B.Teupe, Diabetes-Dorf Althausen, Bad Mergentheim, Germany

Late revenge of analogue insulins among type-1-diabetes (T1D) patients?

►Abstract

Objective (Aim): To collect binding insulin-antibodies (IA)-data status among T1D-patients in relation to their prior and present used insulin sorts (compared with healthy persons, T2D- and MODY-patients)

Methods: Out of 291 consecutive participants from more than 4000 of my CSII-patients 2010 we drew up 277 complete data sets of measured IAs with two different methods (percentage of binding and absolute amounts), last HbA_{1c} and data belonging to their insulin therapy and followed up the development of titers > 7000 nU/mL for maximal 6 years.

Results: At a significance level of $p < 0.05$ we found

- that significant more patients using analogue insulin produce IAs compared with human insulin users,
- significant higher IA-levels in all T1D-patients compared with healthy persons,
- significant and / or borderline significant higher IA-levels in the group of analogue users compared with human insulin users,
- in all cases significant higher IA-levels in the group of the aspart insulin (Novorapid®, Fiasp® not yet available) compared with human-insulin users, users of the other analogue insulins and users of insulin derived from porcine,

Some of them suffers under the paradox hyperglycemia-hypoglycemia-syndrome, up to the need of immuno-adsorption-therapy. Withdrawl of analogue insulin over up to 7 years shows disappearing, declining or persistence of IAs and their clinical impacts.

► Clinical relation

This data analysis was provoked by the plight of a young patient: 1999 type-1-diabetes became manifest in a girl born 1992. Her therapy started with ICT: Actrapid® / Protaphane®, 1 year later additional Humalog®, since 2 / 2001 Semilente®, CSII with Humalog® since 8 / 2004, changing 2008 for a short time to Apidra® + Insuman® Infusat and since 4 / 2010 Novorapid® - in summary: she got porcine-, human- and nearly all available analogue insulin sorts. Since about 2005 she progressively developed daily hyperglycemia up to 600 mg/dl. She suffered several ketoacidosis comas and several times a week also late postprandial severe hypoglycemia often with comas especially in the early morning hours. To rule out insulin deficiency as a cause, the insulin dose was increased up to 600 IU, whereby no improvement resulted. During 5 years her HbA_{1c} worsened from minimal 6.4% (46 mmol/l) to at last 10.3% (89 mmol/l) [normal: 4.2 - 6.1% (22 - 43 mmol/l)], which is unfortunately not uncommon in other adolescents during puberty either.

She had already been examined and treated in some specialised diabetic institutions with different procedures (educational programs, multiple injections therapy, CSII, CGMS, experimentally intravenous therapy, psychological and psychiatric explorations, family therapy) with no ameliorating effect.

17 years old, she was referred to me with the question: Do you have any idea how to help her? I could confirm the diagnosis of type-1-diabetes (C-peptide negative), but also found MODY-3-genes and extreme amounts of insulin-antibodies (23 210 nU/mL, 81%-binding, normal < 400 nU/mL) and at first also extreme amounts of insulin-receptor-antibodies, but the latter seemed to be a methodological problem (free circulating IA-IRA-complexes). Since "all" immune suppressive therapies have failed (glucocorticoids, azathioprin, mycophenolat[3], Rituximab), we have started immunoadsorption dialysis. Because of the urgency at first 4 sessions in about 6 weeks. Until today she is still treated as a precautionary measure for 2 days every six weeks via a vascular access (Cimino-shunt similar to dialysis with a IgA-

column, ADASorb®)[4]. After extracting the IAs into the normal range, her total daily dose has dropped to about 50 I.U./ d / 57 kg and her glucose metabolism could be stabilized near normality, but her antibody production remains. Now we are waiting to see if the I(A)As will decline - which has not happend the last 7 years - and are looking for a definitely less invasive therapy, if possible, to avoid bone marrow transplantation. She has not the genecode of Hirata-disease[1,2].

► Abbreviations

BMI (body mass index); **C-peptide** (connecting peptide); **CSII** (continuously subcutaneous insulin infusion = insulin pump treatment); **CGMS** (continuous glucose monitoring system); **HbA_{1c}** (percentage of N-terminal glycated ß-chains of haemoglobin A1); **IA** (insulin antibody); **IAA** (insulin-auto-antibody); **ICT** (intensified insulin therapy = multiple injections); **IgA** (immunoglobulin class A); **i.U.** (international Units); **m** (male); **MIT** (multiple injection therapy); **MODY** (maturity onset diabetes in young people, here only type 2 and 3); **nU / mL** (nano units per millilitre); **SD** (standard deviation); **T1D** (type-1-diabetes); **T2D** (type-2-diabetes)

► Introduction

Besides the long known rare insulinoma there is growing knowledge in the last years to the rarer hyperinsulinemic hypoglycemia without insulinoma and not induced by artificial insulin injection as insulin autoimmune syndrome (Hirata disease[1,2]) produced by insulin(receptor)-antibodies in non-diabetic and type-2-diabetic persons[5,6,7,8]. Despite some methodological problems[9] there exists some knowledge of insulin antibodies both as auto-antibodies of type-1-diabetics[10] (accompanying the ß-cell-destruction) and as acquired binding antibodies (also allergic) provoked by insulin therapy itself in diabetics. The purification of insulin in the 1970th and the introduction of human insulin 1982 should and could reduce problems with bovine and porcine insulin antibodies[11]. But recently there have been reports of higher levels of antibodies against analogue insulin but seeming without clinical relevance[12,13]. Affected by the course of the disease of the above mentioned young woman, I found more patients in my collective with similar although weaker but therapy relevant problems with high insulin antibodies. Therefore I check all my patients routinely since October 2012 for I(A)As for a better understanding of metabolism problems. The evaluation of this *cross-sectional study* (survey) revealed the strong suspicion that insulin aspart triggers comparatively strong IA production. Therefore we have followed the course of I(A)As for up to 7 years after change from insulin aspart to human insulin in a *longitudinal study* (follow-up). Included were 21 type-1-diabetics, whose I(A)A titers were above 7000 nU/mL at the first measurement: 5 after fresh manifestation, 3 formerly treated with bovine insulin.

