Maternal–fetal interactions, predictive markers for preeclampsia, and programming

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ABSTRACT
During pregnancy close interactions between the maternal system and the fetal system via the placenta exist that result in a powerful crosstalk between both individuals. Looking for predictive biomarkers in maternal blood is extremely difficult because of this crosstalk as such markers may be derived from only maternal sources, only placental sources or both. In particular, the concentrations of markers derived from both sources may vary because of the huge variety of reasons and sources. During the last decade this has misled a number of scientists and clinicians who tried to decipher the sources of markers and the impact of the placenta and/or the maternal vascular system. A few examples for predictive biomarkers are presented, the placenta-specific marker placental protein 13 (PP13) and the angiogenic marker PlGF being released from both mother and placenta. Finally, a further reason why biomarkers may not be successful in predicting all cases of preeclampsia is that different causative routes lead to the development of preeclampsia. The differences in the development of preeclampsia not only explain why markers may or may not have a predictive value, but also why some mothers and/or children may display long-term effects later in life.

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1. Introduction

During pregnancy in the human, fetal cells come into direct contact with maternal cells and tissues. This close contact brings together cells of two genetically different individuals. While this type of contact is typically associated with rejection processes, during normal pregnancy there is neither harm to the mother nor rejection of the baby.

Looking at the sites of contact, it becomes obvious that contact with the maternal cells is facilitated by fetal trophoblast, which is derived from the trophectoderm of the blastocyst. The trophoblast develops into two main subtypes, villous and extravillous trophoblast. The villous trophoblast develops into the epithelial cover of all placental villi with the syncytiotrophoblast as the outermost layer in direct contact with the maternal plasma in the first trimester of pregnancy and the maternal blood during the rest of gestation. The second layer, composed of villous cytotrophoblasts, does not come into direct contact with maternal tissues. If this happens accidentally, e.g., by local damage of the covering syncytiotrophoblast, then such cells are covered by fibrin-type fibrinoid and start to secrete their own matrix-type fibrinoid, similar to extravillous trophoblasts (Kaufmann et al., 1996). Extravillous trophoblasts invade into maternal uterine tissues, crossing the decidua and finally reaching the myometrium. On their way, extravillous trophoblasts...
also migrate toward decidual arteries (spiral arteries) and toward uterine glands, opening both systems toward the intervillous space of the placenta (Burton et al., 2002; Moser et al., 2010, 2011). Opening of the uterine glands enables histiotrophic nutrition during the first trimester of pregnancy, when spiral arteries are still not opened toward the placenta or plugged by endovascular trophoblast. With the onset of maternal blood flow toward the placenta at the beginning of the second trimester, the opening of the spiral arteries ensures hemotrophic nutrition of the fetus until delivery (Burton et al., 2002; Moser et al., 2010).

2. Preeclampsia

Preeclampsia remains one of the major reasons for maternal, fetal, and neonatal mortality and morbidity. Worldwide, the rate of preeclampsia is about 2–8% of all pregnancies, the higher rates are mostly present in developing countries (ACOG, 2002; WHO, 2005). Here, preeclampsia accounts for 10–15% of maternal deaths, 12% of infants born small for gestational age (SGA), and up to 25% of stillbirths and neonatal mortality rates (Duley, 2009). Although preeclampsia is a pregnancy-specific syndrome, it has long-term consequences for those women who experience preeclampsia during pregnancy. Such women are at increased risk of a variety of diseases, including chronic hypertension, diabetes mellitus, ischemic heart disease, cerebrovascular disease, kidney disease, thromboembolism, hypothyroidism, and even impaired memory (Williams, 2011).

There is a multitude of pregnancy-related pathological conditions that cause morbidities and mortality of mothers and children. One of the most common syndromes, preeclampsia, is still the syndrome of hypotheses as its etiology and progress are still unclear in a number of facets. At the same time, it is common knowledge today that the placenta is essential for the development of the clinical symptoms of preeclampsia, while a fetus is not necessarily needed. Hence, in preeclampsia the normal interplay between mother and placenta (and thus the fetus) is dysregulated, finally culminating in the maternal symptoms specific for preeclampsia. While the placenta is releasing factors even during normal pregnancy, it has been shown that in most cases of preeclampsia the release of trophoblastic factors from the placenta is altered because of a dysfunctioning syncytiotrophoblast. On the other hand, the response of the mother depends on her susceptibility to factors derived from the syncytiotrophoblast. The complex interplay between factors released from the placenta and the response of the maternal vascular system finally defines whether or not a pregnant woman develops preeclampsia. Specific features of the subtypes of preeclampsia, including severity, duration of progression, time of onset, and progress to eclampsia, can be explained by the fine tuning of the interactions between the maternal and the placental systems. This very much complicates the search for the etiology of preeclampsia and poses the need to broaden the view and include the interactions among fetus, placenta, and mother, even if the origin of preeclampsia can clearly be attributed to the placenta.

