Elevated complement C3 and C4 levels are associated with postnatal pregnancy related venous thrombosis

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Running head: Complement and pregnancy related venous thrombosis

Summary table

What is known on this topic	What this paper ads
• High levels of complement C3 is associated with venous thrombosis in the general population • Laboratory studies have shown considerable cross-talk between the complement and coagulation systems	• Levels of C3 and C4 are associated with several coagulation factors and inhibitors in fertile women, partly explained by an association with C-reactive protein • High levels of C3 and C4 are associated to postnatal, but not antenatal, venous thrombosis • The association with postnatal venous thrombosis is partly explained by high BMI and high factor IX

Abstract

High levels of complement C3 are associated with venous thrombosis (VT) in the general population. We investigated if high C3 and C4 levels were associated with pregnancy related VT. We undertook the Norwegian VIP study, a case-control study of VT in pregnancy or within 3 months postpartum (cases, n=313) and women without pregnancy related VT (controls, n=353). Determinants of C3 and C4 in the control women were investigated with linear regression and the odds ratio (OR) for pregnancy related VT was calculated with logistic regression. We found that levels of C3 and C4 were associated with body mass index (BMI), C-reactive protein (CRP); with the coagulation factors (F) fibrinogen, FVIII, and FIX; and with the coagulation inhibitors antithrombin, protein C, protein S, and tissue factor pathway inhibitor. These associations were influenced by CRP levels. The crude OR for pregnancy related VT was 1.8 (95% confidence interval (CI) 1.1-3.0) for C3 above the 90th percentile and 2.0 (95% CI 1.2-3.2) for C4 above the 90th percentile. Stratification in antenatal and postnatal VT showed that C3 and C4 were only associated with postnatal VT with an OR for high C3 of 3.0 (95% CI 1.8-5.0), and for high C4 of 2.6 (95% CI 1.5-4.6). Adjustment for high factor IX and BMI reduced the odds ratios. We conclude that the association between postnatal VT and C3 and C4 suggests that there is clinically relevant cross-talk between the complement and the coagulation system.

Keywords: Venous Thromboembolism, Pregnancy, Complement System Proteins, Blood coagulation, Case-Control Studies

Background

The incidence of venous thrombosis (VT) is about 1-2/1000 person-years (1), and is much more common among the elderly. Pregnancy is a major risk factors for VT in young women and is associated with an approximately 5-fold increased risk of VT during pregnancy and a 60-fold increased risk during the first 3 months after delivery (2, 3). Both clinical and genetic risk factors contribute to the development of pregnancy-related VT but the importance of each risk factor differs between ante- and postnatal VT (4, 5), suggesting that there might be different mechanisms causing ante- and postnatal VT.

The complement system belongs to the first line of the innate immune system. It consists of a network of proteinases activating each other, much like the coagulation system. The interplay between coagulation and complement, frequently termed thromboinflammation, is evident in systemic infections where complement activation is linked to disseminated intravascular coagulation, and in some rare diseases like atypical hemolytic uremic syndrome (aHUS) or paroxysmal nocturnal hemoglobinuria (PNH). In the latter two the anti-C5 antibody eculizumab is an important treatment option for complement-mediated inflammation, but with beneficial effects also on the related thrombotic complications (6, 7). In the antiphospholipid syndrome (APS), complement is also assumed to play an important role (8), and there are reports that eculizumab in catastrophic APS can be lifesaving (9). The complement system may play a role for the development of VT also in individuals without APS, and several mechanisms for how the complement system may initiate the thrombotic process have been proposed (10).

Many laboratory studies indicate that proteases and protease inhibitors, that traditionally are considered to be part of the complement system, also have effects in the coagulation system, and vice versa. For example, mannose binding lectin associated serine protease 1 and 2 may activate prothrombin, fibrinogen and factor (F) XIII (11). Furthermore, an association between

high classical pathway activity and low MBL and VT has been suggested (12). Others have shown that thrombin, FIXa, FXa, FXIa, and plasmin may activate the complement system (13-15), although this hypothesis was recently challenged (16). Several complement components and activation products, including C3, C4, factor B and C5a, are found in thrombi (17). C3 binds to fibrin, enhancing clot stability and mice lacking C3 have a prolonged bleeding time and decreased platelet aggregation after vascular injury (18). There is also evidence from mice models that C3 plays a role in platelet activation and C5 in fibrin formation (19).