► Subjects, Materials and Methods

This study consists of 2 parts: at first the greater cross-sectional study, followed by a smaller longitudinal study.

Participants in the cross-sectional study: From a cohort of 291 consecutive participants (13.10.12 – 24.1.13) we got 277 complete records: 245 T1D-patients (83 m), 241 of them with pump treatment, [2 within this group with additional MODY-Genes (2 m)]; 5 T2D-patients (4 m); 9 pure MODY-patients (6 m); 18 non diabetic persons (6 m) (= no insulin treatment).

Participants of the longitudinal study: From February to April 2018 we checked the most of those patients, whose I(A)As initially were elevated > 7000 nU/mL, independent whether or not they were also included in the cross-sectional study.

Measurements and data collection: Insulin antibodies absolute (Normal range: < 400 nU/mL; gray zone: up to 600 nU/mL) performed by MVZ Labor PD Dr. Volkmann und Kollegen GbR, Karlsruhe, Germany, according to the following analysis specification:

200 µL serum will be acidified with 250 µL 0,085M HCl, to dissociate immune complexes out of I(A)A und endogenous insulin. Afterwards add 100 µL of a mixture of activated carbon, dextran and methylcellulose in buffer A (Na-phosphate buffer with 0,33 M NaCl, 1,5% BSA), to bind endogenous insulin. After neutralization centrifuge the assays 10 min, 3750 x g. The supernatant solution will be used in the further radio immune precipitation assay. 100 µL of the supernatant solution will be diluted with 50 µL buffer A and 100 µL in buffer A diluted 125I-Insulin (PerkinElmer, Rodgau). Parallel replace 50 µL buffer A by Insuman® rapid (Sanofi-Aventis, Frankfurt) for determination the unspecific binding of ^{125}J-Insulin. After incubation the assays overnight at 2-8°C, add 1,5 mL buffer B (0,1 M Tris-HCl, pH 8.0 with 17% PEG 6000). After further 20 min. incubation at 2-8°C centrifuge the assays (30 min., 3750 x g,10°C). Add 1,5 mL buffer C (0,1 M Tris-HCl, pH 8.0 with 12,5% PEG 6000) to the aspirated supernatant solution and mix it thoroughly. After 20 min. of incubation at 2-8°C centrifuge the assays (30 min., 3750 x g, 10°C), aspirate the supernatant solution and measure the activity of the precipitated pellets in a gamma-counter. The difference (activities of the precipitates of the specific and the unspecific test) will be converted into nU/mL taking the date of calibration of ^{125}J-Insulin into account. The test result as nU/mL is an absolute quantity, specificity and cross reactivity are unknown.

Percentage insulin binding levels by radio-immunoassay product of DIAsource, formerly BioSource, Belgium (AIA RIA kit, KIP0091; normal range: < 10 %; borderline: 10 - 15 %) performed by Laboratory Limbach Heidelberg Germany, according to the following analysis specification:

The presence of circulating anti-insulin antibodies in patients treated with insulin will be assessed on a semi-quantitative basis by the determination of the binding of ^{125}J-Tyr-A14-insulin in the serum fraction by the polyethylene glycol (PEG) (gammaglobulins) was precipitated. The used kit determined if the samples contain free insulin antibodies, that are not bound to the insulin. The concentration of insulin in the tracer is 0.42 nmol / 100 µl of tracer. The origin of insulin is human recombinant with a purity > 99 % by HPLC ordered at Fitzgerald (USA). Item 1302129.

The insulin is mono-iodated on position A14 and purified by HPLC. Intra- and inter-assay CVs: 1,6-20% (lower CVs in higher IA-concentrations); Mean of 80 healthy persons: 5,5 %; mean + 3 SD (= 8,2%) I(A)As positive. This test does not measure the bound insulin in patients but in his serum in vitro under special conditions. The test result as percentage of binding is a relative number and not an absolute quantity, more a qualitative (antibody yes or no), less a quantitative (=”semi quantitative”) determination, specificity and cross reactivity unknown.

The HbA_{1c}-measurements were done with affinity chromatography [Alere AfinionTM (Netherland), Abbot (Germany)], performed by ourselves. Normal range: < 6 % (< 42 mmol/mol); coefficient of variation CV < 1,4%.

Since October 2012 we have extended our routine blood test with the measurement of the I(A)As in 2 different laboratories in all T1D-patients in our ambulatory and our CSII courses. We routinely asked them for their medical history. The data of age, gender, weight, height, diabetes duration, insulin treatment duration, total daily dose, all previous and present insulin sorts and their application duration, autoimmune state by the patients and their relatives, immune suppressive therapy (present and past) and last HbA_{1c} were filled in a questionnaire and in any doubt or obvious contradiction in the questionnaire we checked stored data and made interviews, using if necessary the services of medical reports. To control the details of the normal values of the laboratories, we invited all healthy escorts of the T1D-patients, T2D-patients and MODY-patients from our ambulatory to the same procedure.

