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DIABETE MELLITO NEL CANE: TERAPIA, MONITORAGGIO E ASPETTI PROGNOSTICI

Presentata da: Dott.ssa Francesca Del Baldo

Coordinatore Dottorato **Supervisore**

Prof. Arcangelo Gentile **Prof. Federico Fracassi**

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ABSTRACT

La presente tesi di dottorato affronta il tema del diabete mellito (DM) nel cane, la più comune disendocrinia di tale specie. Il DM è caratterizzato da una carenza assoluta o relativa di insulina cui consegue iperglicemia cronica e segni clinici caratteristici quali poliuria, polidipsia, polifagia e perdita di peso. I cani con DM necessitano di un trattamento insulinico e richiedono uno stretto monitoraggio terapeutico al fine di garantire un adeguato controllo della patologia. La presente tesi si articola in 7 studi incentrati su terapia, strumenti di monitoraggio e prognosi dei cani affetti da DM.

Il **capitolo 2** costituisce un'introduzione all'argomento e riassume l'attuale stato dell'arte sulla terapia e il monitoraggio del DM, due aspetti fondamentali di questa patologia su cui vertono la maggior parte degli studi presenti in letteratura. Successivamente è stato riportato uno studio il cui scopo è stato quello di comparare l'efficacia e la sicurezza di due tipi di insuline (Neutral Protamine Hagedorn ed insulina veterinaria lenta di origine suina), dimostrando che il loro impiego è equiparabile in termini di raggiungimento di un buon controllo glicemico e sicurezza **(capitolo 3).**

Il monitoraggio dei cani affetti da DM prevede l'utilizzo di metodiche dirette ed indirette. I sistemi di monitoraggio diretto includono le curve glicemiche, tipicamente eseguite misurando la glicemia a partire da una goccia di sangue capillare attraverso l'utilizzo di glucometri portatili, e il monitoraggio continuo del glucosio interstiziale attraverso i *Continuous Glucose Monitoring System* (CGMS). I metodi indiretti comprendono invece la valutazione dei segni clinici e del peso corporeo, la misurazione del glucosio e dei chetoni urinari, nonché la misurazione delle proteine glicate.

Nel **capitolo 4** è stato esposto uno studio finalizzato ad indagare l'accuratezza e la precisione di un glucometro (Gluco Calea, WellionVet) e di un glucometro/chetometro (Belua, Wellion Vet) nella specie canina. Nonostante tali dispositivi siano stati progettati per l'utilizzo in medicina veterinaria, nessuno dei due dispositivi è risultato essere sufficientemente accurato da consentirne l'utilizzo in tale specie.

Il **capitolo 5** riporta uno studio che indaga le performance cliniche del FreeStyle Libre (Flash Glucose Monitoring System, FGMS) nel monitoraggio di 20 cani diabetici. Questo dispositivo è un nuovo CGMS che, rispetto a quelli precedentemente studiati in medicina veterinaria, presenta diversi vantaggi tra cui il fatto di non necessitare calibrazione e la prolungata durata di azione (fino a 14 giorni). Dai risultati è emerso che, in più del 75% dei casi, le modifiche della dose insulinica basate su profili glicemici ottenuti tramite l'utilizzo del FGMS sono in accordo con quelle ottenute tramite l'utilizzo dei comuni glucometri portatili. Tuttavia, il FGMS consente una più accurata identificazione degli episodi ipoglicemici e dei nadir del glucosio rispetto all'uso dei glucometri portatili e permette inoltre il monitoraggio dei valori glicemici di più giorni consecutivi. Per tali ragioni può essere considerato uno strumento estremamente utile e vantaggioso per il monitoraggio di questi pazienti.

A seguire è stato esposto uno studio che ha valutato le performance di due metodiche per la misurazione di emoglobina glicata (HbA1c) e fruttosamine sieriche ed ha comparato l'abilità delle due proteine glicate nel classificare il controllo glicemico, utilizzando come gold standard uno score clinico **(capitolo 6).** Dai risultati è emerso che le due metodiche studiate sono precise e lineari, pertanto possono essere utilizzate routinariamente per la misurazione delle proteine glicate nella specie canina. Né le fruttosamine sieriche, né l'HbA1c, sono tuttavia risultate sufficientemente adeguate nel classificare correttamente il controllo glicemico.

La tesi si conclude con uno studio il cui obiettivo è stato quello di valutare la sopravvivenza e il significato prognostico di diverse variabili cliniche e clinico-patologiche in cani affetti da DM. Dai risultati è emerso che il tempo di sopravvivenza medio dei cani diabetici in terapia presso un centro di referenza è di 964 giorni e che la presenza di iperfosfatemia al momento della diagnosi di DM rappresenta un fattore prognostico negativo **(capitolo 7).**

Nel **capitolo 8** sono riassunte le discussioni e le conclusioni della presente tesi.

Sommario

Capitolo 1

INTRODUZIONE E OBIETTIVI DELLA TESI

1. Introduzione e obiettivi della tesi

Il diabete mellito (DM) è una comune endocrinopatia del cane che si instaura conseguentemente ad un deficit nella produzione e/o nell'azione di insulina. In tale specie, la forma più comune di DM è il diabete insulino-dipendente di tipo 1, il quale è caratterizzato da uno stato di ipoinsulinemia permanente e dalla assoluta necessità di ricevere insulina esogena per mantenere un adeguato controllo glicemico. L'eziologia del DM è tuttora oggetto di studio, ma sembra essere indubbiamente multifattoriale. La degenerazione delle cellule beta tende a verificarsi in modo rapido e progressivo ed è generalmente conseguente a distruzione immunomediata, degenerazione vacuolare o pancreatite. I fattori di rischio identificati includono obesità, patologie concomitanti (ipercortisolismo, ipotiroidismo, ipertrigliceridemia, patologie dentali, infezioni sistemiche, pancreatite e gravidanza/diestro) o farmaci che antagonizzano l'azione dell'insulina quali steroidi e progestinici. Infine, la genetica è probabilmente un altro importante fattore di rischio poiché certe razze risultano essere più suscettibili.

Indipendentemente dall'eziologia, l'iperglicemia protratta e la glicosuria che si instaurano causano i segni clinici tipici della patologia quali poliuria, polidipsia, polifagia e perdita di peso. La diagnosi di tale endocrinopatia si deve basare sulla presenza dei segni clinici caratteristici, associati ad uno stato di iperglicemia persistente a digiuno e glicosuria. A differenza della diagnosi, che risulta essere relativamente semplice, la terapia del DM è complessa e richiede un attento approccio sistematico, oltre ad una ottimale collaborazione tra veterinario e proprietario. Il primo obiettivo del trattamento è l'eliminazione dei segni clinici evitando nel contempo l'ipoglicemia. Nel cane diabetico questo si ottiene attraverso la terapia insulinica, l'utilizzo di una dieta appropriata, la promozione di un costante esercizio fisico, l'identificazione e il controllo di patologie concomitanti nonché attraverso un attento monitoraggio glicemico **(Capitolo 2).**

La terapia insulinica rappresenta l'elemento cardine del trattamento del DM. Le insuline disponibili in commercio vengono classificate sulla base della durata d'azione e potenza in insuline ad azione intermedia (insulina lenta di origine suina ed insulina Neutral Protamine Hagedorn[NPH]) ed insuline a rilascio prolungato (insulina zinco protamina, insulina glargine, ed insulina detemir). Le attuali linee

guida per il trattamento del DM nei cani neo-diagnosticati raccomandano l'uso di preparazioni insuliniche a durata d'azione intermedia e indicano come prima scelta l'insulina lenta di origine suina. Tale indicazione deriva dal fatto che l'insulina NPH non è approvata dalla *Food and Drug Administration* per l'utilizzo nel cane ed ha rivelato, in alcuni soggetti, una breve durata d'azione. Tuttavia, non esistono studi che abbiano comparato l'attività delle due insuline nel cane pertanto, è stato eseguito uno studio finalizzato a comparare l'efficacia e la sicurezza dell'insulina lenta di origine suina e dell'insulina NPH in cani con DM neo-diagnosticato **(capitolo 3).**

Al fine di ottenere un buon controllo della patologia, il monitoraggio dei cani diabetici è un aspetto di sostanziale importanza. Attualmente, le curve glicemiche (Blood Glucose Curve [BGCs]) sono uno degli strumenti più utilizzati nella pratica clinica per effettuare gli adeguamenti del dosaggio insulinico. Le BGCs vengono tipicamente eseguite in ospedale o a casa misurando la glicemia ogni 2 ore per 10-12 ore avvalendosi di glucometri portatili (Portable Blood Glucose Meter [PBGM]) che consentono di misurare la glicemia a partire da una goccia di sangue capillare. L'accuratezza dei PBGM può essere estremamente variabile, soprattutto se si tratta di strumenti progettati per l'utilizzo in medicina umana. Per tale ragione, l'utilizzo di nuovi dispositivi rende necessaria la validazione dello strumento. A tale proposito, è stato effettuato uno studio con l'obiettivo di stabilire l'accuratezza e la precisione di un glucometro (Gluco Calea, WellionVet) e di un glucometro/chetometro (Belua, WellionVet) nella specie canina basandosi sui requisiti stabiliti dalla norma ISO 15197:2013 e valutando l'interferenza esercitata dal *packed cell volume* (PCV) sull'accuratezza dei due dispositivi **(capitolo 4)**.

La valutazione della curva glicemica consente di determinare l'efficacia e la durata d'azione dell'insulina, così come l'entità delle fluttuazioni glicemiche. Tuttavia, questo metodo è associato a diversi svantaggi quali la necessità di eseguire ripetuti prelievi di sangue capillare che può essere doloroso e stressante per il paziente e il rischio di non identificare significative fluttuazioni della glicemia se queste ricadono tra due misurazioni. Inoltre, le BGCs non permettono di monitorare la glicemia in giorni consecutivi, pertanto non consentono di tenere in considerazione la variabilità

glicemica tra giorni consecutivi nel momento in cui si intraprende una determinata decisione terapeutica. Negli ultimi decenni in medicina umana sono stati introdotti i dispositivi per il monitoraggio continuo della glicemia (Continuous glucose monitoring systems [CGMS]) che misurano il glucosio interstiziale il quale si è rivelato essere ben correlato al glucosio ematico. Le prime generazioni di questi strumenti possedevano diversi limiti che sono stati superati dai dispositivi di più recente introduzione come il FreeStyle Libre (Abbott, UK). Tale dispositivo è stato recentemente validato per l'utilizzo nel cane diabetico, tuttavia non sono presenti studi che ne valutino l'impiego nel monitoraggio a lungo termine dei cani con DM non complicato. Pertanto, è stato eseguito uno studio con gli obiettivi di 1) comparare la decisione terapeutica dedotta valutando curve glicemiche eseguite tramite l'utilizzo del FreeStyle Libre e quelle ottenute attraverso l'impiego di un PBGM validato per l'utilizzo nella specie canina; 2) comparare la dose insulinica dedotta valutando curve glicemiche eseguite tramite l'utilizzo del FreeStyle Libre in due giorni consecutivi nello stesso ambiente (casa) e in due ambienti diversi (casa e ospedale); 3) valutare l'abilità del FreeStyle Libre di identificare il nadir glicemico e gli episodi ipoglicemici ed infine 4) comparare l'andamento glicemico tra giorno e notte prendendo in considerazione i nadir glicemici identificati tramite i grafici forniti dal software del FreeStyle Libre **(capitolo 5)**.

Le proteine glicate (fruttosamine sieriche [SF] ed emoglobina glicata [HbA1c]) rappresentano un ulteriore strumento di cui il clinico dispone per monitorare i pazienti diabetici in terapia insulinica. Esse forniscono un'indicazione della glicemia delle settimane (SF) o mesi (HbA1c) precedenti al prelievo e sono usate per chiarire discrepanze tra i risultati della curva glicemica e il quadro clinico. A differenza della medicina umana dove l'HbA1c è considerata il migliore metodo per definire il controllo glicemico, in medicina veterinaria vengono più comunemente usate le SF poiché la loro concentrazione si modifica più rapidamente in risposta alle variazioni della terapia insulinica. Solo pochi e datati studi hanno tuttavia valutato l'utilità dell'HbA1c per il monitoraggio dei cani con DM e non esistono studi che abbiano confrontato l'abilità delle due proteine glicate nel classificare il controllo glicemico. Per tali ragioni, è stato eseguito uno studio con gli obiettivi di valutare le

perfomance di due metodiche di laboratorio per la misurazione di fruttosamine ed emoglobina glicata e comparare l'uso di tali proteine glicate nel classificare il controllo glicemico nei cani con DM **(capitolo 6)**.

Nonostante il DM sia una delle patologie endocrine più studiate nel cane, solo pochi studi hanno valutato l'aspettativa di vita e/o i fattori prognostici nei cani affetti da tale patologia. Poiché la gestione del cane diabetico richiede un importante impegno da parte del proprietario dell'animale, essere in grado di fornire informazioni prognostiche al momento della diagnosi potrebbe determinare una maggiore *compliance* da parte del proprietario. Pertanto, è stato effettuato uno studio con l'obiettivo di identificare il valore prognostico di diverse variabili cliniche e laboratoristiche nonché di determinare l'aspettativa di vita dei cani con DM sottoposti a terapia insulinica **(capitolo 7)**

Capitolo 2

IL DIABETE MELLITO NEL CANE: TERAPIA E MONITORAGGIO

Francesca Del Baldo, Federico Fracassi

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Dipartimento di Scienze Mediche Veterinarie,

Scuola di Agraria e Medicina Veterinaria,

Bologna

Riassunto

Il diabete mellito è una delle più frequenti endocrinopatie del cane. In seguito alla diagnosi, è necessario iniziare una terapia insulinica, nonché una dieta appropriata, al fine di controllare le concentrazioni di glucosio ematiche e conseguentemente la sintomatologia clinica. Il fabbisogno insulinico è influenzato da numerosi fattori. E' consigliabile iniziare la terapia con dosaggi insulinici ridotti che devono essere gradualmente incrementati a seguito di frequenti monitoraggi. Nella presente revisione della letteratura si evidenziano i principali aspetti terapeutici e metodi di monitoraggio glicemico in cani affetti da diabete mellito.

Abstract

Diabetes mellitus is one of the most common endocrine diseases in the dog. After diagnosis, it is necessary to start an insulin treatment and an appropriate dietetic management, in order to control blood glucose levels and consequently the clinical signs. The insulin requirements are affected by several factors. It is recommended to start insulin therapy with a low dose that has to be subsequently gradually increased on the basis of frequent re-evaluations. In the present review we illustrate the main therapeutic aspects and monitoring methods of canine diabetes mellitus.

Introduzione

Il diabete mellito (DM) è una delle endocrinopatie più comuni nel cane ed è dovuto a un deficit nella produzione e/o nell'azione di insulina[1]. Il conseguente sviluppo di iperglicemia e glicosuria è responsabile della comparsa dei segni clinici caratteristici quali poliuria, polidipsia, polifagia e perdita di peso[2] (figura 1). La prevalenza della disendocrinia nel cane varia dallo 0,32% all'1,33% [3,4,5,6] ed uno studio condotto in Italia ha evidenziato che le razze maggiormente colpite risultano essere il Setter irlandese, il Barbone, lo Yorkshire Terrier e il Setter inglese[4]. La diagnosi richiede la concomitante presenza dei segni clinici caratteristici in associazione ad iperglicemia persistente a digiuno e glicosuria[2]. Al fine di riuscire ad ottenere un buon controllo della patologia sono di fondamentale importanza la terapia insulinica, la dieta e un attento monitoraggio glicemico.

Figura 1 - Cataratta bilaterale completa conseguente al diabete mellito

Terapia

La terapia del DM deve porsi come obiettivi la risoluzione dei segni clinici, la prevenzione delle complicazioni quali ipoglicemia e chetosi, il mantenimento di un peso corporeo stabile e quindi il raggiungimento di una buona qualità di vita[1]. A differenza della medicina umana, dove uno stretto controllo glicemico è fondamentale per prevenire complicazioni a lungo termine[7,8,9], nel cane non è stato dimostrato un eguale vantaggio nel mantenere la glicemia entro gli intervalli fisiologici (60-130 mg/dl)[10]; nel cane si considera pertanto ottimale una glicemia che viene mantenuta in corso di terapia tra 90 e 250 mg/dl[1.]

Un aspetto fondamentale nella gestione del DM canino è il raggiungimento di un'ottimale "*compliance*" da parte del proprietario. Un recente studio ha riscontrato che al momento della diagnosi di DM molti proprietari richiedono addirittura l'eutanasia del proprio animale[11]. Questo dato rispecchia quanto la prospettiva di una terapia impegnativa e costosa, per tutta la vita dell'animale, possa spaventare o essere incompatibile con le abitudini del proprietario; nonostante ciò, i tempi di sopravvivenza dalla diagnosi risultano solitamente buoni (17 mesi e 2 anni dalla diagnosi)[11,6] e spesso sovrapponibili a quelli di un cane sano della medesima età[12]. Emerge quindi come sia importante, al fine di ottenere un successo terapeutico a lungo termine, ottimizzare il più possibile la gestione terapeutica del DM senza tuttavia influenzare negativamente la qualità di vita del proprietario[2].

Nel cane diabetico, il controllo glicemico può essere ottenuto tramite terapia insulinica, appropriata dieta, esercizio fisico, trattamento e prevenzione delle patologie concomitanti e sospensione di eventuali farmaci che causino insulinoresistenza[2].

Terapia Insulinica

Preparazioni insuliniche

Le preparazioni insuliniche comunemente usate per il trattamento del DM nel cane includono due classi di insuline: le insuline ad azione intermedia, quali NPH (Humulin I®) e insulina lenta (Caninsulin®); e le insuline a rilascio prolungato, insulina zinco-protamina (PZI) (ProZinc®), insulina glargina (Lantus ®) ed insulina detemir (Levemir®)[2] (Tabella 1 e 2).

Tabella1. Preparati insulinici comunemente usati per il trattamento del DM nel cane e nel gatto

			Somministrazione		**Durata d'azione (h)**	
Insulina	Origine	Indicazioni	Via di somministrazione	Dose iniziale e frequenza	Cane	Problemi comuni
Cristallina amorfa	Ricombinante umana	Chetoacidosi diabetica	IV IM SC SC	CRI Ogni ora Ogni 6-8 h Ogni 8 h	-- 4-6 h 6-8 h 6-8 h	Rapida riduzione della glicemia, può causare ipokaliemia
Lispro	Analogo umano ricombinante	Chetoacidosi diabetica	IV SC	CRI	--	Rapida riduzione della glicemia, ipokaliemia
NPH	Ricombinante umana	Diabete mellito	SC	0,25 U/kg ogni 12 h	6-12h	Possibile breve durata d'azione nel cane e soprattutto nel gatto, iperglicemia post prandiale nel cane
Lenta	100% suina	Diabete mellito, valida scelta iniziale nel cane	SC	0,25 U/kg ogni 12 h	8-14h	Breve durata d'azione nel cane e soprattutto nel gatto
PZI	Ricombinante umana	Diabete mellito, valida scelta iniziale nel gatto	SC	0,25-0,5 U/kg ogni 12 h	10-16h	Durata d'azione > 12h in alcuni cani; tempo del NADIR non prevedibile in alcuni cani
Glargine	Analogo umano ricombinante	Diabete mellito, valida scelta iniziale nel gatto	SC	0,3 U/kg ogni 12-24 h	8-16h	Durata d'azione > 12h in alcuni cani e gatti, debole capacità di ridurre la glicemia e tempo del NADIR solitamente non prevedibile
Detemir	Analogo umano ricombinante	Diabete mellito	SC	0,1 U/kg ogni 12-24 h	8-16h	Durata d'azione > 12h in alcuni cani e gatti, nel cane dosaggi insulinici richiesti considerevolmente più ridotti rispetto alle altre insuline, rischi di ipoglicemia

IM= intramuscolare; EV=endovenosa; SC= sottocutanea; CRI= continuous rate infusion; NPH= neutral protamine Hagedorn, PZI=protamine zinc insulin,

La NPH è una sospensione di insulina umana isofano, ottenuta tramite tecnologia a DNA ricombinante. La sospensione risulta dalla combinazione tra insulina ricombinante umana e protamina, una proteina estratta dal pesce che permette di ritardarne l'assorbimento e prolungarne la durata d'azione[13].

L'insulina lenta è una sospensione insulina-zinco di origine suina altamente purificata che ha il vantaggio di essere antigenicamente identica a quella canina[14]. A differenza della precedente insulina, l'insulina lenta non contiene la protamina, ma grazie alle grandi dimensioni dei suoi cristalli di zinco, riesce ad ottenere un lento assorbimento dal sito di inoculo sottocutaneo ed una durata d'azione prolungata[2]. E' una miscela composta per il 30% da una componente amorfa ad azione rapida e per il 70% da una componente cristallina ad azione protratta. La frazione amorfa raggiunge il picco d'azione a 3 ore dalla somministrazione SC e ha effetto per 8 ore, viceversa la frazione cristallina ha un'insorgenza più lenta con un massimo effetto a 8-14 ore e una durata di circa 24 ore[15].

