Search for Amniotic Fluid-Specific Markers: Novel Biomarker Candidates for Amniotic Fluid Embolism

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Abstract: Objective: Amniotic fluid embolism (AFE) is a catastrophic syndrome. The amniotic fluid (AF)-specific antigens might be assessed in maternal serum when these proteins abruptly enter maternal circulation. The aims of this study were 1) to review a conventional marker for diagnosis of AFE, and 2) to find AF-specific proteins.

Study design: This article reviews the English language literature for identification of proteins specifically or exclusively present in AF. The genome-wide gene expression profiling studies and proteomics-based approaches have been reported to identify the AF-specific proteins.

Results: Maternal serum sialosyl Tn (STN), zinc-coproporphyrin-1 (ZnCP-1), tryptase and complement activation are clinically used as biomarkers for detecting AFE. However, these tests are quite limited. With advances in proteomics technology, together with the considerable efforts to find novel diagnostic biomarkers, many candidate proteins have been discovered and reported. Among 44 candidate markers identified in the present review, interleukin(IL)-6, squamous cell carcinoma (SCC), insulin-like growth factor-binding protein (IGFBP)-1, CA125 and osteopontin may be unique AF-specific markers.

Conclusion: This paper reviews recent advances in proteomics-based technology providing a significant resource for AFE research and a framework for biomarker discovery. Further research is needed to evaluate the potential functional biomarkers for the diagnosis of AFE.

Keywords: Amniotic fluid embolism, Biomarker, Proteomics, Squamous cell carcinoma antigen.

INTRODUCTION

Amniotic fluid (AF) protects the fetus from mechanical stress, possesses anti-microbial action, and contains nutritional factors and growth factors. The proteins and peptides present in AF enter the maternal circulation during pregnancy, particularly during labor. At least there are three possible sources for elevation of these proteins in AF; AF cells, placentas and fetal membranes, as well as urine and meconium from the fetus. During the pregnancy, AF cells produce a number of factors, including cytokines, lipids, prostaglandins and growth factors [1]. Human placenta, decidua, and fetal membranes are the major sites of production and secretion of many substances in maternal serum (MS), AF, and umbilical cord blood. Thousands of proteins are secreted into AF.

Amniotic fluid embolism (AFE) is a rare and serious condition that occurs during labor and delivery. It occurs in 7.7 per 100,000 deliveries and has a case fatality rate of 22% [2]. The diagnosis of AFE so far is based on the clinical criteria, including sudden onset of cardiovascular collapse, hypoxia, sustained tachycardia, disseminated intravascular coagulation, and absence of other illnesses that could explain the signs and symptoms [3]. Although the early pathogenesis of AFE is not understood, the entrance of AF into the maternal systemic circulation might lead to an initial phase of AFE. Accurate and early diagnosis of AFE would facilitate timelier and more appropriate interventions.

The AF-specific antigens might be assessed in MS when these proteins abruptly enter maternal circulation. These markers can shed light on additional criteria for the diagnosis of AFE. The genome-wide gene expression profiling studies and proteomics-based approaches have been used to identify specific markers and diagnostic profiles for this disorder [1,4-8]. Several investigators used surface-enhanced laser desorption ionization-time of flight-mass spectrometry (SELDI-TOF-MS) and matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) to characterize the AF-specific proteins [9]. Integrated computational analysis of the AF proteome combined with several recently published proteomic data sets of maternal serum/plasma results in a list of several putative biomarkers. The AF contains more than 1000 unique gene sequences that correspond to approximately 850 distinct proteins [10]. Proteomic analysis of AF can serve as a valuable tool in the search for biomarkers of AFE.

The aims of this study were 1) to review a conventional marker for diagnosis of AFE, and 2) to find AF-specific proteins.