Table 1: Characterisation of the participants

			T1D		T2D		MODY		Healthy
		animal in comb.*	no analogues**	analogue in comb.***		animal*	human**	analogue***	
	number total	245 (241 CSII)			5 (2 analog, 2 human, 1 no insulin)	9			18
number total all	**277**	**133**	**58**	**185**	**5**	**0**	**6**	**6**	**18**
male	**99**	**47**	**18**	**64**	**4**	**0**	**3**	**5**	**6**
female	**178**	**86**	**40**	**121**	**1**	**0**	**3**	**1**	**12**
age (y)	**45,4**	**50,2**	**49,3**	**42,7**	**70,2**		**57**	**54,0**	**47,2**
± SD	±15,4	± 11,6	± 14,9	± 15,3	± 6,5		± 13,9	± 10,3	± 12,6
min - max	6-78	16-77	12-75	6-77	60-78	0	39-76	39-71	29-74
BMI	**25,7**	**26,2**	**24,9**	**25,6**	**33,3**		**32,4**	**32,1**	**24,7**
± SD	± 5,1	± 4,9	± 3,9	± 5,1	± 6,5		± 7,9	± 8,9	± 3,1
min - max	13,8-47,8	19,5-47,8	16,2-38,3	13,9-47,8	26,0-42,9	0	21,2-47,6	22,6-47,6	20,1-29,8
total daily dose (I.U./day)	**46,8**	**46,6**	**44,1**	**46,3**	**41**		**94,7**	**68,2**	
± SD	± 25,3	± 26,7	± 21,3	± 24,3	± 23,3		± 50,1	± 46,5	
min - max	12-160	12-160	12-100	15-160	15-60	0	34-150	25-150	-
diabetes duration (y)	**26,5**	**35,3**	**33,1**	**24,4**	**26,6**		**30,2**	**24,0**	
± SD	± 13,8	± 10,7	± 12,8	± 13,6	± 10,3		± 12,8	± 14,0	
min - max	1-67	11-67	8-67	1-56	12-37	0	17-48	4-44	-
insulin duration (y)	**26,1**	**35,3**	**33,1**	**24,3**	**16**		**20,7**	**11,2**	
± SD	± 13,9	± 10,8	± 12,9	± 13,6	± 8,5		± 13,8	± 6,4	
min - max	1-67	11-67	8-67	1-56	10-22	0	7-44	3-22	-
last HbA_{1c} in % (mmol/l)									
± SD %	**6,9 (52)**	**7,0(53)**	**6,7(50)**	**7,0(53)**	**7,4(57)**		**7,4(57)**	**7,9**	
(mmol/l)	± 1,0(10,9)	± 1,0(10,9)	± 0,7(7,7)	± 1,0(10,9)	± 1,0(10,9)		±1,2(13.1)	±1,3(14.2)	
min - max	4,6-11,0	4,8-11,0	4,6-8,7	5,0-11,0	6,6-9,0	0	6,5-9,6	6,5-9,6	-
insulin binding in %	**16,2**	**16,1**	**16,0**	**18,2**	**4,8**		**6,8**	**7,8**	**4,9**
± SD	± 14,9	± 15,8	± 15,3	± 15,4	± 0,8		± 1,6	± 1,7	± 0,8
min - max	4-81	4-81	4-81	4-81	4-6	0	5-9	5-10	4-6
IA (nU/mL)	**2151,8**	**1827,4**	**1789,3**	**2635,6**	**54,4**		**105,2**	**209,7**	**58,6**
± SD	± 3682,4	± 3414,3	± 3234,6	± 4005,0	± 9,8		± 95,8	±246	± 16,7
min - max	50-23210	50-14902	50-14902	50-23210	50-72	0	50-296	50-637	50-107

* all patients treated prior with animal derived insulins (porcine and bovine), 2 present with porcine, with possible combination with other insulins, 37 without analogue contact (see Table 2)

** patients never treated with analogue insulins

***patients treated with analogues, possible contact with animal derived or human insulins

Table 2: Patients using animal insulin without analogues (2 present with porcine insulin) compared with patients using animal insulin and analogues too

	animal without analogues [± SD; min – max]	animal with analogues [± SD; min – max]
number total all	38	95
male	11	36
female	27	59
age (y)	54,6 [± 11,4, 16-75]	48,4 [± 11,3; 20-77]
BMI	24,9 [± 3,1; 20,0-31,3]	26,8 [± 5,3; 19,5-47,8]
total daily dose (I.U./day)	40,1 [± 17,6; 12-90]	49,3 [± 29,2; 15-160]
diabetes duration (y)	39,1 [± 10,3; 15-67]	33,8 [± 10,6; 11-56]
Insulin duration (y)	39,2 [± 10,4; 15-67]	33,7 [± 10,6; 11-56]
last HbA_{1c} in % (mmol/l)	6,7(50) [± 0,7(7.7); 4,8(29)-8,7(72)]	7,0(53) [± 1,0(10.9); 5(31)-11(97)]
Insulin binding in %	15,0 [± 16,5; 4-81]	16,5 [± 15,6; 4-81]
IA (nU/mL)	1603,4 [± 3251,5; 50-14902]	1917 [± 3490,0; 50-23210]

These data were transferred in an Excel data file (Microsoft®, table calculation program), controlled by 2 persons and imported for statistical analyses into the statistic program Systat® 10.2 (www.systat.com), controlled by 3 persons, 2 of them are statistic professionals.

As the data were not normal distributed we used the Mann-Whitney U test as a non-parametric statistical hypothesis test that is used to compare two sample means that come from the same population, and used to test whether two sample means are equal or not. With the Kruskal-Wallis one-way analysis of variance test by ranks we assessed whether samples originate from the same distribution, if we compare more than two samples. With Pearson´s chi-squared test we tested some null hypotheses: We define the limit for significance level to p = 0,05. Descriptive statistics (mean values, standard deviations, correlations) were done by Excel, non parametric tests were performed by Systat®.