La PZI è costituita da molecole di insulina strutturalmente identiche a quelle dell'insulina amorfa addizionate di zinco e protamina. Il complesso che ne deriva precipita a pH neutro[16,17] e, se somministrato come sospensione, porta ad una graduale dissociazione e quindi ad un lento rilascio dei monomeri o dimeri di insulina nella circolazione sistemica[18]. E' comunemente usata per il trattamento del DM nei gatti, mentre studi che ne valutino l'utilizzo nel cane sono limitati[2].

L'insulina lenta e la PZI sono approvate dalla Food and Drug Administration (FDA) per il trattamento del DM rispettivamente nel cane e nel gatto, pertanto sono formulate ad una concentrazione di 40 U/ml e richiedono l'utilizzo di siringhe apposite[2].

Gli analoghi insulinici (glargina e detemir) sono forme di insulina modificate che hanno come obiettivo principale quello di mimare la secrezione fisiologica dell'insulina[19,20]. In medicina umana queste preparazioni hanno rivoluzionato il trattamento del diabete mellito e possono rappresentare una potenziale scelta anche per il trattamento del DM nel cane e nel gatto[21].

L'insulina glargina è stata ottenuta sostituendo l'amminoacido asparagina con la glicina nella posizione A21 della catena A, e 2 arginine sono state aggiunte nella posizione C-terminale della catena B dell'insulina, modifiche che hanno spostato il pH isoelettrico dell'insulina da 5,4 verso un pH neutro[22]. Questo cambiamento la rende quindi più solubile a pH lievemente acido e meno solubile a pH fisiologico rispetto all'insulina nativa umana. La soluzione nel flacone di glargina è acida e questo consente di mantenere l'insulina solubile e sospesa (la soluzione è limpida e non è necessario

agitarla prima dell'uso). A causa di questa caratteristica la glargina non può essere diluita e/o mischiata con qualsiasi sostanza che potrebbe modificare il pH della soluzione[2]. Questo preparato insulinico forma dei microprecipitati sottocutanei a livello del sito di inoculo, dal quale piccole quantità di insulina solo lentamente rilasciate in circolo e quindi assorbite[23].

L'insulina detemir è stata ottenuta tramite rimozione dell'amminoacido treonina in posizione B30 e tramite l'acilazione, con acido miristico, della lisina in posizione B29. L'azione prolungata deriva dalla tendenza di questa preparazione ad auto aggregarsi a livello sottocutaneo e dal legame tra l'albumina e l'acido grasso; quest'ultimo infatti riduce le concentrazioni di insulina libera in circolo e garantisce una distribuzione più lenta ai tessuti target[2]. Nel cane questa insulina è molto più potente rispetto alle precedenti, pertanto deve essere usata con cautela nei cani di piccola taglia e sono richieste dosi più basse per ottenere un buon controllo glicemico (dose di partenza 0,1 U/Kg)[21].

Tabella 2. Comparazione dei dosaggi insulinici richiesti per ottenere un buon controllo glicemico

		Dosaggio insulinico (U/Kg/iniezione)		
Preparazione insulinica	Numero di cani	Mediana o media	Range o DS	Studio
NPH	54	0.8* 0.4⁺	0.4-1.9 0.3-0.8	Lorenzen, 1992
NPH	15	0.47	±0.14	Fracassi et al, 2016
Lenta	35	0.8	0.3-1.4	Monroe et al, 2005
Lenta	15	0.6	±0.14	Fracassi et al, 2016
PZI	17	0.9	0.4-1.5	Della-Maggiore et al, 2012
Glargine	12	0.6	0.1-1.1	Fracassi et al, 2012
Detemir	10	0.12	0.05-0.34	Fracassi et al, 2015

NPH, Neutral protamine Hagedorn; NR, non riportato; PZI, protamine zinc insulin
*Peso< 15 kg
⁺Peso>15 kg

Conservazione e diluizione dell'insulina

E' consigliabile conservare il flacone dell'insulina in frigorifero per garantire un ambiente costante e al riparo dalla luce. La conservazione del flacone a temperatura ambiente non disattiva tuttavia l'insulina, mentre congelamento e riscaldamento eccessivo possono inattivarla. Alcuni veterinari raccomandano di sostituire il flacone dell'insulina una volta al mese per evitarne l'inattivazione o la perdita di sterilità. Tuttavia, se l'insulina è correttamente conservata in frigo e correttamente manipolata, non subisce alcuna perdita significativa in termini di efficacia e i problemi legati all'assenza di sterilità sono rari e trascurabili[1].Pertanto non è necessario sostituire il flacone mensilmente soprattutto se il cane non manifesta sintomi[2,1]. Il flacone deve invece essere subito sostituito nel caso in cui vengano evidenziate delle variazioni nel colore dell'insulina. Il proprietario deve essere istruito riguardo la preparazione corretta del prodotto insulinico al fine di effettuare una somministrazione efficace. I produttori dell'insulina ProZinc® consigliano di farla scivolare dolcemente tra le mani per rendere il prodotto omogeneo, l'insulina Caninsulin® deve invece essere agitata vigorosamente, Lantus® e Levemir® infine, non essendo sospensioni, non necessitano di essere agitate prima dell'uso. È importante assicurarsi che il proprietario utilizzi siringhe idonee alla concentrazione della preparazione insulinica usata; errori in questo senso sono estremamente comuni e possono portare a sovra o sotto-dosaggio. Humulin I®, Lantus® e Levemir® hanno una concentrazione di 100 U/ml, mentre Prozinc® e Caninsulin® di 40 U/ml. È consigliabile evitare diluizioni a meno che non si utilizzino diluenti approvati dalla casa farmaceutica produttrice.

Penne per insulina

Le penne per la somministrazione di insulina sono comunemente utilizzate dai pazienti diabetici umani e numerosi studi ne hanno evidenziato i vantaggi. Il loro utilizzo consente di ottenere una somministrazione più semplice e meno dolorosa oltre che più accurata nel dosaggio. Inoltre, rispetto alla tradizionali siringhe e fiale, questi dispositivi comportano un minor disagio durante l'iniezione di insulina in pubblico e di conseguenza risultano più confortevoli per il paziente[24].

In medicina veterinaria l'utilizzo delle penne per la somministrazione di insulina è ancora poco studiato. L'unica insulina veterinaria che può essere utilizzata con uno di questi dispositivi (VetPen®) è l'insulina lenta (Caninsulin®). La VetPen® è disponibile in commercio in due tipologie di formati: uno consente una erogazione massima di 8 U (0,5-8 U) tramite incrementi di 0,5 U, l'altro consente una erogazione massima di 16 U (1-16 U) attraverso incrementi di 1 U. I proprietari che decidono di utilizzare questo dispositivo devono essere accuratamente istruiti sulla modalità di utilizzo dello stesso.[1]

Raccomandazioni iniziali per il trattamento insulinico

Tutti i cani affetti da DM devono essere considerati insulino-dipendenti, quindi una volta confermata la diagnosi, è importante non ritardare il trattamento insulinico per più di qualche giorno. Potrebbero infatti insorgere chetosi e chetoacidosi in grado di complicare il quadro clinico del paziente[25]. Le insuline di prima scelta per il trattamento dei soggetti neo-diagnosticati sono l'insulina lenta (Caninsulin®) o la NPH, anche se quest'ultima può essere associata a un problema di durata d'azione eccessivamente breve[2]. Un recente studio comparativo ha osservato che entrambe queste insuline sono risultate efficaci nel controllo del DM nel cane; in tale studio il dosaggio insulinico finale richiesto per ottenere un buon controllo della patologia era di 0,6 U/Kg nei cani trattati con insulina lenta e di 0,47 U/Kg nei cani trattati con insulina NPH[26.] Poiché uno degli obiettivi principali nel periodo iniziale della terapia è evitare l'ipoglicemia, è consigliabile iniziare la terapia con un dosaggio basso (es. 0,25 U/Kg)[27,2] e somministrare l'insulina due volte al giorno (idealmente ogni 12 ore)[2].

La PZI, l'insulina glargina e la detemir sono anch'esse risultate efficaci nel garantire un adeguato controllo glicemico in cani diabetici[28,29,30,21]. Queste insuline risultano ancora poco studiate nel cane e vengono solitamente considerate di seconda scelta. Vengono solitamente prese in considerazione qualora le insuline di prima scelta diano problemi di scarsa efficacia o di breve durata d'azione[2].

Non è solitamente necessario ospedalizzare i cani diabetici; alcuni autori suggeriscono tuttavia che dopo la diagnosi i soggetti possono essere ospedalizzati per 24-48 ore per completare l'iter diagnostico e iniziare la terapia insulinica. Nel caso di ospedalizzazione è consigliabile controllare la

glicemia 2-3 volte al giorno per evidenziare eventuali ipoglicemie che richiedono una riduzione del dosaggio; viceversa, se le glicemie permangono elevate, non è opportuno aumentare la dose di insulina poiché è spesso necessario qualche giorno di adattamento per ottenere il cosiddetto equilibrio[28].

Dieta

Costanza è la parola chiave per una buona gestione dietetica del cane diabetico. I cani con diabete mellito devono ricevere quotidianamente la stessa quantità e la stessa tipologia di alimento (stessa marca oppure dieta casalinga preparata sempre allo stesso modo). La dose giornaliera deve essere suddivisa in due pasti di uguale quantità da somministrare subito prima o subito dopo l'iniezione di insulina[2,1]. La scelta del tipo di dieta deve tenere in considerazione il peso dell'animale, l'eventuale presenza di patologie concomitanti e le preferenze del cane. Nei pazienti obesi la correzione dello stato di nutrizione è il primo obiettivo da intraprendere, in quanto l'obesità può causare resistenza all'insulina e incostanti risposte alla terapia[32,33]. La perdita di peso può essere ottenuta attraverso l'utilizzo di diete a bassa densità calorica e attraverso un aumento del dispendio energetico con l'esercizio fisico. La riduzione di peso dovrebbe essere pari all'1% a settimana[28]. Per trattare l'obesità e garantire un buon controllo glicemico è opportuno aumentare il contenuto di fibra nella dieta. Le ditte produttrici offrono diverse diete create appositamente per la gestione del cane diabetico. Queste sono caratterizzate dalla presenza di una miscela di fibra solubile e insolubile, che determina un rallentamento dell'assorbimento del glucosio a livello intestinale, contribuendo a ridurre al minimo l'iperglicemia postprandiale. È possibile inoltre optare per una dieta casalinga (Tabella 3). Le diete che favoriscono la perdita di peso, invece, contengono una più elevata quantità di fibra insolubile e hanno una quantità di grassi inferiore. Nei soggetti diabetici con un basso *body condition score* è opportuno innanzitutto raggiungere un peso corporeo adeguato, somministrando una dieta di mantenimento a densità calorica più elevata e contenuto di fibra inferiore, da sostituire successivamente con una dieta a più alto contenuto di fibra. Nel caso in cui sia presente una seconda patologia che richieda una dieta specifica, questa deve avere la precedenza rispetto al diabete.

Tabella 3. Esempi di razione giornaliera casalinga per cani diabetici normopeso di 10, 20 e 30 kg

	10 kg	20 kg	30 kg
Carne di tacchino	250 g	420 g	550 g
Orzo	70 g	120 g	160 g
Piselli	70 g	120 g	160 g
Olio di soia	10 g	16 g	24 g
Olio di salmone	2 g	4 g	4 g
Essential Cane Adult (Cliffi)	6 g	10 g	15 g
Carbonato di calcio	1 g	1 g	1 g

Esercizio fisico

L'esercizio fisico contribuisce a promuovere la perdita di peso e ad eliminare l'insulino-resistenza indotta dall'obesità. Riduce inoltre i livelli glicemici promuovendo la diffusione dell'insulina dal sito d'iniezione, incrementando la perfusione ematica muscolare durante il movimento e stimolando i trasportatori del glucosio nelle cellule muscolari[34,35]. L'attività fisica per un cane diabetico dovrebbe essere quotidiana ed avvenire alla stessa ora, preferibilmente non vicino al momento in cui si verifica la massima azione dell'insulina. L'esercizio sporadico e intenso andrebbe evitato in quanto potrebbe causare ipoglicemia. Nel caso in cui sia inevitabile, è bene raccomandare ai proprietari di somministrare una dose di insulina ridotta del 50%, ed apportare ulteriori aggiustamenti qualora dovessero comparire segni clinici di ipoglicemia o di diabete non controllato. E' bene inoltre consigliare ai proprietari di portare con sé delle fonti di glucosio (es: miele) da somministrare nel caso in cui l'animale manifesti segni d'ipoglicemia[2].

Controllo delle patologie concomitanti

L'identificazione e il trattamento delle patologie concomitanti giocano un ruolo fondamentale nella gestione del DM del cane in quanto, al pari della somministrazione di alcuni farmaci (es. glucocorticoidi e progestinici) , possono causare una resistenza all'insulina[2]. L'insulino-resistenza può essere conseguente ad un alterato metabolismo dell'ormone (problema pre-recettoriale), ad una ridotta concentrazione e affinità di legame dei recettori per l'insulina sulle membrane cellulari (problema recettoriale), ad una interferenza con il segnale intracellulare indotto dall'insulina (problema post-recettoriale) o una combinazione di questi[2]. L'insulino-resistenza che ne deriva può variare da lieve a grave o può subire fluttuazioni nel tempo[1] (Tabella 4).

Per questi motivi nei cani neodiagnosticati è importante raccogliere una dettagliata anamnesi, effettuare un esame fisico accurato e delle indagini collaterali complete (esami ematochimici, esame delle urine e, se indicati, ecografia addominale e radiografie toraciche), al fine di scovare e poter trattare eventuali patologie concomitanti. Nel caso in cui vengano riscontrate patologie concomitanti, il proprietario deve essere informato riguardo la necessità di eseguire monitoraggi più frequenti e riguardo al fatto che il DM risulterà presumibilmente di più difficile controllo [1].

Nel caso in cui siano in corso dei trattamenti a base di glucocorticoidi e progestinici, se possibile, devono essere immediatamente interrotti ed eventualmente sostituiti con altri farmaci[1].

Nelle cagne intere che hanno sviluppato il diabete durante il diestro, è necessario eseguire l'ovariectomia il più rapidamente possibile, idealmente entro pochi giorni dalla diagnosi. In alcune occasioni, la repentina sterilizzazione permette di ottenere la remissione del diabete, pertanto dopo la sterilizzazione è necessario monitorare strettamente il paziente per adeguare la dose insulinica[1]. L'ovariectomia è indispensabile in tutte le cagne diabetiche per evitare la secrezione di GH mammario-progesterone indotta e l'insulino-resistenza che ne deriva[28]. Nel caso in cui non sia possibile eseguire la sterilizzazione, è possibile ricorrere all'utilizzo di farmaci antagonisti del progesterone (aglepristone)[36].

Tabella 4. Cause di insulino-resistenza in cani e gatti diabetici

Patologie che causano una grave insulino-resistenza	Patologie che causano lieve o fluttuante insulino-resistenza
• Sindrome di Cushing • Diestro nelle femmine intere • Tumore surrenalico secernente progesterone • Farmaci diabetogeni • Glucocorticoidi • Progestinici • Ipotiroidismo	• Obesità • Infezioni • Infiammazioni croniche • Pancreatite cronica • IBD • Patologie del cavo orale • Patologia renale cronica • Patologie epatobiliari • Patologie cardiache • Ipertiroidismo • Insufficienza pancreatica esocrina • Iperlipidemia • Neoplasie • Glucagonoma • Feocromocitoma

Metodi di monitoraggio del diabete mellito

Per il monitoraggio dei cani con DM è fondamentale affidarsi, almeno in un primo periodo, ai controlli periodici eseguiti in clinica. In questa sede, oltre alla raccolta dell'anamnesi, risulta importante monitorare il peso corporeo, eseguire un esame fisico, produrre una curva glicemica nonché valutare la concentrazione delle fruttosamine sieriche[1].

Per raggiungere un adeguato controllo glicemico sono solitamente necessari circa 2-3 mesi, durante i quali dovranno essere eseguiti monitoraggi frequenti; successivamente è possibile ridurre la frequenza delle rilevazioni; va tuttavia ricordato al proprietario che i cani diabetici necessitano di periodici controlli per tutta la vita. Presso la struttura degli autori vengono eseguiti controlli a 1, 2-3, 6-8, e 10-12 settimane dopo la diagnosi e successivamente ogni 4 mesi circa[1] (Box 1).

Box 1: Protocollo per il trattamento del diabete mellito nel cane

- Diagnosi di diabete mellito (anamnesi, esame fisico, iperglicemia, glicosuria, aumento delle fruttosamine sieriche)
- Indagini laboratoristiche (esami emocromocitometrico, biochimico, chimico-fisico delle urine e batteriologico delle urine)
- Ecografia addominale, cPLI (se indicato)
- Interrompere eventuali farmaci che possono causare insulino-resistenza
- Somministrare insulina ad azione intermedia/rilascio prolungato (Caninsulin, NPH, Lantus): 0,25 U/Kg q12h
- Trattare la/e eventuale/i patologie concomitanti; se la diagnosi di DM viene eseguite a femmine intere misurare il progesterone sierico e programmare l'intervento di ovariectomia il prima possibile
- Prescrivere una dieta commerciale per cani diabetici. La razione giornaliera deve essere suddivisa in due pasti della stessa quantità e deve essere somministrato preferibilmente immediatamente prima della somministrazione di insulina. Se il cane è in sovrappeso, mirare ad una perdita di peso pari all'1-2% a settimana. Viceversa se il cane è emaciato, prescrivere una dieta di mantenimento fino al raggiungimento di un adeguato peso corporeo e di un adeguato controllo glicemico. Se è presente una patologia concomitante, il trattamento dietetico specifico per questa deve avere la priorità.
- Istruire il proprietario (richiede circa 1 ora) e fornirgli istruzioni scritte

Primo controllo: 1 settimana dopo la diagnosi

- Anamnesi, esame fisico, peso corporeo
- Somministrare il pasto e l'insulina in clinica oppure se il cane è riluttante a mangiare in clinica, fare somministrare al proprietario pasto e insulina a casa e la curva glicemica inizierà al momento dell'arrivo in clinica (il prima possibile)
- Misurare la glicemia tramite PBGM ogni 2 ore (curva glicemica)
- Misurare la concentrazione di fruttosamine sieriche
- Se necessario, modificare il dosaggio insulinico del 10-25%

Secondo controllo: 2-3 settimane dopo la diagnosi

- Ripetere tutte le procedure eseguite al primo controllo (anamnesi, esame fisico, peso corporeo, curva glicemica, fruttosamine, eventuale modifica del dosaggio insulinico)
- Introdurre al proprietario l'eventualità del monitoraggio glicemico a casa e istruirlo riguardo agli aspetti tecnici (richiede almeno mezz'ora)
- Monitoraggio a casa: il proprietario può misurare la glicemia a digiuno 2 volte a settimana ed eseguire una curva glicemica due volte al mese

Terzo controllo: 6-8 settimane dopo la diagnosi

- Ripetere tutte le procedure eseguire al primo controllo (anamnesi, esame fisico, peso corporeo, curva glicemica, fruttosamine, eventuale modifica del dosaggio insulinico). Se il cane clinicamente sembra ben controllato, la glicemia misurata in prossimità della somministrazione di insulina è compresa tra 180-250 mg/dl e le fruttosamine sono compresa tra 350-450 µmol/L la curva glicemica potrebbe non essere necessaria
- Se il proprietario esegue il monitoraggio a casa valutare se la tecnica di somministrazione è corretta

Quarto controllo: 10-12 settimane dopo la diagnosi

- Ripetere tutte le procedure eseguite a 6-8 settimane dopo la diagnosi

Ulteriori controlli (ogni 4 mesi)

- Ripetere tutte le procedure eseguite a 6-8 settimane dopo la diagnosi

Obiettivo della terapia

- Risoluzione dei segni clinici: PU/PD, polifagia, raggiungimento di un peso corporeo adeguato
- Concentrazione di glucosio ematico compresa tra 90 e 250 mg/dl
- Concentrazione di fruttosamine sieriche compresa tra 350 e 450 µmol/L (meno importanti)

Anamnesi ed esame fisico

I dati anamnestici, i reperti dell'esame fisico diretto e il peso corporeo sono i primi parametri da prendere in considerazione per valutare il controllo della patologia[1]. E' opportuno educare il proprietario a rilevare i segni clinici associati ad uno scarso controllo del DM, ad esempio valutando il consumo di acqua e la frequenza/entità delle minzioni[37]. Quando il proprietario non riporta sintomi, l'esame fisico risulta nella norma e il peso corporeo è stabile è verosimile che la patologia sia ben controllata[38].

