

SCIENTIFIC LETTERS

Relevance of insulin immunoassay characteristics in factitious hypoglycemia[☆]



Relevancia de las características del inmunoanálisis para insulina en la hipoglucemia facticia

The measurement of human insulin in plasma became feasible thanks to the introduction of immunoassay technology in the 1950s and 1960s. In clinical practice, measuring plasma insulin is an important part of the diagnostic assessment of hypoglycemia in non-diabetic patients. The introduction of insulin analogs with antigenic determinants different from those of human insulin poses technical limitations in the quantification of insulinemia, due to their different cross-reactivity profiles in immunoassays.^{1,2} We present a case in which the specificity of the immunoassay used for measuring plasma insulin proved essential for establishing the diagnosis of factitious hypoglycemia through exogenous insulin administration.

An 81-year-old woman with no known diabetes presented with a history of arterial hypertension and Alzheimer's disease. She was referred to the emergency room due to diminished consciousness, and severe hypoglycemia was detected, with a capillary blood glucose concentration of 1.2 mmol/L. Laboratory testing confirmed plasma glucose 2.1 mmol/L, with no alterations to the other parameters. Initially, 50% hypertonic glucose solution was administered via the intravenous route, with partial recovery of consciousness. Over the next few hours, and despite continuous 10% glucose infusion (4000 ml in 24 h) and 8 loading doses of 20 ml of 50% hypertonic glucose solution, the capillary blood glucose controls remained in the range of 1.1–3.8 mmol/L. In view of the duration and severity of hypoglycemia, respective blood and urine samplings were performed after 16 and 20 h in the emergency room, with the suspicion of drug-induced hypoglycemia. The results of the tests were obtained on a deferred basis. Both samples showed hypoglycemia associated with very high insulinemia, undetectable C peptide, and negative anti-insulin antibodies (Table 1). Testing for sulfonylureas in urine proved negative. From 24 h after the start of treatment,

the patient maintained normal blood glucose levels. Her relatives reported no prior episodes of diminished consciousness or hypoglycemia. The patient was living with a relative (a healthcare professional). No relatives were using blood glucose-lowering drugs. A review of the previous laboratory tests of the patient revealed blood glucose levels of 7.2 and 6.7 mmol/L. The patient was admitted for observation and clarification of the diagnosis of hypoglycemia. A 48-hour fasting test proved negative. There were no further hypoglycemia episodes. Based on the clinical and laboratory data, severe hypoglycemia secondary to insulin administration was diagnosed.

A diagnosis of factitious hypoglycemia secondary to insulin administration requires the simultaneous determination of hypoglycemia, high plasma insulin levels and undetectable C peptide.^{3,4} A number of immunoassays can be used for recording insulinemia, including chemiluminometric or electrochemiluminescence techniques, Enzyme-Linked Immunosorbent Assay (ELISA) and radioimmunoassay. Immunoassays have traditional limitations such as cross-reactions with proinsulin and C peptide; interference with anti-insulin antibodies; and the lack of standardized methodology. These assays are based on the use of antibodies that recognize the C-terminal portion of the B chain, the sequence of which is altered in the insulin analogs, thus resulting in variable cross-reactivity ranging from 0 to 264%. As an example, for insulin glargine, which upon injection undergoes rapid conversion in plasma to metabolites (M) M1 and M2, almost 100% cross-reactivity has been established in 5 immunoassays versus <5% in two other assays.^{5,6} Although specific immunoassays have been developed for insulin analogs, they are primarily used in preclinical and research studies and not in clinical laboratories.² The currently most commonly used assays are Elecsys[®] E170 (Roche Diagnostics, Indianapolis, IN, USA), Access[®] (Beckman), ARCHITECT[®] (Abbott), ADVIA Centaur[®] (Siemens), IMMULITE[®] 2000 (Siemens) and Coat-A-Count[®] (Siemens). Most of them cross-react to varying degrees with insulin analogs, with the exception of insulin glulisine, which is only detectable by ARCHITECT[®].^{3–5} The Elecsys[®] E170 immunoassay is available at our center, and is based on the electrochemiluminescence technique.⁷ This immunoassay is the least cross-reactive assay (<0.02%) for insulin lispro, aspart, glulisine and detemir analogs.^{8,9} It does not detect insulin glargine, with a low cross-reactivity for M1 (22%) and no cross-reactivity for M2 (0%)^{3,5}; it is therefore specific for determining human insulin. In our patient, detection during the hypoglycemia episode of very high insulin levels with undetectable C peptide allowed us to confirm the diagnostic suspicion of factitious hypoglycemia due to insulin

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Table 1 Results of the laboratory tests performed during the hypoglycemia episode and after normalization.

Sampling (hours from admission)	Plasma glucose (mmol/l)	Insulin (pmol/l)	C peptide (nmol/l)	Anti-insulin antibodies	Sulfonylureas (urine)
16 ^a	0.9	14,510	<0.03	4.7%	Negative
20 ^a	1.7	13,860	<0.03	4.5%	–
72 ^b	4.5	12.6	0.2	ND	ND

Normal values: glucose (4.1–6.9 mmol/l), insulin (17–120 pmol/l), C peptide (0.26–1.44 nmol/l), anti-insulin antibodies (<8.20%). ND: not determined.

^a Sampling performed with continuous infusion of 10% glucose saline solution.

^b Sampling without intravenous glucose.

administration. Considering the duration of hypoglycemia and the characteristics of the immunoassay, we considered the most likely explanation to be the administration of a long-acting human insulin such as human insulin isophane (NPH).

Understanding the characteristics of the immunoassay used is important for the etiological diagnosis of hypoglycemia, and is crucial when factitious hypoglycemia secondary to exogenous insulin administration is suspected. Chemmanam et al.³ and Krull et al.¹⁰ reported two cases in which the insulin levels were undetectable with the Elecsys[®] assay, but where subsequent determination using the ADVIA Centaur[®] immunoassay, exhibiting 89% cross-reactivity for insulin lispro and 143% for glargine, indicated high insulin levels, thereby allowing for a diagnosis of factitious hypoglycemia due to exogenous insulin administration. This shows that combining two immunoassays for the evaluation of these cases may be decisive.^{2,4}

In sum, the diagnostic study of hypoglycemia secondary to exogenous insulin administration requires knowledge of the cross-reactivity of the different insulin analogs conditioned to the concrete immunoassay used, since such assays exhibit variable sensitivity to these analogs.

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