

Biomarkers In Prediction Of Preeclampsia

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Abstract – Preeclampsia is being pregnant-specific, and notably contributes to maternal, and perinatal morbidity and mortality worldwide. An effective predictive test for preeclampsia could facilitate early diagnosis, focused surveillance and well timed delivery; however, restrained alternatives presently exist. A first-trimester screening algorithms has been evolved and demonstrated to expect preterm preeclampsia, with poor utility for term disease, wherein the greatest burden lies. Biomarkers consisting of sFlt-1 and placental growth factor also are now getting used clinically in cases of suspected preterm preeclampsia; their high negative predictive value allows assured exclusion of disease in women with normal results, however sensitivity is modest. There has been a concerted attempt to become aware of ability novel biomarkers that could enhance prediction. These in large part originate from organs concerned in preeclampsia's pathogenesis, which includes placental, cardiovascular and urinary biomarkers. This review outlines the clinical imperative for an effective test and those already in use and summarises modern-day preeclampsia biomarker studies.

Keywords – preeclampsia, Placental transcriptomics, placental genes, Liquid biopsy, proteomics.

I. INTRODUCTION

1. Preeclampsia syndrome

Preeclampsia includes dire effects for the mother and fetus, subsequently one of the major goals of prenatal follow-up is the early detection of the development of this syndrome. Its onset is multifactorial, it could arise at diverse gestational ages, and it could show different grades of severity [1–6]. The modern-day classification is primarily based totally at the onset and the severity of signs; however, it does now no longer as it should reflect the underlying pathophysiological processes. Based on this classification, we distinguish early-onset (<34 weeks) and late-onset (≥34 weeks), or preterm (<37 weeks) and term (≥37 weeks), preeclampsia [2]. Early-onset or preterm preeclampsia is more often complicated by fetal growth restriction (FGR) and more severe symptoms as compared to late-onset or term preeclampsia [1,2,4,5].

In preterm preeclampsia, the extravillous trophoblast dysfunction and the ensuing impairment of spiral artery remodelling are of paramount significance in its pathogenesis. Decreased uteroplacental perfusion and ischemic stress cause an imbalance in angiogenic and antiangiogenic factors, ensuing in endothelial damage, systemic infection, and multiorgan failure [3,7–20]. In term period preeclampsia, the impact of diverse chronic stressors consisting of obesity, diabetes, and kidney, metabolic or autoimmune disease is more dominant [21–30] and maternal vascular and endothelial reaction can also be more sensitive to placental factors [31,32]. Genetic elements related to angiogenesis and immune interactions among the mother and the fetus also are key for the susceptibility to preeclampsia [33–41]. Due to those pathophysiological variations, early-onset or preterm preeclampsia may be greater as it should be expected within the first trimester via way of means of an aggregate of maternal characteristics and biophysical and biochemical markers as compared to late-onset preeclampsia [42]. Improved prediction can probable be expected if the heterogeneous pathophysiological pathways and their specific biomarkers are recognized. Although considerable progress has been made with the expertise of preeclampsia the use of clinical epidemiology, astute observations by clinicians, and

hypothesis-driven studies, the advent of hypothesis-free studies and post-genomic tools (additionally referred to as excessive-dimensional biology or “omics” sciences) [43] enabled us to in addition address the complexity of the ailment pathways and the heterogeneity of this severe syndrome.

2. High-dimensional biology research in preeclampsia

High-throughput “omics” strategies have revolutionized systems biology procedures to disease from the molecular to the clinical levels. With modern-day automation, “omics” properties of as much as tens of thousands of samples may be stacked, and studies aren't restrained to only the set of markers which might be regarded to be clinically applicable however novel ailment biomarkers may be discovered. The assessment of these data may be performed using multidimensional statistical and machine learning methods, that may work as it should be and offer a right picture of the studied ailment only, the use of a large number of samples and well annotated databases. Another mission for this discipline is the need for a universal platform that lets in the assessment of exclusive “omics” statistics. Upon the presence of these conditions, these hypothesis-free exam strategies permit us to locate molecular pattern and to find out about pathological changes of their complexity on the systemic level. Consequently, our findings will now no longer be restrained and biased via way of means of our presumptions or hypotheses [44].

Regardless of the kind of samples concerned (e.g. placenta, blood) or kind of molecular profiling (global, single cell, cell free, etc.), high- throughput experiments in preeclampsia may be extensively grouped in 3 varieties of applications [45]. The first application is known as a class comparison. It ambitions to evaluate molecular profiles among cases with clinically defined phenotypes (e.g. all, early-onset or late-onset preeclampsia cases) versus controls. This approach allows the inference of pathways and biological processes perturbed in cases which might be related to the determined phenotypes and, possibly, the identity of therapeutic targets. The second type of application is a class prediction. This makes use of discriminant analysis and machine learning to develop strategies to broaden ailment prediction models. The focus is on maximizing the prediction accuracy and parsimony in preference to interpretation of discovered variations in molecular profiles. Unsurprisingly, the syndromic nature of preeclampsia, that's manifested via way of means of excessive heterogeneity in expression profiles, has delivered challenging situations to each class-comparison and class-prediction applications, subsequently the need for class discovery. The intention of this remaining kind of application is to discover ailment subtypes using of data driven clustering of affected patient samples without assuming a specific number and pathology of ailment subtypes. This method is absolutely hypothesis-free and independent in the diagnostic criteria, which is key since the categorization of patients primarily based on the onset of clinical symptoms right into a pre-set of two groups (i.e. early-onset vs late-onset) are prone to bias at numerous levels [44].

3. Placental transcriptomics

The placenta, which represents inherent fetal traits and responses to the intrauterine environment, has an important function within the pathophysiology of preeclampsia [3,4]. Therefore, it isn't unexpected that genome-extensive profiling of the human placental transcriptome has become the primary independent method within the study of normal maternal–placental–fetal physiology and of the pathology in preeclampsia. A latest complete assessment [46] summarized human placental transcriptome studies from the cellular to tissue levels even as addressing essential elements of study layout so can promote data sharing and meta-analyses. Yong and Chan summarized 179 studies since 2004 into 4 themes, with one that specialized in pregnancy complications, which includes preeclampsia. Results supplied by those transcriptomics research progressed our expertise of healthy placental development. Also, placenta- derived biomarkers secreted into the maternal circulation in preeclampsia (e.g. sFLT1, sEng) had been discovered [11,12,47], and the biological processes and molecular pathways related to clinical preeclampsia phenotypes had been detected, as a consequence imparting clues into the underlying mechanisms of placental pathologies [46].

