Review: placental biomarkers for assessing fetal health

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Abstract

The placenta is a multifunctional organ that regulates key aspects of pregnancy maintenance and fetal development. As the placenta is in direct contact with maternal blood, cellular products (DNA, RNA, proteins, etc.) from the placenta can enter maternal circulation by a variety of ways. The application of serum proteins and circulating placental derived DNA has been well demonstrated for the diagnosis of aneuploidy, and there is great interest in exploring the use of placental biomarkers for the prediction of a range of fetal health parameters. In this review, we discuss how placental biomarkers might be used for the diagnosis and early detection of preeclampsia, fetal growth restriction and inflammation associated with preterm birth. We emphasize how increased understanding of the underlying placental biology can aid in the interpretation of such approaches and development of new biomarkers that can help predict the onset of pregnancy and neonatal health concerns before they manifest.

Introduction

Fetal development is completely dependent on the placenta, which provides for immune protection, nutrient delivery, endocrine function, and limits harmful exposures, among other roles. Placental dysfunction impacting one or more of these processes can lead to preterm birth (PTB), poor fetal growth and other complications associated with fetal/neonatal morbidity and mortality. Therefore, assessment of placental health during pregnancy is an important part of obstetric care. However, the number of tools in current use for assessing placental health is largely limited to placental imaging and a handful of maternal serum markers.

Unlike the fetus, the placenta is in direct contact with maternal blood during pregnancy. As a consequence, a placenta-specific signature is easily detectable in maternal circulation due to passive release of large multinuclear structures, cells, and subcellular fragments, which all contain trophoblast-specific nucleic acids; or from actively secreted products, including hormones and growth factors, exosomes, or signal molecules. The utility of these placenta-derived products for the prenatal diagnosis of chromosome abnormalities has been well-demonstrated (1). Furthermore, recent advances in high-throughput approaches to biomarker identification provide many new avenues to pursue the improved prenatal assessment of fetal well-being. However, there are still gaps in our knowledge that need to be addressed before such approaches significantly impact the detection of perinatal and newborn health outcomes. These gaps include a need for improved characterization of: 1) normal variation in placental structure and gene expression; 2) the distinct etiologies that underlie the conditions we want to predict; and 3) the factors that influence the measured levels of these biomarkers in maternal fluids.

Placental-mediated Complications

There are multiple obstetrical concerns for which placental biomarkers can have beneficial clinical applications (Fig. 1). Early prediction of poor fetal growth, premature delivery and
maternal preeclampsia (PE) is important, as careful monitoring and interventions can improve outcomes and save the lives of babies and mothers. However, these are heterogeneous conditions for which a variety of genetic and environmental influences (e.g., maternal obesity, diabetes, low socioeconomic status, and poor nutrition) can contribute to risk. Defining abnormal placental health is also challenging as, even in normal pregnancies, there is extensive within and between placenta variation in terms of gross pathology and molecular changes (2).

The term ‘placental insufficiency’ encompasses several conditions in which the placenta does not function adequately, but most commonly refers to intrauterine growth restriction (IUGR) and maternal PE. IUGR is defined as low birth weight due to an underlying pathology. PE is defined by maternal hypertension and proteinuria and is itself a major cause of IUGR and preterm birth. Despite numerous maternal serum protein markers being evaluated for prediction of PE and/or IUGR, few have consistently shown positive predictive value, and the benefits obtained beyond using clinical measures and uterine Doppler ultrasound are limited (3). This may be because the best markers for early detection have not yet been identified, the confounding factors not all accounted for, or because there are multiple placental phenotypes associated with these problems.

Preterm birth (PTB), defined as delivery prior to 37 weeks, can occur for many different reasons, including iatrogenic delivery for IUGR or PE. However, spontaneous PTB most commonly results from the interaction between maternal health, genetic variants, and in utero exposures that can alter the inflammatory signaling pathways in the placenta. At least 40% of spontaneous PTB is associated with chorioamnionitis, an inflammation of the placenta and its membranes that is typically associated with polymicrobial infection and characterized by an infiltration of maternal immune cells (typically neutrophils or T-cells) into the chorioamniotic membranes (4,5). The inflammation may be diffuse or focal and present in variable ways (6). While acute chorioamnionitis is often associated with infection, it can even occur in the absence of demonstrable microorganisms, being triggered by non-microbial “danger signals” including cellular stress (7). Inflammation appears to break down the normal barriers, and may increase the ability of cells, cell fragments, exosomes, miRNAs and other products to move through the placenta from mother to fetus (8).

