Preterm Birth Prediction: Association of Cervical Electrical Impedance Spectroscopy and Cervicovaginal Metabolite Composition of Women at High-risk

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Background

Preterm birth (PTB, delivery before 37 weeks) remains a major global health challenge: 15 million babies are born prematurely annually (approximately 60000 in the UK). It costs about £1 billion to care for preterm babies due to increasing morbidity and disabilities with lifelong consequences. Cervical Electrical impedance spectroscopy (EIS) is a potential clinically useful alternative to the currently employed methods: cervical ultrasound and fetal fibronectin measurements that are less effective in women without symptoms of preterm labour. Several metabolites are produced by vaginal bacteria (microflora) in collaboration with the host cells, which can determine the risk of infection and PTB. This proposal aims to analyse in depth the correlations between cervical EIS and cervicovaginal microbial-metabolite of asymptomatic pregnant women at high risk of PTB employing statistical hypothesis testing methods.

Methods

- Data from the ECCLIPPX study comprising of a cohort of 297 pregnant women (preterm = 44) was analysed. The data consisted of EIS obtained using the Sheffield Mark 5.0 device, which passes an AC at 14 frequencies through the cervix, and cervicovaginal fluid metabolites obtained by nuclear magnetic resonance spectroscopy (1H-NMR).
- The distributions of the EIS and metabolite features were analysed using histograms.
- Then, the significance of the EIS and metabolite features in predicting PTB was analysed using 2-sample t-test and Mann-Whitney U test.
- Finally, the significant associations between EIS and metabolite features were analysed using Pearson's correlation and the Apriori association rule algorithm. The p-values of the associations were computed using Chi-Squared test and Fisher's exact test with the significance level 0.05 and the strength of associations measured using Cramer's V.

Results I

The distributions of the EIS and metabolite features followed a non-normal distribution, according to visual examination and Shapiro-Wilk tests for normality ($p < 0.05$) (Fig.1 and Fig.2).

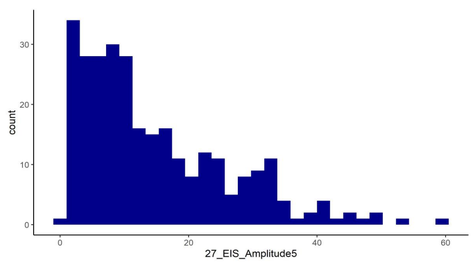

Fig. 1. Distribution of frequency 5 amplitude values (EIS).

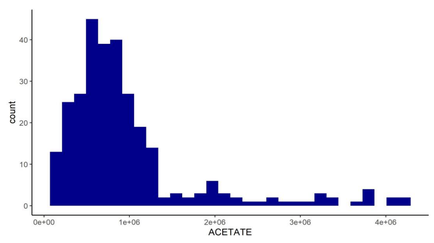

Fig. 2. Distribution of acetate normalised integrals.

Significant differences were seen in 19 EIS features (Mann-Whitney U tests and t-tests, $p < 0.05$) and none for metabolites ($p > 0.05$) (Fig.3 and Fig.4)

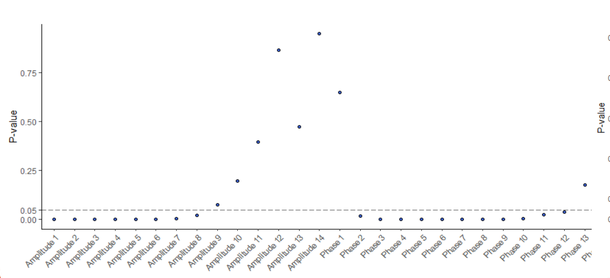

Fig. 3. P-value of EIS features as computed by Mann-Whitney U tests and T-tests. The dotted line shows the significance level of the P-value at 0.05.

