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Angiogenic factors and the lectin pathway of complement in women with secondary recurrent pregnancy loss

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ABSTRACT

The poor remodeling of placental spiral arteries seen in preeclampsia is also discussed to contribute to recurrent pregnancy loss (RPL) preceded by abnormal angiogenesis and excessive complement activation. Low levels of Mannose-binding-lectin (MBL), a pattern recognition molecule (PRM) of the lectin pathway, have been found in women with RPL. We propose that pregnancy loss is connected to defective angiogenesis with reperfusion damage in the placenta and decreased levels of PRM in the lectin pathway in women with RPL. In this cohort study, we investigate the angiogenic factors and the lectin complement pathway in early pregnancy and their time-dependent relationship with pregnancy outcomes in 76 women with secondary RPL (sRPL) who have at least four prior pregnancy losses and a live birth. We evaluated levels of Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2), Vascular Endothelial Growth Factor (VEGF), soluble fms-like tyrosine kinase-1 (sFlt-1), and the PRMs, MBL, ficolin-1, −2, −3 and an additional soluble PRM, Pentraxin-3, during the 5th, 6th, and 7th gestational weeks. Our results showed that, compared to live births, pregnancies that ended in loss were associated with elevated VEGF levels and decreased levels of the Ang-2/Ang-1 ratio. Also, increasing levels of ficolin-2 were significantly associated with pregnancy loss, with MBL showing no association. Our research suggests that women with sRPL may have inadequate placentation with impaired angiogenesis in pregnancies ending in a loss.

1. Introduction

Placentation remains one of the critical factors in the establishment of a successful pregnancy. An altered placentation has been linked to obstetric complications, such as preeclampsia, placental abruption, intrauterine growth restriction, stillbirth, and post-partum bleeding (Brosens et al., 2019). Women who deliver their first child and later experience recurrent pregnancy losses (secondary recurrent pregnancy loss, sRPL) show a higher prevalence of these complications during their first pregnancy, indicating altered placentation in these patients (Nielsen, 2011; Nielsen et al., 2010; Svarre Nielsen et al., 2008). According to the European Society of Human Reproduction and Embryology

Abbreviations: RPL, Recurrent Pregnancy Loss; SRPL, secondary RPL; PRPL;, primary RPL; EVT;, extravillous trophoblast; Ang-1, Angiopoietin-1; Ang-2, Angiopoietin-2; VEGF, Vascular Endothelial Growth Factor; SFlt-1;, soluble fms-like tyrosine kinase-1; MBL;, Mannose-binding-lectin; PTX3;, Pentraxin 3.

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(ESHRE), Recurrent Pregnancy Loss (RPL) is defined as having two pregnancy losses and affects 1–3% of all women trying to conceive (Bender Atik et al., 2023; Practice Committee of the American Society for Reproductive Medicine, 2012).

A prerequisite of proper placentation is the correct decidualization of the endometrium, which involves a dramatic differentiation of endometrial stromal cells orchestrated by estrogen and progesterone (Okada et al., 2018). Implantation and placentation depend on a complex series of events, including vasculogenesis and angiogenesis, ultimately resulting in spiral artery remodeling with a loss of vascular smooth muscle and deposition of fibrinoid and extravillous trophoblasts (EVTs) enlarging the lumen. This ensures a high flow, low-resistance circulation, critical for a well-functioning placenta. Angiopoietin-1 (Ang-1) supports angiogenesis by stabilising the vascular wall and preventing leakage (Thurston et al., 2000). Ang-1 has also been shown to act as a chemotaxic agent for trophoblast cells (Dunk et al., 2000). During the first trimester, upregulation of Angiopoietin-2 (Ang-2) is reported in healthy pregnancies and may be involved in separating vascular smooth cells during spiral artery remodeling (Kappou et al., 2015; Woolnough et al., 2012). Also, in hypoxic situations, the angiogenic growth factors such as Vascular Endothelial Growth Factor (VEGF) are secreted by uterine NK-cells (uNK), which upregulates the secretion of Ang-2 by the placenta. VEGF, together with Ang-2, acts as a mitogen for trophoblast cells and stimulates the release of nitric oxide (Dunk et al., 2000; Hou et al., 2021). The placenta secretes soluble fms-like tyrosine kinase-1 (sFlt-1), which antagonises VEGF and is found in high concentrations in preeclamptic women in the third trimester (Clark et al., 1998; Maynard et al., 2003). Alterations in the early interplay of angiogenic factors may result in insufficient spiral artery remodeling of the placenta, which is believed to be involved in preeclampsia and intrauterine growth restriction (Brosens et al., 2019) and could play a role in pregnancy loss.

