latter. These discrepant findings may be due to differences in the employed preeclampsia criteria, the laboratory assays used or the statistical power across studies.

Excess placental infarction and IUGR were associated with lower MASP-3 and H-ficolin concentrations, both in the overall study population and in women with preeclampsia alone. This contrasts with the results of Halmos et al who found no association between serum H-

ficolin and IUGR in women with preeclampsia;<sup>21</sup> however, the number of women with IUGR in their study was low (n=11). No previous study investigated associations between MASP-3 concentrations and IUGR or placental infarction. Other authors reported an association between serum MBL or *MBL2* polymorphisms and IUGR;<sup>17,19,29</sup> Sziller et al found that the *MBL2* codon 54 polymorphism associated with lower MBL serum concentrations was associated with lower incidence of IUGR in women with preeclampsia,<sup>19</sup> and Celik et al reported a negative correlation between MBL serum concentrations and birth weight.<sup>17</sup> These findings suggest a protective effect of low serum MBL concentration on the risk of IUGR. By contrast, Csuka et al found no difference in MBL-MASP activity between women with preeclampsia with and without IUGR;<sup>29</sup> however, their study included few IUGR events (n=11). We chose a relatively strict definition of IUGR (2 standard deviations below mean weight for GA). This definition was chosen since it better reflects an increased risk of adverse perinatal outcomes<sup>44-47</sup> and reflects the clinical cut-off for intensified antenatal surveillance of pregnancies in Denmark, and thus it was deemed more clinically relevant in the context of the present study. The use of different IUGR definitions may explain different findings between the present study and the work of others.

Lower concentrations of lectin pathway proteins may reflect decreased synthesis or increased consumption. The liver is the main synthesis site for the majority of lectin pathway proteins, with the exception of M-ficolin which is synthesised mainly in leukocytes<sup>48</sup> and MASP-3 which is synthesised throughout the body.<sup>49</sup> Lower concentrations of lectin pathway proteins may thus be explained by impaired liver function in women with preeclampsia; however, it would be expected that all liver-synthesised lectin pathway proteins were lower in preeclampsia than in normotensive pregnancy, which was not the case in our study. Thus, impaired synthesis cannot fully explain our findings. Wang et al described low serum H- and L-ficolin concentrations coupled with evidence of increased placental deposition of H- and L-ficolin in women with preeclampsia.<sup>27</sup> Decreased serum complement factor 4 (C4)<sup>50</sup> and increased C4d deposition in placentae from women with preeclampsia<sup>51,52</sup> has also been described. These findings indicate that increased placental complement activation

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contributes to preeclampsia, as argued by Buurma et al.<sup>51</sup> Interestingly, MASP-3 is emerging as an activator of the alternative complement pathway,<sup>53,54</sup> and Hoffman et al found evidence of increased alternative pathway activation in preeclampsia compared with normotensive pregnancy.<sup>55</sup> We found low H-ficolin and MASP-3 to be associated with preeclampsia but also with signs of placental dysfunction such as placental infarction and IUGR. These results could be interpreted as a sign of increased placental H-ficolin and MASP-3 deposition in women with preeclampsia, placental infarction and IUGR, in accordance with the findings of Wang et al.<sup>27</sup> However, we do not have data on placental H-ficolin or MASP-3 deposition to support this interpretation. This could be a focus point for future studies. Other perspectives could be the use of H-ficolin or MASP-3 as early predictive or prognostic markers for preeclampsia and related placental dysfunction. These lectin pathway proteins could have advantages over e.g. MBL as biomarkers, since their interindividual variation is lower,<sup>33,56</sup> and MASP-3 shows a low diurnal variation.<sup>33</sup> However, H-ficolin shows some diurnal variation<sup>33</sup> and is subjective to changes due to infection and inflammatory conditions,<sup>57</sup> which limits its clinical usefulness.

