SFLT-1/PIGF NEW BIOMARKERS FOR MULTIPLE ADVERSE PREGNANCY OUTCOMES

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SFLT-1/PIGF NEW BIOMARKERS FOR MULTIPLE ADVERSE PREGNANCY OUTCOMES (Abstract): Placenta insufficiency and some related pregnancy outcomes are the result of the imbalance between angiogenic and antiangiogenic factors expressed at placenta level. From the angiogenesis factors, only a few have found practical applications, such as the serum soluble fms-like tyrosine kinase-1 (SFlt-1) / PIGF (Placenta growth factor) ratio, named also the angiogenic fraction, which is increased in pregnant women before the clinical onset of a Preeclampsia (PE), intrauterine growth restriction (IUGR) or uterine apoplexy. PROGNOSIS study demonstrated that sFlt-1 / PIGF ratio<38 predicts the absence of PE, eclampsia and HELLP (Hemolysis, Elevated Liver enzymes , Low Platelet count) syndrome within four weeks with a PPV(Positive Predictive value), (95%CI range) of 99.3% (97.9-99.9).Other approaches used different cutoffs for early and late preeclampsia (onset before or after 34 weeks), to enhance the diagnostic accuracy, or included the sFlt-1 / PIGF ratio in a statistical model, along with maternal risk factors, mean arterial pressure (MAP) measurement and uterine artery pulsatility index (UtA-PI), to improve first trimester prediction of PE. Considering the therapeutic applications of angiogenic factors, the use of statins (pravastatin) on animal models to treat PE seems to be promising, as well as the administration of (Vascular endothelial growth factor) VEGF 121 or extracorporeal removal of sVEGF-1.
Keywords: PREECLAMPSIA, ANGIOGENIC PLACENTAL FACTORS, ANTIANGIOGENIC PLACENTAL FACTORS, SFLT-1 / PIGF RATIO.

Placental insufficiency is responsive for many pregnancy pathological conditions as: preeclampsia (PE) (a pregnancy related disease associated with hypertension and proteinuria); uterine apoplexy (UA) or placental abruption (representing early separation of the placenta from the uterus before delivery). These can lead to maternal complications such as: convulsions, disseminated intravascular coagulopathy, hepatic and kidney failure and fetal reserved outcomes such as: fetal growth restriction (FGR), preterm delivery and stillbirth (1).

Placental insufficiency is due to alterations that start during placental development, when vessel formation occurs initially by vasculogenesis followed by branching and nonbranching angiogenesis. Vasculo-

genesis involves de novo formation of blood vessels from mesodermally derived precursor cells, while angiogenesis means new vessels creation from pre-existing vessels (2) and is classified in branching and nonbranching stages.

Branching angiogenesis occurs mainly in the first and early second trimesters and leads to the formation of an immature villous tree. During this phase, a dramatic decrease of the vascular resistance and increase in the blood flow through the placenta is produced by the progressive loss of the musculo-elastic media in the walls of the maternal spiral arterioles. The villous vascular branching adds this process on the fetal side, allowing the maternal and fetal circulations to convert to low resistance high -capacitance vascular bed circulations. Nonbranching angiogenesis stage starts at about 26 weeks of gestation and continues until term. During this phase, a new villous type arises, the mature intermediate villus that is specialized in gas exchange. In this type of villous tree, the tips of the villi are long and slender, and the capillary network creates several loops, the final capillary loop exceeding 4,000 m in length (3). Placentation process requires the involvement of angiogenic growth factors, but also of other molecules such as cytokines, extracellular matrix metalloproteinases, hormones and transcription factors (4). Immunohistochemistry studies shows that human placenta expresses angiogenic growth factors and their receptors, as well as antiangiogenic factors (5).

The angiogenic factors

The expression of angiogenic factors is, itself, regulated by oxygen pressure and mechanical stimuli (5). Placenta insufficiency and some abnormal pregnancy outcomes which derive from this insufficiency, are the result of the imbalance between angiogenic and antiangiogenic factors at the placenta level together with local inflammation.

Different studies described the expression of angiogenic factors at placenta level (2, 5, 6):

- fibroblast growth factor (FGF),
- hepatocyte growth factor, (HGF) and its receptor, c-met,
-vascular endothelial growth factor, (VEGF) and its receptors: VEGFR-1(Flt-1) and VEGFR-2 (flk-1/KDR),
- placenta growth factor (PIGF).

