Antibody Analysis of Pre-eclamptic Plasma

Antigen Analysis of Pre-Eclamptic Plasma Antibodies Using *Escherichia Coli* Proteome Chips

Te-Yao Hsu\(^1\)*, Jyun-Mu Lin\(^2,3\), Mai-Huong T. Nguyen\(^2,3\), Feng-Hsiang Chung\(^2,3\), Ching-Chang Tsai\(^1\), Hsin-Hsin Cheng\(^1\), Yun-Ju Lai\(^1\), Hsuan-Ning Hung\(^1\) and Chien-Sheng Chen\(^2,3\)*

1 Department of Obstetrics and Gynecology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan

2 Graduate Institute of Systems Biology and Bioinformatics, National Central University, Jhongli 32001, Taiwan

3 Department of Biomedical Science and Engineering, National Central University, Jhongli 32001, Taiwan

* Address correspondence to:

Te-Yao Hsu.

Department of Obstetrics and Gynecology

Kaohsiung Chang Gung Memorial Hospital

Chang Gung University College of Medicine, Kaohsiung, Taiwan

E-mail: tyhsu@adm.cgmh.org.tw

Chien-Sheng Chen

505 5th Science Building,

Graduate Institute of Systems Biology and Bioinformatics

Department of Biomedical Science and Engineering
Antibody Analysis of Pre-eclamptic Plasma

National Central University

300, Jhongda Rd., Jhongli 32001, Taiwan

Phone: +886-3-4227151 ext. 36103. Fax: +886-3-4273822

E-mail: ccchen103@gmail.com
Antibody Analysis of Pre-eclamptic Plasma

Running title

Antibody Analysis of Pre-Eclampsia Plasma
Antibody Analysis of Pre-eclamptic Plasma

**Abbreviations**

| Abbrev. | Definition                                      |
|--------|-------------------------------------------------|
| PE     | Pre-eclampsia                                   |
| IgG    | Immunoglobulin G                                |
| IgM    | Immunoglobulin M                                |
| FDR    | false discovery rate                            |
| GO     | Gene Ontology                                   |
| KEGG   | Kyoto Encyclopedia of Genes and Genomes        |
| LIMMA  | Linear Models for Microarray                    |
| GLAM2  | Gapped Local Alignment of Motifs                |
| $k$-TSP| $k$-top scoring pair                            |
| SVM    | Support vector machines                         |
| $k$NN  | $k$-nearest neighbor                             |
| CS     | Caesarian section                               |
| VD     | Vaginal delivery                                |
| PP-13  | Placental protein 13                             |
| sEng   | Soluble endoglin                                |
Antibody Analysis of Pre-eclamptic Plasma

sFlt-1  Soluble fms-like tyrosine kinase 1

PIGF  Placental growth factor
Antibody Analysis of Pre-eclamptic Plasma

Summary

Pre-eclampsia is one of the main causes of perinatal mortality and morbidity. Many biomarkers for diagnosing pre-eclampsia have been found but most have low accuracy. Therefore, a potential marker that can detect pre-eclampsia with high accuracy is required. Infection has been reported as a cause of pre-eclampsia. In recent years, protein microarray chips have been recognized as a strong and robust tool for profiling antibodies for infection diagnoses. The purpose of the present study was to profile antibodies in the human plasma of healthy and pre-eclamptic pregnancies to identify suitable biomarkers. In this study, an *Escherichia coli* chip was probed with samples from 29 individuals (16 pre-eclamptic women and 13 healthy pregnant women) to profile plasma antibodies. Bioinformatics tools were used to analyze the results, discover conserved motifs, compare against the entire human proteome, and perform protein functional analysis. An antibody classifier was identified using $k$-top scoring pairs and additional samples for a blinded test were collected. The findings indicated that compared with the healthy women, the pre-eclamptic women exhibited 108 and 130 differentially immunogenic proteins against human immunoglobulins G and M, respectively. In addition, pre-eclamptic women developed more immunoglobulin G but less immunoglobulin M against bacterial surface proteins compared with healthy women. The $k$-top scoring pairs identified five pairs of immunogenic proteins as classifiers with a high accuracy of 90% in the blind test. [AG] [ISV] GV [AE] L [LF] and [IV] [IV] RI [AG] [AD] E were the consensus motifs observed in immunogenic proteins in the immunoglobulin G and immunoglobulin M of pre-eclamptic women, respectively, whereas GA [AG] [AL] L [LF] and [SRY] [IQML] [ILV] [ILV] [ACG] GI [GH] [AEF] [AK] [ATY] [RG] N [IV] were observed in the immunoglobulins G and immunoglobulin M of healthy women, respectively.
Introduction

Pre-eclampsia (PE) is a systemic disorder affecting 2%-8% of all pregnancies (4–6) and a notable cause of maternal and fetal morbidity and mortality (7). PE is characterized by hypertension and proteinuria noticeable after 20 weeks of gestation (5). Cardiovascular disease might be caused by PE in women (6) (8) and children born to women with PE may have a high chance of developing stroke, coronary heart disease, and metabolic syndrome in adulthood (9–11). Patients with PE share some common symptoms, such as headache and edema. The cause of PE is unknown. Currently, angiogenesis disorder (11–13) or immunological changes (13–15) might be related to PE. Maternal age, family history, and pre-existing disease all increase the risk of PE.

Many agents have been tested to decrease the likelihood of PE development. Supplemental nutrients such as vitamin C and vitamin E do not reduce PE risk (15). Aspirin has a strong preventative effect on PE when consumed from the early stages of pregnancy (16) and daily low-level dietary calcium intake can improve outcomes in high-risk groups (17). By contrast, magnesium supplementation during gestation does not improve outcomes (18). PE treatment has not changed substantially over the last 50 years (19). However, early treatment can have strong preventative effects, making early diagnosis a priority.

Although imaging techniques are the most common method for diagnosing PE, their accuracy is inconsistent. No tests have accurately predicted the onset of PE because of the heterogeneity of its clinical presentation (20). Labor induction is the only definitive treatment for the dangerous symptom manifestations of PE. A potential biomarker should have the ability to detect PE with high accuracy at the earliest stage. Widespread implementation of such a biomarker may be able to show who is at low or high risk. Soluble fms-like tyrosine kinase 1 (sFlt-1), soluble endoglin (sEng), placental growth factor (PIGF), and placental protein 13 (PP-13) have been considered as biomarkers for PE. However, all of these proteins
Antibody Analysis of Pre-eclamptic Plasma

exhibited low accuracy or inconsistent accuracy between different groups (21–29). Many studies have focused on possible PE biomarkers but an effective one remains to be discovered.

