OBSTETRICS

Proteomic identification of serum peptides predicting subsequent spontaneous preterm birth

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OBJECTIVE: We sought to identify serum markers of subsequent spontaneous preterm birth (SPTB) in asymptomatic women prior to labor.
STUDY DESIGN: Serum proteomics was applied to sera from 80 pregnant women sampled at 24 weeks and an additional 80 pregnant women sampled at 28 weeks. Half had uncomplicated pregnancies and half had SPTB.
RESULTS: Three specific peptides arising from inter-alpha-trypsin inhibitor heavy chain 4 protein were significantly reduced in women at 24 and 28 weeks having subsequent SPTB. The most discriminating peptide had a sensitivity of 65.0% and specificity of 82.5%; odds ratio, 8.8; and 95% confidence interval, 3.1–24.8. A combination of the 3 new biomarkers and 6 previously studied biomarkers increased sensitivity to 86.5%, with a specificity of 80.6% at 28 weeks.
CONCLUSION: Three novel serum markers of SPTB have been identified using serum proteomics. Using a combination of these new markers with additional markers, women at risk of SPTB can be identified weeks prior to SPTB.

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Background and Objective

Spontaneous preterm birth (SPTB) is the leading cause of perinatal morbidity and mortality in the United States. Several proteins present in maternal serum or cervical secretions have been proposed as markers that may predict SPTB; however, none of the current SPTB markers alone or in combination provides adequate specificity or sensitivity to be used in clinical prediction.

Serum proteomic analysis, consisting of chromatographic separation followed by mass spectrometry to identify peptides and proteins by mass, can provide an extensive inventory of peptides and/or proteins present at any given time. The use of proteomic analysis to identify phenotypic molecular characteristics of women who experience SPTB or infection has been attempted in amniotic fluid and cervical secretions, but serum proteomic analysis has not been reported.

We hypothesized that proteomic differences exist in maternal serum several weeks prior to the onset of clinical symptoms in women destined to develop SPTB. Our aim was to use serum proteomics to differentiate women having a subsequent SPTB from those having term deliveries. Moreover, we hoped to identify all peptides that are found to be increased or decreased in the serum of women who go on to have an SPTB compared with those who deliver at term.

Materials and Methods

This study represents a nested case-control study that used samples and data that were collected during the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network Preterm Prediction Study, a multicenter observational investigation of 2929 symptom-free women evaluated longitudinally to determine risk factors for SPTB. An SPTB was defined as a preterm birth <35 weeks' gestation occurring as the result of the spontaneous onset of labor or spontaneous rupture of membranes.

