

The significance of amniotic fluid immunological analysis for the prediction of intrauterine infection

G. Balciuniene^{1,2,*}, L. Jakubauskiene², G.S. Drasutiene^{1,2}, A. Meskauskiene², D. Ramasauskaite^{1,2}

¹Institute of Clinical Medicine of the Faculty of Medicine of Vilnius University, Clinic of Obstetrics, and Gynaecology, Vilnius (Lithuania)

²Vilnius University Hospital Santaros Klinikos, Center of Obstetrics and Gynaecology, Vilnius (Lithuania)

Summary

Intrauterine infection, a dangerous condition for a pregnant woman and her fetus, increases the risk of neonatal death and the incidence of severe diseases like cerebral palsy, chronic lung illnesses and psychomotor disorders. Better prediction of intrauterine infection would support the choice of an appropriate treatment plan during pregnancy and suitable healthcare for the mother and newborn after birth. Herein, we review the immunological analysis of amniotic fluid for prediction of intrauterine infection and survey advances in the field that are bringing us closer to clinical implementation.

Key words: Intrauterine infection; Chorioamnionitis; Preterm birth.

Introduction

Intrauterine infection, also known as chorioamnionitis, is a dangerous condition for the health of a pregnant woman and her fetus. It complicates about 40%-70% of preterm births with premature rupture of membranes (PROM) or spontaneous labor [1]. Intrauterine infection is associated with fetal inflammatory response syndrome, which leads to severe multiorgan (brain, lungs, kidney, heart) injury and a more than 2.4-fold increase in the risk of neonatal death [2]. If intrauterine infection is suspected, it is recommended to discontinue the pregnancy due to the aforementioned health problems. However, preterm newborns are born morphologically and functionally immature. Fetal respiratory function is the last to mature, so there is a higher risk of neonatal respiratory distress syndrome because of immature lungs in preterm birth [3]. For this reason, the management of preterm premature rupture of membranes requires balancing the benefits of pregnancy prolongation and the risk of intrauterine infection.

Diagnosis of chorioamnionitis is based on the Gibbs criteria, introduced more than 40 years ago. Gibbs criteria consist of maternal fever (> 37.8 °C) and at least two of the following criteria: maternal tachycardia (> 100 beats per minute), maternal leukocytosis (white blood cell count), uterine tenderness, fetal tachycardia (> 160 beats per minute) and foul-smelling amniotic fluid [4]. Many studies have indicated that the conventional criteria are limited because of low sensitivity and specificity for detecting chorioamnionitis [5-7] (Table 1).

Imprecise diagnostic criteria have led to a continued search for more specific methods to detect intrauterine infection. Nowadays, more attention is given to how changes of immunological markers in amniotic fluid can predict

intrauterine infection since many immune system components that protect the fetus against infection can be found in amniotic fluid.

Immunological Markers in Amniotic Fluid

Cytokines are small proteins, secreted by cells, that regulate intracellular functions [8]. Specific membrane receptors mediate their action and activate intracellular pathways. Cytokines also regulate the immune response to infection helping to preserve pregnancy [9]. However, some inflammatory cytokines are responsible for the development of preterm labor.

Interleukins are the group of cytokines that can predict intra-amniotic infection. There are several interleukins that could be associated with the inflammatory process, but interleukin- 1β (IL- 1β) seems to be the dominant one for prediction of preterm as well as term labor associated with infectious progression and can be useful for a diagnosis of intra-amniotic infection [10]. Puchner *et al.* reported that for every increased unit of amniotic fluid IL- 1β women were 7.2 times more likely to deliver preterm [11].

Most IL-6 found in amniotic fluid is produced by the amnion and is released in response to infectious stimuli [12, 13]. When compared to non-laboring women, IL-6 is found to be increased in both preterm and term births. A systematic review revealed that elevated mid-trimester amniotic fluid IL-6 levels were associated with spontaneous preterm birth when no prior symptoms manifested [14]. Some studies analyzed IL-6 levels in cases complicated with preterm PROM. Some of these authors state that IL-6 alone cannot significantly predict intra-amniotic inflammation (IAI) in patients with preterm PROM and are only useful in prediction of preterm labor in cases with intact membranes [15].