Infection may be related to PE development (30–32) and urinary tract infections may be a risk factor leading to PE (33–35). One study found that treatment with antibiotics reduced PE rates by 53% (32). Proteome microarrays are a powerful and effective tool for profiling antibodies and identifying biomarkers of infection (38–41). In the present study, human plasma was probed with *Escherichia coli* (*E. coli*) proteome microarrays to discover potential biomarkers for PE detection. Bioinformatics tools were applied to analyze the differential binding proteins for the discovery of conserved motifs, which were queried against the entire human proteome. Additionally, an antibody classifier was identified by using *k*-top scoring pairs (*k*-TSPs) and additional samples for a blinded test.

**Experimental Procedures**

**Patient information**

Twenty-three blood samples from women with PE (ICD 10:O1413) and 23 from women with normotensive pregnancies were collected and stored in ethylenediaminetetraacetic acid tubes. The plasma was subsequently collected immediately using centrifugation at 3000 rmp and 4 °C and frozen immediately at −80 °C until use. The total IgG concentration in each plasma sample was determined using an ELISA kit (eBioscience, 88-50550).
Antibody Analysis of Pre-eclamptic Plasma

Proteome chip assay

An *E. coli* proteome chip was retrieved from a −80 °C freezer and blocked with 3% BSA in a PBS buffer for 1 hour. After gently rinsing the chip with PBS-T buffer, all 4-mg/mL PE and healthy plasma samples were individually probed with an *E. coli* proteome chip. A DyLight 650-labeled antihuman IgG antibody and DyLight 550-labeled antihuman IgM antibody were probed to detect the IgG and IgM antibodies in the plasma samples, respectively. Finally, the chips were washed three times with PBS-T buffer and scanned using a microarray scanner (LuxScanTM 10K Microarray Scanner; CapitalBio Corporation).

Data preprocessing

The binding signals of protein spots were aligned and extracted using GenePix Pro 6.0 software (Molecular Devices, Sunnyvale, CA, US). PCA was performed to exclude the outliers of samples that showed evident deviation from the main group. The global median scaling approach was employed to normalize the protein intensities of all samples. Signals below 128 were regarded as missing values and represented by the minimum value of 128.

Identification of differentially binding proteins

We conducted a two-sample student’s *t* test and multiple testing correction to identify significantly binding proteins by using the LIMMA software package in the R environment. LIMMA is a package for analyzing gene expression data arising from microarrays. The stringency cutoff was controlled by an FDR no higher than 0.05. A more stringent threshold was controlled by an FDR lower than 0.05 and a fold change higher than 2-fold. The proteins were further analyzed and demonstrated using a two-way hierarchical clustering heatmap. The accuracies in the classification of women with PE and healthy pregnant women were
Antibody Analysis of Pre-eclamptic Plasma

examined though a comparison between real labels on samples and hierarchical clusters. Only clusters with accuracies higher than 0.7 were selected.

Functional enrichment analysis of PE binding proteins

The proteins selected from the less stringent threshold of FDR < 0.05 were used as a query set to study their biological roles in the PE and healthy control groups. Official gene symbols converted from the identified proteins for both groups were queried using DAVID bioinformatics resources (58). We chose three functional categories for further investigation of our protein hits, namely cellular component, molecular function, and biological process in level 3 of the GO categories and KEGG pathways. The $p$ values of the top five significantly functional terms in each group are visualized in a bar plot (Fig. 5). Functions with $p$ values lower than 0.05 were defined as significantly enriched terms.

Motif search with GLAM2

The identified proteins with the more stringent threshold of an FDR lower than 0.05 and a fold change greater than 2-fold were converted to FASTA format and queried for consensus motifs across these protein sequences by using the GLAM2 tool (59). The parameters of the GLAM2 analysis were the default settings. The resultant motifs were further surveyed in the entire human proteome by using GLAM2SCAN (59).

Antibody classifier discovery

We performed three supervised learning methods to discover the antibody classifiers of the PE samples. The $k$-TSP is a published algorithm used to compute the $k$ best pairs of proteins
Antibody Analysis of Pre-eclamptic Plasma

or genes to classify samples based on the relative ratio of the expression levels within each profile (60). The appearance of a protein in the $k$-TSP is restricted to at most one $k$-TSP pair. The value of $k$ is automatically determined through cross validation. The $k$-TSP method was implemented using the “ktspair” package in the R environment (61). The LIMMA package was initially applied to select protein features in the training group under the threshold of an FDR lower than 0.01. We used the original samples taken from 16 women with PE and 13 healthy pregnant women as the training group and an additional five PE and five healthy plasma samples as the testing group. The performance of these antibody classifiers selected through three methods was further evaluated for accuracy, specificity, and sensitivity based on the following formulae: sensitivity = true positive (TP) / [TP + false negative (FN)]; specificity = true negative (TN) / [TN + false positive (FP)]; and accuracy = TP + TN / (TP + TN + FP + FN).

Results

Overall strategies for profiling plasma antibodies in PE patients

Plasma samples were taken from 46 individuals (23 women with PE and 23 healthy pregnant women) at Chang Gung Memorial Hospital, Taiwan. Following the policy of the Chang Gung Memorial Hospital Institutional Review Board (102-5739B). To determine whether total plasma immunoglobulin G (IgG) levels differed between the women with PE and healthy pregnant women, we measured the total IgG concentrations before the chip assay. The results showed that the total IgG concentrations in the healthy women were significantly higher than in the PE patient group ($p < 0.0007$), and this echoed findings reported in several previous studies (42–44). Only samples with an IgG concentration near the average were further tested because high IgG concentrations can cause nonspecific binding. Because we
diluted all samples by a certain fold, the extremely high IgG level could cause nonspecific binding, thereby misleading the result. Seven samples (five healthy plasma and two PE plasma samples) were removed and final quantities of 16 PE and 13 healthy samples were used to profile plasma antibodies. The 10 remaining samples (five PE and five healthy samples) were used for blinded tests. Because IgG levels differed between the PE and healthy groups, we adjusted IgG levels to the same level before probing with the *E. coli* microarray. To identify potential biomarkers for PE pathogenesis, we performed chip assays to profile the antibodies in human plasma using *E. coli* proteome chips containing more than 4200 nonredundant *E. coli* proteins (Fig. 1). Table 1 lists the demographic data of the chosen samples. To identify antibodies in human plasma and observe interactions between plasma antibodies and the *E. coli* proteome, we probed chips with antihuman IgG conjugated with DyLight 650 and antihuman IgM conjugated with DyLight 550 (assay designs in Fig. 2). Sixteen 16 PE and 13 healthy plasma samples from pregnant women were probed with *E. coli* proteome chips to investigate globally the differences of immunogenic profiles in response to *E. coli* proteins between women with PE and healthy women. Because of the heterogeneities of human plasma and potential experimental errors, we performed principle component analysis (PCA) as a quality assessment approach to exclude outliers not belonging to the main group (Figs. S1 and S2 for IgG and IgM probing results, respectively). Eight samples, namely six women with PE and two healthy women, were excluded. After median normalization, we performed two analyses to describe the immunogenic responsive profiles. First, we identified differentially responsive proteins by using the Linear Models for Microarray (LIMMA) software package, scanned the potential binding motifs with Gapped Local Alignment of Motifs (GLAM2), searched the human proteome using GLAM2SCAN, and studied their biological functions using the Database for Annotation, Visualization and Integrated Discovery (DAVID). Next, we constructed a robust antibody classifier by using $k$-
Antibody Analysis of Pre-eclamptic Plasma

TSPs (Fig. 1). The representative images of *E. coli* proteome chips are shown in Fig. 2B. The representative proteins flagellar hook protein (flgE) and L-threonine deaminase (ilvA) exhibited higher binding signals in PE plasma than in the healthy controls against IgG and IgM antibodies, respectively. Lipoprotein 28 (nlpA) and yhbG exhibited inverse behaviors against IgG and IgM antibodies, respectively. These results demonstrated that our approach can identify highly immunogenic proteins in women with PE and healthy women.

