Journal of Reproductive Immunology 161 (2024) 104172

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

Classification of preeclampsia according to molecular clusters with the goal of achieving personalized prevention

Nándor Gábor Than [a,b,c,d,e,*], Roberto Romero [f,g,h,1], Máté Posta [a,e,i], Dániel Györffy [a,e,j], Gábor Szalai [a,e,k], Simona W. Rossi [e], András Szilágyi [a], Petronella Hupuczi [c,l], Sándor Nagy [m], Olga Török [n], Adi L. Tarca [e,f,o,p,1], Offer Erez [e,f,o,q,1], Nándor Ács [b], Zoltán Papp [b,c]

[a] Systems Biology of Reproduction Research Group, Institute of Enzymology, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary
[b] Department of Obstetrics and Gynecology, School of Medicine, Semmelweis University, Budapest, Hungary
[c] Maternity Private Clinic of Obstetrics and Gynecology, Budapest, Hungary
[d] Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
[e] Genesis Theranostix Group, Budapest, Hungary
[f] Pregnancy Research Branch[1], NICHD/NIH/DHHS, Bethesda, MD, USA
[g] Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA
[h] Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, USA
[i] Semmelweis University Doctoral School, Budapest, Hungary
[j] Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary
[k] Department of Surgery, School of Medicine, University of Pécs, Pécs, Hungary
[l] Department of Anesthesiology and Intensive Therapy, Semmelweis University, Budapest, Hungary
[m] Faculty of Health and Sport Sciences, Széchenyi István University, Győr, Hungary
[n] Department of Obstetrics and Gynecology, School of Medicine, University of Debrecen, Debrecen, Hungary
[o] Department of Obstetrics and Gynecology, School of Medicine, Wayne State University School of Medicine, Detroit, MI, USA
[p] Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA
[q] Department of Obstetrics and Gynecology, Soroka University Medical Center, Ben Gurion University of the Negev, Be'er Sheva, Israel

ARTICLE INFO

Keywords:
Extracellular matrix-related pre-eclampsia
Liquid biopsy
Maternal anti-fetal rejection-type pre-eclampsia
Metabolic pre-eclampsia
Personalized medicine
Placental pre-eclampsia
Prenatal diagnosis
Proteomics
Screening

ABSTRACT

The prevention of pre-eclampsia is difficult due to the syndromic nature and multiple underlying mechanisms of this severe complication of pregnancy. The current clinical distinction between early- and late-onset disease, although clinically useful, does not reflect the true nature and complexity of the pathologic processes leading to pre-eclampsia. The current gaps in knowledge on the heterogeneous molecular pathways of this syndrome and the lack of adequate, specific diagnostic methods are major obstacles to early screening and tailored preventive strategies. The development of novel diagnostic tools for detecting the activation of the identified disease pathways would enable early, accurate screening and personalized preventive therapies. We implemented a holistic approach that includes the utilization of different proteomic profiling methods of maternal plasma samples collected from various ethnic populations and the application of systems biology analysis to plasma proteomic, maternal demographic, clinical characteristic, and placental histopathologic data. This approach enabled the identification of four molecular subclasses of pre-eclampsia in which distinct and shared disease mechanisms are activated. The current review summarizes the results and conclusions from these studies and the research and clinical implications of our findings.

1. Introduction

Pre-eclampsia, a syndrome with a heterogeneous etiologic and pathologic background, is a leading cause of maternal and perinatal morbidity and mortality (Ness and Roberts, 1996; von Dadelszen et al., 2003; Myatt and Roberts, 2015; Erez et al., 2022; Jung et al., 2022). The gaps in knowledge on the heterogeneous background of this syndrome and the limited first-trimester screening methods are major obstacles to

* Correspondence to: Department of Obstetrics and Gynecology, Semmelweis University, 27 Baross Street, H-1088, Budapest, Hungary.
E-mail address: than.gabor@semmelweis.hu (N.G. Than).

[1] The Perinatology Research Branch, NICHD/NIH/DHHS, has been renamed as the Pregnancy Research Branch, NICHD/NIH/DHHS.

https://doi.org/10.1016/j.jri.2023.104172
Received 10 July 2023; Received in revised form 13 October 2023; Accepted 23 November 2023
Available online 30 November 2023

early pre-eclampsia screening and to effective, tailored prophylaxis (Hahn et al., 2015; Malone, Haj Yahya and Kane, 2022). Currently, pre-eclampsia is classified according to its severity and to gestational age at diagnosis. "Early-onset" pre-eclampsia (~10% of cases), often referred to as "placental pre-eclampsia" that develops before the 34th week of gestation, is more likely to be associated with severe maternal symptoms, a higher incidence of fetal growth restriction, indicated preterm birth, and perinatal morbidity and mortality as well as a high incidence of placental, developmental, functional, and histopathological abnormalities. On the other hand, "late-onset" pre-eclampsia (~90% of cases), referred to as "maternal pre-eclampsia", develops after 34 weeks of gestation due to the high incidence of maternal metabolic or chronic diseases (e.g. obesity, diabetes mellitus, autoimmune diseases). Generally, it presents with a milder maternal or fetal disease and with scarce or missing placental histopathological abnormalities characteristic of early-onset pre-eclampsia, including lesions of maternal vascular malperfusion. Yet, this clinical form of pre-eclampsia still poses a significant risk for maternal and fetal short- and long-term morbidity (Roberts et al., 1989; ACOG Committee on Practice Bulletins-Obstetrics, 2002; von Dadelszen et al., 2003; Maynard et al., 2003; Matthiesen et al., 2005; Venkatesha et al., 2006; Crispi et al., 2008; Soto et al., 2012; Verlohren et al., 2014; Hansson et al., 2014; Redman et al., 2014; Myatt and Roberts, 2015; Scioscia et al., 2015; Blois et al., 2015; Robillard et al., 2019; Birukov et al., 2020; Lekva et al., 2020; Roberts et al., 2021; Staff et al., 2022; Tamás et al., 2022; Robillard et al., 2022; Rana et al., 2022).

However, these classifications do not reflect the underlying mechanisms of disease that affect the maternal, fetal, and placental compartments that, in combination, lead to a wide variety of individual patterns of maternal and fetal disease progression (Than et al., 2018; Beernink et al., 2022) (Fig. 1). Therefore, an important and challenging task is to subclassify patients into homogenous groups based on their molecular profiles that reflect the underlying mechanisms of disease. This would facilitate the discovery and development of disease pathway-specific biomarkers, improve early risk assessment and prediction, and, subsequently, the development of pathway-specific, individualized preventive therapies (Romero et al., 2022; Than et al., 2023).

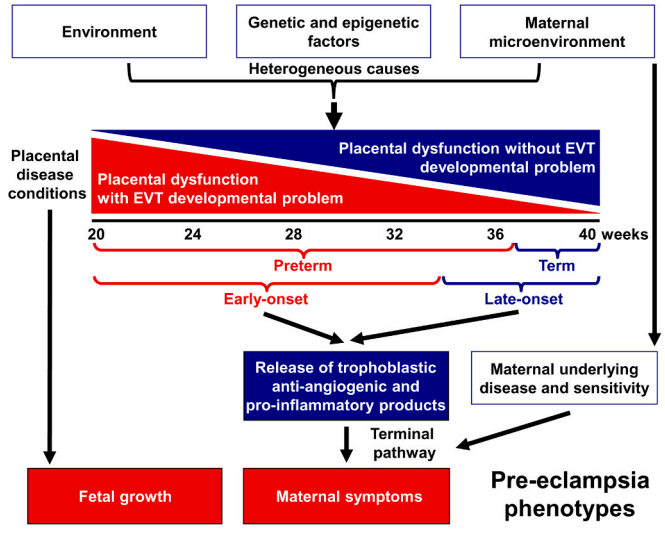

Fig. 1. The heterogeneity of pre-eclampsia. Pre-eclampsia is syndromic in nature with a heterogeneous etiology and a spectrum of phenotypes. The clinical practice has arbitrarily divided pre-eclampsia into early-onset versus late-onset or preterm versus term clinical subgroups. However, a growing body of evidence has shown that there is no clear separation between these arbitrary subgroups based on maternal, fetal, or placental phenotypes and pathologies. The sum of placental disease conditions determines fetal development and disease progression while the combination of maternal underlying disease and sensitivity with the placental release of "toxins" (anti-angiogenic, pro-inflammatory, or other products) will induce the terminal pathway of pre-eclampsia, leading to the characteristic maternal clinical symptoms. The reuse of the figure from (Than et al., 2018) was permitted by the Creative Commons Attribution License. EVT, extravillous trophoblast.