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1874-2912/11

MATERIALS AND METHODS

A Conventional Test to Establish the Diagnosis of AFE

For studies that reported data on the obstetric disorders, only data pertaining to AFE were included. A computerized literature search was performed to identify relevant studies reported in the English language. MEDLINE updates were conducted monthly, and all abstracts were reviewed by 2 investigators (K.N. and H.K.) to identify papers for full-text review. We searched PubMed MEDLINE electronic databases (http://www.ncbi.nlm.nih.gov/sites/entrez) published until September 2010, combining the keywords "amniotic fluid embolism" "marker" and "diagnosis". Each gene is also linked to NCBI Entrez Gene pages (http://www.ncbi.nlm.nih.gov/sites/entrez). Additionally, references in each article were searched to identify potentially missed studies. A priori, case reports and abstracts were not included, since abstracts do not undergo a stringent peer review process. A difficulty in interpreting these literatures is that analyses, results and objects (fatal or nonfatal) are reported differently among the studies. Here, we discuss promising molecular candidates.

Identification of Amniotic Fluid-Specific Antigens (Proteins Present in Amniotic Fluid at Concentrations Extremely Higher than those in the Maternal Serum)

We searched MEDLINE databases, combining the keywords "amniotic fluid embolism" "amniotic fluid" "genome-wide" "proteomics" "mass spectrometry" with specific expression profiles of gene products in AF. This review includes proteins identified as being specifically present in AF (proteins present in AF at concentrations extremely higher than those in the maternal serum (MS) or not present in MS by proteomics-based approach). The proteins and peptides can be classified into several groups.

We discuss the conventional marker for AFE diagnosis and novel biomarker candidates. Optimization of advanced bioinformatics approaches may yield informative biomarker signatures discriminating women with AFE from the related disease.

RESULTS

Article Selection, Data Extraction and Assessment

As the main interest is AFE obtained from human samples, we have not yet included animal model alone in the knowledgebase. However, we included the animal studied performed to support clinical data. Initially, 126 potentially relevant studies were identified by screening electronic databases. 41 peer-reviewed journal articles were additionally identified from references in each article.

Conventional Diagnostic Tests for AFE

The diagnosis of AFE is currently symptom-based. Clinical findings include sudden onset of acute respiratory distress, circulatory distress and fulminant DIC. The diagnosis is based on unfortunate post-mortem pathological investigations. AFE can also be diagnosed by histological and immunological confirmation of amniotic fluid contents and fetal debris in the pulmonary vasculature, although, in some cases, fetal materials could not be found in pathologic staining. The serum markers are unreliable, and their detection generally requires a long time intervals for result. Notwithstanding these limitations, there are at least two ways to diagnose AFE: 1) measurement of amniotic fluid contents and fetal materials in the maternal circulation, and 2) determination of specific markers that can diagnose a subset of patients who have an immunological reaction which is activated by products of the amniotic fluid that enter the maternal circulation.

Firstly, AFE is believed to occur when the constituents of AF enter the maternal circulation. The diagnosis of AFE remains a clinical challenge, but can be supported by the presence of fetal components and amniotic cells in the pulmonary artery, aspirated through a pulmonary artery catheter. Therefore, amniotic fluid contents and fetal materials detected in the maternal circulation appear to be a marker of AFE.

A reliable and useful biomarker must i) come from a readily attainable source, such as maternal blood and urine, ii) provide a rich source for investigation, iii) release into the AF, but not into the maternal circulation in normal pregnancy, iv) have significantly increased AF/MS ratio from the 2^{nd} to the 3^{rd} trimester, v) have sufficient sensitivity to correctly identify affected individuals, vi) have sufficient specificity to avoid incorrect labeling of unaffected women, and vii) result in a notable benefit for the patient through intervention, such as survival or life quality improvement.

Different methods based on clinical evaluation and biological tests have been developed to diagnose AFE. Many investigators have tried to identify proteins present in AF at concentrations extremely higher than those in the MS or not present in MS to provide novel biomarker candidates for AFE. The AF-specific proteins would be used as a routine test in clinical laboratory practice. Clinical laboratory tests used for identification of AFE in Japan at present include MS zinc-coproporphyrin-1 (ZnCP-1) and Sialosyl Tn (STN) tests [11,12].