With the exception of APS, PNH and aHUS, there are almost no data demonstrating a link between VT and the complement system in humans. Recently, a Danish study found that high levels of complement C3 increases the risk for VT in the general population (20). The Danish study also showed that smoking, C-reactive protein (CRP) and body mass index (BMI) were associated with the levels of C3 in blood, as others have shown previously (21, 22).

In the current study we investigated if high levels of complement C3 and C4 were associated with pregnancy-related VT.

Materials and Methods

Patient population

Details on the selection of cases and controls have been reported (23), see also **Table 1** for a flowchart. The cases were identified from a source population comprising 377155 women with 613232 pregnancies. We searched the Norwegian Patient Registry for selected ICD-9 and -10 codes to identify women with a diagnosis of VT in pregnancy or within three months postpartum at 18 Norwegian hospitals during 1990-2003. We included only women who had completed 23 weeks of gestation. The diagnosis of VT was validated through review of medical

records [4]. A total of 559 cases were identified. As controls the Norwegian Medical Birth Registry selected four women who gave birth at Oslo University Hospital Ullevål at the same time as a case. We utilized the two first women selected from the registry as controls, but if their medical records were not retrievable, we included the third or fourth selection. A total of 1229 women were identified as controls. After exclusion of cases and controls who were foreign, had emigrated, or were dead, the remaining cases (n=531) and controls (n=1092) were invited to donate a blood sample and to answer a detailed questionnaire. The final study population of women who agreed to donate a blood sample comprised 313 cases and 353 controls naive of VT prior to index pregnancy. For the current study C3 and C4 were analyzed in 313 cases and 352 controls. Information about BMI was registered early in pregnancy.

The study was approved by the eastern Norway Regional Committee for Medical and Health Research Ethics. All attending women signed a written consent.

Blood sampling

Venous blood samples were collected from fasting women at a single time point during 2006, median eight years (range 3–16 years) after **index** pregnancy, in 5 mL Vacutainer vacuum tubes (Becton-Dickinson, Plymouth, UK) containing 0.5 mL buffered sodium citrate (0.129 M), and in 4.9 mL Monovette tubes (Sarstedt AG, Nümbrecht, Germany) containing potassium-ethylene-diamine-tetra-acetic acid (EDTA). Citrated blood was kept at room temperature and centrifuged at 2000 g for 15 min within 1 h. Platelet-poor plasma aliquots and EDTA-blood were stored at -70 °C until assayed. Serum was made by collecting blood samples into Vacutainer gel tubes, allowing them to clot for at least 30 minutes, then centrifuged at 2500 g for 15 min at 22 °C before being aliquoted.

At the time of blood sampling self-reported morbidity was low, and none of the women had hematologic disease, active cancer, thyroid disorder, or inflammatory bowel disease. Anti-

allergy drugs were used in approximately 10% of both cases and controls. Less than 5% of the cases and the controls used other daily medications, such as antihypertensives, insulin, or antidepressants (24) .

Assays

Complement C3 and C4 were measured in serum with the instrument Behring Nephelometer II (BN II) by immunonephelometry at the Department of Immunology and Transfusion medicine at Oslo University Hospital using antisera to Human Complement C3c and C4 (Siemens Healthcare Diagnostics, Deerfield, IL, USA) as directed by the manufacturer. The laboratory reference intervals for C3 and C4 were 0.67-1.29 g/l and 0.13-0.32 g/l, respectively.