3. Predictive biomarkers for preeclampsia

The hype in identifying new biomarkers to predict preeclampsia is leaving us with not a single biomarker on the market that is of any predictive value. The last decade has seen a huge number of different biomarkers coming and going, leaving only a trace in the scientific literature. Generally, a marker should only be named predictive and is only valuable if the detectable changes of this biomarker occur prior to the onset of clinical symptoms of a disease or syndrome. It would be best to focus on only those markers that display significant differences as early as possible and as specifically as possible. In the case of preeclampsia a biomarker should show such alterations as early as the first trimester of pregnancy – or even prior to pregnancy. Early prediction of preeclampsia enables the planning of an appropriate management strategy and offers close surveillance of pregnant women at risk (Cetin et al., 2011).

Besides showing significant changes as early as possible, a predictive biomarker for preeclampsia should fulfill the following criteria (Cetin et al., 2011):

The marker should be specific for the syndrome and should not show a clear influence of other syndromes, such as IUGR or diabetes.
The marker should be obtained following a non- or minimally invasive procedure, such as blood, urine or saliva.
The marker should be stable in the sample or simple protocols should be available to maintain the marker’s stability.
The marker should be unaffected by changes in other components in the sample such as hemoglobin, uric acid or lipids.
The test should be accurate and sensitive and recognize only the marker of interest.
The test should be able to predict preeclampsia before clinical symptoms are evident.
The test should be specifically for preeclampsia and not interfere with other pathological conditions such as IUGR or diabetes.

Since most of the biomarkers available today are based on proteins found in the blood/plasma/serum samples of pregnant women, kits have been developed to measure even very low concentrations (up to pg/ml) in such samples. However, what is still lacking for most if not all such biomarkers are the kinetics and knowledge of the composition of these biomarkers in maternal blood. The markers may only be of maternal or only of fetal (placental) origin, or they may be derived from both individuals.

If a marker is only derived from the placenta, a direct correlation with placental development and physiology may be possible. Such factors derived from the placenta may subsequently induce the release of maternal factors, leading to alterations in their blood levels as well. Such a maternal biomarker may be used as a biomarker, without anything being known about the signaling pathways leading to such alterations. The only marker known today to be of pure placental/fetal origin is placental protein 13 (PP13 or galectin 13), which is expressed in the placenta and released from the syncytiotrophoblast into maternal blood (Huppertz et al., 2008, 2013). Recently, it has been
shown that PP13 may induce vasodilatation and decrease blood pressure in pregnant rats (Gizurarson et al., 2013). If the carbohydrate binding site of PP13 was deleted specific effects of PP13 were no longer present (Sammar et al., 2014). These data show that a biomarker derived from the placenta may well have direct biological effects on maternal physiology. If a factor is only derived from the mother and not present or released from the placenta, it may give an idea of the response of the mother to pregnancy. Such markers from maternal sources could be altered because of an inflammatory or metabolic response of the mother to the changing environment during pregnancy and especially pregnancy-related pathological conditions. At the same time, such markers may simply be derived from damaged and dying maternal cells. To the best of my knowledge, no such marker is known today.

Most of the markers in use today are derived from both sides: from the mother and the placenta/fetus. Here, a direct correlation with mother and/or fetus/placenta is extremely difficult as it is not clear how much of this marker is derived from which individual. It also needs to be taken into account that interactions between maternal and placental factors may lead to an increase or a decrease in specific markers in the maternal blood. Hence, even if a marker increases in placental tissues, it may decrease in maternal blood as most of this serum marker is released from maternal sources. Below, some examples are shown to illustrate the joint production of markers.

3.2. Angiogenic factors

Quite a number of pro- and anti-angiogenic factors together with their receptors are expressed and active in the placenta throughout gestation. These factors include vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and endoglin as well as VEGF-receptor 1 (Flt-1) and VEGF-receptor 2 (Flk-1). The normal action of these factors and receptors in the placenta is mandatory for normal development and changes in placental vessels throughout pregnancy (Clark et al., 1998). To use such factors as predictive markers of preeclampsia they need to be released into maternal blood rather than into fetal blood, where they act on placental angiogenesis. However, only a fraction of angiogenic factors and receptors are released from the placenta into the maternal circulation, and only those being released can be used as markers in maternal blood, such as PIGF.