The anti-angiogenic factors

- placenta antiangiogenic factors are represented mainly by serum soluble fms-like tyrosine kinase-1 (s-Flt-1) and soluble endoglin (7).

Angiogenic and antiangiogenic placenta factors: Structure, Expression and Receptor-Binding Properties

Cysteine-knot proteins are a group of structurally related dimeric proteins comprised of eight characteristically spaced cysteine residues with intra- and interchain disulfide bonds between them. PlGF, VEGF(VEGF-A), VEGF-B, VEGF-C, VEGF-D, VEGF-E, and Fos-induced growth factor (FIGF), are the members of said group of growth factors and have in common several biochemical and functional features (i.e. PlGF and VEGF have the ability of forming heterodimeric molecules in the cells where both genes are expressed. VEGF was the first member of the above-mentioned family of growth factors to be identified and isolated. The VEGF-A homodimer exerts its biological activities

through activation of two distinct tyrosine kinase receptors: *fms*-like tyrosine kinase receptor-1 (Flt-1; also known as VEGFR-1) and the kinase domain-containing receptor/fetal liver kinase receptor (KDR/Flk-1; also known as VEGFR-2). Endothelial cells are the main target of this factor, because of their high expression of VEGF receptor-1 (VEGFR-1 or Flt-1) and VEGF receptor-2 (VEGFR-2 or Flk-1/KDR); the effect of VEGF over endothelial cells is to stimulate their proliferation (2, 6).

PlGF is a member like VEGF, more recently identified and described, and has, together with VEGF an important role in the stimulation of endothelial cell growth and, thus, angiogenesis. Placenta growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, is one of the key regulators of pathological angiogenesis, as demonstrated by gene inactivation studies. The PlGF primary transcript gives birth to four forms of the mature PlGF proteins (8) of which PlGF-1 and PlGF-2 (also denominated as PlGF-131 and PlGF-152, respectively), are the predominant forms, which differ in terms of structure by the insertion of a highly basic 21-aminoacid stretch at the carboxyl end of the protein, which consecutively gives the ability of binding to heparin to PlGF-2.

Antiangiogenic factors activated in preeclampsia are: soluble endoglin and sFlt-1(7). Endoglin is a protein that is abundantly expressed in the cell membrane of the syncytiotrophoblastic and vascular endothelium and acts as a co-receptor of transforming growth factor β1 and β3 (TGF-β1 and TGF-β3) (7). In consequence, soluble endoglin acts as an inhibitor to TGF-β1 signaling in the vascular endothelium, resulting in an antiangiogenic effect (18,19). In preeclampsia, soluble endoglin is released into maternal circulation, due to an up-regulation of placental endoglin (8). Circulating soluble endoglin level was shown to increase 2-3 months before onset of PE. Soluble fms-like tyrosine kinase 1 (sFlt 1) is a splice variant of the above-mentioned VEGF receptor, that is lacking cytoplasmic and transmembrane domains, thus acting like an important VEGF and PlGF antagonist (8).

According to one study found in literature, on a rodent model, modest hypertension without significant proteinuria and increased vascular permeability were obtained because of overexpression of soluble endoglin, by means of adenoviral vectors (8). In addition, overexpression of sFlt 1 and endoglin, caused, in a similar fashion, fetal growth restriction and severe vascular damage, nephrotic-range proteinuria, severe hypertension, thus a syndrome like HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets).

In conclusion, endoglin and sFlt 1 are two proteins with similar antiangiogenic effects, obtained through different mechanisms, which may combine producing endothelial dysfunction and severe preeclampsia.

Correlations of angiogenesis with fetal hypoxia

There are three different types of hypoxia that may occur in the fetoplacental unit and may influence fetoplacental angiogenesis: *pre-placental hypoxia, uteroplacental hypoxia and post-placental hypoxia* (5).

1. Pre-placental hypoxia. The mother, the placenta and the fetus are hypoxic, as in pregnancy at high altitude, maternal anemia and cyanotic maternal cardiac diseases. In this condition the peripheral placental villi show increased branching angiogenesis with formation of richly branched but

shorter terminal capillary loops.