Due to barriers in placental sample collections and the late clinical onset of preeclampsia symptoms, most of those researches focused on the third trimester placental transcriptome, wherein the molecular patterns representative of oxidative stress and inflammatory pathways had been frequently seen. Of significance, one study [48] of first trimester placental tissues, left over from chorionic villus sampling, assessed the placental transcriptome of four women who later developed preeclampsia (preterm and term period) and 8 healthy controls. Despite the low pattern size, the study confirmed that the dysregulation of genes concerned in cell motility, immune modulation, and infection become already present at this early stage of gestation; however, the gene dysregulation feature of hypoxia or ischemia become now no longer determined. Another difficulty of most researches become that they did now no longer or couldn't deal with the cellular heterogeneity of the placenta. This is a really heterogeneous organ

with cell varieties of diverse origins and differing gene expression profiles [49,50]. Therefore, global or focused expression research with use of bulk tissues couldn't correctly dissect the pathological mechanisms, missing cell-stage information, and cellular interactions inside this organ. As mentioned later, a notable development got here with the rise of single cell transcriptomics research, which solved this bottleneck and have become distinguished for the study of placental gene expression in healthy and diseased states [49,50].

4. Distinct placental gene modules

A study of “class comparison” microarray study on third trimester placentas, it was determined that the transcriptome of women with severe preterm preeclampsia related to the clinical presentation of “haemolysis, elevated liver enzymes, low platelet count” (HELLP) syndrome is much like women with preterm preeclampsia without HELLP syndrome [51]. Differentially expressed (DE) genes in preterm preeclampsia as compared to controls had been much like the ones formerly stated on this preeclampsia subtype [52–55], and among the DE genes encoded proteins which had in advance been proposed as biomarkers for preeclampsia (e.g. FLT1, LEP, PAPP2). Although comparable organic approaches, cellular compartments, and signaling pathways had been enriched in preterm preeclampsia, with or without the presence of HELLP syndrome, there has been greater engagement of the cytokine-cytokine receptor pathway in cases related to HELLP syndrome, reflecting a greater suggested systemic maternal inflammatory reaction. A further systems biology evaluation of this data set recognized major gene co-expression network modules and their hub transcription regulatory genes in the third trimester placentas of women with preterm preeclampsia [56]. The biggest module contained genes concerned in fetal growth (CSH1, HSD11B2), and hub transcription regulatory genes (ESRRG, POU5F1, ZNF554) had been implicated in the regulation of trophoblast metabolism, stemness, differentiation, and invasion [57, 58]. Genes in the 2d biggest module had been related to maternal blood pressure (e.g. FLT1), and their hub transcription regulatory genes (BCL6, BHLHE40, ARNT2) had been implicated in the hypoxia reaction. In vitro functional experiments proven that the trophoblastic overexpression of transcription factors BCL6 or ARNT2 sensitizes the trophoblast to hypoxia and ends in FLT1 overexpression upon hypoxic-ischemic pressure. The expression of the “blood pressure module” biomarker genes become undoubtedly related to the maternal vascular malperfusion score of the placenta, and the amount of their secreted protein products (sFlt-1, sEng, leptin) began out to growth in the maternal circulation after 12 weeks of gestation. These observations in shape the general idea that maternal vascular malperfusion in the first trimester ends subsequent placental oxidative stress, increased placental expression of FLT1, and an anti-angiogenic state beginning from the late first and the early 2d trimesters [7,13,59]. A set of transcription regulatory genes (e.g. BCL6, BHLHE40, JUNB) had been DE in the placenta in preeclampsia, in a path contrary to that determined at some stage in villous trophoblast differentiation, as discovered via way of means of our subsequent microarray study [60]. Five of these transcription regulatory genes are central members of the “blood pressure module”, suggesting links among problems of trophoblast differentiation, maternal vascular malperfusion, placental oxidative stress, an anti-angiogenic state, and preterm preeclampsia.

5. Uncovering the molecular subclasses of preeclampsia via way of means of placental transcriptomics

Although the preliminary research of the placental transcriptome as it should be characterised the severe clinical subtype of preterm preeclampsia, the heterogeneity of cases and the underlying molecular subclasses had been unknown till 2015. This hiatus become filled first via class discovery research at the placental transcriptome by means of Leavey et al. [61,62]. The authors performed unsupervised analyses of placental transcriptomes to offer insights into the molecular taxonomy of preeclampsia. They recognized 5 clusters amongst all cases and controls in the larger study : 1) the first included, in large part, patients who delivered at term; 2) the second cluster become composed predominantly of patients with preterm preeclampsia; 3) the third cluster included a subset of patients with preeclampsia and different complications of pregnancy; 4) the fourth cluster consisted, primarily, of patients with spontaneous preterm delivery; and 5) the 5th cluster included women with placental chromosomal abnormalities with and without preeclampsia, because of the confined placental mosaicisms found in this group additionally detected by means of different studies [63,64]. The 3 major subclasses of preeclampsia recognized in those studies are offered in Fig. 1.

1) “canonical/placental preeclampsia”: The clinical characteristics consisted of preterm preeclampsia, abnormal Doppler velocimetry (several vessels), birthweight <fiftieth centile, and, in some cases, HELLP syndrome. Gene expression for sFlt-1 and endoglin become specifically high for this group of patients. This molecular phenotype has been primarily characterised in previous class comparison researches [51]; 2) “maternal preeclampsia”: This group of patients presented with preeclampsia, most at term period or near-term period delivery, an appropriate-for-gestational-age (AGA) neonate, and known maternal risk factors,

e.g. null parity or prior hypertensive pregnancy. The placentas typically did no longer have any maternal vascular lesions; and 3) “immunological preeclampsia”: This group of patients delivered small-for-gestational-age (SGA) neonates among 30 and 37 weeks of gestation, confirmed low placental weights, had a transcriptome enriched by the expression of genes concerned in the immune reaction, and exhibited poor maternal-fetal tolerance to the fetoplacental unit (e.g. CXCL-10). Fig. 1.

