

Insulin autoimmune syndrome: not just one but two different diseases with therapeutic implications

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Summary

We present a young woman with treatment resistant insulin autoimmune syndrome (IAS) with a protracted course. Her serum insulin level was 6945 pmol/l (<160), C-peptide 4042 pmol/L (<1480), anti-insulin antibodies 5305 U/mL (<0.4) were monoclonal IgG kappa. After 12 h of fasting, her blood glucose fell to 1.2 mmol/L. Post-meal blood glucose peaked at 12.2 mmol/L with reactive hypoglycaemia below 2 mmol/L. Frequent meals and continuous blood glucose monitoring were helpful, but further treatments advocated in the literature with prednisolone, rituximab, plasmapheresis, cyclophosphamide and ciclosporin were without beneficial effect.

Based on this case and a review of the literature, we propose that IAS is not one but two different diseases with different therapeutic strategies. The first disease, polyclonal IAS, predominates in Asia and is characterized by polyclonal anti-insulin antibodies, association with certain HLA genotypes and other autoimmune conditions, medications and viral infections possibly triggering the disease, a possible female predominance among young patients and a tendency towards spontaneous remission. The other disease, monoclonal IAS, predominates in Caucasians. Typical features are monoclonal anti-insulin antibodies, only weak HLA association, no drug predisposition, no sex difference, rare remission and conventional therapy often being without any clinical effect. We suggest that monoclonal IAS with IgG or IgA anti-insulin antibodies should receive therapy targeting plasma cells rather than lymphocytes.

Learning points

- IAS may be considered as two separate diseases, polyclonal and monoclonal.
- The presence of either polyclonal or monoclonal antibodies should determine the choice of treatment for IAS.
- In polyclonal IAS, discontinuation of a triggering medication and treatment of triggering conditions should be the backbone of therapy.
- Monoclonal IAS should receive treatment targeting plasma cells.

Background

Insulin autoimmune syndrome (IAS) is a rare cause of hypoglycaemia, first documented by Hirata and colleagues in 1970 (1) and the mechanisms described

by Følling and Norman in 1972 (2). Its characteristics are episodes of spontaneous hypoglycaemia and very high anti-insulin antibody titres in individuals not previously



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exposed to exogenous insulin. The clinical presentation is similar to hypoglycaemia caused by insulinoma, and the diagnosis is easily overlooked.

In most reported cases, the hypoglycaemic episodes stop spontaneously, by discontinuing drugs that trigger the condition (3) or by the treatment of triggering conditions. There is no consensus on a treatment protocol for IAS, and short reviews (3) and case reports provide suggestions for treatment. No clinical trials exist.

Case presentation

A 24-year-old woman presented to the emergency department after she was found unconscious in bed. Her blood glucose was 1.5 mmol/L. She received intravenous glucose and was conscious on arrival. The clinical examination was unremarkable.

Her chief complaints were profuse night sweats and an intense feeling of hunger starting 9 months earlier. She added extra daytime meals and ate immediately before bedtime to alleviate her nightly complaints. She frequently also had to eat during the night. Remarkably, she had no weight gain but undertook vigorous exercise to avoid this.

She had no coexisting illness. Six months prior to the onset of symptoms, she had vaccinations against hepatitis A, cholera, diphtheria, tetanus, pertussis and typhoid fever.

Investigations and results

After 12 h of fasting, her blood glucose levels fell to 1.7 mmol/L (4.0–6.0) (Fig. 1). When she resumed normal eating, she had episodes where her blood glucose peaked

at 12.2 mmol/L and again fell below 2.0 mmol/L within short time intervals (Fig. 2).

Serum insulin was >6945 pmol/L (fasting <160), and C-peptide 4042 pmol/L (fasting 300–1480). There was no radiological evidence of insulinoma. Anti-insulin antibodies were 5305 U/mL (<0.4), analysed with immunoprecipitation (RSR, Cardiff, UK) with an analytical measuring range from 0.30 to 50 U/mL (CV% 20 at 1.1 U/mL). Samples with higher values were diluted to measure the exact level of antibodies. The cut-off for positivity was adjusted according to Islet Autoantibody Standardization Program (IASP).