Global immunogenic profiles of PE patients against *E. coli* proteome

To identify specifically the differentially immunogenic proteins between PE and the healthy controls, we used LIMMA for statistical analysis. Under the threshold of a false discovery rate (FDR) lower than 0.05, which is the probability of false positive callings in multiple comparisons, we discovered 108 differentially immunogenic proteins against human IgG when comparing women with PE with healthy women and 130 differentially immunogenic proteins against human IgM. Among the differentiated immunogenic proteins against IgG, 75 proteins were highly immunogenic in the women with PE and 33 were highly immunogenic in the healthy women. Among the differentiated immunogenic proteins against IgM, 67 and 63 proteins were highly immunogenic in the women with PE and healthy women, respectively. A two-way hierarchical clustering heatmap illustrated the significant differences in immunogenic responses in each phenotype (Fig. 3A). The immunogenic profiles of these differentiated immunogenic proteins showed effective discrimination between the PE and healthy groups. The accuracies for classification of the two groups obtained using the hierarchical clustering method were 95.2% and 95.2% for the IgG and IgM profiles, respectively (Fig. 3A). A Venn diagram was plotted to demonstrate that the overlaps between these differentiated immunogenic proteins against the human IgG and IgM antibodies were few (Fig. 3B). In general, these results indicated that many differentiated immunogenic
Antibody Analysis of Pre-eclamptic Plasma

proteins can be thoroughly discovered in plasma samples, which can subsequently be regarded as good classifiers for differentiating PE samples from those of healthy women based on their immunogenic profiles.

Functionality and localization of immunogenic proteins discovered in PE plasma

To depict the global features in PE-derived immunogenicity, we assigned the immunogenic proteins into different functional groups based on the classification of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The 75 highly anti-IgG immunogenic proteins in PE, 33 highly anti-IgG immunogenic proteins in healthy women against PE, 67 highly anti-IgM immunogenic proteins in PE, and 63 highly anti-IgM immunogenic proteins in healthy women were individually queried using the DAVID database to classify their functional groups. Unmatched protein entries were removed. Here, we chose the third level of cellular components in GO and focused on six major categories (cytoplasm, ribosome, membrane, cell wall, pilus, and others) to describe the subcellular localization of these immunogenic proteins. We further conducted enrichment analysis and applied hypergeometric statistic tests to compute the probability of the number of proteins involved in each category compared with the appearance by chance. The proportion of subcellular localization distribution showed a higher percentage of proteins localized in the cell membrane and cell wall compartment in IgG-derived highly immunogenic proteins in women with PE than in healthy women (29% vs. 23% for the cell membrane and 15% vs. 13% for the cell wall compartment; Fig. 4A and B). The opposite was true for IgM-derived highly expressed immunogenic proteins; the percentages were 23% versus 34% for the cell membrane and 7% versus 17% for the cell wall when we compared the PE samples with the healthy controls (Fig. 4C and D). These results indicated that the women with PE developed more IgG but less IgM against bacterial surface proteins than did the healthy women.
Regarding the biological process and molecular function in GO terms, most enriched terms in biological processes from IgG-derived immunogenic proteins in the women with PE were related to iron transport and the generation of precursor metabolites and energy, neither of which were enriched in the healthy women ($p = 0.0048$ and $0.0084$ for iron transport and generation of precursor metabolites and energy, respectively; Fig. 5A). Contrasting results were obtained during IgM probing, which revealed that transport-related processes were discovered in IgM-derived immunogenic proteins in the healthy women ($p = 0.0056$ for amino acid transport; Fig. 5B). In the cellular localization analysis of the differentially immunogenic proteins, surface proteins played a key role. We found that most surface proteins were transporters with an especially high proportion related to iron transport. We obtained similar results through KEGG enrichment analysis ($p = 0.0045$ and $0.00007$ for ABC transporters in IgG-derived PE samples and IgM-derived healthy controls, respectively; Fig. S3A). However, the IgM-derived immunogenic proteins played greater roles in the carbohydrate metabolic processes in the women with PE ($p = 0.023$ for the carbohydrate metabolic process; Fig. 5B). The unique feature of molecular functions in GO was cation binding activities being significantly overrepresented on IgG-derived immunogenic proteins in women with PE ($p = 0.009$ for cation binding activities; Fig. S4). We also conducted an analysis using the Pfam protein family database to discover overrepresented protein families among these immunogenic proteins. We discovered that tRNA-synt_2d and Phe_tRNA-synt_N were significantly enriched in the IgM-derived healthy control group ($p = 0.000007$ for PF01409:tRNA-synt_2d and PF02912:Phe_tRNA-synt_N; Fig. S5). These results indicated functional relationships and differences between IgG- and IgM-derived immunogenic proteins and that these highly expressed immunogenic proteins in women with PE play a major role in the ion transport processes in the cellular transmembrane region, especially in response to human IgG.
Consensus motif searching with gapped local alignment

