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Compartmentalization of acute phase reactants interleukin-6, C-reactive protein and procalcitonin as biomarkers of intra-amniotic infection and chorioamnionitis

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Abstract

Background—The arsenal of maternal and amniotic fluid (AF) immune response to local or systemic infection includes among others the acute-phase reactants IL-6, C-reactive protein (CRP) and procalcitonin (PCT). If these molecules can be used as non-invasive biomarkers of intra-amniotic infection (IAI) in the subclinical phase of the disease remains incompletely known.

Methods—We used time-matched maternal serum, urine and AF from 100 pregnant women who had an amniocentesis to rule out IAI in the setting of preterm labor, PPRM or systemic inflammatory response (SIR: pyelonephritis, appendicitis, pneumonia) to infection. Cord blood was analyzed in a subgroup of cases. We used sensitive immunoassays to quantify the levels of inflammatory markers in the maternal blood, urine and AF compartment. Microbiological testing and placental pathology was used to establish infection and histological chorioamnionitis.

Results—PCT was not a useful biomarker of IAI in any of the studied compartments. Maternal blood IL-6 and CRP levels were elevated in women with subclinical IAI. Compared to clinically

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Potential conflict of interest

None of the authors have a commercial or other association that might pose a conflict of interest.

Institution affiliation

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manifest chorioamnionitis group, women with SIR have higher maternal blood IL-6 levels rendering some marginal diagnostic benefit for this condition. Urine was not a useful biological sample for assessment of IAI using either of these three inflammatory biomarkers.

Conclusions—In women with subclinical IAI, the large overlapping confidence intervals and different cut-offs for the maternal blood levels of IL-6, CRP and PCT likely make interpretation of their absolute values difficult for clinical decision-making.

Graphical Abstract

Keywords

Intra-amniotic infection; non-invasive biomarkers; preterm birth; inflammatory response syndrome

1. INTRODUCTION

Pregnancy can be thought of as a compartmentalized state: the mother, the amniotic fluid (AF) cavity, and the fetus *per se*. A successful pregnancy involves fine-tuning of both the maternal and fetal compartments, individually and together [1].

The conundrum of preterm birth (PTB) lies in its multiple etiologies, of which, infection and inflammation play a major role [2]. The dictum of treating the underlying cause of infection in order to combat associated risks of PTB can be complicated by the uncertainty of knowing from which part of the maternal-fetal unit does the infectious process arise. Often times, mothers present with clinical signs and symptoms that do not allow for easy classification into a maternal infectious process (i.e., appendicitis, pancreatitis, pyelonephritis) versus true intra-amniotic infection (IAI). Traditionally, clinical chorioamnionitis is diagnosed by maternal fever, tachycardia, abdominal tenderness, and leukocytosis [3]. However, differentiation from other maternal systemic and abdominal infectious conditions is a challenge during the symptomatic stage of IAI. This is because the aforementioned signs and symptoms are non-specific and occur in only 18% of pregnancies complicated by IAI [4]. Moreover, the spectrum of signs and symptoms is so heterogeneous that just 30% of cases categorized as clinical chorioamnionitis display histological inflammation of the amniochorion and umbilical cord [5]. Equally challenging is diagnosing IAI during the asymptomatic phase of the disease. By far, most cases of IAI deliver preterm and present initially with irregular uterine contractions, cervical dilatation

and/or preterm premature rupture of membranes (PPROM), in the absence of signs or symptoms of clinical chorioamnionitis [6].

In both symptomatic and asymptomatic mothers, initial diagnostic modalities available to aid in decreasing or increasing suspicion of other infectious sources versus IAI include amniocentesis. This procedure is invasive and could be technically difficult. Thus, a lot of attention has been focused on discovery of maternal serum biomarkers that can accurately predict IAI [7, 8]. Yet, despite significant effort, no maternal serum markers alone or in combination offers acceptable sensitivity and specificity for the diagnosis of IAI [1,9,10].

Following microbial invasion of the AF, the inflammatory process of the gestational sac, fetal membranes, choriondecidua, fetus and several maternal compartments can be viewed as independent but also related events. Therefore, the chance of discovering a maternal serum or urine biomarker of IAI that can be decisive for clinical decision making such as immediate delivery of the fetus can be significantly increased through a comparative analysis of the expression levels of the same candidate biomarkers in the AF versus various maternal compartments. The list of potential AF and maternal serum candidate biomarkers is exhaustive and their identity was recently reviewed [1]. Herein, we focused only on IL-6, C-Reactive Protein (CRP) and procalcitonin (PCT) as prototype infection induced inflammatory biomarkers. Understanding how maternal systemic infectious conditions, other than IAI, impact on the levels of the same proposed inflammatory biomarkers, may aid to the process. The current study was conducted to fill this knowledge gap and determine if the three selected acute phase reactive proteins can serve as independent biomarkers of IAI in the symptomatic and asymptomatic stage of the disease.