2. Molecular subclassification of pre-eclampsia

2.1. Placental transcriptomics

In recent years, there has been a growing emphasis on the molecular subclassification of pre-eclampsia and the identification of patient subgroups that present with different molecular patterns. Leavey *et al.*, the first to categorize patients with pre-eclampsia based on their placental whole-genome transcriptomic profile, conducted two aggregate microarray studies of 173 and 330 cases, respectively (Leavey et al., 2015, 2016). These large studies identified three molecular disease subclasses associated with distinct clinical manifestations: 1) *"canonical pre-eclampsia"* was consistent with abnormal uterine and umbilical artery Doppler indices, low neonatal birthweight, and an anti-angiogenic state in which the balance between angiogenic factors [e.g. placental growth factor (PlGF)] and anti-angiogenic factors (e.g. soluble vascular endothelial growth factor receptor-1 [sVEGFR-1, or sFLT-1] and/or soluble endoglin [sENG]) is disturbed; 2) *"immunologic pre-eclampsia"* was characterized by low placental and neonatal weights and the enrichment of differentially expressed genes involved in immune pathways; while 3) *"maternal pre-eclampsia"* was associated with maternal risk factors (chronic hypertension, nulliparity) and term neonates with a normal birthweight. In a subsequent study, these authors also identified placental histopathological lesions characteristic of these subtypes (Benton et al., 2018).

Abnormal placental histologic and molecular findings observed after delivery represent the end-stage of pathophysiological processes in pregnancy rather than in early disease mechanisms. Therefore, it is important to use other analytic and diagnostic methods that can identify those disease pathways and their biomarkers that drive the development of pre-eclampsia in earlier stages of pregnancy. Such a progression would allow the implementation of personalized prevention strategies early in gestation and alleviate the burden of pre-eclampsia and its associated maternal-fetal morbidities. We addressed this key issue by utilizing liquid biopsy to investigate placental and maternal disease pathways reflected by changes in the maternal plasma proteome throughout pregnancy in two separate studies that revealed similar results.

2.2. First-trimester maternal blood proteomics

First, we conducted a targeted proteomics analysis designed as a nested case-control study derived from a cohort of 2545 predominantly Caucasian pregnant women enrolled between 2010 and 2012 in Hungary. We included 164 first-trimester (11–14 gestational weeks) maternal plasma samples from 11 women with subsequent early-onset pre-eclampsia and 71 samples from women with late-onset pre-eclampsia who were matched by gestational age at blood draw with 82 healthy controls who delivered at term a healthy neonate. Nulliparity was more frequent among women who subsequently developed pre-eclampsia (73%) than among controls (45%). On the basis of the targeted mass spectrometry-based proteomics method, we detected 59 proteins (Than et al., 2022), that had earlier been identified by us (Than et al., 2018) or others as biomarker candidates and were selected for their biological plausibility in known pathways of pre-eclampsia. Proteomics data were analyzed by a vigorous feature-selection method, followed by robust, unsupervised consensus clustering, by using 1000x resampling that ensured cluster stability and optimal cluster numbers.

The 82 preeclamptic patients clustered into four distinct molecular groups (Fig. 2A). Three groups (*"placental", "immunological", and "maternal"*) had molecular profiles similar to those in the placental transcriptome reported earlier as *"canonical"*, *"immunologic"*, and

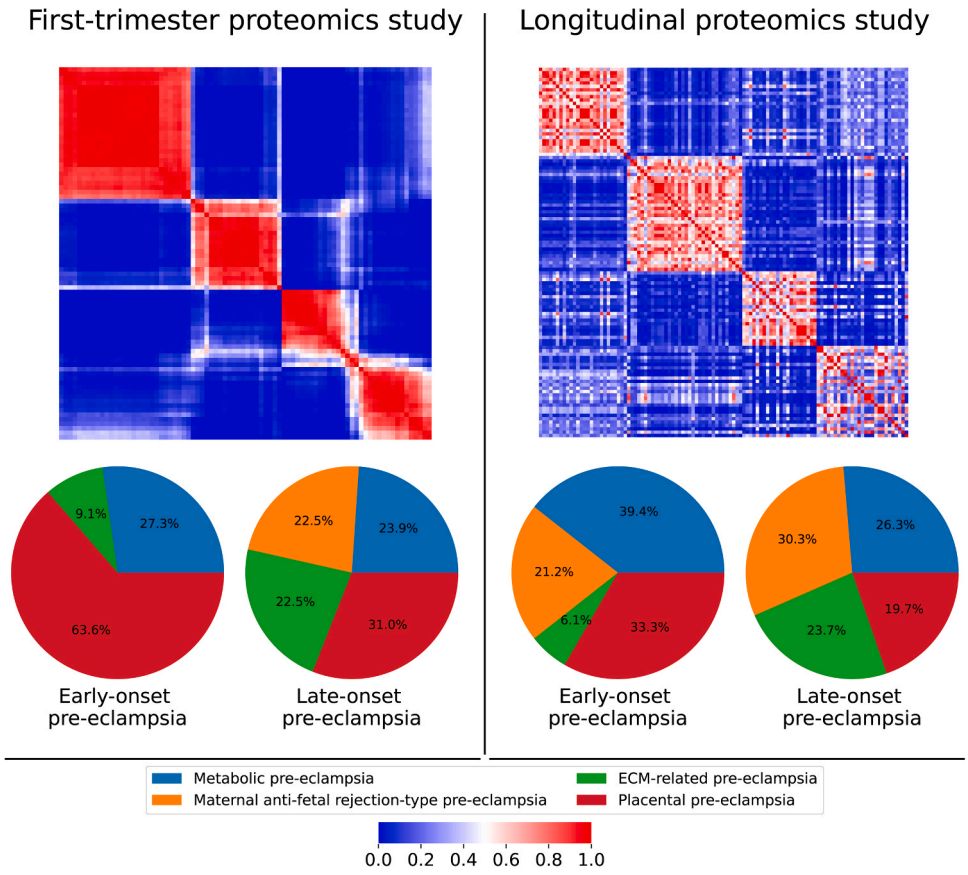

Fig. 2. Molecular subclasses of pre-eclampsia. Consensus matrices represented on the heatmaps show the probability of patients to appear in the same molecular subclasses. The consensus matrices were generated using different types of proteomic data from distinct ethnic populations. Left panel: A cross-sectional case-control study of 82 Caucasian patients detected 59 proteins with the mass spectrometry-based method in first-trimester maternal blood samples (Than et al., 2022). Right panel: A longitudinal case-control study of 109 mainly Afro-American patients detected 1125 proteins with the SOMAmer-based method in longitudinally collected maternal blood samples (Than et al., 2023). In both studies, patients stably clustered into four molecular groups. The color spectrum depicted on the bar below the panels indicates clustering similarity. The reuse of the figures from (Than et al., 2022) and (Than et al., 2023) was permitted by the Creative Commons Attribution License and DeGruyter publishers, respectively. Pie charts show the distribution of the four pre-eclampsia subclasses in early-onset and late-onset pre-eclampsia patients in both studies. In the cross-sectional proteomic study of 82 patients, more than 90% of early-onset cases belonged to *"metabolic"* and *"placental"* pre-eclampsia while the four clusters were distributed more evenly in late-onset pre-eclampsia. In the longitudinal proteomic study of 109 patients, almost 75% of early-onset cases belonged to *"metabolic"* and *"placental"* pre-eclampsia, while the four clusters were distributed more evenly in late-onset pre-eclampsia. *"Maternal anti-fetal rejection-type"* and *"ECM-related"* pre-eclampsia in (Than et al., 2023) resembled *"immunological"* and *"maternal"* pre-eclampsia, respectively, in Than et al. (2022). ECM, extracellular matrix.