The ZnCP-1 is a characteristic component of fetal urine and meconium [13,14]. Therefore, measuring ZnCP-1 in maternal plasma by fluorometry on high performance liquid chromatography (HPLC) may be a noninvasive and sensitive, but not a rapid, method for diagnosing AFE [12]. Usta *et al.*, reported that MS ZnCP-1 might be a promising test for prediction of intrauterine passage of meconium in selected patients [15]. As expected, plasma ZnCP-1 levels were significantly elevated in patients with AFE [11-13]. However, ZnCP-1 levels could not be a prognostic fatality factor. Further efforts are needed to validate ZnCP-1 as a potential diagnostic marker in patients with AFE.

Moreover, the sialosyl Tn structure (NeuAc alpha 2-6GalNAc alpha 1-O-Ser/Thr, STN) is also a characteristic component in meconium- and amniotic fluid-derived mucin [16]. The method for detecting STN antigen in the serum of patients with AFE is a direct way to demonstrate the release of mucin into the maternal circulation and is also a simple and sensitive method for diagnosis of this disorder. Oi *et al.*, have recently identified factors leading to fatality of patients with AFE, demonstrating that serum STN levels could be a possible prognostic fatality factor [17]. Furthermore, TKH-2 is the specific antibody clearly directed to STN and reacts with meconium- and amniotic fluid-derived mucin-type glycoprotein. Therefore, TKH-2 immunostaining is the sensitive

method to detect mucin in the lung sections of patients with AFE at autopsy [18]. Although knowledge about STN levels in survivors is scarce, STN has been found to be a sensitive marker of AFE in fatal cases [11]. Although the diagnosis of AFE relies on both tests that were incorporated into clinical practice one decade ago in Japan [11,12], these available noninvasive diagnostic tests have limited predictive value.

Furthermore, the preliminary results by Van Cortenbosch *et al*., pointed out the interest to measure maternal serum alpha-fetoprotein (AFP), insulin-like growth factor binding protein-1 (IGFBP-1) and fetal fibronectin (fFN) to confirm AFE [19]. Even in healthy pregnant women, however, the vascular lumen in the uterine myometrium contains amniotic squamous cells and mucin material during labor [20]. Obstetricians need to be aware of the sensitivity and specificity of these markers associated with AFE.

Secondly, the immunologic mechanisms have been studied to date. Several substances that are activated by products of the amniotic fluid that enter the maternal circulation could explain the symptoms that are present in AFE. Several investigators suggested that AFE is an anaphylactoid reaction to fetal antigens [21]. With a complex pathophysiology, AFE might actually lead to anaphylaxis to fetal material leaking into the maternal circulation. Allergic anaphylaxis may be inseparable from AFE in terms of the clinical presentation. If this disorder is a type I hypersensitivity reaction, serum tryptase and urinary histamine levels may therefore serve as a marker of mast cell degranulation in AFE cases [22,23]. An increase of pulmonary mast cells was observed in the subjects who died of AFE [24]. Elevated serum tryptase have been reported in some cases of AFE [25-27], but cases have also been described where serum tryptase level has remained normal [28,29].

Finally, other groups reported that anaphylaxis reaction appears to be doubtful while accumulating evidence supports a complement activation as an element of its pathophysiology [23,30,31]. AFE patients had abnormally low levels of complement, C3 and C4, suggesting a role for complement activation in the mechanism of AFE [23,30,31]. The complement system undergoes activation as a main column of innate immunity and the coagulation system. AFE may occur as the result of complement activation initiated by fetal antigen leaking into the maternal circulation [23]. Since meconium-derived serine proteases belonging to the coagulation system are able to activate the complement cascade, the transient decreases in serum complement levels cannot be used diagnostically per se.

