PROGENS-HbA$_{1c}$ study: safety and effectiveness of premixed recombinant human insulin (Gensulin M30)

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Abstract

Introduction: Insulin analogues have gained widespread popularity. However, in many countries the use of these drugs is limited by their relatively high cost, so there is still a need for more cost-effective human insulin therapies. The aim of the study was to assess the effectiveness and safety of the premixed recombinant human insulin (rhuI) Gensulin M30 in a real-life setting.
Material and methods: The study group consisted of 4257 patients (2196 female, 2061 male) with type 2 diabetes, aged 63.7 ±9.4, with body mass index (BMI) 30.3 ±4.5 kg/m^2 and diabetes duration 9 ±5.5 years. All patients were treated with premixed rhuI Gensulin M30. In 91.7% of patients, insulin was used in combination with metformin. In 3.7% of patients, it was used with sulphonylureas. The patients were observed for a period of 6 months.
Results: The total insulin dose on visit 1 was 36.1 ±18.7 U (0.42 ±0.22 U/kg), and by the end of the study it reached 40.3 ±18.9 U (0.48 ±0.22 U/kg). A significant, continuous decrease of the levels of glycated hemoglobin (HbA$_{1c}$), along with fasting and postprandial plasma glucose, was observed during the study period. The frequency of hypoglycemia increased slightly during the study, although these figures remained low, especially with regard to severe hypoglycemic episodes (0.02 episodes/patient/year). The lowest number of hypoglycemic episodes occurred in patients treated with insulin and metformin, while the highest number of episodes was observed in patients treated with insulin alone. No weight changes were noted in the patients during the study.
Conclusions: This study shows rhuI Gensulin M30 to be effective and safe in a real-life setting.

Key words: type 2 diabetes, hypoglycemic episodes, glycated hemoglobin.

Introduction

Type 1 and type 2 diabetes are both increasingly prevalent throughout the world (type 2 diabetes is the most common). According to the International Diabetes Federation (IDF) and World Health Organization (WHO) data, in the year 2014–2015 there were over 400 million adults in the world estimated to have diabetes [1, 2]. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population [2]. The prevalence of diabetes in Poland in the years 2010–2014, determined on the basis of the number of subjects recorded by the National Health Fund as having diabetes, was found to be 4.47% [3].

The disease is not well controlled in many of these patients: according to recent studies, 45% of adults diagnosed with diabetes in the U.S. do not achieve the recommended level of glycated hemoglobin (HbA_{1c}) [4]. In Poland, up to 75% of diabetic patients may have insufficient glycemic control [5]. One potential solution to this problem is the development and marketing of new drugs, parallel to intensively addressing "therapeutic inertia". However, new treatments increase costs for health care systems, and, especially in developing countries, the costs of new and existing drugs may limit their use. These facts play a significant role in motivating the search for cost-effective treatments [6].

Type 2 diabetes is a progressive disease. The gradual development of pathophysiological disturbances, particularly the β-cell defect, makes it necessary to intensify treatment with insulin therapy. In accordance with the Polish Diabetes Association guidelines, insulin therapy in type 2 diabetes is indicated in a newly recognized disease with blood glucose > 300 mg/dl (16.7 mmol/l) and with concomitant clinical symptoms of hyperglycemia. Another indication is lack of efficacy of oral hypoglycemic medications (HbA_{1c} > 7% despite the intensification of therapy) [7]. A premixed insulin regimen is frequently used in clinical practice, especially in elderly patients who maintain a regular lifestyle.

Gensulin is a recombinant human insulin produced by Bioton, a pharmaceutical company based in Poland. The active pharmaceutical ingredient in Gensulin is recombinant human insulin, with an amino acid sequence and structure identical to those of the native human hormone. The insulin is produced by recombinant DNA technology using a special non-disease-producing strain of *Escherichia coli* bacteria. The product meets pharmacopoeial requirements (United States Pharmacopeia, European Pharmacopoeia) and strict internal (company-specific) controls (requirements regarding the levels of contaminants and related substances, as well as substances produced during product decomposition).