2. METHODS

2.1. Study population

In a case-control study we analyzed time-matched AF, maternal blood, and urine of 100 women pregnant with singletons in the following groups: **i**) (+)IAI & PTB (n=48, gestational age (GA): median [interquartile range] 26 [24–28] weeks) of which 11 women had clinical symptoms of chorioamnionitis; **ii**) (–)IAI & PTB (n=20, GA: 29 [25–32] weeks); **iii**) (–)IAI & Term Birth (n=14, GA 28 [24–31] weeks) comprised of women with symptoms of preterm labor that delivered at healthy baby at term; **iv**) (–)IAI & systemic inflammatory response (SIR, n=18, GA: 30 [27–34] weeks) comprised of women with medical inflammatory conditions unrelated to pregnancy. Consecutive cases were selected from a biorepository based on criteria for inclusion in one of the 4 groups described above and availability of the time-matched specimens. The enrollment prior for the cases in the study was between June 2004 and June 2010. This study was approved by the Human Investigation Committee of Yale University. All patients provided written informed consent. Human experimentation guidelines of the United States Department of Health and Human Services and those of Yale University were followed in the conduct of our research.

IAI was defined by either positive Gram stain or positive culture results. GA was established based on clinical and ultrasonographic criteria [11]. Persistent regular uterine contractions, advanced cervical dilatation (≥ 3 cm) or effacement at <37 weeks GA defined preterm labor.

PPROM was confirmed either by a positive amnio-dye test or by “pooling” on speculum examination, positive “nitrazine” and “ferning” tests. Exclusion criteria consisted of maternal complications such as preeclampsia, thyroid disease, cholestasis, connective-tissue-disease, diabetes, viral infections (i.e. HIV, hepatitis B or C), anhydramnios, or fetal growth restriction (sonographic estimated fetal weight <10th percentile). Clinical chorioamnionitis was established in the presence of maternal fever (>37.8°C), maternal or fetal tachycardia, leukocytosis (> 15,000 cells/mm³), uterine tenderness, foul smelling AF or visualization of pus at the time of the speculum exam [12].

All women with SIR presented for evaluation in the setting of maternal fever (>37.8°C), accompanied or not by abdominal or back pain. Given the uncertainty of the diagnosis, an amniocentesis was clinically recommended to rule-out chorioamnionitis. At the end of the clinical, laboratory and imaging work-up the cause of maternal SIR was established for the majority of the patients as follows: urinary tract infection/pyelonephritis n=6, viral syndrome n=3, gastrointestinal conditions/appendicitis n=3, sepsis n=2, pneumonia n=1, Takayasu arteritis n=1. Despite a comprehensive work-up the cause of maternal SIR could not be established in 2 patients.

Clinical management of all patients was left up to the discretion of the medical provider independent of our study.

2.2. Specimen collection and handling

Maternal blood, urine and AF were collected within 1–2 hours of each other. Maternal blood samples were retrieved by venipuncture as part of clinical evaluation. A urine sample (5–10 mL) was collected by standard use of sterile containers using “straight cath” or “clean catch” sterile techniques. In all women AF samples were retrieved by a clinically indicated amniocentesis performed with sterile technique as part of the diagnostic work-up. Cord blood was retrieved under sterile conditions immediately after delivery, for all women that delivered preterm.

The clinical laboratory assessed the AF glucose, lactate dehydrogenase levels (LDH), and performed a white blood cell count (WBC). A glucose level of > 15 mg/dL, LDH activity > 419 U/L, WBC count > 30 cells/μL were considered indicative of IAI [13,14]. AF was concurrently examined for the presence of infection using Gram staining and culturing methods for aerobic and anaerobic bacteria, *Ureaplasma*, and *Mycoplasma* species. Excess AF was used for research purpose.

Maternal and cord blood was allowed to clot. Serum, urine and AF samples were spun at 3000g at 4°C for 20 minutes, the supernatant aliquoted and stored at –80°C until performance of the immunoassays by investigators unaware of the diagnosis. The median duration from case enrollment to immunoassay was 3.2 [1.6–4.3] years. There were no differences in the time of sample storage among groups ($P=0.336$)

2.3. Laboratory methods

Human levels of maternal blood, urine, AF and cord blood IL-6 (eBioscience, San Diego, CA), CRP (R&D Systems, Minneapolis, MN) and PCT (RayBiotech, Norcross, GA) were

assessed by ELISA. The assays were run in duplicate according to the manufacturer's protocols. The high sensitivity IL-6 ELISA minimal detectable concentration was 0.039 pg/ml. The minimal detectable concentration for CRP, and PCT were 10 pg/ml, and 30 pg/ml, respectively. For all assays, samples were diluted from 1:1 to 1:10,000 to fall within the range of the standard curves as appropriate to the sample type. The inter- and intra-assay coefficients of variation was <10% for all analytes. IL-6 and CRP were measured in all samples. PCT levels measured below the minimal detectable concentration in 80% (80/100) of AF, 97% (97/100) of maternal blood, 100% of urine (43/43) and 62% (32/52) of undiluted cord blood samples.

