Update on the Pathophysiological Implications and Clinical Role of Angiogenic Factors in Pregnancy

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Angiogenesis · Uteroplacental insufficiency · Preeclampsia · Intrauterine growth restriction · Congenital heart defects

Abstract

Angiogenic markers are now being incorporated into clinical practice for the screening, diagnosing, and monitoring of preeclampsia. Pregnancy requires both vasculogenesis and angiogenesis in the fetal compartment and angiogenesis in the maternal compartment. Abnormal angiogenesis in the placenta determines impaired remodeling of the maternal spiral arteries and placental underperfusion that may ultimately lead to fetal growth restriction and maternal preeclampsia. The dysregulation of angiogenesis in the placenta and maternal-fetal circulation has emerged as one of the main pathophysiological features in the development of placental insufficiency and its clinical consequences. Abnormal angiogenesis has also been related to other obstetric and fetal conditions such as peripartum cardiomyopathy and fetal cardiac defects. This opens up new challenges for our understanding of angiogenic involvement in maternal cardiovascular function and fetal cardiac development, and it of-

fers new clinical opportunities. This review summarizes the current knowledge of the pathophysiological implications and the clinical role of angiogenic factors in pregnancy.

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Physiological and Pathophysiological Roles in Humans in Pregnancy

The Role of Endoglin, PlGF, VEGF, and sVEGFR-1 in Normal Placenta

Extravillous cytotrophoblasts invade the uterine spiral arteries of the decidua and myometrium in early normal embryonic development. Fetal cells replace the endothelial layer of uterine vessels, transforming them into high-capacity vessels that permit an increase in uterine blood flow.

Similar to tumor growth, a balance of pro-angiogenic and anti-angiogenic factors is established in the developing placenta [1]. The vascular endothelial growth factor (VEGF) family consists of VEGF-A, placental growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D; their receptors VEGF-I (also called fms-like-tyrosine-kinase receptor

I, Flt-1), VEGFR-2 (also called kinase insert domain receptor, KDR), and VEGFR-3 (Flt-4); and their co-receptors, neuropilin-1 (NRP-1) and NRP-2. The key molecules regulating early placental vascular changes are VEGF-A, PlGF, and the receptors Flt-1 and KDR [2]. In addition to the two membrane-bound receptors, a splice variant of Flt-1, called soluble Flt-1 (sFlt-1), is expressed in the placenta and is known to have an anti-angiogenic effect.

Vasculogenesis and angiogenesis are two essential processes in the establishment of the utero-placental circulation when placental expressions of VEGF-A, Flt-1, and KDR are high and PlGF expression is moderate [3]. In vitro experiments have shown that binding of VEGF-A to Flt-1 and KDR stimulates branching angiogenesis [4]. PlGF, which is expressed in trophoblasts throughout gestation by acting on Flt-1, may play a supplementary role in vasculogenesis by recruiting macrophages. From 25 weeks of gestation, angiogenesis switches from branching to non-branching and is accompanied by a decline in VEGF-A and KDR and an increase in the expression of PlGF, Flt-1, and sFlt-1. PlGF may have a role in non-branching angiogenesis that continues until term [5].

Angiogenesis Imbalance in Placental Vascular Impairment and Underperfusion

Both preeclampsia (PE) and intrauterine growth restriction (IUGR) are associated with pathogenic evidence of placental underperfusion and ischemia [6], including acute atherosclerosis, intimal thickening, necrosis, atherosclerosis and endothelial damage, and placental infarction. In these cases, there is impairment of the perivascular and endovascular trophoblastic invasion into the spiral arteries that fail to become low-resistance vessels [7]. Abnormal angiogenesis in the placenta determines impaired remodeling of the maternal spiral arteries and placental underperfusion that may ultimately lead to fetal growth restriction and maternal preeclampsia [8–10]. Evidence shows that angiogenic factors are important in the regulation of placental vasculogenesis.

