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A new biochemical assay to measure plasmalogens with CLIR disease differentiation

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Introduction: The biochemical diagnosis of peroxisomal disorders, including rhizomelic chondrodysplasia punctata (RCDP) and Zellweger spectrum disorders (ZSD), requires the quantitation of C16-C18 plasmalogens (PG) and fatty acids (FA). Plasmalogen analysis in erythrocytes (RBC) is well established, but measurement in dried blood spots (DBS) could be useful for critically ill infants, symptomatic patients in remote areas, and as a 2nd tier test in newborn screening for X-linked adrenoleukodystrophy (X-ALD) to distinguish between X-ALD and ZSD.
Methods: Gas chromatography mass spectrometry (GC-MS) was used to separate and quantitate PG and FA. One 3/16" punch from DBS or 6uL of washed RBC were used to prepare samples for analysis by methylation with 3N methanolic HCl. PG and FA were extracted with hexane, dried and reconstituted in toluene for analysis. Reference ranges for measured markers, C16:0, C18:0 and C18:1 PG and palmitic (C16:0) and stearic (C18:0) FA and 10 analyte ratios were established by the analysis of 720 and 476 control DBS and RBC samples, respectively. Preliminary disease ranges were established for RCDP1 (N=18 DBS, 6 RBC), RCDP2 (N=4 DBS), RCDP4 (N=2 RBC) and ZSD (N=2 DBS, 1 RBC). Post-analytical decision support tools were created using Collaborative Laboratory Integrated Reports (CLIR, https://clir.mayo.edu) post-analytical interpretive tools were created to generate an integrated score and a likelihood of disease for each condition and to provide a conclusive differential diagnosis between paired condition (for example, RCDP1 vs. RCDP2).
Results: Precision, accuracy, sensitivity, specificity, reportable range and sample stability were acceptable to allow for implementation of the analysis as a clinical test. Unadjusted peripheral (99th and 1st) percentiles of reference data were calculated by CLIR software after removal of outliers greater than 5 times the multiple of the reference median (MoM) or less than 0.2 MoM. Single Condition Tools (SCT) and Dual Scatter Plots (DSP) were used to calculate integrated scores of markers and ratios and to provide a differential diagnosis between paired conditions, respectively. DSP facilitated differential diagnoses between ZSD, RCDP1, and RCDP2 in DBS. In RBC, CLIR DSP tools can differentiate between ZSD, RCDP4, RCDP 1.
Conclusion: We developed a new assay for the simultaneous quantitation of C16-C18 plasmalogens and fatty acids in DBS and RBC using GC-MS. Control and disease specific reference ranges were established, and postanalytical decision support tools were developed in CLIR to enhance accurate identification and differentiation of patients with X-ALD and ZSD. DBS specimens are not only easier to collect, transport, store, and analyze, but also provide the opportunity for home collection and for use as a potential second tier screening marker for conditions associated with elevated C26:0 fatty acids, such as X-ALD.

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