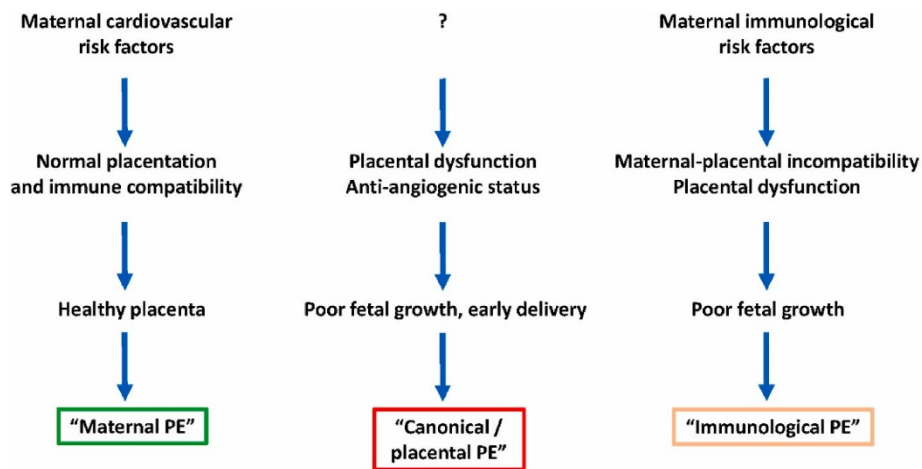


Fig. 1. Molecular subclasses of preeclampsia derived from placental transcriptomics data. Major clinical and placental characteristics are depicted. PE, preeclampsia.

The same authors stated eventually that a high degree of concordance may be determined among the effects of gene expression clustering of the placentas and the histopathologic features of this fetal organ [65]. “Placental preeclampsia” become related to maternal vascular lesions of under perfusion, while “immunological preeclampsia” become characterised by means of chronic inflammatory lesions of the placenta, intervillous thrombi, and maternal vascular lesions of malperfusion. By contrast, “maternal preeclampsia” generally had minimal placental histologic findings. In a subsequent study [66], “immunological preeclampsia” become related to an enrichment in monocytes (positive for CD68) and neutrophils (positive for myeloperoxidase) in the intervillous area even as “canonical preeclampsia” had a notably much less wide variety of these cells. It is essential to be aware that the gene expression profiles of placentas with “placental preeclampsia” and “immunological preeclampsia” have additionally been determined in FGR without preeclampsia [67], indicating that the pattern of gene expression in the placenta isn't enough to outline the clinical phenotype. This indicates that placental ailment may cause hypertension in a woman only if susceptible, and some women may be resistant against the hypertensive state precipitated by means of placental maldevelopment and/or dysfunction. Eventually, the maternal and fetal compartments can also additionally have a degree of independence, and preeclampsia could be precipitated by means of both of these compartments or by their synergy or poor complementarity. As such, it transpired that the molecular investigations of maternal blood, which additionally displays changes in The maternal compartment, is essential in depicting the interplay among the fetus and the maternal environment as each placental and maternal molecular factors decide the development of preeclampsia and its medical phenotype.

6. Liquid biopsy of the placenta

Liquid biopsy, a fast-growing area in diagnostics, includes sampling physical fluids in a minimally invasive manner (e.g. serum, plasma, urine) to derive information about the functional and molecular status of organs [68]. As such, liquid biopsy has emerged as a key in tumor diagnostics as a method to reveal circulating tumor cells and DNA [69]. Historically, the primary tries to non-invasively detect placental function in maternal blood may be connected to the systematic discovery and characterization of placenta-derived proteins and to their study as a potential biomarkers of placental function, pregnant complications, and fetal genetic disorders [70]. For example, the quantification of blood hCG, PP13/galectin-13, and PSG1 has emerged as crucial in the detection of pregnancy or being pregnancy complications, together with preeclampsia, from maternal blood [71,72]. Since the discovery of cell-free fetal DNA (cffDNA) in the maternal circulation by Lo et al. in 1997 [73], the fast-evolving non-invasive prenatal diagnostics (NIPT) technologies have revolutionized prenatal screening of genetic defects primarily based on the

detection of cfDNA in a small amount of maternal blood [74]. Shortly after, Lo et al. additionally identified circulating placental/fetal RNA (cpRNA) in the maternal circulation [75] and determined the earliest gestational age (4th week) at which these cpRNAs are present in the maternal circulation. Their abundance will increase with advancing gestation and reaches 10–15% of overall RNA in the maternal circulation [76]. These discoveries paved the way for the quantification of cpRNAs to non-invasively investigate the placental transcriptome and are predicting pregnancy complications or to monitor high risk pregnancies without endangering the fetus [77,78]. In addition, diverse circulating microparticles are launched from the syncytiotrophoblast throughout pregnancy into the maternal circulation, together with exosomes, which comprise diverse factors of placental origin, which include proteins, lipids, mRNAs, and miRNAs. The molecular signatures of trophoblastic microparticles might also additionally offer crucial information about the condition of the placenta while non-placental microparticles, including exosomes, might also additionally mirror maternal health or disease state. In line with those findings, latest research identified potential biomarkers of preeclampsia with the examining the changes in the type, amount, and content material of these exosomes [79–82].

7. Maternal blood proteomics

The study of proteomics yields vital molecular records concerning maternal and fetal health/disease states. A latest review [83] summarized 69 unbiased quantitative proteomics class comparison studies on preeclampsia, performed since 2004, and on proteins determined to be DE in this syndrome, additionally, the non-stop technical evolution to attain unified outcomes. Most of the studies focused maternal serum/plasma, placenta, or urine proteomics, making it the largest compilation of quantitative proteomics information on preeclampsia. The overall number of DE proteins in the placenta, serum/plasma, and urine have been 912, 559, and 132, respectively. After considering only those proteins which have been defined with the aid of using independent studies with inter-study agreement in control/preeclamptic ratio of protein abundance, they determined a cluster of 18, 29, and 16 proteins constantly DE in preeclampsia in the placenta, serum/plasma, and urine, respectively. Of interest, the various 18 proteins with a robust up- or down- regulation in the placenta in preeclampsia at the time of the disease, 23 studies pronounced Flt1 and PAPP2 on the RNA and protein levels, as a result validating the earlier findings and underlining the up-regulation of the “blood pressure gene module” in the placenta in preeclampsia. Among the 29 proteins with a robust dysregulation in the serum/plasma in preeclampsia in the course of gestation, sEng was constantly determined to be up-regulated and PlGF to be down- regulated, proving the systemic anti-angiogenic state in preeclampsia with proteomics techniques. Moreover, 14 proteins in the maternal circulation, together with sEng, PlGF, MMP7 and plenty of immune-associated proteins.