VEGF, PlGF, and Flt1 are highly expressed by invasive cytotrophoblasts, and it has been shown that their expression is altered in placenta tissue of women with preeclampsia and that sFlt1 reduces cytotrophoblast invasiveness in vitro [11]. In addition, mice deficient in these receptors have impaired vascular development and die in utero. Moreover, recent studies in an animal model of spontaneous preeclampsia (BPH/5 mice) showed that adenoviral-mediated delivery of VEGF early in pregnancy prevents the spontaneous development of preeclampsia and incidence of fetal resorptions [12].

The factors regulating sFlt1 and sEng expression are just beginning to be elucidated. It has been suggested that abnormal oxygen tension and the hypoxia reoxygenation caused by intermittent perfusion-reperfusion can cause oxidative stress [13]. It has been shown that this feature is likely to trigger apoptosis, release of sFlt1 and sEng and pro-inflammatory cytokines such as IL-6 (fig. 1). In addition to this, in women with pre-eclampsia, a lower HO-1 activity has been observed and it may mediate increased sFlt1 and sFlt1 activity [14, 15].

Maternal Consequences of Angiogenesis Deregulation

Endothelial Dysfunction and PE

The Two-Stage Model of preeclampsia proposes that a poorly perfused placenta (Stage 1) produces factor(s) leading to the clinical manifestations of preeclampsia [16] (Stage 2). However, the cause of impaired vascular remodeling occurs earlier in pregnancy. Therefore, we suggest preeclampsia as a three-stage model (fig. 1).

There is experimental evidence that supports the hypothesis that interference with VEGF/PlGF signaling could mediate endothelial dysfunction in preeclampsia. VEGF is important in the stabilization of endothelial cells in mature blood vessels. Organs severely affected by preeclampsia, renal glomerulus, brain, and liver, are characterized by a fenestrated endothelium and VEGF regulation is especially important in these kind of vessels. VEGF signals through two major receptors: Flk and Flt1. Placental expression of sFlt1 is increased in PE, and it is associated with a marked increase in maternal circulating sFlt1 levels [17]. sFlt1 antagonizes both VEGF and PlGF by binding them in the circulation and preventing interaction with their endogenous receptors [18, 19] (fig. 2). In vitro effects of sFlt1 include vasoconstriction and endothelial dysfunction. Studies in gravid mice demonstrated that either with the direct injection of the protein or by injecting adenovirus expressing the sFlt messenger, RNA produces hypertension, proteinuria, and glomerular endotheliosis [20]. Moreover, other experiments, which reduced uterine perfusion, increased circulating and placental sFlt1 [21]. Reduction in circulating soluble Flt-1 alleviated pre-eclampsia-like symptoms in a mouse model [22]. Therefore, over-expression of sFlt1 is a key feature that links placental dysfunction and endothelial disease.

PlGF is also an important contributor to endothelial dysfunction, although its physiological role is less understood. It is thought to amplify VEGF signaling by dis-

Fig. 1. Preeclampsia is a three-stage disorder: In preeclamptic decidua, deficient expression of pro-angiogenic factors (VEGF and PlGF) and hypoxia-inducible factors (HO1) triggers abnormal remodeling of spiral arteries and trophoblast invasion (Stage 1). Impaired placental perfusion leads to hypoxia and oxidative damage (Stage 2). Pathologic placenta induces apoptosis, inflammation, and release of anti-angiogenic factors (sFlt1 and sEnd) that promote systemic endothelial dysfunction, with vasoconstriction and end-organ ischemia, that finally leads to preeclampsia signs and symptoms (Stage 3).

Fig. 2. sFlt1 and sEnd caused endothelial dysfunction by antagonizing VEGF and TGF- β 1 signaling in the vasculature. During normal pregnancy, endothelial synthesis of prostacyclin and expression of nitric oxide is enhanced by physiological levels of VEGF and TGF- β throughout their receptors. In preeclampsia, high levels of circulating sFlt1 and sEnd bind VEGF and TGF- β , antagonizing their actions and resulting in endothelial dysfunction (adapted from Karumanchi SA, Epstein FH: Placental ischemia and soluble fms-like tyrosine kinase 1: cause or consequence of preeclampsia? *Kidney Int* 2007;71:959–961.).

placing VEGF from the Flt1 receptor and instead allowing it to bind to the more potent KDR receptor. During pregnancy, inhibition of both PlGF and VEGF is necessary to produce preeclampsia-like changes in pregnant rats. Soluble endoglin, a truncate form of endoglin, which binds and antagonizes TGF- β , is up-regulated in preeclampsia. In pregnant rats, sEnd amplifies the vascular damage mediated by sFlt1 [20]. The inhibition of

TGF- β and VEGF signaling in the vasculature results in endothelial dysfunction, including decreased prostacyclin, nitric oxide production, and release of pro-coagulant proteins (fig. 2).

