MAYO CLINIC

Explaining Unexplained Hypoglycemia Due To Insulin Analogs

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Hypoglycemia is a potentially life-threatening condition that requires urgent medical attention. Most hypoglycemia in patients with diabetes is related to medication use, and recent studies have indicated the contributions of insulin analogs in cases of hypoglycemia.[1,2] Even in patients without diabetes, it is important to exclude insulin administration as a cause of hypoglycemia, yet detection of insulin analogs is not straightforward. The small structural changes that result in favorable pharmacokinetics of insulin analogs also affect the detection of analog insulin by immunoassays, culminating in varying results from different commercially available assays.[3] Consequently, hypoglycemia caused by surreptitious administration of analog insulin can be difficult to diagnose; identifying the particular type of insulin causing hypoglycemia is a further challenge. Furthermore, not all clinicians may be aware of the limitations of currently available insulin immunoassays to detect analog insulins.

With great sensitivity and particularly high specificity, liquid chromatography with tandem mass spectrometry (LC-MS/MS) has been shown to be a valuable platform in detecting insulin, insulin analogs, and even C-peptides.[4-7] In this issue of *Mayo Clinic Proceedings*, the article by Egan et al[8] reports use of liquid chromatography high-resolution accurate mass (LC-HRAM) immunoassay to detect insulin and insulin analogs. In addition, it highlights the value and potential challenges in detecting insulin analogs using an LC-MS/MS—based assay.

The article describes detection of 6 analog insulins on LC-HRAM immunoassay, 3 rapidacting and 3 longacting. Preprandial administration of rapid-acting insulin analogs (lispro, aspart, and glulisine) address the anticipated rise in blood glucose concentration after carbohydrate ingestion. Long-acting analogs or basal insulins (glargine, detemir, and degludec) address hepatic glucose production by maintaining a steady insulin concentration. These 6 analog insulins are currently widely used clinically for management of diabetes. For patients with type 2 diabetes in the United States, analog insulins accounted for more than 80% of all insulin used in outpatient visits between 2016 and 2020.[9]

In addition to analytical validation, this article describes clinical validation based on real patient samples. An assessment was performed with 75 samples from patients treated with insulin and from 3 control patients (not using insulin). The samples were collected from routine patient testing. As expected, samples from the control patients tested positive for human insulin but not for analog insulins. However, a significant percentage of the samples from patients using analog insulin did not test positive for analog insulin. Specifically, LC-HRAM immunoassay confirmed detectable concentrations of insulin analog in 9 of 27 patients using aspart, 10 of 19 patients using lispro, 3 of 4 patients using glulisine, 31 of 40 patients using glargine, and 11 of 14 patients using detemir. Of the patients using human insulin, 9 of 10 patients tested positive. One patient had detectable glulisine without documented use.

Considering the scarcity of details about the timing of both insulin administration and blood draws, it is unclear whether negative test results are due to the kinetics of the insulin/insulin analogs or assay failure. Assay validation was performed on samples spiked with insulin. It is not clear that the same methods would effectively detect insulin in samples of patients who administered insulin and insulin analogs because of reasons such as matrix effects. Recovery

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information would be conducive to assessing the effectiveness of the assay, although not indicated in this article. Another possible explanation is the timing of sample collection. In patients with normal renal function, rapid-acting insulin analogs are cleared within hours of administration. Sample collection within the expected insulin analog duration of action would be valuable for evaluating the performance of the assay. Nonetheless, this finding raises awareness of the challenges in testing and interpretation of results for insulin and insulin analogs, even using an LC-MS/MS–based assay.

Interestingly, more than 20% of the patients taking long-acting insulins (glargine and detemir) did not have insulin confirmed by immunoassay. At commonly used doses, these basal insulins are expected to have a duration of action of 24 hours; this finding raises concerns about the immunoassay's limitations for detection of analog insulins at clinically relevant doses. One possible explanation: the insulin may have been largely metabolized. If this were the case, the assay may need to be modified to detect metabolites of the analog insulins in addition to the analog itself.[4]

The LC-HRAM assay was also evaluated on samples from 8 patients with insulin-mediated hypoglycemia. Although patients initially denied use of any glucose-lowering agents, exogenous insulin administration was the suspected cause of hypoglycemia. The same samples were also tested using commercially available Roche and Siemens immunoassays. The cases had varying results on the commercial immunoassays (Table 3), as expected. All 8 samples had positive results on LC-HRAM immunoassay. This underscores the value of the LC-MS/MS–based assay in patients with hypoglycemia.

Liquid chromatography with tandem mass spectrometry is an expensive and sophisticated platform. It requires a significant amount of professional experience and expertise to develop the assay described in the article. Many clinical laboratories would most likely send the sample to a larger reference laboratory for LC-MS/MS evaluation. Unfortunately, turnaround time can be longer than desirable for immediate patient care, yet the results can be instrumental for providing an accurate diagnosis, avoiding unnecessary workup, and helping to guide future patient management. As more analog insulins are in development, another advantage of LC-MS/MS is that new analogs can be added to the panel after the LC-MS/MS system is established in the laboratory.[10]

In summary, this research illustrates the value of LC-MS/MS in identification of human insulin and the 6 commonly used insulin analogs. It also demonstrates the challenge in interpreting the results for both immunoassays and the LC-MS/MS–based assays. Importantly, the study highlights the importance of a multidisciplinary team (of clinical providers and laboratory professionals) needed to identify and to provide correct interpretation for insulin analog test results, especially in cases of unexplained hypoglycemia.

POTENTIAL COMPETING INTERESTS

The authors report no competing interests.

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