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.

- Nulliparity is a significant risk factor (RR = 2.1; 95%CI 1.9 – 2.4)[1], and accounts for the greatest population attributable fraction of pre-eclampsia (PAF = 32.3%; 95% CI 27.4– 37.0)[1],
- **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 (PlGF), soluble Fms-like tyrosine kinase-1 (s-Flt1) and soluble Endoglin (s-ENG) have been extensively studied in pre-eclampsia prognosis and detection. **Low levels of circulating PlGF** early in pregnancy have some prognostic performance, yet PlGF-based prognosis is **insufficient to warrants its use in a single-marker test**.
- Thus far, most attempts to find additional biomarkers to improve the prediction of pre-eclampsia risk in nulliparous, did not progress beyond the biomarker discovery phase.
- We established MetDxSCOUT™, a translational research workflow, to **elicit genuine metabolite biomarker potential within discovered biomarker candidates studies**

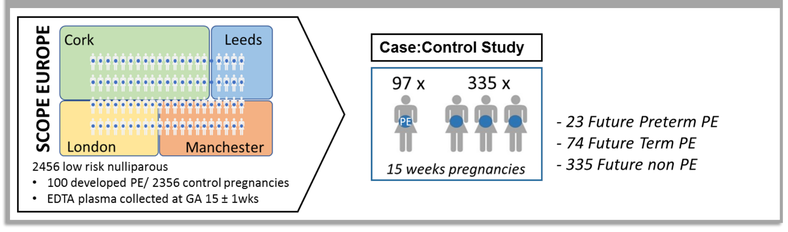
[1]Bartsch et al; doi:10.1136/bmj.i1753

Objectives

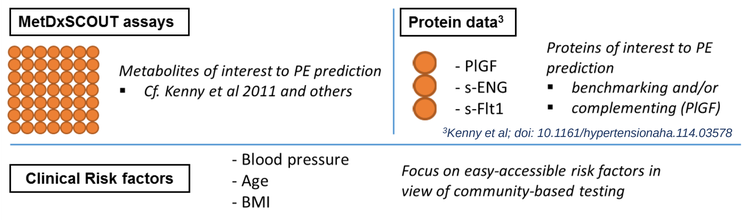
- **Develop a library of quantitative mass spectrometry assays for metabolites implicated in pre-eclampsia (PE),** whereby metabolites discovered in the New Zealand/Australian SCOPE study samples[2] were prioritised.
- **Verify the biomarker potential for prediction** of either low or high **risk of 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/or term-PE.

[2]Kenny et al; doi:10.1161/hypertensionaha.110.157297

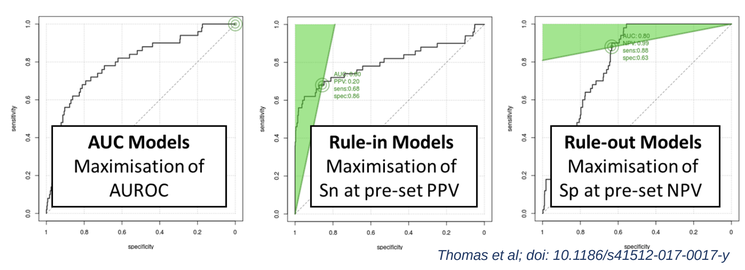
Study samples



Variables for in prognostic analysis



Prognostic viewpoints considered

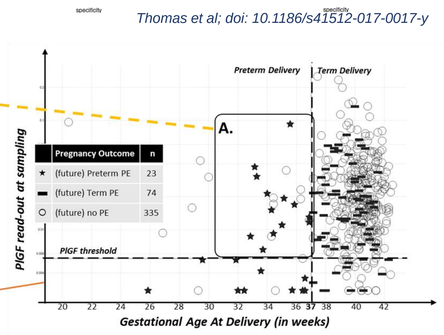


Thomas et al; doi: 10.1186/s41512-017-0017-y

Prediction of Preterm PE in nulliparous:
Identify the False Negatives (**A**) following "PlGF-only" risk classification

Low PlGF early in pregnancy has some prognostic performance for preterm PE.