Fibrinogen, FVIII and FIX, antithrombin and protein C activities, and free protein S antigen were analysed in citrated blood using commercial reagents or kits from Instrumentation Laboratory (Lexington, MA, USA). All assays were run on an ACL Advanced TOP automated coagulometer (Instrumentation Laboratory). Free tissue factor pathway inhibitor (TFPI) antigen was analysed using commercial enzyme-linked immunosorbent assay kits (Asserachrom® FREE TFPI) from Diagnostica Stago, Asnières, France as described earlier (25). The assays were performed at the Haematological Research Laboratory at the Department of Haematology, Oslo University Hospital.

High sensitivity CRP in serum was measured by a latex-enhanced immunoturbidimetric assay (Tina-quant Cardiac C-reactive Protein High Sensitive, Roche Diagnostics GmbH, Mannheim, Germany) using the Roche/Hitachi Modular P-analyser. All analyses were performed examiner blind, and the samples were run in batch using a balanced set-up with equal number of cases and controls in each run.

Statistics

The statistical analyses were performed in two parts. First, the determinants of C3 and C4 were investigated in the control population of women, since they represent the general population of healthy women giving birth. The aim of these analyses was to find factors that could possibly confound the analyses of C3 and C4 as risk factors for pregnancy-related VT. Analyses of determinants of C3 and C4 were done by t-tests, simple scatterplots, univariate and multivariate linear regression in the control women. We report the standardized regression coefficient beta. The interpretation of a standardized regression coefficient beta of, e.g. 0.5, is that an increase of 1 standard deviation of the independent variable (x-axis) is associated with a mean increase of 0.5 standard deviation of the dependent variable (y-axis). For the multiple linear regression analyses, the variables were entered as continuous variables. Secondly, we calculated if pregnancy-related VT was associated with C3, C4 or the possible confounding factors found in the first part. The risk for VT was calculated as odds ratios (OR) with 95% confidence intervals (CI) using logistic regression. Adjusted ORs were calculated with multiple logistic regression. The variables were dichotomized so that levels of C3, C4, CRP, fibrinogen, FVIII, and FIX above the 90^{th} percentile in the controls were defined as "high levels", levels of antithrombin, protein S, protein C and free TFPI below the 10^{th} percentile in the controls were defined as "low levels", and BMI above 25 kg/m^2 was defined as high BMI. For the multiple logistic regression analyses, the variables were entered as dichotomized variables. IBM® SPSS® Statistics version 24 was used for all calculations.

For the analyses of coagulation factors and inhibitors, women who were pregnant at the time of blood sampling (10 cases and 13 controls), used oral contraceptives (5 cases and 25 controls) or anticoagulation (20 cases, no controls) were excluded, since these conditions are known to influence several coagulation parameters (26). Variables that were not normally distributed were log-transformed, these variables were fibrinogen, FIX, protein C, free TFPI, and CRP.

Results

Determinants of C3 and C4 in healthy control women

Pregnant women at blood sampling had slightly higher C3 than non-pregnant women (mean C3 in pregnant women 1.21 g/L vs 1.03 g/L in non-pregnant, $p < 0.01$), whereas there were no differences for C4. Use of combined oral contraceptives at blood sampling did not influence the levels of C3 or C4. The levels of both C3 and C4 were associated with BMI (standardized regression coefficient 0.60 for C3 and 0.35 for C4, **Table 2**). Age at blood sampling was only weakly associated with C3 (-0.14), and not associated with C4 (-0.047) (**Table 2**).

We further investigated if C3 or C4 were associated with CRP; the coagulation factors fibrinogen, FVIII or FIX; or with the coagulation inhibitors antithrombin, protein C, protein S, or free TFPI. Both C3 and C4 were associated with all the investigated factors. Generally, the association was stronger with C3 than with C4. Both C3 and C4 had a stronger association with CRP, fibrinogen, factor IX, protein C, and free TFPI, than with the other variables investigated (**Table 2** and **Supplementary Fig. S1**).