A team of researchers from Poland conducted a comparative analysis of the bioavailability and hypoglycemic activity of the new recombined insulin Gensulin M30 and a reference preparation of premixed insulin after subcutaneous application [8]. They found no differences in the bioavailability between the two preparations and no statistically significant differences between their pharmacokinetic parameters (including AUC – area under the curve of insulin concentrations in the blood serum as a function of time, C_{max} – the maximum insulin concentration in the blood serum, T_{max} – time for the maximum insulin concentration in the blood serum, $T_{1/2}$ – half-life). The lack of significant differences between the pharmacokinetic parameters for glucose and C-peptide concentration in plasma for Gensulin M30 and the reference preparation suggests that the physiological and pharmacodynamic responses are also comparable. The pharmacokinetic profile of Gensulin M30 corresponds to the theoretical profile for premixed insulin preparations, consisting of 30% regular insulin and 70% NPH insulin, like the reference preparation. The above results indicate the bioequivalence of recombined human insulin – Gensulin M30 – and the reference preparation. Furthermore, Gensulin M30 did not differ from the reference preparation in the scope of safety, besides a slight increase in the frequency of hypoglycemic episodes and their intensity.

However, the product was not tested in a clinical study conducted in a large group of patients. Therefore, the objective of this study was to assess the effectiveness and safety of this recombinant human insulin in real-life settings.

Material and methods

The PROGENS HbA_{1c} study was an observational study sponsored by Bioton, a pharmaceutical company based in Warsaw, Poland. The study protocol was prepared according to the GCP and the Declaration of Helsinki and approved by the Committee for Surveillance of Clinical and Animal Research of the MSW Central Clinical Hospital, Warsaw, Poland. All subjects signed an informed consent form. This article was prepared according to STROBE guidelines [9].

The study included 4257 patients with type 2 diabetes, aged 18 years and older, with a body mass index (BMI) < 40 kg/m^2, who had been receiving biosynthetic human premixed insulin Gensulin M30 from 2–4 weeks before enrolment in the trial, alone or in combination with the oral antidiabetic drug Avamina (metformin) or Avaron (glimepiride). Exclusion criteria included: diabetes other than type 2, a history or presence of serious cardiovascular disease (myocardial infarction/acute coronary event or stroke in the last

3 months, NYHA stage IV heart failure, stage III or IV coronary heart disease according to CCS), unstable or high (> 180/100 mm Hg) blood pressure in spite of proper medication, severe hepatic dysfunction (aspartate transaminase (AST), alanine transaminase (ALT) > 3× above the upper limit of the normal range), medication with systemic glucocorticosteroids (excluding inhaled preparations), ACTH or interferon, chronic mental disorders, alcohol and/or substance abuse, participation in other clinical trials or studies in the preceding 3 months, allergy to insulin or any other compound of the formulation, pregnancy or breastfeeding, and other conditions or diseases that could be considered a contraindication for participating in the trial.

After the first visit, there were two follow-up visits, scheduled every 3 months, so that the patients were observed over a total of 6 months. A flow chart is shown in Figure 1. The study was a multicenter one, conducted in 150 diabetological centers in Poland. The insulin dose was modified during the visits according to the HbA$_{1c}$ level (the target HbA$_{1c}$ level was determined individually for each patient). Insulin was administered through the GensuPen automatic injection system. Patients were advised to inject the insulin according to the drug description – 15 min before mealtime.

The primary objective of the study was to assess the effectiveness and safety of the drug. Effectiveness was evaluated based on the levels of HbA$_{1c}$, along with fasting and postprandial glucose. The average glucose reading from the 90 preceding days was also calculated automatically from all values measured by a Glucocard 01-mini plus glucose meter (Arkray, Japan). All patients were advised to monitor glycemia once or twice a day (if the insulin dose was stable) and to perform a four-point blood glucose profile at least once a week. If the insulin dose was changed (per protocol at visit one or two) more intensive control was advised during 2 consecutive weeks (daily fasting plasma glucose and at least two postprandial values measured 2 h after a meal, at least one seven-point glycemic profile). At each visit the advice on diet and exercise was repeated and HbA$_{1c}$ was measured with an NGSP certified (www.ngsp.org) A1cNow+ point of care device (Bayer Health Care, Leverkusen, Germany).

Every investigator was provided with a web-site address where serious adverse events (SAE) were to be reported within 24 h. In the case report form (CRF), special attention was paid to drug-related adverse events, especially hypoglycemia, local adverse reaction at the injection site, or lipoatrophy. Hypoglycemic episodes were classified as severe, documented symptomatic, asymptomatic, probable symptomatic, or relative.

