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Preterm Intraamniotic Infection and Inflammation: Search for Protein Biomarkers via Proteomics Approach

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ABSTRACT

Preterm delivery (PTD) is commonly caused by intraamniotic infection and inflammation (IAI) and is one of the leading causes of maternal and neonatal morbidity or mortality worldwide. IAI in pregnant women with high risk of PTD can be classified according to the membrane status; preterm labour with intact membranes (PLIM) and preterm pre-labour rupture of membranes (PPROM). At present, the treatment for PTD-associated IAI is principally based on antibiotic therapy. However, this therapy is found to be only beneficial to PPROM. Several IAI related protein biomarkers have been identified. Unfortunately, most of the studies that discovered the biomarkers focused on categorising term delivery and PTD-associated IAI, and to a lesser extent on the membrane status. Therefore, identifying a panel of highly sensitive and specific protein biomarkers that are able to discriminate IAI pregnant women at high risk of PTD with PLIM from PPROM are urgently needed to improve the treatment benefits. Over the past decade, proteomics technologies have been applied widely in identifying PTD-associated IAI biomarkers. This review brings together information on PTD-associated IAI protein biomarkers and touches on the combination of multivariable biomarkers to develop a more sensitive and specific biomarkers to discriminate PTD-associated IAI with PLIM from PPROM. Proteomics technologies and its workflow on protein biomarkers discovery are also included in this review.

INTRODUCTION

Intraamniotic infection and inflammation (IAI) contributes a significant healthcare burden to women with spontaneous preterm labour (PTL) and preterm delivery (PTD). The majority of IAI cases leading to PTD are caused by infections of the amnion, chorion, amniotic fluid (AF) and/or placenta [1-4]. However, maternal nutritional status [4], past obstetric history [4], psychological factors [5], pregnancy factors [6], medical condition [7,8], genetic factors [9], hostile behaviours [4,10], multiple pregnancies [11], social and demographic factors as well as unidentified factor [6,12-14] also contributes to PTD worldwide but to a lesser extent.

Spontaneous PTD-associated infections causes over 65-85% of PTD [4]. The most common pathway leading to IAI is the migration of pathogens from the vaginal tract upwards to the cervix, while transplacental infection, anterograde/retrograde infection from the fallopian tubes or iatrogenic introduction takes place to a lesser extent [15-18]. Vaginal flora migrates from the vagina upwards to the cervix via several pathways. Selected vaginal microflora are able to migrate from the vagina to the cervix via ascending mechanisms [19,20] or are attached

to transport vectors (spermatozoids or trichomonas) [21,22] and penetrate through a series of cervical barriers [23]. Eventually, the vaginal microflora spread into the intrauterine cavity [24,25] regardless of fully intact membranes with closed cervix [26,27]. Moreover, cervical ripening process might accelerate the passage of vaginal microflora [28-30].

Bacteria culture is used as the standard method to diagnose IAI in PTL. This technique is very time consuming and lacks the sensitivity and specificity in determining low concentrations of viable microorganisms that are very difficult to culture [31]. Technologies, such as polymerase chain reaction (PCR), have been used to detect bacteria presence in AF and enable the detection of difficult to culture and macrophage-associated microorganisms [32]. Despite the advances in diagnostic techniques, the rate of IAI-caused spontaneous PTL and PTD remains unchanged. This might be due to the absence of clinical signs and symptoms following initial infections [33], difficulties in isolating the fastidious pathogens [17] and/or the lack of treatment benefits [34]. Studies have shown that broad spectrum antibiotic treatments are beneficial to IAI-associated PTD with preterm premature rupture of membranes (PPROM) [35,36], but not with PTL with intact membranes (PLIM) [37,38]. Therefore, it is crucial to distinguish the membrane status (PPROM or PLIM) of women suffering from IAI leading to PTD for treatment benefits.

Omic technologies have also been applied to search for potential inflammation and infection biomarkers which might aid rapid diagnostic tests applicable in the clinical setting. Initially, genomic and transcriptomic are used to discover the biomarkers of IAI. Due to the fact that these omic technologies only study the expression of all genes and mRNA within a cell, therefore they only offer a summary of gene activities without post-translational modifications (PTMs) [39,40]. Later, novel IAI biomarkers search is dominated by proteomics. Proteomics enables the study of protein structure and function within a cell or system, which provides more meaningful information between the gene sequence and cellular environment [41], which is more likely to be responsible for infection and inflammation.