To examine whether a consensus motif existed in the highly immunogenic protein from women with PE and healthy women, we conducted a GLAM2 analysis. For this purpose, we reduced the number of proteins by increasing the stringency of thresholds to discover differentially immunogenic proteins. Under FDR thresholds lower than 0.05 and an absolute fold change higher than 1.7 to 2, we identified seven and six IgG-derived highly immunogenic proteins for women with PE and healthy women, respectively, and 9 and 10 IgM-derived highly immunogenic proteins for women with PE and healthy women, respectively. The list of proteins is summarized in Supplementary Tables 1–4. All selected protein sets exhibited higher than 70% accuracy for classification of the two groups through the hierarchical clustering method. The specific accuracies were 76% and 71% for the IgG-derived women with PE and healthy women, respectively, and 81% and 90% for the IgM-derived women with PE and healthy women, respectively (Fig. 6). From 32 proteins, 18 annotated proteins had cellular component functions and more than one-third of these proteins played roles in cell membrane or flagellum compartments (13 proteins, 72.2%; Supplementary Tables 1–4). Because these proteins had similar binding patterns for immunogenic responses and high accuracy in terms of sample discrimination, they were further used to perform the GLAM2 analysis for motif enrichment. Thus, we found that the consensus motifs among them were [AG] [ISV] GV [AE] L [LF] and [IV] [IV] RI [AG] [AD] E in PE IgG and IgM, respectively, and GA [AG] [AL] L [LF] and [SRY] [IQML] [ILV] [ILV] [ACG] GI [GH] [AEF] [AK] [ATY] [RG] N [IV] for healthy women (Fig. 7). The specific antibodies that bound only in PE were likely to bind to the same motif. Thus, we searched for the consensus motif among the specific protein hits, which exhibited different signals between the PE and healthy control groups. Because human proteins may contain the
same consensus motif, we also looked for human proteins containing these motifs, used such motifs to search the entire human proteome through GLAM2SCAN, and retrieved subcellular locations for proteins from the LOCATE database. For each group, consensus human proteins were identified as containing one or more of the aforementioned consensus motifs. From these consensus proteins, we observed membrane proteins and secreted proteins in human cells in the PE group (Supplementary Tables 5–8). These results were consistent with the GO and KEGG enrichment analysis results. We also observed that two secreted proteins (trio Rho guanine nucleotide exchange factor and matrilin 1) were highly immunogenic in the women with PE and three secreted proteins (cartilage associated protein, N-acetylated alpha-linked acidic dipeptidase-like 1, and hepatocyte growth factor activator) were lowly immunogenic in the women with PE. These findings suggested that these immunogenic signatures in human secreted proteins may also be involved in PE pathogenesis.

Identification of antibody classifiers for diagnosis through k-TSP and blinded tests

Because these immunogenic profiles exhibited accurate classification ability, we wondered whether one or two superantibody classifiers existed and could demonstrate accurate discrimination between samples. For this purpose, we applied the supervised machine learning approach and included an additional 10 plasma samples (five women with PE and five healthy pregnant women) for blinded testing of classifier performance. We constructed the antibody classifiers for differentiating PE samples from healthy pregnant women by using k-TSP, a published classification algorithm for the prediction of biomarkers from high throughput data based on a set of pair-wise measurements (45). Five pairs of immunogenic proteins were generated and are shown in dot plots (Fig. 8). These five pairs were IgM_yiaB with IgM_yhcD, IgM_ilvA with IgM_ybgE, IgG_clpA with IgG_csgF, IgG_rbsC with IgM_yhbG, and IgG_srlA with IgG_trpC, all of which exhibited accurate performance in the training group (accuracies ranged from 95% to 100% and that for all five pairs was 100%);
Antibody Analysis of Pre-eclamptic Plasma

Fig. 8). For the blinded test, accuracy was 90%, specificity was 83%, and sensitivity was 100% for all five pairs after an additional 10 plasma samples were used (Fig. 9). These results indicated that our proteome chip-based approach not only explored specific immunogenic profiles against *E. coli* proteins but also discovered robust antibody classifiers for PE diagnoses. However, this is a preliminary study; further validation is required for use in clinical diagnosis.

Discussion

The identification of novel biomarkers for the early diagnosis of diseases is essential for human health. Protein microarrays have been recognized as a powerful and robust tool that can be used to discover valuable biomarkers (46) (41). In this study, *E. coli* proteome chips were used to probe human plasma to identify PE biomarkers. This study was the first examination to investigate the differences in human immunoglobulin responses to the entire bacterial proteome in women with PE and healthy women. The results showed that IgG levels in the women with PE were considerably lower when compared with those of the healthy group. This may be a partial result of the lower concentrations of estrogen and progestogen (42), higher concentrations of steroids during pregnancy, transference of maternal antibodies to the fetus, and proteinuria or physiological hydremia (44). Several reports have found that PE is caused by infectious agents. In one study, women who had been seronegative for antibodies against infection from sexual partners, including herpes simplex virus type-2 and cytomegalovirus, and subsequently developed primary infections during pregnancy, were deemed more likely to develop PE (31). Moreover, the presence of infectious agents such as *Bacillus, Anaerobacillus, Staphylococcus, Listeria, Nocardiopsis, Escherichia, Shigella, Salmonella, Klebsiella, Lactobacillus, Dialister, and Pediococcus* in the placenta of women with PE increases the risk of developing PE (47) (48). These infectious agents could activate
Antibody Analysis of Pre-eclamptic Plasma

Inflammatory processes and promote antiangiogenic factors that may cause PE (47) (49) (50). IgM is the first antibody secreted during infection. Surface proteins on bacteria are the only targets of IgM in the early stages of infection. This study showed that women with PE have less IgM to fight against bacterial membrane and cell wall proteins than do healthy pregnant women. This finding indicated that the women with PE had less effective IgM–bacteria binding in the early stages of infection, and thus had fewer defensive capabilities against bacterial infections. By contrast, more IgG in the women with PE exhibited strong binding to proteins located in the cell wall and membrane. Live bacteria present more surface antigens than intracellular antigens, and this suggests that the women with PE developed more IgG against live bacteria as a long-term defense than did the healthy pregnant women because of the ineffectiveness of IgM in the early stages of infection in women with PE. This long-term immunity against live bacteria may result in abnormal inflammation in women with PE (51). Further experimentation is required to confirm the relationship between bacterial-associated inflammation and PE. If bacterial-associated inflammation causes PE, antibiotics and anti-inflammatory therapies may prevent PE.

Table 2 summarizes the reported PE biomarkers. Although its accuracy was high, PP-13 was considered a biomarker for not only PE but also intrauterine growth restriction (28), indicating that PP-13 is nonspecific. Another biomarker, sEng, a transmembrane glycoprotein, has been studied extensively (52) (53) (54); however, its sensitivity, specificity, and accuracy were inconsistent across different studies. Although some studies have shown high accuracy for sEng, others have reported low accuracy. For the PlGF biomarker, most studies have shown low sensitivity, specificity, and accuracy (23) (24) (26) (27). Although one study showed that podocyturia presented high accuracy (55), another showed very low accuracy (56). Doppler sonography also exhibited low accuracy (57). In summary, previously reported PE biomarkers have low or inconsistent accuracy. Compared with these, our
Antibody Analysis of Pre-eclamptic Plasma

biomarkers exhibited favorable performance. However, the present study is a preliminary study. More samples are required for further validation.