Study of Post-Delivery Placentae to Refine Diagnosis

Examination of the placenta after delivery is important for defining the types of changes that occur in association with adverse clinical outcomes, while molecular profiling can provide additional insight into the underlying mechanisms. The type and extent of molecular changes in the placenta will ultimately affect our understanding of the mechanisms, the selection of biomarkers, and the strength of any associations. Just as different types of chromosomal aneuploidy (Trisomy 13, 18, and 21) are associated with differing maternal serum profile scores, one can expect that the ability to predict PE or PTB, will first require identifying the distinct placental phenotypes we are trying to predict.

Although findings are variable from case to case, anomalies that reflect maternal vascular malperfusion are found more often when PE and/or IUGR are present; these include: infarcts, retroplacental haemorrhage, distal villous hypoplasia, accelerated villous maturation (usually accompanied by syncytialtrophoblast knots), among other findings (9). Such changes are more common when PE is diagnosed early (<34 weeks) and/or severe, but these changes may not affect the placenta uniformly. The extent of observed pathological changes can affect the sensitivity of screening tests. For example, amongst cases of prenatally-suspected growth restriction (based on obstetric measures), low maternal serum placental growth factor (PIGF) was able to predict only the subset of IUGR cases with significant placental pathology; however, these also corresponded with those cases with the worst prognosis (10).

Gene expression profiling provides a complementary way to subgroup placentas and refine the clinical diagnoses. Using a transcriptomics approach on a large collection of normal and abnormal placentas, Leavey et al. were able to identify 5 distinct placental phenotypes, 3 of which were associated with PE (11). Most cases of preterm (<37 weeks) PE clustered in a group that was associated with expression changes in classical PE-associated angiogenesis genes (i.e. VEGF, FLT1 etc). However, two other clusters consisted of PE and non-PE cases, one of which was associated with alterations related to immune function likely related to maternal-fetal intolerance. Due to its relevance to cell differentiation and gene expression, studying placental DNA methylation (DNAm) after delivery can improve our understanding of placental development in the context of pathology or environmental exposure and also be used to reveal new potential biomarkers (52). DNAm has been reported to be altered in the post-delivery placenta in response to maternal stress, obesity, and smoke exposure (53–55), and widespread...
changes are found in the context of early onset PE (12,56,57,58). As yet, here have been few studies on differential DNAm or gene expression in placentas associated with spontaneous PTB and rarely have the same candidates been reported (13). There is a pressing need for more research in this area in order to improve our understanding of the distinct phenotypes that we want to predict.

**Placenta as a Source of Biomarkers**

While distinct molecular changes in the placenta may be associated with specific pathologies, these changes may not directly correspond to biomarkers present in maternal blood (14). Further, the ability to predict certain conditions before their clinical presentation, depends upon the time in pregnancy when the underlying pathology originates (Fig. 2). Pregnancy complications can result from failure of key processes, such as i) trophoblast proliferation, migration, and invasion; ii) maternal-fetal tolerance; and iii) endocrine signaling important for nutrient transfer. For example, both PE and placental IUGR are associated with insufficient invasion and remodeling of maternal spiral arteries that then leads to downstream effects resulting from reduced perfusion and oxidative stress (15). While the early embryo is relatively immune from infection due to physical barriers and innate placental immune response mechanisms, the onset of maternal perfusion at 11–12 weeks and the fusion of amnion and chorion at 18–20 weeks provide new viral or bacterial exposure routes (6). Hence, early indicators of susceptibility may be non-specific, e.g. maternal pro-inflammatory signals, but more accurate detection may only be possible later when inflammation is more pronounced.