Table 1. — Sensitivity and specificity of Gibbs criteria were evaluated by Sung *et al.* [5] and diagnostic performance of each clinical parameter was evaluated by Romero *et al.* [6].

Criteria or clinical parameter	Result suggesting intrauterine infection (chorioamnionitis)	Sensitivity (%)	Specificity (%)
Gibbs criteria [5]	Fever + 2 following criteria	15.3	92.3
Maternal tachycardia [6]	> 100 beats/min	88.0	5.0
Fetal tachycardia [6]	> 160 beats/min	80.0	30.0
Uterus tenderness [6]	Tenderness on palpation	12.0	95.0
White blood cells [6]	> 15,000 cells/mm ³	76.0	30.0
Vaginal discharge [6]	Foul-smelling	8.0	95.0

Table 2. — Cut-off values of immunological markers in amniotic fluid for prediction of intrauterine infection.

Immunological marker	Cut-off value	Sensitivity	Specificity
MMP-8 [24]	Point of care test: > 10 ng/mL	85	72.8
IL-6 [16, 17]	ELISA: > 2.6 ng/mL	90	76.2
	Point of care test: > 745 pg/mL	85.7	64.1
TNF- α [21]	Point of care test: > 1000 pg/mL	85.7	68.9
	ELISA: > 6.3 pg/mL	73.8	70.1
Soluble Toll-like receptor 2 [37]	ELISA: > 222.7 ng/mL	63	98
Human neutrophil defensin 1-3 [32]	ELISA: > 7.8 ng/mL	80	79

Clinicians have suggested an amniotic fluid IL-6 cut-off value ≥ 745 pg/mL for the detection of IAI in patients with preterm PROM [16]. Moreover, an amniotic fluid IL-6 cut-off value $> 1,000$ pg/mL is also useful in the prediction of microbial invasion of the amniotic cavity (MIAC) or histologic chorioamnionitis (HCA) [17] (Table 2).

Tumor necrosis factor- α (TNF- α) is an inflammatory cytokine that plays an important role in the initiation of labor. TNF- α normally is not detected in amniotic fluid during the second and third trimester. This cytokine is important in the pathogenesis of infection-associated preterm labor, while the presence of infection induces the production of this cytokine [18]. TNF- α is also a valuable predictor of chorioamnionitis in cases with preterm PROM [15, 19]. A study by Thomakos *et al.* found that amniotic fluid TNF- α concentration > 6.3 pg/mL could be a good predictive factor for a positive amniotic fluid culture in mid-trimester pregnancy [20] (Table 2).

Matrix metalloproteases (MMP) are a group of extracellular matrix mediators that can be found in amniotic fluid and are responsible for rupture of membranes. Cytokines are mainly responsible for the control of MMP functions [21]. Elevation of MMP-8 concentrations in the second trimester of pregnancy can strongly predict intra-amniotic inflammation, spontaneous preterm delivery and adverse neonatal outcomes in pregnancies complicated with PPROM [21, 22]. Chaemsaitong *et al.* in their study of a rapid test of amniotic fluid MMP-8 found that a cut-off value of 10 ng/mL has enough high sensitivity and specificity (85.7% and 72.8%, respectively) for the detection of IAI [23] (Table 2).

Human beta defensins (HBD) are a group of antimicrobial peptides that are synthesized by epithelial cells and neutrophils [24]. Two main defensins are HBD-2 and HBD-3. They act like attractants facilitating the interaction between the acquired and innate immune system [25]. Moreover, HMB2 has antimicrobial activity against Gram-negative bacteria and, to a lesser extent, Gram-positive bacteria [26]. Both main HBD are found in higher concentrations in pregnancies with intra-amniotic infection [27, 28]. Iavazzo *et al.* investigated HBD-2 in amniotic fluid and their findings demonstrated that HBD-2 was associated with PPROM, but not with preterm labor [29]. The study of Lucovnik *et al.* demonstrated that elevated levels of amniotic fluid neutrophil defensins (HNP1-3) were associated with histological chorioamnionitis and can predict infant death or neurological impairment [30]. The study of Espinoza *et al.* showed that amniotic fluid HNP1-3 levels with a cut-off value of 7.8 ng/mL could be a good predictive factor for MIAC [31] (Table 2).

