**Monitoring with flash glucose monitoring system to investigate the efficacy of protamine zinc insulin administred once daily in dogs with diabetes mellitus with and without concurrent disorders**

**ABSTRACT**

Insulin is the mainstay of treatment in canine diabetic patients, often combined with dietary modification, exercise, and weight control. Although many insulins are available on the market, the most common insulins historically used in dogs are porcine Lente and NPH, which are intermediate-actin insulin recommended for BID use. Protamine zinc recombinant human insulin (PZIR; ProZinc, Boehringer Ingelheim, Germany) was licensed in Europe for dogs in 2019. PZIR is a recombinant human insulin, which is precipitated with protamine and zinc. It is generally considered to be long-acting insulin due to prolonged absorption from subcutaneous tissue since the insulin/zinc/ protamine complexes cause a slow release of insulin monomers or dimers into the systemic circulation. In support of this, a recent study showed that 71% of diabetic dogs receiving PZIR once daily showed clinical and glycemic improvement. This is a relevant finding considering that the impact of DM treatment on the owner’s lifestyle was cited as a reason for euthanasia in 32% of cases. Therefore, the possibility to use a veterinary insulin that guarantees good glycemic control even if administered only once daily, might be life-saving when it comes to the decision on therapy vs euthanasia.

 The main objective of this study is to further examine the efficacy and safety of PZIR administered once daily in three groups of diabetic dogs monitored with a flash glucose monitoring system (FGMS; FreeStyle Libre; Abbott Laboratories Ltd, Chicago, Illinois). The secondary aims are to evaluate the time required to achieve good glycemic control and to evaluate the effect of the diet type (specific diabetic diet or not) on glycemic control in dogs treated with PZIR. The study is going to be a two-year ongoing study and is going to include three groups of diabetic dogs (group 1: naïve and pre-treated diabetic dogs without concurrent diseases fed with a diabetic diet; group 2: diabetic dogs with concurrent diseases fed with a diabetic diet; group 3: diabetic dogs with or without concurrent diseases that, for several reasons, are not fed with a diabetic diet). This study will evaluate how many dogs are well controlled with PZIR SID and if there are differences in relation to the type of diet fed and the presence of concurrent diseases. Moreover, the study will determine the time required to achieve good glycemic control using the FGMS that allows rapid and, at the same time, safe adjustments of the insulin dose.

**INTRODUCTION AND SCIENTIFIC BACKGROUND**

Diabetes mellitus (DM) is a common disease in dogs with a reported worldwide prevalence of 0.3%–0.6%.1-3 Insulin is the mainstay of treatment in canine diabetic patients, often combined with dietary modification, exercise, and weight control.4 Commonly used insulin preparations for diabetes in dogs include recombinant human neutral protamine Hagedorn (NPH) insulin and purified pork source lente insulin.4,5 The use of protamine zinc recombinant human insulin (PZIR), detemir, and glargine insulins has also been reported.4-7 The lack of large controlled studies in dogs has left the practitioner without a clear choice in optimal insulin therapy, relying instead on product familiarity or licensing requirements. Regardless of which insulin is chosen, previous studies indicate that optimal DM control requires twice-daily (BID) injection.4,8 PZIR (ProZinc, Boehringer Ingelheim, Germany) insulin was licensed in Europe for dogs in 2019. PZIR is a recombinant human insulin, which is precipitated with protamine and zinc. It is generally considered to be long-acting insulin due to prolonged absorption from subcutaneous tissue since the insulin/zinc/ protamine complexes cause a slow release of insulin monomers or dimers into the systemic circulation.9 A preliminary study, showed that PZIR insulin was able to control hyperglycemia and clinical signs associated with DM in dogs.10 Moreover, in a recent trial in diabetic dogs, PZIR safely and effectively improved glycemic parameters and clinical signs in diabetic dogs, many of whom were improved using PZIR once-daily.11 Treatment of DM is time and labor-intensive and owner compliance is essential to achieve good glycemic control. In a recent worldwide study, 20% of pets diagnosed with DM were euthanized at or within the first year after diagnosis.12 The impact of DM treatment on the owner’s lifestyle was cited as a reason for euthanasia in 32% of cases. The need to give insulin BID can have a negative impact on the owner’s quality of life, thereby making once-daily (SID) insulin therapy an appealing alternative.

In recent years, glucose monitoring has been revolutionized by the development of continuous glucose monitoring systems (CGMS), which are wearable non/minimally‐invasive devices that measure glucose concentration almost continuously for several consecutive days/weeks. The Abbott FreeStyle Libre® (flash glucose monitoring system; FGMS) is the CGMS used most commonly. It provides detailed glucose profiles, allowing accurate identification of glycemic excursions occurring throughout the day, as well as of glucose variations during consecutive days, thus enabling the clinician to make a more informed insulin treatment decision.13,14 One of the main advantages of using the Abbott FreeStyle Libre® is that it might allow more frequent insulin dose adjustments, enabling a faster achievement of good glycemic control.