Aberrant complement activation has also been implicated in deranged placentation. The remodeling of spiral arteries and debris clearing depends on the complement system, an essential component of the innate immune system (Girardi et al., 2020). The complement system has three activating pathways: the classical, the alternative, and the lectin pathway. Ficolins and mannose-binding-lectin (MBL) are pattern recognition molecules (PRMs) that activate the lectin pathway. The ficolin family consists of three soluble molecules: ficolin-1 (M-ficolin), ficolin-2 (L-ficolin) and ficolin-3 (H-ficolin) that recognise carbohydrate molecules on pathogens, apoptotic, or necrotic cells, and thereby work both as opsonins and facilitators of the removal of dead cells (Garred et al., 2016). Pentraxin 3 (PTX3) is an acute-phase-reactant and activates the classical pathway and can form complexes with MBL and ficolins to increase complement activation (Ma and Garred, 2018).

We hypothesised that if the placentation is altered in women with sRPL, angiogenic factors and the lectin pathway could be changed in women with sRPL in early pregnancy ending in pregnancy loss compared to pregnancies ending in a live birth. Therefore, levels of PRMs of the lectin pathway and angiogenic factors were measured during early pregnancy in gestational weeks 5, 6 and 7 in sRPL patients to investigate the time-dependent variation in biomarkers in early pregnancy and the association with pregnancy outcome.

2. Materials and methods

Serum samples were taken consecutively during early pregnancy in 76 women with sRPL and a minimum of 4 pregnancy losses with at least three consecutive losses after the birth of a child. The women attended the Recurrent Pregnancy Loss Unit at Copenhagen University Hospital in Denmark and were between 25 and 41 years. They participated in a single-centre, double-blinded, placebo-controlled trial between August 2008 and April 2014 (Christiansen et al., 2015). The 82 women who participated in the trial were contacted again in 2016 and asked to participate in this study with their previous serum samples collected during the trial, and 77 gave their informed consent. One woman was excluded because she had an extrauterine pregnancy in the trial; in total, 76 women were included in the study.

In the previous trial, they were randomised between either infusion with immunoglobulin or placebo. They were block randomised 1:1 in two blocks according to having either four or $\geq$ 5 previous pregnancy losses. The active drug was "Immunoglobulin human CSL Behring®" 120 mg/ml or "Privigen®, CSL Behring" 100 mg/ml and the placebo infusions was "Human Albumin, CSL Behring", 5% infusion administered eight times between gestational week 5–16 weeks. If a pregnancy results in a pregnancy loss the patient will not receive the planned infusions. The patients were randomised if s-hCG was increasing sufficiently ($\geq$30%/24 hours) at two consecutive days and at least above 50 IU/ml and received the first infusion the following day, where the first serum sample also was taken. The second infusion was given after 3–6 days, the following three infusions with 6–8 days intervals and the remaining three infusions with 12–16 days intervals. Serum samples were collected in gestational weeks 5, 6, and 7, concomitantly with infusion numbers 1, 2, and 3. The serum samples were not collected as planned if the woman experienced a pregnancy loss.

2.1. Inclusion and exclusion criteria

The inclusion criteria in the trial were 1) $\geq$ four confirmed pregnancy losses (before GA 14) and a minimum of one live-born child after GA 28, 2) at least three pregnancy losses should be consecutive after birth and with the same male partner. The exclusion criteria in the trial were 1) female age below 18 or above 42, 2) uterine abnormalities, 3) abnormal chromosomal status of couple, 4) irregular menstrual cycle (below 23 days, above 35 days), 5) repeated measurements of plasma homocysteine $\geq$ 25 ug/l, plasma IgG anticardiolipin $\geq$ 40 GPL kU/l or positive lupus anticoagulant, 6) Positive HIV, hepatitis B or C, 7) plasma IgA deficiency, 8) allergy towards immunoglobulin or albumin, 9) using anti-inflammatory or anti-coagulant drugs regularly, 10) conception by oocyte or sperm donation, 11) using gestagen or estrogen drugs during pregnancy, 12) previous participation in trial.

2.2. Analyses

Whole blood was drawn from a venipuncture in a serum gel separator tube, centrifuged, and serum was aliquoted and frozen at −20 °C at each pregnancy visit in gestational weeks 5, 6 and 7. In 2017 the serum samples were thawed and the following biomarkers were measured: VEGF, sFlt-1,Ang-1 and Ang-2 on a Luminex platform at the Multiplex Core Facility in the Division of Pediatrics, Laboratory of Translational Immunology at Wilhelmina Children's Hospital part of UMC Utrecht in the Netherlands, as previously described (Scholman et al., 2018). At the Laboratory of Molecular Medicine, Department of Clinical Immunology, Copenhagen University Hospital, Denmark, the serum levels of the PRM of the lectin complement pathway: ficolin-1, ficolin-2, ficolin-3 and MBL and PTX3 were quantified by use of monoclonal antibodies in a specific sandwich enzyme-linked immunosorbent assays (ELISAs), as previously described (Bastrup-Birk et al., 2013; Garred et al., 1992; Munthe-Fog et al., 2012, 2008, 2007).