Taken together, new diagnostic markers, other than ZnCP-1, STN, tryptase, or complements, are needed for the early prediction of AFE.

A Search for AF-Specific Markers

A rational approach to diagnose AFE is to identify the AF-specific proteins/peptides. For this, we have to identify gene products that are specifically present only in AF but not present in the MS or proteins that are present in AF at concentrations extremely higher than those in the MS. The previously available markers for AFE such as ZnCP-1 and STN do not completely fit this definition. The release of AF-specific proteins into the maternal circulation is suitable for diagnosis of AFE.

The MEDLINE was searched for English-language articles, relating to AF-specific proteins. Biomarker discovery is one of the newly emerging innovations in the early diagnosis of AFE. Many technologies, including genomics and proteomics, are used to identify biomarkers. With advances in proteomics technology, together with the considerable efforts to find novel diagnostic biomarkers, many candidates have been discovered and reported (Table **1**). Enriched protein functions were tumor markers, cell proliferation and embryonic development, metabolism, nervous system, cytokines, immune or complement processes, signaling, cell adhesion and motility, hormones, detoxification system, and metal carrier.

Lists of AF-Specific Proteins

Proteins Associated with Tumor Markers

After our search, eight tumor markers are suggested to be unique to AF; Sialosyl Tn (STN), squamous cell carcinoma (SCC) antigen, carcinoembryonic antigen (CEA), CA125, mucin-like carcinoma-associated antigen (MCA), prostate-specific antigen (PSA), tissue polypeptide specific antigen (TPS), and breast carcinoma amplified sequence 1 (BCAS1).

Sialosyl Tn (STN)

STN is a characteristic component in meconium- and/or amniotic fluid-derived mucin. MS STN antigen levels in women with meconium-stained AF (20.3 ± 15.4 U/ml) at term delivery were higher than those in women with clear AF (11.8 ± 5.6 U/ml). It has been reported that the MS STN levels (mean ± SEM) in patients with AFE (110.8 ± 48.1 U/ml) showed significantly higher concentrations compared with those of patients with non-AFE (17.3 ± 2.6 U/ml) (11). Seventeen of 19 sera (89%) were recognized as AFE by MS STN level. Remarkable positive TKH-2 immunostaining was easily seen within the pulmonary vasculature in 14 of the 15 (93%) patients with AFE. The method for detecting STN antigen in the MS of patients with AFE is a direct way to demonstrate the release of meconium- or AF-derived mucin into the maternal circulation and is a simple, noninvasive, sensitive method for diagnosis of AFE [16]. In addition, TKH-2 immunostaining in the affected lung is a sensitive method to diagnose AFE patients [18].

Squamous Cell Carcinoma (SCC)

SCC antigen is a tumor-associated protein of squamous cell carcinoma of various organs [32]. SCC may be released from fetal epidermis. Extremely high antigen levels were found in AF samples (median, 710 ng/ml) compared to MS (1.7 ng/ml) [13,14]. AF/MS ratio = 400.

Carcinoembryonic Antigen (CEA)

CEA values in MS were below cut-off (< 5 ng/ml). CEA is independent of gestation. Very high antigen levels were found in AF samples (median, 124 ng/ml) compared to MS (0.6 ng/ml). AF CEA with meconium had higher values [14]. AF/MS ratio = 200.

CA125

CA125 is an oncofetal antigen and expressed by coelomic epithelium of fetal tissues. High antigen levels were found in AF samples (median, 700 U/ml) compared to MS (6 U/ml). It is likely that the amnion cell is a major

