Preeclampsia Risk Stratification: Moving from Translational Research to a Dedicated Quantitative LC-MS/MS Assay for Future Clinical Application.

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INTRODUCTION

The risk prediction of pre-eclampsia (PE) in pregnancy remains a challenge in prenatal care. Although low levels of Placental growth factor (PlGF) are associated with the development of preterm PE later in pregnancy, a risk prediction test solely based on PlGF has limited sensitivity, hampering the roll-out of a much-needed risk stratification screening test for preeclampsia in prenatal care pathways [1].

We applied our translational LC-MS/MS based research platform M-Scout™ (highly multiplexed semi-quantitative assays of putative metabolite biomarker candidates) to a cohort of pregnant women including cases of preeclampsia as well as controls. This approach delivered four metabolite biomarkers with the ability to complement PlGF to improve risk prediction of Preterm PE.

Subsequently, we aimed to develop a prototype clinical research assay for the fast, precise and accurate quantification of these four endogenous metabolites from different metabolite classes. Replicate samples of the original cohort were rerun with the new assay to verify it delivers the same predictive power as found with M-Scout™.

OBJECTIVE

Development of a quantitative LC-MS/MS assay for four pre-selected endogenous metabolites in plasma from pregnant women for integration in a future preeclampsia risk IVD product.

METHODS

A sample set of 298 EDTA plasma samples collected at 15±1 weeks in pregnancy were analysed with the new dedicated LC-MS/MS assay. The sample set corresponded a single-centre nested case control study within the European SCOPE cohort (n=298, controls = 231, cases PE = 67, thereof preterm PE = 17) previously analysed with M-Scout™ to allow correlation of results between assays. The samples were organised in four batches distributed over two instruments to assess robustness. Method development was performed on two HPLC-QQQ-MS (Agilent).

The following analytical figures of merits were considered: linearity, measuring interval, imprecision (intra-batch, inter-batch and inter-instrument) and turnaround time.

Correlation of M-Scout and the new LC-MS/MS assay was analysed using weighted Deming regression. ROC analysis and comparison of AUROC (DeLong) were used to assess conservation of risk prediction at univariable level.

RESULTS

We successfully developed a quantitative multiplex LC-MS assay for the four biomarker candidates, reducing the total run time from 20 min to 5 min/injection. Streamlining of the original semi-automated sample preparation resulted in a preparation time of < 2 hours per 96 well plate.

An improvement of the semiquantitative approach in M-Scout™ is the calibration system using matching Stable Isotope labelled Internal Standards for each metabolite. The 7 calibrator levels in a surrogate matrix resulted in $r^2 > 0.99$ and the measuring interval covered all subject samples.

Imprecision metrics were determined using matrix-matched controls (Pool QC plasma pool from all study samples; QC High spiked commercial plasma; QC Low commercial plasma diluted with surrogate matrix). Intra- and inter-batch imprecision as well as and inter-instrument imprecision was assessed, see Table 1. The new assay improved or maintained the imprecision of M-Scout™.

For the four metabolites, good correlation between the new LC-MS/MS assay quantifications and the original M-Scout™ (relative) quantifications was found: slopes between 0.93 and 1.07 (and r > 0.9) were obtained in weighted Deming regression analysis; see Figure 1 for details.

Figure 2 shows the prediction of preterm PE at univariate level for the four metabolites based on the new assay (green) and the original discovery study using M-Scout™ (blue). The biomarker prediction was confirmed for the four metabolites following assay transfer (results see Table 2).

[1] Agrawal, S. et al. *Hypertension* **74,** 1124–1135 (2019).