References

Uncategorized References

[1] A.X. da Silveira Dos Santos, I. Riezman, M.A. Aguilera-Romero, F. David, M. Piccolis, R. Loewith, O. Schaad, H. Riezman, Systematic lipidomic analysis of yeast protein kinase and phosphatase mutants reveals novel insights into regulation of lipid homeostasis, Mol Biol Cell, 25 (2014) 3234-3246.
[2] J.F. Chich, B. Schaeffer, A.P. Bouin, F. Mouthon, V. Labas, C. Larramendy, J.P. Deslys, J. Grosclaude, Prion infection-impaired functional blocks identified by proteomics enlighten the targets and the curing pathways of an anti-prion drug, Biochim Biophys Acta, 1774 (2007) 154-167.
[3] C.T. Chu, J. Ji, R.K. Dagda, J.F. Jiang, Y.Y. Tyurina, A.A. Kapralov, V.A. Tyurin, N. Yanamala, I.H. Shrivastava, D. Mohammadyani, K.Z.Q. Wang, J. Zhu, J. Klein-Seetharaman, K. Balasubramanian, A.A. Amoscato, G. Borisenco, Z. Huang, A.M. Gusdon, A. Cheikhi, E.K. Steer, R. Wang, C. Baty, S. Watkins, I. Bahar, H. Bayir, V.E. Kagan, Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells, Nat Cell Biol, 15 (2013) 1197-1205.
[4] Y. Zhou, C.H. Damsky, S.J. Fisher, Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome?, The Journal of clinical investigation, 99 (1997) 2152-2164.
[5] F. Milne, C. Redman, J. Walker, P. Baker, J. Bradley, C. Cooper, M. de Swiet, G. Fletcher, M. Jokinen, D. Murphy, C. Nelson-Piercy, V. Osgood, S. Robson, A. Shennan, A. Tuffnell, S. Twaddle, J. Waugh, The pre-eclampsia community guideline (PRECOG): how to screen for and detect onset of pre-eclampsia in the community, BMJ, 330 (2005) 576-580.
[6] C.A. Meads, J.S. Cnossen, S. Meher, A. Juarez-Garcia, G. ter Riet, L. Duley, T.E. Roberts, B.W. Mol, J.A. van der Post, M.M. Leeflang, P.M. Barton, C.J. Hyde, J.K. Gupta, K.S. Khan, Methods of prediction and prevention of pre-eclampsia: systematic reviews of accuracy and effectiveness literature with economic modelling, Health Technol Assess, 12 (2008) iii-iv, 1-270.
[7] J. Jarvenpaa, J.T. Vuoristo, E.R. Savolainen, O. Ukkola, T. Vaskivuo, M. Ryynanen, Altered expression of angiogenesis-related placental genes in pre-eclampsia associated with intrauterine growth restriction, Gynecol Endocrinol, 23 (2007) 351-355.
[8] J. Uzan, M. Carbonnel, O. Piccone, R. Asmar, J.M. Ayoubi, Pre-eclampsia: pathophysiology, diagnosis, and management, Vasc Health Risk Manag, 7 (2011) 467-474.
[9] C. Osmond, E. Kajantie, T.J. Forsen, J.G. Eriksson, D.J. Barker, Infant growth and stroke in adult life: the Helsinki birth cohort study, Stroke, 38 (2007) 264-270.
[10] D.J. Barker, C.N. Martyn, C. Osmond, C.N. Hales, C.H. Fall, Growth in utero and serum cholesterol concentrations in adult life, BMJ, 307 (1993) 1524-1527.
[11] A. Rajakumar, K.A. Whitelock, L.A. Weissfeld, A.R. Daftary, N. Markovic, K.P. Conrad, Selective overexpression of the hypoxia-inducible transcription factor, HIF-2alpha, in placentas from women with preeclampsia, Biol Reprod, 64 (2001) 499-506.
[12] S. Rana, S.A. Karumanchi, R.J. Levine, S. Venkatesha, J.A. Rauh-Hain, H. Tamez, R. Thadhani, Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia, Hypertension, 50 (2007) 137-142.

[13] C.W. Redman, I.L. Sargent, Latest advances in understanding preeclampsia, Science, 308 (2005) 1592-1594.

[14] I.L. Sargent, A.M. Borzychowski, C.W. Redman, Immunoregulation in normal pregnancy and pre-eclampsia: an overview, Reprod Biomed Online, 13 (2006) 680-686.

[15] L. Poston, A.L. Briley, P.T. Seed, F.J. Kelly, A.H. Shennan, C. Vitamins in Pre-eclampsia Trial, Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial, Lancet, 367 (2006) 1145-1154.

[16] L. Duley, D.J. Henderson-Smart, S. Meher, J.F. King, Antiplatelet agents for preventing pre-eclampsia and its complications, Cochrane Database Syst Rev, (2007) CD004659.

[17] G.J. Hofmeyr, A.N. Atallah, L. Duley, Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems, Cochrane Database Syst Rev, (2006) CD001059.

[18] M. Makrides, D.D. Crosby, E. Bain, C.A. Crowther, Magnesium supplementation in pregnancy, Cochrane Database Syst Rev, (2014) CD000937.

[19] M. Noris, N. Perico, G. Remuzzi, Mechanisms of disease: Pre-eclampsia, Nat Clin Pract Nephrol, 1 (2005) 98-114; quiz 120.

[20] J.A. Turner, Diagnosis and management of pre-eclampsia: an update, Int J Womens Health, 2 (2010) 327-337.

[21] H. Stepan, A. Geipel, F. Schwarz, T. Kramer, N. Wessel, R. Faber, Circulatory soluble endoglin and its predictive value for preeclampsia in second-trimester pregnancies with abnormal uterine perfusion, Am J Obstet Gynecol, 198 (2008) 175 e171-176.

[22] J.H. Lim, S.Y. Kim, S.Y. Park, M.H. Lee, J.H. Yang, M.Y. Kim, J.H. Chung, S.W. Lee, H.M. Ryu, Soluble endoglin and transforming growth factor-betal in women who subsequently developed preeclampsia, Prenat Diagn, 29 (2009) 471-476.

[23] C.E. Kleinrouweler, M.M. Wiegerinck, C. Ris-Stalpers, P.M. Bossuyt, J.A. van der Post, P. von Dadelszen, B.W. Mol, E. Pajkrt, E.C. Collaboration, Accuracy of circulating placental growth factor, vascular endothelial growth factor, soluble fms-like tyrosine kinase 1 and soluble endoglin in the prediction of pre-eclampsia: a systematic review and meta-analysis, BJOG, 119 (2012) 778-787.

[24] A. De Vivo, G. Baviera, D. Giordano, G. Todarello, F. Corrado, R. D’Anna, Endoglin, PlGF and sFlt-1 as markers for predicting pre-eclampsia, Acta Obstet Gynecol Scand, 87 (2008) 837-842.

[25] A. Hertig, N. Berkane, G. Lefevre, K. Toumi, H.P. Marti, J. Capeau, S. Uzan, E. Rondeau, Maternal serum sFlt1 concentration is an early and reliable predictive marker of preeclampsia, Clin Chem, 50 (2004) 1702-1703.

[26] S.C. Tidwell, H.N. Ho, W.H. Chiu, R.J. Torry, D.S. Torry, Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia, Am J Obstet Gynecol, 184 (2001) 1267-1272.

[27] J. Espinoza, R. Romero, J.K. Nien, R. Gomez, J.P. Kusanovic, L.F. Goncalves, L. Medina, S. Edwin, S. Hassan, M. Carstens, R. Gonzalez, Identification of patients at risk for early onset and/or severe preeclampsia with the use of uterine artery Doppler velocimetry and placental growth factor, Am J Obstet Gynecol, 196 (2007) 326 e321-313.

