

Discriminative capacity of ¹H MRS spectrum binning for preterm delivery-associated Lactobacilli-dominated vaginal microbiota

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Introduction:

Identification of vaginal microbiota community state types (CSTs) and concentrations of metabolites, e.g. lactate, glutamate, glucose, may offer better identification of asymptomatic women at risk of preterm delivery (PTD). Therefore, we sought to determine whether spectrum binning of proton magnetic resonance spectroscopy (¹H MRS) of cervicovaginal fluid (CVF) metabolite spectra improves the determination of specific vaginal microbiota CSTs and the prediction of PTD.

Methods:

¹H MRS spectra of CVF from 301 asymptomatic women (studied at 20⁺⁰- 22⁺⁶ weeks gestation) were analysed using Matlab. A subset of CVF from 91 women were analyzed for the dominant vaginal bacterial species (CST) by amplifying and sequencing the V1-V3 region of the 16S rRNA gene. The ability of ¹H MRS spectrum binning to discriminate between preterm and term outcomes and CSTs was performed by 2-way ANOVA, Receiver Operator Characteristics curve, and PLS-DA.

Results:

From the total cohort 250 women delivered at term and 51 preterm. Women with *L. jensenii*-dominated CST V had significantly higher PTD rate (53.8%) compared to the total cohort (17%, $p=0.004$, OR=5.7 (CI=2.0-18.1)), and women with *L. crispatus*-dominated CST I (7.4%, $p=0.002$, OR=14.6 (CI=2.6-75.9)). Generally, CST I demonstrated higher spectrum bins containing lactate (1.28 ppm, 1.30 ppm and 4.11 ppm, $p<0.0001$) and glutamate peaks (2.38 ppm, $p = 0.02$) while CST V demonstrated higher glucose peaks (3.67 ppm and 3.69 ppm, $p<0.05$). Additionally, the spectrum bin at 1.32 ppm, (lactate peak), was predictive of PTD in both the CST I (AUC=0.98, CI=0.93-1.00) and CST V groups (AUC=0.78, CI=0.52-1.00). Furthermore, women with CST I were discriminated from those with CST V by the spectrum bins containing the lactate: 1.28 ppm (AUC=0.84, CI=0.69-0.94), 1.30 ppm (AUC=0.83, CI=0.67-0.93), 4.11 ppm (AUC=0.85, CI=0.70-0.94), glutamate peaks: 2.83 ppm (AUC=0.76, CI=0.60-0.88), and a combination of lactate and glutamate spectrum bins (AUC=0.90, CI=0.76-0.97). Though there were significant differences in the bins containing lactate and glucose peaks in the preterm vs. term women with CST II (*L. gasseri*) and CST III (*L. iners*) dominated microbiota, none showed a good predictive capacity for PTD.

Conclusions:

¹H MRS spectrum binning improves the determination of specific vaginal microbiota CSTs from CVF metabolite spectra and the prediction of PTD. *L. crispatus* appears to be more frequently associated with stable vaginal microbiota, absence of inflammation and healthy pregnancy outcome, whereas *L. jensenii* appears to be associated with PTD in approximately 50% of cases. The consequences of the functional activities of these species in relation to the host as indicated by their relative abundance and metabolic by-products can be detected by metabolite profiling of CVF in clinical, research and low-income settings.