8. Maternal blood transcriptomics as a prediction device for preeclampsia

A comprehensive review [77] identified 24 studies between 2003 and 2014 which measured cpRNA in maternal whole peripheral blood or in maternal plasma are expecting and/or reveal preeclampsia. Multiple research on cpRNAs confirmed congruent findings with placental transcriptomics research in that many placenta-unique gene transcripts dysregulated in the placenta in preeclampsia have been determined in addition dysregulated in the maternal circulation (e.g. CRH, FLT1, ENG upregulated, hPL, PP13 downregulated). Like placental transcriptomic changes in preeclampsia, alterations in the maternal blood transcriptome in preeclampsia contemplated disturbances with angiogenesis in addition to hypoxia and oxidative stress reaction. There have been extensive variations concerning cpRNA expression with the medical phenotype of preeclampsia, as high levels of unique cpRNA transcripts have been discovered in early-onset vs late-onset preeclampsia, and in more severe forms, mainly the ones complicated with HELLP syndrome. This finding is consistent with the bigger gene expression changes and improved particles output by the placenta in those clinical forms [77]. In a recent large maternal blood cfRNA profiling study, the later onset of preeclampsia may be anticipated in mid trimester with a sensitivity of 75% and an effective predictive value of 32.3% [84].

Of interest, by measuring panels of cpRNAs as early as in the first trimester, substantially good prediction models may be built for preeclampsia. Farina et al. determined that the aggregate of endoglin, FLT1, and TGFβ1 transcripts had a detection rate of 72.3% at a 5% false-positive rate (FPR) at 10–14 weeks of gestation [85]. The same group confirmed that a panel of transcripts, together with FLT1, had a detection rate of 84% at a 5% FPR at 15–20 weeks of gestation [86]. Since 2011, extracellular miRNAs have additionally acquired interest as potential biomarkers. Although their role in the pathophysiology of preeclampsia continues to be unclear, the altered expression of those nucleic acids has been observed. Their advantage over mRNAs is that they're shorter, have fewer species, and are more cost-effective of their analysis. In addition, miRNAs are more extracellularly stable, in order that they may be used each as tools and as therapeutic targets in the future [87]. A recent study at now no longer discovered and tested peripheral miRNAs as a preeclampsia biomarker in mid trimester however additionally confirmed that the placenta contributes the most changes in the miRNA sample in preeclampsia, and that miR-155–5p, which

negatively regulates NO synthase expression, has a relevant position in the pathogenesis [88]. In order to evaluate the maternal compartment as well and to expose variations and similarities in the molecular basis of the 2 principal medical phenotypes at the time of diagnosis, we investigated the maternal entire-blood transcriptome in early-onset and late-onset preeclampsia with microarrays [89]. This study at exposed common features of two of those phenotypes together with the dysregulation of genes involved in host defence (e.g. DEFA4, BPI), tight junctions (EMP1) and liver regeneration (ECT2). While DE genes in women with early-onset preeclampsia have been involved in coagulation (SERPINI2), immune regulation (CD24, VSIG4), developmental process (H19) and inflammation (S100A10), the ones genes DE in late-onset preeclampsia have been implicated in innate immunity (LTF, ELANE) and cell-to-cell recognition in the nervous system (CNTNAP3) [92,93].

A follow-up longitudinal transcriptomics study at exposed that the mRNA whole blood signature of preeclampsia discovered at the time of diagnosis is likewise improved in earlier in gestation at 22–28 weeks [90]. The aggregate of 4 genes from this signature, together with an imprinted long non-protein coding RNA (H19), fibronectin 1 (FN1), tubulin beta-6 elegance V (TUBB6), and formyl peptide receptor three (FPR3), had a sensitivity of 85% and a specificity of 92% for the prediction of early-onset preeclampsia [89]. A principal advancement in the field has been the usage of single-cell transcriptomics to dissect the cell heterogeneity of the normal human placenta and to outline character cell-specific gene signatures [50,91]. This technology additionally enabled the reconstruction of the differentiation trajectory of normal trophoblasts in addition to the discovery of new cells in the placenta and the identity of cell type-specific molecular changes in the placenta of patients with preeclampsia. Of interest, the single-cell transcriptomics signature of extravillous trophoblasts was found to be improved in the maternal blood of patients in early-onset preeclampsia as compared to normal pregnant women at the time of disease [90]. Studies recommended that increased RNA expression in early-onset preeclampsia isn't restricted to the extravillous trophoblasts however that the transcriptomics signatures of different placental cell types also are heightened. The rise in circulating RNA expression of placental signatures turned into diagnosed at the time of disease in addition to identify patients at risk to develop early-onset preeclampsia. The similarity of cpRNA- and cellular RNA-primarily based findings was tested not only whilst studying preeclampsia however additionally throughout independent studies assessing changes with gestational age in normal pregnancies [92,93].

9. First trimester proteomics profiles of preterm and time period

Preeclampsia Initially, we achieved a category assessment evaluation with two - dimensional deference gel electrophoresis (2D-DIGE) proteomics of first trimester maternal blood which diagnosed novel early maternal pathways of preeclampsia [56]. Proteins enriched in term preeclampsia have diagnosed pathways just like the ones determined in early-onset preeclampsia, however the detected changes have been smaller in extent. Subsequent research identified molecular networks that link the 19 DE proteins detected in the maternal circulation in the first trimester to the 1409 DE genes determined in the placentas of patients with preterm preeclampsia [56], suggesting that the changes in the maternal proteome might also additionally have an impact on placental features and gene expression. Indeed, we tested those in silico findings with the aid of using in vitro experiments, wherein number one villous trophoblasts have been cultured with first-trimester maternal serum. The serum from the preterm preeclampsia group vs the healthful control group induced the up-law of many genes in villous trophoblasts, which have been additionally up-regulated in the placentas of preterm preeclampsia patients and related to blood pressure elevation (e.g. LEP, FLT1). Our information pointed to split maternal and placental disease pathways and their interplay in the development of preeclampsia. Several maternal protein biomarkers we identified [56] in early gestation had already been implicated at a later disease level with the aid of using different research whilst their dysregulation is greater mentioned and even as a restricted connection nonetheless exists among the maternal circulation and the placenta [94]. This shows the early activation of maternal disease pathways both in term and preterm preeclampsia, upstream of placental dysfunction, in all likelihood because of pre-existing maternal disease or perturbed maternal-fetal-placental immune interactions [95–97].