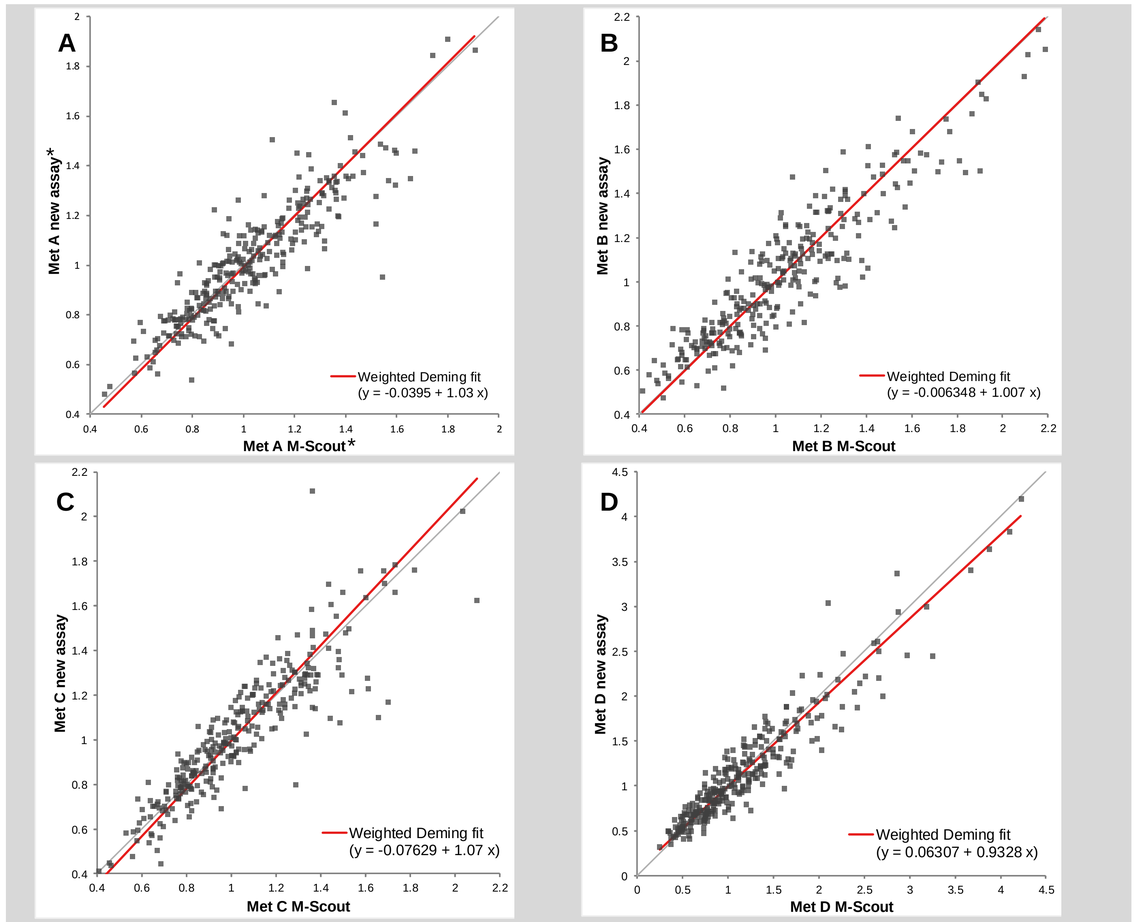

FIGURE 1: Correlation of results for each metabolite for M-Scout and new assay (n= 298, normalised to respective median) including results for weighted Deming regression * one outlier excluded for Met A