"maternal" subtypes, respectively (Leavey et al., 2015, 2016; Benton et al., 2018): 1) the *"placental"* subclass we identified was consistent with oxidative stress, an anti-angiogenic state, most abnormal Doppler indices, and most early-onset and small-for-gestational-age cases; 2) the *"immunological"* subclass was characterized by a high prevalence of recurrent pre-eclampsia in patients and a systemic pro-inflammatory protein profile; and 3) the *"maternal"* subclass was associated with maternal risk factors (nulliparity), a protein profile that differed least from controls, and mostly term neonates with normal birthweight; and 4) the *"metabolic"* subclass identified in our study was novel and contained a substantial proportion of early-onset cases and a high prevalence of maternal metabolic or chronic diseases [high body mass index (BMI), chronic hypertension, diabetes mellitus) as well as pro-inflammatory and vascular changes reflected by the proteomic profile.

The profiles of the majority of women with early-onset pre-eclampsia belonged in either the *"placental"* (64%) or the *"metabolic"* (27%) subclass, corresponding altogether to 91% of the cases. Among women with late-onset pre-eclampsia, the frequency of the four subclasses was similar (Fig. 2B). Since all cases were included from the cohort, this distribution reflected the frequency of pre-eclampsia subclasses among the Hungarian patient population. Because the study included the largest patient population among proteomics studies in pre-eclampsia, a robust bioinformatics pipeline was used, and because the subclass-specific traits corresponded well with the placental transcriptome-defined patient clusters, our findings prompted validation.

2.3. Longitudinal maternal blood proteomics

To expand our maternal blood proteomics class-discovery analysis, we combined and reanalyzed two published (Erez et al., 2017; Tarca et al., 2019) longitudinal proteomics datasets from 109 patients with pre-eclampsia and 90 matched controls. Samples of these 199 women were selected retrospectively from a cohort enrolled at Wayne State University between 2004 and 2013 and comprised mostly African-American women. Since there were no differences in storage time between samples within this cohort, the same proteomics technology was used, and samples were profiled in the same batch; these parameters did not affect analysis (Than et al., 2023). Despite the large number of cases (33 early-onset disease; 76 late-onset disease), and due to the combination of data from two case-control studies, the composition of patients did not completely reflect the entire cohort. We re-used proteomics data generated from 629 samples collected at five gestational-age intervals between 8 and 32 weeks of pregnancy. The

SOMAmer-based proteomics method allowed a deeper investigation of the proteome, and the trajectory of 1125 plasma protein intensities permitted the determination of cluster-specific perturbed pathways, using the most advanced pathway analysis platform (Draghici et al., 2007; Tarca et al., 2009). Detailed results of placental histopathologic examinations, according to international standardized protocols (Langston et al., 1997; Redline et al., 2005; Khong et al., 2016), were also available for all patients, enabling the correlation of clinical, proteomic, and placental histopathologic data. Similar to our previous study (Than et al., 2022), proteomics data underwent feature-selection followed by robust, unsupervised consensus clustering for each gestational-age interval using 1000x resampling. Then, we calculated a longitudinal consensus matrix from all gestational-age intervals to reveal stable patient clusters, which we named on the basis of disease mechanisms inferred from clinical, demographic, proteomic, and placental characteristics.

Like the mass spectrometry-based proteomics study, herein we also revealed four pre-eclampsia subclasses (Fig. 2C) that had distinct characteristics: 1) the *"placental"* subclass included a high rate of early-onset cases and placental lesions of maternal vascular malperfusion and an anti-angiogenic state; 2) the *"maternal anti-fetal rejection-type"* subclass was characterized by the highest occurrence of recurrent pre-eclampsia, predominantly late-onset disease, and an anti-fetal rejection mechanism, supported by evidence from placental histopathology and maternal blood proteomics; 3) the *"extracellular matrix-related"* subclass had the highest rate of late-onset and mild pre-eclampsia cases, featured mostly by the dysregulation of extracellular matrix (ECM) proteins; and 4) the *"metabolic"* subclass was typified by a high frequency of early-onset cases and low birthweight-percentile neonates as well as metabolic perturbations and a maternal prothrombotic state. The names of all proteins with changes specific to each of the four subclasses were listed in Supplementary Table 1 of (Than et al., 2023).

Of importance, the analysis of the SOMAmer-based proteomics data, like the targeted mass spectrometry data, revealed that the *"placental"* (33%) and *"metabolic"* (39%) subclasses predominated among cases of early-onset pre-eclampsia and corresponded altogether to 72% of the cases, while all four subclasses had similar frequency among cases of late-onset pre-eclampsia (Fig. 2D). The resemblance of the findings in the two proteomics studies and their correspondence with results of other omics studies (Leavey et al., 2015, 2016; McElrath et al., 2020) suggest the generalizability of these findings and point to important future research and clinical implications.