10. Plasma proteomic changes in the course of gestation in early- onset and late-onset preeclampsia

To find out extra disease biomarkers and to detect the dynamic changes in the maternal proteome in the course of pregnancy, 2 longitudinal case control studies of 1125 plasma proteins through aptamer- based assays have been performed in women who developed early-onset or late- onset preeclampsia [98,99]. The excellent predictors for the following development of early-onset preeclampsia have been indicated: 1) an excessive abundance of MMP7 and of glycoprotein IIBIIIA complex at 16-22 weeks of gestation; and 2) a low abundance of PIGF and of VEGF-121, and elevated siglec-6 and activin-A, at 22-28 weeks of gestation. At 22–28 weeks, the increased abundance in siglec-6, activin-A, and VEGF-121 differentiated women who sooner developed early-onset preeclampsia from individuals who either developed the late-onset syndrome or had a normal pregnancy. In agreement with

in earlier research, the sensitivity of risk models was higher for early-onset preeclampsia with placental histologic signs of maternal vascular malperfusion than for entire early-onset preeclampsia group, potentially due to the fact those models are sensitive to the pathway of preeclampsia related to the malperfusion of uteroplacental circulation. Biological processes dysregulated in preeclampsia covered the following: 1) “cell adhesion” and “reaction to hypoxia”, apparently unique to early-onset preeclampsia; 2) “small molecule metabolic process” and “positive regulation of apoptotic process,” specific to late-onset preeclampsia; and 3) “extracellular matrix organization”, “positive regulation of VEGFR signaling pathway”, and “positive regulation of cell adhesion” were common for both phenotypes of this syndrome [98,99]. As implied from these and different proteomic discovery research, an anti-angiogenic state, though to a specific extent, displays the common pathway of preeclampsia in all phenotypes.

II. CONCLUSION

Preeclampsia, is a heterogeneous syndrome with multiple subtypes, can be investigated with “omics” and bioinformatics tools. Class discovery placental transcriptomics research earlierly discovered 3 molecular subtypes, so-called, “canonical/placental”, “immunological”, and “maternal” preeclampsia. However, those transcriptomic signatures may also be detected in FGR without preeclampsia, suggesting that placental gene expression styles aren't enough to outline the medical phenotype. As such, molecular investigation of maternal blood, which additionally displays changes in the maternal compartment, may be much more beneficial in detecting each placental and maternal molecular factors that define the development of preeclampsia and its clinical phenotypes. Our proteomics investigations of maternal blood both either in the first trimester or longitudinally in the course of gestation in ethnic populations both revealed 4 distinct patients clusters in preeclampsia, supporting the existence of the “placental”, “maternal” and “immunological” subclasses, and the presence of a novel “metabolic” subclass. It has become clear that PIGF, formerly used as a gold standard biomarker, is only effective for the prediction of “placental” preeclampsia, the only subclass in which the feature drop in PIGF levels was observed. In this subgroup, preventive aspirin remedy is mainly powerful [42,101, 102]. Another important conclusion is that the molecular subclasses do not determine certain clinical phenotypes, which ought to be the complex interplay of maternal, placental, fetal, and environmental factors.

Our data support the concept that the maternal and fetal compartments have a degree of independence and that three different disease origins might also additionally exist: 1) the placental compartment, 2) the maternal compartment, and 3) the synergy or poor complementarity of those two compartments. Of importance, placental transcriptomics research have determined three preeclampsia subclasses at the end of pregnancy, even as our research confirmed that four distinct subclasses and their distinct disease pathways exist in the first trimester. This can be on account of originating subclasses, e.g. “placental” and “metabolic,” that attain a similar end stage and become indistinguishable when viewed from the third trimester placenta. These findings are paramount to our improved understanding of the early pathways of preeclampsia, and they may promote the development of novel diagnostic tools, permitting the early detection and follow-up of patients as well as their tailored therapies with aspirin or other potential preventive treatments under testing [29,103–106].

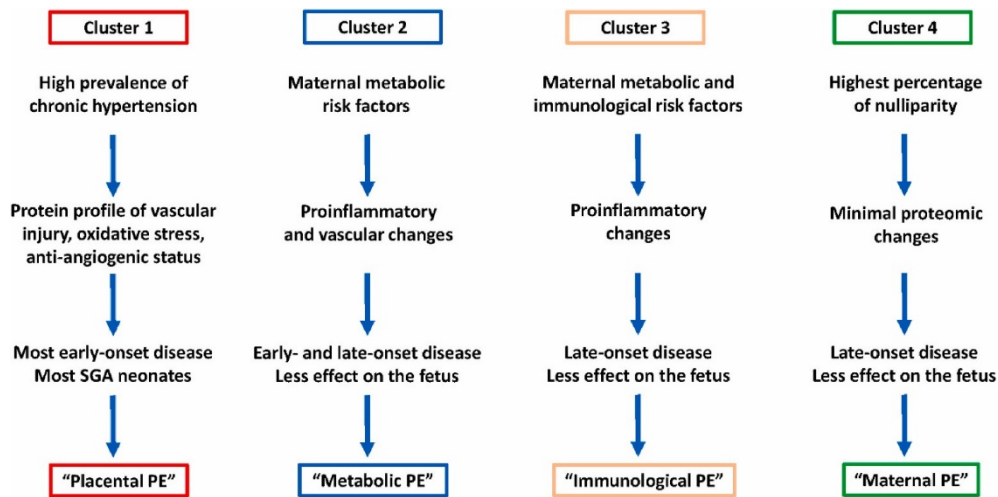


Fig. 2. Molecular subclasses of preeclampsia derived from maternal blood proteomics data. Major clinical and placental characteristics are depicted.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

Authors have equally participated and shared every item of the work.

REFERENCES

- [1] Ness, Roberta B., and James M. Roberts. "Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications." *American journal of obstetrics and gynecology* 175.5 (1996): 1365-1370.
- [2] Von Dadelszen, Peter, Laura A. Magee, and James M. Roberts. "Subclassification of preeclampsia." *Hypertension in pregnancy* 22.2 (2003): 143-148.
- [3] Brosens, Ivo, et al. "The "Great Obstetrical Syndromes" are associated with disorders of deep placentation." *American journal of obstetrics and gynecology* 204.3 (2011): 193-201.
- [4] Chaiworapongsa, Tinnakorn, et al. "Pre-eclampsia part 1: current understanding of its pathophysiology." *Nature Reviews Nephrology* 10.8 (2014): 466-480.
- [5] Jung, Eunjung, et al. "The etiology of preeclampsia." *American Journal of Obstetrics and Gynecology* 226.2 (2022): S844-S866.
- [6] Myatt, Leslie, and James M. Roberts. "Preeclampsia: syndrome or disease?" *Current hypertension reports* 17.11 (2015): 1-8.
- [7] Burton, G. J., et al. "Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy." *Placenta* 30.6 (2009): 473-482.
- [8] Moldenhauer, Julie S., et al. "The frequency and severity of placental findings in women with preeclampsia are gestational age dependent." *American journal of obstetrics and gynecology* 189.4 (2003): 1173-1177.
- [9] Ogge, Giovanna, et al. "Placental lesions associated with maternal under perfusion are more frequent in early-onset than in late-onset preeclampsia." *Journal of perinatal medicine* 39.6 (2011): 641-652.
- [10] Matthiesen, Leif, et al. "Immunology of preeclampsia." *Immunology of Pregnancy* 89 (2005): 49-61.
- [11] Maynard, Sharon E., et al. "Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia." *The Journal of clinical*

