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CFTR protein quantification as a cystic fibrosis diagnostic biomarker in dried blood spots using multiple reaction monitoring tandem mass spectrometry

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Abstract: The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel found on the apical surface of epithelial cells in the airway and gastrointestinal tract. A mutation in the CFTR protein is responsible for developing cystic fibrosis (CF) disease. Therefore, circulating CFTR protein could be a promising biomarker of CF disease. Multiple methodological challenges are associated with CF's available diagnostic and screening methods, such as low specificity and potential false discovery rate, mainly for ethnic groups whose CFTR mutations are not covered in the mutation panels. Herein, we have developed an absolute quantification (AQUA) method based on two CFTR signature peptides (SPs). A liquid chromatography-tandem spectrometry (LC-MS/MS) method in multiple reaction monitoring (MRM) mode (MRM transitions 1168.90 > 85.929 and 707.19 > 85.93 of SP1 and SP2, respectively) enabled the accurate quantification of CFTR protein in a dried blood spot (DBS). The method was validated successfully based on international guidelines in terms of signal linearity, precision (within-run CV 3.37?8.54%; between-run CV 5.15?11.06% for the selected SPs), and accuracy (within-run 93.4?105.59%; between-run 97.45?103.28% for the selected SPs). The level of soluble CFTR protein was evaluated as a potential biomarker for CF using patients (n = 39) and healthy controls (n = 30), were found to be in CF patients lower than controls. For instant, the level of signature peptide 1 (SP1) was 2.09 ? 0.55 nM, 68.77 ? 1.40 nM in CF patients compared to Ctrl, respectively; $p < 0.0001$. This study is the first to report CFTR levels in DBS using signature peptides by LC-MS/MS as a diagnostic marker for CF. The receiver operating characteristic (ROC) for CFTR SP1 and SP2 showed a significant area under the curves (AUC) 0.7714 (99% CI, $p < 0.0001$), and 0.8234 (99% CI, $p < 0.0001$), respectively. The presented MRM method provides a highly specific and sensitive approach to CFTR quantif