In the previous studies in which the PZIR was used in diabetic dogs, the glycemic control was defined on the basis of clinical signs, results of BGCs and serum fructosamine (SF) concentration. Moreover, only dogs without concurrent diseases were included. A study to evaluate the efficacy of PZIR administered SID in several groups of diabetic dogs (naïve and pretreated, with and without concurrent diseases, fed with a diabetic diet or not) monitored with FGMS might be helpful in better objectivating the effectiveness of this type of insulin and in providing more practical insulin recommendations for the use of PZIR in dogs.

**DESCRIPTION OF THE RESEARCH PLAN**

**Objectives:**

* The purpose of this study is to examine the efficacy and safety of PZIR administred SID in three groups of diabetic dogs monitored with FGMS (group 1: naïve or pre-treated diabetic dogs without relevant concurrent disorders fed with a diabetic diet; group 2: diabetic dogs with concurrent disorders fed with a diabetic diet; group 3: diabetic dogs with or without concurrent disorders that, for several reasons, are not fed with a diabetic diet) .
* The secondary aims are to evaluate the time required to achieve good glycemic control and to evaluate the effect of the diet type (specific diabetic diet or not) on glycemic control in dogs treated with PZIR SID.

**Hypothesis:**

* The hypothesis are that:
* PZIR given SID would be safe and efficacious for the treatment of most dogs with only DM and probably also dogs with DM and concurrent disorders that cause higher insulin requirements.
* Thanks to the use of FGMS, which provides continuous glucose monitoring allowing more rapid adjustments of the insulin dose, dogs will achieve good glycemic control more rapidly than previously described using the blood glucose curves (BGCs).
* Dogs fed with a diabetic diet have better glycemic control and lower glucose fluctuations (glucose variability) compared to dogs fed with a different diet.

**Study design:**

* Prospective longitudinal study
* Duration: 24 months
* Inclusion criteria:
	+ Client-owned dogs with newly diagnosed DM or already on insulin treatment
	+ Diagnosis of DM based on the ALIVE criteria15
	+ Dogs without or with concurrent diseases (group 1 and group 2, respectively)
	+ Both dogs fed with a diabetic diet or with other types of diet (group 3)
* Exclusion criteria:
	+ Dogs with concurrent IRIS stage ≥ 2 chronic kidney diseases
	+ Aggressive dogs and dogs that do not tolerate the FGMS
	+ Dogs with severe skin diseases that preclude the FGMS application
* Number of dogs\*:
* Group 1: naïve and pre-treated diabetic dogs without concurrent diseases fed with a diabetic diet (low in simple carbohydrates and high in protein content) (15-20 dogs)
* Group 2: diabetic dogs with concurrent diseases fed with a diabetic diet (10-15 dogs)
* Group 3: diabetic dogs with or without concurrent diseases that, for several reasons (e.g. food allergy/intolerance, specific dietary requirements for other concomitant disorders), are not fed a diabetic diet (10-15 dogs)

\*sample size was extrapolated from similar studies5,16

* Study design:
* T0 (time of the inclusion)
	+ - Obtain owner consent for the inclusion in the study and blood testing
		- Anamnesis
		- Physical examination
		- Blood and urine sample collection for hematology, biochemistry, serum fructosamine (SF) and HbA1c concentration, urinalysis
		- Classification of the clinical score based on the ALIVE criteria15
		- Application of FGMS

In newly diagnosed diabetic dogs, PZIR will be started at 0.5 U/Kg once daily and FGMS will be immediately applied.

In pre-treated dogs, PZIR will be started once daily at a dose equal to 25% less than their previous total 24-hour dose, rounding down to the nearest half or whole unit (e.g. if the dog was getting 5 units of the previous insulin BID, it would be transitioned to 7.5 units of PZIR insulin SID). In these dogs, the switch from the previous insulin to PZIR will be performed one week after FGMS application.

The insulin will be administered subcutaneously during or immediately following the morning meal.

* T2 (2 weeks post T0), T4 (4 weeks post T0), T6 (6 weeks post T0), T8 (8 weeks post T0: end of the study)
	+ - Anamnesis
		- Physical examination
		- Blood sample collection for SF and HbA1c concentration
		- Classification of the clinical score based on the ALIVE criteria23
		- Application of a new FGMS\*
		- Evaluation of the AGP report and extrapolation of the glucose data

\*If during the study the dog self-removes the sensor, a new sensor will be applied within 24 h to ensure continuous monitoring until the conclusion of the study period.