This review provides an overview of the discovery of IAI protein biomarkers as well as proteomics technologies workflow that are being utilised for detection of biomarkers.

Diagnostic Specimens for IAI Biomarkers Identification

Since IAI is an acute inflammation of the AF, membrane, placenta or a combination [42], its infection sites are often surrounded by cytokines and chemokines [43-45]. Researchers had discovered many potential cytokine and chemokine biomarkers using proteomics-based approaches [46-48]. However, only a few have been validated in the clinical research setting to classify the causes of PTD for optimal management [49-51].

Numerous diagnostic specimens can be applied as sources of biomarker discoveries in predicting PTL-associated IAI, for example, serum, vaginal fluid, saliva, urine, AF and placentary tissues. AF and placentary tissues are the more promising specimens, since they are the primary representation of the inflammatory microenvironment leading to PTL and/or embryonic abnormalities [52]. It was shown that proteins involved in IAI are present at the highest concentration at/near the infected or inflamed area [53]. Several studies showed that AF is rich in proteins that are involved in the mechanism of inflammation responsible for PTL. These proteins enable the characterisation of intraamniotic inflammation [54-56], determination of inflammatory response intensity [57] and to differentiate between inflammation caused by intrauterine bleeding and secondary bacterial infection [58]. Placentary tissues offer a better source for a more accurate diagnosis when low pathogenicity microbes such as *Ureaplasma* spp. may form choriodecidual colonisation, and infect without involving in inflammatory responses [59]. Therefore, identified protein biomarkers from AF and placentary tissues, which might also present in vaginal fluid and serum will allow the development of more sensitive and specific biomarkers in predicting PTL-associated IAI.

Cytokines and Chemokines

Bacterial infection in maternal and foetal tissues causes the release of endotoxins and exotoxins that triggers the production of cytokines and chemokines [29,60-64], making cytokines and chemokines the central topic in searching for protein biomarkers [65]. Furthermore, pregnant women with IAI leading to spontaneous PTL are commonly caused by intraamniotic inflammation and to a lesser extent by intraamniotic infection [43,66,67]. Several studies have reported the coordination of the immune and inflammatory responses by cytokines and chemokines to resolve infections of bacteria and viruses by regulating further defences, suggesting their participation in the intraamniotic inflammatory process [68-70].

Interleukins (IL) and C-reactive protein (CRP)

Some of these early pro-inflammatory response markers have been evaluated as protein biomarkers present in AF, vaginal fluid and serum of women at high and low risk for IAI [71,72]. IL-6 is one of the most thoroughly studied early pro-inflammatory cytokines in relation to IAI regardless of its clinical subtype, and it is also used to predict delivery within a week, as well as to predict neonatal complications [43,44,73,74]. Wei et al. [75] showed that elevations of AF-IL-6 and CRP, as well as cervicovaginal fluid-IL-6, are strongly associated with spontaneous PTD in asymptomatic women. However, this was not observed in maternal serum samples. In addition, pregnant women with exalted vaginal fluid IL-6 levels at mid-trimester are strongly associated with spontaneous PTD [72,75]. Moreover, Hitti et al., [76] reported that bacteria was found via PCR in 75% of PTL pregnancies with intact membranes and AF-IL-6 concentration of >2ng/ml. Although AF-IL-6 showed an excellent sensitivity to predict PTD in both asymptomatic and symptomatic patients, unfortunately this marker does not meet the clinical criteria for discriminating pregnant women at risk of spontaneous PTD [77]. Interestingly, Jacobsson [78] reported that elevated IL-18 levels in AF were observed in IAI in preterm pregnant women with PLIM, and not in women with PPROM, suggesting that AF-IL-18 can be used to differentiate PLIM from PPROM.

Endogenous Enzymes

Matrix metaloproteinases (MMPs)

It has been shown that the release of pro-inflammatory cytokines and chemokines by uterine epithelial cells, leukocytes, maternal and foetal tissues triggers the production of prostaglandins. The presence of prostaglandins activates MMPs, which are endogenous enzymes that are responsible for the initial degradation of amniochorion connective tissues leading to cervical ripening for successful birth [79-82]. MMP has been evaluated as a potential marker for IAI leading to PLIM. Angus et al. [83] reported an increase in the risk of

spontaneous PLIM in patients with IAI with elevated AF-MMP-8 concentration. It was able to identify patients with PLIM who are at risk for IAI regardless of positive [84] or negative [85] microbial culture. AF-MMP-8 is also used to predict patients with spontaneous PTD with IAI within 7 days and 14 days and allow evaluation of the inflammatory status of the amniotic cavity to predict whether patients are at high risk of impending PTD [85]. It can be concluded that AF-MMP-8 assay is more accurate than AF-IL-6 assay.