Later Cardiovascular Risk

The cardiovascular implications of preeclampsia do not end with the birth of the infant and placenta.

Common risk factors between CVD and preeclampsia lead us to hypothesize that increased CVD in women after preeclampsia is the result of pre-existing conditions. Multiple studies and meta-analysis confirm that women, whose pregnancy was complicated by preeclampsia, have higher susceptibility to CVD later in life. Whether preeclampsia directly influences the development of maternal cardiovascular disease later in life or preeclampsia uncovers a pre-existing condition that could lead to CVS later in life anyway remains undetermined [23].

Women with preeclampsia have markedly abnormal cardiac function [24, 25]. It has been shown that myocardial performance index (MPI) correlated with levels of circulating sFlt1, whereas elevated blood pressure (BP) was not associated with MPI in women with preeclampsia. Therefore, these authors suggested that elevated sFlt1 causes the diastolic dysfunction in preeclampsia [26]. At 1 year postpartum, asymptomatic left ventricular moderate-severe dysfunction or hypertrophy was significantly higher in preterm preeclampsia (56%) compared with term preeclampsia (14%) or matched controls (8%). The risk of developing essential hypertension within 2 years was significantly higher in both preterm preeclamptic women and those with persistent left ventricular moderate-severe abnormal function/geometry [27].

Anti-VEGF therapies given to adult animals cause glomerular endothelial damage with proteinuria. Anti-angiogenic therapies, including antibodies that neutralize VEGF and small-molecule VEGF receptor inhibitors, are being increasingly used in the oncological and ophthalmological settings in which cardiomyopathy and heart failure have recently been recognized as major side effects [28, 29]. Angiogenic factors are involved in the development of atherosclerosis and show pronounced changes during acute myocardial infarction (AMI). High sFlt-1 levels proved to be a good predictor of mortality during a 1-year follow-up of AMI, regardless of information provided by troponin T and N-terminal pro-B-type natriuretic peptide (NT-proBNP) [30]. PlGF has emerged as a central mediator in both coordination of cardiomyocyte growth and neo-angiogenesis [31]. Genetic and pharmacological studies identified PlGF as a novel cardioprotective factor [14].

Peripartum cardiomyopathy (PPCM) is characterized by systolic heart failure presenting in the last month of pregnancy and the first months post-partum. Half of these women with PPCM progress to chronic heart failure, cardiac transplantation, or death. Women who develop preeclampsia or multiple gestations are known risk factors. Patten et al. have recently associated PPCM with

a systemic angiogenic imbalance [26]. At 4–6 weeks postpartum, women who developed PPMC had higher levels of sFlt1, remaining till 5- or 10-fold higher than the levels in control women.

Overall, these studies indicated that an anti-angiogenic state could be harmful to the human heart. Further studies are necessary to ascertain the contribution of angiogenic status during pregnancy to cardiovascular disease later in life.

Prediction of Complications of Placental Disease

Evidence of Prediction of Preeclampsia/IUGR/IUFD with Angiogenic Factors in the First and Second Trimester of Pregnancy

Circulating levels of sFlt1 and PlGF are altered several weeks before the onset of clinical disease and correlated with the severity of disease [32]. sFlt-1 concentrations seemed to be increased throughout gestation in women destined to develop PE, a significant difference usually detectable at 5–6 weeks before presentation [33]. Serum concentrations of PlGF tend to be lower in women who develop the disease, and therefore, sFlt1/PlGF ratio has been proposed as an index of angiogenic imbalance.

With the ability of sFlt-1 and PlGF to accurately diagnose the disease, many studies have focused on the use of the markers as early predictors. Here, different approaches have been addressed about when to test and which other biophysical tests to adjunct in order to reach a maximum detection rate for the later onset of the disease.