3. Research implications of the molecular subclasses

3.1. Disease mechanisms

1) The inferred pathomechanism in the *"placental"* subclass of the second proteomics study corresponds with the classical two-stage model of pre-eclampsia development. This includes defective extracellular trophoblast invasion that leads to the failure of physiologic transformation of the spiral arteries, maternal vascular malperfusion, consequent placental ischemic stress, an anti-angiogenic state with low levels of angiogenic proteins (e.g. PlGF, VEGF), and exaggerated maternal systemic inflammation, severely affecting maternal health as well as placental and fetal growth (Roberts et al., 1989; Maynard et al., 2003; Venkatesha et al., 2006; Cindrova-Davies, 2009; Burton et al., 2009; Hahn et al., 2012; Redman e al., 2014; Hansson et al., 2014; Than et al., 2018; Han et al., 2019; Pierik et al., 2019; Erez et al., 2022; Staff et al., 2022; Rana et al., 2022; Erez et al., 2022; Romao-Veiga et al., 2022; Wang et al., 2022; Stepan et al., 2023). Further studies are required to address the question of how preexisting maternal chronic disease conditions (e.g. high BMI, chronic hypertension) (Robillard et al., 2019), placental implantation, placentation, and trophoblast development problems (Than et al., 2018; Huppertz, 2018; Szilagyi et al., 2020) or their interplay may contribute to this severe pathogenesis.
2) In the *"metabolic"* subclass, our findings were consistent with a hyper-coagulable state, also reflected by an increased abundance of prothrombin, leading to thrombotic vascular lesions in the placenta, the highest rates of maternal (55%) and fetal (24%) vascular malperfusion and placental abruption (9%) among all subclasses, consequent placental ischemia, and fetal growth restriction (Shamshirsaz et al., 2012), frequently necessitating indicated preterm delivery. Maternal vascular malperfusion was mostly manifested in recent villous infarctions, increased syncytial knots, and increased intervillous fibrin deposition; fetal vascular malperfusion also included hyalinized avascular villi, and placental abruption was present only in this subclass. These findings were in accord with the excessive activation of thrombin and the coagulation pathway earlier implicated in the development of pre-eclampsia (Chaiworapongsa et al., 2002; Said and Dekker, 2003; Erez et al., 2008, 2018, 2023). A key question remains to be answered: how may the observed perturbation of certain metabolic pathways (e.g. glucagon, insulin, and apelin signaling) (Rademacher et al., 2007; Scioscia et al., 2015; Wang et al., 2017; Villalobos-Labra et al., 2017; Ho et al., 2017; Para et al., 2022; Moldenhauer et al., 2022) lead to a hyper-coagulable state and severe placental pathogenesis besides thrombophilia (e.g. Leiden mutation) (Dekker et al., 1995; Roberts et al., 2013)?
3) The *"maternal anti-fetal rejection-type"* subclass resembled the *"immunological"* subclass in our Hungarian proteomics study (Than et al., 2022). It was characterized by CXCL10 overexpression, which induces maternal cytotoxic T-cell infiltration into the placenta and fetal membranes, resulting in anti-fetal rejection (Kim et al., 2015), consistent with the high rate of major chronic inflammatory lesions (i.e. villitis of unknown etiology and chronic chorioamnionitis) seen in this subclass. Maternal anti-fetal rejection often occurs after 34 weeks of gestation and can explain the high rate of late-onset pre--eclampsia observed in this subclass of patients. There are three key questions: Is genetic incompatibility (e.g. natural killer cell receptors vs. human leukocyte antigen haplotypes) (Moffett and Colucci, 2015; Lakatos et al., 2022) reflected by the highest prevalence of cases of recurrent pre-eclampsia in this group? May genetic factors alone or in combination with the observed high BMI-associated systemic inflammation (Fink et al., 2019) result in the perturbed atherosclerosis pathway (Priest and Tontonoz, 2019) and in acute atherosis of the decidual arterioles (Staff et al., 2022)? Which treatment modalities can be utilized to inhibit this pathway activation and to prevent the development of pre-eclampsia in these patients?
4) The *"extracellular matrix-related"* subclass of patients had the highest rate of late-onset pre-eclampsia, the lowest mean arterial pressure, modest placental histopathologic changes, and neonates with the highest mean birthweight percentiles. This subclass mainly resembled the *"maternal"* subclass in our Hungarian proteomics study (Than et al., 2022) and the same subclass in the placental transcriptomics studies (Leavey et al., 2015, 2016). In this subclass, the proteomic changes started only in mid-gestation, and differentially abundant proteins included those involved in ECM–cell interactions [e.g. collagen receptor and interstitial collagenase (matrix metalloproteinase-1)], while perturbed pathways included 'focal adhesion' and 'ECM and ECM–receptor interaction.' These findings pointed to the perturbation of cell–cell and cell–ECM interactions as the mechanism of disease that would affect primarily the endothelium, leading to a late-onset and mild phenotype. Two big questions could be addressed in future investigations: What activates cell–ECM interaction perturbations, and how does this mechanism develop? Which medications can be used to prevent the disease?

3.2. Shared, distinct, and converging disease pathways

The differences in disease mechanisms among the molecular

subclasses in the second proteomics study were clearly observed by the dysregulation of biological pathways, which was more distinct (44 pathways) than shared (19 pathways) among the subclasses. 'Platelet activation' was shared by all subclasses, suggesting that the activation of platelets (Jakobsen et al., 2019; Forstner et al., 2021) and the consequent release of pro-inflammatory and procoagulant mediators (Ghasemzadeh and Hosseini, 2013) may be a central, terminal pathomechanism of pre-eclampsia. This may also explain the beneficial effect of aspirin (an anti-platelet drug) in the prevention of pre-eclampsia.

The two most severe subclasses of pre-eclampsia—"metabolic" and "placental"—involved the most perturbed pathways, i.e., 53 pathways in *"placental"* and 21 in *"metabolic"* pre-eclampsia. Of importance, 14 pathways were shared by these two subclasses, the most by any of the four subclasses. Of interest, at the 24–28 gestational week period, we observed the common clustering of patients with *"metabolic"* and *"placental"* pre-eclampsia (Fig. 3A). Although the shared pathways, the common clustering of patients at a later gestational period, and their placental histopathologic findings suggest that the initial pathologies of these two subclasses differ, the subsequent pathological processes converge in the second half of pregnancy (Fig. 3B). Our data suggest that the pathologies initially include maternal vascular malperfusion due to distinct reasons (prothrombotic processes in *"metabolic"* subclass vs abnormal trophoblast invasion in *"placental"* subclass), which lead to subsequent placental hypoxia/ischemia and secondary placental pathologies (e.g. villous infarcts). The placental transcriptomic profile of these two subclasses may also converge and cannot be differentiated at the end of pregnancy, which is substantiated by the class-discovery placental transcriptomic studies (Leavey et al., 2015, 2016). Our study also revealed that while trophoblast invasion problems affect only the maternal compartment in the *"placental"* subclass, coagulation problems in the *"metabolic"* subclass lead to maternal and fetal thrombotic vascular lesions as well as placental abruption. Herein, we are left with the questions of how are all of them activated and how does it happen.

3.3. The timing of differential protein abundance and disease onset

We reported that differentially abundant proteins change dynamically during gestation, reflecting the activation or inhibition of disease pathways in various subclasses. There were remarkable proteomic changes between 16 and 19.9 weeks of gestation in two subclasses with the highest rate of early-onset pre-eclampsia (*"placental"* and *"metabolic"*), in line with reports that early-onset disease progression cannot be prevented beyond 16 weeks of gestation (Poon et al., 2019; Chaemsaithong et al., 2022; Rolnik et al., 2022; Stefanovic, 2023). Another peak of changes in these two subclasses occurred after 28 weeks of gestation, reflecting the late activation of disease pathways in late-onset cases in these subclasses.

By contrast, in the *"maternal anti-fetal rejection-type"* and *"extracellular matrix-related"* subclasses, which consisted of predominantly late-onset cases, the peak of proteomic changes occurred between 20 and 23.9 weeks of gestation, in line with the later and milder pathogenesis in these subclasses. It would be important to investigate at the level of individual patients in longitudinal studies how and when pathway