PPV based PlGF level threshold (preset PPV = 0.07; comparator Preterm PE rate in Screen Positive group as observed in ASPRE study: PPV=0.051[5])



[5]Rolnik et al; doi: 10.1002/uog.18816

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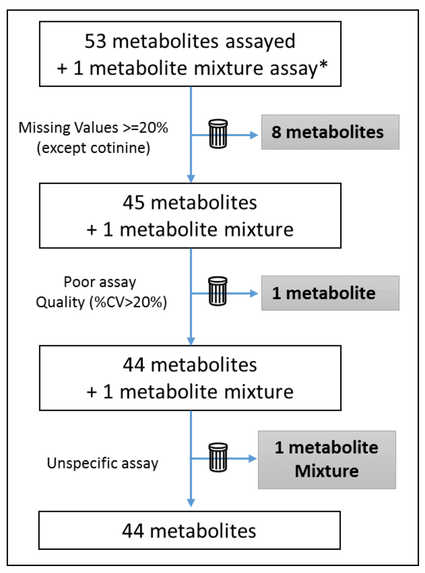
Methods

Data Pre-processing



Metabolite quantitation data was selected for biomarker analysis when missingness 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, or gestational age at sampling were normalised using Multiple of the Median (MoM) methodology. Both normalised and non-normalised were considered.

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

**except for cotinine; **based on replicate analyses*

Univariable analysis

- Predictive performance: The prognostic performance of single variables for all-, preterm- and term-PE was assessed using AUROC.
- (Bio)marker selection: Variables with AUROC >0.6 (and lower limit of the AUROC 95% CI ≥0.5) are considered promsing 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 <=0.1. 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 prognosing generic-, high-(rule-in) or low PE risk (rule-out) are given ↓→.

Generic performance	Rule-in performance	Rule-out performance
AUROC	Sn at 20% FPR	Sp at 20% FNR
	Sn at 10% FPR	Sp at 20% FNR
	Sn at pre-set PPV	Sp at pre-set NPV

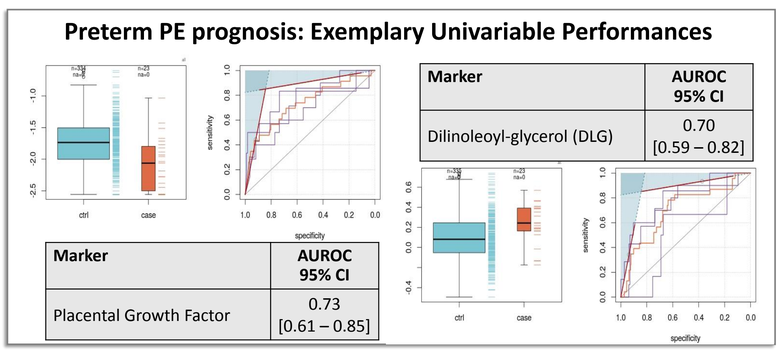
Outcome	Prevalence in SCOPE[3]	PPV cut-off	NPV cut-off	Cut-off rationale
All PE	0.05	1/7.5= 0.133	1-(1/90)= 0.988	Equal to PE risk in multiparous with previous PE (PPV) or without previous PE (NPV)[4]
Preterm PE (<37 wks)	0.014	1/14= 0.0714	1-(1/400)= 0.9975	PPV and NPV targets based on Preterm predictor as reported in[6]
Term PE (>=37)	0.037	1/6.5= 0.154	1-1/160 = 0.99375	Arbitrary: 5-fold increase in risk (PPV); 5-fold decrease in risk (NPV)

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

Results

Verified 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 verified.