As IAS can be associated with other autoimmune conditions we performed a thyroid antibody test, celiac screen, anti-GAD, anti-IA2, anti-zinc transporter 8 antibodies, anti-nuclear antibody screen, rheumatoid factor (IgM), anti-intrinsic factor, anti-parietal cells, anti-mitochondrial antibodies, and anti-glomerular base membrane, which have all been within normal range. She was HLA B27 negative and DRB1*0404 positive.

Plasma IgA was slightly increased at 4.3 g/L (0.7–3.7 g/L). P-IgG and p-IgM were normal. Serum electrophoresis and immune fixation showed no monoclonal component. Free kappa and lambda chains were also normal, and the modest IgA elevation seemed to be unspecific and not representing clinical illness.

Her anti-insulin antibodies were characterized using two in-house methods:

Method 1: A three-step manual assay. Biotinylated recombinant human insulin (Sigma) was fixed to streptavidin-coated plastic wells. Serum was applied so that her anti-insulin antibodies could bind. Characterization was performed by applying anti-IgG, anti-IgA, anti-IgM, anti-kappa and anti-lambda

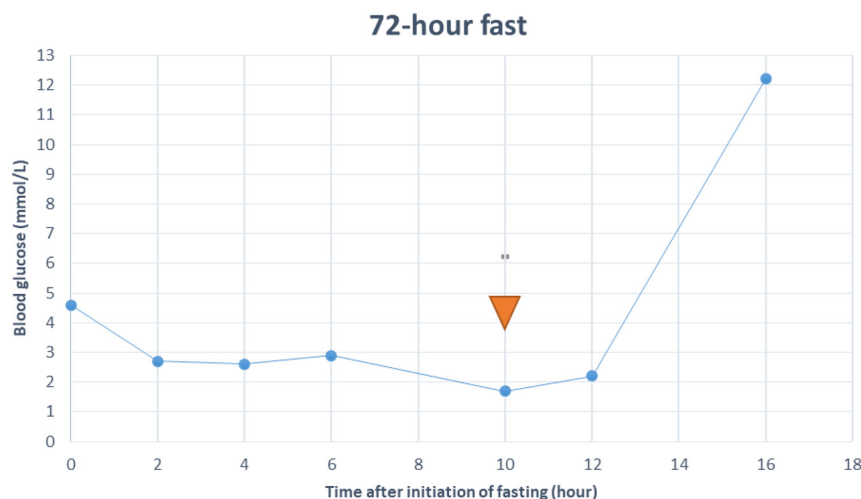


Figure 1
We planned a 72-h fast but stopped after 10 h due to symptomatic hypoglycaemia.

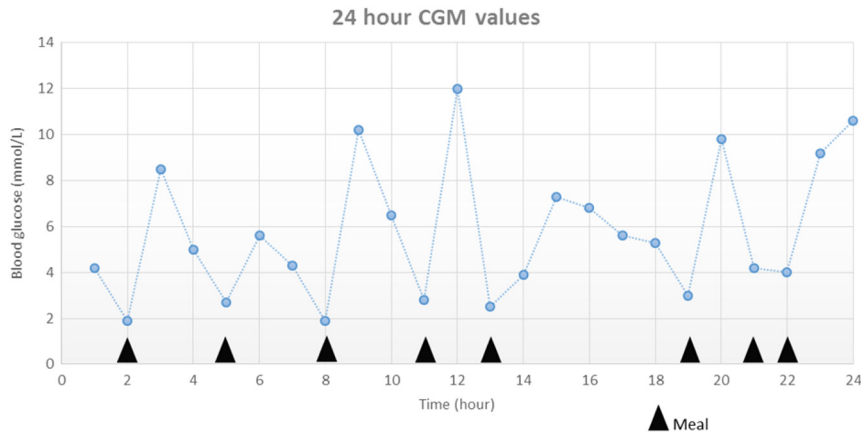


Figure 2
CGM readings for 24 h.