2. Uteroplacental hypoxia. In this situation, maternal oxygenation is normal, but the placenta and the fetus are hypoxic, due to an impaired utero-placental circulation. This condition characterizes the preeclampsia with preserved end diastolic flow at umbilical artery.

In this case, the peripheral placental villi show formation of richly branching nests, as in the previous situation, and fetal blood flow is normal or slightly reduced.

Semi-quantitative Western blotting analysis shows in placentae of this type compared to normal placentae, an increased expression of VEGF and reduced expression of PlGF (5), suggesting that placental hypoxia, upregulates VEGF *in vivo* and causes changes in angiogenesis.

3.Post-placental hypoxia.

In this situation, the fetus is hypoxic, whereas the mother is normoxic and the placenta may show higher PO_2 levels than normal, a situation described as 'placental hyperoxia'.

The histopathological analyses show terminal capillaries that are poorly developed and capillary branching that are virtually absent. At ultrasound, the end diastolic flow at umbilical artery is zero or negative.

In this circumstance, the perinatal mortality is around 40% and survivors of neonatal intensive care are at risk of neurodevelopmental handicap. In these cases, PIGF is increased, while VEGF is decreased, suggesting that early onset of placental hyperoxia by enhancing PIGF, induces decrease of branching angiogenesis and failure of terminal villi formation.

Clinical applications of placental angiogenic/antiangiogenic factors

Preeclampsia is an imbalance between pro-angiogenic and anti-angiogenic factors. The ratio sFlt-1/PIGF is elevated in pregnant women before the clinical onset of preeclampsia, and many studies have been conducted, to establish the best sFlt-1: PlGF ratio cutoff to predict the clinical onset of PE in women with risk factors (9).

PROGNOSIS (Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia *Study*), was a multicenter, prospective, double-blind, non-interventional trial, evaluating the short-term prediction of preeclampsia, eclampsia (maternal convulsions due to complicated PE) and HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome (PE complicated with hepatic insufficiency and coagulation disorders), in pregnant women with suspected preeclampsia. In this study, more than 1,270 pregnant women were enrolled at 30 sites in 14 countries, between December 2010 and January 2014. Results of the PROGNOSIS study were published by Zeisler *et al.* (10).

This study demonstrated that a ratio of the proteins sFlt-1/PlGF of 38 and below predicts an absence of preeclampsia, eclampsia and HELLP syndrome for one week , with a negative predictive value (i.e., no preeclampsia in the subsequent week) of 99.3% (95% confidence interval [CI], 97.9 to 99.9), whilst an sFlt-1/PlGF ratio above 38 predicts onset of preeclampsia, eclampsia and HELLP syndrome within four weeks, with a positive predictive value of 36.7% (95% CI, 28.4 to 45.7), with 66.2% sensitivity (95% CI, 54.0 to 77.0) and 83.1% specificity (95% CI, 79.4 to 86.3).

In 2013, the same team separated early preeclampsia (onset between 20 and 34 weeks of pregnancy) from late preeclampsia (onset between 34 and 37 weeks of pregnan-

cy). For each of the 2 gestational periods, 2 independent cutoffs framing an equivocal zone were determined: the first cutoff with focus on high sensitivity, and the second focusing on high specificity (11).

Between 20 and 34 weeks, the cutoffs at ≤33 and ≥85 resulted in a sensitivity/specificity of 95%/94% and 88%/99.5%, respectively. After 34 weeks, the cutoffs at ≤33 and ≥110 yielded a sensitivity/specificity of 89.6%/73.1% and 58.2%/95.5%, respectively. The approach to use multiple cutoffs for the early and late preeclampsia phases, enhances the diagnostic accuracy of the sFlt-1/PlGF ratio as a diagnostic tool for hypertensive complication during pregnancy (11).

Some teams, specialized in analyzing different statistical models in the screening of preeclampsia, included biomarkers of angiogenesis (Sflt/PIGF) in a combination of other markers and examined the performance of screening for PE by maternal factors alone and by maternal factors with the addition of each biomarker individually and combinations of biomarkers (12).

