Key Summary of Published Article

Antibody-Free Quantification of Serum Chromogranin A by Targeted Mass Spectrometry

Background

- Chromogranin-A (CgA) is a diagnostic and prognostic serum protein biomarker for neuroendocrine tumors.[1]
- Currently, immunoassays are typically used to measure CgA levels in serum. However, immunoassays have limitations, including non-specific binding, limited dynamic ranges, varying diagnostic sensitivities, and lack of standardization across assays.[2,3]
- An alternative method used to quantify serum protein levels is liquid chromatography-tandem mass spectrometry (LC-MS/M). This method is antibody-free and highly specific; it has a large dynamic range and is not impeded by protein modifications that occur post-translationally.
- **Objective:** In this study, the investigators developed and validated an LC-MS/MS assay to precisely quantify levels of CgA in serum specimens in a clinical laboratory.

Methods

- For sample preparation, CgA and internal standard were extracted from serum specimens with a mixed-mode anion exchange solid-phase extraction plate and then digested into peptides using the protease trypsin.
  - Calibration standards and quality control samples were prepared from reference material well-characterized by quantitative amino acid analysis.
- CgA peptides were first analytically separated by high-performance liquid chromatography and then quantified using a Sciex 6500+ QTrap mass spectrometer.
- Analytical performance characteristics of the LC-MS/MS assay were assessed.
- Additionally, the correlation and concordance between the LC-MS/MS assay and a commercially available immunoassay were measured across 200 patient specimens.
  - The specimens that had conflicting results between the 2 assays were re-analyzed with high-resolution mass spectrometry (HRMS) to examine whether the presence of truncated and/or post-translationally modified forms of CgA contributed to the disagreements between assays.

Results

- The LC-MS/MS assay for quantifying levels of CgA in serum demonstrated linearity across a wide range of concentrations (50-50,000 ng/mL), as well as inter- and intra-assay imprecision of <10%.
- Comparison of the CgA measurements across the 200 patient specimens of the LC-MS/MS assay to those of the immunoassay method showed good correlation (Pearson's correlation = 0.953) and a concordance of 80.9% (CI: 72.8%-89.2%).
  - CgA measurements were on average 2 to 4 times higher for the LC-MS/MS assay.
- The HRMS analysis of conflicting results between the LC-MS/MS assay and immunoassay indicated that truncated and post-translationally modified forms of CgA did not likely directly contribute to assay disagreements.

Conclusions

- This LC-MS/MS assay demonstrated high analytical sensitivity for measurement of CgA levels in serum specimens.
- The assay overcomes many of the limitations of immunoassays and offers an alternative option for use in clinical laboratories.

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