Next, to investigate acute phase reaction as a possible cause of the associations, we adjusted the standardized regression coefficients for levels of CRP. We found that the association with both C3 and C4 was weakened for BMI, fibrinogen, factor VIII, factor IX, protein C and free TFPI, but significant associations still remained (**Table 2**). We then added the factors in a multiple regression model to find which variables that best explained the variation in C3 and C4. A model with C3 as the dependent variable, containing CRP, BMI, fibrinogen, and protein C as independent variables resulted in an R^2 of 0.64 as compared with R^2 of 0.45 with CRP alone. Increasing the number of independent variables did not increase the R^2 above 0.68. For multiple linear regression with C4 as the dependent variable, a model with CRP, protein C and fibrinogen as independent variables resulted in an R^2 of 0.29. Adding more variables did not increase the

R^2 above 0.32. In comparison, a model with CRP as the only independent variable had an R^2 of 0.24 (**Table 3**).

High levels of C3 and C4 and the risk of pregnancy related VT

The crude OR for pregnancy-related VT was 1.8 (95% CI 1.1-3.0) for C3 above the 90th percentile and 2.0 (95% CI 1.2-3.2) for C4 above the 90th percentile. After exclusion of women pregnant at blood draw, the odds ratio was 2.1 (95% CI 1.2-3.5) for C3 above the 90th percentile and 1.9 (95% CI 1.2-3.2) for C4 above the 90th percentile. Further stratification in ante- and postnatal VT showed that the OR of C3 above the 90th percentile for antenatal VT was 0.87 (95% CI 0.43 to 1.7), and for C4 1.4 (95% CI 0.73-2.6). For postnatal VT, the OR for C3 above the 90th percentile was 3.0 (95% CI 1.8-5.0), and for C4 2.6 (95% CI 1.5-4.6) (**Table 4**). Exclusion of pregnant women at blood draw did not materially change the ORs for antenatal or postnatal VT.

Since CRP and BMI, fibrinogen, FVIII and FIX, and antithrombin, protein C, protein S, and free TFPI, were all associated with C3 and C4, we investigated if these variables also influenced the risk of postnatal VT. We found that CRP above the 90th percentile, BMI above 25 kg/m^2, protein S below the 10th percentile, and fibrinogen, FVIII and FIX above the 90th percentiles increased the risk for postnatal VT. FVIII and BMI also increased the risk for antenatal VT. Antithrombin, protein C and TFPI below the 10th percentile were not associated with VT (**Table 5**).

We then investigated if any of the variables associated with both C3 and C4 and with postnatal VT could explain the association of postnatal VT with C3 and C4. Thus, we adjusted the ORs found for high C3 and high C4 for each of these possible confounders, but this only marginally changed the ORs. We also adjusted for women with two or more positive tests for antiphospholipid antibodies, women with postnatal infection, women with preeclampsia and

women with more than 1000 mL postnatal blood loss, but none of these adjustments changed the ORs (**Table 6**).

Finally, we did multiple logistic regression with the variables associated with C3 and C4 and with postnatal VT (BMI, CRP, protein S, fibrinogen, factor VIII, factor IX). We found that a model with factor IX above the 90th percentile and BMI above 25 explained some of the risk of postnatal VT associated with high levels of C3 and C4. The OR for high C3 was in this model reduced from 3.0 to 1.5 (95% CI 0.69-3.4) and the OR for high C4 was reduced from 2.6 to 1.9 (95% CI 0.94-3.7).

Discussion

The current report shows that high levels of C3 and C4 were associated with postnatal, but not antenatal VT. Adjustment for other variables that were associated with postnatal VT and C3 and C4 did not change the risk estimates, although a multiple logistic regression model containing factor IX above the 90th percentile and BMI above 25 appeared to partly explain the risk associated with high levels of C3 and C4. In addition, we showed that the level of C3 and C4 in blood was associated with several coagulation factors and inhibitors, although some of this association is probably explained by active phase.

