Detection of IGF-1 Protein Variants by Use of LC-MS with High-Resolution Accurate Mass in Routine Clinical Analysis

To the Editor:

Low or increased serum insulin-like growth factor 1 (IGF-1) concentrations might indicate growth hormone (GH) deficiency or overproduction, respectively. Currently, IGF-1 is mostly measured by automated immunometric assays. However, reagent availability issues prompted us to evaluate LC-MS with high-resolution accurate mass measurement (HRAM) as an alternative.

We developed and validated an IGF-1 LC-MS HRAM method on a Q Exactive mass spectrometer (Thermo Scientific) using 1:70000 resolution (at m/z 200) with a mass accuracy of 5 ppm. A similar method has been published for a qTOF instrument (Agilent) (1). We compared our LC-MS-HRAM method initially in 459 patient samples with the iSYS IGF-1 automated immunoassay (IDS). This yielded a least square linear fit of LC-MS HRAM / iSYS = 0.84, with an r² of 0.966. During further studies involving 1720 samples to generate reference ranges, the 2 methods continued to be highly correlated, with a reproducible systematic bias. However, we also identified 16 outliers for which the LC-MS HRAM method gave dramatically lower results than the iSYS immunoassay (Fig. 1). Of the 16 samples examined, 15 had LC-MS HRAM IGF-1 concentrations of approximately 50% of those obtained by the iSYS assay, whereas 1 sample had a MS result of <5% of the immunoassay measurement. Reexamination of the HRAM data of these 16 samples led to the discovery of a protein variant with a mass m/z 1098 that showed a spectrum with similar isotopic ratio as the monitored m/z 1093 charge envelope for IGF-1 (most-abundant observed m/z 1093.5214, [M+7H]7+). When the protein variant peak was quantitated and summed with the IGF-1 results, the LC-MS HRAM IGF-1 (method I) values closely matched those of the iSYS immunoassay, leading us to hypoth- esize that the protein variant was related to IGF-1. Additional studies included a comparison with a reference LC-MS HRAM method (method II) (2) (Quest Diagnostics), which showed good correlation with our m/z 1093 data in the normal population and in the variant samples (both approximately 50% of iSYS in those with 1098 present) (Fig. 1). Ongoing LC-MS HRAM IGF-1 testing revealed that this phenomenon occurred in approximately 0.6% of our patient population.

We also confirmed that the mass spectrum for the IGF-1 variant was identical between various patients with as little as 2 ppm error. Reduction studies with dithiothreitol revealed no difference of mass between reduced and nonreduced protein. Fragmentation of wild-type IGF-1 (wtIGF1) predominantly produced b65 and b68 ions. The fragments from the wtIGF1 and IGF-1 variant were identical, implying that the difference in IGF-1 species mass most likely came from a C-terminal variant with possible changes in amino acids at positions 69 or 70. The difference between the observed monoisotopic mass of 1093[M+7H]7+ (1093.0917) and 1098[M+7H]7+ (1097.3802) was 4.2885. We performed in silico analysis of all variant possibilities for IGF-1. Only 1 of these variants, A70T, was likely among the possible

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1 Nonstandard abbreviations: IGF-1, insulin-like growth factor 1; GH, growth hormone; HRAM, high-resolution accurate mass measurement; wtIGF1, wild-type IGF-1; SNP, single nucleotide polymorphism.
changes at the 69th or 70th amino acids. Theoretical analysis of the monoisotopic [M+7H]⁺⁻ A70T compared to the wild-type yielded a predicted difference of m/z 4,287.1, which is within approximately 0.5 ppm mass error of the experimental data. We have also confirmed the A70T amino acid substitution (c. 208G>A; p.70A>T) on the genetic level in 3 patients who had sufficient residual serum sample to allow extraction of enough cell-free DNA for PCR and Sanger DNA sequencing (data not shown). The A70T single nucleotide polymorphism (SNP) is catalogued in the NCBI SNP database (rs151098426) (3).

In conclusion, we have identified a fairly common variant of IGF-1, A70T-IGF1, of unknown clinical significance in our clinical LC-MS HRAM assay, which cannot be distinguished from wild-type by the current market-leading immunoassay. When known pathogenic mutations exist or discordant results with established immunoassays are discovered, variant protein sequences should be considered and evaluated. The corollary is also true. When mass spectrometric assays give unexpectedly low results, one should contemplate the possibility of the presence of variants and scan for them. One reason for doing this is that a “missed” pathogenic variant would give the clinically correct result for the wrong reason, which might result in a failed opportunity for definitive genetic family testing (4, 5). The other reason is that several of the variants are either non-pathogenic or of uncertain significance, leading to a potentially falsely low IGF-1 result being reported.

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