Clinical Mass Spectrometry 3 (2017) 25–38

Contents lists available at ScienceDirect

Clinical Mass Spectrometry

journal homepage: www.elsevier.com/locate/clinms

Analytical validation of protein biomarkers for risk of spontaneous preterm birth

Chad Bradford [a,*], Rob Severinsen [a], Trina Pugmire [a], Matison Rasmussen [a], Kathryn Stoddard [a], Yuta Uemura [a], Spencer Wheelwright [a], Marija Mentinova [b], Daniel Chelsky [b], Stephen W. Hunsucker [c], Paul Kearney [c], Durlin Hickok [a], Tracey C. Fleischer [a], Ilia Ichetovkin [a], J. Jay Boniface [a], Gregory C. Critchfield [a], John M. Peltier [a]

[a] Sera Prognostics, Inc., 2749 East Parleys Way, Suite 200, Salt Lake City, UT 84109, USA
[b] Caprion Biosciences, 201 Avenue Président Kennedy, Suite 3900, Montréal, Québec H2X 3Y7, Canada
[c] Integrated Diagnostics, Inc., 219 Terry Avenue North, Suite 110, Seattle, WA 98101, USA

ARTICLE INFO

Article history:
Received 23 February 2017
Received in revised form 9 June 2017
Accepted 10 June 2017
Available online 12 June 2017

Keywords:
Spontaneous preterm birth
Proteomics
Mass spectrometry
Insulin-like growth factor-binding protein 4
Sex hormone-binding globulin
Validation
Clinical

ABSTRACT

Presented are the validation results of a second-generation assay for determining the relative abundances of two protein biomarkers found in maternal serum that predict an individual's risk of spontaneous preterm birth. The sample preparation workflow is complex, consisting of immuno-depletion of high-abundance serum proteins, tryptic digestion of the immuno-depleted fraction to generate surrogate peptide analytes, and detection by tandem mass spectrometry. The method was determined to be robust on observation of the following characteristics: classifier peptide detection precision was excellent; results were accurate when compared to a reference method; results were linear over a clinically relevant range; the limits of quantitation encompassed the range of expected results; and the method demonstrated analytical specificity and resilience to differences in patient serum and common endogenous interferents.
© 2017 The Association for Mass Spectrometry: Applications to the Clinical Lab (MSACL). Published by Elsevier B.V. All rights reserved.

1. Introduction

Preterm birth (PTB), defined as delivery at fewer than 37 weeks of gestation, is the leading cause of mortality and morbidity in neonates [1]. Worldwide, PTB impacts 15 million deliveries annually and results in over one million infant deaths [2,3]. Spontaneous onset of preterm birth (sPTB) represents a high percentage of all PTB cases [4]. Until recently, sPTB lacked an adequate prognostic test. The development of such a test was complicated by the variety of etiologic associations described for sPTB, including infection, inflammation, placental complications, and uterine distension [4]. The complex etiology requires that an effective prognostic test must have the ability to interrogate multiple biological pathways.

Increasingly, proteomics is being used in clinical diagnostic testing as a predictors for a variety of complex diseases and conditions (e.g., spontaneous preterm birth [5], lung cancer [6], therapeutic targeting of breast cancer [7]). These conditions are often characterized by numerous and diverse interconnecting biological pathways, requiring systematic approaches for the development of comprehensive clinical diagnostic tests. Tandem mass spectrometry is not only capable of multiplexing assays, but it can also be rigorously validated on a wide variety of analytes, including proteins [8–13].

We applied a targeted proteomics workflow, coupled with highly multiplexed tandem mass spectrometry detection, to simultaneously monitor peptides from candidate signature proteins and quality control proteins in subsets of clinical study serum samples. Insulin-like growth factor-binding protein 4 (IBP4) and sex hormone-binding globulin (SHBG) were previously shown by our group to perform well as biomarkers for discriminating sPTB from term birth [5]. Additionally, we utilized this technology to develop and clinically validate a bivariate protein biomarker assay for the qualitative prediction of individual risk of spontaneous preterm birth [5].

We previously validated the first-generation assay using an Agilent 6490 and a 30-min liquid chromatography gradient. This method established acceptable measures of analytical validation, including precision, carryover, limit of detection, and analytical specificity, and was used to assay blinded samples during a

* Corresponding author.
E-mail address: cbradford@seraprognostics.com (C. Bradford).

http://dx.doi.org/10.1016/j.clinms.2017.06.002
2376-9998/© 2017 The Association for Mass Spectrometry: Applications to the Clinical Lab (MSACL). Published by Elsevier B.V. All rights reserved.

previously published clinical validation [5] and commercial samples later. As with any new assay, performance requirements change over time, either to improve analytical performance or reduce cost. To increase the laboratory's throughput and to enable integration of a current-generation mass spectrometer, a plan was developed to validate the method after migration to an Agilent 6495 triple quadrupole mass spectrometer with a 15-min liquid chromatography gradient. The validation plan required that this novel second-generation assay demonstrate analytical performance equivalent to that of the previously validated [5] first-generation method, prior to being placed into clinical practice. Validation of the second-generation clinical diagnostic assay for the IBP4 and SHBG signature proteins, which measured precision, alternative method comparison, linearity, limits of quantitation, carryover, analytical specificity, interference, and stability [14], is described herein.