Endogenous Danger Ligands

There has been an increase in the findings of endogenous danger ligands [high mobility group box 1 (HMBG1), damage-associated molecular patterns (DAMPs), endogenous secretory-receptor for advanced glycation end products (esRAGE), and soluble-RAGE (sRAGE)] that bind to innate immune cells receptors (toll like-receptors -TLRs, RAGE, IL-18R and IL-23R). These endogenous danger ligands induce the production of cytokines that are responsible for activation of the inflammation cascade events leading to PTL [86]. DAMPs (aka alarmins) act as endogenous danger ligands, which contribute to chronic pro-inflammatory state through the initiation of TLRs and receptor for advanced glycation end products (RAGE) [87]. Moreover, patients with IAI were more likely to have elevated levels of AF-esRAGE and sRAGE than their respective controls [88]. HMGB1 is another member of alarmin family. This protein is secreted by stressed cells during injury [89,90], and induces pro-inflammatory cytokines and chemokines through binding to RAGE [91-93]. Studies show that AF-HMGB1 concentration was higher in subjects with PPROM than those with intact membranes [94,95]. As a whole, it is becoming clearer that several candidate alarmins are involved in triggering an immune response due to tissue injury leading to PTD, including HMGB1, calgranulins, amyloid and beta sheet fibril [96].

Antimicrobial Peptides and Calgranulin Proteins

Elevation of inflammatory cytokines and chemokines levels recruits neutrophils and macrophages leading to further production of downstream inflammatory mediators such as antimicrobial peptides and calgranulin that may serve as potential diagnostic biomarkers [97,98]. Human neutrophil peptide (HNP) is an α-defensin that is produced by the neutrophils. Neutrophils play a vital role in innate immunity and protect the host from microbial invasion by punching pores in the outer membrane of bacteria [99,100], whereas calgranulin forms a leukocyte L1 complex and contains antimicrobial properties that play a vital role in the inflammatory disorder [101,102]. Additionally, maternal serum-calgranulin B, a protein involved in inflammatory processes and placenta development [103], was demonstrated to be present in PTD-associated IAI subjects with PLIM when compared to subjects with PTL delivered at term [104,105].

A study by Ruetschi et al. [106] demonstrated 17 protein peaks were differentially expressed and identified in IAI patients' AF samples compared to non-IAI patients, with 5 proteins strongly expressed (HNP 1-3, calgranulins A and B). In a related study [107], HNPs and calgranulins were also found to be elevated on a proteomic chip in AF of patients with PTL and intraamniotic inflammation. Another study by Buhimschi et al. [108] identified HNP-1, HNP-2, calgranulin A and calgranulin C that were specifically overexpressed in AF obtained from patients with PTD-associated IAI regardless of membrane status compared with patients with PTL and delivered at term. Recently, Romero et al. [109] have proven that the mass restricted (MR) score of AF HNP-1,-2, calgranulins A and C obtained have a similar diagnostic values compared to AF-IL-6 immunoassay in identifying PTD associated IAI.

Combinations of Biomarkers

Several studies have reported the potential of individual vaginal fluid protein marker such as MMP-9 [110], foetal fibronectin [111] and human neutrophil defensins [112] as important diagnostic protein markers to discriminate between women with IAI-associated PTD and term birth. Due to the marked heterogeneity aetiology and pathogenesis of spontaneous PTD, therefore it is unlikely that an individual biomarker can categorise spontaneous PTD caused by spontaneous PLIM or labour induced by PPROM. Hitti et al. [113] suggested that combination of several protein biomarkers with different functional groups provide better diagnostic values in disease status.