La perdita di peso e la persistenza dei segni clinici sono indicativi di uno scarso controllo glicemico o della presenza di patologie concomitanti. Per caratterizzare il problema e adeguare la dose insulinica, risulta quindi importante eseguire una curva glicemica e misurare le fruttosamine sieriche. Per indagare eventuali patologie concomitanti è inoltre opportuno eseguire ulteriori test diagnostici (Tabella 5).

Il proprietario deve essere inoltre ben informato su come si manifestino i sintomi di ipoglicemia quali ad esempio, tremori, andatura incerta, incapacità a mantenere la stazione fino ad arrivare alle crisi convulsive. Nonostante i segni clinici risultino un valido strumento per identificare uno scarso controllo glicemico, solitamente non sono altrettanto efficaci nell'individuare cani a rischio di ipoglicemia. L'incremento del dosaggio insulinico esclusivamente basato sui segni clinici può pertanto risultare molto rischioso[28].

Tabella 5. Test diagnostici da considerare per identificare la causa di insulino-resistenza nei cani diabetici

- Esami emocromocitometrico, biochimico e chimico-fisico delle urine
- Esame batteriologico delle urine
- CPL (pancreatite)
- TLI (insufficienza pancreatica esocrina)
- Test di funzionalità surrenalica
    1. Rapporto cortisolo/creatinina urinaria
    2. Test di soppressione con desametasone a basse dosi
    3. Test di stimolazione con ACTH
- Test di funzionalità tiroidea
    1. T4 e fT4 sierico
    2. TSH sierico
    3. Test di stimolazione con rhTSH
- Concentrazione di progesterone sierico (diestro nelle femmine intere)
- Concentrazione delle IGF-1 (acromegalia)
- Anticorpi anti-insulina
- Concentrazione di trigliceridi a digiuno (iperlipidemia)
- Ecografia addominale (adrenomegalia, masse surrenaliche, pancreatite, neoplasie)
- Radiografie torace (cardiomegalia, neoplasie)
- Tomografia Computerizzata o Risonanza Magnetica Nucleare (massa ipofisaria)

CPLI, canine pancreatic-specific lipase; TLI, trypsin-like immuno-reactivity; TSH, thyroid-stimulating hormone; IGF-1, insulin-like growth factor

Fruttosamine sieriche

Le fruttosamine sieriche sono proteine glicate che si formano a seguito di un legame non enzimatico ed irreversibile tra glucosio ematico e gruppi amminici delle proteine del sangue[39,40,41]. La loro concentrazione dipende dall'entità della glicemia e dall'emivita delle proteine plasmatiche stesse, pertanto le fruttosamine rispecchiano la concentrazione media del glucosio ematico delle 2-3 settimane precedenti[42] e non sono influenzate da variazioni rapide della glicemia.

In generale la concentrazione di fruttosamine aumenta quando il controllo glicemico peggiora e diminuisce quando il controllo glicemico migliora[1]. E' importante tuttavia considerare che il loro livello in circolo può risultare diminuito in corso di ipoproteinemia o ipoalbuminemia, iperlipidemia, iperazotemia, emolisi, ma anche per inadeguata conservazione del campione[43,44,45,46]. Al contrario, si può riscontrare un aumento delle concentrazioni in cani ipotiroidei e in cani con iperglobulinemia conseguente a mieloma multiplo[47,48]. I range di riferimento variano lievemente tra i vari laboratori, ma generalmente sono compresi fra 200 e 360 μmol/l[1]. Nei soggetti neo diagnosticati la

concentrazione di fruttosamine varia solitamente da 320 a 850 µmol/l[2]. L'interpretazione delle fruttosamine nei cani diabetici deve tenere in considerazione che, anche i soggetti ben controllati, risultano iperglicemici per buona parte della giornata[2] (Tabella 6).

Le fruttosamine non devono mai essere considerate l'unico indicatore del controllo glicemico poiché esistono differenze sostanziali nel processo di glicazione tra individui diversi[49]. Alcuni cani diabetici presentano inoltre una marcata discrepanza fra controllo glicemico e valore di fruttosamine. Le fruttosamine sieriche vanno pertanto sempre interpretate all'interno del quadro complessivo rappresentato dai dati anamnestici e clinici, in associazione ai valori ottenuti dalla curva glicemica[1].

Tabella 6: Interpretazione delle fruttosamine sieriche nei cani diabetici

Range normale: 225-365 µmol/L
Eccellente controllo: 350-400 µmol/L
Buon controllo: 400-450 µmol/L
Adeguato controllo: 450-500 µmol/L
Scarso controllo: > 500 µmol/L
Ipoglicemia prolungata: < 300 µmol/L
Remissione: < 300 µmol/L

Fattori che possono influenzare i risultati:

- Ipoalbuminemia (↓)
- Ipotiroidismo (↑)
- Iperlipidemia (lieve ↓)
- Azotemia (lieve ↓)
- Prolungato stoccaggio del campione a temperatura ambiente (↓)
- Emolisi (↓)

Esame delle urine

Il monitoraggio occasionale delle urine è consigliato in soggetti diabetici che manifestano chetosi o ipoglicemie e permette di valutare, rispettivamente, la presenza di chetonuria o la persistente assenza di glicosuria. Quest'ultima potrebbe essere indicativa di un sovradosaggio insulinico, mentre la presenza di chetonuria è indicativa di carenza insulinica o insulino-resistenza e quindi suggerisce la necessità di ulteriori indagini[50]. Il proprietario può essere istruito a monitorare il glucosio urinario tramite il dipstick. In caso di positività, non deve modificare il dosaggio insulinico per tentare di

eliminare/ridurre la glicosuria in quanto, tale modo di agire, è stato identificato come una delle più comuni cause dell'effetto Somogyi[1]. Al contrario, può essere opportuno ridurre il dosaggio insulinico in soggetti che manifestano episodi di ipoglicemia ricorrenti e assenza di glicosuria persistente[1].

Misurazione del glucosio ematico

Misurazione singola

La singola misurazione glicemica non è sufficiente per definire il controllo glicemico. Le uniche due eccezioni sono rappresentate dal suo utilizzo nei pazienti ben controllati e dall'eventuale riscontro di ipoglicemia[1]. Nel primo caso, se la glicemia in prossimità della somministrazione insulinica risulta tra 180-250 mg/dl e il paziente non manifesta sintomi, è verosimile che il diabete sia ben controllato e non siano necessarie ulteriori misurazioni glicemiche. Il riscontro di un'ipoglicemia, invece, è segno di sovradosaggio e implica la necessità di ridurre il quantitativo insulinico[1].

Misurazione seriale o curve glicemica

La curva glicemica rappresenta lo strumento più importante per effettuare degli adeguamenti del dosaggio insulinico in modo razionale[2]. La generazione di una curva glicemica prevede che il paziente rispetti le quotidiane abitudini riguardo l'assunzione di alimento e insulina. In corso di ospedalizzazione, nelle 10-12 ore successive alla somministrazione di insulina, vengono misurati i valori glicemici ogni 2 ore[50]. È consigliabile intensificare le misurazioni se la glicemia diminuisce rapidamente o in caso di ipoglicemia, in modo da aumentare la probabilità di identificare con precisione il nadir. La glicemia viene misurata utilizzando il sangue capillare proveniente da una goccia ottenuta mediante dispositivi appositi, potenzialmente utilizzabili in varie zone corporee, quali il padiglione auricolare (figura 2), i polpastrelli o la mucosa labiale[50]. Le concentrazioni di glucosio ematico ottenute da sangue capillare non risultano significativamente differenti da quelle ottenute da sangue venoso[51,52]. La glicemia viene generalmente misurata attraverso glucometri portatili (Portable Blood Glucose Meter, PBGM), la cui accuratezza per l'utilizzo nel cane è fortemente variabile, specialmente nel caso di PBGM sviluppati per l'uomo[53,54,55]. Alcuni PBGM ad uso umano sono sufficientemente accurati e precisi se utilizzati nel cane, tuttavia molti di essi tendono a sottostimare

i valori di glucosio plasmatico[1]. Un recente studio condotto nel cane ha valutato accuratezza e precisione di 9 PBGM ad uso umano secondo le nuove norme ISO 15197:2013. Nessuno dei dispositivi valutati soddisfaceva pienamente i requisiti della norma, ma l'AccuChek Aviva Nano è risultato essere la migliore opzione[56]. Da qualche anno sono disponibili sul mercato anche PBGM appositamente prodotti per l'utilizzo in medicina veterinaria. Tra questi l'AlphaTRAK®, prodotto da Abbott Laboratories, ha mostrato una buona accuratezza e inoltre necessita di un campione di sangue estremamente piccolo, pari a 0,3 μL.[55] Le variazioni di ematocrito possono influenzare questi dispositivi, nel caso dell'AlphaTRAK®, questo ha mostrato una ridotta accuratezza in pazienti con ematocrito < 30%; al contrario, PBGM di derivazione umana hanno mostrato un calo di accuratezza all'aumentare dell'ematocrito[57].

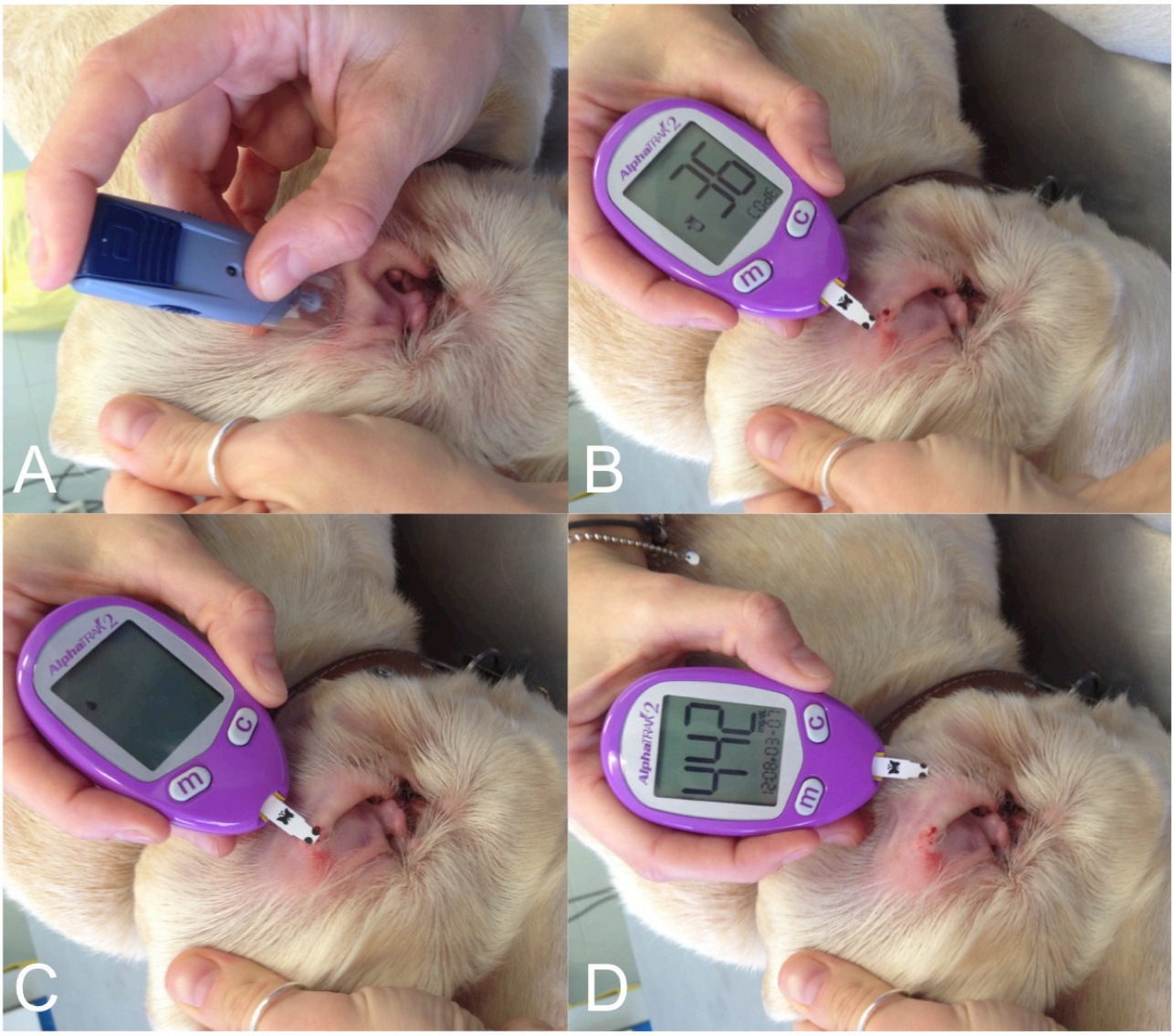


Figura 2 - Utilizzo di AlphaTRAK® per la misurazione della glicemia da sangue capillare. **A)** manualità per il prelievo di sangue capillare; **B)** goccia di sangue capillare; il numero rappresentato nel display del glucometro si riferisce al codice relativo alla specie canina; **C)** misurazione della glicemia; **D)** risultato ottenuto.

Monitoraggio glicemico a casa

Il principale limite di una curva glicemica eseguita in clinica è rappresentato dall'influenza che lo stress legato al ricovero può avere sui valori glicemici. Il mancato riconoscimento dell'iperglicemia da stress può portare ad una erronea interpretazione di scarso controllo glicemico e potenzialmente ad un incremento del dosaggio insulinico, con un maggiore rischio di indurre stati di ipoglicemia o effetto Somogyi[2]. Un altro limite delle curve glicemiche effettuate in clinica è il costo. Per questi motivi una valida alternativa all'ospedalizzazione può essere l'esecuzione della curve glicemiche a

casa ad opera del proprietario[58]. Il monitoraggio domestico non dovrebbe essere proposto prima delle 3-4 settimane dall'inizio della terapia insulinica, in modo da lasciare tempo al proprietario di prendere dimestichezza con la patologia e con la somministrazione insulinica[1]. È di fondamentale importanza istruire il proprietario riguardo tecniche e strumentazioni richieste per eseguire correttamente una curva glicemica. I risultati ottenuti, assieme alla valutazione dei segni clinici e la stabilità del peso corporeo, vengono inviati al veterinario per l'interpretazione degli stessi. Il monitoraggio casalingo consente valutazioni glicemiche più frequenti, associate a ripetuti adeguamenti del dosaggio insulinico e di conseguenza un migliore controllo glicemico[59]. Uno dei problemi che si potrebbe riscontrare è la variazione del dosaggio insulinico da parte del proprietario senza consultare il veterinario, pratica che frequentemente porta ad un sovradosaggio e quindi potenzialmente all'effetto Somogyi[1].

Interpretazione della curva glicemica

Le curve glicemiche permettono di determinare l'efficacia dell'insulina, il nadir del glucosio, il picco e la durata d'azione dell'insulina ed infine le fluttuazioni della glicemia[1]. Nei soggetti ben controllati le glicemie dovrebbero essere comprese tra 90-250 mg/dl.

L'efficacia dell'insulina viene definita come la differenza tra il valore glicemico maggiore e quello minore e deve essere interpretata alla luce del più alto valore glicemico. Una piccola differenza (es. 50 mg/dl) è accettabile se la glicemia più alta è < 220 mg/dl ma non se questa è > 300 mg/dl[28].

Il nadir del glucosio è il valore minimo registrato durante la curva glicemica e idealmente dovrebbe essere compreso tra 90-150 mg/dl. Un nadir inferiore ai 90 mg/dl può indicare una ridotta assunzione di alimento, esercizio intenso, un sovradosaggio insulinico o una sovrapposizione dell'effetto dell'insulina tra le due somministrazioni giornaliere. Un nadir >160 mg/dl può indicare un errore nella somministrazione, un sottodosaggio dell'insulina, oppure si sta verificando la fase iperglicemica della risposta Somogyi.

La durata d'azione dell'insulina viene definita come il tempo che intercorre tra la somministrazione della stessa e il ritorno della glicemia a valori compresi tra 180 e 270 mg/dL, passando per il nadir.

Se la durata è troppo breve (< 8 ore), l'animale può manifestare i segni clinici del DM; se invece risulta molto prolungata (> 14 ore), potrebbe verificarsi una sovrapposizione dell'effetto e manifestarsi ipoglicemia o la risposta Somogyi[1].

In base ai risultati della curva glicemica può essere modificato il dosaggio insulinico o il tipo di insulina.

La modifica della dose insulinica deve essere dell'ordine del 10-25%, sebbene nei casi di ipoglicemia sintomatica la dose debba essere ridotta di almeno il 50%[1].

Monitoraggio continuo della glicemia

I sistemi per il monitoraggio continuo della glicemia (Continuous Glucose Monitoring System, CGMS) sono comunemente utilizzati in medicina umana ed il loro utilizzo si sta diffondendo anche in medicina vetrinaria[60]. I CGMS sono dispositivi che, attraverso specifici sensori, rilevano per più giorni la concentrazione del glucosio interstiziale, che ben si correla con la concentrazione del glucosio sierico nel cane e nel gatto[61,62,63]. Essi consentono di indagare la glicemia senza dover ricorrere ai prelievi di sangue capillare. Diversi dispositivi sono stati studiati in medicina veterinaria[61,64,62,65,66,63,67,68,69]. Il principale vantaggio nell'utilizzo di questi dispositivi consiste nella capacità di identificare periodi di ipoglicemia, anche notturni, ed effetto Somogyi. Consentono inoltre di ridurre le manualità sul paziente, comportando minori sprechi di tempo per il personale, minore stress per il paziente e quindi una minore incidenza dell'iperglicemia stress-indotta. Tra i principali svantaggi vi sono invece il costo elevato, la necessità di frequenti calibrazioni, la scarsa accuratezza nei pazienti disidratati e la portata wireless limitata[70]. Recentemente è stato introdotto in Europa un nuovo CGMS (Freestyle Libre, Abbott) che, rispetto ai precedenti, presenta diversi vantaggi tra cui le dimensioni ridotte, il costo più contenuto, una durata maggiore di lettura una volta applicato (14 giorni) ed infine non richiede calibrazioni. Nel cane si raccomanda l'applicazione del sensore sul collo (previa tricotomia) (figura 3) o a livello di garrese. E' bene che il sensore venga protetto da un bendaggio. Un recente studio ha dimostrato l'accuratezza clinica di tale strumento nei cani iperglicemici e normoglicemici, mentre è risultato meno accurato per valori glicemici <100 mg/dl[71].

Nei soggetti con cute particolarmente spessa il sensore può non riuscire a rilevare il glucosio interstiziale.

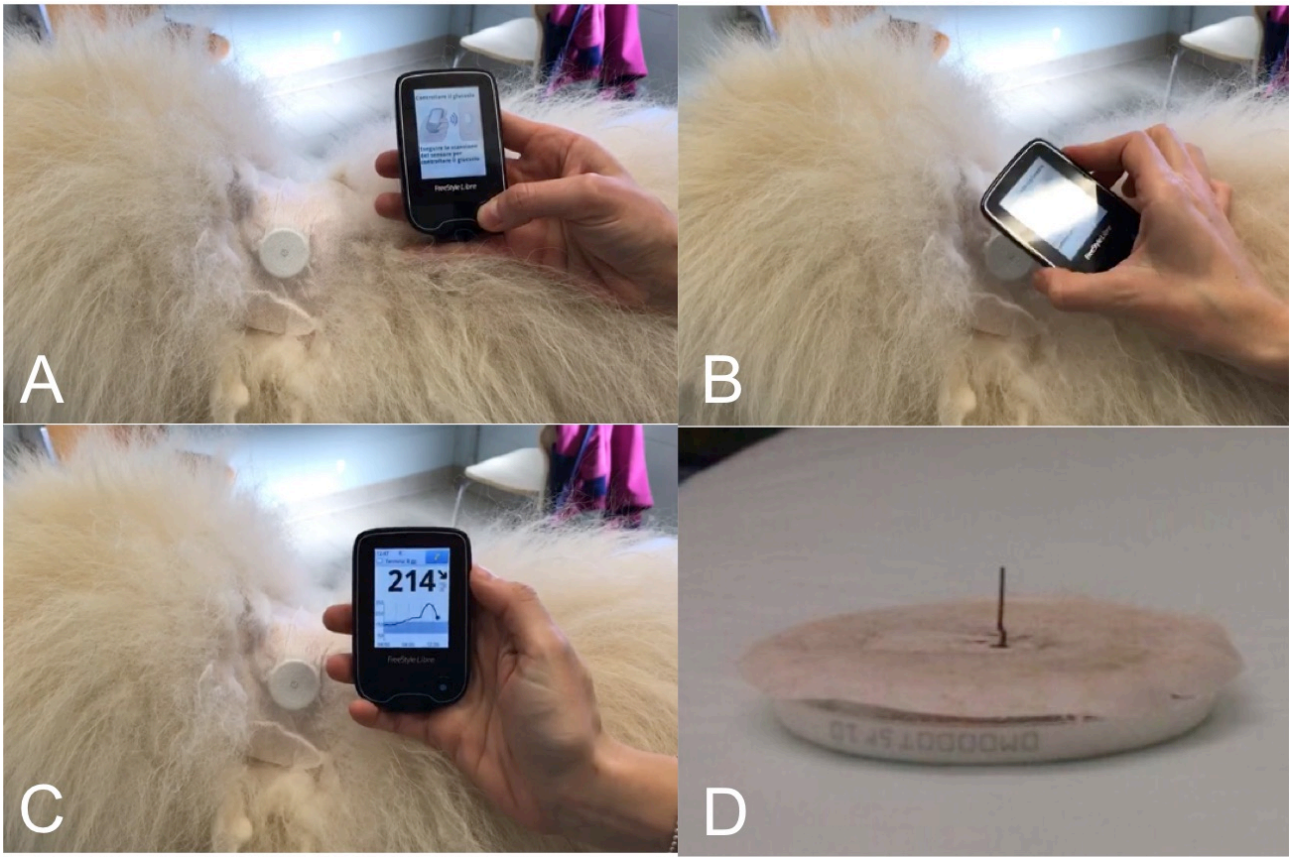


Figura 3: Utilizzo di FreeStyle Libre® come metodo di monitoraggio continuo della glicemia. **A)** paziente diabetico con sensore e cerotto di rinforzo applicato; **B)** avvicinamento del lettore al sensore; **C)** scansione dei dati; **D)** sensore rimosso dopo 14 giorni di applicazione.