It is not obvious that high levels of C3 or C4 would increase the risk of VT. We do, however, know from the coagulation system that high levels of procoagulants, e.g., prothrombin, factor VIII, factor IX, factor XI increase thrombin generation in laboratory studies (27, 28) and also increase the risk for VT (29-32). A similar mechanism may be postulated for the complement system, i.e., high levels of the circulating inactive zymogens complement factors, such as C3 and C4, may increase the production of activated end products both under basal physiological turnover and in situations where the complement system is pathologically activated. If any of

the active proteinases in the complement system then can activate, or contribute to the activation, of the coagulation system, high levels of C3 and C4 may play a role.

A possible cause for the difference in risk between ante- and postnatal VT associated with C3 and C4 could be that postnatal infection increases the risk for postnatal VT, but adjustment for postnatal infection with multiple logistic regression did not change the ORs for postnatal VT. A more probable explanation is that tissue injury may lead to complement activation (33). A link between complement and coagulation in trauma patients could be the factor VII activating protease (FSAP). A study found a large increase in FSAP activity immediately after a trauma and that FSAP were able to activate C3 and C5 (34). In labor there is a natural blood loss of 200-500 ml in average caused by placental separation. Further hemorrhage is prevented by activated coagulation and contraction of the uterus. Thus, the trauma of the birth in itself may activate the coagulation system. Notably, blood loss per se, even without any trauma, may lead to complement activation (35), and if the complement system is able to activate parts of the coagulation system, one may speculate that this might be a link between complement and VT. In the acute situation this normally leads to decreased levels of C3, due both to consumption and dilution. The samples for C3 and C4 measurement were, were however obtained long time after delivery, and it could not be excluded that a higher C3 prior to delivery would lead to a more pronounced activation during delivery-mediated trauma and blood loss. Our study, like a previous study (36), showed that C3 were higher in women during pregnancy. Thus, one may speculate that women with already high levels of C3 before pregnancy get even higher levels during pregnancy, which again increase the risk for postnatal VT.

The risk of VT associated with high C3 and C4 was independent of blood loss above 1000 mL, although we know that increased blood loss is associated with postnatal VT in this study (4).

Thus, if the trauma of the birth is an eliciting factor for complement associated VT, large blood loss does not appear worse than normal blood loss.

One previous study, a large Danish general cohort study, has investigated the level of C3 as a risk factor for VT in the general population (20). We confirm the association between C3 and BMI/CRP/age reported by the Danish investigators. Our results do, however, suggest that high levels of C3 or C4 do not increase the risk for all types of VT, since we did not find any association with antenatal VT.

It was unexpected that C3 and C4 were associated with all investigated coagulation factors and inhibitors, in addition to BMI, pregnancy and CRP. There were, however, differences in the degree of association. BMI, CRP, protein C, free TFPI, fibrinogen, and FIX had a stronger association than the other variables. One possible explanation for the almost global association between complement C3/C4 and the coagulation variables is the "acute phase reaction", which influences the blood level of many proteins. This was supported by the strong association between CRP and both C3 and C4. When we adjusted the standardized regression coefficients for CRP we found that CRP partly influenced the associations of BMI, fibrinogen, factor VIII, factor IX, protein C, and free TFPI, but could not fully explain the associations. Complement C3, C4, CRP, fibrinogen, FVIII and protein S are all well-known acute phase reactants. Protein C, FIX and free TFPI are however not known as acute phase proteins. An additional explanation could be that low grade inflammation activates both the complement system and the coagulation system, resulting in increasing levels of factors from both systems (37). We are anyway not discussing acute phase as in a full inflammatory reaction in this study since the blood samples were taken long after the VT. We do not suggest that the birth or the VT have influenced the level of coagulation variables, CRP or complement, but the results may be consistent with a subclinical acute phase.

In a multiple linear regression model, the most important determinants of C3 were BMI, CRP, fibrinogen, and protein C. While the most important determinants for C4 were CRP, fibrinogen, and protein C. This also shows that blood levels of C3 and C4 are determined by factors related to inflammation as well as coagulation.