Many groups have shown that the serum measurement of sFlt-1 and PlGF and other factors such as soluble endoglin in the second trimester of pregnancy allow for a precise prediction of the later onset of the disease [34–36]. Differences in asymptomatic patients who develop these complications with regard to healthy pregnancies are considerably more pronounced in early-onset cases, whereas in late-onset disease there is a substantial overlap with controls [37] (fig. 3).

The predictive value of sFlt1, PlGF, and sEng for the prediction of PE has consistently been demonstrated in the second trimester of pregnancy. Several studies evaluated a combined approach with the Doppler sonography of the uterine artery in the second trimester. The sensitivity and positive predictive value of uterine artery Doppler, known to be low, has been shown to increase when combined with measurement of angiogenic and anti-angiogenic factors [38, 39]; the combination of angiogenic factors and uterine artery Doppler at 20–24 weeks of ges-

Fig. 3. PI GF (pg/ml) levels and PI GF/sFlt1 ratio in asymptomatic patients at 20–24 weeks of gestation according to pregnancy outcomes (CO utN = Healthy patients with normal uterine artery Doppler; CO utA = healthy patients with abnormal uterine artery Dop-

pler at 20 weeks of gestation; SGA = small for gestational age cases; Late PE/IUGR = late preeclampsia or/and IUGR cases; Early PE/IUGR = early preeclampsia or/and IUGR cases). * $p < 0.05$ compared with CO utN and CO utA and SGA cases (ANOVA test).

tation have 89.5% sensitivity with 95% specificity for identifying early-onset preeclampsia. Conversely, the sensitivity for late-onset PE was below 11% [37]. These observations are in line with the idea that late-onset PE constitutes a heterogeneous condition with either minimal or no placental involvement, and, therefore, tests based on the identification of signs of abnormal placentation are of limited value in predicting this entity. In addition to these, the group consisting of Roberto Romero and others has highlighted the importance of repeated measurements, as the slope of the markers indicates those at risk for the condition [40–42].

The evidence from meta-analysis shows that prevention of preeclampsia with low-dose aspirin may be possible if treatment is started before 16 weeks of gestation [43]. The efforts for the prediction of preeclampsia have shifted from the second to the first trimester of pregnancy. Data on PI GF occur early in the first trimester and have been tested alone or in combination with other biomarkers as a potential predictive test. Recently, the group of Kypros Nicolaides has evaluated the ability of serum markers to predict preeclampsia already in the first trimester. In an combined screening approach, he has shown that a combination of maternal history, uterine artery Doppler, blood pressure measurement, and the serum measurement of PAPP-A and PI GF is able to detect the later onset of early-onset preeclampsia with a sensitivity till 93% at a false-positive rate till 5% [4, 44–46]. Recent data showed that not only PI GF but also sFlt-1 substantially improved the prediction for late PE [47].

Usefulness of Angiogenic Factors in the Third Trimester of Pregnancy for Adverse Outcomes

Most efforts have been concentrated in predicting early PE/IUGR, as it denotes the highest severity. However, most cases of PE and IUGR occur at or near term, including a substantial proportion of maternal morbidity and medically indicated late preterm births [48–50]. Consequently, late PE and IUGR and its early prediction/diagnosis represent a public healthcare priority.

First- and second-trimester strategies show poorer predictive values for late-onset forms of PE and IUGR [51–57]. This may be explained by the milder nature of the disease, where changes can possibly not be picked up that early in gestation, together with the heterogeneity of these clinical conditions late in pregnancy. However, a different approach that comes closer in time to the onset of the disease could improve the detection rates.