Serum was collected at 24 and 28 weeks' gestation and pregnancy out-

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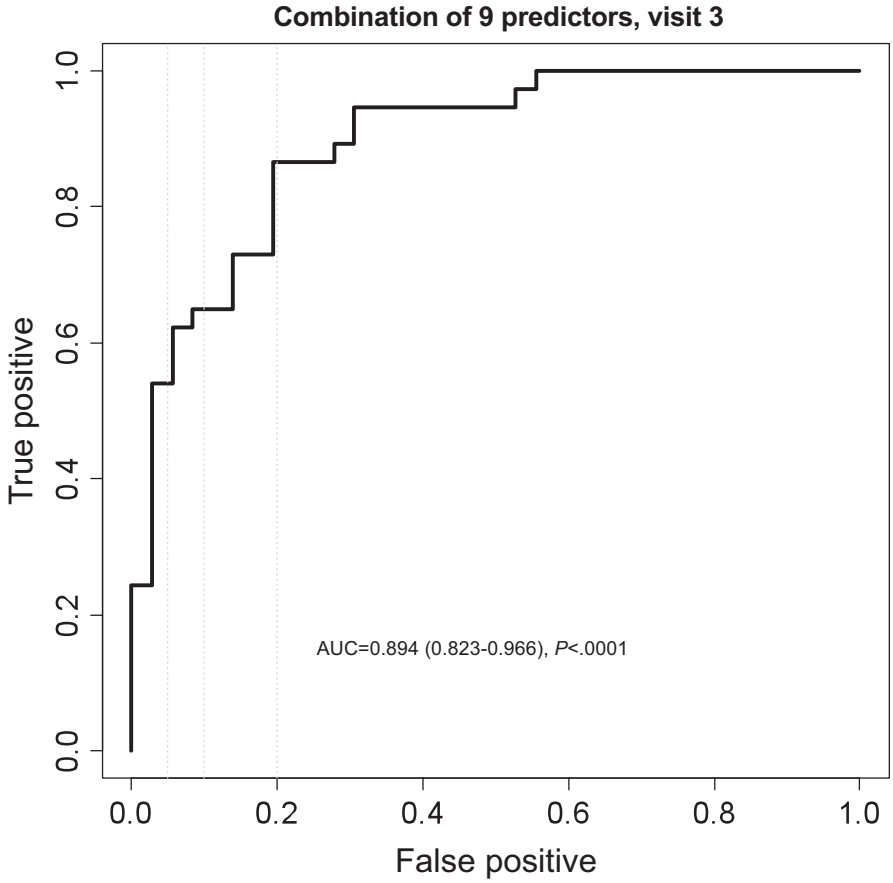
FIGURE
Receiver operator curve for the marker panel



Receiver operating characteristic curve demonstrating predictive capability of combination of 9 predictors (peak 677, peak 857, peak 860, corticotrophin-releasing factor, defensin, ferritin, lactoferrin, thrombin antithrombin complex, and tumor necrosis factor-α receptor type 1) to predict subsequent spontaneous preterm birth after sampling at 28 weeks. Area under curve (AUC) and 95% confidence intervals are also reported.

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comes were obtained. Serum from 40 subjects who experienced a subsequent SPTB and 40 subjects having uncomplicated pregnancies was obtained at 24 weeks' gestation and submitted to proteomic analysis. Serum from a separate group of 40 subjects who experienced a subsequent SPTB and 40 subjects having uncomplicated pregnancies, who ultimately delivered at term after spontaneous onset of labor was obtained at 28 weeks' gestation and was similarly analyzed. High-molecular-weight proteins were removed by acetonitrile precipitation following an established protocol.

Capillary liquid chromatography was interfaced with a mass spectrometer, allowing for the continuous direct delivery of fractionated, protein-depleted serum to the mass detector.

Effluent from the capillary liquid chromatography was directed into a quadrupole orthogonal time-of-flight mass spectrometer through an IonSpray source. Mass spectra were collected every second for m/z 500-2500 from 5- to 55-minute elution. Specimens from cases and controls were analyzed together in random order.

One reference peak, observable in all specimens, near the center of each interval, and which did not demonstrate differences in abundance between the 2 groups was used to align time in that elution region. The initial review of mass spectra was accomplished by overlaying 2-minute summary spectra from cases and controls distinguished by color and visual review. Each candidate marker, appearing quantitatively different between groups, was further evaluated.

The abundance (peak height) of each candidate and its relevant reference peak was quantified (extracted) by the instrument's software and tabulated, as was the calculated ratio of each candidate marker abundance relative to the abundance of the reference within each patient. The log of that ratio was also determined because abundance varied substantially. The data were subjected to statistical analysis. Candidate markers demonstrating statistically different abundances between cases and controls were further analyzed in an effort to chemically identify the candidate molecule.

Plasma corticotropin–releasing factor, defensin, ferritin, lactoferrin, thrombin antithrombin complex, and tumor necrosis factor-α receptor type 1 assays have been previously analyzed by immunoassays and reported. The results from those previous assays were reevaluated statistically for the subjects included in this study and combined with the newly identified markers to improve the markers' predictive capability.