Persistenza o ricorrenza dei segni clinici

La persistenza o la ricomparsa dei segni clinici è un'eventualità piuttosto comune nella gestione dei cani con DM. Nel box 2 e nell'algoritmo 1 vengono riportate rispettivamente le cause più comuni e l'approccio corretto a questo tipo di problematica.

Box 2: Cause di persistenza o ricorrenza dei segni clinici

Problemi tecnici

- Errori nella manipolazione e somministrazione di insulina (es: uso di un diluente inappropriato, tecnica di miscelazione dell'insulina sbagliata, uso di un'insulina scaduta, congelata o riscaldata, tecnica di iniezione scorretta, utilizzo di siringhe inappropriate (siringhe da 40 U/ml devono essere utilizzate per le insuline veterinarie –Caninsulin® e PZI (ProZinc®)-, viceversa siringhe da100 U/ml per le insuline umane)
- Identificazione del problema: chiedere al proprietario di portare le siringhe che utilizza e farsi mostrare la tecnica da lui utilizzata, sia per la preparazione che per la somministrazione di insulina
- Se viene identificato l'errore spiegare di nuovo al proprietario l'intera procedura e fornire assistenza fino a che non prende dimestichezza con la procedura

Sottodosaggio insulinico

- Molti cani con DM sono ben controllati con un dosaggio di insulina ≤ 1U/kg somministrata ogni 12 ore
- Se la dose insulinica somministrata è ≤1U/Kg q12h e il cane manifesta i sintomi di uno scarso controllo glicemico è probabile che la dose di insulina non sia sufficiente
- Aumentare il dosaggio insulinico gradualmente del 10-25% a settimana

Sovradosaggio insulinico e risposta Somogyi

- Si deve sospettare quando in anamnesi viene riportato un buon controllo glicemico per 1-3 giorni, seguiti da diversi giorni di scarso controllo glicemico
- Diagnosi: richiede l'evidenza di ipoglicemia o una riduzione rapida della glicemia seguita da iperglicemia (>300 mg/dl) nelle 12 ore successive (può durare fino 72h); le fruttosmaine sieriche in genere risultano elevate (>500 µmol/l)
- Identificazione del problema: eseguire curve glicemiche seriali , meglio se eseguite a casa o tramite l'uso di CGMS (NB: non confondere con una breve durata d'azione dell'insulina)
- Se viene identificata la risposta Somogyi la dose insulinica deve essere ridotta gradualmente (1-5 UI in relazione a taglia del cane e dose di insulina) e il proprietario deve monitorare i sintomi nei successivi 2-5 giorni
- Se non si riscontrano miglioramenti è necessaria una ulteriore riduzione del dosaggio
- Se i sintomi peggiorano: considerare altre cause di inefficacia dell'insulina (es: breve durata)

Breve durata d'azione dell'insulina

- Le insuline NPH e lenta (Caninsulin®) in alcuni soggetti possono avere una durata d'azione < 8h
- Identificazione del problema: eseguire una curva glicemica
- Passare ad una insulina a rilascio prolungato (es: glargine, detemir, PZI) q12h (tabella 1)

Prolungata durata d'azione dell'insulina

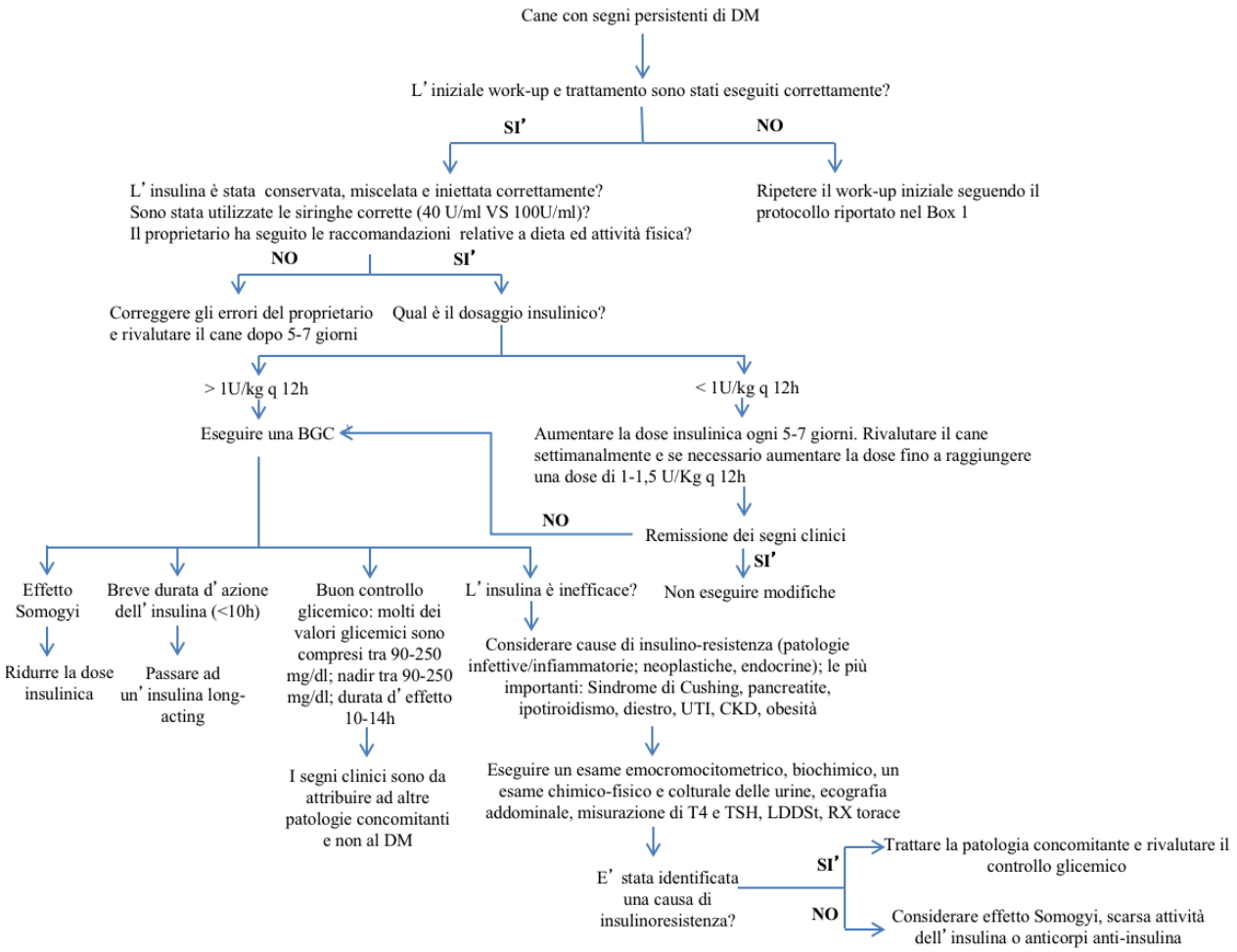
- Si osserva quando il nadir del glucosio si verifica a 10 ore o oltre la somministrazione di insulina
- Identificazione del problema: eseguire una curva glicemica
- Passare ad un'insulina a durata d'azione più breve o utilizzare un'insulina a rilascio prolungato q24h

Anticorpi anti-insulina

- La loro formazione si osserva più frequentemente se vengono utilizzate insuline di origine bovina o bovina/suina (oggi poco diffuse) ma può avvenire anche in cani trattati con insulina ricombinate umana, pertanto la loro formazione deve essere sospettata quando, a fronte di uno scarso controllo glicemico, non si evidenziano altre cause
- Gli anticorpi anti-insulina possono influenzare farmacocinetica e farmacodinamica dell'insulina esogena causando così uno scarso controllo glicemico
- Identificazione del problema: documentare la presenza degli anticorpi tramite test validati
- Passare ad un'insulina di origine suina (strutturalmente identica a quella canina)

Patologie concomitanti

- Si devono sospettare se la dose insulinica richiesta è elevata (>1,5 U/kg) e il cane non manifesta un adeguato controllo glicemico; molte patologie possono causare insulino-resistenza (tabella 3)
- Identificazione del problema: anamnesi, esame fisico, ed eventuali indagini collaterali
- Trattare, se possibile, la patologia concomitante e considerare che il trattamento di essa può ridurre l'insulino-resistenza e quindi richiedere un aggiustamento della dose insulinica



Algoritmo 1 - Approccio ai cani diabetici in terapia che manifestano segni clinici persistenti. UTI, urinary tract infection; CKD, chronic kidney disease; T4, tiroxina; TSH, thyroid-stimulating hormone; LDDSt, low-dose - dexamethasone suppression test

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Capitolo 3

COMPARISON OF LENTE INSULIN AND NPH INSULIN THERAPY FOR THE TREATMENT OF NEWLY DIAGNOSED DIABETIC DOGS

Federico Fracassi, Guido Linari, Francesca Del Baldo, Antonella Di Cunzolo, Simona D'Angelo, Eleonora Malerba, Gaia Carotenuto, Ugo Bonfanti, Sara Corradini

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Dipartimento di Scienze Mediche Veterinarie,

Scuola di Agraria e Medicina Veterinaria,

Bologna

3. Comparison of lente insulin and NPH insulin therapy for the treatment of newly diagnosed diabetic dogs

Abstract

Objective: Clinical studies that compare the efficacy and safety of Lente insulin and Neutral Protamine Hagedorn (NPH) insulin in diabetic dogs are lacking. To compare the efficacy and safety of lente insulin and NPH insulin in diabetic dogs.

Design: Prospective, randomized, controlled clinical study.

Animals: Thirty client-owned, newly diagnosed diabetic dogs.

Procedures: Dogs were fed q12h with the same commercial diet. Animals were randomized into two groups such as lente insulin and NPH insulin administered q12h. Follow-up re-evaluations were done at 1, 2, 4, 6, 8, and 12 weeks. At each re-evaluation, a physical exam, blood glucose curve (BGC), and serum fructosamine concentrations were performed.

Results: All dogs completed the trial. At the end of the study, the median insulin dose was 0.61 U/kg (0.28 U/lb; range, 0.34 to 0.92 U/kg [0.15 to 0.42 U/lb], SC, q12h) and 0.49 U/kg (0.22 U/lb; range, 0.23 to 0.68 U/kg [0.10 to 0.31 U/lb], SC, q 12 h) in the lente and NPH groups, respectively. There was a significant improvement of polyuria and polydipsia and glucose concentrations in both groups but serum fructosamine concentrations decreased significantly only in the NPH group. At the end of the study, the glycemic control was considered good in 9/15 (60%) and 11/15 (73%) in the lente and NPH group, respectively. These differences were not significant.

Conclusions and Clinical Relevance: Lente insulin and NPH insulin are effective in the treatment of dogs with DM. The success rate with NPH insulin seems somewhat higher than with lente insulin.

Introduction

Various types of insulin are used to treat diabetes mellitus (DM) long-term.[1-8] Based on duration of action and potency, they include intermediate-acting (i.e. lente, Neutral Protamine Hagedorn [NPH]) and long-acting insulins (i.e. protamine zinc insulin [PZI], insulin glargine, and insulin detemir). Current guidelines for dogs with newly diagnosed DM recommend the use of insulin preparations with an intermediate duration of action.[9]

Lente is a porcine–origin zinc 40 U/mL insulin that consists of 30% short-acting amorphous insulin and 70% long-acting, microcrystalline insulin. Lente is approved by the Food and Drug Administration (FDA) for use in dogs and allows a good glycemic control in most treated diabetic dogs.[3] NPH (100 U/mL) is recombinant human insulin, usually administered q12h. Some studies have demonstrated a good efficacy of this insulin in the treatment of canine DM.[1,4] One study observed that with NPH insulin, postprandial hyperglycemia could occur in some well-regulated dogs.[4]

Several clinical studies evaluated single insulin products for the treatment of dogs with DM but clinical articles comparing the efficacy and safety of different insulin preparations are uncommonly reported in the veterinary literature.

The aim of the present study is to compare the efficacy and safety of lente insulin and NPH insulin in newly diagnosed diabetic dogs.

Materials and Methods

Dogs

Thirty client-owned newly diagnosed diabetic dogs were prospectively enrolled in the study between November 2014 and September 2016. DM was diagnosed based on clinical signs such as polyuria, polydipsia (pu/pd), weakness, weight loss, blood glucose concentration > 200 mg/dL after food had been withheld for at least 10 h, glucosuria, and serum fructosamine concentration > 340 µmol/L. To identify any concurrent disorders, complete blood count (CBC), serum biochemical profile, and urinalysis were performed in all dogs at the time of enrollment in the study. Additional testing were

done if clinically indicated. Dogs with a relevant concurrent disease (e.g., renal insufficiency, neoplasia, hypothyroidism, or hypercortisolism), that received insulin for > 7 days before admission and dogs that had received glucocorticoids or progestagens within the previous 60 days were not enrolled. Dogs with diabetic ketoacidosis (DKA) requiring aggressive management were used if their condition had been stabilized by medical treatment, including regular insulin therapy.

The recruitment of dogs in the study was voluntary and the only cost for the owner was the purchase of insulin, also the food was provided for free. The protocol and informed consent forms were approved by the Scientific Ethics Committee of the University of Bologna. All owners signed the written informed consent before enrollment in the study.

Study design

The trial was designed as a prospective, randomized, and controlled 3-month clinical study. Before treatment (day 0), anamnesis and physical examination were obtained as well as a CBC, serum biochemical profile (that included measurement of serum fructosamine concentration), and urinalysis were performed. At the time of diagnosis each dog was randomly assigned to receive lente insulin (Caninsulin, MSD, Boxmeer, The Netherlands) or NPH insulin (Humulin I, Eli Lilly Italia S.p.A., Sesto Fiorentino, Italy). The randomization was performed using a computer-generated randomization program based on the Fisher–Yates shuffle algorithm. All dogs received the same prescription diet (Diabetic Royal Canin, Royal Canin SAS, Milano, Italy), which was low in simple carbohydrates and high in protein content. The diet was dry, canned, or a mixture of both based on the preferences of the dog. The diet and the formulation (dry/canned) were maintained for the entire duration of the study. The prescription diet was introduced as the dog's only food with a transition of 2–3 days from the dog's previous diet at the time of enrollment. The initial insulin dose for both products was 0.25–0.5 U/kg (0.11-0.23 U/lb) administered SC every 12 h. Six follow-up re-evaluations were performed 1, 2, 4, 6, 8, and 12 weeks after the initial evaluation. These evaluations included an assessment of clinical signs and determination of serum fructosamine concentration and BGCs. During each re-evaluation, food and insulin were given at home and blood glucose

concentrations were measured after the dog arrived at the clinic (≤ 1 hour after insulin administration). To generate the blood glucose curves, blood capillary glucose was obtained from the pinna and was measured after 1, 2, 4, 6, 8, 10, and 12 h from insulin injection. The adjustment of the insulin dose was based increasing from 0.5 to 2.0 U/dog the insulin dose at each evaluation as required; the aim was to maintain blood glucose concentrations between 90 and 270 mg/dL. Insulin dosage adjustments were made by the attending veterinarian and were based on the owner's perception of clinical signs in response to treatment (including evidence of hypoglycemic episodes, body weight, and physical examination results), BGC, and serum fructosamine concentration. Hypoglycemia was defined as blood glucose concentration < 80 mg/dL.

Analytical Methods

Blood glucose concentrations were measured in capillary blood obtained from the inner surface of the pinna using a hand-held glucometer produced for the dog (Glucocalea Wellionvet, Isomedic srl, (LO), Italy). Detectable blood glucose concentrations ranged from 20 to 600 mg/dL. When blood glucose concentrations were <20 mg/dL and >600 mg/dL, registered as "LO" and "HI" on the glucometer were arbitrary given a value of 20 mg/dL and 600 mg/dL, respectively. Fructosamine analyses were performed using a colorimetric nitroblue tetrazolium reduction method (Fructosamine, Olimpus, Milano, Italy). CBC (Advia 2120 Hematology System, Siemens Healthcare Diagnostics, Tarrytown, New York, USA), serum biochemical profiles (AU2700 Beckman-Coulter, Lismeehan O'Callaghan's Mills, Co. Clare, Ireland) including lipase (1,2-diglyceride enzymatic/colorimetric assay) (Lipase, OSR 6130, Olympus/Beckman Coulter, Lismeehan O'Callaghan's Mills, Co. Clare, Ireland) and urinalyses were performed by standard laboratory methods in a reference laboratory (Mylav Laboratorio Lavallonea, Alessano, Italy).

Assessment of Efficacy

In order to objectively evaluate the glycemic control, the following parameters were used: body weight, presence of polyuria/polydipsia, median glucose of the BGC, blood glucose nadir of the BGC,

overall evaluation of the BGC, and serum fructosamine concentration. For each parameter, a score was arbitrarily assigned: 2 = good, 1 = moderate, and 0 = poor. Maintaining or increases of body weight was considered good (score = 2), conversely a decrease (> 5%) of the body weight was judged as poor (score = 0). Absent, improved, and present/unchanged-present/worsen pu/pd was considered good (score = 2), moderate (score = 1), and poor (score = 0), respectively. Median glucose of the BGC < 230 mg/dL, between 230–300 mg/dL, and > 300 mg/dL was considered good (score = 2), moderate (score = 1), and poor (score = 0), respectively. Glucose nadir of the BGC was considered good (score = 2), moderate (score = 1), and poor (score = 0), if it was < 180 mg/dL, between 180–250 mg/dL, and >250 mg/dL, respectively. The overall evaluation of the BGC was considered good (score = 2) if ≥50% of blood glucose measurements were between 80–270 mg/dL or poor (score = 0) if < 50% of the glucose measurements were between 80–270 mg/dL. Serum fructosamine concentration < 450 µmol/L, between 450–550 µmol/L, and > 550 µmol/L were considered good (score = 2), moderate (score = 1), and poor (score = 0), respectively.

A total clinical score between 0 and 12 was obtained adding all the scores. A total clinical score between 8–12, 4–7, and 0–3 points was suggestive of good, moderate, and poor glycemic control, respectively.

Data Analysis

Statistical analysis was performed with commercially available software[1].

The distribution of data was assessed by using the D'Agostino and Pearson tests. The parameters normally distributed were expressed as mean ± SD, while the data without a normal distribution were expressed as median (minimum and maximum value). Proportions and percentages were used to describe categorical variables. Parametric and non-parametric tests were used to analyze data based on the distribution. Categorical variables were compared using the Fisher's exact test. Differences between groups for age, body weight, laboratory results, and insulin dose, recorded at admission and body weight, laboratory results, and insulin dose over the 3 months study period were analyzed using the Mann-Whitney U-test or t-test. Within each group, differences in body weight, insulin dose, blood

glucose, and fructosamine concentrations between baseline or first re-evaluation and the end of the study were evaluated using the Wilcoxon signed rank test or paired t-test. Differences were considered significant at $P < .05$.