In this case control study the blood samples were obtained long after the index pregnancies. This was done to avoid that the pregnancy or the VT should influence the blood analyses. We then assume that the level of the factor measured after the index pregnancy is the same as before the pregnancy. It is, nevertheless, a weakness that we do not have samples from before the pregnancy. On the other hand, such a study would be difficult to conduct since it would require inclusion of several 100 000 of fertile women because only 1/1000 pregnancies result in VT(2). Some of the women were pregnant again when the blood samples were taken, and although the numbers were small, this gave us the opportunity to see how pregnancy influenced complement C3 and C4.

Although acute phase may explain some of the association between complement C3 and C4 and the coagulation variables, it does not seem to explain the risk for postnatal VT found for high levels of C3 and C4 since adjustment of CRP did not substantially change the odds ratios. In a multiple logistic regression analyses high levels of factor IX and BMI above 25 did, however, seem to explain some of the risk associated with C3 and C4. This suggests a connection between complement C3 and C4 and factor IX not previously described.

The current study investigated complement, coagulation and pregnancy related VT, and it shows an increased risk for postnatal VT associated with high levels of complement C3 and C4. Although the study is restricted to women with pregnancy-related VT, it would be interesting to test if these results also are valid for VT in the general population, e.g., trauma related VT. The study reveals many unexpected associations between coagulation factors and C3 and C4 where some can be explained by a subclinical acute phase, but others, like the association with

protein C and factor IX, we cannot explain. Furthermore, the levels of single complement components like C3 and C4 gives limited functional information on the degree of complement activation going on *in vivo*. Analysis of complement activation in these patients will add further to the understanding of a novel cross talk between complement and coagulation.

Addendum

AD did the statistics and wrote the first draft, AFJ collected the data and reviewed the manuscript, PMS designed the study and reviewed the manuscript. TEM contributed intellectually with complement knowledge and interpretation of data. , HSW, SMK and EMJ edited the manuscript. All authors critically revised the manuscript and approved the final version.

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Disclosure of Conflict of Interests

A. Dahm reports grants and personal fees from Pfizer AS, and personal fees from Bristol-Mayer Squibb, Novartis Norway AS, and Bayer outside the submitted work.

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Table 1 Flowchart of inclusions and exclusions in the VIP study.

Cases			**Controls**	
559			**1229**	
			Controls emigrated	38
5	Emigrated cases		Controls excluded	10
2	Foreign cases		Controls excluded	4
9	Dead cases		Controls excluded	18
			Controls dead	4
543		**Eligible for study participation**		**1155**
12		Invalid address		63
531		**Invited**		**1092**
215		Non-responders after two reminders		736
			No questionnaire	3
1	No blood sampling			
2	Previous VT (questionnaire data)			
313		**Final study population**		**353**
313		**Complement C3 and C4 analysed**		**352**

Table 2 Determinants of complement C3 and C4 in healthy controls.

Factor	**C3**		**C4**	
	Unadjusted	Adjusted for CRP	Unadjusted	Adjusted for CRP
Fibrinogen	**0.56**	0.25	**0.40**	0.18
Factor VIII	0.25	0.10	0.21	0.11
Factor IX	**0.56**	0.29	**0.37**	0.16
Antitrombin	0.19	0.18	0.18	0.17
Protein C	**0.49**	**0.31**	**0.33**	0.20
Protein S	0.21	0.16	0.13	0.10
Free TFPI	**0.40**	0.20	**0.30**	0.16
CRP	**0.67**	n.a.	**0.49**	n.a.
BMI	**0.60**	**0.40**	**0.35**	0.18
Age at blood sampling	-0.14	n.a.	-0.047	n.a.

Abbreviatons: n.a. denotes “not applicable”.

Note: Numbers are standardized regression coefficients. All the associations in the table are significant with $p < 0.05$ except for the age-C4 association. Bold indicates standardized regression coefficients above 0.30.

Table 3 Linear regression models with complement C3 and C4 as dependent variables.