PIGF is expressed by all populations of trophoblast, including villous and extravillous trophoblast (Clark et al., 1998). Mostly neglected but important to note is that PIGF is also expressed in endothelial cells and smooth muscle cells of healthy adults (Pan et al., 2010). Hence, the concentration of PIGF detected in the blood of pregnant women is the summation of the release from placental sources plus the release from different maternal sources.

Unfortunately, the concentration of PIGF in maternal blood is not only the summation of the release from placental and maternal sources, but rather becomes even more complex since the soluble form of a receptor to PIGF and VEGF (soluble Flt-1, sFlt-1) is released (again from placental and maternal sources) into maternal blood and is circulating in the blood of pregnant women. This scavenging receptor binds to VEGF and PIGF circulating in maternal blood and thus decreases the fraction of PIGF that is freely floating in maternal blood. The assays available today only bind to and recognize nonbound and freely available PIGF. Hence, even in the presence of a stable release of PIGF from maternal and placental sources, simply an increase in the release of sFlt-1 (Levine et al., 2004) will lead to a virtual reduction of PIGF (Akolekar et al., 2008) owing to the binding of PIGF to sFlt-1.

As can be seen from the above, predicting the levels of a predictive marker such as PIGF in maternal blood is extremely difficult and may lead to incorrect assumptions and hypotheses (Staff et al., 2013). These authors assumed that PIGF is solely expressed in the placenta and built their hypotheses on these grounds, always referring to PIGF as a placenta-derived factor. However, there are at least two reasons why this cannot be as easy as the authors proposed. First, comparing PIGF levels in non-pregnant cancer patients with those in pregnant women revealed the following picture. In pregnant women PIGF levels before 28
weeks’ gestation were 18.4 pg/ml (Meler et al., 2014), while in patients with oral squamous cell carcinoma the levels increased from 10.1 pg/ml in healthy non-pregnant controls to 19.1 in cancer patients prior to surgery (Cheng et al., 2012). Second, it has been shown that the protein expression of PI GF in the placenta and that in CD105- and CD34-positive endothelial cells are very similar (Su et al., 2004). Assuming a similar release of PI GF from the two sources expressing PI GF, the surface area of these sources comes into play. The surface area of a term placenta was estimated to be 12–15 m² (Benirschke et al., 2006), while the endothelial surface area of blood vessels in an adult was estimated to be about 4000–7000 m² (Aird, 2005). Both reasons make it very unlikely that the sole source for PI GF in maternal blood is the placenta. However, so far it is totally unclear what the placental and maternal contributions of PI GF are to the total pool of PI GF in maternal blood.

The angiogenic markers, including PI GF, suffer from another major disadvantage. It has become clear during the last few years that angiogenic factors are not at all specific for preeclampsia (Robinson et al., 2006), but rather show an even better predictive value for patients suffering from pure IUGR (Stepan et al., 2007; Cowans et al., 2010). This is why angiogenic markers have a good predictive value for early onset patients suffering from preeclampsia and IUGR, while they have no predictive value for more than 80% of all preeclampsia cases, i.e., those at term without growth restriction (Smith et al., 2007) (Fig. 1).

4. Etiology of preeclampsia

After defining the needs for predictive biomarkers, the final chapter will clarify why a single marker or even a combination of markers may still fail to explicitly predict preeclampsia. We know that the placenta is mandatory for the development of preeclampsia; however, only the interplay between mother and fetus/placenta finally decides whether or not the mother develops clinical symptoms. Hence, the impact of each side on the clinical onset of the syndrome needs to be considered (Huppertz, 2008).

Taking into account the close interactions between maternal and feto-placental factors, at least three different scenarios can be developed to explain the different types and times of the onset of preeclampsia as well as the different effects on the health of the mother and fetus later in life (Fig. 2):

1. Cases consisting of a normal mother before pregnancy and a dysfunctioning placenta

Normally, the syncytiotrophoblast releases factors by controlled secretion and apoptotic shedding. In this set of cases developing preeclampsia, necrosis, and
markers such as PP13 can identify such cases already during the first trimester of pregnancy (Huppertz et al., 2008). In this scenario, the fetus mostly develops into a normally grown baby (no growth restriction).