2.3. Statistical analyses

Continuous data are presented as medians, interquartile range, and categorical data as absolute numbers and percentages. To evaluate the differences between outcome groups in Table 1, the Mann-Whitney *U*-test, Chi-square test and Fisher's exact test were used where appropriate.

We divided exposure measures (VEGF, sFlt-1, Ang-1, Ang-2, Ang-2/Ang-1 ratio, ficolin-1, ficolin-2, ficolin-3, MBL, PTX3) into tertiles at each visit/measure time and used these as time-dependent covariates in a time-to-event analysis (loss of fetus as an event). Time was calculated from the first visit to the loss of the fetus, birth, or end of follow-up, whichever came first. Time was divided into at most three periods

Table 1
Background characteristics according to the outcome of the trial pregnancy.

	Live birth in the trial pregnancy n= 41	Pregnancy loss in the trial pregnancy n= 35	P-value
Age, years median (range)	35 (26;41)	34 (25;41)	0.409
BMI, kg/m^2 median (IQR)	24 (22;26)	26 (22;30)	0.224
BMI group, n (%)			
Normal weight (BMI<25)	28 (70)	17 (49)	0.059
Overweight/Obese (BMI>25)	12 (30)	18 (51)	
Current smoker n (%)	6 (15)	2 (6)	0.275
Reproductive history			
Total number of pregnancy losses after last birth before inclusion, median (range)	4 (3;7)	4 (3;9)	0.401
Firstborn boy, n(%)	20 (49)	18 (51)	1.000
Birth weight of first child, grams, median (IQR)	3420 (3148;3625)	3325 (2653;3737)	0.729
Trial pregnancy			
IVIG intervention, n(%)	22 (54)	17 (49)	0.832
Gestational age at loss in trial pregnancy (n)	NA		
Week			
5		3	
6		5	
7		7	
8		12	
9		4	
≥10		4	

BMI: Bone Mass Index, IQR: Inter quartile range, IVIG: Immunoglobulin.

according to number of visits obtained by each patient. A possible effect of IVIG on the biomarkers was investigated by adding an interaction term of IVIG and the biomarker in question to the statistical model and testing the significance of this term.

Results from univariate analyses are presented as Kaplan-Meier curves with 95%-confidence bands and a p-value derived from a similar Cox regression with the lowest tertile as reference. The assumption of proportional hazards was investigated by plotting the Log of Negative Log survival function.

A significance level of 0.05 was used throughout. All analyses were performed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 25.0. Armonk, NY: IBM Corp) or R version 3.4.4 (https://www.r-project.org/).

2.4. Ethics approval

The study is approved by the Regional Ethics Committee (Journal no. H-15010934) and by the Data Protection Agency (Journal no.2012–58–0004) in the Capital Region of Denmark. According to the Declaration of Helsinki, all patients received oral and written information about the study before signing an informed consent.

3. Results

Of the 76 included pregnant women, 41 women had a live birth, and 35 women experienced another pregnancy loss. The background demographics and reproductive history are presented in Table 1 and were part of the previously published trial(Christiansen et al., 2015). There were no differences in median age or BMI between the two pregnancy outcome groups (Table 1). There were more women in the pregnancy loss group with BMI>25, but this was not significantly different from the live birth group. An overall time-dependent illustration of pregnancy outcome after inclusion is depicted in Fig. 1. Treatment with IVIG vs placebo did not affect pregnancy outcome, as previously reported in the trial(Christiansen et al., 2015). In this study, the IVIG intervention did not affect the measured biomarkers, except for one interaction with Ang-2, p=0.038. This interaction was not regarded biologically justifiable in the absence of related affected biomarkers. In this study, the randomized patients were therefore compiled. The medians (IQR) of the measured biomarkers in gestational week 5, 6 and 7 are shown in Table 2

By time-to-event analysis, the angiogenesis-related factors Ang-1 (Fig. 2a) and Ang-2 were evaluated concerning pregnancy outcome. There was a trend with the lowest tertile of Ang-2 being related to pregnancies ending in pregnancy loss (Fig. 2b), p=0.081. The tertiles of the Ang-2/Ang-1 ratio were significantly associated with pregnancy loss in a dose-dependent relationship, with the lowest ratio of Ang-2/Ang-1 related to pregnancy loss (Fig. 2c), p=0.022. The highest tertile of VEGF