Understanding how biomarkers can enter maternal circulation and reflect placental health also requires knowledge of placental biology. The mature placenta is a highly vascularized tree-like structure of chorionic villi that are the functional units of maternal-fetal oxygen exchange and nutrient transport. The villi are composed of three component layers: 1) a syncytiotrophoblast/cytotrophoblast bilayer that covers the entire surface of the villous tree; 2) a mesenchyme-derived core that includes fibroblast cells, macrophages (Hofbauer cells) and pockets of blood islands; 3) fetal vessels that include vascular smooth muscle cells, perivascular cells (pericytes), and endothelial cells. It is the outer layer of multinucleated syncytium that is directly bathed in maternal blood. This syncytium provides a barrier to the migration of maternal cells and bacteria, while allowing substances to be transported from maternal blood to fetal blood (and vice versa) across this interface through both active and passive processes. Syncytial trophoblast is also the main source of many placenta-specific growth factors and hormones that are key indicators of placental growth and health (16). These include, for example, human chorionic gonadotropin (hCG), progesterone, human placent lactogen, vascular endothelial growth factor (VEGF), VEGF-receptor (FLT1), placental growth factor (PlGF). Syncytiotrophoblast-derived particles can also enter maternal circulation through apoptosis/necrosis at the placental surface or through the production of microvesicles. Deported trophoblast structures are found in the uterine vein blood of normal pregnancies and may be transcriptionally active and synthesize a significant proportion of placental mRNA and proteins (17).

From the tips of the chorionic villi, invasive extra-villous trophoblasts (EVTs) migrate into and remodel the uterine spiral arteries leading to loss of the endothelial lining and muscular elastic tissue in these vessels. Because invasion of the maternal uterine vasculature is a normal property of EVTs, they can be found at low rates in maternal circulation (~ 1 fetal/placental cell to 100,000 maternal cells) (18). This invasion process is dependent on the health of the maternal uterine natural killer cells and macrophages at the placental interface (19), and is often incomplete in early-onset/severe PE, causing reduced blood flow to the maternal-fetal interface. While the mesenchymal cells from the inner core of the villi are less studied, they can produce substances that may be transported through the trophoblast layer into maternal blood. Early in development, Hofbauer cells modulate trophoblast migration and villous tree branching by producing angiogenic factors including VEGF, fetal growth factor, vasculotropin, and vascular endothelial cell proliferation factor (16), while later they provide immune protection (20) and may be altered in number in placenta exhibiting inflammation (21).

![Figure 2](https://academic.oup.com/hmg/article-abstract/26/R2/R237/3865112)
Placental Signature in the Maternal Circulation

Protein-based biomarkers

The levels of serum proteins such as hCG, INHA, PAPP-A, PI GF and sFLT1 have been widely investigated as predictive markers for adverse outcomes (22). For example, screening for PE using maternal serum levels of PI GF and PAPP-A, in a combined model with maternal risk factors (e.g. maternal age, obesity, hypertension, family health history, etc.) and uterine artery measures by Doppler ultrasound, is suggested to detect 95% of early onset PE (diagnosed < 34wks) with a 5% false detection rate in a Caucasian population (23). These risk factors though can vary by population. Amongst different studies, PIGF has most consistently been shown to have predictive value (24). For the prediction of PTB prior to onset of symptoms, there has been some promise in utilizing maternal serum biomarkers (25) including C-reactive protein (26,27), corticotropin releasing hormone (CRH (28)), neutrophil to lymphocyte ratio (29), and HLA panel reactive antibodies (30). Biomarkers of inflammation, such as levels of amniotic fluid IL-6 show improved sensitivities compared to amniotic fluid culture methods. Using proteomics approaches, a number of new classes of proteins have been identified as differentially expressed in pregnancies affected by PE. This profile differs in pre-symptomatic PE from that observed when PE ultimately manifests, with differentially expressed proteins in the first trimester including placental, vascular, transport, matrix, and acute phase proteins (32). A signature for predicting IUGR based on mass spectrometry was reported, with apolipoprotein C-II and C-III, demonstrating the strongest associations (33). Similarly, a proteomic approach was used to identify an altered protein signature in the maternal serum that could predict spontaneous PTB in asymptomatic women (34). These studies are largely still exploratory, but there is an enormous potential for improvement of integrated screening tools as more-high throughput and efficient approaches are developed for screening a range of proteins.