Because bacterial cultures and Gram stain have known limitations in the diagnosis of chorioamnionitis, we confirmed the presence of intra-amniotic inflammation secondary to IAI by using mass spectrometry [4, 15]. The methodology for generation of the proteomic Mass Restricted (MR) score has been previously described. Briefly, the MR score provides qualitative information regarding the presence or absence of intra-amniotic inflammation. The MR score ranges from 0 to 4, depending upon the presence or absence of each of the four protein biomarkers. A categorical value of 1 was assigned if a biomarker peak was present and 0 if absent. IAI was characterized by a degree of severity which varied from an MR score 0 ("absent" inflammation) to MR score 1–2 ("minimal" inflammation) to MR score 3–4 ("severe" inflammation).

2.4. Statistical analysis

Data distribution was tested by using the Shapiro-Wilk test, and reported as median and interquartile [IQR] range. Immunoassay results were logarithmically transformed prior to statistical analysis. Data were compared with one-way ANOVA followed by Student Newman Keuls tests (parametric) or Kruskal-Wallis on ranks followed by Dunn's tests (non-parametric). Proportions were compared with Chi-square test. Relationships between variables were explored using Spearman's Rank order correlations. Strength of correlations was calculated based on z statistic. We used Receiver Operating Characteristic (ROC) curve analysis and calculated diagnostic performance of the analytes by using sensitivity, specificity, overall accuracy (ratio of cases correctly classified/total cases), likelihood ratios (LRs) and positive and negative predictive values. A probability level of <.05 was considered statistically significant. Med-Calc (Mariakerke, Belgium), SigmaStat 12 (RockWare, Golden, CO) and GraphPad Prism (Dan Diego, CA) statistical softwares were used for data analysis.

3. RESULTS

3.1. Demographics and clinical characteristics for the study population

Demographics and clinical characteristics are presented in Table 1. Among all cases 5 women had PPRM at the time of amniocentesis. The remainder of cases in the (+)IAI or (–)IAI groups presented with symptoms of preterm labor and intact membranes. Women with (+)IAI presented at a lower GA compared to women with SRI. Women who delivered preterm and those evaluated preterm but ultimately delivering at term had a higher frequency of steroid exposure and uterine contractions. Antibiotic exposure occurred more frequently

in PTB and SIR mothers. PTB mothers more often had cervical dilation ≥ 3 cm at the time of amniocentesis. Women with (+)IAI and SIR more regularly displayed signs and symptoms of chorioamnionitis. Women with (+)IAI delivered at an earlier GA and had neonates of lower birth weight. In Table 2 we show that women with (+)IAI and SIR had significantly higher maternal WBC than the other groups. Lastly, AF of women with (+)IAI&PTB had significantly lower glucose, higher LDH, WBC, MR scores and degree of histological chorioamnionitis ($P < .001$ for all).

3.2. Intra-compartment analyses for IL-6, CRP and PCT

3.2.1. Interleukin-6—In Fig. 1 we present the results of IL-6 levels in AF (A), maternal blood (B), maternal urine (C) and umbilical cord blood (D) in our study groups. Women with (+)IAI has significantly elevated AF IL-6 levels compared to (–)IAI or SIR women ($P < .001$ for both, Fig. 1A). Among women with (+)IAI, AF IL-6 levels were similar irrespective of presence or absence of clinical chorioamnionitis ($P = .130$). In maternal blood (Fig. 1B), the highest level of IL-6 were reached in the context of SIR. Women with (+)IAI had higher level of IL-6 in maternal blood compared to women with (–)IAI ($P < .001$) but these could not reach the level noted in SIR. In maternal urine there were no significant differences in IL-6 among groups (ANOVA, $P = .365$, Fig. 1C). Women with (+)IAI had higher cord blood IL-6 levels compared to both (–)IAI ($P < .001$) and SIR ($P < .001$) cases where cord blood was available for analysis (preterm delivery $n = 3$, amniocentesis to delivery interval median [IQR]: 2 [1–3] days) or term delivery ($n = 3$, amniocentesis to delivery interval 72 [8–89] days) (Fig. 1D).