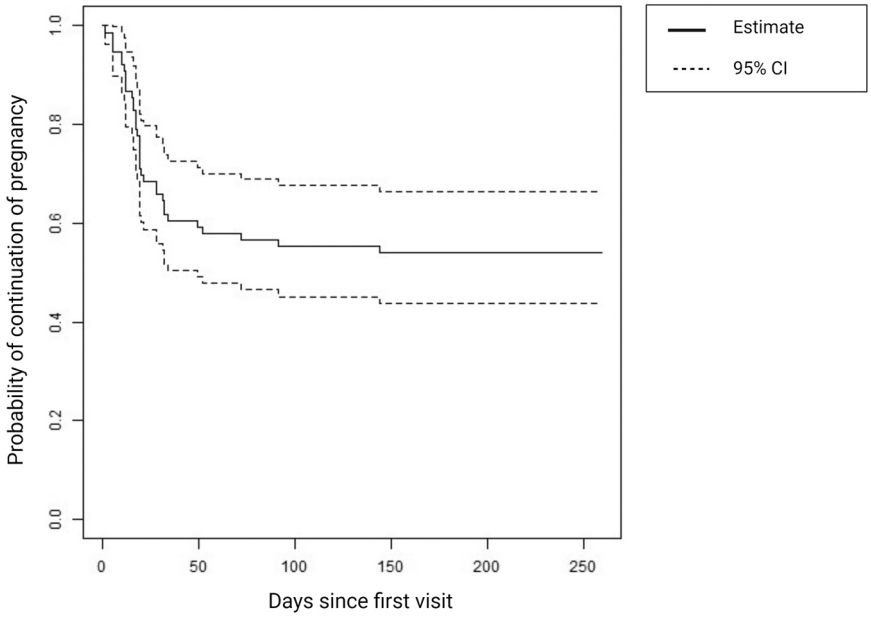

Fig. 1. Kaplan-Meier curve of pregnancy status according to time after inclusion. The probability of staying pregnant according to time after inclusion (continuous line). The dotted lines indicate the 95% CI.

Table 2
Medians (IQR) of the measured biomarkers at the three visits in gestational weeks 5, 6 and 7.

	Live birth in the trial n= 41		Pregnancy loss in the trial n= 35	
VEGF, pg/ml	n=	57 (27;100)	n=	84 (37;151)
GW 5	41	49 (8;86)	35	69 (35;142)
GW 6	40	6 (1;16)	34	51 (19;89)
GW 7	41		27	
sFlt-1, pg/ml	41	1906 (1513;2375)	35	2071 (1846;2640)
GW 5	40	2008 (1703;2409)	34	2109 (1669;2897)
GW 6	41	2701 (2006;3480)	27	2735 (2155;3600)
GW 7				
Angiopoietin-1, pg/	41	50744	35	49178
ml	40	(39778;60896)	34	(42175;53252)
GW 5	41	46963	27	45630
GW 6		(36387;54315)		(39450;55957)
GW 7		43915		45547
		(36006;52753)		(39346;50433)
Angiopoietin-2, pg/	41	760 (356;1231)	35	868 (356;1231)
ml	40	802 (194;1768)	34	929 (398;1321)
GW 5	41	2219 (1226;3696)	27	1141 (700;2066)
GW 6				
GW 7				
Ficolin-1, ng/ml	40	449 (314;706)	35	430 (257;521)
GW 5	40	554 (268;805)	34	418 (312;593)
GW 6	40	400 (250;616)	26	383 (311;703)
GW 7				
Ficolin-2, ug/ml	40	1.5 (1.1;1.8)	35	1.6 (1.3;2.1)
GW 5	40	1.4 (1.0;2.0)	34	1.6 (1.2;2.2)
GW 6	40	1.2 (1.0;1.5)	26	1.7 (1.0;2.0)
GW 7				
Ficolin-3, ug/ml	38	28 (21;42)	35	25 (22;35)
GW 5	36	29 (22;47)	33	27 (22;39)
GW 6	40	33 (24;48)	25	24 (20;42)
GW 7				
MBL, ng/ml	38	812 (417;1593)	35	866 (480;1484)
GW 5	36	963 (432;2494)	33	1017 (267;1839)
GW 6	40	1074 (479;2142)	25	1122 (590;1857)
GW 7				
PTX3, ng/ml	40	2 (2;2)	35	2 (2;2)
GW 5	40	2 (2;2)	34	2 (2;2)
GW 6	40	2 (2;2)	26	2 (2;2)
GW 7				

GW: Gestational week, VEGF: Vascular Endothelial Growth Factor, sFlt-1: Soluble fms-like tyrosine kinase-1, MBL: Mannose-binding Lectin, PTX3: Pentraxin 3.

was significantly associated with pregnancy loss (Fig. 3a), $p<0.001$, whereas s-Flt-1 did not show any time-dependent association with pregnancy loss in this cohort, $p=0.993$ (Fig. 3b).