Results

Thirty dogs were enrolled in this study. Fifteen dogs were treated with lente insulin and 15 with NPH insulin. Mean age was 9.6 years (SD, ± 1.9 years). There were 17 mixed-breed dogs, 5 English Setters, 3 Labrador Retrievers, 2 Yorkshire Terriers, 1 Maltese, 1 Cocker Spaniel, and 1 Yugoslavian Shepherd Dog. Thirteen were spayed females, 3 intact females, 5 neutered males, and 9 intact males. All 3 intact female dogs were spayed within 4 weeks after inclusion in the study. Median body weight was 17.8 kg (39.2 lb; range, 4.2 to 59.8 kg [9.2 to 131.8 lb]). At the time of the enrollment no significant differences between dogs assigned to lente or NPH group considering age, sex, or body weight were observed (Table 1).

Table 1 Baseline characteristics of 30 dogs included in the study

Variable	Lente Group	NPH Group	*P* Value
N° of Dogs	15	15	
Female	8 (1 intact, 7 spayed)	8 (2 intact, 6 spayed)	1
Male	7 (4 intact, 3 neutered)	7 (5 intact, 2 neutered)	1
Age (years)	9 (6–12)	10 (7–13)	0.35
Body weight (Kg)	12.5 (4.2–59.8)	16.0 (4.4–50.0)	0.60
Serum glucose mg/dL	400 (64–616)	384 (132–824)	0.69
Serum fructosamine (µmol/L)	455 (224–849)	607 (288–880)	0.08
Serum lipase activity (IU/L)	373 (179-2795)	410 (107-1343)	0.79

Six dogs were enrolled after resolution of DKA, 3 were in the lente insulin group, and 3 in the NPH insulin group. No differences considering serum glucose and fructosamine concentrations at the time of enrollment in the study between the two groups were detected (Table 1). All dogs accepted the new diet and in all subjects, it was maintained throughout the study. Of the expected 180 follow-up re-evaluations (30 dogs for 6 follow up re-evaluations) only 170 were performed. 10 re-evaluations were lost because owners did not come to the clinic, i.e. skipped the appointment. Two dogs lost 2 re-evaluations and 6 dogs lost one re-evaluation. The last re-evaluation (at the 3rd month) was

performed on all animals. Mean insulin dosages per injection at the beginning and at the end of the study were 0.36±0.08 U/kg (0.16±0.04 U/lb) and 0.6 ± 0.14 U/kg (0.28±0.07 U/lb) in lente group and 0.32± 0.07 U/kg (0.14±0.03 U/lb) and 0.47±0.14 U/kg (0.22± 0.06 U/lb) in dogs treated with NPH insulin. The increase of the insulin dose throughout the study was significant in both groups and at the end of the study the insulin dose was significantly lower in the NPH group when compared to the lente group (P = 0.0206).

Blood glucose concentrations < 80 mg/dL were identified in 3/86 (3.5%) and 6/84 (7.1%) of total BGCs performed by dogs treated with lente and NPH insulin, respectively. Such difference was not significant. Symptomatic hypoglycemia was not recorded in both groups and no reactions at the site of insulin administration were reported.

Evaluating all the BGCs, the glucose nadir was observed more commonly 4-6 h and 2-4 h after insulin injection in the lente group and in the NPH group, respectively (Figure 1).

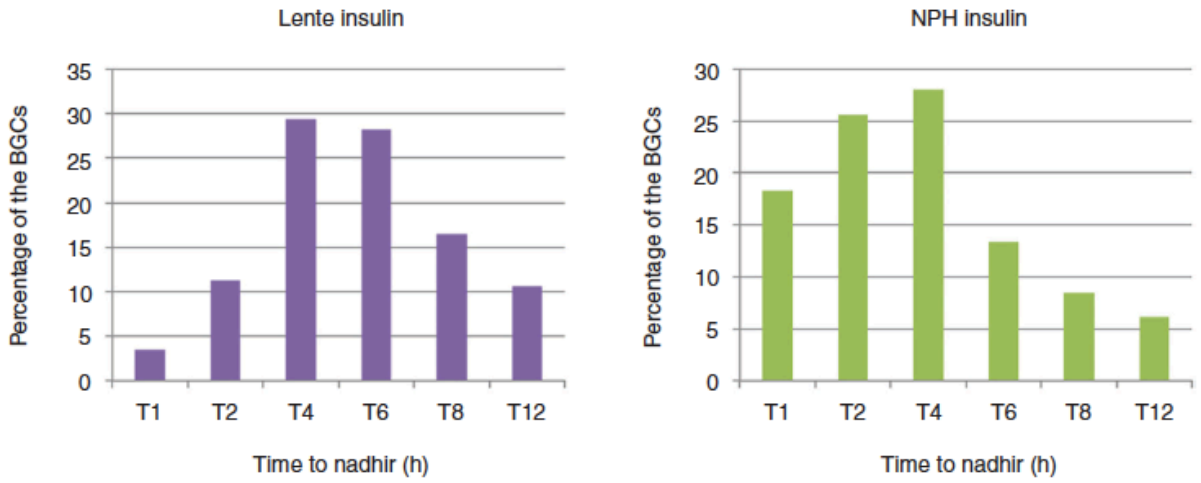

Figure 1. Histograms indicate the number of blood glucose curves (%) from dogs with diabetes mellitus treated by administration of lente insulin (n = 15) or Neutral Protamine Hagedorn (NPH) insulin (n = 15) twice daily for 3 months where the glucose nadir was observed at 1 or 2, 4, 6, 8, 10, and 12 h after insulin injection, respectively

Throughout the study, body weight did not change significantly either in the lente group (P = 0.85) nor in the NPH group (P = 0.95). Median blood glucose concentrations of the BGCs at the end of the

study, compared with the first re-evaluation (1 week), were significantly decreased in both groups: from 415 mg/dL (range, 173–533) to 250 mg/dL (range, 90–407) in the lente group (P=0.009); and from 357 mg/dL (140–506) to 211 mg/dL (83–417) in the NPH group (P = 0.04). Serum fructosamine concentrations at the end of the study were significantly decreased compared with the evaluation before treatment only in the group treated with NPH insulin: from 607 µmol/L (288–880) to 418 µmol/L (292–848) in the NPH group (P = 0.005); and from 455 µmol/L (224–849) to 457 µmol/L (329–749) in the lente group (0.854).

Table 2 reports the assessment of the glycemic control in the 2 groups at the end of the study and considers body weight, polyuria-polydipsia, median blood glucose concentration of the BGCs, blood glucose nadir, overall evaluation of the BGCs, and the serum fructosamine concentrations.

Table 2. Assessment of the glycemic control using different parameters in 30 diabetic dogs treated for 3 months (results at the end of the study) with lente insulin (n = 15) or NPH insulin (n = 15). BCG = blood glucose curve

Method of assessment	Score	Lente Group	NPH Group	*P* value
Body weight	Good	13/15 (87%)	15/15 (100%)	0.80
	Poor	2/15 (13%)	0/15 (0%)	0.49
Polyuria-polydipsia	Good	10/15 (67%)	13/15 (87%)	0.78
	Moderate	1/15 (7%)	2/15 (13%)	1.00
	Poor	4/15 (27%)	0/15 (0%)	0.11
Median blood glucose concentration (BGC)	Good	7/15 (47%)	9/15 (60%)	0.76
	Moderate	3/15 (20%)	3/15 (20%)	1.00
	Poor	5/15 (33%)	3/15 (20%)	0.70
Glucose nadir (BGC)	Good	8/15 (53%)	10/15 (67%)	0.77
	Moderate	3/15 (20%)	3/15 (20%)	1.00
	Poor	4/15 (27%)	2/15 (13%)	0.66
Overall evaluation of the blood glucose curve	Good	9/15 (60%)	11/15 (73%)	0.78
	Poor	6/15 (40%)	4/15 (27%)	0.72
Serum fructosamine concentration	Good	6/14 (43%)	10/15 (67%)	0.54
	Moderate	4/14 (29%)	3/15 (20%)	1.00
	Poor	4/14 (29%)	2/15 (13%)	0.66

At the end of the study, the glycemic control as evaluated using the total clinical score was classified as good in 9/15 (60%), moderate in 3/15 (20%), and poor in 3/15 (20%) dogs treated with lente insulin. In the group treated with NPH insulin, the glycemic control was classified as good in 11/15 (73%), moderate in 4/15 (27%), and poor in 0/15 (0%) of dogs. Such differences between the two

groups were not statistically significant. In the 4 dogs treated with NPH insulin that at the end of the study had a moderate glycemic control this was apparently not due to short insulin duration but rather to insufficient glycemic suppression (nadir >250 mg/dL).

The 3 dogs included after the resolution of DKA in the lente group at the end of the study were classified with good (n=2) or moderate (n=1) glycemic control, respectively. The 3 dogs included after the resolution of DKA in the NPH group at the end of the study were all classified with moderate glycemic control. None of the dogs included in the study showed clinical signs (e.g. vomiting, painful abdomen at the physical examination, diarrhea) consistent with pancreatitis. In the group treated with lente insulin serum lipase activity resulted above the reference range in 4/15 dogs at T0 and in 4/15 dogs at T12. In the group treated with NPH insulin 7/15 dogs at T0 and 4/15 dogs at T12 had serum lipase activity above the reference range. In the group treated with lente insulin the 3 dogs classified at the end of the study with moderate glycemic control had lipase activity above the reference range in 2/7, 1/7 and 0/7 re-evaluations, respectively and the 3 dogs classified with poor glycemic control had lipase activity above reference range in 5/7, 1/7 and 5/7 re-evaluations, respectively. In the group treated with NPH insulin the 4 dogs classified with moderate glycemic control had lipase above reference range in 3/7, 0/7, 1/7 and 1/6 re-evaluations, respectively.

Discussion

The results of this study indicate that both lente and NPH insulin are safe and efficacious as treatment for dogs with newly diagnosed DM.

Starting insulin dosage in the lente and NPH groups, according to the treatment protocol, were commonly reported in the veterinary literature.[10] At the end of the study, insulin dosage observed in both groups was similar to what was obtained in previous studies that evaluated lente and NPH insulin in dogs.[3,1] Mean insulin dose after three months of treatment was significantly different between the lente and NPH groups, this is likely related to the greater potency of NPH insulin. Median blood glucose concentrations were significantly reduced after three months of insulin treatment in both groups; whereas the median fructosamine concentration was significantly reduced only in dogs

treated with NPH insulin. This finding must be interpreted with caution because at time of enrollment, despite not significant (P = 0.08), the median fructosamine concentration was higher in the NPH group (607 µmol/L) than in the lente group (455 µmol/L), which was already a value closer to the normal reference range. Blood glucose nadir was identified mostly at 2 and 4 h from the insulin administration in dogs treated with NPH insulin and at 4 and 6 h in dogs treated with lente insulin. These results are similar to those obtained in other studies where time to nadir in dogs treated with NPH insulin resulted at 2, 5, and 4.9 h [11,12,4] and from 4–8 h in dogs treated with lente insulin.[13]

In the NPH group, three of the four dogs classified as having a moderate glycemic control at the end of the study, were enrolled after resolution of DKA. There is no evidence to support that dogs after the resolution of DKA are more difficult to control as diabetic patients. However, it is possible that, despite the complete diagnostic work-up before enrollment, such dogs had an insulin resistance for not clarified reasons (e.g. undiagnosed disease such as subclinical pancreatitis). In both groups no clinical signs consistent with acute pancreatitis were observed; however, the presence of mild acute pancreatitis or chronic pancreatitis cannot be excluded. The diagnosis of chronic pancreatitis can be very challenging because of the nonspecific and often low-grade nature of the clinical signs and the relatively low sensitivity of non-invasive diagnostic tests.[14] At the time of diagnosis no differences in terms of serum lipase activity between the two groups was observed. Two of 3 dogs treated with lente insulin and classified with poor glycemic control showed serum lipase activity above the reference range in most of the re-evaluations. In these dogs a chronic pancreatitis as a cause of insulin resistance cannot be excluded. A limitation of the present study was that serum lipase activity was determined using the 1,2diglyceride enzymatic/colorimetric assay and not the canine pancreatic lipase immunoreactivity that seems to have higher sensitivity in detecting chronic pancreatitis.[15] However, the present study is focused on the comparison of the efficacy and safety of two different insulin products, rather than evaluating the possible causes of insulin resistance.

In terms of hypoglycemic events, this study obtained better results in comparison with other studies evaluating lente and NPH insulin. In a study performed on 53 dogs treated with lente insulin, clinical

hypoglycemic events were reported in 38.6% of patients with total of 24 events (15%) with glucose concentration < 60 mg/dL on 159 BGCs. One possible reason for the high incidence of hypoglycemia was a starting insulin dosage that was high, > 1 U/kg (0.45 U/lb) every 24 h; in such a study, 41% of dogs enrolled had necessity for insulin dose reduction.[3] Another study performed on dogs treated with NPH insulin showed clinical hypoglycemia in four dogs on 57 (7%).[1] The low incidence of hypoglycemic episodes observed in the present study is probably related to low starting insulin doses (0.25–0.5 U/kg [0.11-0.23 U/lb] twice daily), frequent re-evaluations, and consequent frequent dosage adjustments.

All dogs have been fed with the same diet for the entire length of the study, which minimized food dependent glycemic variability. In contrast to similar studies[1-8], only newly diagnosed diabetic dogs were enrolled. This is in accordance with the study's aim to compare two insulin treatment options to investigate if one of the two was more effective and/or safe as a first-line treatment. In this study, the residual endogenous insulin secretion has not been tested. Likely, some of the included dogs had still an insulin production, i.e. the so-called "honeymoon period", and this could have partially influenced the results of this study.

The main limitation of the present study, similarly to other clinical veterinary studies on DM, was the small number of dogs included. This has been a limit in terms of reaching significance when comparing results between the two groups. For example, at the end of the study, glycemic control was classified as good in 11/15 (73%) dogs treated with NPH insulin and in 9/15 (60%) dogs treated with lente insulin; however, such differences were not significant. According to the calculation of statistical power and sample size and assuming the same percentages of glycemic control, 136 instead of 15 dogs in each group would have been necessary to achieve statistical significance.

Some authors consider NPH insulin a second choice in comparison with lente insulin[16]; this is due to a study performed on 10 diabetic dogs in which duration of insulin action was too short.[4] That study evaluated the serum insulin and glucose concentrations for a period of 10 h from insulin administration. In four dogs, the insulin duration of action at the end of the study was 5.5 h; in another

four dogs the duration was longer than 10 h; and in the remaining two dogs, it was not possible to evaluate insulin duration of action because there was not enough blood glucose concentration reduction to assess the duration of action. The authors of that study concluded that more investigations are needed to assess the real duration of action for NPH insulin.[4] This study did not to evaluate NPH insulin's duration of action; however, we observed for dogs in the NPH group that moderate glycemic control was not related to the short duration of insulin action.

Both lente and NPH insulin have demonstrated safety and efficacy in the treatment of dogs with uncomplicated DM. In general, dogs with NPH insulin obtained a higher percentage of better glycemic control; although these differences were mostly not significant. The low incidences of hypoglycemic events were likely obtained because of low insulin starting doses that were gradually increased and frequent re-evaluations. According to this study, NPH and lente insulin must be considered first choice insulins for the treatment of uncomplicated DM in dogs and the success rate with NPH insulin seems somewhat greater than with lente insulin.

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Capitolo 4

EVALUATION OF ONE PORTABLE BLOOD GLUCOSE METER AND ONE PORTABLE GLUCOSE-KETONES METER IN DOGS

F. Del Baldo, E. Malerba, S. Corradini, I. Rovatti, A. Zeppi, F. Dondi, F. Fracassi

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Abstract premiato come miglior poster

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Dipartimento di Scienze Mediche Veterinarie,

Scuola di Agraria e Medicina Veterinaria,

Bologna

4. Evaluation of one portable blood glucose meter and one portable glucose-ketones meter in dogs

Background

Nowadays only few portable blood glucose meters (PBGMs) have been developed specifically for use in dogs and cats. Recently one glucometer (Gluco Calea, WellionVet; GC) and one glucose-ketones meter (Belua, WellionVet; BE) have been developed for use in veterinary medicine (Table 1).

	GLUCOCALEA	BELUA	
		Glucometer	Ketometer
Test principle	Glucose oxidase	Glucose dehydrogenase	3- Hydroxybutyrate dehydrogenase
Detecting range	20 – 600 mg/dL	20 – 600 mg/dL	0.1 – 8 mmol/L
Test time	5 seconds	6 seconds	8 seconds
Sample volume	0.5 µl	0.8 µl	0.8 µl
Blood sample	Capillary blood	Capillary or venous blood	Capillary or venous blood
HCT range	35% – 55%	10% - 70%	30% - 60%

Table 1: Specification of WellionVet® GLUCO CALEA and Belua

Objectives

The aims of this study were to assess the accuracy and precision of these devices in canine venous and capillary blood samples based on ISO 15197:2013 and to evaluate packed cell volume (PCV) interferences.

Materials and methods

Samples were obtained from 45 non anemic dogs (PCV 37-54%) and 10 anemic dogs (PCV<37%) divided into three glycemic ranges: high (>140 mg/dL), medium (90-139 mg/dL), and low (<90 mg/dL). Paired measurements of glucose and 3-ß-hydroxybutyrate (3-HB) from capillary and venous blood samples were determined using the two devices and compared with the results of reference

methods (enzymatic hexokinase and 3-HB-dehydrogenase, respectively) obtained by an automated chemistry analyzer (Beckman-Coulter AU480). Linear regression, Bland-Altman plots and the Parkes error grid analysis (EG) were used to assess the accuracy.1

PCV interferences for glucose measurement were assessed comparing the differences between PBGMs readings and reference method values in anemic and non-anemic dogs. To assess within-run precision, glucose concentrations obtained from 12 samples, belonging to the three glycemic ranges, were measured 10 times within 10 minutes. Between-day precision was assessed by testing each manufacturer's glucose control solution over 10 consecutive days. $P < 0.05$ was considered significant.

Results

Mean differences (mg/dL) between measurements of each PBGM on venous and capillary blood and values measured by the reference method in patients with normal PCV were: GC 44.1±27.2, 37.8±24.2, BE 10.2±25.1 and 20.4±28.6, respectively. A positive significant correlation between all paired samples was found for both devices ($r > 0.89$) (Table 2).

HAND-HELD METER	REFERENCE METHOD	MEAN BIAS (mg/dL)		CORRELATION (r)	
		Venous blood	Capillary blood	Venous blood	Capillary blood
Wellion vet GLUCO CALEA	Hexokinase	44.1 ± 27.2	37.8 ± 24.2	0.94	0.93
Wellion vet BELUA GLUCOMETER	Hexokinase	10.2 ± 25.1	20.4 ± 28.6	0.93	0.89
Wellion vet BELUA KETOMETER	3-HB-dehydrogenase	-0.07 ± 0.79	0.05 ± 0.57	0.59	0.48

Table 2: Mean differences (mg/dL) and correlations between measurements of Gluco Calea and Belua on venous and capillary blood and values measured by the reference method.

However, neither PBGMs fulfilled ISO requirements: 82.21% and 84.08% of glucose values measured respectively on capillary and venous blood using GC fell in zone A+B of EG; 86.7% and 97.8% of glucose values measured respectively on capillary and venous by BE fell in zone A+B of EG (Figure 1, Figure 2).