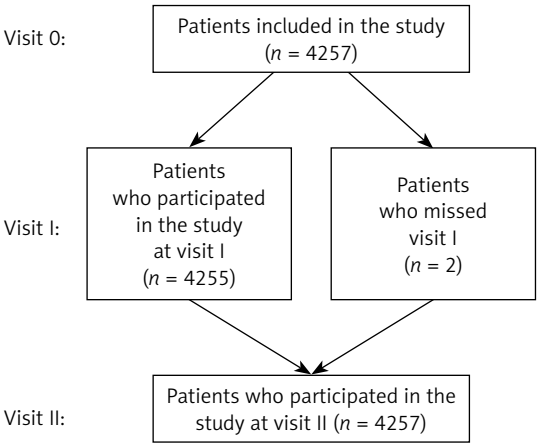

Figure 1. Study diagram

The definitions of hypoglycemic episodes were based on the report from the American Diabetes Association Workgroup on Hypoglycemia [10] and are as follows:

- severe hypoglycemia: an episode requiring the assistance of another person to raise the plasma glucose concentration resulting in a resolution of symptoms, with or without a measured low plasma glucose concentration;
- documented symptomatic hypoglycemia: symptoms consistent with hypoglycemia with a measured plasma glucose concentration < 70 mg/dl (3.9 mmol/l);
- asymptomatic hypoglycemia: a measured plasma glucose concentration < 70 mg/dl (3.9 mmol/l) in the absence of symptoms;
- probable symptomatic hypoglycemia: typical symptoms of hypoglycemia without a measured plasma glucose concentration;
- relative hypoglycemia: typical symptoms of hypoglycemia with a measured plasma glucose concentration > 70 mg/dl (3.9 mmol/l) but approaching the level of hypoglycemia.

A secondary aim of the study was to assess the correlation between average blood glucose values and HbA$_{1c}$ levels in the general population of Polish diabetic patients and in subpopulations with anemia and chronic kidney disease. The results of these secondary analyses will be published in a separate paper.

Statistical analysis

Statistical analysis was performed using the SAS System software (version 9.3). Missing values were not imputed. Statistical significance was set at the level of $\alpha = 0.05$.

Results

The basal characteristics of the patients were as follows: 51.6% (2196) women, mean age of 63.7 ±9.4 years, BMI 30.3 ±4.5 kg/m^2, diabetes duration

9 ±5.5 years, estimated glomerular filtration rate (eGFR) 73.3 ±21.3 ml/min/1.73 m^2, AST 28.6 ±12.5 IU/l, ALT 30 ± 13.6 IU/l, hematocrit value 37.5 ±2.5%, blood hemoglobin concentration 13.7 ±1.5 g/dl, erythrocyte count 4.5 ±0.5 T/l. As the mean duration of diabetes in this group of patients was relatively long, diabetes-related complications were found in many of them: 28.4% of the diabetic patients had retinopathy, 7.2% had nephropathy, 48.4% suffered from coronary heart disease, 12.3% had peripheral vessel disease and 22.9% had neuropathy.

All 4257 patients were treated with recombinant human insulin Gensulin M30, in most cases (91.7% of the group) in combination with metformin. Sulphonylureas were used in only 3.7% of the patients. The mean daily insulin dose at visit 1 was 0.42 ±0.22 U/kg. The dose increased at visit 2 (0.47 ±0.21), and at the end of the study it was 0.48 ±0.22 U/kg. The average dose of metformin at visit 1 was 1700 mg, at visit 2 it was 2000 mg, and at visit 3 it was 2200 mg. The average dose of glimepiride at visit 1 was 2.95 mg, and at visits 2 and 3 it was 2.92 mg.

The results pertaining to glycemic control are shown in Figure 2. As can be seen, during the study there was a significant, continuous decrease of the mean levels of HbA_{1c} and of fasting and postprandial plasma glucose.

During the study no SAE were recorded other than severe hypoglycemia. However, as in many cases the CRFs did not allow for reliable differentiation between particular types of hypoglycemia, this part of the analysis was conducted on 3465 patients only. The frequency of hypoglycemia increased slightly during the study with the intensification of treatment, although it remained low even then (11.6% documented episodes and 0.4% severe episodes at visit 3; accurate percentage data of hypoglycemic episodes during the study are presented in Figure 3), especially with regard to severe hypoglycemic episodes. The lowest number of hypoglycemic episodes occurred in patients treated with metformin, while the highest number was recorded in patients treated with insulin alone. Overall, the number of severe episodes was low in all treatment groups (Table I), with an average of 0.02 episodes/patient/year. Episodes of hypoglycemia were more frequent in patients in whom doses of insulin were changed during the study, regardless of whether the dose was increased or decreased (Figure 4).