3.2.2. C-Reactive Protein—The level of AF CRP was significantly elevated in women with (+)IAI compared to (–)IAI cases ($P < .001$) with levels that were not statistically different between subclinical and clinical chorioamnionitis ($P = .995$) (Fig. 1E). Women in the (–)IAI groups had the lowest AF CRP concentration while the SIR group had intermediate level which was not significantly different from any of the other groups ($P > .05$). In maternal blood, CRP was elevated at a similar extent in both (+)IAI and SIR groups (Fig. 1F). There was a wide range of maternal blood CRP levels among cases with (+)IAI and clinical chorioamnionitis which made separation of this group from those with (–)IAI and SIR impossible based on CRP concentration alone. There was no significant difference in CRP concentration in maternal urine (Fig. 1G). In cord blood, CRP concentrations were the highest in women with (+)IAI. There was a broad range in cord blood CRP levels of women (+)IAI and subclinical chorioamnionitis which rendered this group of newborns statistically non-different from the (–)IAI or SIR group (Fig. 1H).

3.2.3. Procalcitonin—The levels of PCT were not different among groups in either AF (Fig. 1I), maternal blood (Fig. 1J) or urine (Fig. 1K). Among the types of samples analyzed, PCT was detectable in a larger proportion in cord blood samples and in particular in newborns of women with (+)IAI. However, the concentration of cord blood PCT in the subclinical (+)IAI group was not significantly different from that of any other group (Fig. 1L).

3.3. Intra-compartment correlations

In Fig. 2 we present the correlation coefficients for IL-6, CRP and PCT in the 4 studied compartments. Correlations remaining significant after correction for multiple comparisons are presented in red. As shown, there was a significant correlation between levels of IL-6 and CRP in AF, maternal blood and cord blood ($r=.567$, $r=.662$, $r=.698$ respectively, $P<.001$ for all), but not in maternal urine where levels of IL-6 and CRP varied independent of each other ($r=.125$, $P=.428$). In cord blood, but in no other compartment, the concentration of PCT correlated with that of IL-6 ($r=.574$, $P<.001$) and CRP ($r=.638$, $P<.001$).

3.3. Inter-compartment analyses for Interleukin-6, C-Reactive Protein and Procalcitonin

In Fig. 3 we display for each individual subject the levels of the three acute phase reactive proteins in AF, maternal blood and urine of patients with (+)IAI, (-)IAI, and SIR. As shown in Fig. 3A the highest levels of IL-6 were reached in AF of women with (+)IAI, with relatively close-fitting levels in AF but not in maternal blood or urine. In women with (-)IAI, the levels of IL-6 were broadly distributed in all three compartments, albeit at a predominantly lower level compared to (+)IAI. Compared to (+)IAI, the visible clustering of AF IL-6 levels was lost in maternal blood and urine of women with (-)IAI. A relatively high level of overlap was seen in the maternal blood IL-6 levels among the group with (+)IAI vs. (-)IAI. The AF IL-6 levels of most SIR cases clustered together with the (-)IAI group. In maternal blood, SIR cases grouped with or above the level of (+)IAI. A discernable clustering pattern between (+)IAI and (-)IAI cases was lost in urine of women with SIR.

In Fig. 3B we show that the only discernable pattern of distribution of the CRP concentrations was observed in the maternal blood of women with (-)IAI, that was below that of women with (+)IAI and SIR. As displayed in Fig. 3C distribution of the urine, AF, and maternal blood PCT concentrations were scattered with no specific clustering pattern observed.

3.4. Relationships between levels of Interleukin-6, C-Reactive Protein and Procalcitonin in maternal blood and urine compartments and severity of histologic chorioamnionitis

Following correction for GA and amniocentesis-to-delivery interval, there was a significant direct correlation between the maternal blood CRP and severity of histologic amnionitis ($r=.272$, $P=.002$), choriodecidualitis ($r=.384$, $P<.001$) and chorionic plate inflammation ($r=.378$, $P<.001$). There were no relationships with maternal blood IL-6 or PCT. Additionally, all urine analytes varied independently of histologic markers of inflammation in fetal membranes.

3.5. Diagnostic performance of maternal and urine Interleukin-6, C-Reactive Protein and Procalcitonin for diagnosis of intra-amniotic infection

In Table 3 we present the diagnostic characteristics for maternal blood and urine IL-6, CRP and PCT in identifying women with (+)IAI. Only maternal blood IL-6, maternal blood CRP and urine PCT registered ROC areas above 0.5 (Fig. 4). However, none of the studied maternal blood or urine acute phase reactive proteins reached area under the curve values to the level of maternal blood WBC.