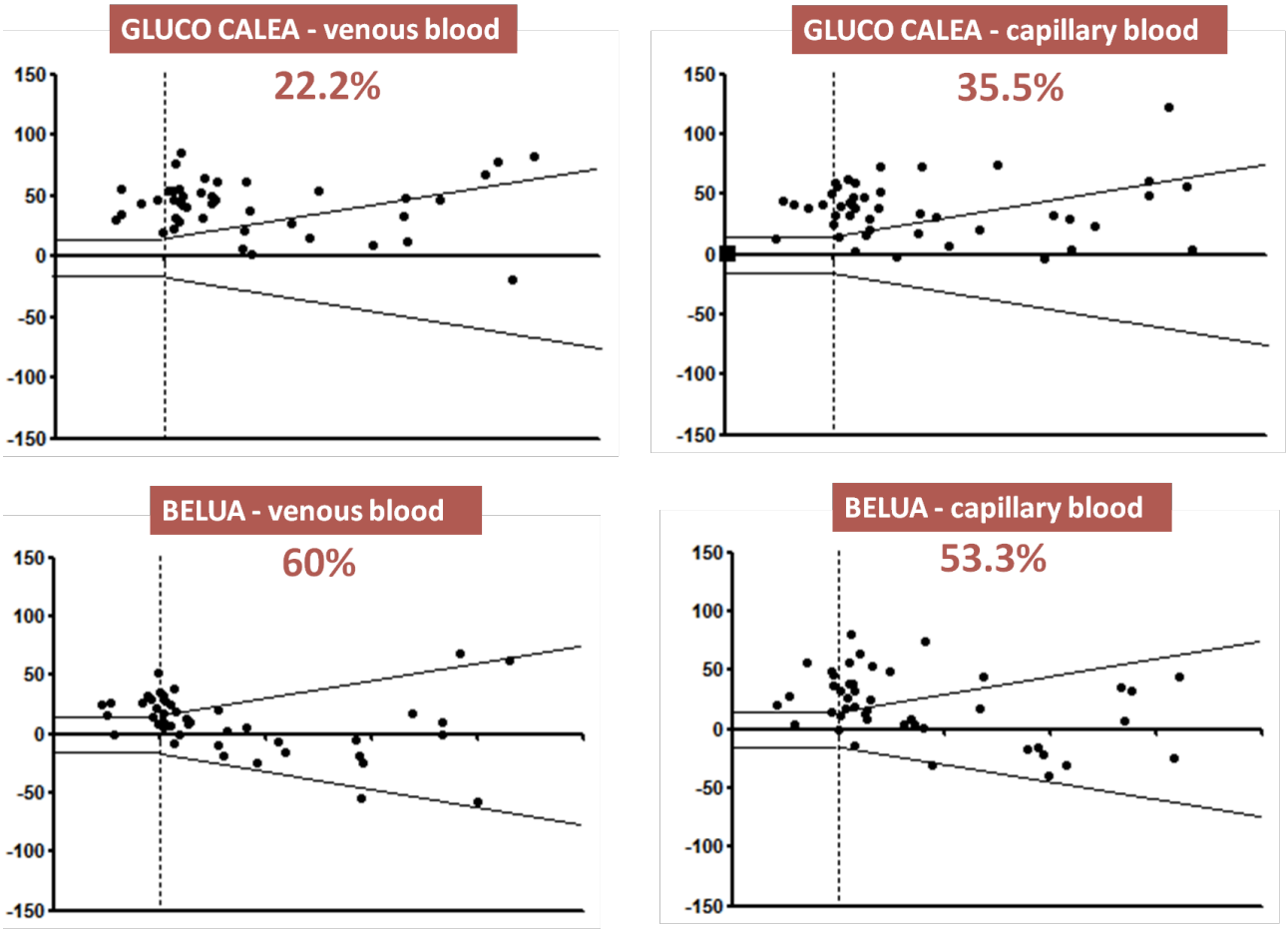

Figure 1: Bland-Altman plots represent the difference between blood glucose measurements obtained by the use of the two glucometer, Gluco Calea and Belua, versus blood glucose concentrations obtained by the reference method (enzymatic hexokinase; Beckman-Coulter AU480). On the x axis are the reference glucose values, plotted against the absolute errors for each corresponding value. The requirements established by ISO 15197:2013 criteria are represented by the 2 solid symmetrical lines: at ±15 mg/dL from the reference value for glucose <100 mg/dL and at ±15% from the reference for glucose ≥100 mg/dL. At the top, are reported the percentages of samples within limits for the total number of measurements.

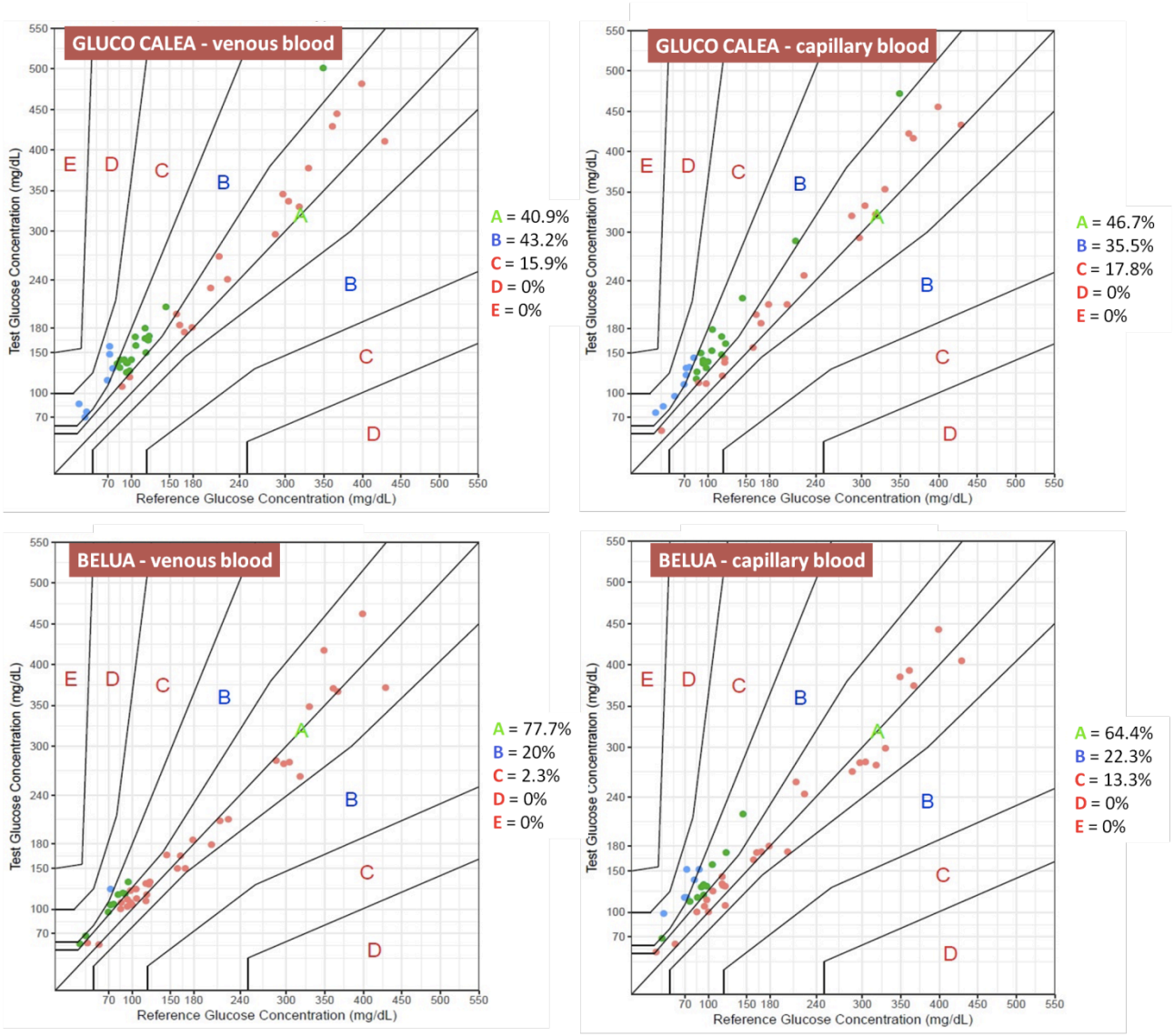

Figure 2: Parkes Consensus Error Grid Analysis representations for venous and capillary blood for each device with the percentages of values within different zones. The reference glucose values (blood glucose obtained by the reference method), on the x axis, are plotted against the blood glucose measurements obtained the two glucometers, Gluco Calea and Belua, on the y axis. The different zones designate the magnitude of risk: no effect on clinical action (zone A), altered clinical action - little or no effect on clinical outcome (zone B), altered clinical action – likely to affect clinical outcome (zone C), altered clinical action – could have significant medical risk (zone D) and altered clinical action – could have dangerous consequences (zone E). ISO 15197:2013 requires that 99% of the values fall within zones A+B for a device to be considered accurate.

Results of within-run and between-day precision are shown in Table 3.

HAND-HELD METER	WRP (mean±SD)	BDP (mean±SD)
Wellion vet GLUCO CALEA	5.66 ± 5.41	13.59 ± 21.17
Wellion vet BELUA GLUCOMETER	2.77 ± 1.34	9.40 ± 12.83
Wellion vet BELUA KETOMETER	4.85 ± 5.04	/

Table 3: Within-run precision (WRP) and between-day precision (BDP) of Gluco Calea glucometer and Belua gluco-ketometer.

The effect of PCV was significant and higher results with lower PCV were observed (Table 4).

HAND-HELD METER	MEAN BIAS (mg/dL) VENOUS BLOOD		P	MEAN BIAS (mg/dL) CAPILLARY BLOOD		P
	Non anemic dogs	Anemic dogs		Non anemic dogs	Anemic dogs	
Wellion vet GLUCO CALEA	44.0	60.0	0.0141	39.0	69.5	0.0029
Wellion vet BELUA GLUCOMETER	10.0	55.5	0.0003	19.0	74.5	0.0008

Table 4: Median of the mean bias on venous and capillary blood for Gluco Calea and Belua glucometers in non anemic and anemic dogs.

The correlations between capillary and venous 3-HB and reference 3-HB were r=0.48 and r=0.59, respectively. Mean differences between capillary and venous 3-HB and reference method were 0.05 (±0.57) and -0.07 (±0.79) respectively (Table 2); within-run precision is shown in Table 3.

Discussion

Our results show that both GC and BE glucometers are not sufficiently accurate and safe for clinical use in dogs. BE ketometer has proven to be less accurate compared with results of other studies in which correlations of 0.96 and 0.97 were found.2,3 However, to date, there are not specific guidelines for quality assurance for ketometers.

4. Evaluation of one portable blood glucose meter and one portable glucose-ketones meter in dogs

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Capitolo 5

CLINICAL PERFORMANCES OF FLASH GLUCOSE MONITORING SYSTEM IN DIABETIC DOGS

F. Del Baldo, C. Canton, S. Testa, H. Swales, S. Golinelli, F. Fracassi

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Dipartimento di Scienze Mediche Veterinarie,

Scuola di Agraria e Medicina Veterinaria,

Bologna

Abstract

Background: Flash glucose monitoring system (FGMS, FreeStyle Libre®) was recently validated for use in diabetic dogs (DD).

Objective: The aim of this study was to evaluate the clinical usefulness of FGMS in monitoring DD.

Methods: Twenty DD on insulin treatment were prospectively enrolled in the study. The FGMS was placed on the neck for up to 14 days. During the 1^{st}-7^{th}-14^{th} days, blood glucose curves (BGCs) have been performed simultaneously in the hospital with FGMS and a validated portable blood glucose meter (PBGM) (Optium Xceed, Abbott®). During the 5^{th}-6^{th} and 12^{th}-13^{th} days the owners performed a BGC using the FGMS at home. The BGCs performed with PBGM and FGMS in hospital and those performed with FGMS at home and in hospital were compared. Each BGCs has been evaluated as optimal considering: 1) 50% of the values between 90-250 mg/dL or 2) glucose nadir between 90-180 mg/dL. The glucose nadirs obtained from the data downloaded by the software (DDS), the FGMS scans and the PBGM were compared. Moreover, the glucose day-time (GDTNs) and night-time nadirs (GNTNs) were compared.

Results: The evaluation of the BGCs performed in hospital with FGMS and PBGM, led to the same decision on insulin adjustment in 77% and 80% of cases considering the percentage of values in the range 90-250 mg/dL and the glucose nadir, respectively.

The evaluation of the BGCs performed at home and the following day in the hospital with the FGMS, led to the same decision of insulin adjustment in 68% and 64% of cases considering the percentage of values in the range 90-250 mg/dL and the glucose nadir, respectively. The glucose nadirs were identified in 81% of cases by the DDS and in 65% and 35% of cases using FGMS scans and PBGM, respectively. The medians of GNTNs were significantly higher than the GDTNs.The hypoglycemic episodes obtained from the DDS were 39% more than those immediately showed on the display of the FGMS.

Conclusions and clinical importance: Adjustments in insulin dose based on BGCs obtained with FGMS and with PBGM are similar. The FGMS detects the nadirs and the hypoglycemic episodes

more frequently than PBGM and it allows the assessment of glucose variations also during different consecutive days.

Introduction

Diabetes mellitus (DM) is a common endocrine disease of dogs characterized by an absolute or relative deficiency of insulin.[1] Dogs with DM are treated with exogenous insulin and require regular monitoring to ensure appropriate dosing. Tools available to veterinarians for monitoring diabetic patients' response to treatment include clinical signs, body weight, glycated proteins levels, and blood glucose curves (BGCs) among others.[2] Typically, BGCs are conducted in a hospital setting or at home by the owners and involve one-to-two hourly blood sampling with a portable blood glucose meter (PBGM) over an 8-12 hour period. Evaluation of the BGCs allows the clinicians to determine the efficacy of the insulin preparation, time to peak effect, duration of effect as well as assessing the degree of variation in blood glucose concentration.[1] The need for repeated venipuncture to obtain a capillary drop of blood can be stressful and painful for the patient and also carries the risk of missing the blood glucose peak or nadir if they fall between two sampling times[3], both of which represent significant disadvantages of the method. Additionally, in hospital BGCs are time consuming and expensive and do not allow the assessment of glycaemia on consecutive days. This last aspect represents a significant limitation as large day-to-day variability of serial blood glucose curve has been demonstrated previously in dogs.[4]

Continuous glucose monitoring systems (CGMS) are routinely used in human diabetic patients and several studies have demonstrated their accuracy and/or clinical utility in veterinary medicine.[3,5-11] CGMS typically consist of a needle-based sensor which measures interstitial glucose (IG); the sensor is paired with a transmitter that relays the recorded measurements. The devices used in previous veterinary studies have a number of limitations not limited to the need for frequent calibration to circulating blood glucose, but also to the limited monitoring period.

Recently, a novel flash glucose monitoring system (FGMS) has been licensed for the use in the European Union (CE mark August 2014). The device measures interstitial glucose (IG) levels on a minute-by-minute basis via a disc shaped sensor with a small catheter inserted under the skin that can be worn for up to 14 days. In contrast to other CGMS, this FGMS does not require calibration and a

recent study demonstrated the accuracy of the device when evaluating IG in dogs with diabetes mellitus as well as showing it to be well tolerated.[11] However, studies evaluating the clinical use of FGMS in the long-term monitoring of dogs with DM are lacking and whether treatment decisions based on glucose profiles obtained with the FGMS differ from those derived using a PBGM in dogs has not been investigated. Therefore, the aims of the present study were 1) to compare the recommended insulin dose based on the evaluation of BGCs obtained by the FGMS with those obtained by a validated PBGM; 2) to compare the recommended insulin dose based on evaluation of BGCs obtained by the FGMS on two consecutive days both in the same environment and in different environments (hospital and home) 3) to assess the ability of the FGMS to detect the glucose nadir as well as hypoglycemic episodes and 4) to compare day-time and night-time glucose nadirs obtained by the use of FGMS.

Materials and methods

Dogs with diabetes mellitus:

Client-owned dogs with diabetes mellitus and admitted to the Veterinary Teaching Hospital of the University of Bologna between May 2015 and March 2018 were prospectively enrolled into the study. DM was diagnosed based on consistent clinical signs including polyuria, polydipsia (PU/PD), weakness, weight loss, blood glucose concentration >180 mg/dL (>11 mmol/L) after food had been withheld for at least 10 hours, glucosuria and serum fructosamine concentration >340 µmol/L.

All dogs had been treated with insulin for at least four weeks prior to enrolment in the study. Owners provided written informed consent for inclusion of their dogs in the study. The study was approved by the Scientific Ethics Committee of the University of Bologna.

Flash Glucose Monitoring System

The Free Style Libre® Flash Glucose Monitoring System (FGMS) was used in this study and is composed of a small, lightweight disc-shaped sensor (35 mm x 5 mm). The sensor measures the IG concentration through a small, subcutaneous catheter (0.4 mm x 5 mm). Glucose detection is based on Wired Enzyme Technology, that consists of both enzymatic (glucose oxidase) and amperometric

(electrodes) systems. Reduction of glucose by glucose oxidase results in generation of an electric current, the intensity of which is proportional to the IG concentration.

The detection limits of the sensor is between 20 and 500 mg/dL and measurements outside of this range are not recorded. The system is factory-calibrated and consequently does not require calibration before or during the wearing period. The sensor begins recording data one hour after its application and automatically reads and records the IG concentration data every minute. Glucose data are transferred from the sensor to a reader when the user brought it into close proximity to the sensor (scanning of the sensor). The hand-held reader then display current sensor glucose level, a glucose trend arrow, and glucose readings over the preceding 8 hours (Figure 1).

Figure 1: Hand-held reader showing current IG value, a glucose trend arrow and the graph representative of the glucose level during the preceding 8 hours.

Scanning can be done as often as is needed for current glucose concentration, otherwise the measurements are automatically captured and stored on the sensor (every 15 minutes) and displayed on the reader when scanned. The reader stores data for 90 days. Data can be uploaded from the reader, using the device software (Ambulatory Glucose Profile, AGP) to generate summary glucose reports. Among these, the daily log report shows glycaemic fluctuations included between 0-350 mg/dL during a 24-hour period (Figure 2) and, as such, it has been used in this study in order to extrapolate the true nadirs and the number of the hypoglycaemic episodes. At the end of wearing period, the sensor is fully disposable, but the reader can be re-used for a new sensor. The sensor was applied as previously described[11] on the dorsal aspect of the neck.

Blood glucose measurements were acquired with a validated PBGM.[12]

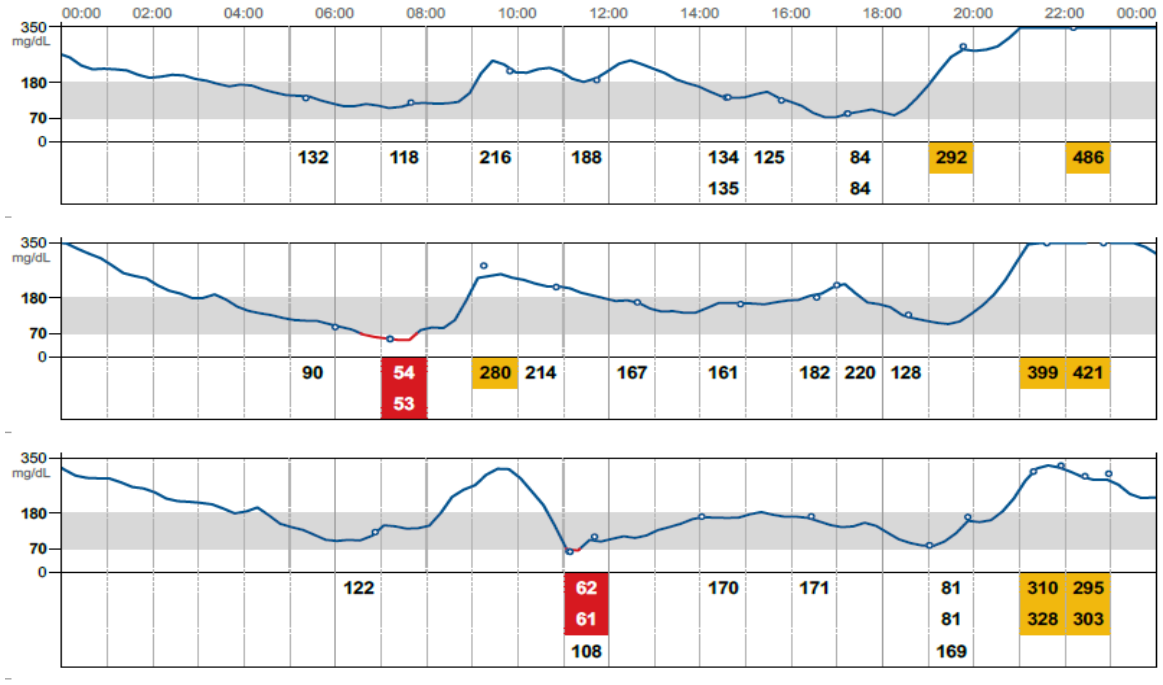

Figure 2: Daily log report showing glycaemic fluctuations during a 24-hour period in 3 different consecutive days. The red and yellow squares show the IG values <70 mg/dL and >240 mg/dL, respectively. The numbers correspond to the IG values detected during the scanning of the sensor by the hand-held reader. Nadirs and hypoglycaemic episodes extrapolated from these graphs have been used as "gold standard" in order to compare the ability of the FGMS and PBGM in detecting the true nadirs and hypoglycaemic episodes. Above 350 mg/dL, the glycaemic fluctuations are not shown.

Study design

Seven separate BGCs were acquired for each dog during the recording period of their respective FGMS (Table 1). On days 1, 7, and 14, paired, in-hospital BGCs were acquired using the FGMS and PBGM devices. On days 5, 6, 12, and 13, home BGCs were acquired using only the FGMS device by the dogs' respective owners. On day one of the study, dogs were hospitalized and the sensor was applied. For a total of 10-12 hours, IG glucose measurements were recorded using the FGMS on a two-hourly basis. Capillary blood glucose was obtained from the pinna every 2 hours using the PBGM during the same period. On days 7 and 14, food and insulin were given at home and the paired BGCs were started after the dog arrived at the clinic (≤1 hour after insulin administration) using the same

protocol. On the remaining days, owners acquired BGCs every 1 to 2 hours using the FGMS. The displayed values were recorded in a diary.