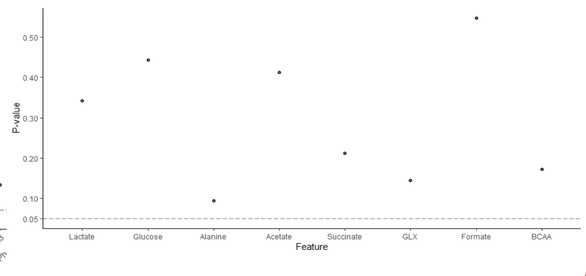

Fig. 4. P-value of metabolite features as computed by Mann-Whitney U tests. The dotted line shows the significance level of the P-value at 0.05.

Results II

Correlation analysis was carried out between metabolite and EIS features, with Succinate, Acetate and Glucose showing the most significant coefficients (Fig. 5). The number of significant associations found using the Apriori algorithm and tested with Chi-Squared or Fisher's exact tests ($p < 0.05$) are shown in Fig.6.

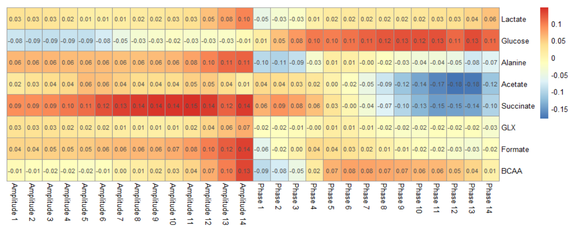

Fig. 5. Correlation matrix between EIS and metabolite features, showing Pearson's correlation coefficients.

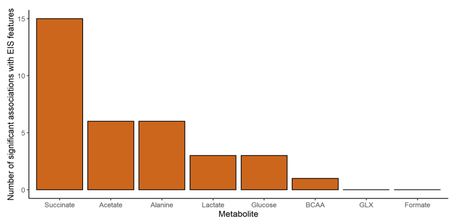

Fig. 6. Bar chart representing number of significant association (y-axis) for each metabolite (x-axis).

Table 1 shows these significant associations between the discretised values of EIS and metabolite features.