After pregnancy, the mother may recover completely without any deleterious effects later in life.

2. Cases consisting of a predisposed mother and a normal placenta

This time, the placenta releases the normal portfolio of fragments, factors, and molecules into the maternal blood. However, the mother’s scavenging system, her vascular system or any other signaling pathway involved in regulating maternal blood pressure and kidney function may not work properly. This may lead to an inadequate response of these systems, resulting in an overload and defect even by the normal quantity and quality of factors released from the placenta. This scenario may be predicted by a number of factors known to predispose a woman to developing preeclampsia, such as chronic hypertension or renal disease.

Since the placenta has normal growth and development and the malfunctions of the maternal systems slowly develop into preeclampsia, this scenario results in the appearance of clinical symptoms late during pregnancy (late onset preeclampsia). Also, the fetus may not be affected, resulting in a baby with no growth restriction. Again, changes in predictive angiogenic markers such as PI GF and sFlt-1 prior to the onset of symptoms are mostly absent (Ohkuchi et al., 2007), and due to a normally developed placenta, also placenta-specific markers may not be able to predict such cases.

Since women in this scenario already suffer from mostly subliminal defects of their vascular system, they may well be at a higher risk of developing cardiovascular diseases later in life.

3. Cases consisting of a predisposed mother and a dysfunctioning placenta

This scenario includes the most severe cases as the combination of defects on the maternal and on the placental side will definitely lead to more severe symptoms in the mother.

Looking at the placenta, there may be a subpopulation only showing alterations of the villous trophoblast, especially the syncytiotrophoblast. Here, the syncytiotrophoblast displays necrotic and aponecrotic shedding of subcellular fragments, resulting in a systemic deterioration of the maternal vascular system, which is already impaired. The damage to the maternal system will develop quickly and clinical symptoms will appear early in pregnancy (early onset preeclampsia) with no effect on the growth of the fetus (no growth restriction). Such cases will be easily predicted by placenta-derived markers such as PP13; however, since fetal growth is not affected these cases will not be able to be predicted by angiogenic markers such as sFlt-1 and PI GF.

There may be a larger subpopulation of cases with a maldeveloped placenta that also show defects in the development of the extravillous trophoblast and thus will display impaired trophoblast invasion (Huppertz, 2011). In those cases the syncytiotrophoblast is affected...
and releases factors by necrotic and aponecrotic shedding, while the maldeveloped extravillous trophoblast will alter maternal blood flow toward the placenta (Burton et al., 2009), subsequently impairing fetal growth. Again, the damage to the maternal system will develop early in pregnancy (early onset preeclampsia) and growth of the fetus will be impaired as well (growth restriction). Such cases will be easily detectable by all markers, placenta-specific (Chafetz et al., 2007) and angiogenic markers (Schaarschmidt et al., 2013).

In this scenario, women may well be at a higher risk of developing cardiovascular diseases and other morbidities later in life. In cases with IUGR the babies may show fetal programming as well (Longtine and Nelson, 2011; Hogg et al., 2013).

5. Conclusions and outlook

The very tight interactions between the maternal system and the fetal system via the placenta enable an intense crosstalk of both individuals during pregnancy. The disadvantage for science is that the calculation of any biomarker detected in maternal blood is extremely difficult as too many parameters need to be taken into account. This has misled quite a number of scientists and clinicians who have tried to hypothesize how markers might be derived and what the impact of the placenta and/or the maternal vascular system might be. Finally, depending on the causative routes leading to the development of preeclampsia, markers may or may not have a predictive value and mother and/or child may display long-term effects later in life.

What can be done to promote a better understanding of the etiology of preeclampsia? There are several answers to this question. Only a few are listed here:

1. The scientific community needs to be more open-minded and to accept more hypotheses, even if they may sound “unusual” in the first instance. Accepting only the current model has not led to success or progress in the last two decades.

2. Scientists working on biomarkers for preeclampsia depend on high-quality samples from pregnant women. During the last decade a number of publications have been published using bad-quality samples and/or assays. This may even lead to a good biomarker being disregarded because the wrong samples have been used. Here, a better understanding of the importance of sample and assay quality is needed.

3. Finally, high-quality samples need to be collected and stored at multiple sites – with a comparable quality standard and respective governance structures. It is insufficient to set up a consortium of existing collections without controlling and harmonizing sample quality and without developing transparent governance mechanisms to access such samples.

Conflict of interest

None declared.

References


