

## Cervical and intra-amniotic markers of preterm birth and infection

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av

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Legitimerad läkare

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Holst R-M, Mattsby-Baltzer I, Wennerholm U-B, Hagberg H, Jacobsson B.  
Interleukin-6 and interleukin-8 in cervical fluid in a population of Swedish women in preterm labor: relationship to microbial invasion of the amniotic fluid, intra-amniotic inflammation, and preterm delivery.  
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Holst R-M, Jacobsson B, Hagberg H, Wennerholm U-B.  
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Holst R-M, Laurini R, Jacobsson B, Samuelsson E, Sävman K, Doverhag C, Wennerholm U-B, Hagberg H.  
Expression of cytokines and chemokines in cervical and amniotic fluid: Relationship to histological chorioamnionitis. J  
*Matern Fetal Neonatal Med* 2007; 20(12): 885-893

Holst R-M, Hagberg H, Wennerholm U-B, Skogstrand K, Thorsen P, Jacobsson B. Prediction of spontaneous preterm delivery in women with preterm labor – analysis of 27 proteins by Luminex xMAP technology in amniotic and cervical fluids.  
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Holst R-M, Hagberg H, Wennerholm U-B, Skogstrand K, Thorsen P, Jacobsson B.  
Prediction of microbial invasion of the amniotic cavity in women with preterm labor based on analysis of multiple proteins in amniotic and cervical fluids with Luminex xMAP technology.  
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# Cervical and intra-amniotic markers of preterm birth and infection

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## Abstract

**Background:** Preterm delivery (PTD; < 37 gestational weeks), is one of the greatest unsolved obstetrical problems worldwide. As much as 80% of the perinatal mortality and 50% of the long-term neurological handicaps are associated with PTD. Spontaneous preterm birth (SPTD), i.e. preterm labor (PTL) or preterm prelabor rupture of membranes (PPROM) is responsible for 55% of PTD. Clinical and experimental evidence suggest that maternal infection and/or inflammation are centre stages in SPTD and the major risk factors for fetal injury. Several cytokines and chemokines play a central role in SPTD. However, in most cases the precise mechanistic pathway leading to SPTD remains unknown and good markers of prediction and therapies are few.

**Aim:** To investigate if cervical and intra-amniotic proteins on their own and/or in combination with each other and/or with clinical characteristics could predict SPTD and intra-uterine infection/inflammation in women with singleton pregnancies in PTL. In particular the purpose was to investigate the predictive value of cervical markers (proteins or sonography) collected less invasively compared with amniotic fluid proteins collected via amniocentesis.

**Material and methods:** A cohort of 134 women in PTL and 30 with PPROM with singleton pregnancies and gestational age less than 34 weeks were studied. Amniotic fluid (AF) was retrieved transabdominally from 107 patients in PTL and in 30 patients with PPROM. Cervical fluid (CF) was sampled from the external cervical os in all PTL women, but from none of the PPROM cases. Transvaginal sonography (TVS) assessing cervical length (CL) was performed in all patients. Polymerase chain reaction analyses for *Ureaplasma urealyticum* and *Mycoplasma hominis* and culture for aerobic and anaerobic bacteria were performed. Interleukin (IL)-6, IL-8, IL-18, monocyte chemoattractant protein (MCP)-1, MCP-2, and MCP-3 were analyzed with enzyme-linked immunosorbent assay. The multiplex sandwich immunoassay, flowmetric Luminex xMAP (multiple analyte profiling) technology analyzed 27 specific proteins, IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, sIL-6 $\alpha$ , IFN- $\gamma$ , TNF- $\alpha$ , TNF- $\beta$ , MCP-1, TGF- $\beta$ , MIP-1 $\alpha$ , MIP-1 $\beta$ , MMP-9, TREM-1, BDNF, GM-CSF, NT-4, NT-3, sTNF RI, MIF and RANTES. Histological examinations of the placentas were performed in 42 cases in PTL and in 30 with PPROM. Maternal, antenatal and intrapartur variables were retrieved from medical records

**Results:** Non-lacto-bacillus dominated flora was detected in CF in 25% (22/89) and 17% had microbial invasion of the amniotic cavity (MIAC) and 45% had intra-amniotic inflammation. High levels of IL-6 and IL-8 were associated with PTD  $\leq$  7 days from assay and  $\leq$  34 weeks of gestation. Cervical length assessed by TVS predicted intra-amniotic inflammation as well as PTD. Intra-amniotic levels of IL-6, IL-8, IL-18, MCP-1 and MCP-3 were all significantly higher in PTL cases with histological chorioamnionitis (HCA) whereas such relationship was not found in the PPROM group. Cervical IL-6 and IL-8 in PTL were associated HCA and an IL-8 value of 10.0 ng/mL was a strong predictor of HCA with sensitivity 100%, specificity 67%, positive predictive value 63%, and negative predicted value 100%.

Several of the proteins analyzed in both AF and CF, by the xMAP technology, were associated with PTD  $\leq$  7 days from assay and MIAC. Novel findings were that amniotic IL-17 and TREM1 and cervical IL-17, sIL-6 $\alpha$ , BDNF, NT4, NT3, IL-4, IL-5, and RANTES were significantly higher in the women delivering within 7 days of assay. We found that cervical IL-17, sIL-6 $\alpha$ , NT3, TNF- $\beta$ , IL-4, and TREM1 were significantly associated with MIAC which has not previously been reported. A multivariate model combining amniotic macrophage inflammatory protein (MIP)-1 $\beta$  with cervical interferon (INF)- $\gamma$  and MCP-1 predicted SPTD  $\leq$  7 days likelihood ratio (LR) 5.6 and area under the ROC-curve (AUC) 0.91 and a non-invasive multivariate model based on CL, cervical INF- $\gamma$ , IL-6 and MCP-1 predicted SPTD  $\leq$  7 days with LR 4.7 and AUC 0.91. The best multivariate model predicting MIAC based on cervical IL-17 and MCP-1 had LR 6.0 and AUC 0.87.

**Conclusions:** In the present studies, we have identified inflammatory markers in both cervical and amniotic fluid that together with cervical length as measured by transvaginal sonography can predict spontaneous preterm delivery, intraamniotic infection and/or inflammation and histological chorioamnionitis. It seems as the non-invasive route of sampling analytes can be used instead of the more commonly used invasive method of amniocentesis.

**Key words:** Spontaneous preterm delivery, preterm labor, preterm prelabor rupture of membranes, intra-amniotic infection/inflammation, inflammatory proteins, histological chorioamnionitis, cervical and amniotic markers.