The levels of the following PRM of the lectin pathway of complement, MBL, ficolin-1, ficolin-2 and ficolin-3 were analysed. The tertiles of MBL did not associate with pregnancy outcome after inclusion, $p=0.633$. Only Ficolin-2 proved a time-dependent association with pregnancy loss ($p=0.026$); however, only for the highest tertile without a dose-dependent relationship (Fig. 4a). PTX3 proved not to be relevant in early pregnancy as the biomarker remained constant throughout the sample time points with no differences between outcome groups. When evaluating the sex of the previously born child concerning the outcome of this pregnancy, no association was found in the biomarkers analysed, $p=0.829$.

4. Discussion

This study provides insights into the dysregulation of angiogenic factors very early in pregnancy of women with sRPL ending in another pregnancy loss compared with those who succeed with a live birth. There was a significant time-dependent association between a higher level of VEGF in patients with a later pregnancy loss concomitantly with a lower level of theAng-2/Ang-1 ratio compared with pregnancies ending in a live birth.

Interestingly, the angiogenic factors such as VEGF, secreted by uNK cells and decidual macrophages, together with Ang-1 and Ang-2, transform the uterine spiral arteries into low-resistance arteries with a larger lumen to provide the growing fetus with a sufficient blood supply (Wang and Lash, 2017b). Ang-2 is a context-dependent regulator of angiogenesis that can either promote or inhibit vessel growth depending on other angiogenic factors present (Lobov et al., 2002; Wang and Lash, 2017b). The results of this study are surprising, as high levels of VEGF usually stimulate the secretion of Ang-2, which in turn promotes angiogenesis by enhancing VEGF-mediated signalling pathways that are crucial in stabilising the blood vessels (Wang and Lash, 2017). The excess levels of VEGF could indicate an underlying pathological process of disrupted angiogenesis, excessive vascular permeability, or hypoxia. Notably, this study investigates angiogenic factors in very early pregnancy when the uteroplacental bed is still plugged by endovascular trophoblast, and the fetus depends on uterine glands for nutrition (Burton et al., 2002). The EVT invasion usually occurs around gestational weeks 8–10 and is one of the critical steps in the spiral artery formation (Brosens et al., 2019). In this study, pregnancies that ended in pregnancy loss had higher levels of VEGF but no apparent increase in Ang-2. This could be biologically plausible when there is poor placentation, supported by a previous study showing significantly lower levels of Ang-2 in the first trimester of pregnancies subsequently complicated by intrauterine growth restriction compared with pregnancies with children born small for gestational age (Wang et al., 2007). A recent study found that Ang-2 alterations can disrupt EVT invasion and spiral artery remodeling (Hou et al., 2021). Interestingly, it has been shown that Ang-2 expression and vascular remodeling were decreased when the progesterone receptor was inhibited in mice (Park et al., 2020), indicating that a low Ang-2 could be due to low progesterone or reduced sensitivity to progesterone preceding pregnancy loss. The observed alterations in angiogenic factors could be linked to the function of the uterine natural killer (uNK) cells. These cells are abundant in the uterus during early pregnancy and are instrumental in spiral artery remodeling. Previous studies found increased levels of perivascular uNK cells surrounding the spiral arteries, with the correlation between high numbers of uNK cells and increased density of vessels in women with RPL (El-Azzamy et al., 2018; Quenby et al., 2009), suggesting a delay in remodeling. They speculated that high numbers of uNK cells result in hyper-coiling of the spiral arteries (El-Azzamy et al., 2018), possibly mediated through increased levels of VEGF, as observed in this study.

Several studies have found elevated or lower levels of VEGF when comparing women with RPL and healthy pregnant women (Atalay et al., 2016; Gupta et al., 2019). In addition, several VEGF polymorphisms have been identified in the RPL population (Su et al., 2011; Sun et al., 2017), where RPL recurring in families may be an essential factor. Elevated levels of VEGF in pregnancies resulting in pregnancy loss, as shown here in our well-defined cohort of sRPL women, may indicate a pro-inflammatory state or local hypoxia. A previous study found that pregnant women who smoke have higher expression of VEGF in the placenta during 10–11 weeks of gestation (Shinjo et al., 2014). Moreover, it was shown that women carrying a male fetus exhibit an increased proinflammatory and proangiogenic profile with increased levels of VEGF (Enninga et al., 2015). This is particularly noteworthy given that women with sRPL are at a higher risk of losing male fetuses (Nielsen et al., 2009; Nørgaard-Pedersen et al., 2021; Svarre Nielsen et al., 2008), which could explain the increased levels of VEGF observed in those who have another pregnancy loss in this study.