At the end of the recording period, the sensor was removed and the FGMS data were downloaded onto a personal computer using the AGP software.[b]

Day	Environment	BGCs
1	Hospital	Paired BGCs with both FGMS and PBGM
5	Home	Single BGC with FGMS
6	Home	Single BGC with FGMS
7	Hospital	Paired BGCs with both FGMS and PBGM
12	Home	Single BGC with FGMS
13	Home	Single BGC with FGMS
14	Hospital	Paired BGCs with both FGMS and PBGM

Table 1: Study design

Assessment of BGCs

Based on assessment of the BGCs, a hypothetical insulin dose recommendation was made with the aim to maintain 50% of BG/IG values between 90-250 mg/dL (Table 2) and a BG/IG nadir between 90-180mg/dL.[13]

Comparison of insulin dose recommendations based on the in-hospital BGCs acquired using FGMS and PBGM was made on days 1, 7 and 14 (study aim 1). Comparison of insulin dose recommendations based on consecutive-day BGCs acquired at home using the FGMS was made on days 5 and 6 as well as days 12 and 13 (study aim 2). Comparison of insulin dose recommendations based on consecutive-day, home vs. hospital BGCs acquired using the FGMS was made on days 6 and 7 as well as days 13 and 14 (study aim 2).

Insulin dosage	% glycaemic values (GV)	Glucose Nadir (GN)
Unchanged (↔)	At least 50% GV between 90-250 mg/dL	GN between 90-180 mg/dL
Increased (↑)	At least 50% GV > 250 mg/dL	GN > 180 mg/dL
Decreased (↓)	At least 50% GV < 90 mg/dL	GN < 90 mg/dL

Table 2: Variables used to define the adjustment of insulin therapy.

Assessment of nadirs

Nadirs extrapolated from the 'daily log report' of the AGP, those scanned by the FGMS reader and those detected by the PBGM on days 1, 7 and 14 were compared. The nadirs extrapolated from the AGP software were considered the 'true' nadirs and they were considered concordant with those obtained by the FGMS scans and by the PBGM if they had the same time interval, in particular if they fell within the same hour from the morning injection of insulin.

Comparison between day-time and night-time nadirs

During the recording period, each day was divided into two time intervals: day-time and night-time. Day-time was defined as the time interval between the morning insulin administration and the evening insulin administration (8:00 am-20:00 pm). Night-time was defined as the time interval between the evening insulin administration and the next morning insulin administration (20:00 pm-8:00 am). The day-time and night-time nadirs extrapolated from the daily log repot of the AGP were compared and were considered concordant if they fell within the same glycaemic range: <90 mg/dL, 90-180 mg/dL, >180 mg/dL.

Assessment of hypoglycemic episodes

The number and duration of hypoglycemic episodes (<70mg/dL) were recorded from the AGP software and were compared with the FGMS scans as well as with the PBGM readings.

Data analysis

Statistical analysis was performed with the aid of a commercially available software (GraphPad Prism 7®). Normality was assessed using D'Agostino and Pearson tests and parametric or non-parametric tests were used accordingly. Non-normal data were reported as median and ranges while normal data were expressed as mean ± standard deviation (SD).

Fisher's exact test was used to compare the percentage of BGCs in which the same insulin dose recommendation was made. Day-time and night-time nadir were compared using Wilcoxon test. Differences were considered significant at $P \leq 0.05$.

Results

Diabetic dogs

Twenty dogs met the inclusion criteria. There were 10 mixed-breeds, 5 English Setters, 1 Springer Spaniel, 1 Yugoslavian Shepherd dog, 1 Pinscher, 1 Maltese and 1 Poodle. Of these, 13 dogs were neutered females, 5 neutered males and 2 entire males.

The median age was 11years and 1 month, the median body weight and BCS were 16.5 kg (range 6-64.1 kg) and 5 (3-8), respectively. Median time from the diagnosis of DM was 7.5 months. Thirteen dogs were treated with porcine insulin zinc suspension[c], 5 with Neutral Protamine Hagedorn (NPH) human analogue insulin[d] and 2 with glargine[e]. All dogs received twice daily insulin administration and all received the same dose morning and evening. Median insulin dose was 0.56 U/kg (0.32-1.62). Six out of 20 dogs had concurrent disease; 3 dogs had pituitary dependent hypercortisolism and were receiving trilostane[f]; 3 dogs had primary hypothyroidism and were receiving levothyroxine[g]. One dog was on enalapril and amlodipine[i] treatment for hypertension and proteinuria.

All sensors reported IG concentrations by sixty minutes post-application. In 8/20 dogs the sensor recorded for 14 days while in 12/20 dogs the sensor stopped recording IG before fourteen days because due to accidental detachment (4/20) or because the hand-held reader showed persistently "LOW" or "ERR" (8/20). In these dogs, the recording period of the sensor was 13 days in 2/20, 11 days in 2/20, 10 days in 4/20, 6 days in 1/20, 4 days in 1/20 and 2 days in 1/20. The median wearing period was 12 days (2-14).

At the end of the wearing period, 3/20 dogs showed mild erythema at the site of application of the sensor. In all dogs the erythema was self-limiting and did not require specific treatment.

One hundred twenty-eight BGCs were obtained: 42/128 BGCs were recorded at home using FGMS. 86/128 BGCs were performed in the hospital of which 43/128 with FGMS and 43/128 with PBGM.

Assessment of BGCs

FGMS vs PBGM

When comparing BGCs acquired using the FGMS and PBGM in the hospital, the insulin dosing recommendation would have been the same in 33/43 cases (77%) cases considering the percentage of values in the ideal range and in 34/43 cases (80%) considering the glycemic nadir. These percentages did not differ significantly (P>0.99) (Table 3 and 4).

Day-day same environment (home)

When comparing BGCs performed on two consecutive days with the same device (FGMS) in the same environment (home) insulin dosing recommendation would have only been the same in 5/14 cases (36%) considering the percentage of values in the ideal range and in in 9/14 cases (64%) considering the glycemic nadir. These percentages did not differ significantly (P>0.25) (Table 3 and 4).

Different environment (home-hospital)

When comparing BGCs performed on two consecutive days using the same device (FGMS) in two different environments (home and hospital) the insulin dosing recommendation would have been the same in 17/25 cases (68%) considering the percentage of values in the ideal range and in 16/25 cases (64%) considering the glycemic nadir. These percentages did not differ significantly (P>0.99) (Table 3 and 4).

A			B			C		
Hospital	**Hospital**		**Home**	**Home**		**Home**	**Hospital**	
FGMS	**PBGM**	**%BGCs**	**FGMS**	**FGMS**	**%BGCs**	**FGMS**	**FGMS**	**%BGCs**
↑	↔	(11%)	↑	↔	(29%)	↑	↔	(8%)
↓	↔	(2 %)	↓	↔	(14%)	↓	↔	(8%)
↔	↑	(5%)	↔	↑	(14%)	↔	↑	(8%)
↔	↓	(5%)	↔	↓	(7%)	↔	↓	(4%)
↑	↓	/	↑	↓	/	↑	↓	(4%)
↔	↔	(30%)	↔	↔	/	↔	↔	(28%)
↑	↑	(42%)	↑	↑	(36%)	↑	↑	(36%)
↓	↓	(5%)	↓	↓	/	↓	↓	(4%)
Same therapeutic recommendation		***77%***			***68%***			***36%***
Different therapeutic recommendation		***23%***			***32%***			***36%***

Table 3: Comparison of BGCs based on the percentage of glycaemic values in the range of 90-250 mg/dL. A) BGCs performed in the same environment (hospital) with two different instruments (PBGM and FGMS); B) BGCs performed at home on two consecutive days with FGMS; C) BGCs performed at home and the following day in the hospital with FGMS. A recommended increase in insulin dosage is represented by ↑, a decreased by ↓, no change by ↔

A			B			C		
Hospital	**Hospital**		**Home**	**Home**		**Home**	**Hospital**	
FGMS	**PBGM**	**%BGCs**	**FGMS**	**FGMS**	**%BGCs**	**FGMS**	**FGMS**	**%BGCs**
↑	↔	(9%)	↑	↔	(8%)	↑	↔	(8%)
↓	↔	(5%)	↓	↔	/	↓	↔	(12%)
↔	↑	(5%)	↔	↑	(14%)	↔	↑	(8%)
↔	↓	(5%)	↔	↓	(14%)	↔	↓	(8%)
↑	↓	/	↑	↓	/	↑	↓	/
↔	↔	(26%)	↔	↔	(21%)	↔	↔	(24%)
↑	↑	(42%)	↑	↑	(29%)	↑	↑	(28%)
↓	↓	(11%)	↓	↓	(14%)	↓	↓	(12%)
Same therapeutic recommendation	***80%***			***64%***			***64%***	
Different therapeutic recommendation	***20%***			***36%***			***36%***	

Table 4. Comparison of BGCs based on glucose nadir between 90-180 mg/dL. A) BGCs performed in the same environment (hospital) with two different instruments (PBGM and FGMS); B) BGCs performed at home on two consecutive days with FGMS; BGCs performed at home and the following day in the hospital with FGMS;
A recommended increase in insulin dosage is represented by ↑, a decreased by ↓ and no change by ↔.

Assessment of nadirs

Using the AGP software, glucose nadirs were identified in 35/43 (81%) of cases, using FGMS scans and PBGM in 28/43 (65%) and 15/43 (35%) of cases, respectively.

In 7/43 (16%) BGCs the nadirs could not be extrapolated from the AGP software because the values were above 350 mg/dL and in 5/43 BGCs (12%) because there was a reading error.

The true nadir occurred between two consecutive PBGM measurements in 9/43 (21%) BGCs and it was recorded after the hospitalization period in 11/43 (26%) BGCs.

Comparison between day-time and night-time nadir

One hundred and fifty-two, paired day-time and night-time nadirs recorded on the AGP software were available for the analysis (Table 3). Day-time and night-time nadirs were within the same

glycaemic range in 82/152 (55%) cases. The night-time nadir was greater than day-time nadir in 46/152 (30%) cases whereas the night-time nadir was lower than day-time nadir in 24/152 (15%) cases (Table 5).

The median day-time nadir was 147 mg/dL (40-470 mg/dL) and the median night-time nadir was 170 mg/dL (40-351 mg/dL). The difference was significant (P=0.02).

Day-time nadir	Night-time nadir	N° nadir (%)
↓	↔	(12%)
↑	↔	(11%)
↔	↓	(3%)
↔	↑	(15%)
↑	↓	(2%)
↓	↑	(4%)
↑	↑	(22%)
↓	↓	(17%)
↔	↔	(14%)
Night-time nadirs > than day-time nadirs		(30%)
Day-time nadirs > than night-time nadirs		(16%)
Day-time and night-time nadirs within the same glycaemic range		(54%)

Table 5. Glucose nadir included in the range of 90-180 mg/dL is represented by ↔, glucose nadir below 90 mg/dL is represented by ↓, glucose nadir above 180 mg/dL is represented by ↑.

Assessments of hypoglycaemic episodes

Using any method, hypoglycaemic episodes were recorded in 13/20 (65%) dogs during the recording period however none of these dogs showed hypoglycemic signs. Analysing the AGP, 66 hypoglycemic episodes were identified whereas using the individual FGMS scans 40 hypoglycemic episodes were detected. During the hospitalization period, the PBGM identified 6 hypoglycemic episodes in 5/20 dogs. All of the hypoglycemic episodes detected by the PBGM were also detected by the FGMS scans.

Discussion

The aim of the present study was to evaluate the clinical usefulness of FGMS in the monitoring of dogs with DM. Although the FGMS has been shown to be accurate in measuring IG in dogs with diabetes mellitus,[11] data evaluating the clinical performance of this device in diabetic dogs are still lacking.

In all dogs, the sensor started to read the IG after 60 minutes following application as reported by the manufacturer. In 4 dogs the sensor detached before the end of the study, likely due to the individual attitude of the dogs or a deficiency to secure the sensor at the body of the dog. Also, in 8 dogs the reader showed persistently "LOW" or "ERR" and this could be caused by the presence of inflammation or the usury/bent of the sensor. All dogs tolerated well the use of a bandage and mild erythema at the site of the sensor occurred in 3/20 (15%) dogs. The incidence of erythema at the site of application was much lower than the 50% reported in a previous study of dogs[11] but similar to the 4%-44% reported human patients with diabetes mellitus.[14-17] It is currently suspected that the dermatological changes represent a reaction to the isobornyl acrylate that is contained in the sensor itself and that can migrate into the adhesive part of the device and come into contact with the skin.[18]

One of the major aims of this study was to compare the BGCs generated using the FGMS with those simultaneously generated using a PBGM. Analysis of the BGCs obtained with the use of FGMS and PBGM in the hospital showed that FGMS led to the same insulin dose recommendation in more than 75% of cases. In the majority of cases the insulin dose deduced from the FGMS profiles was higher than those looking at the PBGM profiles. This most likely reflects the fact that the PGBM used in this study has been previously shown to underestimate the BG concentration.[12] Furthermore, the FGMS measures IG which, although has been shown to accurately estimate plasma glucose, in humans has been previously shown to have a wide time lag of 4-50 minutes.[19] Time lag between plasma and IG appear to differ depending on whether plasma glucose values are rising or falling[20-22] or the type of CGM instrument and sensor algorithm used.[23,24] In a previous veterinary study, FGMS was unable to measure the rapid changes between the peripheral glucose and interstitial fluid glucose

after the injection of a bolus of dextrose IV.[11] It is possible that the lag phase could have influenced the discrepancy between the recommended insulin dose from the two devices.

When analyzing consequent day, home-home, FGMS recorded BGCs, the same insulin dose recommendation was obtained in less than 65% of cases, although these results was not statistically significant. The lack of statistically significant difference could be due to the small sample size. To the authors knowledge, there were no studies so far that have looked at this aspect in a home environment. Indeed, in the study by Fleeman et al, dogs were maintained in the hospital during all the monitoring period, although insulin dose and meal were kept constant. Inter-day glycaemic variability is reported also in 93% of human diabetic patients.[25] It has been associated with daily fluctuations in the postprandial glycemic response to a standard meal[26], variable sensitivity to insulin[27] and variation in the rate of absorption of insulin from the SC injection site, particularly if different anatomic region are used.[28] Additional factors include the level of diabetic instability,[29-31] the amount of residual β-cell function[32,33] and inherent error when measuring insulin doses in a syringe.[34] All of these factors could be expected to influence blood glucose concentrations also in diabetic dogs.[4] Considering this result, it seems that the reproducibility of blood glucose curves produced at home is not better than for those produced in a hospital and highlights the importance of performing serial BGCs before making a certain treatment decision or, even better, consider the use of CGMS that allow the monitoring of glucose concentrations during different consecutive days.

When analysing consequent day, home-hospital, FGMS recorded BGCs, the same insulin dose recommendation was obtained in less than 70% of cases. The objective of this analysis was to evaluate if day-to-day variability between the home and clinic curves existed. In diabetic dogs, serial BGCs performed in hospital show significant day-to-day variability. In the study of Fleeman and Rand (2003), significant differences in blood glucose measurements were noted on 2 consecutive days when insulin dose and meals were kept constant. In that study, comparison of the BGCs obtained in two consecutive days led to the same recommendations regarding insulin dose adjustment in only 57% of cases. This implies important clinical implications, especially in well-controlled dogs.[4] In that

study, however, dogs were maintained in a hospital environment. Differences in the feeding schedules and in the amount of exercise of the diabetic pet, as well as stress due to unfamiliar environment or repeated vein punctures, could have contributed to that findings. Contrary to our expectations, from our results the insulin dose recommended from the FGMS profiles obtained in the hospital setting was higher than those obtained at home only in approximately half of the cases. This result is in line with another study in which, surprisingly, the mean and maximum glucose concentration of the hospital curves were significantly lower than those of the home curves.[35] A possible explanation is that stress hyperglycaemia is a well-recognized problem in cats but it is less frequently identified in dogs with DM (Nelson, 2015). Also, blood glucose concentration in the clinic may be lower than those at home because of reduce appetite.[35] However, in our study dogs were fed at home before the arrival in the hospital, therefore it is unlikely that the food assumption can have influenced this result. Analysing the AGP report of the FGMS, the glycaemic nadir was identified in 81% of cases. In 16% of cases, the true glucose nadirs could not be extrapolated by the AGP software because the IG readings were higher than 350 mg/dL. This is a limitation of the FGMS use because in dogs with poor glycaemic control, the majority of data cannot be extrapolated. Additionally, in 12% of cases it was not possible to analyse the graph because it was not correctly generated by the AGP software. The gaps in the graph generated by the AGP software could be the result of sensor dysfunction. Using PBGM readings, glucose nadirs were detected only in 35% of cases. In the majority of cases, and as expected, the PBGM did not allow the identification of the nadir because it fell between two sampling times or after the period of hospitalization. Similarly, FGMS scans allowed the identification of glucose nadirs in only 65% of cases because nadirs occurred between two scans or after the period in which the BGC was conducted. As the nadir is crucial for determining the appropriate insulin dose, the use of FGMS that measures IG levels every minute likely provides more information regarding glucose patterns and trends, potentially allowing a more correct dose recommendation. However, in those dogs in which the hand-held reader is not able to display the glucose concentrations (because of the reading errors), the use of a PBGM might be useful to confirm the glycaemic value.

Comparison between the day and night-time nadirs showed that in more than half of the cases, the nadirs were in the same glycaemic range. However, in those that were different, the majority of night-time nadirs (30%) were higher compared to day-time nadirs. This was highlighted by the difference between the mean night-time and day-time nadirs (170 mg/dL and 147 mg/dL, respectively). A previous study, in which a different CGMS was used, found no differences between day and night-time mean, maximum and minimum glucose values. However, the dogs in the above mentioned study were maintained under controlled living conditions including room temperature, humidity and light/dark cycle precluding differences in environmental factors.[10] On the contrary, dogs in our study were maintained in their natural environment. Moreover, they were free to exhibit their habitual lifestyle. Circadian hormone secretory patterns might also affect glucose fluctuations in diabetic dogs, although in this species, significant circadian secretory fluctuations of the major counterregulatory hormones such as cortisol and growth hormone have not been demonstrated[36,37] and, as such, weakly, if at all, affected glucose fluctuations in the present study. In human medicine, hypoglycaemic episodes have been shown to be more common at night compared with during the day.[38,39] The risk of nocturnal hypoglycaemia in human diabetic patients has been associated with various factors, including age, insulin dose, site of injection, temperature, and day-to-day intraindividual variation in the rate of insulin absorption, which may vary up to 50%.[40] All dogs in this study and in the study by Mori et al, were fed the same amount of food and were injected with the same doses of insulin twice daily at a 12-h interval. Thus, daily glucose fluctuations might be lower in dogs than in humans who regularly eat three meals each day and inject insulin three or four times each day.[10] Additionally, during the day dogs might perform more physical activity than during the night. It is well established that exercise causes increases in insulin-stimulated whole body glucose disappearance, muscle glucose uptake, and muscle non-oxidative glucose metabolism[41] thus making daily glucose fluctuations lower during the day.

During the study period, 65% of dogs exhibited hypoglycemic episodes but no clinical signs of hypoglycaemia were documented. FGMS scans and PBGM allowed identification of 60% and 9% of

the hypoglycemic episodes. Hypoglycemia is a serious complication in insulin-treated humans and dogs with DM.[1,39] Hypoglycemic episodes can be easily missed when using a PBGM owing to the logistics of frequent blood sampling. Moreover, decreases in blood glucose to below 65 mg/dL or a rapid drop regardless of the nadir can result in the secretion of counterregulatory hormones and the so-called Somogyi response.[42] Diagnosis of a Somogyi response requires identification of hypoglycaemia or a rapid drop of glucose followed within hours by hyperglycemia.[1] If a PBGM is used, significant glucose fluctuations may be missed and it can result in erroneous insulin dose recommendations.[13,43] Also, it has been shown that the use of FGMS significantly reduces time and frequency of hypoglycaemia in patients with type 1 and type 2 DM.[44-47] This is likely due to a combination of on-demand access to real-time sensor glucose results with trend arrows, enabling preventive action and informing behaviour modification, alongside healthcare professional review of glucose reports, to alter the balance of insulins.[45] Considering all the above, FGMS can be a valuable tool in detecting hypoglycaemic episodes. Its usefulness is not limited to the possibility of reviewing retrospectively the graphs showing the 24-hour glucose fluctuations generated by the AGP software during the entire wearing period. Indeed, a real-time glucose level may be obtained as often as every minute by scanning the sensor with the hand-held reader. A glucose trend arrow (indicating rate and direction of change in glucose levels) and a graphical trace of glucose values for the previous 8-hour period are also displayed on the screen (Figure 1).