No significant weight change was noted in the patients during the study. The mean BMI at the beginning of the study was 30.3 ±4.5 kg/m^2 and at the end it was 30.3 ±4.6 kg/m^2. It should be noted that 12 patients with BMI higher than 40 kg/m^2

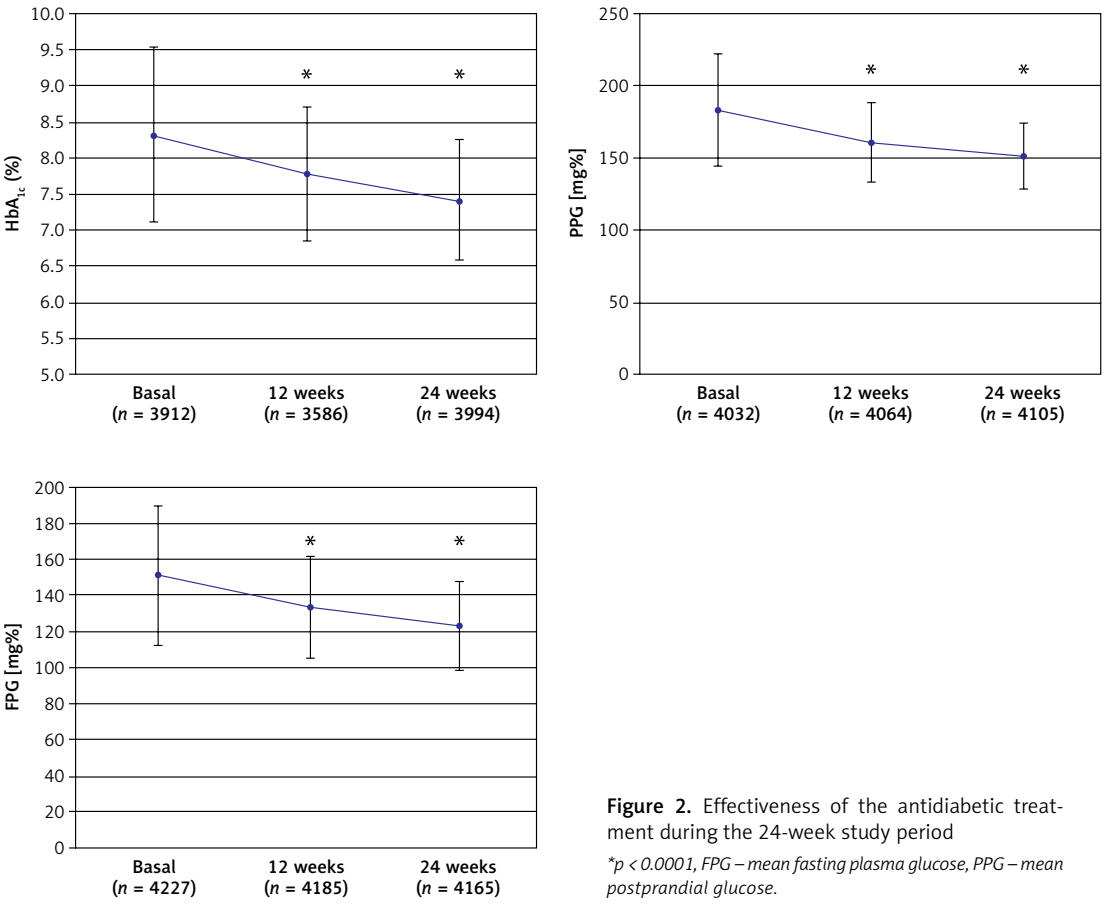

Figure 2. Effectiveness of the antidiabetic treatment during the 24-week study period

**p < 0.0001, FPG – mean fasting plasma glucose, PPG – mean postprandial glucose.*

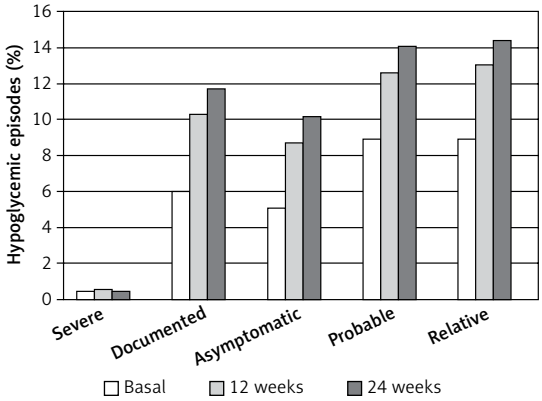

Figure 3. Prevalence of hypoglycemia in the study period (per protocol analysis)

