Abstract T-080

Basic Parturition, Prematurity

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Immunoassay Confirmation of MRM-MS Data Supports a Role for Acute Phase Proteins in the Prediction of Spontaneous Preterm Birth

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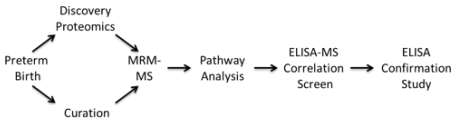
Abstract

Introduction: Preterm birth (PTB), defined as birth prior to 37 weeks gestation, is a leading cause of perinatal morbidity and mortality affecting about 11% of all pregnancies. Factors such as infection, inflammation, placental hemorrhage and stress have been associated with PTB; however, the ability to accurately predict women at risk has been problematic. We sought to identify, by mass spectrometry (MS), biological pathways whose perturbation may be predictive of PTB and confirm results in an immunoassay format that may accelerate the wider application of a diagnostic test.

Methods: MS discovery was combined with immunoassay confirmation. A single multiple reaction monitoring (MRM)-MS assay quantified >200 candidate biomarkers in ~90 PTB case and ~180 control serum samples from our Proteomic Assessment of Preterm Risk (PAPR) trial. This study included 5,500 pregnant women representative of US demographics, enrolled from eleven clinical sites. Proteins with levels differing significantly ($p \leq 0.05$) by t-test between cases and controls were grouped into functional pathways.

Results: Acute phase response (APR) proteins were highly represented and indicate the presence of an underlying infection or the induction of non-pathologic inflammatory signaling cascades in PTB. In an ELISA-MS correlation screen focused on APR, commercial ELISA test kits were used to test sixteen PAPR samples containing "high" and "low" MS levels of each analyte. For twelve APR proteins the ELISA p-values ranged from 0.02 to <0.0001, indicating agreement between MS-peptide and ELISA-protein measurements. A larger ELISA study confirmed the significance of APR proteins in PTB via an independent platform.

Biomarker Discovery Strategy



A large MRM-MS assay of candidate biomarkers was built by discovery and curation. APR proteins, quantified by MRM and classified by pathway analysis, were filtered for robustness and general agreement by an ELISA-MS correlation screen. Qualifying analytes were then tested in a larger confirmation study to measure correlation between ELISA and MRM-MS results.

Proteomic Assessment of Preterm Risk (PAPR) Clinical Trial

Purpose: to collect blood specimens and corresponding clinical data from asymptomatic pregnant women to develop a noninvasive test for prediction of preterm delivery

Primary Outcome Measures: Spontaneous Preterm Birth (SPTB)

Enrollment: 5,500 pregnant women who were receiving prenatal care

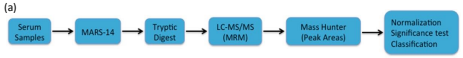
Inclusion Criteria:
- Subject is 18 years or older.
- Subject has a singleton pregnancy.
- Subject is able to provide consent.

Exclusion Criteria:
- Subject is pregnant with more than one fetus.
- There is a known or suspected fetal anomaly.

ClinicalTrials.gov Identifier: NCT01371019

MRM-MS Discovery and Pathway Analysis of Potential PTB Biomarkers

(a)



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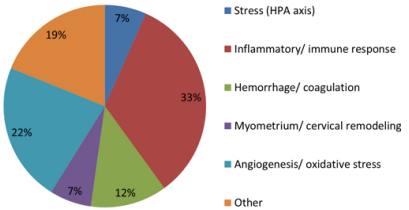
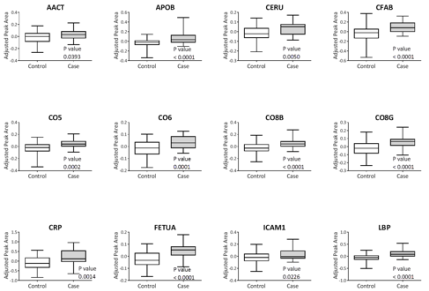


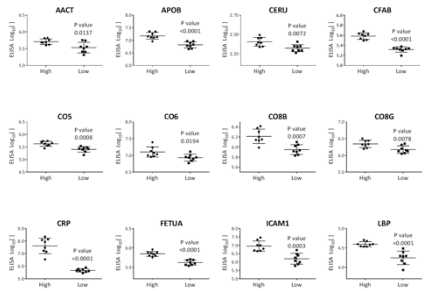
(c)



(a) MRM-MS Workflow. Clinical samples were processed through the workflow above in a 96-well format. Following depletion of the 14 most abundant serum proteins, samples were digested with trypsin overnight. MRM-MS data was acquired on an Agilent 6490 in dMRM mode and peak areas were calculated using Mass Hunter software. Data was corrected for run-order and batch effects. (b) Proteins that were expressed at significantly ($p \leq 0.05$) different levels in SPTB vs. term controls were mapped to pathways implicated in preterm birth. The pie chart illustrates the distribution across designated pathways. (c) MRM-MS results showing adjusted peak areas for a panel of 12 APR and APR-related proteins with significant differences between SPTB cases and controls. Serum drawn from 17 weeks, 0 days to 22 weeks, 6 days from 90 pregnant women (30 SPTB and 60 term controls) were analyzed. P-values were calculated using a standard t-test.