When the analysis was limited to women presenting with nonspecific clinical symptoms of chorioamnionitis where the amniocentesis was necessary to diagnose (+)IAI, only AF IL-6 had statistical significance in differentiating between (+)IAI and SIR as etiologies (ROC area: 0.965 [0.821–0.999], z statistic: 15.958, $P < .001$; optimal criterion >3 ng/mL, sensitivity: 90.9 [58.7–99.8] %, specificity: 94.4 [72.7–99.9] %, +LR: [16.4 [2.4–110.9], –LR: 0.096 [0.01–0.6], PPV: 90.9 [56.6–99.8] %, NPV: 94.4 [72.7–99.9] %).

4. DISCUSSION

The pathophysiology of the inflammatory process during pregnancy has been intensely studied and reviewed [2,6,16]. Overall, the existing data lends support to the theory that genital bacteria invade the uterus and spread to the AF via an ascending route. Hematogenous dissemination of infection via a transplacental pathway is also possible. Finally, microorganisms gain access to the fetus, a key pathophysiologic event because the vast majority of AF neutrophils are of fetal origin [17].

The site and magnitude of the immune response to microbes determine the pregnancy outcome [18]. From this perspective, nature designed mechanisms to protect the mother. Our assertion is supported by the clinical observation that most often IAI is progressing clinically silent with majority of gestations ending as spontaneous PTB in the absence of maternal clinical inflammation. An attractive idea is that the mother becomes clinically symptomatic only after the protective mechanisms set in place to confine the uterine infection/inflammation process are overwhelmed. This model is complex and requires forward-thinking explanations. Studies genotyping the amniochorion neutrophils demonstrated that the initial inflammatory response is maternal in its origin [19, 20, 21]. It remains unknown why the initial wave of decidual inflammation [22] is not associated with signs and symptoms of maternal clinical chorioamnionitis (i.e. fever, maternal leukocytosis). Possible explanations are: 1) bacterial passage in the AF requires local choriodecidualitis, while maternal clinical chorioamnionitis is associated with a more generalized tissue inflammatory process; 2) diverse bacterial organisms interact with the host receptors in different fashion and hence the various character of the inflammatory response; 3) different maternal and fetal genetic makeup predisposes toward either hyper- or a hypo- reactive immune system and diverse clinical manifestations of infection [23]. The concept of pregnancy as multiple coexisting, yet separate compartments - the AF cavity, the maternal circulation, maternal urinary system and the fetal circulation - is important when attempting to utilize a biomarker obtained from one area to predict a disease state in another. Certainly the ability to guide the clinician towards the proper route of evaluation and/or treatment modality using a rapid, non-invasive test is very desirable [7,8,9]. Nevertheless, establishing the presence and the site of infection is essential for survivability of the host. Higher mortality rates were reported when sepsis was the result of gastrointestinal or pulmonary causes relative to a genitourinary source [24].

A relevant clinical question is at what point during the process, inflammatory mediators detected in the maternal serum or other biological samples carries the potential to diagnose non-invasively IAI? As shown, the answer to the above question is complex. As proof of concept we focused on three prototype acute phase reactants IL-6, CRP and PCT. IL-6 is a

participant in the acute phase response to infection and previously proposed as AF and maternal serum biomarker of IAI [7]. Human amnion, choriodecidua and fetus are potential sites of IL-6 synthesis and probable sources of AF IL-6 [22]. CRP is synthesized by the liver in response to IL-6 [25]. The AF and circulatory levels of CRP were previously found elevated in women with IAI [7,26]. Although synthesized by the thyroid's parafollicular cells (C cells) and by the neuroendocrine cells of the lung and the intestine, in the context of systemic inflammation, almost every tissues cell expresses PCT [27,28]. PCT was previously evaluated in critical care setting to distinguish between SIR and sepsis [29,30,31].

Extensive research was conducted to identify if cytokines and acute reactive proteins can be used as non-invasive biomarkers of IAI [7,8,32]. Different from previous studies that search for the clinical usefulness of maternal blood, cervical and vaginal fluid, we explored the urine compartment and analyzed all our data based on the presence or absence of clinical chorioamnionitis. Furthermore, unlike other studies, we analyzed the levels and the diagnostic predictive value of our analytes in patients with SIR, a clinical situation where the differential diagnosis of IAI vs. other surgical and medical conditions is challenging. Several lessons were learned. In IAI, AF IL-6 and CRP levels were significantly elevated, yet alike whether or not maternal clinical chorioamnionitis was present. Likewise, maternal blood IL-6 and CRP levels were higher in the setting of IAI and did not vary with the presence or absence of clinical chorioamnionitis. These observations made us conclude that symptoms of clinical chorioamnionitis are not a reflection of higher pro-inflammatory cytokine levels in the maternal blood compartment. We further observed that in IAI the levels of AF IL-6 are much higher than maternal blood IL-6. Thus, it would be tempting to propose that the cause of elevated maternal blood IL-6 concentration is "leakage" of AF IL-6 in the maternal circulation. However, our data suggest a reverse situation for CRP where the maternal blood levels were higher than those of the AF. This implies that the primary source of IL-6 or CRP in the maternal blood and AF may be different and not just the result of ineffective isolation of the gestational sac. For example, maternal circulatory levels of IL-6 could be the consequence of AF IL-6 transfer toward the mother coupled with an independent IL-6 production process by the fetal membranes, choriodecidua and maternal neutrophils. Furthermore, although significant, we observed a minimal degree of correlation between the maternal blood CRP and severity of histologic amnionitis, choriodecidualitis and chorionic plate inflammation. Absence of a similar relationship between histological chorioamnionitis and maternal blood IL-6 or PCT makes it difficult to support the concept of cytokines/acute phase reactive proteins "spillage" from one compartment into the other. Various clearance mechanisms for different cytokines and proteins can impact as well. From a diagnostic value standpoint multiple sources and many clearance mechanisms are significant challenges.