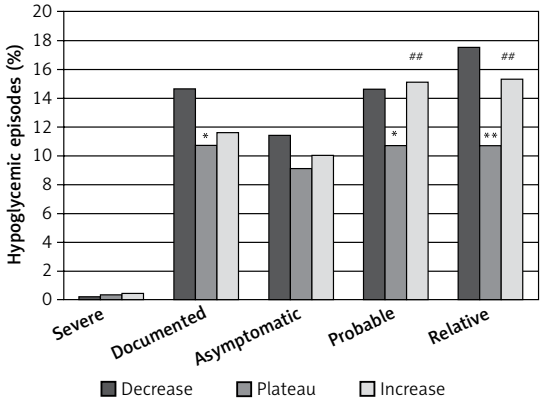

Figure 4. Comparison of frequency of hypoglycemic episodes in patients on stable insulin dose (P) and in those in whom the dose was decreased (D) or increased (I)

**p < 0.05, **p < 0.001 (D vs. P), ##p < 0.001 (I vs. P).*

Table I. Differences in the prevalence of hypoglycemia in various treatment groups in the study period (per protocol analysis) – number (percentage) of patients with at least one event

Event	Insulin, glimepiride, metformin	Insulin, glimepiride	Insulin, metformin	Insulin	χ^2 test for multiple comparisons
Severe hypoglycemia	2 (0.4)	0 (0.0)	14 (0.5)	0 (0.0)	NS
Documented hypoglycemia	75 (14.0)	11 (12.0)	286 (10.5)	29 (26.6)	$p < 0.0001$
Asymptomatic hypoglycemia	49 (9.1)	11 (12.0)	289 (10.6)	19 (17.4)	NS
Probable symptomatic hypoglycemia	72 (13.3)	18 (19.6)	373 (13.7)	20 (18.3)	$p < 0.0001$
Relative hypoglycemia	72 (13.3)	20 (21.7)	400 (14.7)	16 (14.7)	NS

were included in the study, and in spite of that protocol violation it was decided to leave them in the study and include them in the analysis.

Discussion

This study assesses the safety and effectiveness of the human insulin Gensulin M30 in a real-life setting in a large group of patients.

The results show that treatment with premixed Gensulin M30 was effective. The design of the study does not evaluate the true effectiveness of the drug, as insulin treatment was not randomly assigned at visit 1, but rather it had already been started when the patients were included in the study. This study uses a similar design to other observational studies in the literature [11]. Additionally, most of the patients were not insulin naïve, and the decrease of glycemic control parameters was caused not by the insulin itself, but rather by the intensification of treatment through changes in the dose and/or treatment scheme. Most likely, this is the reason why the decrease of HbA$_{1c}$ was not as great as it would be in patients for whom insulin would have been introduced for the first time. For example, in insulin-naïve patients with type 2 diabetes, treatment initiation with premixed Gensulin M30 resulted in a decrease of HbA$_{1c}$ by 1.6% within 6 months [12]. A similar hypoglycemic effect can of course be achieved using another human insulin, as well as human insulin analogues and GLP-1 receptor agonists. However, in the two latter cases the financial cost will be higher, whereas elderly people (study group mean age: 63.7 ±9.4 years) are often lacking sufficient financial resources. Therefore in many cases not only safe and effective but also inexpensive drugs are needed.

One additional factor impacting the assessment of the effectiveness of the drug was the lack of a placebo group. With no placebo group, it is impossible to discern whether the observed decrease of HbA$_{1c}$ and glucose levels was caused by insulin or simply by inclusion in the study. This latter "study" effect (improvement of glucose control in a run-in phase even under placebo treatment) is consistently observed in clinical studies.