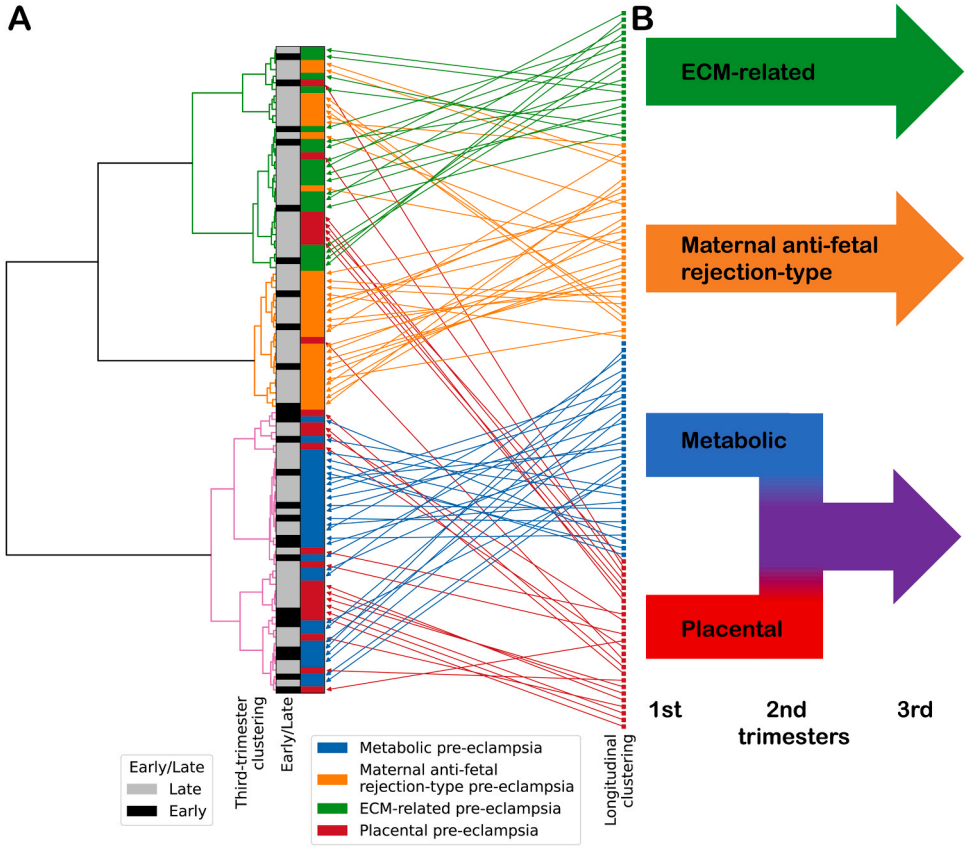

Fig. 3. The convergence/divergence of pathologies in molecular subclasses. **A)** The graph illustrates the assignment of samples to subclasses (clusters) between the 24–28 week period in comparison to their distribution in the longitudinal analysis (Than et al., 2023). The dendrogram was based on the consensus matrix of patients in the 24–28 week interval and reveals the formation of three distinct clusters, with the mixture of patients in the *"placental"* subclass and *"metabolic"* subclass. The color bars indicate the cluster membership in the longitudinal clustering, and the gray/black bars indicate early-onset or late-onset pre-eclampsia samples. The arrows show the connection between cluster membership of the same patients in the longitudinal and in the 24–28 weeks' gestational interval analyses. **B)** The schematic diagram illustrates the observed convergence of *"metabolic"* and *"placental"* subclass pathologies in the second half of pregnancy. ECM, extracellular matrix.

activation occurs and in which timeframe the escalation of the pathomechanisms leads to clinical symptoms in the four subclasses.

4. Clinical implications of the molecular subclasses

The large number of cases in our two studies combined with robust proteomics and bioinformatics methods enabled confident subclass discovery. The deep coverage of the proteome contributed to the identification of pathway perturbation in the longitudinal study. The analysis of available maternal demographic, clinical, and placental histopathologic features also enabled the complex characterization of maternal and placental disease mechanisms. The detection of four similar patient subclasses in two different populations supports the generalization of our findings. The identification of these distinct molecular subclasses is an important advancement in our understanding of the disease mechanisms of pre-eclampsia that may enable a more specific taxonomy as well as subclass-specific diagnostic and personalized preventive options.

4.1. Diagnostic implications

Based on these findings, we propose that preeclamptic patients can reliably be subclassified by liquid biopsy utilizing maternal blood proteomics, and superior biomarkers can be developed to reduce the heterogeneity among patients. Such biomarkers may be informative for a specific molecular subclass but still not be predictive for all cases, which is in line with previous pre-eclampsia studies showing the lack of a single, specific, and sensitive biomarker for this syndrome (Than et al., 2008).

Our data on the larger and earlier proteomic changes in two subclasses with most early-onset cases (*"placental"* and *"metabolic"*) are in agreement with the observations that early-onset pre-eclampsia can be better predicted than late-onset pre-eclampsia using first trimester biomarkers (Erez et al., 2017; Poon et al., 2019; Tarca et al., 2019; Chaemsaithong et al., 2022). The large proportion of patients in these two subclasses among early-onset cases in both proteomics studies (72% and 91%, respectively) are in line with data showing the good sensitivities of existing biomarkers that are associated with pathologies of these two subclasses (*"placental"*: PlGF; *"metabolic"*: thrombin generation assay) in the screening of early-onset pre-eclampsia. The much lesser proportion of women in these two subclasses among late-onset pre-eclampsia cases underscores why PlGF or thrombin generation assay has less screening accuracy for late-onset pre-eclampsia in the first trimester (Crispi et al., 2008; Verlohren et al., 2014; Erez et al., 2017; Tarca et al., 2019; Chaemsaithong et al., 2022; Erez et al., 2023). Our data also imply that a small set of biomarkers for subclasses with the most late-onset disease may still enable good prediction in the first trimester. Alternatively, a two-step screening approach that would target mainly early-onset pre-eclampsia in the first trimester and late-onset disease in the second trimester may also be successful.

4.2. Therapeutic implications

Since our data revealed pathway perturbation specific to each subclass, it suggests that the addition of molecular subclassification to the conventional classification may also inform subclass-specific preventive treatment options (Mastrolia et al., 2016; Romero et al., 2017; Rolnik et al., 2022; Cruz-Lemini et al., 2022; Tong et al., 2022). The "*placental*" subclass can be screened with high sensitivity by using already established biomarkers (e.g. PlGF) and predictive models (Wright, Wright and Nicolaides, 2020; Tarca et al., 2022), among which the one developed by the Fetal Medicine Foundation (FMF) was recommended as best practice by the International Federation of Gynecology and Obstetrics (FIGO) (Poon et al., 2019). In this subclass, prophylactic aspirin therapy is especially effective if administered before the 16th week of gestation, a treatment recommended by FIGO, the International Academy of Perinatal Medicine, and the International Society for the Study of Hypertension in Pregnancy guidelines (Poon et al., 2019; Chaemsaithong et al., 2022; Rolnik et al., 2022; Magee et al., 2022; Stefanovic, 2023). Our data on the proportion of women in the *"placental"* subclass among early-onset and late-onset pre-eclampsia may explain the different effectiveness of aspirin therapy in these clinical subgroups (Poon et al., 2019; Chaemsaithong et al., 2022). We have to note that pre-eclampsia screening and aspirin prophylaxis were still not introduced into the general practice at the time of sample collections in either of the cohorts we analyzed; therefore, in certain populations, the "placental" subtype may have lower frequency nowadays due to effective screening and aspirin prevention in place.

Women with "*metabolic*" pre-eclampsia who have prothrombotic parameters may be good candidates for prophylaxis by low molecular-weight heparin (LMWH) if identified as being at risk during the first trimester (Mastrolia et al., 2016; Cruz-Lemini et al., 2022). Our findings on the heterogeneity of the study populations and the proportion of women in the *"metabolic"* subclass may explain the varying results of clinical trials on LMWH prophylaxis in pre-eclampsia (Mastrolia et al., 2016; Cruz-Lemini et al., 2022). In addition, Roberge *et al.* has found in their meta-analysis that the combination of LMWH and low-dose aspirin was more effective than low-dose aspirin alone for the prevention of early-onset pre-eclampsia in women with a history of pre-eclampsia, underlying our findings on the predominant prevalence of *"placental"* and *"metabolic"* subclasses among early-onset patients (Roberge et al., 2016). A recent study demonstrated that perturbation in the kinetics of thrombin generation can identify a subset of patients that may benefit from LMWH administration for the prevention of pre-eclampsia (Erez et al., 2023). Thus, an effective screening protocol for women in this subclass would require biomarkers specific to this cluster, including thrombin generation assays.

Since women in three subclasses (*"metabolic", "maternal anti-fetal rejection-type," "extracellular matrix-related")* were overweight, they could benefit from changes in lifestyle: specifically, by achieving an optimal gestational weight gain, pre-eclampsia frequency could be halved (Robillard et al., 2023). Alternatively, the tailored therapies in for women in different subclasses could be achieved by other potential preventive treatments that are under testing (e.g. metformin, pravastatin) (Romero et al., 2017; Robertson et al., 2019; de Alwis et al., 2020; Saito et al., 2021; Tong et al., 2022).

5. Summary and conclusions

The analysis of the plasma proteome of pregnant women in the first trimester and longitudinally in pregnancy revealed four distinct subclasses of pre-eclampsia. The parallel analysis of the plasma proteome with maternal demographic, clinical, and placental histopathological data also enabled the inference of cluster-specific perturbed pathways in the maternal and placental compartments. The similarity between the results of these different studies, in spite of the differences in proteomics platforms and populations, strengthens the confidence in these findings. The development of novel diagnostic tools for detecting the activation of the identified disease pathways would enable early and accurate screening and personalized preventive therapies.