► Limitations, Possible Bias and Difficulties

For a better reminder, we gave our patients a list of about hundred historical and actual insulin product names and the names of the insulin producing companies. From this they could choose the origin of insulin (bovine, porcine, human, analogue) or special information as importations or foreign insulin. Sometimes it was easier for the patients to remember the insulin producing companies, years of use and / or the origin of insulin – that enabled reconstructions too. Many of the collected data has been very accurately reconstructed and confirmed by medical prescriptions. The duration of application was less precise, but one could rely on semi quantitative results (shorter or longer than weeks, months, years). Overall, we only had to exclude 4,8% of patients due to lack of reliable data.

We could not discriminate between insulin auto-antibodies and acquired antibodies and non specificity due to cross reactivity between different insulin sorts. But in average the influence of IAAs in our patients should be much smaller than in children cohorts, as their mean age was 45,4 years with an averaged diabetes duration of 26,5 years, in which the IAAs should have been disappeared (Figure 4).

It is difficult to recruit “pure” groups of T1D-patients who have used exclusively only one sort of insulin over a longer time – and this is for the future nearly impossible. Before the introduction of human insulin about 35 years ago, they could have used only animal derived insulin (mostly bovine and porcine, purified in the 1970th). The first analogue insulin starts 1996, about 22 years ago. In the past, the vast majority oft he T1D has had contact with different sorts, different degrees of purity, each with different useful lives. Nowadays, there is a growing group that is only treated with analogues – but we do not have a peer group for comparison purposes. However we can form groups in which one or some sorts are missing and compare them to each other. IA against animal insulin may persist for more than 20 years[11].

We selected our CSII-patients (241 of 245 T1D-patients) from our highly specialised institution with the peculiarity that the influence of any long acting insulin, the analogues, too, stopped mostly many years ago totally.

As some of our significances failed sharply the 5 % level, we urgently need bigger groups. The statistical power of these data suffers from the high dispersion (high standard deviation) of the values.

►Results (cross-sectional study)

Table 3: insulin usage groups in all of our patients

group	**insulin sorts**	**number**	
0	only regular	64	different products
1*	only aspart	80	Novorapid®
2	only glulisin	7	Apidra®
3	only lispro	53	Humalog®,Liprolog®
4*	aspart + lispro	35	
5	glulisin + lispro	4	
6*	aspart + lispro + glulisin	5	
	sum	248	

significant different levels of IA absolute: p = 0,003,
for %-binding p = 0,004, compared to group 0

- Significant more patients (almost twice as many) using analogue insulin produce IAs (p = 0,023; p = 0,04) compared with human insulin users;
- significant higher IA-levels in the group of analogue users compared *with the **rest*** of the group (p < 0,05).
- There are borderline significant higher IA-levels in the group of analogue users compared *with **human** insulin users* (p = 0,07),
- in all cases significant higher IA-levels in the aspart-insulin group (Novorapid®; Fiasp® not yet available in those time) compared with human insulin users, users of other analogue insulins and users of animal insulin (p ≤ 0,003).
- The T2D- and MODY-group is too small to make strong statements. May be, the immunogenic impacts are less pronounced respective an indication that only T1D autoimmune genes promote the IAs, not the genes for T2D or MODYs. Therefore we could not detect IAs among them.
- There was no correlation between HbA_{1c} and amount of IAs in all participants, but the HbA_{1c}-values of patients with hyperglycemic-hypoglycemic phenomenon were higher.
- It exists a weak correlation between I(A)As and diabetes-duration with a declining tendency (see Figure 3). Other interesting relationships related to age, insulin demand and other autoimmune diseases were too speculative or not verifiable due to weak data.

► Follow up (Longitudinal study)

Alerted by the results of this cross-sectional study, we routinely measured the I(A)As of each T1D who contacted us at our outpatient clinic, and we repeated this measurement yearly. In Figure 4 you see the IAA-courses of patients, whose titers were initially above 7000 nU/mL, regardless of whether they were included in the cross-sectional study above or not. You see the averaged titers of the start-I(A)A and of the averaged end-I(A)A about 5 years later, not their fluctuations. The black line represents the development only of those patients, whose aspart-insulin was withdrawn after the start-I(A)A measurement. The blue graph represents 3 patients who were treated only with bovine-insulin in the past, afterwards using human insulin. The orange one shoes 5 newly diagnosed T1D (< 3 years; 3 treated only with human insulin, 2 patients treated some months with aspart insulin, afterwards with human insulin). Not shown is a female who undergo an immuno-adsorption therapy continuously since 7 years about every 6 weeks. One male developed stable titers at about 7000 nU/mL and stable bloodglucose with less insulin demand after 2 immuno-adsorption therapies.