RESULTS

Of the 4 markers considered further, 3 were independently found to be quantitatively significantly reduced. The sensitivity of each of the 3 biomarkers improved generally from 24-28 weeks. The biomarker at m/z 676.7 was the best single predictor of SPTB at 24 or 28 weeks. Combining the 3 markers did not improve sensitivity and specificity, but including the 6 best additional markers previously tested (7 patients excluded due to missing values) improved the sensitivity to 86.5% with a specificity of 80.6% at 28 weeks.

Sequencing by means of a tandem mass spectrometry with intervening fragmentation allowed for the complete amino acid sequence to be determined by amino acid homology to known pep-

tide or protein sequences. All 3 peptides were found to be derived from 1 region of inter-alpha-trypsin inhibitor heavy chain 4, the common parent protein.

When biomarker abundance was plotted as a function of time to delivery, a significant correlation was found for all 3 markers at 28 weeks' gestation and for 2 of the markers at 24 weeks. In each case, abundance of the biomarkers was lower the nearer the delivery.

Comment

We identified 3 peptides in the serum of pregnant women at both 24 and 28 weeks' gestation that were significantly decreased in women who experienced a subsequent SPTB. The changes in peptide concentrations at 24 and 28 weeks predated the SPTB, on average, by a mean of 8.1 and 4.7 weeks, respectively. All 3 identified peptides came from a single protein. The parent compound, inter-alpha-trypsin inhibitor heavy chain 4, is a glycoprotein that is a kallikrein-sensitive acute-phase reactant.

When the current 3 peptides were coupled to 6 previously tested candidate biomarkers, the sensitivity was 86.5%, with a specificity of 80.6% for the prediction of subsequent SPTB. The receiver operating characteristic curve demonstrates the predictive capability of the combination of 9 predictors to predict subsequent SPTB after sampling at 28 weeks (Figure).

CLINICAL IMPLICATIONS

- Serum proteomics can be used to identify potential markers of spontaneous preterm birth (SPTB).
- Three fragments of the glycoprotein inter-alpha-trypsin inhibitor heavy chain 4 are decreased in the serum of pregnant women 4-8 weeks before a subsequent SPTB.
- A combination of 3 new serum markers and 6 additional serum proteins may predict subsequent SPTB with a sensitivity of 86.5% and a specificity of 80.6%. ■

Stage-based outcomes of 682 consecutive cases of twin–twin transfusion syndrome treated with laser surgery: the USFetus experience

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OBJECTIVE: We sought to describe stage-specific perinatal outcomes after selective laser photocoagulation of communicating vessels (SLPCV) for twin-twin transfusion syndrome.

STUDY DESIGN: Patients with twin-twin transfusion syndrome underwent SLPCV preferentially using sequential vs standard laser technique. Patient characteristics and outcome data were examined by Quintero stage.

RESULTS: Of 682 consecutive women studied, the Quintero stage distribution was: 114 stage I (17%), 177 stage II (26%), 328 stage III (48%), and 63 stage IV (9%). Perinatal survival of at least 1 twin did not differ according to stage (I-92%, II-93%, III-88%, IV-92%; $P = .30$). However, dual twin survival differed by stage (I-79%, II-76%, III-59%, IV-68%; $P < .01$), primarily because stage III pregnancies were associated with decreased donor twin survival ($P < .01$). Sequential SLPCV was associated with improved donor survival, independent of stage (odds ratio, 1.67; 95% confidence interval, 1.16–2.40; $P < .01$).

CONCLUSION: Stage-specific perinatal outcomes after laser therapy may assist physicians in patient counseling and in planning future studies.

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Background and Objective

Most studies have shown that selective laser photocoagulation of communicating vessels (SLPCV) is the optimal treatment for twin-twin transfusion syndrome (TTTS). Knowledge of perinatal outcomes of laser-treated patients according to Quintero stage may facilitate patient selection and counseling.

The aim of this study was to describe perinatal outcomes after laser surgery