Label free two-dimensional liquid chromatography mass spectrometry (2D-LC-MS) proteomic analysis of vaginal fluid samples from patients with or without IAI revealed a total of 338 biomarker proteins with 15 differentially expressed proteins, including acute phase reactants (α-1-acid glycoprotein), immune modulators (calgranulin C and cystatin A), high abundance AF proteins (insulin-like growth factor binding protein-1) and extracellular matrix signalling factors (fatty acid binding protein). These biomarkers were then validated with enzyme-linked immunosorbent assay (ELISA) and proved to be useful in identifying patients with IAI [113]. The study was also supported by Holst et al. [114] work, where by six pro-inflammatory cytokine proteins involved in intraamniotic inflammatory pathways that had been identified to be elevated from previous studies to discriminate spontaneous PTD caused by spontaneous PLIM or labour induced by PPROM using ELISA were selected. Five AF-pro-inflammatory cytokines were significantly over-expressed in spontaneous PTD caused by spontaneous PLIM with histological chorioamnionitis and not in PPROM subgroup, with potential applications in spontaneous PTD diagnosis [114].

Romero et al. [115] results showed a total of 39 different AF marker patterns using multiple quantitative proteomics surface-enhanced laser desorption/ionisation Time-Of-Flight-MS (SELDI-TOF-MS), in patients with PTL-associated IAI compared to those with PTL delivered at term and the results were combined to produce a proteomic "fingerprint" using a novel computational approach to identify the changes in protein marker levels in AF. Subjects were then being classified into individuals with PTD and IAI or term delivery by creating an empirical model [115]. This proteomic assay presents the potential in identification of prospective biomarkers which deliver preterm with PTL-associated IAI from PTL-IAI free delivered at term, with 90.3% accuracy [115].

A recently published work by Liong et al. [116] used two-dimensional fluorescence difference gel electrophoresis coupled with liquid chromatography-electrospray ionisation tandem mass spectrometry analysis (2D-DIGE-LC-ESI-MS/MS) to study differentially regulated proteins in cervical vaginal fluid of pregnant women who will eventually experience spontaneous PTL. The authors compared the two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) cervical vaginal fluid protein profile of the women 11-22 days before spontaneous PTL and at term in labour and discovered a total of eight spots

that were significantly differentially expressed. Six proteins were significantly under-expressed (CSTA-dimer, SOD1, FABP5, GGCT, TXN and IL1RN) while two proteins were over-expressed (GSTP1 and GC) in the protein profile of the women. To validate these proteins, the authors selected five proteins of interest (TXN, SOD1, IL1RN, CSTA and GC) and validated them using an independent cohort of women who will eventually experience spontaneous PTL. In addition, these five selected proteins had been previously identified to be associated with spontaneous term labour using proteomic technologies [117,118]. Interestingly, only two cervical vaginal fluid proteins (TXN and IL1RN) were reported to be involved in the progression of IAI leading to PTL and able to discriminate at-risk IAI asymptomatic women with subsequent PTL. TXN is involved in the IAI pathway [119,120] and IL1RN is involved in the extracellular matrix degradation pathway [121,122].

In an effort to study serum and vaginal fluid proteomic profiles in PTL-associated IAI populations, with the aim of applying less invasive test to maximize patients safety, Pereira et al. [123] used the matrix assisted laser desorption ionisation-Time-of-Flight- mass spectrometer (MALDI-TOF-MS) and 2D-LC-MS/MS to construct glycopeptide profiling from maternal serum obtained from women with IAI-associated PLIM, and women with PTL who delivered at term. Eight proteins were identified from 23 peptides generated from MS to distinguish between both mentioned groups with an accuracy of 97.0%.

Esplin et al. [124] used cLC-ESI-TOF-MS to study serum protein profiles in PTD irrespective of membrane status. Three novel inter-α-trypsin inhibitor heavy chain 4 protein derived peptides were identified. These peptides could differentiate pregnant women with spontaneous PTD regardless of membrane status from uncomplicated pregnancies with a sensitivity of 65.0% and specificity of 85.5%. The sensitivity and specificity of the diagnostic assay increased to 86.5% and 80.6%, respectively, after the authors included six more previously potential biomarkers (Corticotropin releasing factor, Defensin, Ferritin, Lactoferrin, Thrombin anti-thrombin, TNF receptor type 1). Bringing together the findings of the above studies, combinations of several PTD-associated IAI biomarkers offer greater sensitivity and specificity compared to single biomarkers.

Strategies for IAI Protein Biomarker Discovery

There are three fundamental phases in discovering protein biomarkers: the experimental design phase, the biomarker candidate screening phase and the biomarker candidate validation phase [125].