The premise of independent regulation of the levels of various biomarkers in different compartments is supported by our data. It is reasonable to propose that increased blood levels of IL-6 augments maternal liver's CRP synthesis. As shown, in IAI, AF had the lowest levels of CRP while the maternal and cord blood concentrations were the highest. In addition, fetuses exposed to IAI displayed significantly elevated levels of PCT with no correspondent elevation in AF or maternal blood and urine PCT levels. The correlation indices among the levels of our cytokines, in maternal blood and AF were modest for IL-6 and CRP and absent for PCT. This can be explained through the complexity of the

regulatory mechanism governing synthesis and possible transfer of one biomarker from one compartment to the other.

Urine does not seem to be a useful biological sample to diagnose IAI. This could reflect the biological response of the glomerular basal membrane to systemic inflammation. It is plausible to propose that in most cases of IAI, the maternal inflammatory process does not frequently leads to damage and transfer of the cytokines in the urine.

Our data analysis demonstrated that out of the chosen biomarkers AF IL-6 had the highest diagnostic value for IAI. The close-fitting levels of IL-6 in the AF compared to maternal blood and urine helps improving its diagnostic performance. In this study we did not focus our attention on cervical or vaginal inflammatory biomarkers of IAI. While several studies reported identification of promising biomarkers [33,34], ours and other's studies [35, 36] suggest that the normal vaginal milieu is rich in inflammatory biomarkers that can potentially confound the results in patients with intact membranes or PPROM. In the current study while AF is a biological sample that can be obtained invasively, none of the studied maternal blood or urine proteins had diagnostic predictive values better than maternal blood WBC. When inflammatory conditions such as SIR were included in the analysis the diagnostic predictive value of maternal blood IL-6, CRP and PCT was of limited clinical use. The large level of overlap among the biomarker levels reached in each biological sample, and for all studied condition made it difficult to diagnose IAI in a non-invasive fashion.

5. CONCLUSIONS

In summary, our results suggest diagnosing IAI and making medical decisions based on maternal blood levels of acute inflammatory biomarkers is of limited clinical value. Until a specific biomarker of IAI will be identified and the ability to assess its presence and level in a non-invasive fashion developed the decision to deliver or continue pregnancy should be based on clinical manifestation or direct sampling of AF, when possible.

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Highlights

- Intra-amniotic infection is an underdiagnosed cause of preterm birth.
- Amniotic fluid infection most often progresses silently.
- Clinical chorioamnionitis does not reflect in elevated maternal cytokinemia.
- Maternal inflammatory markers have limited diagnostic value for chorioamnionitis.
- Decisions for expectant management or delivery require analysis of amniotic fluid.

Fig. 1. Scattergram of the levels of IL-6 (A–D), C-Reactive Protein (CRP, E–H) and Procalcitonin (PCT, I–L) in the amniotic fluid, maternal blood, urine and cord blood in women with positive intra-amniotic infection (+IAI) and negative IAI (-IAI) in the presence or absence of clinical chorioamnionitis (Ch). The levels of IL-6, CRP and PCT were also searched in women with systemic inflammatory response (SIR) of other causes than IAI. Data analyzed by one-way ANOVA. Superscripts with different letters indicate groups with statistically significant differences at $P < .05$.