Funding

The proteomics studies were funded by the Perinatology Research Branch, Division of Obstetrics and Maternal Fetal Medicine, Division of Intramural Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, United States Department of Health and Human Services (NICHD/NIH/DHHS); and with Federal funds from NICHD/NIH/DHHS under contract No. HHSN275201300006C; and the Hungarian Ministry for National Economy, Grant GINOP-2.1.7-15-2016-00415. Data re-analysis and manuscript writing was funded in addition by the Hungarian Research

Network (HUN-REN) SA-83/2021 grant and by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the K128262, 2020-1.1.2-PIACI-KFI-2021-00273, 2020-1.1.5-GYORSÍTÓSÁV-2021-00012 and 2019-2.1.7-ERANET-2020-00014 funding schemes, the latter under the frame of European Union ERA PerMed (2020-346 grant). ALT was supported by the Wayne State University Perinatal Initiative in Maternal, Perinatal and Child Health.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript, have written the manuscript, and approved its submission.

Declaration of Competing Interest

No conflict of interest was reported by the authors, except NGT, ALT, ZP, and RR who are inventors of granted patents on biomarkers of preeclampsia, and by authors affiliated with Genesis Theranostix Group are involved in the industrial utilization of them. The funders had no role in the design of the reported study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results. RR has contributed to this work as part of his official duties as an employee of the United States Federal Government.

Acknowledgments

We thank Maureen McGerty, M.A. (Pregnancy Research Branch, NICHD/NIH/DHHS) for her critical reading of the manuscript and editorial support.

References

ACOG Committee on Practice Bulletins–Obstetrics, 2002. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. Obstet. Gynecol. 99 (1), 159–167. https://doi.org/10.1016/s0029-7844(01)01747-1.

de Alwis, N., et al., 2020. Novel approaches to combat preeclampsia: from new drugs to innovative delivery. Placenta 102, 10–16. https://doi.org/10.1016/j.placenta.2020.08.022.

Beernink, R.H.J., et al., 2022. First trimester serum biomarker discovery study for early onset, preterm onset and preeclampsia at term. Placenta 128, 39–48. https://doi.org/10.1016/j.placenta.2022.08.010.

Benton, S.J., et al., 2018. The clinical heterogeneity of preeclampsia is related to both placental gene expression and placental histopathology. Am. J. Obstet. Gynecol. 219 (6), 604. https://doi.org/10.1016/j.ajog.2018.09.036.

Birukov, A., et al., 2020. Myocardial evaluation of post-preeclamptic women by CMR: is early risk stratification possible? JACC Cardiovasc. Imaging 13 (5), 1291–1293. https://doi.org/10.1016/j.jcmg.2020.01.005.

Blois, S.M., et al., 2015. A potential pathophysiological role for galectins and the renin-angiotensin system in preeclampsia. Cell. Mol. Life Sci. 72 (1), 39–50. https://doi.org/10.1007/s00018-014-1713-1.

Burton, G.J., et al., 2009. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta 30 (6), 473–482. https://doi.org/10.1016/j.placenta.2009.02.009.

Chaemsaithong, P., Sahota, D.S., Poon, L.C., 2022. First trimester preeclampsia screening and prediction. Am. J. Obstet. Gynecol. 226 (2), S1071–S1097. https://doi.org/10.1016/j.ajog.2020.07.020.

Chaiworapongsa, T., et al., 2002. Evidence of in vivo generation of thrombin in patients with small-for-gestational-age fetuses and pre-eclampsia. J. Matern. Fetal Neonatal Med. 11 (6), 362–367. https://doi.org/10.1080/jmf.11.6.362.367.

Cindrova-Davies, T., 2009. Gabor Than Award Lecture 2008: pre-eclampsia—from placental oxidative stress to maternal endothelial dysfunction. Placenta 30 (Suppl A), S55–65. https://doi.org/10.1016/j.placenta.2008.11.020.

Crispi, F., et al., 2008. Predictive value of angiogenic factors and uterine artery Doppler for early—versus late-onset pre-eclampsia and intrauterine growth restriction. Ultrasound Obstet. Gynecol. 31 (3), 303–309. https://doi.org/10.1002/uog.5184.

Cruz-Lemini, M., et al., 2022. Low-molecular-weight heparin for prevention of preeclampsia and other placenta-mediated complications: a systematic review and meta-analysis. Am. J. Obstet. Gynecol. 226 (2), S1126–S1144. https://doi.org/10.1016/j.ajog.2020.11.006.

Dekker, G.A., et al., 1995. Underlying disorders associated with severe early-onset preeclampsia. Am. J. Obstet. Gynecol. 173 (4), 1042–1048. https://doi.org/10.1016/0002-9378(95)91324-6.

Draghici, S., et al., 2007. A systems biology approach for pathway level analysis. Genome Res. 17 (10), 1537–1545. https://doi.org/10.1101/gr.6202607.

Erez, O., et al., 2008. Over-expression of the thrombin receptor (PAR-1) in the placenta in preeclampsia: a mechanism for the intersection of coagulation and inflammation. J. Matern.-Fetal. 21 (6), 345–355. https://doi.org/10.1080/14767050802034859.

Erez, O., et al., 2017. The prediction of late-onset preeclampsia: results from a longitudinal proteomics study. PLoS One 12 (7), e0181468. https://doi.org/10.1371/journal.pone.0181468.

Erez, O., et al., 2018. The pattern and magnitude of "in vivo thrombin generation" differ in women with preeclampsia and in those with SGA fetuses without preeclampsia. J. Matern.-Fetal. 31 (13), 1671–1680. https://doi.org/10.1080/14767058.2017.1323327.

Erez, O., et al., 2022. Preeclampsia and eclampsia: the conceptual evolution of a syndrome. Am. J. Obstet. Gynecol. 226 (2), S786–S803. https://doi.org/10.1016/j.ajog.2021.12.001.

Erez, O., et al., 2023. Perturbations in kinetics of the thrombin generation assay identify women at risk of preeclampsia in the first trimester and provide the rationale for a preventive approach. Am. J. Obstet. Gynecol. 228 (5), 580.e1–580.e17. https://doi.org/10.1016/j.ajog.2022.11.1276.

Fink, N.R., et al., 2019. Levels of systemic low-grade inflammation in pregnant mothers and their offspring are correlated. Sci. Rep. 9 (1), 3043 https://doi.org/10.1038/s41598-019-39620-5.

Forstner, D., Guettler, J., Gauster, M., 2021. Changes in maternal platelet physiology during gestation and their interaction with trophoblasts. Int. J. Mol. Sci. 22 (19), 10732. https://doi.org/10.3390/ijms221910732.

Ghasemzadeh, M., Hosseini, E., 2013. Platelet-leukocyte crosstalk: linking proinflammatory responses to procoagulant state. Thromb. Res. 131 (3), 191–197. https://doi.org/10.1016/j.thromres.2012.11.028.

Hahn, S., et al., 2012. Neutrophil NETs in reproduction: from infertility to preeclampsia and the possibility of fetal loss. Front. Immunol. 3 https://doi.org/10.3389/fimmu.2012.00362.

Hahn, S., Lapaire, O., Than, N.G., 2015. Biomarker development for presymptomatic molecular diagnosis of preeclampsia: feasible, useful or even unnecessary? Expert Rev. Mol. Diagn. 15 (5), 617–629. https://doi.org/10.1586/14737159.2015.1025757.

Han, X., et al., 2019. Differential dynamics of the maternal immune system in healthy pregnancy and preeclampsia. Front. Immunol. 10, 1305 https://doi.org/10.3389/fimmu.2019.01305.

Hansson, S.R., Nääv, Å., Erlandsson, L., 2014. Oxidative stress in preeclampsia and the role of free fetal hemoglobin. Front. Physiol. 5, 516. https://doi.org/10.3389/fphys.2014.00516.