Table 1. AF-Specific Biomarker: Possible Candidate Proteins to Diagnose AFE

AF/MS Ratio	Target Proteins	Protein Ontology Functions
500	IL-6	Cytokines
450	PINP	Metabolism
400	SCC	Tumor Markers
200	CEA	Tumor Markers
150	IGFBP-1	Embryonic Development
100	CA125	Tumor Markers
100	MCA	Tumor Markers
50	BNP	Nervous System
5-50	SOD	Detoxification
20-40	PSA	Tumor Markers
10-20	TPS	Tumor Markers
10-20	IL-8	Cytokines
10	POMC	Nervous System
10	hCGbeta	Hormones
5	ActivinA	Hormones
4	PRL	Hormones
3	sTNFp55	Cytokines
2.5	CgA	Nervous System
2-3	AFP	Hormones
unknown*	BCAS1	Tumor Markers
unknown*	OPN	Embryonic Development
unknown*	Amiloride-sensitive amine oxidase	Embryonic Development
unknown*	Transcriptional regulator ATRX	Embryonic Development
unknown*	Ras GTPase-activating protein 3	Embryonic Development
unknown*	RBM19	Embryonic Development
unknown*	PLAP	Embryonic Development
unknown*	Annexin I	Metabolism
unknown*	Myosin Id	Nervous System
unknown*	Pn-1	Nervous System
unknown*	Agrin	Nervous System
unknown*	CD59	Immune or Complement
unknown*	MDR/TAP	Immune or Complement
unknown*	PAEP	Immune or Complement
unknown*	Keratin, type I cytoskeletal 9	Signaling
unknown*	PKDREJ	Signaling
unknown*	fFN	Cell Adhesion and Motility
unknown*	Perlecan	Cell Adhesion and Motility

(Table 1). Contd…..

AF/MS Ratio	Target Proteins	Protein Ontology Functions
unknown*	Mesothelin precursor	Cell Adhesion and Motility
unknown*	Dynein heavy chain	Cell Adhesion and Motility
unknown*	MAGUK p55 subfamily member 5	Cell Adhesion and Motility
unknown*	Protocadherin 16 precursor	Cell Adhesion and Motility
unknown*	TTR	Detoxificatio
The commercially available markers for prediction of AFE		
10-100<	STN	Tumor Markers
20-100<	ZnCP-1	Metal Carrier

The proteins were exclusively present in AF and not detected in serum/plasma by proteomics-based approach. The expression of proteins up-regulated in AF tightly links to tumor markers, hormones, neuropeptides, embryonic development, adhesion and motility, immune and complement system, and metabolism. AF, amniotic fluid; and MS, maternal serum/plasma. *, no information of its concentrations in AF and MS in human pregnancy is available so far.

source of CA125 in AF [13,14]. Pregnancy has an influence on MS CA125. 10% of MS CA125 values were above cut-off (<35 U/ml). AF/MS ratio = 100.

Mucin-Like Carcinoma-Associated Antigen (MCA)

MS MCA values increased significantly with gestational age, being higher in the 3rd trimester and in labor than in control values [33]. AF/MS ratio = ~100.

Prostate-Specific Antigen (PSA)

Pregnant women had significantly higher MS PSA values than non-pregnant women. AF PSA values at term were higher by a factor of 20-40 [34].

Tissue Polypeptide Specific Antigen (TPS)

MS TPS values increased significantly with gestational age, being significantly higher in the 3rd trimester (180-200 U/L) and during labor (800-1000 U/L) than those in the controls (< 130 U/L) [35]. AF TPS values (greater than 10000 U/L) were markedly elevated, compared with those in MS. AF/MS ratio = 10-20.

Breast Carcinoma Amplified Sequence 1 (BCAS1)

BCAS1, a candidate oncogene, was amplified in a variety of tumor types and associated with more aggressive tumor phenotypes [36]. BCAS1 expression is limited in brain and prostate. Although the proteomics-based approach has demonstrated that this protein was exclusively present in AF and not in MS [7], no information of its concentration in AF in human pregnancy is available so far.

Proteins Associated with Cell Proliferation and Embryonic Development.