[28] I. Chafetz, I. Kuhnreich, M. Sammar, Y. Tal, Y. Gibor, H. Meiri, H. Cuckle, M. Wolf, First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction, Am J Obstet Gynecol, 197 (2007) 35 e31-37.

[29] R. Romero, J.P. Kusanovic, N.G. Than, O. Ereç, F. Gotsch, J. Espinoza, S. Edwin, I. Chafetz, R. Gomez, J.K. Nien, M. Sammar, B. Pineles, S.S. Hassan, H. Meiri, Y. Tal, I.
Antibody Analysis of Pre-eclamptic Plasma

Kuhnreich, Z. Papp, H.S. Cuckle, First-trimester maternal serum PP13 in the risk assessment for preeclampsia, Am J Obstet Gynecol, 199 (2008) 122 e121-122 e111.

[30] A. Conde-Agudelo, J. Villar, M. Lindheimer, Maternal infection and risk of preeclampsia: systematic review and metaanalysis, Am J Obstet Gynecol, 198 (2008) 7-22.

[31] L.I. Trogstad, A. Eskild, A.L. Bruu, S. Jeansson, P.A. Jenum, Is preeclampsia an infectious disease?, Acta Obstet Gynecol Scand, 80 (2001) 1036-1038.

[32] J.A. Herrera, G. Chaudhuri, P. Lopez-Jaramillo, Is infection a major risk factor for preeclampsia?, Med Hypotheses, 57 (2001) 393-397.

[33] C. Minassian, S.L. Thomas, D.J. Williams, O. Campbell, L. Smeeth, Acute maternal infection and risk of pre-eclampsia: a population-based case-control study, PLoS One, 8 (2013) e73047.

[34] R. Mittendorf, K.Y. Lain, M.A. Williams, C.K. Walker, Preeclampsia. A nested, case-control study of risk factors and their interactions, J Reprod Med, 41 (1996) 491-496.

[35] S.R. Easter, D.E. Cantonwine, C.A. Zera, K.H. Lim, S.I. Parry, T.F. McElrath, Urinary tract infection during pregnancy, angiogenic factor profiles, and risk of preeclampsia, Am J Obstet Gynecol, 214 (2016) 387 e381-387.

[36] C.L. Haggerty, M.A. Klebanoff, I. Panum, S.A. Uldum, D.C. Bass, J. Olsen, J.M. Roberts, R.B. Ness, Prenatal Chlamydia trachomatis infection increases the risk of preeclampsia, Pregnancy Hypertens, 3 (2013) 151-154.

[37] K.M. Powis, T.F. McElrath, M.D. Hughes, A. Ogwu, S. Souda, S.A. Datwyler, E. von Widenfelt, S. Moyo, M. Nadas, J. Makhema, E. Machakaire, S. Lockman, M. Essex, R.L. Shapiro, High viral load and elevated angiogenic markers associated with increased risk of preeclampsia among women initiating highly active antiretroviral therapy in pregnancy in the Mma Bana study, Botswana, J Acquir Immune Defic Syndr, 62 (2013) 517-524.

[38] P.C. Chen, G.D. Syu, K.H. Chung, Y.H. Ho, F.H. Chung, P.H. Chen, J.M. Lin, Y.W. Chen, S.Y. Tsai, C.S. Chen, Antibody Profiling of Bipolar Disorder Using Escherichia coli Proteome Microarrays, Mol Cell Proteomics, 14 (2015) 2299.

[39] A.E. Dent, R. Nakajima, L. Liang, E. Baum, A.M. Moormann, P.O. Sumba, J. Vulule, D. Babineau, A. Randall, D.H. Davies, P.L. Felgner, J.W. Kazura, Plasmodium falciparum Protein Microarray Antibody Profiles Correlate With Protection From Symptomatic Malaria in Kenya, J Infect Dis, 212 (2015) 1429-1438.

[40] C.S. Chen, S. Sullivan, T. Anderson, A.C. Tan, P.J. Alex, S.R. Brant, C. Cuffari, T.M. Bayless, M.V. Talor, C.L. Burek, H. Wang, R. Li, L.W. Datta, Y. Wu, R.L. Winslow, H. Zhu, X. Li, Identification of novel serological biomarkers for inflammatory bowel disease using Escherichia coli proteome chip, Mol Cell Proteomics, 8 (2009) 1765-1776.

[41] M. Natesan, R.G. Ulrich, Protein microarrays and biomarkers of infectious disease, Int J Mol Sci, 11 (2010) 5165-5183.

[42] C.H. Horne, P.W. Howie, R.B. Goudie, Serum-alpha2-macroglobulin, transferrin, albumin, and IgG levels in preeclampsia, J Clin Pathol, 23 (1970) 514-516.

[43] G. Arinola, A. Arowojolu, A. Bamgboyce, A. Akinwale, A. Adeniyi, Serum concentrations of immunoglobulins and acute phase proteins in Nigerian women with preeclampsia, Reproductive biology, 6 (2006) 265-274.

[44] R. Sharma, D. Nandi, I. Shukla, V. Maheshwari, Effect of pregnancy associated hypertension on immunoglobulin levels in newborns, Indian Pediatr, 29 (1992) 581-586.

[45] D. Geman, C. d’Avignon, D.Q. Naiman, R.L. Winslow, Classifying gene expression profiles from pairwise mRNA comparisons, Stat Appl Genet Mol Biol, 3 (2004) Article19.

[46] N. Ramachandran, S. Srivastava, J. Labaer, Applications of protein microarrays for biomarker discovery, Proteomics Clin Appl, 2 (2008) 1444-1459.
Antibody Analysis of Pre-eclamptic Plasma