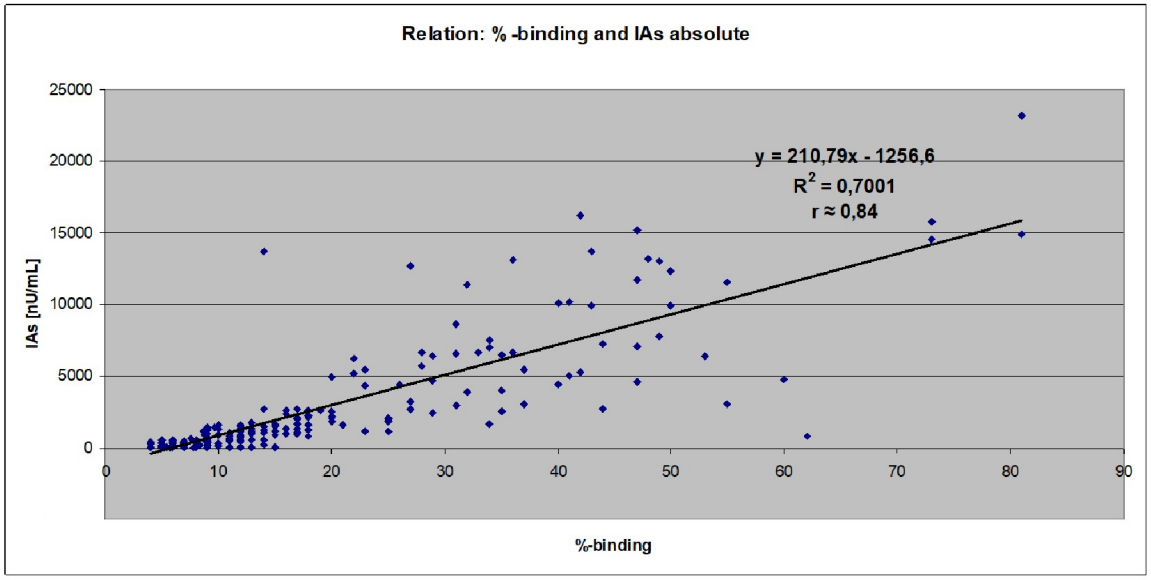
► Discussion and Conclusions

What is fact, what can we hypothesize? Animal derived insulin and also analogue insulin are not produced naturally by the human body and all sorts are administered at unnatural routes. Therefore the human immune system may detect and fend them off with humoral factors as it does it with other foreign proteins, too.

We have measured significant larger titers of IAs between T1D-patients treated with short acting analogue insulin in contrast to T1D-patients treated with short acting human insulin (probably only the effect of insulin aspart). First, no more, no less. We can further conclude from our data that independent of high purity and origin, therapeutic insulins continue to be immunogenic in humans. Finding antibodies is not a normal state, and the extent is surprising - but it is initially only a laboratory value and not (yet) a disease. We should ask: What do they matter?

We measured IAs with 2 different methods (Figure 1): %-IA-binding of insulin and absolute concentrations of IAs (nU/mL).

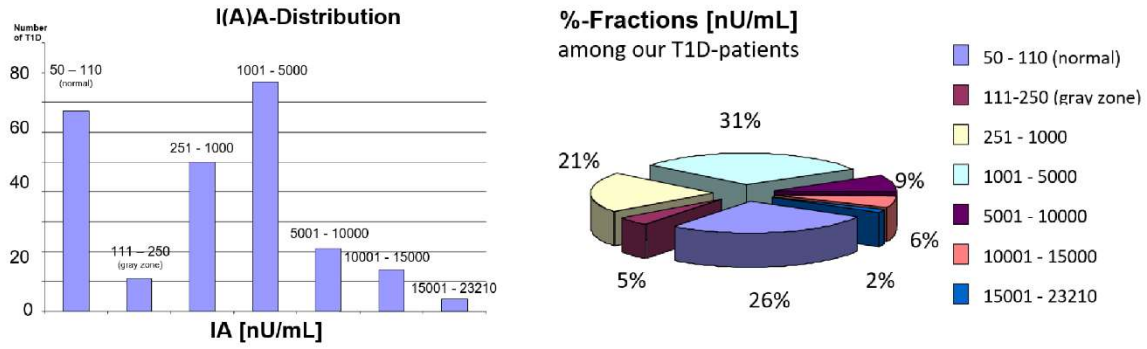
Figure 1: Relationship of the percentage of bound insulin to the absolute amount of insulin antibodies [nU/mL]



- Both IA-values for all healthy subjects were inside the normal ranges and confirmed these normal ranges for our whole collective.
- We found significant higher IA-levels in all T1D-patients compared with healthy persons ($p < 0{,}000$).
- 69 % of all T1D-patients exceed the IA normal range, 60 % the gray zone.

In our data we see that they correlate indeed, but not very strongly ($r \approx 0{,}84$). For some persons both values differ very much, therefore the methods must have different problems or they do not testify the same. We see an unphysiological problem in the fact, that during the analyze procedurte in patients serum sample there is always added the same aliquot of insulin independent from the patients insulin requirement. The in some cases large differences in insulin sensitivity and varying insulin demand for eating and correction are not taken into account. But when it comes to understanding the clinical symptoms of hyperglycemia after administering insulin for eating or correction and late postprandial hypoglycemia as binding and releasing processes from the IA-buffer (due to the law of mass action), then this process is much more dynamic in vivo and not comparable with the percentage of bound insulin in vitro with constant insulin addition. If one considers the clinical consequences (hyperglycemia – hypoglycemia) the measurement of the proportion of bound insulin of unphysiological constant insulin quantities, the same for everybody, therefore seems less relevant. If we want to describe the status of the IA-producing process, it is more reliable to determine absolute amounts or concentrations of IAs.

Figure 2: Distribution of IAs among T1D-patients



Although the frequency and amount of IAs in our cohort may be was biased by several factors (p. e. nearly exclusive CSII-patients, no pure groups, high standard deviations, …), the phenomenon of potentially life-threarening high titers of IAs exists. This has been shown clearly in one patient and 5 other patients suffer from severe therapy problems. This is therefore not as rare as generally assumed up to now.