- investigation* 111.5 (2003): 649-658.
- [12] Venkatesha, Shivalingappa, et al. "Soluble endoglin contributes to the pathogenesis of preeclampsia." *Nature medicine* 12.6 (2006): 642-649.
- [13] Cindrova-Davies, Tereza, et al. "Soluble FLT1 sensitizes endothelial cells to inflammatory cytokines by antagonizing VEGF receptor-mediated signalling." *Cardiovascular research* 89.3 (2011): 671-679.
- [14] Kumar, Ashok, et al. "IL-10, TNF- α & IFN- γ : potential early biomarkers for preeclampsia." *Cellular immunology* 283.1-2 (2013): 70-74.
- [15] Roberts, James M., et al. "Preeclampsia: an endothelial cell disorder." *American journal of obstetrics and gynecology* 161.5 (1989): 1200-1204.
- [16] Hahn, Sinuhe, et al. "Neutrophil NETs in reproduction: from infertility to preeclampsia and the possibility of fetal loss." *Frontiers in immunology* 3 (2012): 362.
- [17] Umaphathy, Anandita, Lawrence W. Chamley, and Joanna L. James. "Reconciling the distinct roles of angiogenic/anti-angiogenic factors in the placenta and maternal circulation of normal and pathological pregnancies." *Angiogenesis* 23.2 (2020): 105-117.
- [18] Fosheim, Ingrid Knutsdotter, et al. "Decidua basalis and acute atherosclerosis: Expression of atherosclerotic foam cell associated proteins." *Placenta* 107 (2021): 1-7.
- [19] Allerkamp, Hanna H., et al. "Something old, something new: Digital quantification of uterine vascular remodelling and trophoblast plugging in historical collections provides new insight into adaptation of the utero-placental circulation." *Human Reproduction* 36.3 (2021): 571-586.
- [20] Blois, Sandra M., et al. "A potential pathophysiological role for galectins and the renin-angiotensin system in preeclampsia." *Cellular and molecular life sciences* 72.1 (2015): 39-50.
- [21] Verlohren, S., et al. "Uterine artery Doppler, birth weight and timing of onset of pre-eclampsia: providing insights into the dual etiology of late-onset pre-eclampsia." *Ultrasound in Obstetrics & Gynecology* 44.3 (2014): 293-298.
- [22] Soto, Eleazar, et al. "Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal under perfusion." *The Journal of Maternal-Fetal & Neonatal Medicine* 25.5 (2012): 498-507.
- [23] Vatten, Lars J., et al. "Changes in circulating level of angiogenic factors from the first to second trimester as predictors of preeclampsia." *American journal of obstetrics and gynecology* 196.3 (2007): 239-e1.
- [24] Crispi, F., et al. "Predictive value of angiogenic factors and uterine artery Doppler for early-versus late-onset pre-eclampsia and intrauterine growth restriction." *Ultrasound in Obstetrics and Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology* 31.3 (2008): 303-309.
- [25] Romero, Roberto, et al. "A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate." *The journal of maternal-fetal & neonatal medicine* 21.1 (2008): 9-23.
- [26] Redman, C. W., I. L. Sargent, and A. C. Staff. "IFPA Senior Award Lecture: making sense of pre-eclampsia—two placental causes of preeclampsia?" *Placenta* 35 (2014): S20-S25.
- [27] Hahn, Sinuhe, Olav Lapaire, and Nandor Gabor Than. "Biomarker development for presymptomatic molecular diagnosis of preeclampsia: feasible, useful or even unnecessary?" *Expert Review of Molecular Diagnostics* 15.5 (2015): 617-629.
- [28] Robillard, Pierre-Yves, et al. "Progress in the understanding of the pathophysiology of immunologic maladaptation related to early-onset preeclampsia and metabolic syndrome related to late-onset preeclampsia." *American Journal of Obstetrics and Gynecology* 226.2 (2022): S867-S875.
- [29] Hürter, Hanna, et al. "Prevention of pre-eclampsia after infertility treatment: preconceptional minimalisation of risk factors." *Best Practice & Research Clinical Endocrinology & Metabolism* 33.1 (2019): 127-132.

