Combining Metabolite Biomarkers and Placental Growth Factor Yields a Prognostic Test for Preterm Pre-eclampsia

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Introduction

Prognosis of pre-eclampsia in nulliparous remains a challenge in prenatal care.

• Nullparity is a significant risk factor (RR = 2.1; 95%CI 1.9 – 2.4), and accounts for the greatest population attributable fraction of pre-eclampsia (PAF = 32.2%; 95% CI 27.4–37.0%).

• Current prenatal care protocols are still largely based on clinical risk factors, rendering them ineffective for predicting pre-eclampsia risk in 1st time pregnant women.

• The protein biomarkers Placental Growth Factor (PIGF), soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble Endoglin (sEng) have been extensively studied in pre-eclampsia prognosis and detection. Low levels of circulating PIGF early in pregnancy have some prognostic performance, yet PIGF-based prognosis is insufficient to warrant its use in a single-marker test.

• Thus far, most attempts to find additional biomarkers to improve the prediction of pre-eclampsia in nulliparous women did not progress beyond the biomarker discovery phase.

• We established MetDiSCOUT1, a translational research workflow, to elicit genuine metabolite biomarker potential within discovered biomarker candidates studies

• Developed a library of quantitative mass spectrometry assays for metabolites implicated in pre-eclampsia (PE), whereby metabolites discovered in the New Zealand/Australian SCOPE study samples1 were prioritised.

• Verify the biomarker potential for prediction of either low or high risk of developing PE, preterm-PE and/or term-PE in early pregnancy specimens from a separate low risk nulliparous cohort, i.e., the European branch of SCOPE.

• Identify core combinations of complementary (bio)markers with the potential of delivering clinical useful prognostic performance for prediction of all, preterm- and term-PE.

Objectives

• Develop a library of quantitative mass spectrometry assays for metabolites implicated in pre-eclampsia (PE), whereby metabolites discovered in the New Zealand/Australian SCOPE study samples1 were prioritised.

• Verify the biomarker potential for prediction of either low or high risk of developing PE, preterm-PE and/or term-PE in early pregnancy specimens from a separate low risk nulliparous cohort, i.e., the European branch of SCOPE.

• Identify core combinations of complementary (bio)markers with the potential of delivering clinical useful prognostic performance for prediction of all, preterm- and term-PE.

Methods

Data Pre-processing

53 metabolites assayed + 1 metabolite mixture assay* Metabolite abbreviated with (except column) Metabolite abbreviated with (except column)

+ 8 metabolites

45 metabolites + 1 metabolite mixture Pre-ease procedure (HPLC/MS/MS)

+ 1 metabolite

44 metabolites + 1 metabolite mixture Unprocessed assay

+ 1 metabolite Metabolite mixture assay

44 metabolites Metabolite quantitation data was selected for biomarker analysis when misssingness was < 20%* and the assay precision** was > CV<25%.

All metabolite and protein quantitation data were log transformed.

Quantitation data showing significant dependency (p<0.01) on collection center, BMI at sampling, age, gestational age at sampling were normalised using Multiple of the Median (MoM) methodology. Both normalised and non-normalised were considered.

• Cotinine data was dichotomised based on presence (1) or absence (0) in a specimen; strong agreement with self-reported smoking status was found.

• For protein biomarkers, referred to as "false negatives". *Excerpt from perspective

Univariable analysis

• Predictive performance: The prognostic performance of single variables for all-, preterm- and PE was assessed using AUROC.

• Biomarker selection: Variables with AUROC >0.6 (and lower limit of the AUROC 95% CI >0.5) are considered promising predictors.

Multivariable analysis

• Modelling: For each possible combination of one to four predictors, a model was trained using one component partial least square analysis (PLS-DA/EDC) across all outcomes. The prognostic performance were derived for a) mean over 3-fold cross validation and b) the entire sample sets. Concordance with models developed using logistic regression was also checked.

• Model selection: Models were selected if 1) lower limit of the 95% CI >0.5 for the AUROC statistic in both the cross-validation and entire set, AND 2) difference between the AUROC statistic over the cross-validation and the entire set < 0.01. Only sparse models were retained by selecting models whose difference of test performance between a given model and all its parent models are greater than a given threshold.

• Test performance: The statistics used to assess biomarker panels for prognostic performance, high (rule-in) or low PE risk (rule-out) are given below.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Prevalence in SCOPE</th>
<th>PVF (cut-off)</th>
<th>NPV (cut-off)</th>
<th>Cut-off rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>All PE</td>
<td>0.014</td>
<td>0.013</td>
<td>0.988</td>
<td>Used to PE risk in multiplex with previous PE (PVF) or without previous PE (NPV)</td>
</tr>
<tr>
<td>Term PE</td>
<td>0.014</td>
<td>0.014</td>
<td>0.875</td>
<td>PVF and NPV targets based on Preterm status as reported in SQU4RE.</td>
</tr>
<tr>
<td>Preterm PE</td>
<td>0.014</td>
<td>0.014</td>
<td>0.875</td>
<td>Term PE cut-off based on Preterm status as reported in SQU4RE.</td>
</tr>
</tbody>
</table>

• Biomarker selection: Predictors were ranked based on the test performance of the selected models they are constituent of.

Confirmed prognostic (bio)markers for PE

• Variables which featured in at least two of the prognostic viewpoints assessed (univariable, multivariable modelling: generic, rule-in, rule-out) across the three outcomes investigated (all-, preterm- and term-PE) are considered confirmed.

Results

Complicating PIGF for Preterm PE prognosis

• Recursive partitioning was applied to further triage the "PIGF-only" False Negatives into a high PE risk group (PVF>0.07) and a low PE risk group (PVN>0.9975).

• Interestingly, the three metabolites map onto complementary pathways. DLG, a diacetylcysteine, may mediate insulin resistance, ergothioneine associates with mitochondrial oxidative stress, and amino acids leucine/isoleucine inform about placental nutrient uptake.

Conclusions

• An extensive list of putative metabolite biomarkers for the prognosis of pre-eclampsia have been subjected to a comprehensive verification exercise, resulting in a verified set of 13 prognostic metabolites. These are being progressed to clinical assay development*.

• Three metabolite biomarkers were found to effectively complement PIGF enabling accurate prediction of preterm PE at 15 weeks’ gestation.

• Taken together with PIGF, a marker for placental insufficiency, the resulting 3+1 panel more comprehensively encapsulates the different aspects of the preterm pre-eclampsia syndrome, thus delivering an accurate biomarker-only preterm PE prognosis in nulliparous, which was unachievable until now.

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Metabolic Diagnostics (MedDiX) developed the MedDiSCOUT™ workflow. All metabolite analyses were performed at MedDiX as part of iPROBEO. MedDiX is developing the clinical assays.