Our MRM-MS results implicated an upregulation of the APR pathway in SPTB.

ELISA High-Low Screen of APR Proteins



Graphs show the log transformed protein concentrations for the indicated APR proteins as measured by ELISA. Each dot represents a different sample, selected based upon the mass spectrometry data, where samples that scored within either the top or bottom quartile across all transitions for a given protein were selected for analysis. P-values were measured using a standard t-test.

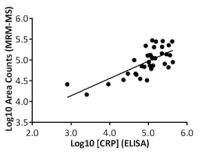
Screens using a small number of patient samples allowed us to identify ELISA kits with performance that paralleled the MRM-MS analyses.

ELISA Confirmation Study for APR Proteins

(a)

Analyte	Pearson's r
AACT	0.05609
APOB	0.2746
CERU	0.3644
CFAB	0.186
CO5	0.7112
CO6	0.1931
CO8B	0.3152
CO8G	0.225
CRP	0.7241
FETUA	0.3241
ICAM1	0.2394
LBP	0.9222

(b)



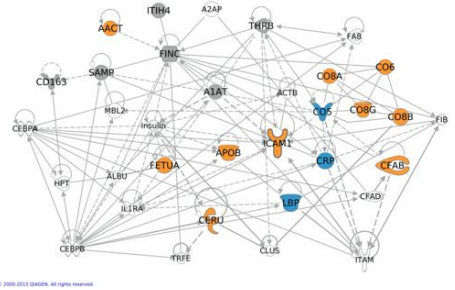
(c)

	CRP_ELISA	Transition 1a	Transition 1b	Transition 2a	Transition 2b
CRP_ELISA	1	0.6869	0.7053	0.7137	0.7241
Transition 1a	0.687	1	0.9914	0.9804	0.9720
Transition 1b	0.7053	0.9914	1	0.974	0.972
Transition 2a	0.7137	0.9804	0.9741	1	0.9889
Transition 2b	0.7241	0.9720	0.9718	0.9889	1

(a) Table summarizing the correlation between MRM-MS and ELISA values for ~20 SPTB case and ~20 term control serum samples. (b) The graph depicts MRM-MS (1 transition) vs. ELISA values for CRP, with a linear regression curve fit. (c) Table summarizes Pearson's correlation values (r) between CRP ELISA values and adjusted peak areas for the different MRM transitions measuring CRP. Correlations between the different transitions measuring CRP by MRM are also shown.

Good correlation between MRM-MS and ELISA was seen for a subset of APR proteins.

Summary of APR Proteins Discovery



The network depicts proteins involved in the acute phase response. The colors of the proteins signify the results of our discovery studies:

- White: not in MRM-MS assay
- Grey: assayed by MRM-MS; p-value >0.05
- Orange: significant by MRM-MS; p-value $\leq$0.05, with ELISA separation of high and low values, but NOT well-correlated with ELISA results in a larger confirmation study
- Blue: significant by MRM-MS; p-value <0.05 AND had a Pearson's r $\geq$0.7 for correlation with the ELISA data

There was good correlation between MRM-MS and ELISA data for a subset of the APR proteins tested, suggesting these would be good candidates for inclusion into an ELISA-based classifier for predicting SPTB.

Conclusions

- Our MRM-MS analyses of serum from 30 women with SPTB and 60 with term deliveries implicated APR proteins as biomarkers for SPTB.
- An ELISA screen was developed to test ELISA kits from multiple vendors and determine if the ELISA could sufficiently separate "high" and "low" values for each APR protein.
- Using a subset of the study samples (~20 SPTB vs. ~20 Term Controls), we demonstrated correlation between MRM-MS results and ELISA measurements for some of the APR proteins.
- Using APR proteins as an example, ~25% of our MRM-MS findings could be translated into an ELISA format.

By extending these screens and correlation studies to other pathways, we expect to build an ELISA-based classifier for SPTB that emulates our MRM-MS performance.