Evidences for Angiogenic Factors in Third-Trimester Screening of Late PE

Recent studies have shown a potential utility of angiogenic factors for the third-trimester prediction of late PE. Chaiworapongsa et al. demonstrated that maternal plas-matic concentrations of PI GF/sEng < 0.3 MoM at 30–34 weeks of gestation have been demonstrated to be associated with late PE (adjusted odds ratio 7) and severe late PE (adjusted odds ratio 16), leading to a sensitivity of 74% for severe late PE with a false-positive rate of 15% [54]. The addition of PI GF/sEng to clinical risk factors increased the area under the receiver-operating characteristic curve from

0.76 to 0.88. Predictive values in the third trimester for late PE are superior to those achieved in the first or second trimester of pregnancy. In addition, several studies from the Fetal Medicine Foundation [55, 56] have also evaluated the predictive value of angiogenic factors together with maternal characteristics and uterine Doppler in the third trimester for late PE. A combination of maternal characteristics and serum PlGF had a detection rate of 86 and 53% for intermediate PE (delivery at 34–37 weeks) and late PE (delivery after 37 weeks), with a 10% false-positive rate.

Placental Underperfusion and Maternal PlGF in IUGR

Intrauterine growth restriction is defined as a failure to achieve the endorsed growth potential and is usually diagnosed by birthweight below the 10th centile. A clinically relevant issue is the distinction of ‘true’ IUGR, associated with placental insufficiency, poorer perinatal and long-term outcome from constitutional small-for-gestational age [57]. It is well known that patients with IUGR are often associated with preeclampsia and exhibit an anti-angiogenic profile [58–60]. The levels of sFlt-1/PlGF-ratio in early-onset IUGR are significantly different from those of controls, as several clinical studies have shown. A dysfunctional placenta is the origin of a risk for either mother and/or fetus. However, it is unknown as to which women with a dysfunctional placenta ‘only’ develop a fetal syndrome and which will develop maternal disease termed preeclampsia. Assessment of angiogenic and anti-angiogenic factors, however, might determine those at risk for complications [61].

Recent studies [62, 63] suggest that differences in maternal PlGF concentrations may have the ability to discriminate between fetuses with placental IUGR and constitutionally small fetuses. Placental vascular insufficiency in late pregnancy may be anticipated by maternal low detectable PlGF levels on discovery of SGA status. Actually, Triunfo et al. [62] demonstrated that maternal PlGF is an independent predictor of placental underperfusion in IUGR. Therefore, angiogenic factors may be pivotal in identifying small fetuses with placental underperfusion and true growth restriction. Future studies are warranted to assess how incorporating these factors into the current clinical criteria defines late-onset IUGR due to placental insufficiency.

Maternal PlGF for the Detection of Perinatal Complications in IUGR

Once the diagnosis of IUGR is established, a second clinically relevant step in IUGR is management and deci-

sion on the optimal timing to finalize the pregnancy. For that aim, it is critical to adequately select those IUGR cases at risk of perinatal complications. Despite most research focalized in feto-placental Doppler, recent data also suggest that angiogenic factors could help in the prediction of complications among IUGR cases. Maternal angiogenic imbalance (lower PlGF and higher sFlt1/PlGF) has shown a significant association with neonatal metabolic acidosis and operative delivery for non-reassuring fetal status. Lobmaier et al. [64] demonstrated a similar performance of maternal PlGF and feto-placental Doppler for the early detection of perinatal complications in IUGR, concluding that maternal angiogenic factors at diagnosis and follow-up with Doppler predict adverse outcomes with a similar performance. This information may be of help in tailoring surveillance and identifying late-onset FGR fetuses who may benefit from elective delivery at term.

Overall, the evidence showing an association among maternal angiogenic factors, placental underperfusion, and adverse perinatal outcome in IUGR supports the future incorporation of PlGF in the clinical protocols for diagnosing and managing IUGR fetuses. The use of angiogenic biomarkers for early identification and risk stratification has a strong potential to reduce both morbidity and healthcare costs in the management of IUGR fetuses.

Evidence for Angiogenic Factors in Third-Trimester Screening for Late IUGR

There is evidence that maternal concentrations of PlGF/sFlt1 and PlGF/sEng are associated with a significant increase in the likelihood of development of SGA. Although a recent work could not demonstrate maternal angiogenic factors improving the identification of IUGR from the models using clinical factors [54], future studies are warranted to assess their potential predictive value in an integrated third-trimester screening for FGR.

Evidence for Angiogenic Factors in Third-Trimester Screening for Late Stillbirth

Promising recent data suggest that very low values of maternal plasmatic PlGF/sFlt1 (<0.12 MoM) have a strong association with subsequent stillbirth, leading to 80% sensitivity and 93% sensitivity for detecting late stillbirth [54].