Variables in the model	**Dependent variable**	**R^2**
BMI, CRP, fibrinogen, protein C	C3	0.64
CRP	C3	0.45
CRP, fibrinogen, protein C	C4	0.29
CRP	C4	0.24

Table 4 Odds ratios (ORs) for pregnancy related venous thrombosis (VT) for C3 and C4 above the 90th percentile.

	All VT	**Controls (n)**	**Cases (n)**	**OR (95% CI)**
		352	312	
C3	≤90th Percentile	321	265	1.8 (1.1-3.0)
	>90th Percentile	31	47	
C4	≤90th Percentile	323	265	2.0 (1.2-3.2)
	>90th Percentile	29	47	
		Controls (n)	**Cases (n)**	**OR (95% CI)**
	Antenatal VT	352	155	
C3	≤90th Percentile	321	143	0.87 (0.43-1.7)
	>90th Percentile	31	12	
C4	≤90th Percentile	323	138	1.4 (0.73-2.6)
	>90th Percentile	29	17	
		Controls (n)	**Cases (n)**	**OR (95% CI)**
	Postnatal VT	352	157	
C3	≤90th Percentile	321	122	3.0 (1.8-5.0)
	>90th Percentile	31	35	
C4	≤90th Percentile	323	127	2.6 (1.5-4.6)
	>90th Percentile	29	30	

Table 5 Odds ratios (OR) with 95% confidence interval (CI) for possible covariates for ante- and postnatal venous thrombosis (VT).

Variable	**Antenatal VT OR (95% CI)**	**Postnatal VT OR (95% CI)**
CRP > 90th percentile	1.0 (0.53-1.9)	**1.7** (1.0-3.0)
BMI > 25 kg/m^2	**1.8** (1.2-2.9)	**2.2** (1.4-3.6)
Factor IX > 90th percentile	1.1 (0.59-2.2)	**3.2** (1.9-5.5)
Factor VIII > 90th percentile	**1.9** (1.1-3.6)	**1.8** (1.0-3.2)
Fibrinogen > 90th percentile	0.93 (0.47-1.8)	1.7 (0.93-3.0)
Protein S < 10th percentile	1.3 (0.65-2.5)	**2.4** (1.3-4.3)
Free TFPI < 10th percentile	0.63 (0.30-1.3)	0.55 (0.25-1.2)
Antithrombin < 10th percentile	0.98 (0.50-1.9)	1.1 (0.60-2.1)
Protein C < 10th percentile	0.70 (0.33-1.5)	1.0 (0.52-1.9)

Note: Bold indicates ORs with confidence interval above 1.0.

Table 6 Adjusted odds ratios (OR) for high C3 and C4 for postnatal venous thrombosis (VT).

Adjusted for	**C3** **OR (95% CI)**	**C4** **OR (95% CI)**
No covariate	3.0 (1.8-5.0)	2.6 (1.5-4.6)
Pregnant women at blood sampling excluded	3.7 (2.1-6.4)	2.7 (1.5-4.7)
Women with two positive APS tests excluded	3.1 (1.8-5.2)	2.7 (1.6-4.7)
BMI > 25 kg/m^2	2.2 (1.1-4.6)	2.1 (1.1-4.0)
Postnatal infection	3.9 (2.2-7.1)	2.5 (1.4-4.5)
Blood loss > 1000 mL	3.3 (1.9-5.7)	2.8 (1.6-4.9)
Preeclampsia	2.7 (1.6-4.6)	2.4 (1.4-4.2)
CRP > 90^{th} percentile	3.5 (1.8-6.7)	2.1 (1.1-3.9)
Factor VIII > 90^{th} percentile	3.4 (1.9-6.1)	2.4 (1.3-4.3)
Factor IX > 90^{th} percentile	2.5 (1.3-4.8)	2.1 (1.1-3.8)
Fibrinogen > 90^{th} percentile	3.7 (2.0-6.9)	2.4 (1.3-4.4)
Protein S < 10^{th} percentile	3.3 (1.9-5.9)	2.5 (1.4-4.4)

Note: The table shows ORs adjusted for one covariate at the time.