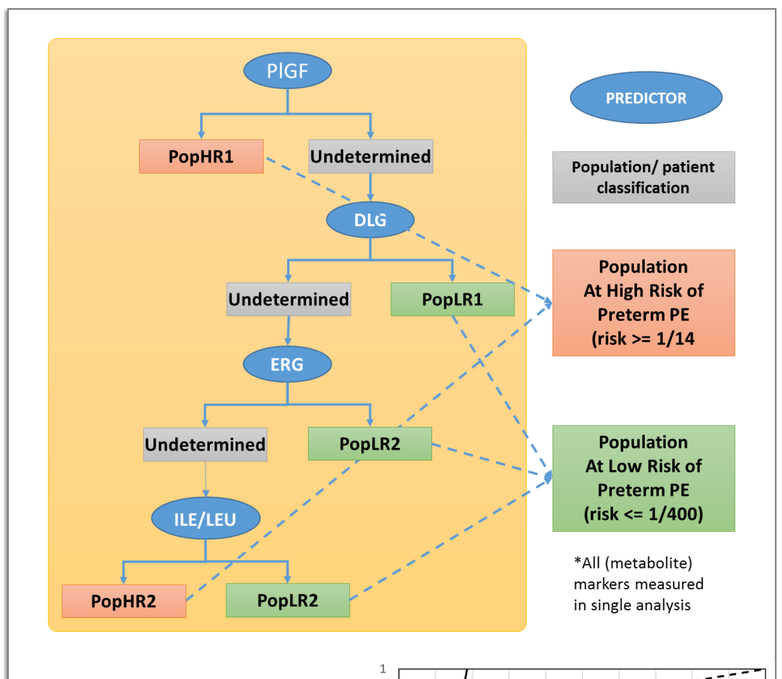


The authors GT & RT also gratefully acknowledge the SCOPE consortium for the collaboration opportunity.

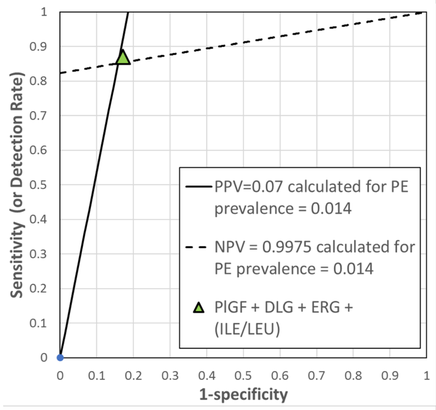
Clinical risk factor			Metabolite		
Bmi	■	■	Dilinoleoyl-glycerol (DLG)	■	■
Blood pressure	■	■	Citrulline		■
Protein			1-heptadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine	■	■
Placental Growth factor	■	■	Isoleucine (ILE)	■	■
s-Endoglin	■	■	Leucine (LEU)		■
			NG-Monomethyl-L-arginine	■	■
			Stearoylcarnitine		■
			Ergothioneine (ERG)	■	■
			2-Hydroxybutanoic acid	■	■
			Decanoylcarnitine	■	■
			Etiocholanolone glucuronide		■
			20-Carboxy-leukotriene B4		■
			25-Hydroxyvitamin D3		■

■ Univariable Performance
■ Complementarity in multivariable models

Complementing PlGF for Preterm PE prognosis



- Recursive partitioning was applied to further triage the "PlGF-only" False Negatives into a high PE risk group (PPV>=0.07) and a low PE risk group (NPV >= 0.9975).



- Interestingly the three metabolites map onto complementary pathways. DLG, a diacylglycerol, 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 PlGF** enabling accurate prediction of preterm PE at 15 weeks' gestation.

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

IMPROvED
SCOPE Study
Screening for Pregnancy Endpoints
SQU4RE
METABOLOMIC DIAGNOSTICS

Metabolomic Diagnostics (MetDx) developed the MetDxSCOUT™ workflow. All metabolite analyses were performed at MetDx as part of IMPROvED. *MetDx is developing the clinical assays.