2. Experimental

2.1. Materials

2.1.1. Validation samples and quality control material

This work used samples derived from the Proteomic Assessment of Preterm Risk (PAPR) study, which enrolled 5501 pregnant women from 11 clinical sites and is broadly representative of the US population [15]. Maternal serum was collected from patients between 17 and 28 weeks of gestation for the purposes of developing a second trimester serum-based test predictive of sPTB risk. Women with singleton pregnancies, aged 18 to 60 years, receiving prenatal care and capable of providing consent, were eligible for the study. Subjects pregnant with more than one fetus or those with a known or suspected fetal anomaly were excluded. Of the 5235 women who continued the study, pre-specified exclusions resulted in the removal of 326 subjects with medically-indicated preterm birth, 109 subjects who had been treated with progesterone, and 28 samples with pre-analytical issues (e.g., hemolysis). Of the remaining samples, 413 were used for analytical validation. The PAPR study followed the Good Clinical Practice guidelines issued by the International Conference on Harmonisation [16].

In addition to the PAPR samples, two pools of serum from female donors were purchased from Golden West Biologicals (Temecula, CA). One serum pool (QC1) was created from equal volumes of serum from 10 non-pregnant female donors. A second serum pool (QC2) was created from equal volumes of serum from 21 pregnant donors. The QC pools were a critical part of quality control for each batch, allowing for long-term trend analysis of assay performance. Non-pooled serum from individual donors was also purchased from Golden West Biologicals. Both the pooled materials and the single donor samples consisted of a large number of identical single-use aliquots that were stored in the same conditions as clinical samples (−80 °C). The pooled materials and single-donor samples were collected using the same protocol used in the PAPR study.

Phosphate buffered saline served as a negative control sample and was purchased from Life Technologies (Carlsbad, CA).

2.1.2. Reagents and consumables

Protein Depletion Buffer A and B were supplied by Agilent Technologies (Santa Clara, CA). Acetonitrile and water were LC-MS grade and were purchased from ThermoFisher (Hampton, NH). Methanol was HPLC grade and was purchased from JT Baker. Dithiothreitol (DTT), iodoacetamide (IAA) and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO). A custom order of solubilized Trypsin Gold was purchased from Promega (Madison, WI). Formic acid was purchased from ThermoFisher (Hampton, NH).

High purity stable isotope standards (SIS) were purchased from New England Peptides (Gardner, MA). The SIS were checked for a minimum purity of 95% by HPLC. A mass spectral analysis was also performed and yielded an acceptable measured mass within 0.1% of the calculated average molecular weight. An amino acid analysis was performed to verify the amino acid composition to within 20% of the theoretical concentration for each amino acid and to determine the molar yield of each peptide. Carboxy-terminal lysine and arginine residues of SIS peptides were uniformly labeled with ^{13}C or ^{15}N resulting in either a +8 or +10 amu mass shift, respectively. Individually synthesized peptides were used to create a pool of high purity SIS containing heavy-labeled analogues of signature and quality control peptides monitored in the PreTRM® assay. SIS peptides were used at a final concentration that approximated the abundance of the endogenous peptides, excepting the linearity and limits of quantitation studies.

2.2. Methods

2.2.1. Initial workflow and assay development

Early assay development involved the creation of an MRM assay to perform relative determination of abundances of 242 proteins chosen because of their association in the literature with preterm birth and other pregnancy complications. Public and proprietary databases were used to identify up to five proteotypic peptides per protein based on previous detection in blood. The assay was further refined by supplementation with novel discoveries, recurrent literature curation, and trimming of the assay size using peptide correlation and analytical performance. Mass spectrometer settings were optimized for each peptide to provide the highest signal-to-noise, the highest precision, or a signal free of chromatographic anomalies. Work flow optimization considered both serum and plasma blood fractions, protein depletion strategies and materials, tryptic digestion conditions, solid phase extraction-based desalting methods, LC-MS/MS gradient separation, and HPLC column performance. Initial assay development utilized separate injections of synthetic unlabeled peptides to confirm analyte identity. Later phases of assay development utilized sample fortification with high purity heavy-labeled peptide standards to confirm analytical specificity.

2.2.2. Batch design

Except for the batch runs to determine linearity and limits of quantitation, a standardized plate batch design was adopted and used for this work. The batch design utilized replicates of the two serum quality control pools (i.e., QC1 and QC2). Two terminal replicates of phosphate buffered saline, named Process Blanks, served to monitor for routine carryover and cross contamination. A maximum of 24 clinical samples were assayed in a single batch, as shown in Table 1, representing a standardized batch design; run order was top to bottom, column 1 to column 4.

2.2.3. Protein depletion chromatography

Clinical serum samples and pooled serum quality control materials were retrieved from −80 °C storage and allowed to thaw while resting on crushed wet ice. Once thawed and mixed by inverting three times, 50 μL of a serum sample was added to a well of a 0.2 μm polypropylene filter plate (Captiva Filter Plate, Agilent Technologies, Santa Clara, CA) containing 150 μL of Protein Depletion Buffer A. The plate was mixed on a plate vortexer for approximately 30 s and then vacuum filtered into a polypropylene deep 96-well plate.

The sample filtrates were depleted of 14 high abundance proteins using a protein depletion system based on Agilent 1260 liquid

Download English Version:

https://daneshyari.com/en/article/8917897

Download Persian Version:

https://daneshyari.com/article/8917897

Daneshyari.com