Experimental design phase

It is generally inferred that sample determination and good experimental designs are crucial aspects to obtain meaningful proteome profile for meaningful analysis. Thus, in good experimental designs researchers must (I) select sample size that is representative of the investigated population, (II) well addressed sample morphology and histopathogy, (III) obtain samples from the same site with adequate amount for several repetitions of proteomic analysis, (IV) establish appropriate sample storage and preparation to maintain the integrity of proteins including sample clean-up, analytes enrichment process, removing of unwanted salts, contaminants and abundant proteins, protein isolation and protein refractionation or fractionation [126].

Biomarker candidate screening phase

The underlying steps for the investigation of any biomarkers in infected and control samples are based on their differential protein expression. There are two proteomic-based approaches for biomarker investigation; global unbiased and specific. Global unbiased approach enables construction of proteomic profiling for both unidentified and identified proteins that enables potential biomarkers identification [127] with an unbiased manner using gel-approaches or gel-free-approaches. Whereas, specific approach allows markers detection by applying samples to ELISA or western blotting, which is then used to detect protein of interest using its' corresponding antibody. Therefore, prior knowledge of the protein is required. The current limitation of specific approach is that only very minimal proteins can be detected at one time, and it often requires large amounts of sample. Clearly, this is not an ideal approach for biomarkers identification [128].

Gel-based-MS techniques

Gel-based technique along with MS technology was widely applied from the early proteomics era until today for protein separation and identification. The two-dimensional gel electrophoresis (2-DE) method separates proteins using two different physiochemical properties. Soluble proteins present in biological samples are first separated according to their isoelectric point (PI) in a mono direction manner across a define pH gradient until they reach a position where the overall net charge is zero. These proteins are solubilized again in sodium-dodecyl-sulphate (SDS) to give them a net negative charge [129] before being subjected to a second separation by their molecular weights via SDS-protein polyacrylamide gel electrophoresis (SDS_PAGE) [130]. Finally, the gel is stained with coomassie blue or silver stain-based visual dye and stained bands are then analysed with commercially available software for differentially stained spots. Coomassie blue is more preferred than silver stain due to its compatibility with downstream MS technology. Spots of interest are then excised, trypsinised into peptides and identified using peptide mass fingerprinting with MALDI-TOF-MS, MALDI-TOF/TOF MS or ESI TOF/TOF MS [131,132] coupled with proteomic software (e.g. MASCOT, SwissProt). MALDI and ESI are the most commonly used ionisation source [133].

2-DE allows excellent separation of protein with a molecular weight between 20-250 kDa and PI of 3-11. However, 2DE gel-to-gel variations make it difficult to compare spot variation. Nonetheless, recent advancements in this field had made efforts to improve the pH gradient, protein resolution, staining method and isoelectric focusing (IEF) range [134,135], as well as the development of 2D-DIGE that allows determination of discriminating spots within a single gel by pre-labelling each protein sample with different Cy-fluorescent dye (Cy-2, Cy-3 and Cy-5) of different excitation and emission wavelengths [136] prior to electrophoretic separation. Although 2D-DIGE is able to overcome the gel-to-gel variations that lead to protein ratio errors compared to 2-DE [137], it is important to note that both 2D-DIGE and 2-DE under represent proteins with too low or too high molecular weights, extreme PI values, various hydrophobic properties and proteins with similar PI and molecular weight co-migrate to the same spot [138]. Owing the potential disadvantages of the gel based techniques, gel-free-MS techniques open new doors for researchers to identify biomarkers [139,140].

Gel-free-MS techniques

In recent years, gel-free-MS-based quantitative proteomics have been largely applied in favour of detecting low and high-

abundant proteins (1000-3000 proteins). Protein identification using this method involves three steps: (1) trypsinising proteins into peptides, (2) peptides separation on LC, and (3) identification and quantification of separated peptides using tandem MS. The quantitative proteomic approach can be divided into two distinct groups, label-free and label-based with chemical isotopes or metabolites [141,142].

Isotope-coded affinity tag (ICAT) and isobaric tags for relative and absolute quantitation (iTRAQ) are chemical isotopes used to label proteins for label-based quantitative MS. Cysteine residues of proteins form sulphide bonds with either light or heavy ICAT [143]. Since ICAT can only ligate to cysteine residue, therefore, identification of proteins that contain few cysteine residues is difficult [144]. iTRAQ binds to peptides' N-terminal and amino groups of lysine via its hydroxyl succinimide ester group [145] and allows sampling of 8 samples simultaneously in one procedure [146].