Overall, a third-trimester approach has demonstrated a high predictive value for late complications when compared with first- and second-trimester strategies. Identification of high-risk cases, even in third trimester, might

have a huge impact on maternal and perinatal outcomes. Selection of high-risk subgroups might allow exploring the impact of strategies of targeted customized follow-up, or clinical trials evaluating the benefits (and risks) of elective delivery at term. Future studies will need to define the best combination of clinical, ultrasonographic, and biochemical factors in an integrated third-trimester screening for late complications of placental disease.

Clinical Role in the Diagnosis and Prediction of Adverse Outcomes in IUGR

Angiogenic factors have a major role in vasculogenesis and angiogenesis in the placenta. An imbalance in angiogenic factors is impaired angiogenesis, placental development, and IUGR. As angiogenic factors are very closely related to placental disease and IUGR, recent studies have evaluated the potentiality of maternal PlGF/sFlt1 for assessing severity and predicting the risk of adverse perinatal outcome in IUGR pregnancies.

Clinical Role in the Diagnosis and Management of Preeclampsia

Angiogenic Factors in the Diagnosis of PE

The gold standard for preeclampsia diagnosis is the measurement of blood pressure and proteinuria. Clinicians and researchers alike are aware of the fact that this 'gold standard' is merely a surrogate definition [65]. It relies on the presence of a vague set of signs and symptoms to diagnose a multisystem disorder that may progress to severe complications involving end-organ damage. The pioneering work by Robertson, Brosens et al., published in 1967 has first shown the central role of the placenta in the etiology of the condition [66]. The knowledge about the impact of a failure in trophoblast invasion and defective spiral artery remodeling in preeclampsia has increased tremendously during the past decade due to meticulous research in that field [67–69]. This understanding, however, is not yet reflected in the clinical definition of the disease.

The current definitions for preeclampsia are poor in predicting preeclampsia-related adverse outcomes. Zhang et al. have shown that a diagnosis of preeclampsia based on blood pressure measurement and assessment of proteinuria has a positive predictive value of approximately 30% in predicting preeclampsia-related adverse outcomes [70]. At a time when suspicion of preeclampsia prompted renal biopsies to detect the preeclampsia defining lesion glomerular endotheliosis, studies have shown

Fig. 4. Maternal serum sFlt-1/PlGF-ratio in early-onset versus late-onset preeclampsia: patients with early-onset preeclampsia <34 weeks of gestation have a significantly elevated sFlt-1/PlGF-ratio as compared with gestational age-matched patients with uneventful pregnancy outcomes. The same holds true for late-onset preeclampsia >34 weeks as compared with late controls. * $p < 0.05$. PE = Preeclampsia; sFlt-1 = soluble fms-like tyrosin kinase-1; PlGF = placental growth factor: modified according to [78].

that 20% of primipara and more than 30% of multipara were falsely classified as having preeclampsia based on the clinical definition [71].

The field of preeclampsia research has been shaken up by the discovery of the role of angiogenic and anti-angiogenic factors by the group of Ananth Karumanchi in 2003 [17]. Since these first publications, a multitude of clinical studies has established the ability of these markers to accurately diagnose and predict preeclampsia [72–76]. Meanwhile, automated tests are available to test for the condition in the clinical setting [77–79] (fig. 4). Recently, the results of a European multicenter study have been published while introducing new cut-offs for the sFlt-1/PlGF-ratio in the clinical setting. It is now possible to diagnose preeclampsia with a sensitivity till 95% and a specificity till 99.5% [80].

Angiogenic Imbalance in the Prediction of Adverse Outcomes in Women with Suspected PE

Most clinical studies have chosen the endpoint preeclampsia according to the current definitions to evaluate new strategies for diagnosing or predicting the disease. However, from a clinical perspective, the question must be raised as to whether that endpoint is of any interest to

the maternal-fetal medicine specialist in the clinic or the obstetrician in the delivery ward. In the end, the aim is to identify patients at risk for complications who are known to be associated with the presence of hypertension and proteinuria in pregnancy.