antibodies described previously (4). These are all (Fab')₂ europium-labelled tracer antibodies for fluorescence detection. The anti-IgM is rabbit polyclonal (Dako), the four others are mouse monoclonal antibodies (in-house reagents). In serum diluted 1:100, strong anti-insulin signals (in counts per second vs background signal) were observed with anti-IgG (466111 vs 26718) and anti-kappa (451625 vs 35719) compared to the low signals for anti-lambda (36677 vs 16530), anti-IgA (5234 vs 5000) and anti-IgM (6626 vs 3069). Even when her serum was diluted 1:100000 the anti-insulin signals using anti-IgG (1820 vs 721) and anti-kappa (1254 vs 351) tracer antibodies were detectable, while anti-lambda gave no signal (307 vs 260).

Method 2: In aliquots of her serum diluted 1:1000 we removed either her kappa or lambda antibodies using magnetic Dynabeads (M280) coated with anti-kappa or anti-lambda antibodies, respectively. The depleted serum aliquots were applied to wells coated with insulin, and the anti-insulin signal was detected using an anti-IgG tracer. In the aliquot where kappa antibodies were removed, the anti-insulin signal disappeared (3343 vs 3251), while the anti-insulin signal remained strong in the aliquot where lambda antibodies were removed (48130 vs 2955). Thus, both methods independently show that her anti-insulin antibodies are monoclonal IgG kappa.

Treatment

With high insulin, high C-peptide, and extremely high anti-insulin antibody titres, we concluded that the patient suffered from IAS, and through one and a half years, we tried the treatment options advocated in the literature. Figure 3 shows the time course. She took frequent small meals low in carbohydrates and

used continuous blood glucose monitoring (CGM) throughout the course of treatment.

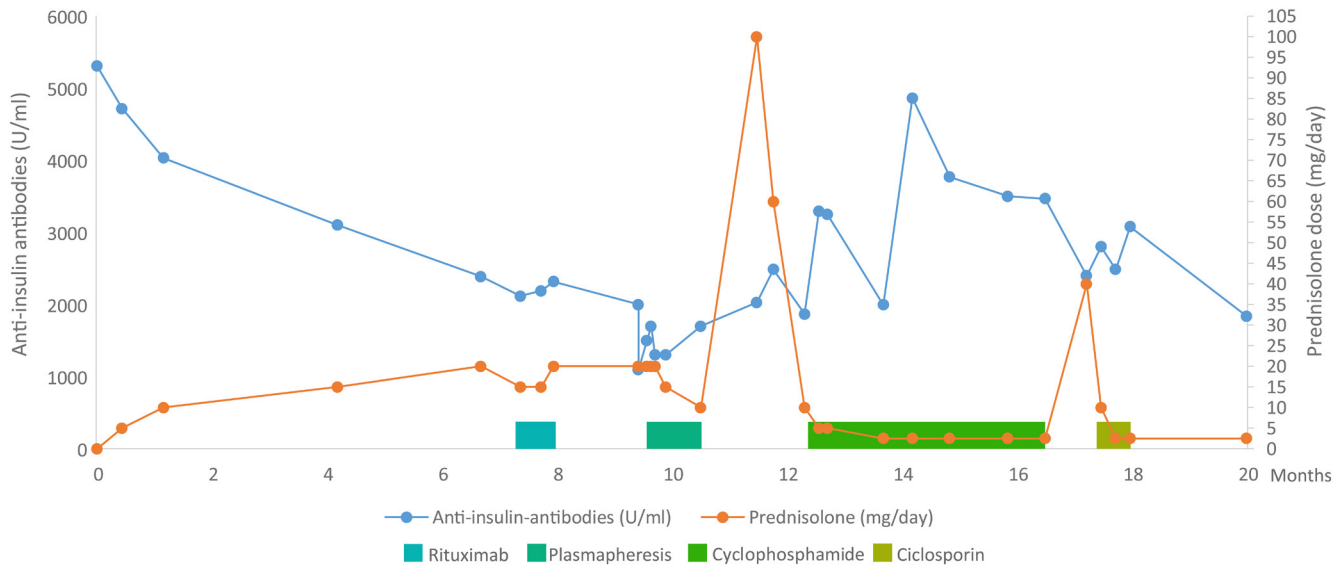
In most cases, the condition seems to be self-limiting with rapid falls in anti-insulin antibodies within 3–6 months (3, 5, 6). This makes it difficult to evaluate if decline of antibodies and clinical improvement are caused by treatment or natural resolution of the condition. Our patient already had symptoms for 9 months and spontaneous recovery seemed unlikely.