The following seven proteins, including insulin-like growth factor binding protein 1 (IGFBP-1), osteopontin precursor (OPN), amiloride-sensitive amine oxidase [copper-containing] precursor, transcriptional regulator ATRX, ras GTPase-activating protein 3, probable RNA-binding protein KIAA0682 (RBM19), and alkaline phosphatase placental like (PLAP), are unique to AF.

Insulin-Like Growth Factor Binding Protein 1 (IGFBP-1)

IGFBP-1 is produced in decidua and exists in high concentration in AF and MS. The median IGFBP-1 levels in non-pregnant adult serum were 18 ng/ml. Extremely high levels were found in AF samples (mean, 13000 ng/ml) compared to MS (75 ng/ml) [37]. AF/MS ratio = 150.

Other proteins (OPN [38], Amiloride-sensitive amine oxidase precursor [39], ATRX, Ras GTPase-activating protein 3 [40], RBM19 [41], and PLAP [42]) are exclusively present in AF and not in MS as described by proteomics-based approach; however, little information is available on the AF and MS concentrations of these proteins so far. Human amniotic membranes appear to be an origin of osteopontin.

Proteins Associated with Metabolism

Three proteins associated with metabolism are specifically present in AF: Procollagen type I N-terminal propeptide (PINP), Pro-early placenta insulin-like peptide (Pro-EPIL), and Annexin I. There are no reports on the annexin I concentrations in AF and MS [7,43].

Procollagen Type I N-Terminal Propeptide (PINP)

The concentration of the 2nd trimester AF was determined to ~25000 ng/ml. The normal range for MS PINP was 56 ng/ml (median) [44]. AF/MS ratio = 450. PIPN is considered to be associated with fetal development and metabolism.

Pro-Early Placenta Insulin-Like Peptide (Pro-EPIL)

Pro-EPIL, a new member of the insulin-related gene family, was detected in placenta. The concentration of the 2nd trimester AF was high (137 ng/ml, mean) [45]. The mean MS PINP value was 13 ng/ml. AF/MS ratio = 10.

Proteins Involved in Nervous System

Placenta produces neuro-endocrine markers. In total, six unique proteins involved in nervous system were identified: brain natriuretic peptide (BNP), pro-opiomelanocortin (POMC), chromogranin A (CgA), myosin Id, glia derived nexin precursor (Pn-1), and agrin. No information of the concentrations of myosin Id, Pn-1 and agrin in AF and MS in human pregnancy is available so far.

Brain Natriuretic Peptide (BNP)

The AF BNP level was much higher (10- to 100-fold) than those in third trimester MS (2-3 pmol/L) [46], demonstrating the AF/MS ratio is 50.

Pro-Opiomelanocortin (POMC)

POMC was present in very high levels in AF (mean, 3400 U/ml). The MS POMC become detectable by the 8th week of pregnancy and reached its maximum at around 20th week, remaining stable thereafter (mean, 310 U/ml) [47]. AF/MS ratio = 10.

Chromogranin A (CgA)

Median CgA level in MS at term tended to be higher (490 pmol/L) than at the 1st trimester (286 pmol/L) or in sera from nonpregnant women (306 pmol/L). In AF, median CgA value was significantly higher at term (1163 pmol/L) than at 2nd trimester (551 pmol/L) [48]. AF/MS ratio = 2.5.

Cytokines

Three unique cytokines were identified predominantly in AF: interleukin-6 (IL-6), IL-8, and tumor necrosis factor-alpha-soluble receptor p55 (sTNFp55).

Interleukin-6 (IL-6)

The origin of IL-6 may be the extra-placental gestational membranes. IL-6 is a useful marker for predicting preterm birth [4]. High IL-6 levels were found in AF samples (median, 1500-2000 pg/ml) [49] compared to MS (~3 pg/ml) [50]. Pregnancy and labor have an influence on IL-6. AF/MS ratio = 500.