A strength of the present study was the longitudinal sample collection which allowed us to assess lectin pathway proteins both during pregnancy and after delivery in a well-described study population. We assessed a broad range of lectin pathway proteins, some of which have not previously been investigated in normal pregnancy or in preeclampsia. We ensured a high validity of the preeclampsia diagnosis through verification by two independent obstetricians. Moreover, experienced perinatal pathologists performed the placental pathology investigations. However, some limitations should be considered. Two women with preeclampsia had gestational diabetes mellitus, which could influence lectin pathway protein serum concentrations, as described for non-pregnant diabetic subjects.<sup>58,59</sup> However, since this was a low proportion of the entire cohort, we assumed that it would not influence our results. Four women received low molecular weight heparin during pregnancy, but we have previously shown that low molecular weight heparin in prophylactic doses does not influence lectin pathway protein plasma concentrations.<sup>60</sup> GA was similar but not identical in the



normotensive group and the preeclampsia group, and we cannot exclude a possible influence on our results. Additionally, for the preeclampsia group, the timing of blood sample collection after delivery varied widely. We cannot exclude that glucocorticoid treatment could have influenced our results. The effect of glucocorticoid treatment on MASP-3 plasma levels is not well investigated; one study found an upregulating effect of dexamethasone on the *MASP1/3* gene *in vitro*.<sup>61</sup> The time from blood sampling to analysis was long. Nevertheless, lectin pathway proteins are stable in serum for several years at -80°C (unpublished results from our laboratory), but we cannot rule out that some evaporation may have occurred, possibly resulting in higher absolute protein concentrations. However, this increased storage time was non-differential across the groups; thus, we consider it unlikely to have influenced the main conclusion of our findings. Differences in lectin pathway proteins between early- and late onset preeclampsia could have been of interest; however, the women were classified according to preeclampsia severity and not onset time; thus it was not possible to investigate this issue. Finally, our sample size was moderate; thus, our study may underestimate associations between placental infarction or IUGR and lectin pathway proteins.

In conclusion, we found that H-ficolin and MASP-3 serum concentrations were lower in the 3<sup>rd</sup> trimester than after delivery and were lower in women with preeclampsia than in normotensive pregnant women in late pregnancy, in contrast to other lectin pathway proteins. Furthermore, low MASP-3 concentrations were associated with both placental infarction and IUGR, and low H-ficolin concentrations were associated with IUGR. Our results indicate a possible involvement of MASP-3 and H-ficolin in the pathogenesis of preeclampsia and related placental dysfunction.

### **Author contributions**

JBL, ASA, CLH, ST, MRL, AMH and ATH contributed to the study design. ASA collected blood samples and clinical data. JBL performed laboratory and statistical analyses, prepared tables and figures and drafted the manuscript. ASA, CLH, ST, MRL, AMH and AT contributed to critical revision of the manuscript.

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## Tables

**Table 1. Clinical and demographic characteristics on normotensive pregnant women and women with preeclampsia.**

	Normotensive pregnant women (n=51)	Preeclampsia (n=54)
<b>Age, years</b>	32.6 ± 3.8	31.6 ± 5.9
<b>GA at birth, weeks</b>	40+3 (39+2-41+3)	36+2 (33+6-38+0)
<b>Blood sampling before delivery</b>		
-GA, weeks	-	35+4 (33+0-37+5)
-days before delivery		2 (1-6)
<b>Blood sampling after delivery</b>	-	
-months after delivery		4.4 (0.25-8)
<b>Parity = 0</b>	26 (51)	39 (72)
<b>Method of birth</b>		
-vaginal	38 (75)	17 (31)
-sectio	13 (25)	37 (69)
<b>Preeclampsia severity</b>		
-mild/moderate	-	11 (20)
-severe		39 (72)
-HELLP		4 (7)
<b>Gestational diabetes mellitus</b>	0	2
<b>LMWH prophylaxis<sup>†</sup></b>	0	4
<b>Betamethasone before birth</b>	0 (0)	23 (43)
<b>Preeclampsia in previous pregnancy</b>		
-no	24 (47)	3 (6)
-yes	1 (2)	12 (22)
-not relevant (parity = 0)	26 (51)	39 (72)
<b>Symptomatic VTE in current pregnancy<sup>‡</sup></b>	0 (0)	0 (0)
<b>Symptomatic VTE in previous pregnancy</b>	0 (0)	1 (2)