Soluble Toll-like receptors (sTLRs) are transmembrane receptors that can recognize and respond to microorganisms and also control the activation of an adaptive immune response [32]. These sTLRs can be activated by ligands from many microbes (bacteria, viruses, fungi, parasites). sTLRs-2 is mainly stimulated by lipoproteins and lipopeptides found in the outer membranes of Gram-positive bacteria [33]. Furthermore, sTLRs-2 is a component of amniotic fluid in healthy pregnancies. Its levels increase up to 30 weeks of gestation and decrease thereafter towards term [34]. A study by Kacerovsky *et al.* investigated sTLRs in amniotic fluid and stated that elevated levels of sTLRs, es-

pecially sTLRs-1, sTLRs-2 and sTLRs-6, predicted MIAC [35]. Moreover, sTLRs-2 was found to be a promising predictor of HCA and MIAC in pregnancies complicated by preterm PROM, using a cut-off value of 222.7 ng/mL [36] (Table 2).

Conclusions

Immunological analysis of amniotic fluid could detect intrauterine infection earlier and improve the selection of appropriate management during pregnancy and suitable healthcare for the mother and newborn after birth. More randomized controlled studies are required to find the best predictive markers and determine their cut-off values that then could be introduced to routine clinical practice.

Acknowledgments

Thanks to all the peer reviewers and editors for their opinions and suggestions.

Conflict of Interest

The authors declare no conflict of interest.

