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P1500 2,3-DIPHOSPHOGLYCERATE DETECTION VIA DIRECT INFUSION HIGH RESOLUTION MASS SPECTROMETRY CORRELATES WITH QUANTITATIVE DETECTION IN BLOOD OF PATIENTS WITH SICKLE CELL DISEASE

Topic: 26. Sickle cell disease

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Background: Sickle cell disease (SCD) is a hereditary and chronic life-threatening disorder, characterized by haemolytic anaemia. Increased 2,3-diphosphoglycerate (2,3-DPG) concentrations, along with decreased oxygen affinity of hemoglobin, may be related to the variability of clinical outcomes in SCD. Furthermore, genomic health data holds promise to improve the prediction of disease severity in SCD. Based on the integration of genomics, metabolomics and clinical data from 1000 SCD patients, to be included in 2022, GenoMED4all aims to develop Artificial Intelligence (AI) based deep learning algorithms to improve the prediction of disease severity and phenotype in SCD.

Aims: To correlate non-quantitative metabolomics data obtained from dried blood spots (DBS), one of the inputs for GenoMED4all, to quantitative measurement of 2,3-DPG. Aiming to improve the potential of non-quantitative metabolomics from DBS to asses 2,3-DPG.

Methods: In snap frozen blood samples from 37 SCD patients and 29 healthy controls, 2,3-DPG was quantified by liquid chromatography mass spectrometry. 2,3-DPG was also detected in DBS from the same subjects by direct infusion high resolution mass spectrometry (DIHRMS). The oxygen tension at 50% Hb saturation (p50)was determined using a Hemox Analyzer (TCS). Statistical analysis were performed by Spearman's correlation coefficients (SPSS v26.0.0.1) and Mann Whitney testing (GraphPad Prism v9.3.0).

Results: After correcting for Hb, 2,3-DPG concentrations were higher in SCD patients than in controls ($p<0.001$) and Z-scores for 2,3-DPG, as assessed by DIHRMS, were similar in patients and controls. The Z-scores positively correlated with 2,3-DPG concentrations (Fig.1A, 0.353, $p=0.004$). Because of the anaemia in SCD, RBCs and plasma make up lower and higher volumes in the DBS, respectively, compared to healthy controls. DIHRMS detects a wide range of RBC and plasma metabolites, whereas the quantitative measurement is restricted to measuring 2,3-DPG of RBCs. To correct for those differences between methods, we applied a correction factor to the DIHRMS data using the formula (1/Ht)*(1-Ht/1), correcting for the RBC volume (1/Ht) and the plasma volume (1-Ht/1). This resulted in a trend towards higher Z-scores in patients than controls ($p=0.0597$). Moreover, the positive correlation with 2,3-DPG concentrations increased to 0.526 (Fig1B, $p<0.001$). As 2,3-DPG affects the oxygen affinity of Hb, all measurements were correlated to p50. Expectedly, 2,3-DPG concentrations positively correlated with p50 (0.842, $p<0.001$). After applying the correction factor to the DIHRMS data, p50 correlations increased from 0.361 ($p=0.003$) to 0.529 ($p<0.001$).

Image:

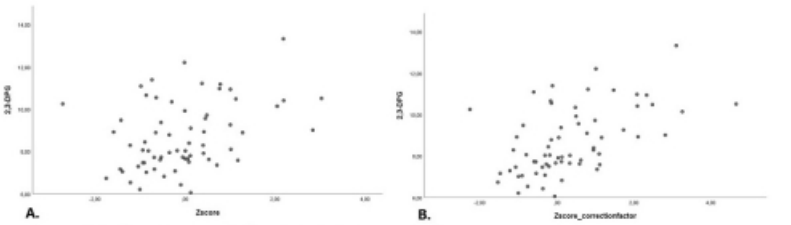

Fig 1. correlations between non-quantitative and quantitative 2,3-DPG detection
A) non-quantitative (Zscore) and quantitative 2,3-DPG detection (2,3-DPG) (0.353, p=0.004) B) non-quantitative 2,3-DPG detection corrected for RBC and plasma volume (Zscore_correctionfactor) and quantitative 2,3-DPG detection (2,3-DPG) (0.526, p<0.001).

Summary/Conclusion: Strongest correlation between quantitative and non-quantitative methods for 2,3-DPG detection were observed after correcting for both the RBC and plasma volume in non-quantitative metabolomics. After correction, DIHRMS can be used to assess 2,3-DPG concentrations in DBS. This translation of non-quantitative to quantitative metabolomics for 2,3-DPG and potentially other RBC metabolites adds significant value to the use of DIHRMS, as DIHRMS is far more efficient in obtaining extensive information about the total metabolome than quantitative methods. The large population size and the AI based deep learning algorithms of GenoMED4all will enable to evaluate the potential of non-quantitative metabolomics in SCD.

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