[47] R. Amarasekara, R.W. Jayasekara, H. Senanayake, V.H. Dissanayake, Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia, J Obstet Gynaecol Res, 41 (2015) 662-669.
[48] S. Barak, O. Oettinger-Barak, E.E. Machtei, H. Sprecher, G. Ohel, Evidence of periopathogenic microorganisms in placentas of women with preeclampsia, J Periodontol, 78 (2007) 670-676.
[49] P. von Dadelszen, L.A. Magee, Could an infectious trigger explain the differential maternal response to the shared placental pathology of preeclampsia and normotensive intrauterine growth restriction?, Acta Obstet Gynecol Scand, 81 (2002) 642-648.
[50] P. von Dadelszen, L.A. Magee, J.C. Marshall, O.D. Rotstein, The Maternal Syndrome of Preeclampsia: A forme fruste of the Systemic Inflammatory Response Syndrome, Sepsis, 4 (2000) 43-47.
[51] W. Ramma, A. Ahmed, Is inflammation the cause of pre-eclampsia?, Biochem Soc Trans, 39 (2011) 1619-1627.
[52] L.T. Petla, R. Chikkala, K.S. Ratnakar, V. Kodati, V. Sritharan, Biomarkers for the management of pre-eclampsia in pregnant women, Indian J Med Res, 138 (2013) 60-67.
[53] D.M. Carty, C. Delles, A.F. Dominiczak, Novel biomarkers for predicting preeclampsia, Trends Cardiovasc Med, 18 (2008) 186-194.
[54] S. Venkatesha, M. Toporsian, C. Lam, J. Hanai, T. Mamamoto, Y.M. Kim, Y. Bdolah, K.H. Lim, H.T. Yuan, T.A. Libermann, I.E. Stillman, D. Roberts, P.A. D’Amore, F.H. Epstein, F.W. Sellke, R. Romero, V.P. Sukhatme, M. Letarte, S.A. Karumanchi, Soluble endoglin contributes to the pathogenesis of preeclampsia, Nat Med, 12 (2006) 642-649.
[55] V.D. Garovic, S.J. Wagner, S.T. Turner, D.W. Rosenthal, W.J. Watson, B.C. Brost, C.H. Rose, L. Gavrilova, P. Craigo, K.R. Bailey, J. Achenbach, M. Schiffer, J.P. Grande, Urinary podocyte excretion as a marker for preeclampsia, Am J Obstet Gynecol, 196 (2007) 320 e321-327.
[56] B. Jim, P. Jean-Louis, A. Qipo, D. Garry, S. Mian, T. Matos, C. Provenzano, A. Acharya, Podocyturia as a diagnostic marker for preeclampsia amongst high-risk pregnant patients, Journal of pregnancy, 2012 (2012).
[57] H. Stepan, A. Unversucht, N. Wessel, R. Faber, Predictive value of maternal angiogenic factors in second trimester pregnancies with abnormal uterine perfusion, Hypertension, 49 (2007) 818-824.
[58] W. Huang da, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nature protocols, 4 (2009) 44-57.
[59] M.C. Frith, N.F. Saunders, B. Kobe, T.L. Bailey, Discovering sequence motifs with arbitrary insertions and deletions, PLoS computational biology, 4 (2008) e1000071.
[60] A.C. Tan, D.Q. Naiman, L. Xu, R.L. Winslow, D. Geman, Simple decision rules for classifying human cancers from gene expression profiles, Bioinformatics, 21 (2005) 3896-3904.
[61] T.L. Jeffrey, tspair: Top Scoring Pairs for Microarray Classification, R package version 1.10.0.
ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Technology Taiwan, MOST 104-2320-B-008-002-MY3 and Grants NMRPG8D0251, and the Veterans General Hospital and University System of Taiwan VGHUST 106-G1-4-2.
Antibody Analysis of Pre-eclamptic Plasma

Tables

Table 1. Demographic data of healthy pregnancies and those of women with severe PE

| Characteristic                               | Normal pregnancies (n=18) | Severe pre-eclampsia (n=21) | P value |
|----------------------------------------------|---------------------------|-----------------------------|---------|
| Maternal age (years)                         | 33.5±4.7                  | 33.4±4.2                    | NS      |
| Gestational age (weeks)                      | 38.7±1                    | 34.1±3.9                    | 0.007   |
| Body weight at delivery (kg)                 | 66±6.6                    | 73.5±12.6                   | 0.05    |
| Body mass index at delivery (kg/m$^2$)       | 26±3.4                    | 28.9±3.3                    | 0.027   |
| Systolic blood pressure (mmHg)               | 113.8±6.1                 | 180.4±18.2                  | < 0.0001|
| Diastolic blood pressure (mmHg)              | 70.1±7.7                  | 115.9±8.7                   | < 0.0001|
| Proteinuria (dipstick)                       | Negative                  | +++                         | < 0.0001|
| Multipara (%)                                | 75% (12/16)               | 53% (8/15)                  | NS      |
| Nullipara (%)                                | 25% (4/16)                | 47% (7/15)                  | NS      |
| Mode of delivery (CS:VD, %)                  | 37.5%:62.5% (6:10)        | 80%:20% (12:3)              | 0.02    |
| Fetal of sex (M:F, %)                        | 75%:25% (12:4)            | 46.7%:53.3% (7:8)           | NS      |
| Infant birth weight (g)                      | 3111.1±274.7              | 1998.3±753.5                | < 0.0001|
Antibody Analysis of Pre-eclamptic Plasma

CS: Caesarian section, VD: Vaginal delivery, M: Male, F: Female, NS: not significant. Data are presented as mean standard deviation. Statistical significance for differences in means among groups was assessed using a student’s $t$ test. $p < 0.05$ was considered statistically significant.

**Table 2. Summary of previous biomarkers**

| Biomarker                              | Sensitivity, % | Specificity, % | Accuracy, % | References |
|----------------------------------------|----------------|----------------|-------------|------------|
| PP-13 (placenta protein 13)            | 79             | 90             | 85.5        | [28]       |
|                                        | 100            | 80             | 90          | [29]       |
| sEng (soluble endoglin)                | 80             | 43.2           | 61.6        | [21]       |
|                                        | 85             | 85             | 85          | [22]       |
|                                        | 18             | 67             | 42.5        | [23]       |
|                                        | 80.8           | 84.6           | 82.7        | [24]       |
| sFlt-1 (soluble fms-like tyrosine kinase 1) | 26            | 72             | 49          | [23]       |
|                                        | 73.1           | 80.8           | 76.9        | [24]       |
|                                        | 80             | 100            | 90          | [25]       |
| PlGF (placental growth factor)         | 32             | 75             | 53.5        | [23]       |
|                                        | 92.3           | 80.8           | 86.5        | [24]       |
|                                        | 88.9           | 64             | 76.5        | [26]       |
|                                        | 69.1           | 51.4           | 60.25       | [27]       |
| Podocytes                              | 100            | 100            | 100         | [55]       |
Antibody Analysis of Pre-eclamptic Plasma

|                | 38 | 70 | 54 | [56] |
|----------------|----|----|----|------|
| Doppler        | 77 | 46 | 61.5 | [57] |
| Our biomarker  | 100| 83 | 90  |      |
Antibody Analysis of Pre-eclamptic Plasma

Figures Legends

Figure 1. Workflow used in this study. Sixteen pre-eclamptic and 13 healthy plasma samples were probed with an *E. coli* proteome chip. After outlier exclusion, median normalization, and missing value imputation, we applied two strategies to analyze the data. One was significant analysis of differential binding proteins. The other was antibody classifier discovery using $k$-TSP and including additional samples for a blinded test.

Figure 2. *E. coli* proteome chip assays. (A) Assay format for screening pre-eclamptic plasma samples by using an *E. coli* proteome chip. The first step was to collect plasma. *E. coli* proteome chips were blocked with BSA and these plasma samples were individually probed with *E. coli* proteome chips. Subsequently, we incubated the chips with antihuman IgG antibody conjugated with DyLight 650 and antihuman IgM antibody conjugated with DyLight 550. (B) Representative image of *E. coli* proteome chips and four proteins for differentially binding spots between pre-eclamptic samples and healthy controls.