Metabolite Features	Association Rules (P-value, Cramer's V)
Succinate	1. Amplitude5=(-∞,20.772367] => SUCCINATE=(-∞,634855.333333] (0.004, 0.16) 2. Amplitude6=(-∞,19.4897] => SUCCINATE=(-∞,634855.333333] (0.013, 0.15) 3. Amplitude5=(-∞,20.772367] and Amplitude6=(-∞,19.4897] => SUCCINATE=(-∞,634855.333333] (0.008, 0.15) 4. Amplitude7=(-∞,15.025433] => SUCCINATE=(-∞,634855.333333] (0.012, 0.15) 5. Amplitude6=(-∞,19.4897] ==> SUCCINATE=(-∞,634855.333333] (0.013, 0.15) 6. Amplitude6=(-∞,19.4897] and Amplitude7=(-∞,15.025433] ==> SUCCINATE=(-∞, 634855.333333] (0.012, 0.15) 7. Amplitude2=(-∞,28.5559] ==> SUCCINATE=(-∞,634855.333333] (0.04, 0.13) 8. Amplitude1=(-∞,28.265867] ==> SUCCINATE=(-∞,634855.333333] (0.045, 0.13) 9. Amplitude1=(-∞,28.265867] and Amplitude2=(-∞,28.5559] ==> SUCCINATE=(-∞,634855.333333] (0.04, 0.13) 10. Phase11=(-22.8283,∞) and Phase7=(-19.871567,∞) ==> SUCCINATE=(-∞,634855.333333] (0.03, 0.13) 11. Phase8=(-20.8575,∞) and Phase11=(-22.8283,∞) ==> SUCCINATE=(-∞,634855.333333] (0.01, 0.14) 12. Phase8=(-20.8575,∞) and Amplitude9=(-∞,6.845567] and Phase11=(-22.8283,∞) ==> SUCCINATE=(-∞,634855.333333] (0.02, 0.14) 13. Phase11=(-22.8283,∞) ==> SUCCINATE=(-∞,634855.333333] (0.01, 0.14) 14. Phase8=(-20.8575,∞) and Amplitude9=(-∞,6.845567] ==> SUCCINATE=(-∞,634855.333333] (0.02, 0.13) 15. Phase8=(-20.8575,∞) ==> SUCCINATE=(-∞,634855.333333] (0.01, 0.14)
Acetate	1. Amplitude1=(-∞,28.265867] and Amplitude4=(-∞,21.413] ==> ACETATE=(-∞,1464358] (0.025,0.14) 2. Amplitude2=(-∞,28.5559] and Amplitude4=(-∞,21.413] ==> ACETATE=(-∞,1464358] (0.039, 0.13) 3. Amplitude1=(-∞,28.265867] and Amplitude2=(-∞,28.5559] and Amplitude4=(-∞,21.413] ==> ACETATE=(-∞,1464358] (0.031, 0.14) 4. Amplitude1=(-∞,28.265867] ==> ACETATE=(-∞,1464358] (0.016, 0.15) 5. Amplitude1=(-∞,28.265867] and Amplitude2=(-∞,28.5559] ==> ACETATE=(-∞,1464358] (0.029, 0.15) 6. Amplitude1=(-∞,28.265867] and Amplitude4=(-∞,21.413] and Amplitude5=(-∞,20.772367] ==> ACETATE=(-∞,1464358] (0.25, 0.14)
Alanine	1. Amplitude9=(-∞,6.845567] and Amplitude5=(-∞,20.772367] ==> ALANINE=(-∞,587480.333333] (0.024, 0.15) 2. Amplitude9=(-∞,6.845567] and Phase9=(-24.022333,∞)==> ALANINE=(-∞,587480.333333] (0.025, 0.14) 3. Amplitude9=(-∞,6.845567] ==> ALANINE=(-∞,587480.333333] (0.004, 0.16) 4. Amplitude5=(-∞,20.772367] and Amplitude6=(-∞,19.4897] and Amplitude9=(-∞,6.845567] ==> ALANINE=(-∞,587480.333333] (0.02, 0.15) 5. Amplitude5=(-∞,20.772367] and Amplitude8=(-∞,10.663133] and Amplitude9=(-∞,6.845567] ==> ALANINE=(-∞,587480.333333] (0.032, 0.14) 6. Amplitude8=(-∞,10.663133] and Amplitude9=(-∞,6.845567] ==> ALANINE=(-∞,587480.333333] (0.027, 0.14)
Lactate	1. Amplitude2=(-∞,78.483933] => LACTATE=(-∞,26662145.666667] (0.022, 0.25) 2. Amplitude3=(-∞,72.047] => LACTATE=(-∞,26662145.666667] (0.022, 0.25) 3. Amplitude2=(-∞,78.483933] and Amplitude3=(-∞,72.047] => LACTATE=(-∞,26662145.666667] (0.022, 0.25)
Glucose	1. Phase11=(-45.1263,-22.8283] ==> GLUCOSE=(-∞,153224.333333] (0.009, 0.15) 2. Phase10=(-48.9336,-24.8443] and Phase11=(-45.1263,-22.8283] ==> GLUCOSE=(-∞,153224.333333] (0.029, 0.14) 3. Phase10=(-48.9336,-24.8443] ==> GLUCOSE=(-∞,153224.333333] (0.049, 0.13)
BCAA	1. Amplitude14=(1.525767,2.135133] ==> BCAA=(-∞,3181598.333333] (0.014, 0.15)
GLX	None
Formate	None

Table. 1. Significant associations between EIS and metabolite features.

Conclusions

This works shows that Succinate, Acetate, Alanine, Lactate, Glucose and BCAA have association with EIS features with Succinate having the largest association with EIS features. Next, we will relate these associations to the structure and function of the pregnant cervix and vagina - to inform future studies and biomarker discovery. Machine learning algorithms will be used to predict PTB based on the most associated metabolite features.

Acknowledgements

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The University Of Sheffield.

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