Placenta secretes sFlt-1 in response to oxidative stress and inflammation and is increased in later pregnancy in patients with preeclampsia (Maynard et al., 2003). sFlt-1 antagonises VEGF, which can result in increased complement activation (Girardi et al., 2020). Impaired spiral artery formation is acknowledged as a shared pathophysiological background for preeclampsia and intrauterine fetal growth restriction, and interestingly, women with these pregnancy complications also share an increased risk of later cardiovascular disease as do women with RPL

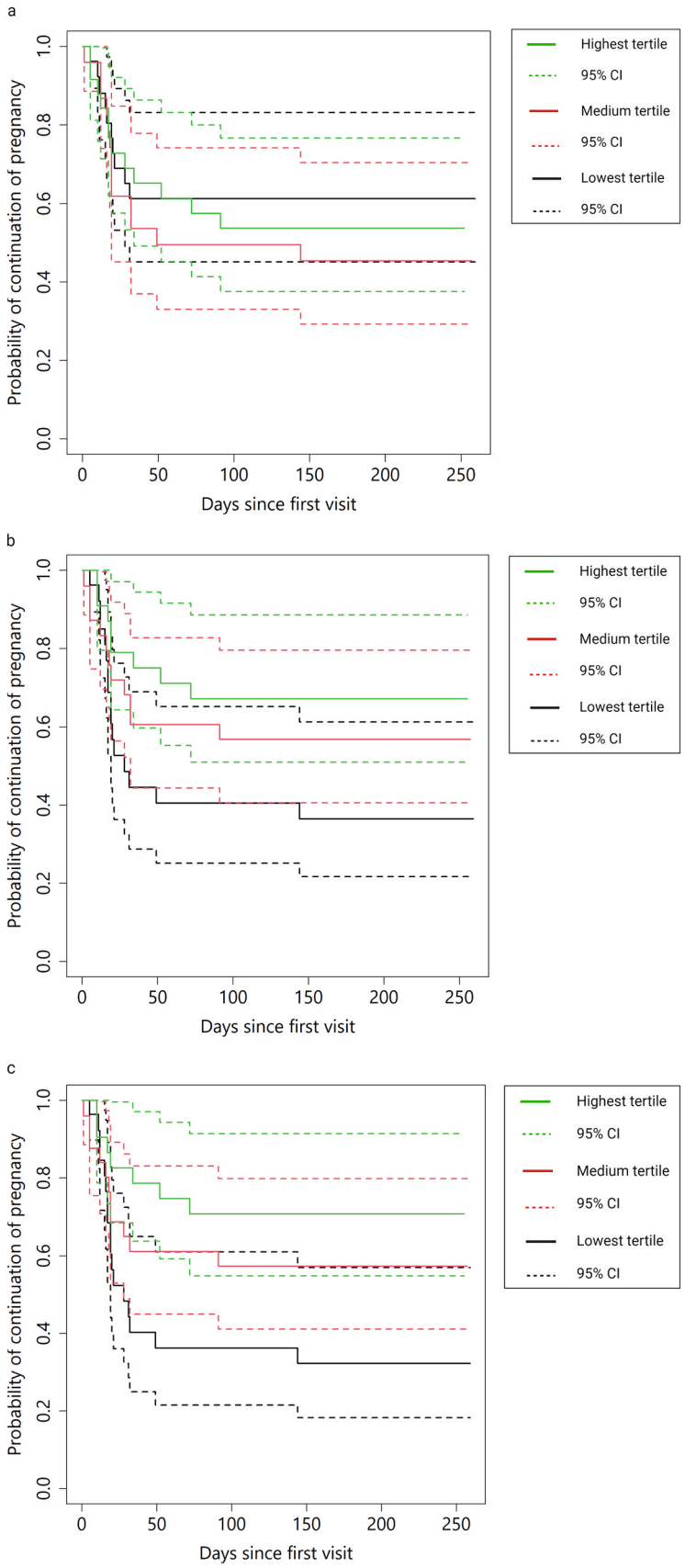

Fig. 2. Tertiles of Ang-1 (2a), Ang-2 (2b) and Ang-2/Ang-1 ratio (2c) levels in gestational weeks 5, 6 and 7 according to the probability of staying pregnant after inclusion in the trial. The lowest tertile of Ang-2/Ang-1 ratio were significantly associated with pregnancy loss.