Many sulfhydryl-containing drugs are associated with the development of IAS (3, 6, 7). Discontinuing these often induces cure (3). Our patient took no such medication. No evidence indicates that vaccinations may trigger the condition. Moreover, vaccines trigger polyclonal immune responses, whereas our patient has monoclonal anti-insulin antibodies.

Therefore, after establishing the diagnosis we started treatments with prednisolone first (for doses see Fig. 3). After 7 months, she got rituximab 375 mg/m² weekly for 4 weeks, then plasmapheresis two to three times weekly, altogether 18 times, and then cyclophosphamide 150 mg daily for 11 weeks and 100 mg daily for another 3 months. Finally, we attempted ciclosporin up to 200 mg twice daily for a few weeks.

Outcome and follow-up

Frequent meals and CGM helped her ameliorate her hypoglycaemic attacks, but her complaints remained severe. During prednisolone treatment, her anti-insulin levels fell to about half of initial values with no significant relief of symptoms. Increasing Cushingoid features prompted the need to reduce the dose. Rituximab did not help. On plasmapheresis, antibody levels fell but resumed initial levels before the next session, as expected. Cyclophosphamide and ciclosporin

**Figure 3**

Throughout the course, frequent small meals along with continuous glucose monitoring has been at the base of the treatment.

improved neither antibody levels nor symptoms. In summary, apart from frequent meals and CGM, the treatments were almost without beneficial effects.

Discussion

We present a patient with IAS. She has suffered from serious and frequent hypoglycaemic attacks for nearly three years due to monoclonal IgG kappa anti-insulin antibodies in high titre. A series of therapies advocated in the literature has been without substantial benefit. Here we explain how the antibodies cause the hypoglycaemic attacks and then argue why IAS should be regarded as two different diseases and how this dichotomy is important for therapeutic strategies.

As shown by Følling and Norman (2), the pathogenesis of the hypoglycaemia is as follows: The antibodies bind large amounts of insulin already in the fasting state, and there is additional binding capacity available. Meals stimulate secretion of insulin, which is then bound to the available antibodies, resulting in greatly prolonged half-life, abolishing metabolic effect and therefore causing postprandial hyperglycaemia. As the total binding capacity is reached, insulin dissociates from this large reservoir causing hypoglycaemia; similar to the release from too large a dose of an insulin injection, it cannot be stopped.

There is no clear correlation between the antibody titre and the clinical symptoms. Most probably, the concentration of anti-insulin antibodies has to be very

low before clinical improvement occurs. The affinity in the binding of insulin also plays a role. The affinity will be heterogeneous in a patient with polyclonal anti-insulin, and homogeneous in a patient with monoclonal antibodies, although different between patients.

We suggested a heterogeneity of the syndrome already in 1993, based both on different HLA genotypes and the difference between polyclonal and monoclonal anti-insulin antibodies (8). In 1995, Ushigata and colleagues (9) confirmed this distinction. Later, however, most reports and reviews pay little attention to this heterogeneity, neither with respect to aetiology nor to its implication for therapeutic strategies. In addition to the heterogeneity based on differences between monoclonal and polyclonal anti-insulin antibodies and between HLA genotypes, several other differences have been reported. This clearly indicates that IAS consists of two different diseases.

The first disease predominates in Asia, first described in 1970 (1). Typical features are polyclonal anti-insulin antibodies (3, 7, 9); association with certain HLA genotypes (3, 6, 7, 9), mainly those with glutamate in position 74 in the HLADR4- β 1 chain (9); association with other autoimmune conditions (3, 6); many drugs containing sulfhydryl groups (3, 6, 7) and possibly viral infections (3) triggering the disease; a possible female predominance among young patients (6); and a tendency towards spontaneous remission (3, 5, 6), making treatment effects difficult to evaluate (5). This disease, for which we suggest the name



'polyclonal IAS', shows many similarities to the autoimmune polyglandular syndrome (10).

