LABORATORY EXPLORATION OF A VERY HIGH LEVEL OF INSULIN MEASURED IN A LONG-STANDING TYPE 1 DIABETIC PATIENT.

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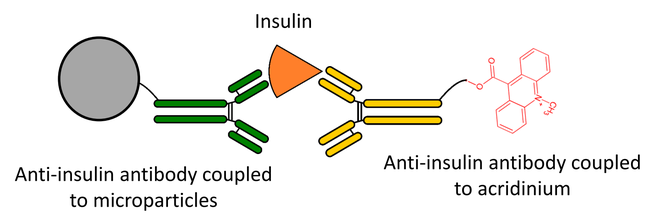
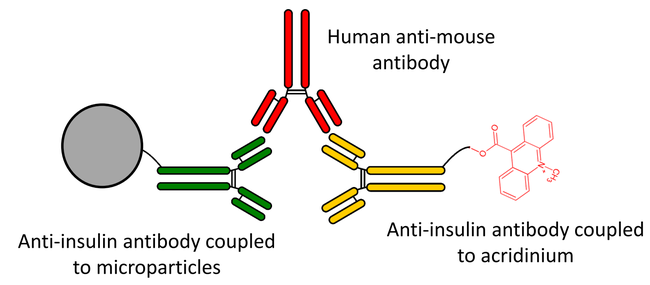
1. Case description

During a routine biological control prescribed by her general practitioner, an insulin concentration of 11850 pmol/L was measured with Abbott Alinity in the serum of a type 1 diabetes 67-year old woman. The disease has been present for 50 years, currently treated with insulin aspart and insulin detemir, and characterized by the occurrence of sudden hypoglycaemia and sometimes significant hyperglycaemia. Despite the very high level of insulin, blood glucose concentration was above the normal range (159 mg/dL) and the patient did not present any signs of hypoglycaemia which, a priori, excluded therapeutic insulin overdose. Therefore, analytical issue was suspected and explored.

2. Laboratory exploration

- The Alinity Insulin assay is a one-step immunoassay where two monoclonal mouse anti-insulin antibodies form a sandwich in presence of insulin. Therefore, the presence of heterophilic antibodies could induce false increase of the insulin level.





  Nevertheless, the use of Veraprep did not significantly change the insulin quantification which exclude the presence of heterophilic antibodies.

- Then we suspected the presence of a ‘macroinsulin’ (a insulin-immunoglobulin complex) that could accumulate in the patient organism. We explored this hypothesis by performing a precipitation with polyethylene glycol 6000 (25%). After precipitation, a recovery of 8% was observed, this was in accordance with the macroinsulin hypothesis. Moreover, the presence of anti-insulin antibodies (16.09 U/mL measured with Medipan Medizym IAA ELISA) was then discovered in the sample which reinforces our hypothesis.

- We also measured insulin (before and after PEG precipitation) on two other commercial kits:

Kit	Level before precipitation	Recovery	Level after precipitation	Recovery	Theorical cross reaction with insulin aspart	Theorical cross reaction with Insulin detemir
Alinity (Abbot)	11320 pmol/L	100 %	856 pmol/L	8 %	110 %	30 %
Cobas (Roche)	6.74 pmol/L	0 %	5.56 pmol/L	0 %	0 %	0 %
Atellica (Siemens)	1590 pmol/L	14 %	566 pmol/L	5 %	12 %	8 %

  The important differences observed between apparatus are consistent with the different cross-reactivity between immunoassays and commercial insulin analogues reported by Parfitt et al., 2015. They demonstrated that Abbot kit is able to detect most of the commercial analogues contrary to Roche and Siemens kits.

3. Discussion

The presence of macroinsulin in patient could have clinical consequences. Indeed, on one hand, anti-insulin antibodies could reduce the efficiency of insulin treatment by inactivating the injected hormone and, on the other hand, insulin could be released from the insulin-antibody complex between 2 injections, potentially inducing hypoglycemia. Poor diabetes control and unexpected hypoglycaemia are indeed observed in our patient.

From an analytical perspective, the exploration of macroinsulin in suspicious samples should better be standardized, the gold standard technique for the exploration of macrohormones is gel permeation chromatography but this method is time consuming and require specific apparatus.

Nevertheless, in patient with poorly controlled diabetes despite a good compliance to treatment, anti-insulin antibodies interfering with the insulinic treatment could be an explanation. The measurement of insulin with a kit cross-reacting with most of the commercial insulin analogues and the exploration of suspicious results with PEG precipitation could be a useful screening tool to highlight such antibodies.

References: Parfitt, C. et al., 2015. Clin Biochem 48, 1354–1357.