DNA-based biomarkers

Cell-free "fetal" DNA in maternal plasma has attracted much attention since its discovery in 1997 as a novel approach for prenatal screening. This DNA is mainly syncytiotrophoblast in origin (35,36) arising mainly from either apoptosis/necrosis or through formation of microvesicles (37). While its low level (~10% of the total cell-free DNA (38)) relative to maternal cell-free DNA is the main technical limitation, it has been successfully used to determine fetal sex, RHD blood group, and carrier status for common monogenic diseases (39). This 'fetal' DNA, more accurately referred to as cell-free placental (cfp)-DNA, is increasingly used in clinical settings for fetal aneuploidy screening based on genomic sequencing data (40,41), yielding high detection rates for T21 (~99%), T18 (96%), T13 (92%), and sex chromosome aneuploidies (90%) (42). The placental origin of this DNA has some disadvantages for prenatal diagnosis, as false-positive results can result from confined placental mosaicism, which occurs in up to 2% of all pregnancies. A detailed overview of possible applications is out of the scope of this review and is available from other sources (39).

As placental insufficiency is associated with increased apoptosis and necrosis, increased levels of cfDNA might be used to detect related conditions. The cfDNA level can be estimated utilizing sites of unique DNA methylation patterns that are unique to placental tissue. The placenta has a unique pseudomalignant pattern of DNA methylation that is quite distinct from that found in somatic tissues, and is characterized by global hypomethylation, with hypermethylation in common tumor-suppressor gene promoters, and a distinctive profile of placental genomic imprinting (43,44). Maternal serum levels of cfDNA have been reported as increased in PE and/or IUGR, but these levels are highly variable and influenced by maternal characteristics (1,45,46). Furthermore, as the level of cfDNA is correlated with maternal serum hCG (51) its independent prognostic value is unclear. While cfDNA might be increased in PTB in the second trimester (47), most studies have reported no association between first-trimester cfDNA fraction and spontaneous PTB (48,49,50). Just as selective quantification of promoter methylation of tumor-suppressor genes, e.g. RASSF1A, has been applied in cancer genetics, there could be placental diseasespecific DNA changes also detectable in maternal blood. However, the reported methylation changes are generally small in magnitude and would require very sensitive detection methods.

RNA-based biomarkers

In addition to DNA, mRNA from the placenta also circulates in the maternal blood and offers a greater range of possibilities to monitor placental function (59,60). Similar to cfDNA, placenta-specific transcripts are detectable in maternal circulation at the first weeks of pregnancy, and are rapidly cleared from plasma in a few hours after delivery (61). This placental mRNA can both act as a messenger or be packed in debris (apoptotic bodies or microvesicles) as a result of cell death (61,62). Many RNA transcripts are placental-specific and these circulating placental RNAs may be differentially expressed in pregnancies complicated by early-onset PE and IUGR (63,64). A limited number of RNA species have also been evaluated for prediction of PTB. IL1R1 transcripts were increased in PTB-associated placentas (65) and were detectable in 60% of maternal plasma samples collected during preterm labour. The proinflammatory cytokine interleukin 1β was also increased in the blood of women with choioamnionitis-associated PTB (66). However, the main limitation to the use of placental mRNA as a biomarker of pregnancy is that mRNA can degrade rapidly and requires strict handling guidelines. Furthermore, the mRNAs evaluated as predictive biomarkers in maternal blood often do not show improved sensitivity as compared to the corresponding protein product (1).

Trophoblast miRNAs are extremely attractive as biomarkers due to their stability, specificity, and relative abundance in maternal plasma (67). They are associated with all types of placental debris, but are thought to have regulatory function when allied with exosomes and protein complexes (68,69). The human placenta has a unique miRNA profile, originating in part from large miRNA clusters on chromosomes 14 and 19 (70). Altered miRNA expression was reported in maternal plasma samples at 20 weeks in pregnancies that went on to spontaneous preterm delivery (28–32 weeks) (71). Using microarray approaches, many miRNAs have been reported to have altered expression levels in PE-associated placentas (72–76), although there is little reproducibility across studies. Upregulation of miR-210 is among the most frequent change reported in PE, but even this is inconsistent across studies (73). The low rate of reproducibility may be
related to our incomplete knowledge of their origin and biological function (67), as well as insufficient study power (77). Furthermore, the alterations in miRNAs may depend on gestational age, population studied, and other clinical measures (e.g. maternal blood pressure and/or the uterine artery Doppler pulsatility index) (73). Larger studies considering potential confounding variables are needed to refine the utility of these placental miRNAs.