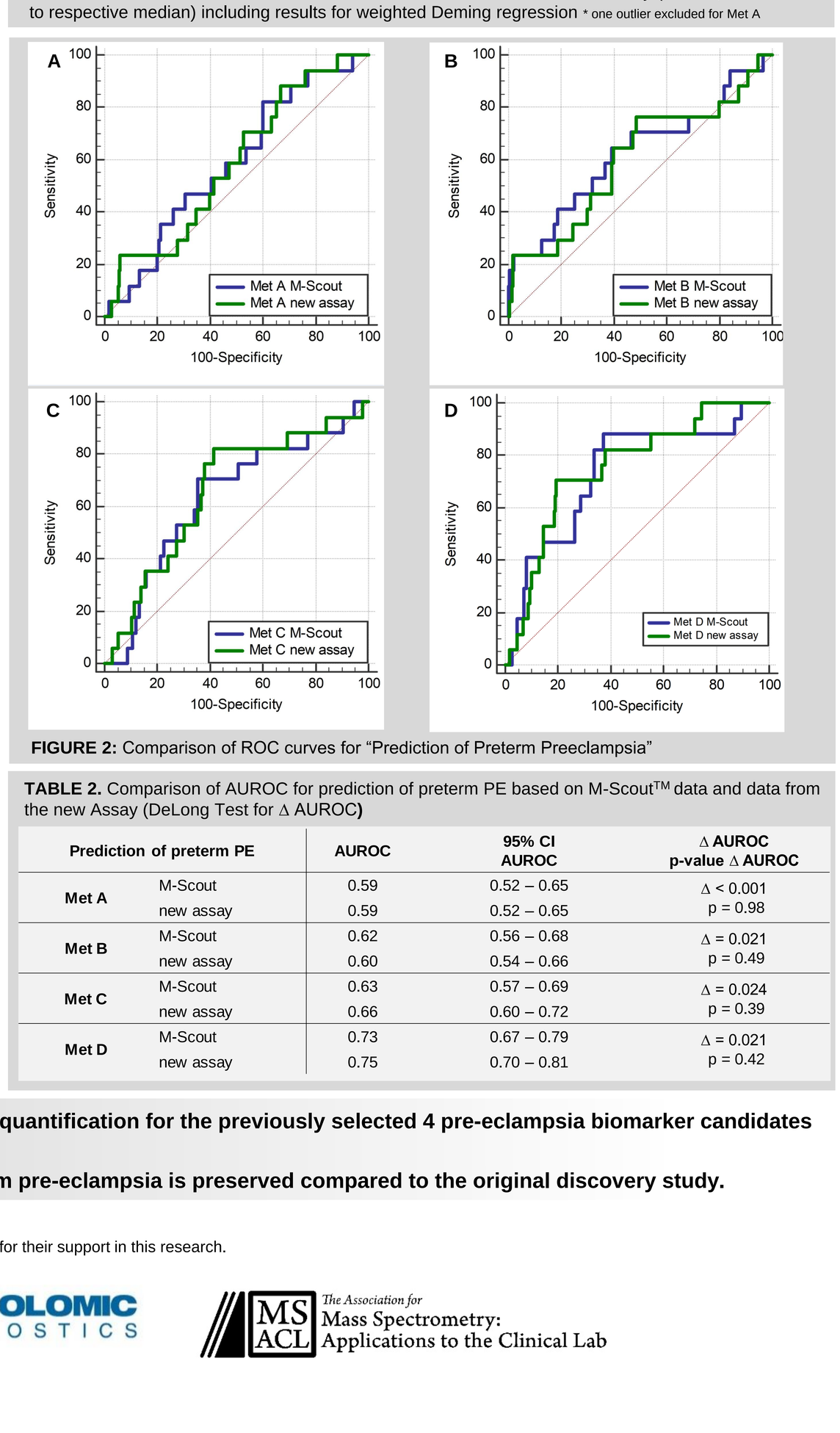

FIGURE 2: Comparison of ROC curves for "Prediction of Preterm Preeclampsia"

TABLE 1. Imprecision for metabolites in new assay and M-Scout, calculated as intra-batch (batch number 1-4), inter-batch (instrument number I, II) and inter-instrument; QC Pool: 9 replicates/batch, QC High/Low: 2 replicates per batch each * 1 outlier excluded

		imprecision		Met A	Met B	Met C	Met D
New Assay	QC Low	inter-batch	I	10%	5%	4%	12%
			II	1%	4%	5%	8%
		inter-instrument		7%	6%	4%	9%
	QC High	inter-batch	I	11%	3%	3%	6%
			II	2%	4%	4%	5%
		inter-instrument		10%	3%	4%	6%
	QC Pool	intra-batch	1	13%	10%	10%	12%*
			2	7%	5%	3%	15%
			3	7%	5%	4%	11%
			4	3%	4%	3%	10%
		inter-batch	I	10%	9%	8%	11%
			II	5%	4%	3%	14%
		inter-instrument		8%	8%	6%	18%
M-Scout	QC Pool	inter-batch		8%	14%	22%	13%

TABLE 2. Comparison of AUROC for prediction of preterm PE based on M-Scout™ data and data from the new Assay (DeLong Test for Δ AUROC)

Prediction of preterm PE		AUROC	95% CI AUROC	Δ AUROC p-value Δ AUROC
Met A	M-Scout	0.59	0.52 – 0.65	Δ < 0.001 p = 0.98
	new assay	0.59	0.52 – 0.65	
Met B	M-Scout	0.62	0.56 – 0.68	Δ = 0.021 p = 0.49
	new assay	0.60	0.54 – 0.66	
Met C	M-Scout	0.63	0.57 – 0.69	Δ = 0.024 p = 0.39
	new assay	0.66	0.60 – 0.72	
Met D	M-Scout	0.73	0.67 – 0.79	Δ = 0.021 p = 0.42
	new assay	0.75	0.70 – 0.81	

CONCLUSIONS

- ✓ **The newly developed LC-MS/MS assay offers precise quantification for the previously selected 4 pre-eclampsia biomarker candidates in pregnant women's plasma.**
- ✓ **The predictive power of the markers regarding preterm pre-eclampsia is preserved compared to the original discovery study.**

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METABOLOMIC DIAGNOSTICS

The Association for Mass Spectrometry: Applications to the Clinical Lab

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