Analyte	Compartment	IL-6	IL-6	IL-6	IL-6	CRP	CRP	CRP	CRP	PCT	PCT	PCT	PCT
		AF	MB	U	CB	AF	MB	U	CB	AF	MB	U	CB
IL-6	AF		0.196	0.201	0.576	0.587	0.332	0.130	0.816	0.097	-0.103	0.275	0.383
IL-6	MB	0.196		0.203	0.083	0.406	0.682	0.097	0.334	-0.168	0.055	-0.198	0.169
IL-6	U	0.201	0.203		0.245	0.183	0.123	0.125	0.176	-0.265	0.074	0.024	0.130
IL-6	CB	0.576	0.083	0.245		0.285	0.095	0.080	0.816	0.044	-0.201	0.012	0.514
CRP	AF	0.567	0.406	0.183	0.285		0.554	0.322	0.299	0.029	-0.066	0.269	0.142
CRP	MB	0.332	0.682	0.123	0.095	0.554		0.410	0.291	-0.136	-0.035	-0.042	0.050
CRP	U	0.130	0.097	0.125	0.080	0.322	0.410		0.134	-0.118	-0.080	-0.064	-0.034
CRP	CB	0.516	0.334	0.176	0.698	0.299	0.291	0.134		0.051	-0.132	0.061	0.619
PCT	AF	0.097	-0.168	-0.265	0.044	0.029	-0.136	-0.118	0.051		0.175	0.150	-0.083
PCT	MB	-0.103	0.055	0.074	-0.201	-0.066	-0.035	-0.080	-0.132	0.175		-0.224	-0.040
PCT	U	0.275	-0.198	0.024	0.012	0.269	-0.042	-0.064	0.061	0.150	-0.224		-0.132
PCT	CB	0.383	0.169	0.130	0.914	0.142	0.050	-0.034	0.816	-0.083	-0.040	-0.132	

Fig. 2.

Colored representation of the correlation indices among the levels of IL-6, C-Reactive Protein (CRP) and Procalcitonin (PCT) in the amniotic fluid (AF), maternal blood (MB), urine (U) and cord blood (CB). Red cells denote statistical significant correlation at $P < .05$, that remained following Šidák corrections for multiple comparisons.

Fig. 3.

Individual display of the concentration of IL-6, C-Reactive Protein (CRP) and Procalcitonin (PCT) in the maternal urine, amniotic fluid (AF), and maternal blood (MB) of women with positive intra-amniotic infection (+IAI), negative IAI (-IAI) and systemic inflammatory response (SIR) of other causes than IAI.

Fig. 4. Receiver operating characteristic (ROC) curve analysis in our study population to diagnose intra-amniotic infection using maternal white blood cell count (WBC), IL-6, C-Reactive Protein (CRP) and Procalcitonin (PCT) in the maternal blood (A) and urine (B).

Table 1

Demographic and clinical characteristics of the study population.

Variable	(+)IAI & PTB n=48	(-)IAI & PTB n=20	(-)IAI & Term birth n=14	Maternal SIR n=18
<i>Characteristics at amniocentesis</i>				
Age, years [†]	26 [21–34]	26 [20–31]	26 [22–29]	26 [20–32]
Parity [†]	0 [0–1]	1 [0–2]	1 [0–1]	1 [0–1]
Gravidity [†]	3 [2–4]	2 [1–4]	2 [2–4]	2 [1–2]
Non-Caucasian race [‡]	41 (85)	14 (70)	12 (86)	12 (67)
Gestational age, weeks ^{†**}	26 [24–28]	29 [25–32]	28 [24–31]	30 [27–34]
Steroid exposure ^{‡***}	39 (81)	18 (90)	9 (64)	4 (22)
Antibiotics exposure ^{‡*}	41 (85)	10 (50)	4 (29)	11 (61)
Uterine contractions ^{‡*}	33 (69)	14 (70)	9 (64)	5 (28)
Cervical dilatation 3 cm ^{‡***}	25 (52)	10 (50)	1 (7)	0 (0)
PPROM [‡]	5 (10)	0 (0)	0 (0)	0 (0)
Clinical chorioamnionitis ^{‡*}	11 (23)	0 (0)	0 (0)	5 (28)
<i>Characteristics at delivery</i>				
Gestational age, weeks ^{†**}	26 [24–28]	30 [28–34]	39 [38–40]	39 [36–40]
PPROM [‡]	7 (15)	2 (10)	0 (0)	1 (6)
Cesarean section [‡]	12 (25)	8 (40)	2 (14)	8 (44)
Birthweight, grams ^{†***}	830 [645–1,152]	1,470 [1,190–2,260]	3,308 [2,829–3,623]	3,015 [2,289–3,420]
Amniocentesis –to-delivery, days ^{†***}	1 [0–1]	6 [0–19]	78 [54–96]	54 [16–70]

Abbreviations: IAI, intra-amniotic infection; PTB, preterm birth; SIR, systemic inflammatory response; PPRM, preterm premature rupture of membranes

[†]Data presented as median [IQR] and analyzed by Kruskal-Wallis 1-Way Analysis of Variance

[‡]Data presented as n (%) and analyzed by Chi square test

* P<0.05;

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P<0.001

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Table 2

Clinical, research and pathology laboratory test results of the study population.