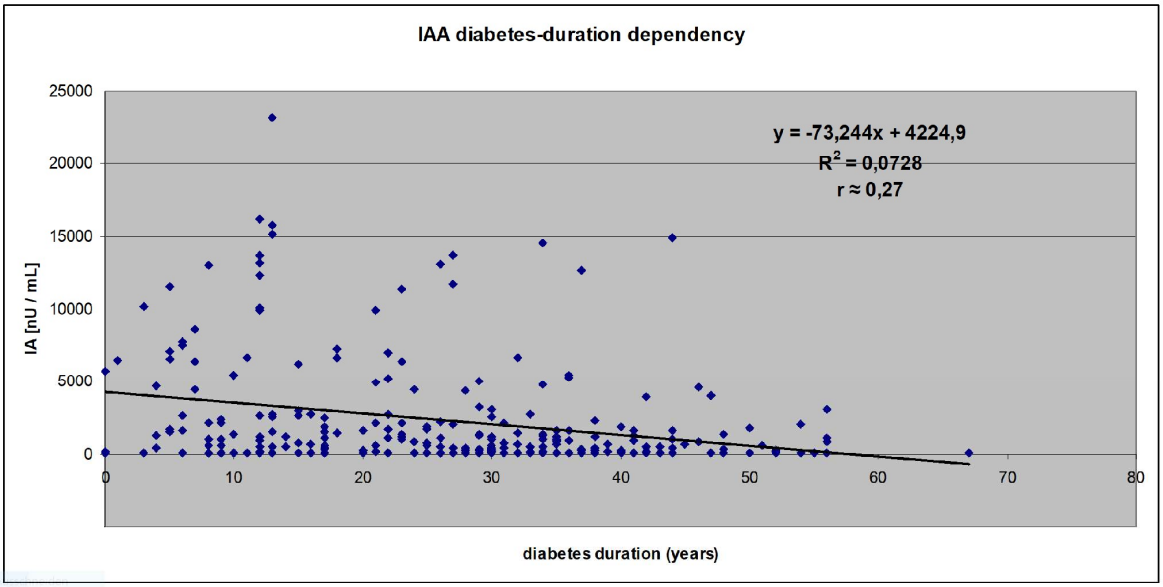
We see correlations, significant differences and are tempted to assume causalities. But we need to separate the early phase of type-1-diabetes with insulin-auto-antibodies (IAA). These IAAs can rise to high values and disappear relatively quickly (Figure 4). This allows them to overlay the subsequent or simultaneous development of acquired insulin-antibodies (IAs), and are are not specifically distinguishable by common assays from the IAAs. However, if there are significant differences between the frequency of the occurrence and / or the amounts of I(A)As between human insulin and analogue insulin, then at least these differences seem to be provoked by the insulin sort.

Furthermore we see in our data that T1D-patients treated with analogues and the longer the higher are the titers [nU/mL]: 1789,3 no analogue contact - 2635,6 with analogue contact, this difference is significant. Remarkable: 1603,4 for treated with porcine insulin (n=2), without any contact to analogues, nor bovine insulin (not listed in Table 1). Due to the small number of cases, it would be premature to characterize porcine insulin as less immunogenic.

The longest treatment period with insulin is usually expected in children with T1D. For this reason, it is particularly important for them to pay attention to possible side effects in longterm use when selecting insulins. We advocate that the development of IAs depending on the type of insulin administered is carefully examined in further serious studies. So far this problem is little known among pediatricians. This is understandable for the following reasons: When pediatricians treat T1D-patients, they experience the outbreak of the disease with different high titers of IAA. Perhaps, in rare cases they control them quantitatively during disappearing, but of course they do not have a good chance to see the development of acquired IAs in the short period of the remaining childhood. If internal specialist then take over the further treatment of the now grown up T1D-patients they experience the phase of decreasing IAAs and possibly simultaneously increasing IAs. In the same time they see the phase of increasing IAAs in their adult patients with newly diagnosedT1D and see the follow-up for decades in both groups – and therefore he is confronted with the increasing / decreasing IAAs and the increasing IAs. The respective effects are then indistinguishable. Over a short period up to 2 years, Mianowska[15] observed an increase of the frequency of semi quantitatively I(A)As from 80% to 97.9% with no difference between the insulin sorts. This is no proof that those children with T1D will not run the risk of developing acquired IAs in large quantities later on, depending only on special insulins. He sees this phenomenon as a consequence of the T1D autoimmune process which would disappear spontaneously within a short time (similiar to the steep drop of the graph `freshly diagnosed´ T1D in Figure 4) or as immunogenic consequences of insulin therapy. However, the diabetes duration of our problematic patients however is much longer. We have detected more than 10000 nU/mL of I(A)As in 16 of our 245 T1D-patients with diabetes duration longer than 10 years, in 8 patients longer than 20 years, in 3 longer than 30 years (Figure 3). Will they persist for a lifetime, at least by some patients? This remind

me of the disastrous discussion of pediatricians in the past that children will not develop late-complications.

Figure 3: Relationship between I(A)As and diabetes duration



We have no data of the influence of the analogue long-acting insulins to IAs. Most of our patients never have had or have contact with analogue long-acting insulin (CSII-therapy), only few just for short periods and / or long time ago. Unfortunately, we cannot describe the course of the further development of IAs longer than for about 5 years so far. The I(A)A courses of our patients who were treated exclusively with human insulin after bovine insulin (n=3) and those with fresh manifestations (n=5) agree with the literature, so that they do not have to be doubted despite the small number of cases. New however, are the IA courses of patients (n=13) who switched to human insulin due to high IA titers (> 7000 nU/mL).