Ho, L., et al., 2017. ELABELA deficiency promotes preeclampsia and cardiovascular malformations in mice. Science 357 (6352), 707–713. https://doi.org/10.1126/science.aam6607.

Huppertz, B., 2018. The critical role of abnormal trophoblast development in the etiology of preeclampsia. Curr. Pharm. Biotechnol. 19 (10), 771–780. https://doi.org/10.2174/1389201019666180427110547.

Jakobsen, C., et al., 2019. Platelet function in preeclampsia—a systematic review and meta-analysis. Platelets 30 (5), 549–562. https://doi.org/10.1080/09537104.2019.1595561.

Jung, E., et al., 2022. The etiology of preeclampsia. Am. J. Obstet. Gynecol. 226 (2S), S844–S866. https://doi.org/10.1016/j.ajog.2021.11.1356.

Khong, T.Y., et al., 2016. Sampling and definitions of placental lesions: Amsterdam placental workshop group consensus statement. Arch. Pathol. Lab. Med. 140 (7), 698–713. https://doi.org/10.5858/arpa.2015-0225-CC.

Kim, C.J., et al., 2015. Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. Am. J. Obstet. Gynecol. 213 (4 Suppl), S53–69. https://doi.org/10.1016/j.ajog.2015.08.041.

Lakatos, K., et al., 2022. The role of natural killer cells in the immune homeostasis of the maternal fetal interface. Orv. Hetil. 163 (19), 734–742. https://doi.org/10.1556/650.2022.32458.

Langston, C., et al., 1997. Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists. Arch. Pathol. Lab. Med. 121 (5), 449–476.

Leavey, K., et al., 2016. Unsupervised placental gene expression profiling identifies clinically relevant subclasses of human preeclampsia. Hypertension 68 (1), 137–147. https://doi.org/10.1161/HYPERTENSIONAHA.116.07293.

Leavey, K., Bainbridge, S.A., Cox, B.J., 2015. Large scale aggregate microarray analysis reveals three distinct molecular subclasses of human preeclampsia. PLoS One 10 (2), e0116508. https://doi.org/10.1371/journal.pone.0116508.

Lekva, T., et al., 2020. Multiplex analysis of circulating maternal cardiovascular biomarkers comparing preeclampsia subtypes. Hypertension 75 (6), 1513–1522. https://doi.org/10.1161/HYPERTENSIONAHA.119.14580.

Magee, L.A., et al., 2022. The 2021 International Society for the Study of Hypertension in Pregnancy classification, diagnosis & management recommendations for international practice. Pregnancy Hypertens. 27, 148–169. https://doi.org/10.1016/j.preghy.2021.09.008.

Malone, S.L., Haj Yahya, R., Kane, S.C., 2022. Reviewing accuracy of first trimester screening for preeclampsia using maternal factors and biomarkers. Int. J. Women's Health 14, 1371–1384. https://doi.org/10.2147/IJWH.S283239.

Mastrolia, S.A., et al., 2016. LMWH in the prevention of preeclampsia and fetal growth restriction in women without thrombophilia. A systematic review and meta-analysis. Thromb. Haemost. 116 (5), 868–878. https://doi.org/10.1160/TH16-02-0169.

Matthiesen, L., et al., 2005. Immunology of preeclampsia. Chem. Immunol. Allergy 89, 49–61. https://doi.org/10.1159/000087912.

Maynard, S.E., et al., 2003. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in

