

Biomarkers in mid-trimester amniotic fluid in relation to gestational duration and spontaneous preterm delivery

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet, kommer att offentlig försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, fredagen den 5 juni 2020 klockan 09.00

av Maria Hallingström

Fakultetsopponent: Helena Strevens, överläkare och PhD, Medicinska fakulteten, Institutionen för kliniska vetenskaper, Avdelningen för obstetrik och gynekologi, Lunds universitet, Lund, Sverige

Avhandlingen baseras på följande delarbeten

- I. Hallingström M, Cobo T, Kacerovský K, Skogstrand K, Hougaard DM, Holst RM, Tsiartas P, Bullarbo M, Carlsson Y, Nilsson S, Jacobsson B. The association between selected mid-trimester amniotic fluid candidate proteins and spontaneous preterm delivery. *J Matern Fetal Neonatal Med.* 2018 Sep 10:1-10.
- II. Hallingström M, Lenčo J, Vajrychová M, Link M, Tambor V, Liman V, Bullarbo M, Nilsson S, Tsiartas P, Cobo T, Kacerovský M, Jacobsson B. Proteomic analysis of early mid-trimester amniotic fluid does not predict spontaneous preterm delivery. *PLoS One.* 2016 May 23;11(5).
- III. Hallingström M*, Zedníková P*, Tambor V, Barman M, Vajrychová M, Lenčo J, Viklund F, Tancred L, Rabe H, Jonsson D, Kachikis A, Nilsson S, Kacerovský M, Adams Waldorf K, Jacobsson B. Mid-trimester amniotic fluid proteome's association with spontaneous preterm delivery and gestational duration. Accepted for publication in *PLoS One*, April 2020.
- IV. Viklund F*, Hallingström M*, Kacerovský M, Cobo T, Skogstrand K, Hougaard D M, Sävman K, Carlsson Y, Tsiartas P, Juodakis J, Nilsson S, Jacobsson B. Protein concentrations of thrombospondin-1, MIP-1 β and S100A8 suggest the reflection of a pregnancy clock in mid-trimester amniotic fluid. Revision submitted to *Reproductive Sciences*, April 2020.
- V. Hallingström M*, Barman M*, Savolinen O, Viklund F, Kacerovský M, Brunius C*, Jacobsson B*. Metabolomic profiles of mid-trimester amniotic fluid are not associated with subsequent spontaneous preterm delivery or gestational duration at delivery. Revision submitted to *Journal of Maternal-Fetal & Neonatal Medicine*, April 2020.

(*Equal contribution)

**SAHLGRENKA AKADEMIN
INSTITUTIONEN FÖR KLINISKA VETENSKAPER**



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Maria Hallingström

Avdelningen för Obstetrik och Gynekologi, Institutionen för Kliniska Vetenskaper, Sahlgrenska akademien, Göteborgs Universitet, Sverige, 2020

Abstract

Background: The biological mechanisms and physiological pathways of pregnancy maintenance and timing of delivery are complex and multifactorial. Pregnancy clocks, partly controlled by timing mechanisms linked to fetal development, which regulate the onset of labor has previously been described. These clocks include inflammatory processes, involving endocrine, mechanical and genetic factors. However, the sequence and timing of events preceding the spontaneous onset of labor, both at term and at preterm, are as yet incompletely identified. Spontaneous preterm delivery, defined as delivery before 37 weeks of gestation, is a serious global health problem accounting for the majority of all perinatal deaths and half of the short- and long-term postnatal morbidity. Identifying women at risk of spontaneous preterm delivery is complicated by its heterogeneous etiology and several different sub-phenotypes. Mid-trimester amniocentesis, clinically performed for prenatal genetic testing, provides a unique opportunity to obtain insight into the intrauterine environment in asymptomatic women early in gestation. However, the complex and dynamic composition of amniotic fluid changes continually as pregnancy progresses, making early identification of factors involved in the process of spontaneous preterm delivery and other pregnancy complications, a major challenge.

Objective: The aim of this thesis and its constituent papers was to identify specific biomarkers related to the development of subsequent spontaneous preterm delivery, by examination of mid-trimester amniotic fluid composition in asymptomatic women. During the period of doctoral studies, new data emerged, indicating that a shift to gestational duration as the main outcome might increase the likelihood of finding associations that could assist in the prediction of spontaneous preterm delivery. The aim thus partly shifted toward investigating associations between mid-trimester amniotic fluid composition and gestational duration.

Material and methods: All constituent papers in this thesis are based on subsets of a single cohort of 1,240 amniotic fluid samples collected from asymptomatic women aged over 18 years with a singleton viable pregnancy, intact membranes, without preterm labor or signs of infection, undergoing genetic amniocentesis at gestational weeks 14-19 at Sahlgrenska University Hospital/Östra, Gothenburg, Sweden during September 2008 to December 2017. Demographics and clinical data were obtained from medical records at inclusion and after delivery. Studies investigating inflammatory, immuno-logical and cellular-metabolic markers were designed to contribute to early identification of women with subsequent spontaneous preterm delivery and to study associations with gestational duration. Amniotic fluid samples were analyzed with targeted hypothesis-driven approaches using multiplex technologies such as Lminex xMAP and Meso-Scale Discovery, as well as with broad, untargeted hypothesis-generating approaches such as proteomics and metabolomics. The proteomics analyses were followed by validation/replication with Enzyme-Linked Immunosorbent Assay, a singleplex technology.

Results: No mid-trimester amniotic fluid biomarkers associated with spontaneous preterm delivery were identified. Thrombospondin-1, macrophage inflammatory protein-1 beta and S100 calcium-binding protein A8, two alarmins and one chemokine, were found to be significantly associated with gestational duration in women with a spontaneous onset of labor at term. Gestational age at sampling was strongly associated with protein concentrations in several of the constituent studies.

Conclusions: I) Biological signals in early mid-trimester amniotic fluid may be of insufficient strength for accurate risk prediction of spontaneous PTD, or the condition may result from acute events not detectable in amniotic fluid as early as at mid-trimester; II) Alarmins and chemokines, which seem to play an essential role in the inflammatory processes preceding the spontaneous onset of labor at term, can be detected in amniotic fluid as early as in the mid-trimester; III) The concept of a pregnancy clock is strengthened by our findings, which also suggest that this is reflected in the amniotic fluid, where deviations from the clock may precede spontaneous preterm delivery; and IV) The results emphasize the importance of adjusting for gestational age at sampling when performing amniotic fluid biomarker studies.

Keywords: amniotic fluid, biomarkers, cytokine, damage-associated molecular pattern, gestation, gestational duration, inflammation, labor, mid-trimester, multiplex, pregnancy clock, proteins, spontaneous preterm delivery, term delivery