IL-8

In AF, IL-8 is not detectable during the second trimester or at term not in labor but is present in significant amounts at preterm and term labor [51]. High IL-8 levels were found in AF samples at term labor (mean, 500-700 pg/ml) [52] compared to MS (30-40 pg/ml). AF/MS ratio = 10-20.

Tumor Necrosis Factor-Alpha-Soluble Receptor p55 (sTNFRp55; also known as sTNF-R1)

sTNFRp55 concentrations increased in AF from the 1st to the 2nd trimester. There was a decrease in this antigen at term (2000-3000 pg/ml [53]). The normal range for MS sTNFp55 at term was 626 pg/ml (mean) [54]. AF/MS ratio = 3.

Proteins Involved in Immune or Complement Processes

Three proteins specifically involved in immune or complement processes are unique to AF: CD59 glycoprotein precursor (CD59) [7,55], antigen peptide transporter 2 (MDR/TAP) [56], and pregnancy-associated endometrial alpha2 globulin (PAEP) [7]. No information of their concentrations in AF and MS in human pregnancy is available so far.

Proteins Involved in Signaling

A comprehensive survey of the proteomic technology showed that two proteins involved in cell signaling (keratin type I cytoskeletal 9 [7,57] and polycystic kidney disease and receptor for egg jelly related protein precursor (PKDREJ) [7,58]) occur in AF but not in MS. There is no information about the AF and MS levels of both markers during pregnancy.

Proteins Associated with Cell Adhesion and Motility

The data set provides a foundation for evaluation of the following proteins associated with cell adhesion and motility as markers for AF: fetal fibronectin (fFN) [19,59], perlecan [60], mesothelin precursor [61], dynein heavy chain, cytosolic (DHCs) [7], MAGUK p55 subfamily member 5 (MPP5) [7], protocadherin 16 precursor [7,62]. There are no reports on the concentrations in AF and MS.

Proteins or Peptides Associated with Hormones

Amniotic fluid is a rich source of peptides associated with hormones. hCG, hPL and SP1 in MS were higher than in AF, while AF values of hCGbeta, AFP and PRL were higher than in MS, but the ratio AF/MS of all hormone values decreased significantly from the 2nd to the 3rd trimester. The hCGbeta concentration of the 2nd trimester AF was determined to 165 ng/ml (mean). The normal range for MS hCGbeta was 17 ng/ml (mean). AF/MS ratio = 10. Both fetal and AF AFP levels decline in a parallel fashion throughout pregnancy, whereas MS AFP rises to a peak at 28 to 30 weeks and declines thereafter [63]. AF/MS ratio at term = 2-3. Furthermore, the AF PRL level was 1000 ng/ml (mean), significantly higher than that of the MS (250 ng/ml) [64]. AF/MS ratio = 4.

Activin

Second-trimester MS and AF levels of activin A increased with gestational age. Activin A levels in MS and AF ranged from 0.66 to 4.33 ng/ml and from 0.72 to 29.19 ng/ml, respectively, in unaffected pregnancies [65,66]. AF/MS ratio = 5.

Proteins Associated with Detoxification System

Third trimester AF may provide a rich source for proteins associated with detoxification enzymes: superoxide dismutase (SOD) [67,68] and oxidized transthyretin (TTR) [4,69]. There have been no reports on oxidized TTR levels in the AF and MS.

Superoxide Dismutase (SOD)

There is a large biological variance of the SOD concentrations in normal pregnancies (range for AF 10-150 U/ml) [67]. The normal range for MS SOD was 2.2 U/ml (median) [68]. AF/MS ratio = 5- 50.

Metal Carrier

Zinc-Coproporphyrin-1 (ZnCP-1)

Plasma ZnCP-1 might be a promising test for prediction of intrauterine passage of meconium in high-risk patients [15]. The plasma ZnCP-1 concentration was 97 nmol/l in the AFE patients, 11 nmol/l in the non-AFE patients, 12 nmol/l during normal pregnancy, and 26 nmol/l shortly after normal delivery. Furthermore, mean AF ZnCP-1 was significantly higher in the meconium-stained AF as compared to the clear AF group. Measuring ZnCP-1 in MS by fluorometry on HPLC is a noninvasive and sensitive method for diagnosing AFE [12].