The other disease predominates in Caucasians, first described in 1972 (2). It is much rarer than polyclonal IAS (11). Typical features are monoclonal anti-insulin antibodies (2, 3); only weak HLA association (3, 9, 11); uncertain or variable drug predisposition (5); no documented sex difference; rare remission (3, 5); and conventional therapy being most often without beneficial effects (3, 5). This disease, for which we suggest the name 'monoclonal IAS', fulfils the criteria of the group of diseases called 'monoclonal gammopathy of clinical significance' (12). In some patients the diagnostic criteria for multiple myeloma or Waldenström's macroglobulinaemia may be fulfilled. Normally an individual produce huge numbers of different antibodies, theoretically up to around 10^{14} (13). Some of them may by chance fit to bind endogenous antigens or drugs. If a cell producing such antibodies proliferates and forms a clone with many cells, the antibodies may cause harm. A clone producing antibodies binding to insulin and causing IAS is a typical example. Other examples are cold agglutinin disease due to antibodies to I/i antigens on erythrocytes (14), and fatal intravascular precipitation due to antibodies to an x-ray contrast agent (15).

This dichotomy into two different diseases should lead to different therapeutic strategies.

The advocated pharmacological therapies target B and T lymphocytes, the resulting release of lymphokines and polyclonal antibody responses (16). This holds for prednisolone, rituximab, cyclophosphamide and ciclosporin. Therefore, it makes sense to use them in polyclonal IAS. However, in monoclonal IAS, the IgG or IgA anti-insulin must be produced by an autonomous plasma cell clone, and hence treatment directed against lymphocytes will be expected to fail. Rituximab targets CD20 on B lymphocytes, which lose this expression when they develop into plasma cell. Ciclosporin target T-cell effects only. Prednisolone and cyclophosphamide exert broad suppression of polyclonal immune responses. They may have a small effect in monoclonal plasma cell diseases when used in combination with other drugs but not as monotherapy. Our present case illustrates the inefficiency of these therapies in monoclonal IAS. We suggest that treatment of monoclonal IAS should target plasma cells primarily, as is done in other diseases with monoclonal gammopathy of clinical significance (12). Targeting CD28, which is abundant on plasma cells, or inhibiting proteasome activity in plasma cells are

examples of such treatment strategies. Because monoclonal IAS is so rare that controlled clinical trials will be difficult to perform, it is reasonable to adopt the principles used in the related monoclonal diseases (12).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the case study reported.

Funding

This research did not receive funding from any agency in the public, commercial or not-for-profit sector.

Patient consent

Written consent for publication of the clinical details has been obtained from the patient.

Author contribution statement

ACP Wammer (MD) is a consultant endocrinologist who was responsible for the management of the patient and for writing the article. I Nermoen (MD, PhD) is a consultant endocrinologist who aided in the management of the patient, reviewed the manuscript and helped with the literature review. I Følling (MD) is an endocrinologist who reviewed the manuscript and provided valuable insight on the mechanisms behind the condition and helpful treatment suggestions. H Tran (MD, PhD) is a consultant hematologist who reviewed the manuscript and was involved in the management of the patient. P M Thorsby (MD, PhD) is a consultant endocrinologist and medical head of department at the Oslo Hormone Laboratory, who reviewed the manuscript and was responsible for quantification of insulin, c-peptide and anti-insulin antibodies. K Lima (MD, PhD) is a consultant endocrinologist who reviewed the manuscript and was involved in the management of the patient. N Bolstad (MD, PhD) is a consultant in medical biochemistry who characterised the anti-insulin antibodies and reviewed the manuscript.

Acknowledgements

We thank Astrid Aandahl, MD, who was in charge of plasmapheresis at Akershus University Hospital. We are grateful to Nina Norstrand, bioengineer at the Hormone Laboratory, Oslo University Hospital.

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Received 22 March 2023

Accepted 4 October 2023

Version of Record Published 17 November 2023