preeclampsia. J. Clin. Investig. 111 (5), 649–658. https://doi.org/10.1172/JCI17189.
McElrath, T.F., et al., 2020. Late first trimester circulating microparticle proteins predict the risk of preeclampsia < 35 weeks and suggest phenotypic differences among affected cases. Sci. Rep. 10 (1), 17353. https://doi.org/10.1038/s41598-020-74078-w.
Moffett, A., Colucci, F., 2015. Co-evolution of NK receptors and HLA ligands in humans is driven by reproduction. Immunol. Rev. 267 (1), 283–297. https://doi.org/10.1111/imr.12323.
Moldenhauer, L.M., et al., 2022. Immune-metabolic interactions and T cell tolerance in pregnancy. J. Immunol. 209 (8), 1426–1436. https://doi.org/10.4049/jimmunol.2200362.
Myatt, L., Roberts, J.M., 2015. Preeclampsia: syndrome or disease? Curr. Hypertens. Rep. 17 (11), 83 https://doi.org/10.1007/s11906-015-0595-4.
Ness, R.B., Roberts, J.M., 1996. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. Am. J. Obstet. Gynecol. 175 (5), 1365–1370. https://doi.org/10.1016/S0002-9378(96)70056-X.
Para, R., et al., 2022. Maternal circulating concentrations of soluble Fas and Elabela in early- and late-onset preeclampsia. J. Matern. -Fetal Neonatal Med. 35 (2), 316–329. https://doi.org/10.1080/14767058.2020.1716720.
Pierik, E., et al., 2019. Dysregulation of complement activation and placental dysfunction: a potential target to treat preeclampsia? Front. Immunol. 10, 3098 https://doi.org/10.3389/fimmu.2019.03098.
Poon, L.C., et al., 2019. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: a pragmatic guide for first-trimester screening and prevention. Int. J. Gynaecol. Obstet. 145 (Suppl 1), 1–33. https://doi.org/10.1002/ijgo.12802.
Priest, C., Tontonoz, P., 2019. Inter-organ cross-talk in metabolic syndrome. Nat. Metab. 1 (12), 1177–1188. https://doi.org/10.1038/s42255-019-0145-5.
Rademacher, T.W., Gumaa, K., Scioscia, M., 2007. Preeclampsia, insulin signalling and immunological dysfunction: a fetal, maternal or placental disorder? J. Reprod. Immunol. 76 (1–2), 78–84. https://doi.org/10.1016/j.jri.2007.03.019.
Rana, S., Burke, S.D., Karumanchi, S.A., 2022. Imbalances in circulating angiogenic factors in the pathophysiology of preeclampsia and related disorders. Am. J. Obstet. Gynecol. 226 (2), S1019–S1034. https://doi.org/10.1016/j.ajog.2020.10.022.
Redline, R.W., et al., 2005. Placental diagnostic criteria and clinical correlation—a workshop report. Placenta 26 (Suppl A), S114–117. https://doi.org/10.1016/j.placenta.2005.02.009.
Redman, C.W., Sargent, I.L., Staff, A.C., 2014. IFPA Senior Award Lecture: making sense of pre-eclampsia—two placental causes of preeclampsia? Placenta 35, S20–S25. https://doi.org/10.1016/j.placenta.2013.12.008.
Roberge, S., et al., 2016. Prevention of pre-eclampsia by low-molecular-weight heparin in addition to aspirin: a meta-analysis. Ultrasound Obstet. Gynecol. 47 (5), 548–553. https://doi.org/10.1002/uog.15789.
Roberts, J.M., et al., 1989. Preeclampsia: an endothelial cell disorder. Am. J. Obstet. Gynecol. 161 (5), 1200–1204. https://doi.org/10.1016/0002-9378(89)90665-0.
Roberts, J.M., et al., 2021. Subtypes of preeclampsia: recognition and determining clinical usefulness. Hypertension 77 (5), 1430–1441. https://doi.org/10.1161/HYPERTENSIONAHA.120.14781.
Roberts, L.N., et al., 2013. African-Caribbean ethnicity is associated with a hypercoagulable state as measured by thrombin generation. Blood Coagul. Fibrinolysis 24 (1), 40–49. https://doi.org/10.1097/MBC.0b013e32835a07fa.
Robertson, S.A., et al., 2019. Therapeutic potential of regulatory T cells in preeclampsia-opportunities and challenges. Front. Immunol. 10, 478 https://doi.org/10.3389/fimmu.2019.00478.
Robillard, P.-Y., et al., 2019. Increased BMI has a linear association with late-onset preeclampsia: a population-based study. PLoS One 14 (10), e0223888. https://doi.org/10.1371/journal.pone.0223888.
Robillard, P.-Y., et al., 2022. Progress in the understanding of the pathophysiology of immunologic maladaptation related to early-onset preeclampsia and metabolic syndrome related to late-onset preeclampsia. Am. J. Obstet. Gynecol. 226 (2), S867–S875. https://doi.org/10.1016/j.ajog.2021.11.019.
Robillard, P.-Y., et al., 2023. Preeclampsia in 2023: time for preventing early onset- and term preeclampsia: the paramount role of gestational weight gain. J. Reprod. Immunol. 158, 103968 https://doi.org/10.1016/j.jri.2023.103968.
Rolnik, D.L., Nicolaides, K.H., Poon, L.C., 2022. Prevention of preeclampsia with aspirin. Am. J. Obstet. Gynecol. 226 (2S), S1108–S1119. https://doi.org/10.1016/j.ajog.2020.08.045.
Romao-Veiga, M., et al., 2022. DAMPs are able to skew CD4+ T cell subsets and increase the inflammatory profile in pregnant women with preeclampsia. J. Reprod. Immunol. 149, 103470 https://doi.org/10.1016/j.jri.2021.103470.
Romero, R., et al., 2017. Metformin, the aspirin of the 21st century: its role in gestational diabetes mellitus, prevention of preeclampsia and cancer, and the promotion of longevity. Am. J. Obstet. Gynecol. 217 (3), 282–302. https://doi.org/10.1016/j.ajog.2017.06.003.
Romero, R., et al., 2022. Toward a new taxonomy of obstetrical disease: improved performance of maternal blood biomarkers for the great obstetrical syndromes when classified according to placental pathology. Am. J. Obstet. Gynecol. 227 (4), 615. https://doi.org/10.1016/j.ajog.2022.04.015.
Said, J., Dekker, G., 2003. Pre-eclampsia and thrombophilia. Best Pract. Res. Clin. Obstet. Gynaecol. 17 (3), 441–458. https://doi.org/10.1016/s1521-6934(03)00008-7.
Saito, S., et al., 2021. A randomized phase 3 trial evaluating antithrombin gamma treatment in Japanese patients with early-onset severe preeclampsia (KOUNO-TORI study): study protocol. Contemp. Clin. Trials 107, 106490. https://doi.org/10.1016/j.cct.2021.106490.
Scioscia, M., et al., 2015. Endothelial dysfunction and metabolic syndrome in preeclampsia: an alternative viewpoint. J. Reprod. Immunol. 108, 42–47. https://doi.org/10.1016/j.jri.2015.01.009.
Shamshirsaz, A.A., Paidas, M., Krikun, G., 2012. Preeclampsia, hypoxia, thrombosis, and inflammation. J. Pregnancy 2012, 374047. https://doi.org/10.1155/2012/374047.
Soto, E., et al., 2012. Late-onset preeclampsia is associated with an imbalance of angiogenic and anti-angiogenic factors in patients with and without placental lesions consistent with maternal underperfusion. J. Matern. -Fetal Neonatal Med. 25 (5), 498–507. https://doi.org/10.3109/14767058.2011.591461.
Staff, A.C., et al., 2022. Failure of physiological transformation and spiral artery atherosis: their roles in preeclampsia. Am. J. Obstet. Gynecol. 226 (2), S895–S906. https://doi.org/10.1016/j.ajog.2020.09.026.
Stefanovic, V., 2023. International Academy of Perinatal Medicine (IAPM) guidelines for screening, prediction, prevention and management of pre-eclampsia to reduce maternal mortality in developing countries. J. Perinat. Med. 51 (2), 164–169. https://doi.org/10.1515/jpm-2021-0636.
Stepan, H., et al., 2023. Clinical utility of sFlt-1 and PlGF in screening, prediction, diagnosis and monitoring of pre-eclampsia and fetal growth restriction. Ultrasound Obstet. Gynecol. 61 (2), 168–180. https://doi.org/10.1002/uog.26032.
Szilagyi, A., et al., 2020. Placenta-specific genes, their regulation during villous trophoblast differentiation and dysregulation in preterm preeclampsia. Int. J. Mol. Sci. 21 (2), E628 https://doi.org/10.3390/ijms21020628.
Tamás, P., et al., 2022. The two faces of preeclampsia. Orv. Hetil. 163 (17), 663–669. https://doi.org/10.1556/650.2022.32427.
Tarca, A.L., et al., 2009. A novel signaling pathway impact analysis. Bioinformatics 25 (1), 75–82. https://doi.org/10.1093/bioinformatics/btn577.
Tarca, A.L., et al., 2019. The prediction of early preeclampsia: results from a longitudinal proteomics study. PloS One 14 (6), e0217273. https://doi.org/10.1371/journal.pone.0217273.
Tarca, A.L., et al., 2022. Prediction of preeclampsia throughout gestation with maternal characteristics and biophysical and biochemical markers: a longitudinal study. Am. J. Obstet. Gynecol. 226 (1), 126. https://doi.org/10.1016/j.ajog.2021.01.020.
Than, N.G., et al., 2008. Prediction of preeclampsia—a workshop report. Placenta 29 (Suppl A), S83–85. https://doi.org/10.1016/j.placenta.2007.10.008.
Than, N.G., et al., 2018. Integrated systems biology approach identifies novel maternal and placental pathways of preeclampsia. Front. Immunol. 9, 1661 https://doi.org/10.3389/fimmu.2018.01661.
Than, N.G., et al., 2022. Early pathways, biomarkers, and four distinct molecular subclasses of preeclampsia: the intersection of clinical, pathological, and high-dimensional biology studies. Placenta 125, 10–19. https://doi.org/10.1016/j.placenta.2022.03.009.
Than, N.G., et al., 2023. Molecular subclasses of preeclampsia characterized by a longitudinal maternal proteomics study: distinct biomarkers, disease pathways and options for prevention. J. Perinat. Med. 51 (1), 51–68. https://doi.org/10.1515/jpm-2022-0433.
Tong, S., et al., 2022. Pravastatin, proton-pump inhibitors, metformin, micronutrients, and biologics: new horizons for the prevention or treatment of preeclampsia. Am. J. Obstet. Gynecol. 226 (2), S1157–S1170. https://doi.org/10.1016/j.ajog.2020.09.014.
Venkatesha, S., et al., 2006. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat. Med. 12 (6), 642–649. https://doi.org/10.1038/nm1429.
Verlohren, S., et al., 2014. Uterine artery Doppler, birth weight and timing of onset of pre-eclampsia: providing insights into the dual etiology of late-onset pre-eclampsia: UtA Doppler, birth weight and pre-eclampsia'. Ultrasound Obstet. Gynecol. 44 (3), 293–298. https://doi.org/10.1002/uog.13310.
Villalobos-Labra, R., et al., 2017. Akt/mTOR Role in human foetoplacental vascular insulin resistance in diseases of pregnancy. J. Diabetes Res. 2017, 5947859 https://doi.org/10.1155/2017/5947859.
von Dadelszen, P., Magee, L.A., Roberts, J.M., 2003. Subclassification of preeclampsia. Hypertens. Pregnancy 22 (2), 143–148. https://doi.org/10.1081/PRG-120021060.
Wang, C., et al., 2017. Apelin as a novel drug for treating preeclampsia. Exp. Ther. Med. 14 (6), 5917–5923. https://doi.org/10.3892/etm.2017.5304.
Wang, L.-L., et al., 2022. Cutting edge: the regulatory mechanisms of macrophage polarization and function during pregnancy. J. Reprod. Immunol. 151, 103627 https://doi.org/10.1016/j.jri.2022.103627.
Wright, D., Wright, A., Nicolaides, K.H., 2020. The competing risk approach for prediction of preeclampsia. Am. J. Obstet. Gynecol. 223 (1), 12. https://doi.org/10.1016/j.ajog.2019.11.1247.