- [30] Than, Nándor Gábor, et al. "Early pathways, biomarkers, and four distinct molecular subclasses of preeclampsia: The intersection of clinical, pathological, and high-dimensional biology studies." *Placenta* (2022).
- [31] Scioscia, Marco, et al. "Endothelial dysfunction and metabolic syndrome in preeclampsia: an alternative viewpoint." *Journal of reproductive immunology* 108 (2015): 42-47.
- [32] Stark, Michael J., et al. "Alterations in the maternal peripheral microvascular response in pregnancies complicated by preeclampsia and the impact of fetal sex." *Journal of the Society for Gynecologic Investigation* 13.8 (2006): 573-578.
- [33] Dijk, Marie van, and Cees Oudejans. "(Epi) genetics of pregnancy-associated diseases." *Frontiers in genetics* 4 (2013): 180.
- [34] Johnson, Matthew P., et al. "Genome-wide association scan identifies a risk locus for preeclampsia on 2q14, near the inhibin, beta B gene." *PloS one* 7.3 (2012): e33666.
- [35] Lau, Sien Yee, et al. "Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 levels are altered in preeclampsia: a systematic review and meta-analysis." *American journal of reproductive immunology* 70.5 (2013): 412-427.
- [36] Dunk, Caroline E., et al. "Functional evaluation of STOX1 (STORKHEAD-BOX PROTEIN 1) in placentation, preeclampsia, and preterm birth." *Hypertension* 77.2 (2021): 475-490.
- [37] Miralles, Francisco, et al. "Long-term cardiovascular disorders in the STOX1 mouse model of preeclampsia." *Scientific reports* 9.1 (2019): 1-13.
- [38] Yong, Hannah EJ, et al. "Genetic approaches in preeclampsia." *Preeclampsia* (2018): 53-72.
- [39] Burton, Graham J., et al. "Pre-eclampsia: pathophysiology and clinical implications." *Bmj* 366 (2019).
- [40] Hahn, Sinuhe, et al. "Feto-maternal microchimerism: the pre-eclampsia conundrum." *Frontiers in Immunology* 10 (2019): 659.
- [41] Jiménez, Karen Marcela, et al. "Identifying new potential genetic biomarkers for HELLP syndrome using massive parallel sequencing." *Pregnancy Hypertension* 22 (2020): 181-190.
- [42] Poon, Liona C., et al. "A literature review and best practice advice for second and third trimester risk stratification, monitoring, and management of pre-eclampsia: Compiled by the Pregnancy and Non-Communicable Diseases Committee of FIGO (the International Federation of Gynecology and Obstetrics)." *International Journal of Gynaecology and Obstetrics* 154.Suppl 1 (2021): 3.
- [43] Loscalzo, Joseph, and Albert-Laszlo Barabasi. "Systems biology and the future of medicine." *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* 3.6 (2011): 619-627.
- [44] Robinson, Scott W., Marco Fernandes, and Holger Husi. "Current advances in systems and integrative biology." *Computational and structural biotechnology journal* 11.18 (2014): 35-46.
- [45] Tarca, Adi L., Roberto Romero, and Sorin Draghici. "Analysis of microarray experiments of gene expression profiling." *American journal of obstetrics and gynecology* 195.2 (2006): 373-388.
- [46] Yong, Hannah EJ, et al. "Significance of the placental barrier in antenatal viral infections." *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1867.12 (2021): 166244.
- [47] Menkhorst, Ellen, et al. "Galectin-7 impairs placentation and causes preeclampsia features in mice." *Hypertension* 76.4 (2020): 1185-1194.
- [48] Founds, Sandra A., et al. "Altered global gene expression in first trimester placentas of women destined to develop preeclampsia." *Placenta* 30.1 (2009): 15-24.
- [49] Li, Hui, et al. "Single cell transcriptome research in human placenta." *Reproduction* 160.6 (2020): R155-R167.
- [50] Pavličev, Mihaela, et al. "Single-cell transcriptomics of the human placenta: inferring the cell communication network of the maternal-fetal interface." *Genome research* 27.3 (2017): 349-361.
- [51] Várkonyi, T1, et al. "Microarray profiling reveals that placental transcriptomes of early-onset HELLP syndrome and preeclampsia are similar." *Placenta* 32 (2011): S21-S29.

- [52] Nishizawa, H., et al. "Microarray analysis of differentially expressed fetal genes in placental tissue derived from early and late onset severe pre-eclampsia." *Placenta* 28.5-6 (2007): 487-497.
- [53] Enquobahrie, Daniel A., et al. "Differential placental gene expression in preeclampsia." *American journal of obstetrics and gynecology* 199.5 (2008): 566-e1.
- [54] Sitras, V., et al. "Differential placental gene expression in severe preeclampsia." *Placenta* 30.5 (2009): 424-433.
- [55] Winn, Virginia D., et al. "Severe preeclampsia-related changes in gene expression at the maternal-fetal interface include sialic acid-binding immunoglobulin-like lectin-6 and pappalysin-2." *Endocrinology* 150.1 (2009): 452-462.
- [56] Than, Nandor Gabor, et al. "Integrated systems biology approach identifies novel maternal and placental pathways of preeclampsia." *Frontiers in immunology* 9 (2018): 1661.
- [57] Knöfler, Martin, and Jürgen Pollheimer. "Human placental trophoblast invasion and differentiation: a particular focus on Wnt signaling." *Frontiers in genetics* 4 (2013): 190.
- [58] E. Davies, Jessica, et al. "Epithelial-mesenchymal transition during extravillous trophoblast differentiation." *Cell adhesion & migration* 10.3 (2016): 310-321.
- [59] Hansson, Stefan R., Åsa Nääv, and Lena Erlandsson. "Oxidative stress in preeclampsia and the role of free fetal hemoglobin." *Frontiers in physiology* (2015): 516.
- [60] Szilagyi, Andras, et al. "Placenta-specific genes, their regulation during villous trophoblast differentiation and dysregulation in preterm preeclampsia." *International journal of molecular sciences* 21.2 (2020): 628.
- [61] Leavey, Katherine, Shannon A. Bainbridge, and Brian J. Cox. "Large scale aggregate microarray analysis reveals three distinct molecular subclasses of human preeclampsia." *PloS one* 10.2 (2015): e0116508.
- [62] Leavey, Katherine, et al. "Unsupervised placental gene expression profiling identifies clinically relevant subclasses of human preeclampsia." *Hypertension* 68.1 (2016): 137-147.
- [63] Yuen, R. K. C., and W. P. Robinson. "A high capacity of the human placenta for genetic and epigenetic variation: Implications for assessing pregnancy outcome." *Placenta* 32 (2011): S136-S141.
- [64] Coorens, Tim HH, et al. "Inherent mosaicism and extensive mutation of human placentas." *Nature* 592.7852 (2021): 80-85.
- [65] Benton, Samantha J., et al. "The clinical heterogeneity of preeclampsia is related to both placental gene expression and placental histopathology." *American journal of obstetrics and gynecology* 219.6 (2018): 604-e1.
- [66] Leavey, Katherine, David Grynspan, and Brian J. Cox. "Both "canonical" and "immunological" preeclampsia subtypes demonstrate changes in placental immune cell composition." *Placenta* 83 (2019): 53-56.
- [67] Gibbs, Isaac, et al. "Placental transcriptional and histologic subtypes of normotensive fetal growth restriction are comparable to preeclampsia." *American Journal of Obstetrics and Gynecology* 220.1 (2019): 110-e1.
- [68] Michela, Battistelli. "Liquid biopsy: a family of possible diagnostic tools." *Diagnostics* 11.8 (2021): 1391.
- [69] Crowley, Emily, et al. "Liquid biopsy: monitoring cancer-genetics in the blood." *Nature reviews Clinical oncology* 10.8 (2013): 472-484.
- [70] Than, Gábor N., Hans Bohn, and Dénes G. Szabó. *Advances in pregnancy-related protein research functional and clinical applications*. CRC Press, 2020.
- [71] Cole, Laurence A. "hCG, the wonder of today's science." *Reproductive Biology and Endocrinology* 10.1 (2012): 1-18.
- [72] Than, Nándor Gábor, et al. "Placental protein 13 (PP13)—a placental immunoregulatory galectin protecting pregnancy." *Frontiers in immunology* 5 (2014): 348.
- [73] LO YM, CorbettaN, et al. "Presence of fetal DNA in maternal plasma and serum." *Lancet* 350.9076 (1997): 485-487.
- [74] Than, Nándor Gábor, et al. "Early pathways, biomarkers, and four distinct molecular subclasses of preeclampsia:

