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# Accurate selection of insulin immunoassay to discern factitious hypoglycemia: a case report

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## **Keywords**

Hypoglycemia · Insulinemia · Insulin analogues · Immunoassays cross-reactivity

Automated immunoassays allow for measuring insulin in the context of hypoglycemia. The broad introduction of insulin analogues raised the problem of discriminating between them and endogenous insulin for the quantification of serum insulin level. Assessing cross-reactivity of the different insulin immunoassays is a key point to decipher recurrent hypoglycemia in persons with diabetes when surreptitious insulin administration is suspected. In this report, we described a clinical case of a 20-year-old woman with a 5-year history of type 2 diabetes, treated with metformin and insulin pump therapy (insulin aspart, Novo-Nordisk, Bagsvaerd, Denmark, infused by Omnipod, Insulet Corporation, Acton, MA, USA). She was admitted for uncontrolled hyperglycemia (blood glucose = 25 mmol/L, HbA1c = 12.2 % (110 mmol/mol)). On day 4 following admission, unexplainable hypoglycemia occurred and became more frequent even after insulin therapy was withdrawn for over 48 h. The corticotropic axis was normal. Concentration of anti-insulin antibodies (ELISA method) was < 10 U/L and IGF-1 and IGF-2 concentrations were in the normal range. During the hypoglycemic episodes, pro-insulin was measured several times and always found below 0.5 pmol/L, hypoglycemic sulfonamides were undetectable and glucagon was normal ( $N < 211$ ). Glucose was constantly monitored in the laboratory (COBAS 8000, Roche Diagnostics, Meylan, France) from day 11 together with insulin and C-peptide measurements. These latter tests were performed in the laboratory with Elecsys assay on COBAS 8000 e602 analyzer (Roche Diagnostics, Meylan, France) and then confirmed on two samples with Immulite 2000 XPi from Siemens Healthcare (Saint-Denis, France). Finally, samples from days 29 and 30 were analyzed by Liquid Chromatography-High Resolution Mass Spectrometry (LC-MS/HRMS, ThermoFisher, Courtaboeuf, France) (Table 1).

The Elecsys assay measurements of serum insulin were highly variable while glucose levels were repeatedly very low. While at days 11 and 14 both insulin and C-peptide were low, the following days (15 and 22) insulin level increased considerably and C-peptide was rather limit (0.61 ng/mL at day 15) and low (0.4 ng/mL at day 22), suggesting the presence of insulin analogues. Surprisingly, at day 34 the level of C-peptide became normal (1.07 ng/mL) despite hypoglycemia (0.8 mmol/L) and insulin level extremely high. One explanation might be that, at such high concentration of insulin, an interference with the C-peptide quantification assay cannot be excluded. The insulin/C-peptide molar ratio was constantly < 1 thus not informative when calculated with the Roche assay measurements. On the contrary, the insulin/C-peptide molar ratio of blood samples from day 15 and day 34 analyzed with the Immulite 2000 XPi system was largely superior to 1, confirming the presence of insulin analogues (Table 1). Indeed, Roche immunoassay is known to specifically detect endogenous insulin and to have a negligible cross-reactivity with recombinant insulin analogues [1], whereas Siemens method recognizes both endogenous insulin and the analogues [2]. Interestingly, suspected insulin analogues concentration was so high in the sample from day 34 (> 900  $\mu$ U/mL) that it was also partially detected by the Roche method, the manufacturer reclaiming having tested concentration up to 1000  $\mu$ U/mL. Two additional samples from days 29 and 30 were sent for specific identification of insulin analogues by LC-MS/HRMS method [3]. Aspart insulin analogue was identified in the two samples at the concentration of 1954  $\mu$ U/mL and 451  $\mu$ U/mL respectively. Endogenous insulin was undetectable as were lispro, glulisine, glargine, glargine M1 metabolite, detemir and degludec. Despite the result in favor of self-administered hidden insulin injections, the patient denied surreptitious insulin use. Psychiatric assessment was requested and did not reveal any suicidal urge or major dysthymia.

**Table 1** Plasma insulin and C-peptide concentration and ratio associated with blood glucose levels

Day	Blood glucose, mmol/L	Insulin, $\mu\text{U/mL}$			C-peptide, ng/mL	Molar Ratio (Insulin, pmol/L / C-peptide, nmol/L)
		Elecsys (ROCHE)	Immulite 2000 XPi (SIEMENS)	LC-MS/HRMS		
11	2.4	1.6	/	/	0.24	0.1
14	1.4	0.8		/	0.09	0.2
15	1.3	12.5	520	/	0.61	0.4; 18.5*
22	1.2	17.4	/	/	0.4	0.9
23	2.8	1.4	/	/	0.09	0.3
29	1.6	/	/	1954	/	/
30	1.5	/	/	451	/	/
34	0.8	43.9	> 900	/	1.07	0.9; 18*

LC-MS/HRMS: Liquid Chromatography-High Resolution Mass Spectrometry

\*Molar ratio calculated with Immulite 2000 insulin measurement

After exclusion of critical illnesses, endocrine deficiencies, auto-immune etiologies and IGF-2-secreting tumors, unexplained hypoglycemia may point toward surreptitious or malicious insulin injections. Nevertheless, asserting such a diagnosis remains challenging and time-consuming. This is partially because routinely used insulin quantification assays provide variable results depending on their cross-reactivity with insulin analogues. In 2014, the pediatric guidelines mentioned the interest in insulin/C-peptide molar ratio for detecting the presence of insulin analogues (value > 1) [4] but this is not the case for all kind of assay. To date manufacturers reported cross-reactivity information for a limited number of insulin analogues despite this knowledge helps making diagnosis more quickly and avoid heavy explorations to rule out all other hypothesis. When hidden insulin injections are suspected, we suggest to consider, early in the diagnosis process, two insulin quantification methods bearing different cross-reactivity for insulin analogues. The discrepancy in measured serum insulin level would immediately elucidate the presence of insulin analogues. Recently, Joshi et al. used a similar approach with two insulin quantification methods (i.e. Architect assay from Abbott Laboratories and Elecsys assay from Roche) to conclude on hidden insulin analogue injections [5]. Subsequently, the interfering analogues can be identified with LC-MS/HRMS system which, in some countries, is still an uncommon and expensive method for this kind of analysis. In conclusion, our results showed that hypoglycemia was explained by the presence of an insulin analogue, which was initially invisible to the Roche Elecsys assay. This clinical case supports the systematic use of a combination of two insulin quantification tests when surreptitious insulin injections are suspected and underlines the importance of the collaboration between medical biologists and clinicians to untangle complex situations.

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## Declarations

Conflict of interest No potential conflicts of interest relevant to this article were reported.

Ethical standard This study was performed according to the principles of the Declaration of Helsinki and approved by the Ethic Committee of Montpellier university hospital. Informed consent The person gave her informed consent prior to the inclusion in the study.

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