Stable isotope labelling by amino acids in cell culture (SILAC) is a metabolic tag for label-based quantitative MS that ligates with peptides through several passages of cells grown in medium contains $^{13}C_6$-arginine and $^{13}C_6$-lysine [147]. This method is claimed to be one of the best quantitative methods to study small alterations in protein levels and protein PTMs due to its ability to ligate itself at the early stage of sample preparation [148].

Large amount of samples, complex biochemical workflows, restricted whole proteome analysis and expensive reagents, makes label-based quantitative proteomic a less economically attractive option compared to label-free quantification [149]. Gel-free quantification-MS strategies are based on comparing two or more experiments by direct comparison of peptide intensities or signal intensities [150,151] obtained from each peptide/protein which is more straightforward. In addition, Filiou et al. [152] have shown that label-based and label-free quantitative proteomic methods work equally well if the experimental design is appropriately outlined.

Biomarker candidate validation phase: Biomarker candidate validation is very important in protein biomarker discovery to determine the diagnostic significance that provides an indication of the confirmatory clinical trials success rate. Conventional ELISA is used as the standard technique during biomarker validation phase. This technique is highly dependent on antibody quality, requires large samples volume, and is limited to a single antigen detection at any one time [153]. Therefore, array-based surface plasmon resonance (SPR) system is more preferred than conventional ELISA due to its high-throughput, minimal sample requirement, label free with real-time detection [154]. This allows identification of beneficial biomarker candidates at the early phase of biomarker discovery.

Perspective and Future Direction

Reducing the rate of neonatal morbidity and mortality caused by spontaneous PTL- and PTD-associated IAI is a great challenge. Studies have shown that pregnant women undergoing spontaneous PTD-associated IAI can be due to PPROM or PLIM. At present, the broad spectrum antibiotics that are administered are only beneficial to PPROM. Researchers have been working towards finding highly specific biomarkers for early detection of preterm associated IAI, however membrane status has been overlooked during the search for biomarkers by most of the studies. Currently, proteomics technologies are preferred in identifying spontaneous preterm associated IAI biomarkers because it provides intense information compared to genomics and transcriptomics approaches. It was proven that screening multiple protein biomarkers gives higher specificity and sensitivity with better diagnostic and prognostic values compared to single biomarker in identifying preterm associated IAI pregnant women. Noteworthy, through proteomics approaches, protein expressions are found to be differentially expressed between PPROM and PLIM. Therefore, it is imperative to focus in finding a combination of multiple protein biomarkers that are able to distinguish women who are suffering from preterm associated IAI due to PPROM or PLIM for better treatment values of preterm associated IAI. This can be achieved by selecting good multivariable biomarkers from previously published studies that are involved in different pathophysiological pathways of IAI in preterm pregnant women associated to PPROM and PLIM, and validate them using on-chip immunoassay in a large multicentre prospective cohort of pregnant women with IAI.

The ultimate success of this methodology also depends on the inclusion and exclusion criteria employed among the selected studies. In addition, closely cooperative and collaborative relationships among clinicians, bioinformaticians, statisticians and mass spectrometricians would lead to successful biomarkers with superlative sensitivity, specificity and high accuracy for IAI-associated PTD/PTL subtype.

DECLARATION OF INTERESTS

The authors report no conflicts of interest.

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Abbreviations

2-DE: two-dimensional gel electrophoresis; **2D-DIGE**: two-dimensional fluorescence difference gel electrophoresis; **2D-LC-MS**: label free two-dimensional liquid chromatography mass spectrometry; **AF**: amniotic fluid; **CRP**: C-reactive protein; **DAMPs**: damage-associated molecular patterns; **ELISA**: enzyme-linked immunosorbent assay; **HMBG1**: high mobility group box 1; **HNP**: human neutrophil peptide; **IAI**: intraamniotic infection and inflammation; **ICAT**: isotope-coded affinity tag; **IL**: interleukins; **iTRAQ**: isobaric tags for relative and absolute quantitation; **MALDI-TOF-MS**: matrix assisted laser desorption ionisation-Time-of-Flight- mass spectrometer; **MMPs**: Matrix metaloproteinases; **PCR**: polymerase chain reaction; **PI**: isoelectric point; **PLIM**: preterm labour with intact membranes; **PPROM**: preterm premature rupture of membranes; **PTD**: preterm delivery; **PTL**: preterm labour; **PTMs**: post-translational modifications; **RAGE**: advanced glycation end products; **esRAGE**: endogenous secretory-receptor for advanced glycation end products; **sRAGE**: soluble-RAGE; **SDS**: sodium-dodecyl-sulphate.

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