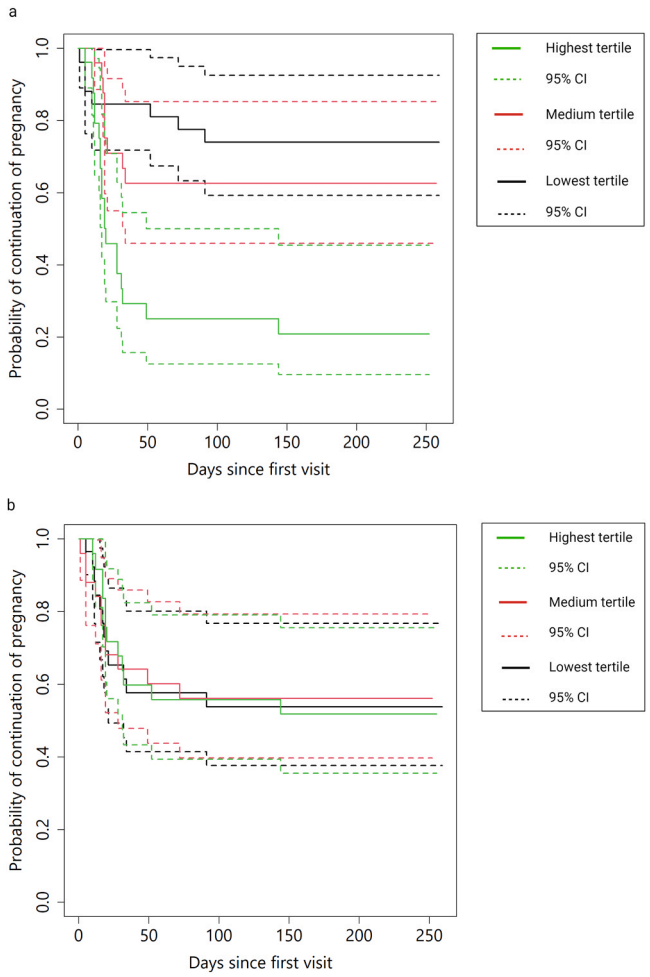

Fig. 3. Tertiles of VEGF (3a) and sFlt-1 (3b) levels in gestational weeks 5, 6 and 7 according to the probability of staying pregnant after inclusion in the trial. Increasing levels of VEGF were significantly associated with pregnancy loss in a dose-dependent manner.

(Petersen et al., 2022; Ranthe et al., 2013), albeit more likely linked to primary RPL (pRPL) (Westergaard et al., 2020). Whether this is due to endothelial dysfunction(Germain et al., 2007) or an underlying altered immune response with a generalised pro-inflammatory state is unknown. This study found no association between the sFlt-1 levels in the early weeks of pregnancy (gestational weeks 5, 6 and 7) and pregnancy loss.

Our analyses also involved the evaluation of PRMs of the lectin pathway of complement. An overly activated complement system has been linked with RPL (Girardi et al., 2020, 2006), and speculations persist about whether this could be due to a hyperinflammatory state, an endometrial infection, or a loss of complement regulation. The human trophoblast expresses numerous regulating proteins, and controlled complement activation may be essential to remodel spiral arteries and clearance of cellular debris (Abeln et al., 2018). In early pregnancy, the trophoblast plugs the spiral arteries, and the intervillous space is a hypoxic environment (Weiss et al., 2016). Incorrect unplugging and reperfusion of the spiral arteries could activate the lectin pathway resulting in cell destruction with uncontrolled complement activation resulting in pregnancy loss. It was recently discovered that the lectin pathway plays a vital role in the ischemia-reperfusion injuries seen after kidney transplantation from deceased donors which involves cold ischemia during transportation (Cernoch and Viklicky, 2017). RPL has long been associated with low levels of MBL (Kruse et al., 2002; Nørgaard-Pedersen et al., 2022) (a PRM of the lectin pathway),