- The intersection of clinical, pathological, and high-dimensional biology studies." *Placenta* (2022).
- [75] Poon, Leo LM, et al. "Presence of fetal RNA in maternal plasma." *Clinical chemistry* 46.11 (2000): 1832-1834.
- [76] Chiu, Rossa WK, et al. "Time profile of appearance and disappearance of circulating placenta-derived mRNA in maternal plasma." *Clinical chemistry* 52.2 (2006): 313-316.
- [77] Whitehead, Clare L., Susan P. Walker, and Stephen Tong. "Measuring circulating placental RNAs to non-invasively assess the placental transcriptome and to predict pregnancy complications." *Prenatal diagnosis* 36.11 (2016): 997-1008.
- [78] Nagy, Bálint, Zoltán Csanádi, and Róbert Póka. "The importance of free nucleic acids in the non-invasive diagnostics." *Orvosi Hetilap* 157.48 (2016): 1900-1909.
- [79] Nair, Soumyalekshmi, and Carlos Salomon. "Extracellular vesicles as critical mediators of maternal-fetal communication during pregnancy and their potential role in maternal metabolism." *Placenta* 98 (2020): 60-68.
- [80] Salomon, Carlos, et al. "Placental exosomes as early biomarker of preeclampsia: potential role of exosomal microRNAs across gestation." *The Journal of Clinical Endocrinology & Metabolism* 102.9 (2017): 3182-3194.
- [81] Kandzija, Neva, et al. "Placental extracellular vesicles express active dipeptidyl peptidase IV; levels are increased in gestational diabetes mellitus." *Journal of extracellular vesicles* 8.1 (2019): 1617000.
- [82] McElrath, Thomas F., et al. "Late first trimester circulating microparticle proteins predict the risk of preeclampsia < 35 weeks and suggest phenotypic differences among affected cases." *Scientific reports* 10.1 (2020): 1-15.
- [83] Than, N. G., et al. "Early pathways, biomarkers, and four distinct molecular subclasses of preeclampsia: The intersection of clinical, pathological, and high-dimensional biology studies." *Placenta* (2022).
- [84] Rasmussen, Morten, et al. "RNA profiles reveal signatures of future health and disease in pregnancy." *Nature* 601.7893 (2022): 422-427.
- [85] Farina, Antonio, et al. "Performance of messenger RNAs circulating in maternal blood in the prediction of preeclampsia at 10-14 weeks." *American journal of obstetrics and gynecology* 203.6 (2010): 575-e1.
- [86] Purwosunu, Yuditiya, et al. "Prediction of preeclampsia by analysis of cell-free messenger RNA in maternal plasma." *American journal of obstetrics and gynecology* 200.4 (2009): 386-e1.
- [87] Lv, Yan, et al. "Roles of microRNAs in preeclampsia." *Journal of cellular physiology* 234.2 (2019): 1052-1061.
- [88] Srinivasan, Srimeenakshi, et al. "Discovery and verification of extracellular miRNA biomarkers for non-invasive prediction of pre-eclampsia in asymptomatic women." *Cell Reports Medicine* 1.2 (2020): 100013.
- [89] Chaiworapongsa, Tinnakorn, et al. "Differences and similarities in the transcriptional profile of peripheral whole blood in early and late-onset preeclampsia: insights into the molecular basis of the phenotype of preeclampsia." *Journal of perinatal medicine* 41.5 (2013): 485-504.
- [90] Tarca, Adi L., et al. "Maternal whole blood mRNA signatures identify women at risk of early preeclampsia: a longitudinal study." *The Journal of Maternal-Fetal & Neonatal Medicine* 34.21 (2021): 3463-3474.
- [91] Tsang, Jason CH, et al. "Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics." *Proceedings of the National Academy of Sciences* 114.37 (2017): E7786-E7795.
- [92] Gomez-Lopez, Nardhy, et al. "The cellular transcriptome in the maternal circulation during normal pregnancy: a longitudinal study." *Frontiers in immunology* 10 (2019): 2863.
- [93] Ngo, Thuy TM, et al. "Noninvasive blood tests for fetal development predict gestational age and preterm delivery." *Science* 360.6393 (2018): 1133-1136.
- [94] Jauniaux, Eric, et al. "Onset of maternal arterial blood flow and placental oxidative stress: a possible factor in human early pregnancy failure." *The American journal of pathology* 157.6 (2000): 2111-2122.
- [95] Moffett, Ashley, and Charlie Loke. "Immunology of placentation in eutherian mammals." *Nature Reviews Immunology* 6.8 (2006): 584-594.
- [96] Saito, Shigeru. "Th17 cells and regulatory T cells: new light on pathophysiology of preeclampsia." *Immunology*

and cell biology 88.6 (2010): 615.

- [97] Saito, Shigeru, and Masatoshi Sakai. "Th1/Th2 balance in preeclampsia." *Journal of reproductive immunology* 59.2 (2003): 161-173.
- [98] Tarca, Adi L., et al. "The prediction of early preeclampsia: Results from a longitudinal proteomics study." *PloS one* 14.6 (2019): e0217273.
- [99] Erez, Offer, et al. "The prediction of late-onset preeclampsia: Results from a longitudinal proteomics study." *PloS one* 12.7 (2017): e0181468.
- [100] Wilkerson, Matthew D., and D. Neil Hayes. "ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking." *Bioinformatics* 26.12 (2010): 1572-1573.
- [101] Roberge, Stéphanie, et al. "Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis." *Fetal diagnosis and therapy* 31.3 (2012): 141-146.
- [102] Legro, Richard S. "Periconception care of the infertile patient: Are we doing enough ?." *Fertility and Sterility* 118.4 (2022): 653-655.
- [103] Romero, Roberto, et al. "Metformin, the aspirin of the 21st century: its role in gestational diabetes mellitus, prevention of preeclampsia and cancer, and the promotion of longevity." *American journal of obstetrics and gynecology* 217.3 (2017): 282-302.
- [104] de Alwis, Natasha, et al. "Novel approaches to combat preeclampsia: from new drugs to innovative delivery." *Placenta* 102 (2020): 10-16.
- [105] Morales-Prieto, Diana M., Rodolfo R. Favaro, and Udo R. Markert. "Placental miRNAs in fetomaternal communication mediated by extracellular vesicles." *Placenta* 102 (2020): 27-33.
- [106] McLaughlin, Kelsey, et al. "Circulating maternal placental growth factor responses to low-molecular-weight heparin in pregnant patients at risk of placental dysfunction." *American Journal of Obstetrics and Gynecology* 226.2 (2022): S1145-S1156.