Figure 3. Identification of significantly binding proteins in PE through LIMMA analysis. (A) Heatmap showing normalized binding signals of differentially expressed immunogenic proteins. With the threshold of FDR < 0.05, we identified 108 significantly highly expressed immunogenic proteins for comparison with healthy controls, including 75 strongly binding proteins in patients and 33 strongly binding proteins in healthy controls for IgG responses. We also identified 130 significantly highly expressed immunogenic proteins for comparison with healthy controls, including 67 strongly binding proteins in patients with
Antibody Analysis of Pre-eclamptic Plasma

PE and 63 strongly binding proteins in the healthy controls for IgM responses. (B) Venn diagram showing the overlaps of protein expression between IgG- and IgM-binding proteins.

**Figure 4. Proportion of protein location distribution for IgG- and IgM-binding proteins.**

(A) Strongly binding proteins in patients with PE probed with IgG antibody. (B) Strongly binding proteins in healthy controls probed with IgG antibody. (C) Strongly binding proteins in patients with PE probed with IgM antibody. (D) Strongly binding proteins in healthy controls probed with IgM antibody. We performed Fisher’s exact test to examine the overrepresentation of the subcellular location (* p < 0.05, ** p < 0.005, *** p < 0.0005).

**Figure 5. Functional analysis of enriched biological processes in patients with PE.** (A) Top five significant processes involved in IgG-binding proteins. Light-blue bars indicate significant biological processes for IgG-binding proteins in patients with PE. Blue bars indicate significant biological processes for IgG-binding proteins in healthy controls. (B) Top five significant processes involved in IgM-binding proteins. Light-blue bars indicate the significant biological processes for IgM-binding proteins in patients with PE. Blue bars indicate the significant biological processes for IgM-binding proteins in healthy controls.

**Figure 6. Subheatmap of specific highly binding proteins for PE.** Proteins with the statistical thresholds of FDR < 0.05 and absolute fold change > 1.7 were selected for motif enrichment analysis. (A) Seven strongly binding proteins in patients with PE probed with IgG were selected and the accuracy for classification was 76%. (B) Six strongly binding proteins in healthy controls probed with IgG were selected and the accuracy for classification was 71%. (C) Nine strongly binding proteins in patients with PE probed with IgM were selected.
Antibody Analysis of Pre-eclamptic Plasma

and the accuracy for classification was 86%. (D) Ten strongly binding proteins in healthy controls probed with IgM were selected and the accuracy for classification was 81%.

**Figure 7. Consensus motif search using GLAM2.** (A) The consensus motif was derived from seven strongly binding proteins in patients with PE probed with IgG. (B) The consensus motif was derived from six strongly binding proteins in healthy controls probed with IgG. (C) The consensus motif was derived from nine strongly binding proteins in patients with PE probed with IgM. (D) The consensus motif was derived from 10 strongly binding proteins in healthy controls probed with IgM.

**Figure 8. Five pairs showing accurate performance on training sets of PE samples.** The red dot represents the patients with PE and the grey dot represents the healthy controls. Using A as an example, the decision rule was that if the binding signal of yiaB of IgM probing was greater than yhcD of IgM probing in the plasma sample, it would be defined as belonging to a patient with PE. Inversely, if the binding signal of yiaB of IgM probing was smaller than yhcD of IgM probing in the plasma sample, it would be defined as belonging to a healthy woman. (A) The first pair using yiaB with IgM and yhcD with IgM probing results showed 100% accuracy in discerning patients with PE from healthy controls. (B) The second pair using ilvA with IgM and ybgE with IgM probing results showed 100% accuracy. (C) The third pair using csgF with IgG and clpA with IgG showed 91% accuracy. (D) The fourth pair using rbsC.1 with IgG and yhbG with IgM showed 91% accuracy. (E) The fifth pair using srlA with IgG and trpC with IgG showed 91% accuracy. (G) The heatmap shows the ratios of binding signals among these five pairs, displaying the highly accurate classification in distinguishing between patients with PE and healthy controls (100% accuracy). Blue
Antibody Analysis of Pre-eclamptic Plasma

represents the ratio of >1, yellow represents the ratio of <1, and white represents the ratio of =0. Outcomes were predicted based on the majority of votes; for example, patient N48 had only one protein pair ratio higher than 1, but had four protein pairs lower than 1. Therefore, patient N48 was classified as healthy because four of five k-TSP pairs (more than half) indicated that she was healthy.

Figure 9. Five pairs showing high performance on testing sets of PE samples. The heatmap shows the ratios of binding signals among the 5-TSP pairs, which show accurate discrimination between patients with PE and healthy controls (90% accuracy). Blue represents the ratio of >1, yellow represents the ratio of <1, and white represents the ratio of =0. Outcomes were predicted based on the majority of votes.
Antibody Analysis of Pre-eclamptic Plasma

Figures

Figure 1

16 pre-eclampsia patients
13 healthy pregnant mothers

E. coli proteome chip

Outlier exclusion
Median normalization

Significant differentially proteins by statistical analysis

Antibody classifier discovery using k-TSP

Consensus motifs discovered by GLAM2

Functional analysis using GO, KEGG, PFAM, etc

Search back to human proteome by GLAM2SCAN

Blind testing of additional samples (5 pre-eclampsia and 5 healthy controls)
Antibody Analysis of Pre-eclamptic Plasma

Figure 2

A

Normal people → Patients with pre-eclampsia

E. Coli proteome chip

Collect serum samples

Hybridize samples to chips

Incorporate with labeled anti-human antibodies

Scan the chips

Legend:
- Human IgM in serum
- Anti-human IgM conjugated with DyLight 560
- Human IgG in serum
- Anti-human IgG conjugated with DyLight 650
- * = Different proteins in E. coli proteome chip

B

Pre-eclampsia → Healthy people

IgM_yhbG (0.006, 0.33)

IgM_IiVA (0.02, 3.88)

IgG_nlpA (0.04, 0.2)

IgG_flgE (0.04, 3.7)
Antibody Analysis of Pre-eclamptic Plasma

Figure 3
Antibody Analysis of Pre-eclamptic Plasma

Figure 4
Antibody Analysis of Pre-eclamptic Plasma

Figure 5
Antibody Analysis of Pre-eclamptic Plasma

Figure 6
Antibody Analysis of Pre-eclamptic Plasma

Figure 7

A  Strongly binding proteins in patients probing with IgG

B  Strongly binding proteins in normal people probing with IgG

C  Strongly binding proteins in patients probing with IgM

D  Strongly binding proteins in patients probing with IgM
Antibody Analysis of Pre-eclamptic Plasma

Figure 8

Accuracy = 1

Accuracy = 0.95
Antibody Analysis of Pre-eclamptic Plasma

Figure 9

Accuracy = 0.9