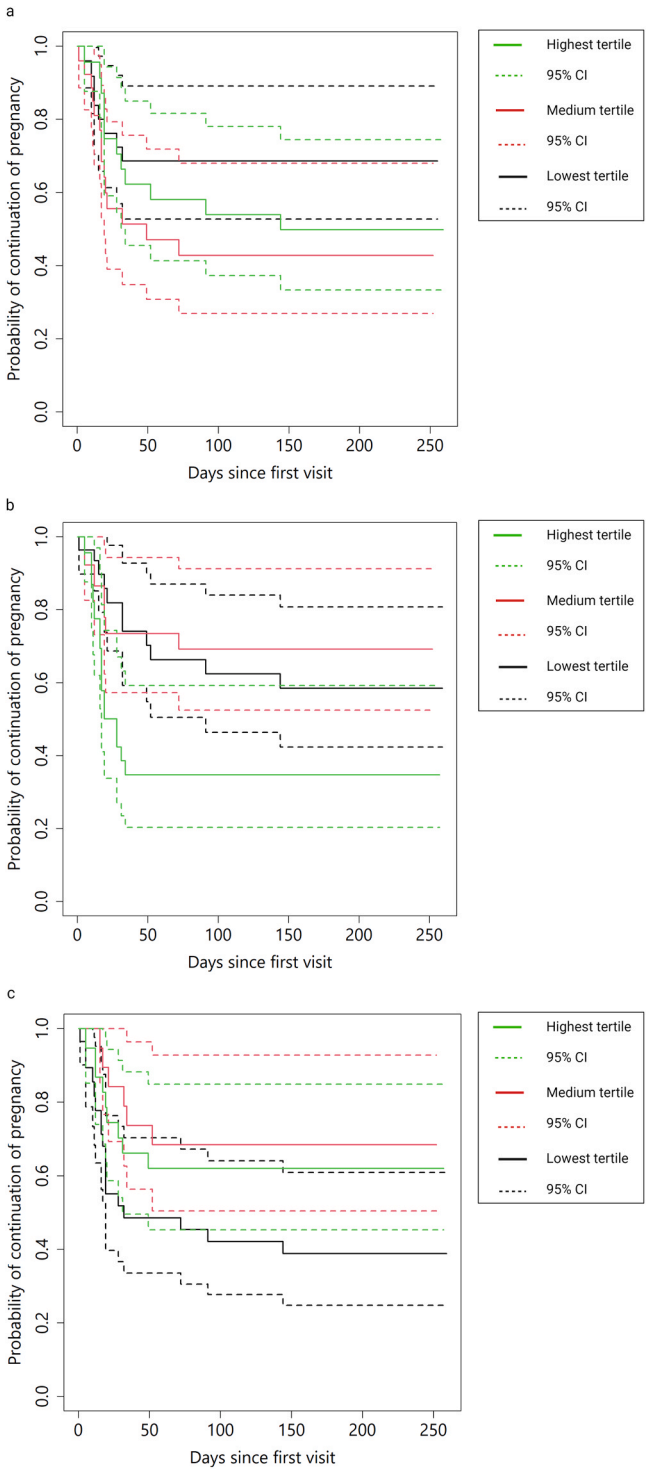

Fig. 4. Tertiles of ficolin-1 (4a), ficolin-2 (4b) and ficolin-3 (4c) levels in gestational weeks 5, 6 and 7 according to the probability of staying pregnant after inclusion in the trial. The highest tertile of ficolin-2 was significantly associated with pregnancy loss.

indicating either a genetic predisposition with deficiency or increased consumption of MBL. In this study, no time-dependent association in early pregnancy between MBL levels and pregnancy outcome was found but increasing levels of ficolin-2 proved a significant association with pregnancy loss. Ficolin-2 has been shown to bind apoptotic cells efficiently and is important in tissue remodeling. A previous study found normal or even lower levels of ficolin-2 in women with RPL (Kilpatrick

et al., 1999); however, it was not documented when the samples were taken (between pregnancies or during pregnancy), and most examined women were pRPL. Nevertheless, the observed association between the highest tertile and ficolin-2 could be a chance finding in this relatively small sample size, which must be confirmed in additional studies.

A limitation when studying pregnancy loss is whether the observed alteration is a cause or a consequence of the failed pregnancy. Even though we carefully examined an effect of the intervention on the investigated biomarkers, we cannot rule out an immune modulatory effect in some women, which is a major limitation. An additional constraint in assessing biomarkers in early pregnancy pertains to not knowing the sex of the fetus of the pregnancy, as previously noted, given that fetal sex could potentially impact the results. The relatively small sample size may limit statistical power and the generalizability of the results. Applying the time-dependent analyses of pregnancy outcomes in a highly selected cohort of women with sRPL enhanced the precision of our findings, despite the small sample size. Most importantly, the study provides the basis for further exploration into poor placentation in RPL.

5. Conclusions

In this study, angiogenesis-related biomarkers were time-dependently altered in early pregnancy in women with sRPL preceding a pregnancy loss compared with live birth. Unlike the rest of the PRMs of the lectin pathway analysed in this study, only ficolin-2 (highest tertile) was associated with pregnancy loss. According to our findings, neither a deficiency nor an increased consumption of the PRMs of the lectin pathway was observed in women who experienced another pregnancy loss. Future research would benefit from investigating complement activation per se to evaluate the role of excessive complement activation in the lectin pathway during early pregnancy. In conclusion, our findings suggest that some women with sRPL may experience altered angiogenesis and poor placentation in early pregnancy preceding another pregnancy loss.

Author contribution

MCK, HSN and PG conceived the concept of the study. MCK planned the study, included patients and obtained consent, performed statistical analyses and wrote the first draft of the manuscript. EMF performed the time-series statistical analyses. WDJ, LM and lab performed the measurements of the angiogenic factors. PG and his laboratory measured the complement factors. OBC performed the treatment trial, collected the serum samples, and wrote the manuscript. All authors critically read, discussed, and approved the final version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jri.2024.104221.

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