Variable	IAI(+)/PTB n=48	IAI(-)/PTB n=20	IAI(-)/Term birth n=14	Maternal SIR n=18
Maternal hematology				
Maternal WBC (x1000/ μ L) ^{†***}	16 [13–20]	11 [8–14]	12 [10–14]	14 [11–21]
Amniotic fluid laboratory results				
Glucose, mg/dL ^{†***}	2 [2–6]	30 [22–39]	31 [25–46]	29 [21–42]
LDH activity, U/L ^{†***}	989 [610–2,135]	183 [118–232]	169 [146–233]	171 [124–331]
WBC count, cells/mm ³ ^{†***}	730 [207–1,765]	1 [1–5]	5 [3–15]	3 [1–22]
Positive Gram stain ^{†*}	39 (81)	0 (0)	0 (0)	0 (0)
IL-6, ng/mL ^{†***}	94 [38–143]	0.3 [0.2–1.9]	0.2 [0.1–0.3]	0.2 [0.1–0.7]
MR Score ^{†***}	4 [3–4]	0 [0–1]	0 [0–1]	0 [0–1]
Placental pathology				
Chorionic plate inflammation (stage 2–3) [†]	42 (98)	0 (0)	NA	3 (33)
Amnionitis (grade 2–4) [†]	36 (84)	0 (0)	NA	0 (0)
Choriodeciduitis (grade 2–4) [†]	43 (100)	5 (25)	NA	5 (56)
Funisitis (grade 1–4) [†]	27 (63)	1 (5)	NA	2 (22)

Abbreviations: IAI, intra-amniotic infection; PTB, preterm birth; LDH, lactate dehydrogenase activity; WBC, white blood cell count; MR, Mass Restricted score

[†]Data presented as median [IQR] and analyzed by Kruskal-Wallis 1-Way Analysis of Variance

^{†*}Data presented as n (%) and analyzed by Chi square test

* $P<0.05$;

** $P<0.001$

Comparative diagnostic performance for prediction of Intra-amniotic infection (IAD) in maternal blood and urine (non-invasive) in the study population.

Table 3

Variable	AUC [95%CI]	Z statistic P value	Optimal cut-off*	Sens. (%) [95%CI]	Spec. (%) [95%CI]	+LR [95%CI]	-LR [95%CI]	PPV (%) [95%CI]	NPV (%) [95%CI]
Maternal blood IL-6	0.626 [0.524–0.721]	2.159 <i>P</i> =0.031	>3.7 pg/mL	75.0 [60.4 – 86.4]	59.6 [45.1 – 73.0]	1.86 [1.3 – 2.7]	0.42 [0.2 – 0.7]	63.2 [49.3 – 75.6]	72.1 [56.3 – 84.7]
Maternal blood CRP	0.692 [0.592–0.781]	3.478 <i>P</i> <0.001	>25.5 µg/mL	79.2 [65.0 – 89.5]	63.5 [49.0 – 76.4]	2.17 [1.5 – 3.2]	0.33 [0.2 – 0.6]	66.7 [52.9 – 78.6]	76.7 [61.4 – 88.2]
Maternal blood PCT	0.553 [0.450–0.653]	1.325 <i>P</i> =0.185	1.2 pg/mL	89.6 [77.3 – 96.5]	23.1 [12.5 – 36.8]	1.16 [1.0 – 1.4]	0.45 [0.2 – 1.2]	51.8 [40.6 – 62.9]	70.6 [44.0 – 89.7]
Urine IL-6	0.567 [0.403–0.720]	0.935 <i>P</i> =0.350	>6.0 pg/mL	54.6 [32.2 – 75.6]	80.0 [56.3 – 94.3]	2.73 [1.0– 7.1]	0.57 [0.3 – 0.9]	75.0 [46.6 – 93.1]	61.5 [40.6 – 79.8]
Urine CRP	0.640 [0.476–0.784]	1.432 <i>P</i> =0.152	>1.7 ng/mL	87.0 [66.4 – 97.2]	40.0 [19.1 – 63.9]	1.45 [1.0 – 2.1]	0.33 [0.1 – 1.1]	62.5 [43.4 – 79.1]	72.7 [39.0 – 94.0]
Urine PCT	0.714 [0.552–0.844]	3.761 <i>P</i> <0.001	>0.0 pg/mL	39.1 [19.7 – 61.5]	100.0 [83.2 – 100.0]	NA	0.61 [0.4 – 0.8]	100 [66.4 – 100]	58.8 [40.7 – 75.4]
Maternal WBC	0.716 [0.617–0.802]	4.171 <i>P</i> <0.001	>13,500 cells/mm ³	70.8 [55.9 – 83.0]	65.4 [50.9 – 78.0]	2.05 [1.4 – 3.1]	0.45 [0.3 – 0.7]	65.4 [50.9 – 78.0]	70.8 [55.9 – 83.0]