COMMENT

Amniotic fluid constitutes a potential rich source of biomarkers for diagnosis of maternal and fetal disorders. We initially performed a comprehensive literature survey of the proteins specifically expressed in AF. Two antigens, ZnCP-1 and STN, preferentially overexpressed in AF have been identified so far. Therefore, both tests are commonly used

for the prediction of AFE in Japan [11,12,18]. Despite the existence of both tests on this subject, their efficacy remains very far from what we wish. At present, there is no clear evidence to support the use of STN and ZnCP-1 for the early diagnosis of AFE. A downside of ZnCP-1- and STN-based diagnosis is their low sensitivities and specificities. Notwithstanding these limitations, STN levels could be a possible prognostic fatality factor [17]. Further research is required to address the contradictory findings of diagnostic accuracy. However, there is no opportunity for future large-scale studies on the usefulness of these markers since AFE is a rare obstetric catastrophe.

The second goal of this study was to identify the most robustly detected AF-specific proteins. The search for new and improved biomarkers for AFE will continue. We review the published articles and relevant bibliographies following a systematic search of MEDLINE for English language articles. We analyzed changes in expression of gene products in humans only. Many investigators have performed genome-wide and proteomics analyses, including Northern blot, comparative proteomic-based technology such as SELDI-TOF-MS, MALDI-TOF-MS, Western blot, immunohistochemistry, and ELISA on biological fluids taken from pregnant women. Proteins specifically found in human AF, but not detected in MS, might play a role as putative AFE diagnostic biomarkers.

This review summarizes our current knowledge regarding the AF-specific proteins. Proteomic analysis of AF may provide an opportunity for early recognition of AFE. This methodology may in the future identify candidates for not only specific diagnostic markers but also therapeutic interventions. In total, 44 unique proteins and peptides have been recognized. The entire set is available in Table **1**. Systematic analysis allowed their further grouping into functional categories such as tumor markers, cell growth, embryonic development and metabolism. Some of the proteins reviewed have previously been reported as the AF-enriched proteins in small-scale analyses of human samples. These results point out the potential interest to assay biomarkers to confirm AFE using rapid laboratory tests. These proteins will be evaluated as being specifically present in AF by the future study. Among the 44 proteins, one may select five antigens that can be assessed by commercially available ELISA kits: interleukin (IL)-6, squamous cell carcinoma (SCC), osteopontin, CA125, and insulin-like growth factor-binding protein (IGFBP)-1. These markers might exhibit the higher AF/MS ratio.

At present, we have been examining if these proteins indeed present in AF at concentrations extremely higher than those in the MS. So far, we have no clinical data demonstrating that these markers work well to diagnose AFE. The next purpose is to determine whether AFE could be detected by quantification of these antigens in maternal serum.

In conclusion, we present a panel of 44 proteins, robustly detected in AF, possibly including novel biomarkers for future AFE investigation.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author indicated a financial interest.

Research Funds

Hiroshi Kobayashi and Katsuhiko Naruse, Alfresa Pharma Corporation, Employment: N/A, Leadership: N/A, Consultant: N/A, Stock: N/A, Honoraria: N/A, Testimony: N/A, and Other: N/A.

ACKNOWLEDGEMENTS

Grant Support

Supported by Grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan to the Department of Obstetrics and Gynecology, Nara Medical University (H. Kobayashi, H Oi and K Naruse); and by Grant from Alfresa-pharma Corporation (H. Kobayashi and K. Naruse).

CONDENSATION

This review article summarizes recent advances in proteomics-based technology providing a significant resource for amniotic fluid embolism research and a framework for biomarker discovery.

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Received: August 06, 2011 Revised: October 06, 2011 Accepted: October 24, 2011