Submitted: January 17, 2020

Accepted: June 22, 2020

Published: December 15, 2020

References

- [1] Yoon B.H., Romero R., Moon J.B., Shim S.S., Kim M., Kim G., et al.: "Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes". *Am. J. Obstet. Gynecol.*, 2001, 185, 1130-1136.
- [2] Mittendorf R., Montag A.G., MacMillan W., Janeczek S., Pryde P.G., Besinger R.E., et al.: "Components of the systemic fetal inflammatory response syndrome as predictors of impaired neurologic outcomes in children". *Am. J. Obstet. Gynecol.*, 2003, 188, 1438-1446.
- [3] Zanardo V., Vedovato S., Cosmi E., Litta P., Cavallin F., Trevisanuto D., et al.: "Preterm premature rupture of membranes, chorioamnion inflammatory scores and neonatal respiratory outcome: PPROM, chorioamnionitis and neonatal respiratory outcome". *BJOG.*, 2010, 117, 94-98.
- [4] Gibbs R.S.: "Diagnosis of intra-amniotic infection". *Semin. Perinatol.*, 1977, 1, 71-77.
- [5] Sung J.H., Choi S.J., Oh S.Y., Roh C.R., Kim J.H.: "Revisiting the diagnostic criteria of clinical chorioamnionitis in preterm birth". *BJOG.*, 2017, 5, 775-783.
- [6] Romero R., Chaemsaihong P., Korzeniewski S.J., Kusanovic J.P., Docheva N., Martinez-Varea A., et al.: "Clinical chorioamnionitis at term III: how well do clinical criteria perform in the identification of proven intra-amniotic infection?". *J. Perinat. Med.*, 2016, 44, 23-32.
- [7] Oh K.J., Kim S.M., Hong J.S., Maymon E., Erez O., Panaitescu B., et al.: "Twenty-four percent of patients with clinical chorioamnionitis in preterm gestations have no evidence of either culture-proven intraamniotic infection or intraamniotic inflammation". *Am. J. Obstet. Gynecol.*, 2017, 216, 604.e1-11.
- [8] Zhang J.M., An J.: "Cytokines, Inflammation and Pain". *Int Anesthesiol Clin.*, 2007, 45, 27-37.
- [9] Vrachnis N., Karavolos S., Iliodromiti Z., Sifakis S., Siristatidis C., Mastorakos G., et al.: "Impact of mediators present in amniotic fluid on preterm labour". *In Vivo*, 2012, 26, 799-812.
- [10] Nadeau-Vallée M., Obari D., Quiniou C., Lubell W.D., Olson D.M., Girard S., et al.: "A critical role of interleukin-1 in preterm labor". *Cytokine Growth Factor Rev.*, 2016, 28, 37-51.
- [11] Christiaens I., Zaragoza D.B., Guilbert L., Robertson S.A., Mitchell B.F., Olson D.M.: "Inflammatory processes in preterm and term parturition". *J. Reprod. Immunol.*, 2008, 79, 50-57.
- [12] Le J.M., Vilcek J.: "Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response". *Lab. Investig. J. Tech. Methods Pathol.*, 1989, 61, 588-602.
- [13] Keeelan J., Sato T., Gupta K.D., Marvin W.K., Mitchell D.M.: "Prostanoid stimulation of cytokine production in an amnion-derived cell line: Evidence of a feed-forward mechanism with implications for term and preterm labor". *J. Soc. Gynecol. Investig.*, 2000, 7, 37-44.
- [14] Wei S.Q., Fraser W., Luo Z.C.: "Inflammatory cytokines and spontaneous preterm birth in asymptomatic women: a systematic review". *Obstet. Gynecol.*, 2010, 116, 393-401.
- [15] Kunze M., Klar M., Morfeld C.A., Thorns B., Schild R.L., Markfeld-Erol F., et al.: "Cytokines in noninvasively obtained amniotic fluid as predictors of fetal inflammatory response syndrome". *Am. J. Obstet. Gynecol.*, 2016, 215, 96.e1-8.
- [16] Cobo T., Palacio M., Martínez-Terrón M., Navarro-Sastre A., Bosch J., Filella X., et al.: "Clinical and inflammatory markers in amniotic fluid as predictors of adverse outcomes in preterm premature rupture of membranes". *Am. J. Obstet. Gynecol.*, 2011, 205, 126.e1-8.
- [17] Kacerovsky M., Musilova I., Stepan M., Andrys C., Drahosova M., Jacobsson B.: "Detection of intraamniotic inflammation in fresh and processed amniotic fluid samples with the interleukin-6 point of care test". *Am. J. Obstet. Gynecol.*, 2015, 213, 435-436.
- [18] Hillier S.L., Witkin S.S., Krohn M.A., Watts D.H., Kiviat N.B., Eschenbach D.A.: "The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection". *Obstet. Gynecol.*, 1993, 81, 941-948.
- [19] Weiyuan Z., Li W.: "Study of interleukin-6 and tumor necrosis factor-alpha levels in maternal serum and amniotic fluid of patients with premature rupture of membranes". *J. Perinat. Med.*, 1998, 26, 491-494.
- [20] Thomakos N., Daskalakis G., Papapanagioutou A., Papantoniou N., Mesogitis S., Antsaklis A.: "Amniotic fluid interleukin-6 and tumor necrosis factor-a at mid-trimester genetic amniocentesis: Relationship to intra-amniotic microbial invasion and preterm delivery". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2010, 148, 147-151.
- [21] Yoon B.H., Oh S.Y., Romero R., Shim S.S., Han S.Y., Park J.S., et al.: "An elevated amniotic fluid matrix metalloproteinase-8 level at the time of mid-trimester genetic amniocentesis is a risk factor for spontaneous preterm delivery". *Am. J. Obstet. Gynecol.*, 2001, 185, 1162-1167.
- [22] Weiss A., Goldman S., Shalev E.: "The matrix metalloproteinases (MMPS) in the decidua and fetal membranes". *Front. Biosci. J. Virtual. Libr.*, 2007, 12, 649-659.
- [23] Chaemsaihong P., Romero R., Docheva N., Chaiyasit N., Bhatti G., Pacora P., et al.: "Comparison of rapid MMP-8 and interleukin-6 point-of-care tests to identify intra-amniotic inflammation/infection and impending preterm delivery in patients with preterm labor and intact membranes". *J. Matern. Fetal. Neonatal. Med.*, 2018, 31, 228-244.
- [24] Hancock R.E., Diamond G.: "The role of cationic antimicrobial peptides in innate host defences". *Trends Microbiol.*, 2000, 8, 402-410.
- [25] Garcia J.R., Jaumann F., Schulz S., Krause A., Rodriguez-Jimenez J., Forssmann U., et al.: "Identification of a novel, multifunctional beta-defensin (human beta-defensin 3) with specific antimicrobial activity. Its interaction with plasma membranes of *Xenopus* oocytes and the induction of macrophage chemoattraction". *Cell Tissue Res.*, 2001, 306, 257-264.
- [26] Harder J., Bartels J., Christophers E., Schroder J.M.: "A peptide antibiotic from human skin". *Nature*, 1997, 387, 861.
- [27] Heine R.P., Wiesenfeld H., Mortimer L., Greig P.C.: "Amniotic fluid defensins: potential markers of subclinical intrauterine infection". *Clin. Infect. Dis.*, 1998, 27, 513-518.
- [28] Soto E., Espinoza J., Nien J.K., Kusanovic J.P., Erez O., Richani K., et al.: "Human β -defensin-2: A natural antimicrobial peptide present in amniotic fluid participates in the host response to microbial invasion of the amniotic cavity". *J. Matern. Fetal Neonatal. Med.*, 2007, 20, 15-22.
- [29] Iavazzo C., Tassis K., Gourgiotis D., Boutsikou M., Baka S., Hasiakos D., et al.: "The role of human beta defensins 2 and 3 in the second trimester amniotic fluid in predicting preterm labor and premature rupture of membranes". *Arch. Gynecol. Obstet.*, 2010, 281, 793-799.

