Preeclampsia risk stratification early in pregnancy: Conversion of a promising metabolomics discovery into a LC-MS based clinical assay

Liz Bond1,4, Charline Lenaerts3, Christopher Benton3, Phil Baker4,6, Louise Kenny5,6, Robin Tuytten1,6

Metabolomic Diagnostics, Little Island, Cork, Ireland; 1. Lab. of Pharmaceutical Analysis, Faculty of Medicine & Pharmacy, University of Mons, Belgium; 2. Agilent Technologies, Life Sciences & Chemical Analysis Group, Stockport, UK; 3. School of National Centre for Growth and Development, The University of Auckland, New Zealand; 4. The Irish Centre for Fetal and Neonatal Translational Research, Cork University Maternity Hospital, Cork, Ireland; 5. on behalf of the IMPROvEd Pregnancy Outcomes by Early Detection consortium (IMPROvED)

Abstract

Basic metabolomics research has uncovered that combinations of blood borne metabolites can risk-stratify women early in pregnancy according to their risk of developing preeclampsia later in their pregnancy. Since then, a company has been established which is dedicated to translating this finding into a tool for health care providers and pregnant women. A targeted approach is being developed whereby ca. 40 metabolites are (semi-) quantified using liquid chromatography-tandem mass spectrometry. An update on the method development process as well as an overview of the clinical studies lined-up to verify and validate the preeclampsia risk stratification test will be discussed.

Fig. 1. Overview Metabolomic Diagnostics' Preeclampsia Risk Stratification product

Background

• Preeclampsia is mostly a syndrome of late pregnancy characterised by concomitant (new onset of) hypertension (high blood pressure) accompanied by new onset proteinuria (elevated protein in the urine) or signs of multi-systemic impairment. Currently there is no cure for preeclampsia other than delivery.

• Preeclampsia has been the most significant cause of maternal death over recent decades: between 70,000 and 80,000 women die every year from preeclampsia and in excess of half a million new born babies die annually as a direct result of the condition.

• Efficient prediction of preeclampsia is considered a crucial step stone to deliver more effective prenatal care, minimise preeclampsia related complications and per result reduce health care costs.

The Challenge

• Thus far preeclampsia prediction is largely depending on clinical risk factors, but these are marginally useful in healthy first time pregnant women who account for >50% of preeclampsia cases.

• Prediction of preeclampsia in first time pregnant women requires a panel of biomarkers in order to encapsulate the complex pathogenesis of the syndrome.

• Research and (commercial) test development requires prospectively collected 1st pregnancy biobanks, whereby >100’000 of women need to be longitudinally monitored and sampled throughout pregnancy.

The Opportunity

• Kenny et al. from the University College Cork, Ireland found that accurate prediction of preeclampsia in 1st time pregnant women is possible using a panel of blood-borne metabolite plasma in presence of ~15 weeks pregnant women.

• In recent years, there is an increased demand for the rationalisation of healthcare: identification of 1st time women at risk of preeclampsia will allow health-care workers to better administer the right prenatal care to the right women.

• Metabolite analysis using LC-MS is well established in Pharma (bioanalysis) and neonatal screening (“heel prick”). LC-MS is therefore an obvious platform choice to port a potentially disruptive preeclampsia risk stratification test into the clinical laboratory.

• Together the above present an entrepreneurial opportunity to develop a disruptive, first-in-class diagnostic tool to stratify 1st time pregnant women early in pregnancy according to their preeclampsia risk.

The Approach

• Collaboration between Company, technology inventors and other clinical experts.

• Creation of Public-Private partnerships to lever transnational funding to propel both academic research and SME-based product development.

• Seminal partnership with dedicated instrument manufacturers.

• Understanding of funding mechanisms - what funding to apply / seek at what stage of company / product development.

Method Development

Selection of Target Metabolites

• Primarily based on metabolites identified in Kenny et al. (2010); 41 Target Metabolites selected

• Selection of corresponding stable isotopically labelled standards chosen based on the metabolite classes; 16 ISTDs selected

Development of multiplex LC-MRM assay for target metabolites and selection of ISTDs

• Metabolite extraction procedure from plasma developed and optimised for all metabolites (and ISTDs) and compatible with LC-MS analysis methodology.

• LC: 10 minute gradient elution - Agilent PFP Pump coil; separation of hydrophilic and hydrophobic compounds - MS: Unique Qual and Quant transitions for 41 Target Metabolites and 16 ISTDs: 112 MRM transitions / run (dMRM)

• Development of a simple, comprehensive plasma extraction procedure

• Metabolite extraction procedure from plasma developed and optimised for all metabolites (and ISTDs) and compatible with LC-MS analysis methodology.

• Selection of ISTDs for 41 target metabolites

• Quantitative analysis by metabolite; used to monitor precision of measurements.

• Selection of relative quantitation for ISTDs

• Selection of ISTDs for target metabolites

• Selection of isotopic label for ISTDs

• Selection of quantitative approach for target metabolites

• Selection of quantitative approach for ISTDs

• Selection of LC-MS method dependent on target metabolite

• Selection of LC gradient run time: 10 minutes

• Selection of Quantitative approach for ISTDs

• Selection of Quality control procedures for ISTDs

• Selection of Quality control procedures for target metabolites

Fig. 3. Composition of metabolite panel

Development of a multiplex analytical workflow

Fig. 4. Metabolite sample processing pipeline

Proof of Principle: Determination of target metabolites in pregnancy blood

• 41 metabolites of interest are assay-able in pregnancy blood (15wks) with good precision

• Semi quantitative data generated using Relative ISTD quantitation

• Current throughput: 435 samples / week (QC samples excluded)

• Ongoing: stability testing batch processing and reference range determinations

Fig. 5. Precision metrics 40 metabolite assays: * 5 independently prepared samples - %CV for whole sample processing pipeline

The Near Future

• Case Control study - SCOPE (n = 700): Metabolite candidate confirmation and prediction algorithm refinement.

• Final Standard Operation Procedures for processing pipeline and data analysis.

• Field testing of SOPs and verification of algorithm in full SCOPE Europe (n ~2500). Technical and clinical validation of PrepSia v1.0 in IMPROvED (n ~5000)1,2

Conclusions

• Within Metabolomic Diagnostics, a single step metabolite extraction and a targeted LC-QqQ-MS approach using stable isotope labelled metabolites for relative quantification has been successfully developed.

• All major components are in place to commence processing the clinical samples as available in SCOPE and IMPROvED biobanks.

References


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Fig. 2. Overview of the interplay between funding bodies, academia and company