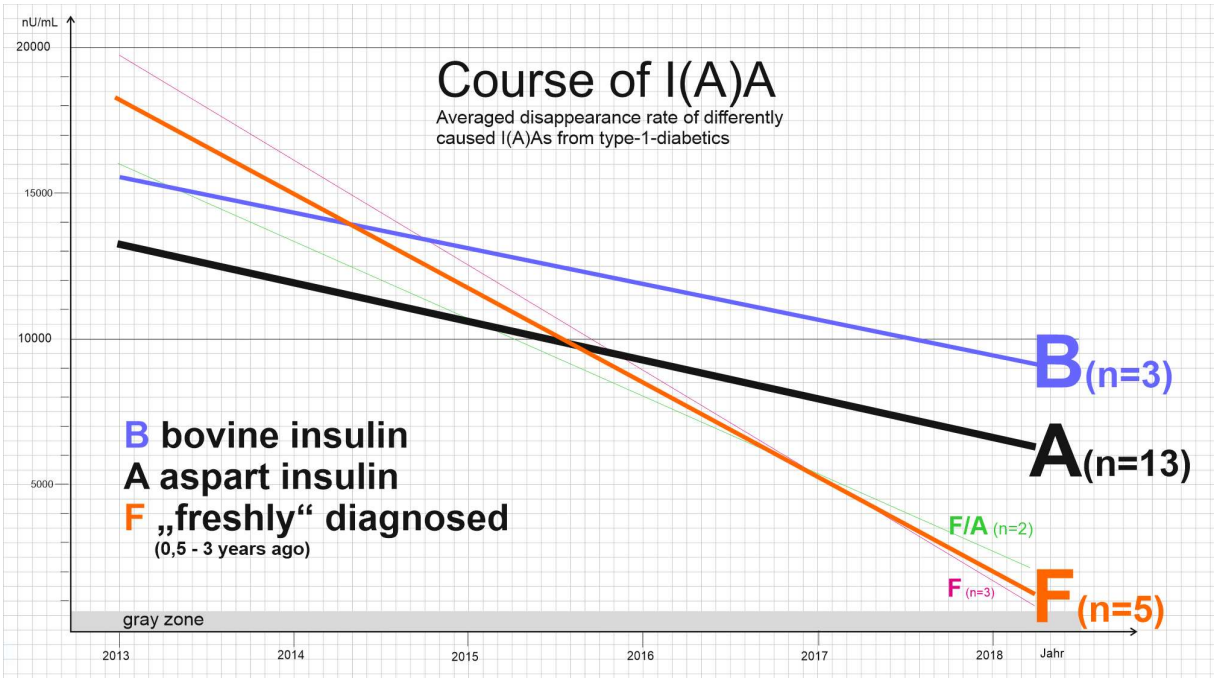


Figure 4 clearly shows that IAAs disappear faster and the acquired IAs remain (very) long: The parallelism of the disappearance rates of bovine insulin and aspart insulin induced antibodies is apparent (both induced by foreign proteins). Once triggered, can they be boostered again by newly exponation, or by administering another analogue or human insulin (insulin hopping)? The fact that IAAs and IAs are degraded or post-produced at different rates is an indication that they are different proteins.

When we critically discuss human insulin and short acting analogue insulins with regard to their direct effect at bloodglucose and their suitability for everyday life, we see advantages

and disadvantages for both insulin sorts in different patients and in different life circumstances. Therefore it is not necessary to start therapy principally with analogues. It is too simple to call IAs the price of a modern therapy or lifestyle. If we prescribe analogue insulin, we should inform the patient to this risk and control their IA-status routinely. As we announcing “empowerment” and “informed decision”, we should require warning notices in the enclosed package inserts of insulins, if our results should be confirmed.

Furthermore we should be more vigilant when we see daily long stretched hyperglycemias followed by late postprandial severe hypoglycemias, especially if happening in the early morning. Then we should take IAs in consideration, especially in cases of growing total daily dosages of insulin and should observay their course and therapeutic impacts in the near future.

When Fineberg et al summarize: “Little proof exists, however, that the development of insulin antibodies (IAs) to exogenous insulin therapy affects integrated glucose control, insulin dose requirements, and incidence of hypoglycemia…” then we cite Ishizuka T et al.[14] “encountered two patients who developed daytime hyperglycemia and early morning hypoglycemia because of insulin antibody (IA) that the affinity was extremely lower and the capacity extremely higher than those of IA in the insulin autoimmune syndrome, after their insulin treatment were changed from human insulin to analogue insulin.”

And I encountered a young patient with type-1-diabetes, who can only survive by continuous immune adsorption therapy due to high IAs (maximum of 23210 nU/mL, 81-% binding) since then of more than 7 years, likely caused by analogue insulin, but not by aspart insulin (because she only has had contact with aspart insulin after the diagnosis of extreme I(A)A-values). At this extreme endpoint, we can proof the life-threatening clinical impact of I(A)As. In such cases, no standard therapy exists. There is an analogy to the seldom autoimmune Hirata disease, first described by Hirata et al. 1970. He reported a patient with insulin autoimmune syndrome, followed 1994 by reports of 197 Japanese persons with the same disease[1,2,5,6,8] and T2D-person[7], now in T1D-patients?

But at least 5 further T1D-patients of my collective have bigger therapy-problems (daytime long stretched hyperglycemias, nocturnal severe hypoglycemias) probably due to high IA-levels (> 7000 nU/mL), but some other patients with similar quantitative concentrations of nU/mL have obviously no therapy problems.

We also do not know whether patients with high IA titers suffer from further negative effects.

I fear that we have seen only the tip of the iceberg – or have I and my colleagues systematically checked our patients regarding to this phenomenon so far?

As an attempt to answer the question of the title, we need to extend it: Late revenge of analogue insulins and/or footprints of T1D autoimmune process and/or unavoidable immunogenic consequences of insulin therapy and/or Hirata-disease among T1D-patients? So far we cannot say exactly which of these options or mixtures are applicated for our patients in individual cases. Depending on the patient we search at the outbreak of T1D to the diagnostic markers of autoimmune process, later we should observay unavoidable immunogenic consequences of insulin therapy. May be it is also the seldom Hirata-disease (associated with HLA-DRB1*0406/DQA1*0301/DQB1*0302?). However the suspicion that one or more analogue insulins may play a triggering or amplifying role is particulary worrying. Now we can make mistakes in two opposite directions: Optimistic appeasing or fear mongering. A better consequence is to eliminate this uncertainty through serious investigations. We have many undecided questions and should avoid a false sense of security. One of the most urgent question is: will this phenomenon remain an exception or will there be an avalanche in the long term? We should investigate it immediately – may be we have a chance to avert disaster for some T1D.