- [30] Lucovnik M., Kornhauser-Cerar L., Premru-Srsen T., Gmeiner-Stopar T., Dergan M.: "Neutrophil defensins but not interleukin-6 in vaginal fluid after preterm premature rupture of membranes predict fetal/neonatal inflammation and infant neurological impairment". *Acta. Obstet. Gynecol. Scand.*, 2011, 90, 908-916.
- [31] Espinoza J., Chaiworapongsa T., Romero R., Edwin S., Rathnasabapathy C., Gomez R., *et al.*: "Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes". *J. Matern. Fetal Neonatal. Med.*, 2003, 13, 22-21
- [32] Gay N.J., Gangloff M.: "Structure and function of toll receptors and their ligands". *Annu. Rev. Biochem.*, 2007, 76, 141-165.
- [33] Oliveira-Nascimento L., Massari P., Wetzler L.M.: "The role of TLR2 in infection and immunity". *Front. Immunol.*, 2012, 3, 79.
- [34] Dulay A.T., Buhimschi C.S., Zhao G., Oliver E.A., Mbele A., Jing S., *et al.*: "Soluble TLR2 is present in human amniotic fluid and modulates the intraamniotic inflammatory response to infection". *J. Immunol.*, 2009, 182, 7244-7253.
- [35] Kacerovsky M., Andrys C., Drahosova M., Musilova I., Hornychova H., Lesko D., *et al.*: "Soluble Toll-like receptor 1 family members in the amniotic fluid of women with preterm prelabor rupture of the membranes". *J. Matern. Fetal Neonatal. Med.*, 2012, 25, 1699-1704.
- [36] Andrys C., Kacerovsky M., Drahosova M., Musilova I., Pliskova L., Hornychova H., *et al.*: "Amniotic fluid soluble Toll-like receptor 2 in pregnancies complicated by preterm prelabor rupture of membranes". *J. Matern. Fetal Neonatal. Med.*, 2013, 26, 520-527.

Corresponding Author:

GRETA BALCIUNIENE, M.D., Ph.D.

Institute of Clinical Medicine of the Faculty of Medicine of Vilnius University,

Clinic of Obstetrics, and Gynaecology, Santariskiu st. 2, Vilnius (Lithuania)

e-mail: greta.balciuniene@gmail.com