

# **Resolution of hypoglycemia and cardiovascular dysfunction after rituximab treatment of insulin autoimmune syndrome**

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## History & Examination

A 37 year-old female presented to the emergency department having collapsed; venous glucose measured 50mg/dL. She reported recurrent dizziness since the birth of her fifth child 10 months previously and had recorded low capillary blood glucose (36-52mg/dL) when symptomatic. Her past history included gestational diabetes during the most recent pregnancy, treated with insulin aspart, insulin glargine and metformin. These therapies had been discontinued 1 month post-partum.

## Investigation

Blood count and renal, liver, thyroid and adrenal function were all normal. HbA<sub>1c</sub> was 41mmol/mol (5.9%). Continuous glucose monitoring (CGM) demonstrated early-morning (asymptomatic) hypoglycemia and post-prandial hyperglycemia. During hypoglycemia she had high circulating insulin (39,181pmol/L; C-peptide 1,046pmol/L). Insulin assay was performed as previously described.(1) Insulin eluted from a gel filtration column in high molecular-weight fractions (**Figure**). Serum anti-insulin IgG concentration was 171mg/L (0-5).

We diagnosed insulin autoimmune syndrome (IAS), a term commonly used in patients without previous exposure to exogenous insulin. Here, we cannot exclude that the insulin antibodies generated were in response to exogenous insulin, but severe hypoglycemia did not develop until many months after cessation of insulin therapy. Moreover, antibodies developing after exposure to exogenous insulin rarely bind insulin with sufficient capacity or affinity to perturb glycemia.(2)

The patient was fitted with CGM with hypoglycemia alarm and prescribed a diet of frequent low glycemic-index carbohydrate meals. As significant hypoglycemia continued, prednisone was commenced (1mg/kg/day) and titrated according to CBG readings to 10mg/day over three months. To reduce anti-insulin antibodies, we gave the anti-CD20 monoclonal antibody rituximab.

Repeat CGM after six weeks revealed intermittent hypoglycemia and sustained daytime hyperglycemia. Over months, there were reductions in total insulin, anti-insulin antibody concentration and antibody-bound insulin (**Figure**). These were associated with reduced

hypoglycemia and improved hypoglycemic awareness. By 6 months, hypoglycemia was rare and post-prandial hyperglycemia had improved (CGM peak glucose 162.0mg/dL). There were no adverse events and prednisone was discontinued after 10 months, after which no further hypoglycemia was recorded.

Recurrent hypoglycemia is associated with endothelial dysfunction, inflammation and increased cardiovascular risk.(3) Thus, we explored the cardiovascular phenotype associated with IAS, before and after treatment. Carotid-femoral pulse wave velocity (PWV) was 7.6m/s at presentation and 5.2m/s at 6 months (5.2-8.0m/s); there was no change in blood pressure over this period. This suggests elevated arterial stiffness at presentation, associated with cardiovascular risk. Other surrogate markers of endothelial dysfunction were all higher at disease presentation than at 6 months (**Figure**). Circulating miR-126 rose following treatment (**Figure**). This microRNA is endothelial-enriched and is thought to maintain endothelial homeostasis and promote vasculogenesis.(4) There was minimal change in control miR-122-5p.

## **Conclusions**

Treatment of IAS is poorly defined. Historically, glucocorticoids and plasmapheresis were used for refractory cases. We show that B-cell depletion with rituximab induces a sustained reduction in anti-insulin antibodies, circulating insulin and the frequency of hypoglycemia. Rituximab has been used successfully in two other cases of IAS.(5,6) However in one, concomitant use of plasmapheresis, methotrexate and intravenous immunoglobulin make it difficult to ascribe the beneficial therapeutic effect to rituximab.

Our report is novel in providing data to suggest an adverse vascular phenotype in IAS that is reversible when dysglycemia resolves. We speculate that recurrent dysglycemia may contribute to vascular dysfunction in IAS, but further study is required to determine the underlying mechanism.

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### *Duality of interest*

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

### *Author contributions*

DC, RWH and ML performed the study, analyzed the data and drafted the manuscript. CC, ADBV, JWD, RS, ND and AD analyzed the results. All authors revised the manuscript and approved the final version.

### *Guarantor's statement*

AD is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Figure: (A) Reportable insulin concentration, total insulin estimation & free insulin estimation against time.** Day 0 is the day of first presentation; prednisone was commenced on day 44; rituximab was administered on days 44 and 58. Insulin concentrations shown here were measured using the DiaSorin LIAISON® assay; an alternative assay (Abbot Architect®) gave consistent results. Follow-up insulin determination was undertaken on neat plasma ('measured insulin') and after 1:49 dilution in 0.9% saline to promote insulin-antibody dissociation and reduce negative assay interference by antibodies ('total insulin') as well as in supernatant following polyethylene glycol precipitation ('free' insulin). ○ represents measured insulin concentration >3000pmol/L in neat plasma. **(B) Serum anti-insulin IgG concentration** (in-house ImmunoCAP® assay; reference range 0-5). **(C) Changes in plasma macroinsulin in response to immunosuppressive therapy.** At presentation, only 4% of total immunoreactive insulin was recovered from plasma supernatant following polyethylene glycol precipitation, consistent with the presence of high molecular-weight insulin immunoreactivity. Predominantly high molecular-weight insulin consistent with macroinsulin was demonstrable using gel filtration chromatography at presentation. The elution volumes of immunoglobulin and monomeric insulin are shown by the black and white arrows respectively; the majority of insulin co-eluted with immunoglobulins. Follow-up investigations on day 271 confirmed a decrease in macroinsulin. **(D) Changes in circulating markers of endothelial / vascular function** at presentation ('pre') and at 6 months ('post'). ADMA, asymmetric dimethylarginine; ET-1, endothelin-1.