Rana et al. have recently shown that the sFlt-1/PlGF-ratio is able to predict preeclampsia-associated adverse outcomes with a high accuracy [81]. In a prospective multicenter study, they have enrolled 616 patients with signs and symptoms for preeclampsia. The endpoint of this study was not 'gold-standard preeclampsia' but the onset of preeclampsia-associated maternal or fetal adverse outcomes within two weeks. This was a composite outcome of either maternal complications such as a combination of hypertension and pulmonary edema, disseminated intravascular coagulation, and placental abruption (or else) or fetal complications such as indicated early delivery, fetal growth restriction, or fetal death. These patients who developed adverse outcomes have a significantly higher sFlt-1/PlGF-ratio with a median of 4 (25th–75th centile, 2–14) in patients without and a median of 226 (50–547) in patients with early-onset preeclampsia-associated adverse outcomes. Furthermore, the authors were able to show that these patients who presented with an sFlt-1/PlGF-ratio of more than 85 had a significantly reduced remaining pregnancy duration. The predictive accuracy for preeclampsia-associated complications was best for the sFlt-1/PlGF with an area under the receiver operating characteristics curve of 0.89 and significantly better than other clinical tools such as blood pressure or serum markers for HELLP syndrome such as alanine aspartate transferase. In twins, similar results were shown [82].

Is There a Need to Revise Our Clinical Definition and Management of Preeclampsia?

In the light of the increasing understanding of the central role of the placenta in the pathophysiology of preeclampsia, the current 'gold standard' of preeclampsia diagnosis must be revised. Till a few years ago, no biomarker was available to reliably assess the placental function in the clinical setting. Revising the gold standard does not simply mean to change the diagnostic criteria for 'preeclampsia'. Future interest must be directed toward a re-definition of this condition that is now called preeclampsia. Hypertension and proteinuria are just arbitrary markers for a syndrome that we did not have the means to previously describe better. Possibly, this newly defined disease will include a slightly different cohort of patients: those at risk for adverse outcomes. A cohort of patients who were diagnosed with increased blood pressure and proteinuria will

not be classified at risk for adverse outcomes based on the measurements of the angiogenic factors, whereas others not fulfilling the current diagnostic criteria for preeclampsia will be based on their biochemical test result. It has been shown that patients with 'non angiogenic preeclampsia' are not at risk for adverse outcomes, whereas those with an increased sFlt-1/PlGF-ratio are at risk [83].

A limitation to this approach is the role of late-onset disease. Several authors have proposed a 'two disease theory' of preeclampsia, based on the clinical observation that early-onset preeclampsia has a predominant placental component and late-onset preeclampsia often coincides with preexisting maternal disease such as features of metabolic syndrome [84]. This dichotomization of placental (early onset) and maternal (late onset) preeclampsia is likely to be simplistic. The levels of angiogenic and antiangiogenic factors, however, are less disparate when comparing late-onset preeclamptic patients with term controls [85]. This again might relate to the nature of the (case-control) studies: new diagnostic markers have to be evaluated with the current gold standard. Hence, the 'control group' in these studies might be, in fact, a heterogeneous group involving patients with features of (late onset) placental dysfunction or decreasing placental function, as levels of sFlt-1 are higher and those of PlGF are lower in normal pregnant women near term than before 34 weeks. Likewise, the late-onset preeclampsia group without angiogenic dysbalance might not be a homogeneous group. When looking at birth weight as a proxy for placental function in late-onset preeclampsia patients, a bimodal, skewed distribution is observed, highlighting a component next to placental insufficiency in late-onset disease [86].