<b>IUGR</b>	4 (8)	17 (31)
<b>Placental infarction <math>\geq 10\%</math></b>		
<b>-no</b>	38 (74)	42 (78)
<b>-yes</b>	1 (2)	11 (20)
<b>-data not available</b>	12 (24)	1 (2)

Data are presented as either mean  $\pm$  standard deviation, median (interquartile range) or n (%). Abbreviations: GA, gestational age (weeks); HELLP, haemolysis, elevated liver enzymes, low platelets; IUGR, intrauterine growth restriction; LMWH, low molecular weight heparin; VTE, venous thromboembolism. <sup>†</sup>Indication for LMWH prophylaxis: known thrombophilia, n=2, other indications (immobilisation, previous obstetric complication), n=2. <sup>\*</sup>Data on venous thromboembolism were obtained from medical records. Participants were followed until 6 weeks after delivery.

## Figure legends

### Figure 1

Figure 1. Lectin pathway protein concentration in normotensive pregnant women at gestational week 16, 33 and 37 (n=51) and 1 month after delivery (n=37).

Median with interquartile range is displayed. Dotted lines: Lectin pathway protein concentrations in healthy non-pregnant women (n=149), interquartile range. <sup>a</sup>Friedman's test, <sup>b</sup>Repeated measures analysis of variance with Geisser-Greenhouse correction, <sup>c</sup>Wilcoxon's matched-pairs signed-rank test, <sup>d</sup>Paired t-test. Abbreviations: CL-L1, collectin liver 1; GA, gestational age (weeks); MAp19/-44, MBL-associated protein of 19/44 kDa; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; p.p., post partum (after delivery)

### Figure 2

Figure 2. Lectin pathway protein concentrations in women with preeclampsia at gestational week 36 and after delivery (n=54) and in normotensive pregnant women at gestational week 37 (n=51).

Median with interquartile range is displayed. Dotted lines: Lectin pathway protein concentrations in healthy non-pregnant women (n=149), interquartile range. <sup>a</sup>Mann-Whitney test, <sup>b</sup>Unpaired t-test with/without Welch's correction, <sup>c</sup>Wilcoxon's matched-pairs signed-rank test, <sup>d</sup>Paired t-test. Abbreviations: CL-L1, collectin liver 1; GA, gestational age (weeks); MAp19/-44, MBL-associated protein of 19/44 kDa; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; NP, normotensive pregnant women; PE, women with preeclampsia.

### Figure 3

Figure 3. Lectin pathway protein concentrations in women with placental infarction  $\geq 10\%$  (n=12) and no placental infarction or infarction  $< 10\%$  (n=80).

Median with interquartile range is displayed. <sup>a</sup>Mann-Whitney test, <sup>b</sup>Unpaired t-test with/without Welch's correction. Abbreviations: CL-L1, collectin liver 1; MAp19/-44, MBL-associated protein of 19/44 kDa; MASP, MBL-associated serine protease; MBL, mannose-binding lectin.

### Figure 4

Figure 4. Lectin pathway protein concentrations in women with (n=21) and without (n=84) intrauterine growth restriction.

Median with interquartile range is displayed. <sup>a</sup>Mann-Whitney test, <sup>b</sup>Unpaired t-test with/without Welch's correction. Abbreviations: CL-L1, collectin liver 1; MAp19/-44, MBL-associated protein of 19/44 kDa; MASP, MBL-associated serine protease; MBL, mannose-binding lectin.