The results show that human premixed insulin Gensulin M30 is safe. As can be seen in Figure 3 and Table I, the frequency of hypoglycemia was relatively low. This is especially relevant in

case of severe hypoglycemic episodes, which are associated with an increased risk of cardiovascular incidents and other adverse events, including death [13–15]. Severe hypoglycemia is also a risk factor for automobile accidents [16]. As even minor episodes are associated with clinical symptoms and deterioration in quality of life, avoiding hypoglycemia in general is advised. The frequency of documented, probable symptomatic and other hypoglycemic episodes in the PROGENS study was low. In general, the frequency of hypoglycemia was comparable or lower than in other studies. For example, the rate of major hypoglycemic episodes in patients treated with premixed human insulin at baseline of the IMPROVE study was 0.355 events/patient/year [17], and in the PRESENT study it was 0.7 events/patient/year [18]. The doses of insulin in those studies were also comparable to those in our study. In the PRESENT study the baseline dose was 41.18 U/day, and in the IMPROVE study it was 33.4 U/day (which most likely accounted for the lower rate of hypoglycemic episodes).

Interestingly, patients whose insulin dose either increased or decreased had more hypoglycemic episodes than those who maintained the same dose throughout the study (Figure 4). In the first group (with increased doses), the episodes were probably a result of the implemented changes, while in the second group it was the reverse: the decrease in dosage was a response to an episode. However, in both groups the risk of hypoglycemia is higher, and they should be treated with special attention by the diabetologist.

No significant weight gain was observed in the patients during the study. This result was somewhat surprising, as we would have expected an increase of body weight of about 1–4 kg during these 6 months [19]. One explanation for the lack of weight gain might be that many patients were treated with insulin earlier. Additionally, the patients were advised to change their lifestyle, which might compensate for the insulin effect and explain this finding. Better control and self-control during participation in the study, which is commonly observed in patients participating in medical studies, could account for the effect. A third factor may be that the increase in insulin doses during the study was rather small.

About 90% of the patients in this study were treated with metformin. This finding in an observational study seems to confirm that the doctors followed guidelines, which consistently advise the use of metformin at every stage of treatment [20–22]. The investigators were not asked about the reasons why metformin was not used in some patients, but given the small number of these patients it seems that these cases are likely accounted for by adverse events or contraindications.

On the other hand, less than 5% of patients were treated with sulphonylureas in combination with insulin. Given that it appears that in most cases such a combination is not only unnecessary, but also increases the number of hypoglycemic episodes [23], this fact most likely reflects the real-life behavior of physicians and diabetologists taking part in this study, where the treatment was in fact left to their discretion.

Conventional premixed human insulin preparations have an onset of action of approximately 0.5 to 1 h, usually plateau at 3 to 6 h, and last up to 24 h [24]. Gensulin M30 should be administered within 15 min before a meal, according to the drug label. This instruction was introduced as a result of the study "The timing of injection of premixed insulin 30/70 and glucose profile in patients with type 2 diabetes mellitus" [25]. The authors concluded that administration of Gensulin M30 5, 15 and 30 min before a meal in patients with type 2 diabetes induced a similar postprandial glucose increase and 24-hour profile. Comparable conclusions were made by Müller: an injection-to-meal interval in patients with type 2 diabetes mellitus and preprandial human insulin therapy is not necessary [26].

Muller's survey also highlights that the reported pharmacological properties of a single insulin preparation depend largely on the method used. For example, in the 22 studies analyzed in Muller's paper, the onset of action after subcutaneous injection of human regular insulin ranged from 0.08 to 0.5 h, with peak action from 0.75 to 4 h, and duration of action from 4 to 12 h [27].

The present study has some limitations. For example, the fact that patients included in the study began insulin treatment before randomization, which was necessary to ascertain the observational character of the study, precludes a precise assessment of the influence of insulin on the magnitude of the decrease of glycated hemoglobin or glycemia. However, it must be assumed that the actual decrease of HbA_{1c} in the course of insulin treatment was even greater. As in many observational studies, the characteristics of patients are rather scarce and post-hoc analyses are impossible to perform. Another limitation is missing data. However, in spite of this, the number of analyzed patients was sufficient to draw conclusions (Figure 1).

In conclusion, the PROGENS study shows that the recombinant human insulin Gensulin was effective in terms of lowering glucose and HbA_{1c} levels. The number of hypoglycemic episodes was low and no weight gain was observed in the studied group.

Conflict of interest

The study was sponsored by Bioton. MW, JJ, JR, GZL, AZ declare no conflict of interest. PB was former employee of Bioton, current is employee of Teva. MM is employee of Bioton.

EF – lectures: Bioton, Novo Nordisk, MSD, Berlin-Chemie, Sanofi, Merk, Servier, advisory board: Bioton, Berlin-Chemie, Novo Nordisk.

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