Options for Treatment

Till now, the only causative treatment for preeclampsia is delivery. In case of early-onset disease, this contributes to neonatal morbidity due to preterm delivery. The research on the pathophysiological role of the angiogenic imbalance for preeclampsia opened up the field for a novel therapeutical option. An excessive placental release and abundant serum concentration of sFlt-1 has been identified as a pacemaker of the disease. Ravi Thadhani and colleagues now hypothesized that lowering the circulating sFlt-1 concentrations in the maternal blood might ameliorate the disease and prolong pregnancy. With the use of dextran sulfate apheresis, a treatment method that has been previously shown to be safe and feasible in pregnancy for other indications such as familiar hypercholesterinemia [87], a removal of sFlt-1 from the maternal

blood was performed. In a pilot study involving 8 women with severe early-onset preeclampsia, removal of sFlt-1 led to reduced proteinuria, stabilized blood pressure without any adverse events for mother and fetus. Most of all, a prolongation of pregnancy till 23 days was achieved [88]. If the results of this pilot study are confirmed by larger randomized trials, a therapeutic option for severe early-onset preeclampsia might contribute to an improvement of maternal and fetal preeclampsia-related morbidity and mortality.

New Evidence in Congenital Cardiac Defects

Animal studies [89–91] suggest that VEGF signaling plays a critical role throughout cardiac formation and that its levels must be tightly regulated for normal cardiac formation to occur [92]. Abnormal expression of angiogenic markers in heart tissue of human fetuses with CHD has been demonstrated, which showed increased VEGF-A and sFlt-1 expression [93].

Moreover, in isolated major fetal heart defects, maternal serum PlGF levels at 11–13 weeks of gestation were decreased, suggesting impaired placental angiogenesis [94]. In addition, at 18–37 weeks of gestation, maternal serum PlGF was decreased and sFlt-1 was increased in women carrying a fetus with isolated major heart defects. In both studies, this angiogenic impairment was observed in the presence of conotruncal and septal-valve defects but not in the presence of left heart defects [93, 94]. These findings suggest that abnormal angiogenesis may also be deleterious for embryogenesis of the human heart.

Further studies are warranted to ascertain whether these fetuses with CHD have an intrinsically altered angiogenesis, leading to an abnormal formation of the heart, that may be also present in trophoblastic cells or alternatively, low PlGF may be associated with a lesser degree of trophoblast invasion of the spiral arteries and therefore placental hypoxia that triggers abnormal heart development [95, 96].

Conclusions

The discovery of the role of angiogenic and anti-angiogenic factors in 2003 [17] has increased the acknowledgment of the pathophysiology of preeclampsia. This evidence leads to an exploration of the potential usefulness of angiogenic factors as markers for the prediction of the disease. The combination of maternal risk factors, angio-

genic factors, and uterine artery Doppler velocimetry in the first trimester can predict subsequent development of early-onset preeclampsia with a sensitivity till 93% for a 5% false-positive rate. More recently, third-trimester screening has emerged as another important hallmark in the stratification of patient risk; thus, most cases of PE and IUGR occur at or near term, including a substantial proportion of maternal morbidity and medically indicated late preterm births. Angiogenic factors have also shown good sensitivity for the third-trimester prediction of late PE and stillbirth. Early estimation of patient-specific risks for preeclampsia and other placental insufficiency complications could potentially improve the outcome by directing such patients to specialist clinics for close surveillance and potentially, as well as early pharmacological interventions, such as aspirin, starting from the first trimester of pregnancy.

In the light of the increasing understanding of the central role of the placenta in the pathophysiology of preeclampsia, the question must be raised as to whether this information must be included in diagnostic algorithms. Future interest must be directed toward a re-definition of this condition that is now called preeclampsia. It is possible that angiogenic status would be useful to improve detection, define prognosis, and provide directions for best clinical management of hypertensive diseases of pregnancy and established preeclampsia.

Finally, angiogenic factors are also involved in other maternal pathologic conditions of pregnancy such as peri-partum cardiomyopathy and fetal cardiac defects. Abnormal angiogenesis in the heart tissue of human fetuses with CHD has been demonstrated. Moreover, in isolated major fetal heart defects, maternal serum PlGF levels were decreased and sFlt-1 was increased in women carrying a fetus with isolated major heart defects. We believe that evaluating the relationship between CHD and placental-related complications is an important hypothesis to explore the subject of future research.

Future studies focusing on the regulation of angiogenic gene products and their role in placental angiogenesis and systemic vascular health will lead to a better understanding of the relationship between preeclampsia and fetal programming in intrauterine growth restriction with the development of cardiovascular disease [97].

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E.L.L. and F.C. declare no conflicts of interest.

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