Biomarkers to Predict Fetal Outcomes

Beyond what the placenta can tell us about fetal health, there is a keen interest in using placental biomarkers at birth to predict postnatal neurodevelopment, metabolic syndrome, and other health outcomes. Although the placenta is developmentally distinct from somatic tissues, the molecular profile of the placenta may offer a glimpse into the exposures that lead to developmental defects of the baby. Cord blood may also provide a source of biomarkers for fetal outcomes, but can be affected by transient variability in blood cell composition associated with perinatal factors, that must be accounted for.

A link between neurodevelopment and placental health is indicated by several observations. Toddlers with a history of preterm birth, chorioamnionitis and/or very low birth weight were more likely exhibit autism spectrum features (78). Autism and other neurodevelopmental outcomes have also been linked with maternal metabolic disorder, including obesity and diabetes (79–83), and PE (82). Also, trophoblast inclusions were reported at a higher frequency in placentas from babies at high risk of developing autism (83). DNAm alterations in the placenta have been linked to infant neurodevelopmental outcomes (84–90), but there are as yet no independently confirmed associations. Although requiring further validation, these studies highlight the potential of predicting postnatal development from placental molecular profiling.

Altered placental morphology has also been linked to metabolic disorders such as high blood pressure in children (91) and adults (92), and with chronic heart failure in adults (93). Placental biomarkers in maternal serum or the placenta at delivery have also been linked with these adverse outcomes. For example, placental CRH level was associated with childhood BMI trajectories (94) and reduced BMI but increased central adiposity in children (95). Altered expression of 8 miRNAs in the placenta was also linked to maternal obesity, of which several of these were linked to weight gain parameters in the offspring (96). As with fetal neurodevelopmental outcomes, exposure in utero to maternal metabolic disorder and PE are also risk factors for the development of metabolic disorder later in life (97,98). Thus, it is difficult to separate out the effects of exposure during pregnancy from inherited genetic factors that might predispose to both. These studies in infant neurodevelopment and child and adult metabolic disease may also reflect placental pathologies such as those associated with PTB or PE and it is important to consider these co-occurring clinical conditions in any predictive modelling.

Future Directions

Recent advances in genomic technologies have led to the identification of novel nucleic acid and protein biomarkers for the assessment of placental and fetal health outcomes. The use of a combination of biomarkers along with clinical parameters would be expected to have more sensitivity than analyzing individual ones, and genomic approaches should be integrated with clinical measurements, relevant maternal parameters (maternal BMI, ethnicity, past pregnancy history), fetal parameters (sex, gestational age) and the pathological understanding of disease, for optimal risk prediction. This requires large sample sizes to integrate all the relevant molecular and clinical factors of interest. Data reduction approaches can also be employed to increase power and isolate unique correlated networks of changes. Using a transcriptomics approach and weighted-gene co-expression network analysis to identify clusters of coordinately altered gene sets, 12 placental gene expression clusters were identified amongst normal and abnormal placentas, one of which was linked to PE and characterized by hub genes: PVR/L4, INHBA, and INHA (99). Importantly, these co-expression networks were conserved across gestation. Applying similar data reduction methods to maternal blood profiles would likely increase power to assess altered processes. Importantly, trying to identify a single set of markers that can predict all IUGR or all PTB is unrealistic when we know that multiple distinct etiologies can lead to these same outcomes. As our knowledge of basic placental biology improves, we increase our ability to use the abundance of candidate biomarkers in constructive ways to diagnose fetal health early on when interventions and monitoring will have the greatest impact.

Acknowledgements

WPR receives salary support through an investigator award from BC Children’s Hospital Research Institute. SLW is funded through the University of British Columbia 4-year doctoral fellowship.

Conflict of Interest statement. None declared.

Funding

BC Children’s Hospital Research Institute and University of British Columbia.

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