**Adjustments of the insulin dose:**

PZIR dose will be increased by 10-30% every 2 days when the IG nadir is >350 mg/dL (>19 mmol/L), until a nadir <350 mg/dL (<19 mmol/L) is achieved.

When the IG nadir is <350 mg/dL (<19 mmol/L), no further change will be done, and continued monitoring for 3-5 days will be carried on. The goal will be to observe for any consistent pattern in the daily glucose excursions. A key point is that the overall pattern will be considered, rather than the micromanagement of individual IG results.

The following recommendations will provide a guide to the assessment of glucose excursions during this period:

* Nadir 150-300 mg/dL (8.3-17 mmol/L): the q24h PZIR dose will be increased by 10-30%, or by 1 U for dogs receiving <7 U per injection.
* Nadir 80-150 mg/dL (4.4-8.3 mmol/L), or nadir <80 mg/dL (<4.4 mmol/L) and average IG >120 mg/dL (>6.7 mmol/L): the current q24h PZIR dose will be continued, but also it will be considered if glycemic control might be improved by switching to q12h dosing with PZIR\*
* Nadir <80 mg/dL (<4.4 mmol/L) and average IG <120 mg/dL (< 6.6 mmol/L): the q24h PZIR dose will be decreased by 10-30%, or by 1 U for dogs receiving <7 U per injection.

**\*Switching from once daily to twice daily PZIR administration**

When there is a CONSISTENT PATTERN with IG nadir of 80-150 mg/dL (4.4-8.3 mmol/L), or nadir <80 mg/dL (<4.4 mmol/L) and average IG >120 mg/dL (>6.7 mmol/L) AND a period of about 12 h during each 24 h period when IG results are all >300 mg/dL (>17 mmol/L), PZIR dosing will be switched from q24h to q12h. When switching from q24h to q12h dosing, PZIR dose will be decreased by about 30% per injection (i.e., the total daily dose is therefore increased by 40%), and the first q12h dose will be administered 24 h after the previous injection. The dog’s response will be then monitored for the following 3-5 days before re-evaluating the dose.

**Flash glucose monitoring system**

The sensor will be applied in a clipped and sterile area on the dorsal aspect of the neck of each diabetic dog. After positioning, the sensor will be fixed with extra tape and a body bandage will be used to secure the sensor at the body. The detection limits of the sensor are between 20 and 500 mg/dL. The sensor begins recording data 1 hour after its application and automatically measures the IGc every minute. The IGc is transferred from the sensor to the FreeStyle LibreLink mobile app, when the user brings the mobile phone into close proximity to the sensor. The FreeStyle LibreLink mobile app then displays the current sensor IGc and an IG trend arrow as well as the IGc over the preceding 8 hours. The measurements are automatically recorded and stored on the sensor (every 15 minutes) and displayed on the FreeStyle LibreLink app, when scanned. The data are automatically uploaded to LibreView when the phone is connected to the internet. This is a free, secure, cloud-based diabetes management system provided by Abbott. The system generates summary glucose reports from the uploaded sensor data, including the AGP and the Daily Log, and provides a secure repository for data.

**Samples collection**

* A blood sample (1.5 ml) for the CBC will be collected into EDTA-coated plastic tubes. The sample will be analyzed within one hour. This sample will be also used for the HbA1c measurement.
* For the biochemistry profile, the same serum sample for the measurement of SF will be used. The sample will be analyzed within 3 hours.

**Analytical methods**

* Complete blood count (Advia 2120, Siemens Healthcare Diagnostics, Erlangen, Germany), chemistry profile (AU 480, Beckman Coulter/Olympus, Brea, CA) and urinalyses will be performed by standard laboratory methods at the medical laboratory of the referral institution.
* Serum fructosamine concentrations will be assessed with a colorimetric nitroblue tetrazolium reduction method.17 The HbA1c values will be assessed with an immunoturbidimetric method,17 by which total hemoglobin concentration was colorimetrically measured. All measurements for both assays will be performed by use of an automated chemistry analyzer in the same laboratory of the referral institution.

***Statistical analysis***

The glycemic control will be assessed using ALIVE clinical score, glycated proteins (fructosamine and HbA1c%) and the glucose values extrapolated from the AGP report of the FGMS.

Normality will be assessed with the Shapiro–Wilk test and parametric or nonparametric tests to compare the different groups will be used accordingly. Results will be reported as mean (SD) or median (range) for normally or non-normally distributed data, respectively.

Significance will be set at P<0.05. Confidence interval and mean difference will be reported.

**Inclusion of details of appropriate ethical approval or exemption from same**

Owners will provide informed consent for the inclusion of their dogs in the study. The study protocol will be submitted for approval by the Scientific Ethics Committee of the University of Bologna. This study does not involve invasive procedures and therefore there should be no problems with approval by the ethics committee.

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