► Conclusion

It must urgently be investigated whether the start of therapy with analogue insulins or the switch to analogue insulins leads to a dangerous development towards an impairment of the blood glucose metabolism by IAs. Until this is clarified, the indication for the prescription of analogue insulin should not be given too quickly. Even if the the metabolic state is chronically unsatisfactory, it should be first carefully examined for construction errors of the therapy and insufficient adherence with the rules before changing the insulin sort. The hastily prescription of insulin analogues also the change from one type of insulin to the other („insulin hopping“) must be reconsidered under these results. In addition all clinical diabetologists should routinely check and track their type-1-diabetics of their IA-status.

► Acknowledgments

Dr. Jochen Selbach, nephrologist of Caritas Krankenhaus carried out the immuno-adsorptions.
Laboratory Dr. Volkmann and colleagues GbR (Chief of the autoimmune diagnostic department: Dr. Martin Blüthner) performed all IA-measurements as nU / mL and advised me on the laboratory method and described it in this article.
Bettina Teupe thoroughly revised the English texts.

► Authors address

Dr. med. Bernhard Teupe, Diabetes-Dorf Althausen, Im Brunnental 10 – 18, D-97980 Bad Mergentheim; Email: Teupe@DiabetesDorfAlthausen.de.

► Conflict of interest

No financial or advisory connections to the insulin producing industry, no sponsoring from any side.

► References

Literature research by PUBMED.DE, DIMDI, Literature-Appendix at publications, spontaneous

[1] Hirata Y; Uchigata Y: Insulin autoimmune syndrome in Japan. Diabetes research and clinical practice 1994; 24 Suppl: 153-7

[2] Uchigata Y , Eguchi Y, Takayama-Hasumi S, Omori : Insulin autoimmune syndrome (Hirata Disease): clinical features and epidemiology in Japan Review Article. Diabetes Research and Clinical Practice 1994, 22, Issues 2–3: 89-94

[3] Segal, Allgrove et al.: Severgarde insulin resistance secondary to insulin antibodies: successful treatment with immunsupressant MMF. Pediatric Diabetes 2008, 9, 250 - 254

[4] Koyama R; Nakanishi K; Kato M; Yamashita S; Kuwahara H; Katori H: Hypoglycemia and hyperglycemia due to insulin antibodies against therapeutic human insulin: treatment with double filtration plasmapheresis and prednisolone. The American journal of the medical sciences 2005; 329 (5): 259-64

[5] Yeung CW; Mak CM; Lam KS; Tam S: Hypoglycemia due to autoimmune insulin syndrome in a 78-year-old Chinese man. British journal of biomedical science 2012; 69 (2): 80-82

[6] Nasu T; Suzuki R; Okamoto Y; Miyata K; Uno A; Nakao R; Kawashima A; Nakao T; Kondo M: Late postprandial hypoglycemia due to bioactive insulin dissociation from autoantibody

leading to unconsciousness in a patient with insulin autoimmune syndrome. Internal medicine (Tokyo, Japan) 2011; 50 (4): 339-43

[7] Zhao TY; Li F; Xiong ZY: Frequent reoccurrence of hypoglycemia in a type 2 diabetic patient with insulin antibodies. Molecular diagnosis & therapy 2010; 14 (4): 237-41

[8] Paiva ES; Pereira AE; Lombardi MT; Nishida SK; Tachibana TT; Ferrer C; Hauache OM; Vieira JG; Reis AF: Insulin autoimmune syndrome (Hirata disease) as differential diagnosis in patients with hyperinsulinemic hypoglycemia. Pancreas 2006; 32 (4): 431-2

[9] Kim S; Yun YM; Hur M; Moon HW; Kim JQ: The effects of anti-insulin antibodies and crossreactivity with human recombinant insulin analogues in the E170 insulin immunometric assay. The Korean journal of laboratory medicine 2011; 31 (1): 22-9

[10] Eisenbarth GS; Jeffrey J: The natural history of type 1A diabetes. Arquivos brasileiros de endocrinologia e metabiologia 2008; 52 (2): 146-55

[11] Oak S; Phan TH; Gilliam LK; Hirsch IB; Hampe CS: Animal insulin therapy induces a biased insulin antibody response that persists for years after introduction of human insulin. Acta diabetologica 2010; 47 (2): 131-5

[12] Klingensmith GJ: Insulin antibodies - are they still with us? Do they matter? Pediatric diabetes 2011; 12 (2): 75-7

[13] Chen JW; Frystyk J; Lauritzen T; Christiansen JS: Impact of insulin antibodies on insulin aspart pharmacokinetics and pharmacodynamics after 12-week treatment with multiple daily injections of biphasic insulin aspart 30 in patients with type 1 diabetes. European journal of endocrinology / European Federation of Endocrine Societies 2005; 153 (6): p. 907-13

[14] Ishizuka T; Ogawa S; Mori T; Nako K; Nakamichi T; Oka Y; Ito S: Characteristics of the antibodies of two patients who developed daytime hyperglycemia and morning hypoglycemia because of insulin antibodies. Diabetes research and clinical practice 2009; 84 (2): e21-3

[15] Mianowska B; Szadkowska A; Pietrzak I; Zmyslowska A; Wegner O; Tomczonek J; Bodalski J; Mlynarski W: Immunogenicity of different brands of human insulin and rapid-acting insulin analogs in insulin-naive children with type 1 diabetes. Pediatric diabetes 2011; 12 (2): 78-84

[16] Fineberg SE; Kawabata TT; Finco-Kent D; Fountaine RJ; Finch GL; Krasner AS: Immunological responses to exogenous insulin. Endocrine reviews (2007); 28 (6): 625-52