Using maternal risk factors, mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF), this combination could identify, at a 5% false-positive rate (FPR), 98% of cases of early PE, but only 70% and 33% of intermediate and term PE, respectively. Screening at around 32 weeks by a combination of maternal factors with MAP, UtA-PI, PlGF and serum soluble fms-like tyrosine kinase-1 (sFlt-1) could identify, at a 5% FPR, 98% of cases of intermediate PE, but only 54% of term PE (12). PlGF and sFlt-1 levels are simultaneously affected in PE (especially in early pre-eclampsia); thus, the sFlt-1/PlGF ratio represents now a predictor element superior to the individual analysis of these markers.

Another approach in assessing these markers might consist in the sequential determination of serum levels, an approach that has provided better predictive results than the punctual assessment in of the pregnancy a certain moment. The sensitivity of sequential determinations of the sFlt-1/PlGF ratio is of 100% and its specificity is of 98-99% (13).

In the original article, sFlt-1: PlGF ratio, named also "preeclampsia fraction", was performed on Elecsys® Roche Platform (14). The sFlt-1/PlGF ratio is well described and it is a marker for prediction of the PE. Burke *et al*. (14) demonstrated that measurements of PlGF are reliable if we use different techniques and different platforms (R&D Systems, AlereTriage, Roche Elecsys or Abbott Architect).

Future therapeutic uses of angiogenic-antiangiogenic factors

We are waiting for the moment when angiogenic factors will be used as therapy for the prevention of adverse pregnancy outcomes due to placental insufficiency: preeclampsia, uterine apoplexy, fetal growth restriction, in utero fetal death and some cases of preterm delivery. For the moment, there are some promising studies on animals. Sulistyowati *et al*. (15) demonstrated that injection of recombinant VEGF121 has the potential to prevent fetal growth restriction in a newly proposed preeclampsia mouse model (*Mus musculus*).

There are studies on samples of placenta and blood vessels, as well as animal experiments suggesting that statins can reduce the level of abnormalities in angiogenic/anti-angiogenic balance, and perhaps reduce or eliminate the effects and risks of pre-eclampsia. This has been previously

demonstrated in stillbirths of unknown etiology. Statins (HMG CoA reductase inhibitors), seem to positively influence the inflammatory, anti-angiogenic milieu of pregnancies with underlying placental ischemia by their pleiotropic effects. This might prevent, ameliorate and delay preeclampsia (16). Esteve-Valverde *et al*. (17), in a systematic search to summarize the role of statins for preventing and treating severe preeclampsia, found 13 studies about statin's specific use during pregnancy. The review shows a potential beneficial role of statins in preventing and treating severe preeclampsia, meanwhile the rate of major congenital abnormalities in the newborn exposed to statins during pregnancy is no higher than the overall risk population.

Other proposed therapeutic interventions to reverse an anti-angiogenic status during pregnancy include the administration of VEGF 121 or extracorporeal removal of sVEGFR-1(18).

CONCLUSIONS

Our study wishes to be an update concerning the factors of placental angiogenesis and their role in the diagnosis and treatment of preeclampsia and other associated pathologies.

The structure of angiogenesis factors has been known since the '70s, but their clinical applications in placental insufficiency screening and the main obstetric syndromes induced by it, have only emerged in the last few years.

The ratio of variable pro-angiogenic and anti-angiogenic factors characterizes different situations of fetal hypoxia, which could be used in the clinical practice.

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NEWS

DIAGNOSIS IN MENINGITIS: AT THE BORDER BETWEEN MEDICINE AND COMPUTER SCIENCE

We all live in a world marked by the stamp of technology and information. In these circumstances, the biggest challenge for the information era is to establish some diagnoses using only computer science, without the help of human mind. In other words, the final goal is to develop performing programs which can diagnose a disease using just a set of parameters. For the first time, the researchers developed an original method that can differentiate between bacterial and viral meningitis. This is based on the DRSA methodology and it is constituted by a set of decision rules, like "if…then...". The method had results with a coverage factor between 2 and 95% and this can maintain the interest on the topic. Although the establishment of the diagnosis by computer seems to be utopian at this moment, this study opens the gate of a new universe of research with big chances of feasibility and practicability in future medicine (Gowin E, Januszkiewicz-Lewandowska D, Słowinski R, Błaszczynski J, Michalak M, Wysocki J. With a little help from a computer: discriminating between bacterial and viral meningitis based on dominance-based rough set approach analysis. *Medicine* 2017; 96: 32(e7635).

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