Some of the limitations of the FGMS are related to the fact that the sensor is designed for human patients with DM where stricter glyaemic control is required. For example, the graphs generated by the AGP software show glycaemic values up to 350 mg/dL and above this, values are not reported (Figure 2). This aspect can limit the utility of the sensor in dogs with poorly controlled DM. Moreover, to ensure uploading of recorded IG measurements the sensor need to be scanned at least every 8 hours by the hand-held reader which may not always be possible. Finally, during the last days of the wearing period, we noted that the hand-held reader can display falsely low values or reading errors, likely due to the usury or the bent of the sensor.

There were limitations in the present study. First, the numbers of dogs included was relatively small and in most of the dogs the sensor lasted less than 14 days. Second, it is authors' impression that the sensor could underestimates glucose values at the end of the wearing period. However, we did not perform paired glucose measurements using a PBGM or the reference hexochinase method to confirm this.

In summary, insulin dose adjustments based on BGCs generated with FGMS and PBGM are similar, suggesting that FGMS is a valuable tool for obtaining BGCs in diabetic dogs in a clinical setting. Moreover, FGMS allows a more accurate identification of the glucose nadirs and hypoglycemic episodes compared to the use of a PBGM and it allows the assessment of glucose variations during consecutive days, enabling the clinician a more careful decision of the insulin dose. Indeed, as already demonstrated, a marked inter-day glycemic variability was seen in this study. Therefore, insulin treatment decision should never be made on the basis of a single BGC. Further studies are needed to investigate whether long-term use of FGMS during follow-up examinations improves glycemic control in diabetic dogs as demonstrated in human diabetic patients.

Footnotes:

a. Optium Xceed, Abbott Laboratories, Witney, England.
b. Abbott. FreeStyle Libre Software. http://www.FreeStyleLibre.com (accessed July 18, 2016).
c. Caninsulin, MSD, Boxmeer, Netherlands.
d. Humulin I, Eli Lilly Italia S.p.A., Sesto Fiorentino, Italy.
e. Lantus, Sanofi-Aventis US LLC, Bridgewater, NJ.
f. Vetoryl, Dechra Pharmaceuticals, Northwich, England.
g. Canitroid, Dechra Pharmaceuticals, Northwich, England.
h. Enacard, Merial, Milan, Italy
i. Amodip, Ceva Salute Animale, Agrate Brianza, Italy

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Capitolo 6

GLYCATED HEMOGLOBIN (HBA1C) AND SERUM FRUCTOSAMINE: COMPARISON OF THE TWO GLYCATED PROTEINS FOR THE ASSESSMENT OF GLYCEMIC CONTROL IN DOGS WITH DIABETES MELLITUS

Francesca Del Baldo, Luca Magna, Francesco Dondi, Paolo Maramieri, Oana M. Catrina, Sara Corradini, Guido Linari, Stefania Golinelli, Antonio M. Tardo, Ugo Bonfanti, Federico Fracassi

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Dipartimento di Scienze Mediche Veterinarie,

Scuola di Agraria e Medicina Veterinaria,

Bologna

6. Glycated hemoglobin (HbA1c) and serum fructosamine: comparison of the two glycated proteins for the assessment of glycemic control in dogs with diabetes mellitus

ABSTRACT

OBJECTIVE To evaluate the performance of 2 assays for the measurement of serum frucostamine (SF) and glycated hemoglobin (HbA1c) values in dogs and to compare the use of SF and HbA1c to assess glycemic control in dogs with diabetes mellitus (DM).

SAMPLE Blood samples from 40 healthy dogs and 23 anemic normoglycemic nondiabetic dogs and 200 reevaluations for assessments of glycemic control in 46 diabetic dogs.

PROCEDURES Colorimetric and immunoturbidimetric methods were used for measurment of SF and HbA1c, respectively. Linearity and precision were determined. Use of SF and HbA1c values to assess glycemic control were evaluated with a clinical score as the reference method. Correlation among SF and HbA1c values and the clinical score was evaluated; cut-off values obtained from receiver operating characteristic curves were used to identify the percentage of dogs categorized as well classified by SF and HbA1c values.

RESULTS Mean intra-assay and interassay coefficients of variation (CV) were 3.8% and 2.5%, respectively, for SF, and 1.2% and 1.8%, respectively, for HbA1c. Excellent linearity ($R^2 > 0.99$) was obtained for both assays. Values for SF and HbA1c were significantly correlated ($R^2 = –0.40$ and –0.33, respectively) with the clinical score and correctly identified glycemic control in 99 of 200 (50%) and 88 of 200 (44%) reevaluations, respectively.

CONCLUSIONS AND CLINICAL RELEVANCE The SF and HbA1c assays were precise, had good linearity, and appeared to be suitable for routine use in veterinary medicine. However, they performed poorly for classifying glycemic control in diabetic dogs.

6. Glycated hemoglobin (HbA1c) and serum fructosamine: comparison of the two glycated proteins for the assessment of glycemic control in dogs with diabetes mellitus

Introduction

Serum fructosamine and HbA1c are 2 glycated proteins that result from the irreversible nonenzymatic binding of glucose to serum proteins or hemoglobin in RBCs, respectively. In dogs, SF reflects the mean BG concentration during the preceding 1 to 2 weeks,[1–3] whereas HbA1c can be considered as an index of the average plasma glucose concentration over the preceding 2 to 3 months.[4]

Concentrations of SF can be measured quickly, accurately, and economically by use of the nitroblue tetrazolium reduction method; however, only a few studies have been conducte to validate this assay for use in samples obtained from dogs,[1,3,5] and the reference interval for SF concentrations differs among laboratories.[6] In dogs, HbA1c values have been measured by use of several methods developed for humans, including affinity chromatography,[7,8] colorimetric analysis,[9] ion-exchange high-performance liquid chromatography,[10] immunoturbidimetric assays,[11–13] capillary electrophoresis,[14] and point-of-care devices that involve the use of boronate-affinity chromatography and immunoassay, respectively.[15,16] Because HbA1c testing is used infrequently and is widely unavailable,[14,15] measurement of HbA1c values is not commonly performed in clinical practice.

The SF concentrations in dogs do not depend only on the BG concentration. They also are affected by several pathological conditions, including hypoproteinemia, hyperlipidemia, azotemia, hypothyroidism, and hyperglobulinemia caused by multiple myeloma.[17–19]

In human medicine, in contrast to SF concentrations, HbA1c values appear to be less affected by diseases other than DM.[20] However, HbA1c values in humans and other animals may decrease in pathological conditions that shorten erythrocyte lifespan or are associated with increased erythrocyte turnover (eg, blood-loss anemia, hemolytic anemia, splenomegaly, and pregnancy).[21–23] Falsely increased HbA1c values might occur under conditions that prolong erythrocyte lifespan or are associated with decreased erythrocyte turnover (eg, iron deficiency anemia and splenic disorders). Moreover, falsely elevated HbA1c values attributable to interference with the assay has been described in patients with extreme hypertriglyceridemia and hyperbilirubinemia.[21–23]

Currently, SF concentrations are commonly used to monitor long-term diabetic control in both dogs and cats[3]; SF concentrations can be measured during routine evaluation of diabetic dogs to clarify discrepancies between the clinical condition and serial BG concentrations, which thus allows clinicians to assess the effectiveness of changes in insulin treatments.[24] Because SF concentrations changes more rapidly than HbA1c values in response to alterations in insulin treatments, the SF concentration is considered better for assessing glycemic control in diabetic canine patients.[24] However, discrepancies between SF concentrations and the clinical condition can remain ambiguous, and there may be individual differences with regard to protein glycation.[25]

Since 1988, the American Diabetes Association has recommended measurement of HbA1c values for routine monitoring of human patients with diabetes mellitus,[26] and it is generally accepted as the best method for assessing glycemic control.[27] In situations when HbA1c values may not provide an accurate assessment of glycemic status, SF concentrations may act as a surrogate marker. Some of the potential uses of SF concentrations are diagnosing gestational DM (although not recommended) and monitoring glycemic status during end-stage renal disease, certain types of anemia, transfusions,[23] and recent acute changes in glycemic control (ie, treatment with glucocorticoids[22]).

Studies comparing the ability of SF and HbA1c to reflect glycemic control in dogs with DM are lacking. Therefore, the objectives of the study reported here were to evaluate the performance of 2 assays for the measurement of SF and HbA1c in canine samples, obtain specific reference intervals for both glycated proteins, and compare the use of SF concentrations and HbA1c values to assess glycemic control in dogs with DM.

Materials and Methods

Sample

All samples were collected from dogs admitted to the Veterinary Teaching Hospital of the University of Bologna. Blood samples were collected from 40 healthy blood donors and student- or staff-owned dogs; dogs were considered healthy on the basis that no abnormalities were detected during physical examination clinicopathological testing. Blood samples were also collected from 13 client-owned

diabetic dogs. Diabetes mellitus was diagnosed on the basis of clinical signs (eg, polyuria and polydipsia, weakness, and weight loss) and a fasting BG concentration > 200 mg/dL combined with glycosuria. Eight dogs had DM that was newly diagnosed, and 5 dogs were receiving insulin treatment; the 8 dogs with newly diagnosed DM were assumed to have extremely high amounts of glycated proteins. Finally, samples were obtained from 23 anemic (Hct < 35%) normoglycemic nondiabetic dogs that were recruited to evaluate the effect of anemia on HbA1c concentrations. Of these, 9 dogs had regenerative anemia (> 71,000 reticulocytes/μL) and 14 had nonregenerative anemia (< 71,000 reticulocytes/μL). All analyses were performed on samples collected for diagnostic or monitoring purposes. Owners provided informed consent for inclusion of their dogs in the study. The study was approved by the Scientific Ethics Committee of the University of Bologna.

SF and HbA1c assays

Analytic methods—Concentration of SF was assessed with a colorimetric nitroblue tetrazolium reduction method (Fructosamine 17350H, Sentinel Diagnostic, Milano, Italy). Coagulated blood samples were centrifuged for 10 minutes at 3,000 X *g*, and the serum was collected and stored at –80°C until analyses were performed. The HbA1c value was assessed with an immunoturbidimetric method (HbA1c, B00389, Beckman CoulterInc, Brea, Calif.), where total hemoglobin concentration was measured with a colorimetric method. All EDTA-anticoagulated samples of whole blood were stored at –80°C and thawed (15-25°C for approximately 1 hour) prior to analysis. An aliquot (10 μL) of each sample was manually mixed with 1,000 μL of hemolyzing reagent provided by the manufacturer, and hemolysis was allowed to proceed for at least 1 minute. Hemolyzed samples were mixed thoroughly immediately before the assay was conducted. The HbA1c-to-total hemoglobin ratio was expressed as the HbA1c percentage. The following equation, which was provided by the manufacturer, was used for correction of the HbA1c percentage: ([HbA1c value/total hemoglobin concentration] X 91.5) + 2.15.

All measurements were performed in an automated chemistry analyzer (AU 480, Beckman Coulter Inc, Brea, Calif.). Precision and linearity were assessed for both methods. A CBC was performed with a flow cytometry–based hematology system (ADVIA 2120, Siemens Healthcare Diagnostics, Tarrytown, NY). Reticulocytes counts were performed by use of oxazine 750 perchlorate (autoRetic, Siemens Healthcare Diagnostics, Tarrytown, NY) on the automated analyzer.

Precision—Intra-assay (within-run) variability was estimated by determining the SF concentration in 10 canine serum samples (5 samples from healthy dogs with a low concentration of the analyte and 5 samples from dogs with poorly controlled DM with a high concentration of the analyte). Analyses were repeated 10 times within the same assay. To determine intra-assay variability of the HbA1c assay, the same procedures were used for 7 canine EDTA-anticoagulated blood samples (3 samples harvested from healthy dogs with a low concentration of the analyte and 4 samples from dogs with poorly controlled DM with a high concentration of the analyte).

Interassay (between-run) variability of the SF assay was estimated by determining the SF concentration in 6 canine serum samples (3 from healthy dogs with a low concentration of the analyte and 3 from dogs with poorly controlled DM with a high concentration of the analyte) twice daily over 5 days. All analyses were performed in duplicate. To determine interassay variability of the HbA1c assay, the same procedures were used for 2 canine EDTA-anticoagulated blood samples (1 from a healthy dog with a low concentration of the analyte and 1 from a dog with poorly controlled DM with a high concentration of the analyte).

Storage stability of SF and HbA1c assays was evaluated by use of samples from 3 dogs with poorly controlled DM (with a high concentration of the analyte) and 3 healthy patients (with a low concentration of the analyte) stored at 3 temperatures (–80°C, 4°C, and 15-25°C) for 5 consecutive days. Samples stored at –80°C were thawed (15-25°C for approximately 1 hour) on the day of the analysis, whereas samples stored at 4°C and room temperature were thawed the first day of the analysis and maintained at 4°C and room temperature, respectively, until the end of the study period.

Linearity—Linearity of the SF assay was determined by use of 2 canine serum samples from 2 dogs with poorly controlled DM with a high concentration of the analyte. Sera were diluted to obtain 75%, 50%, 25%, 12.5% and 6.25% of the starting analyte concentration. Linearity of the HbA1c assay was assessed by use of 1 canine EDTA-anticoagulated blood sample collected from a dog with poorly controlled DM and 1 quality control human sample (CQ2 extendSURE level 2, B12396, Canterbury Scientific Ltd, Christchurch, New Zealand), both with high HbA1c values. These samples were used undiluted and also diluted with distilled water to obtain dilutions (canine sample, 75%, 50%, 25%, and 12.5%; human sample, 75%, 62.5%, 50%, 37.5% and 25%) of the starting analyte concentration. All analyses were performed in duplicate.

Reference interval—The reference interval for SF and the HbA1c value in dogs was established. Serum and EDTA-anticoagulated blood samples obtained from the aforementioned 40 healthy dogs (various breeds and ages and both sexes) were used.

Use of HbA1c and SF values to classify glycemic control

Diabetic dogs receiving insulin treatment and that were admitted for routine monitoring at the Veterinary Teaching Hospital of the University of Bologna were enrolled in the study if the medical record, including the medical history, physical examination findings, body weight, BG curve, biochemical profile, and values for glycated proteins (HbA1c and SF) was complete. To generate BG curves, capillary blood was obtained from the pinna and the blood glucose concentration was measured 1, 2, 4, 6, 8, 10, and 12 hours after the morning meal and insulin injection. Dogs that had their insulin dose modified up to 1 week before the reevaluation were also included. Dogs were excluded from the study when the medical records were unavailable or missing information.

Assessment of glycemic control—To objectively evaluate glycemic control, a clinical score for diabetic dogs was used.[28] This clinical score considered the following variables: body weight, presence of polyuria and polydipsia, median glucose concentration of the BG curve, nadir BG concentration of the BG curve, and overall evaluation of the BG curve. For each variable, a score was assigned as follows: 0 = poor, 1 = moderate, and 2 = good. Maintaining or increasing body weight

was considered good (score = 2), conversely a decrease (> 5%) in body weight was judged as poor (score = 0). For obese dogs, weight loss needed to achieve an optimal body condition score was not considered as a negative. Such dogs were given a score of 2, even if they were losing weight. Polyuria and polydipsia were scored as absent (good = 2), improved (moderate = 1), and present, unchanged, or worse (poor = 0). Median glucose concentration of the BG curve < 230 mg/dL, between 230 and 300 mg/dL, and > 300 mg/dL was considered good (score = 2), moderate (score = 1), and poor (score = 0), respectively. Glucose nadir of the BG curve < 180 mg/dL, between 180 and 250 mg/dL, and > 250 mg/dL was considered good (score = 2), moderate (score = 1), and poor (score = 0), respectively. Overall evaluation of the BG curve was considered good (score = 2) when ≥ 50% of BG concentrations were between 80 and 270 mg/dL and poor (score = 0) when < 50% of BG concentrations were between 80 and 270 mg/dL.

Total clinical score (0 to 10) was obtained by summing the scores for each variable. The entire population was grouped on the basis of the total score into a good glycemic control group (score, 7 to 10), moderate glycemic control group (score, 4 to 6), and poor glycemic control group (score, 0 to 3).

Analytic methods—The BG concentrations were measured in capillary blood obtained from the inner surface of the pinna by use of 2 portable BG meters (glucometer A (Gluco Calea, WellionVet, Med Trust, Marz,Austria) and glucometer B (Optium Xceed, Abbott Laboratories, Witney, England)).[29,30] Detectable BG concentrations for glucometer A ranged from 20 to 600 mg/dL, whereas BG concentrations for glucometer B ranged from 20 to 500 mg/dL. When BG concentrations registered as low and high on the glucometer, values of 20 mg/dL and 600 or 500 mg/dL were arbitrarily assigned. The above mentioned assays were used to measure SF and HbA1c in serum and EDTA-anticoagulated blood samples, respectively, collected by venipuncture on the same days as the BG curves were obtained.

Data analysis

Statistical analysis was performed with commercially available software (Prism, version 7.0d, GraphPad Software Inc, San Diego, Calif.; Microsoft Excel, version 2016, Microsoft Corp, Redmond, Wash.). Distribution of the data was assessed graphically and by use of the D'Agostino-Pearson test. Normally distributed variables were reported as mean ± SD, and data without a normal distribution were reported as median and range (minimum and maximum values). Proportions and percentages were used to describe categorical variables. Parametric and nonparametric tests were used to analyze data on the basis of their distribution.

To assess the intra-assay and interassay precision of both assays, the CV for each sample was calculated. The overall CV was calculated as the mean of the CVs of each sample as follows: CV= SD/mean X 100. To assess linearity, measured values were plotted against expected values in a linear regression analysis. For both precision and linearity, the mean of 2 determinations was used when duplicate measurements were performed. The reference intervals for SF and HbA1c values were established by use of the robust method.

To compare HbA1c values in anemic and nonanemic dogs and to compare HbA1c values in dogs with regenerative and nonregenerative anemia, a *t* test or Mann-Whitney test was used.

The correlation coefficient between SF concentration and clinical score, HbA1c value and clinical score, SF concentration and HbA1c value, and HbA1c value and Hct was obtained by use of Spearman rank correlations.

The Kruskal-Wallis test was used to compare the SF and HbA1c values among the 3 categories of clinical control (good glycemic control, moderate glycemic control, and poor glycemic control). When a significant difference was detected, a Mann-Whitney *U* test or unpaired *t* test was performed. When the SF and HbA1c results differed significantly between groups, ROC curves were constructed to enable us to determine optimal cut-off values with the maximum sum of sensitivity and specificity for SF and HbA1c values for discriminating between the groups. On the basis of the established cut-off values, each reevaluation was categorized as well classified or misclassified, with the clinical score considered the criterion-referenced standard. This type of analysis was performed by first

considering all reevaluations and then separating reevaluations for dogs receiving insulin treatment for < 3 months from the reevaluations for dogs receiving insulin treatment for > 3 months as well as separating dogs that had the insulin dose modified during the past 3 months from dogs on a stable insulin dose (not modified during the past 3 months).

The χ^2 test was used to compare the percentage of reevaluations for SF and HbA1c values categorized as well classified. For all statistical tests, significance was set at values of $P < 0.05$.

Results

Evaluation of SF and HbA1c assays

The overall mean intra-assay and interassay CVs for SF were 3.8% and 2.5%, respectively, whereas the overall mean intra-assay and interassay CVs for HbA1c were 1.2% and 1.8%, respectively. The CVs for samples stored frozen, refrigerated, and at room temperature were 1.8%, 1.9% and 2.5%, respectively.

Linear regression analysis of the SF assay provided R^2 values of 0.997 and 0.996 for the diluted serum samples from the 2 dogs with poorly controlled DM. Linear regression analysis of the HbA1c assay provided R^2 values of 0.993 for the canine EDTA-anticoagulated blood sample diluted to 12.5% of the starting analyte concentration and r values of 0.999 and 0.998 for the human quality control sample diluted to 12.5% and 25% of the starting analyte concentration, respectively.

The reference interval for SF was 222 to 382 μmol/L (median, 286 μmol/L; range, 211 to 367 μmol/L). The 95% CI of the lower limit was 209 to 235 μmol/L, and the 95% CI of the upper limit was 357 to 402 μmol/L. The reference interval for HbA1c was 1.6% to 4.5% (median, 2.61%; range, 2.0% to 4.7%). The 95% CI of the lower limit was 1.4% to 1.7%, and the 95% CI of the upper limit was 4.8% to 5.2%.

Among dogs with regenerative anemia, 7 had blood loss and 2 had immune-mediated hemolytic anemia. Among dogs with nonregenerative anemia, 7 had chronic kidney disease, 1 had chronic gastrointestinal blood loss, 1 had hypothyroidism, 2 had nonregenerative immune-mediated

hemolytic anemia, 2 had neoplastic disease, and 1 had inflammatory disease. The overall mean ± SD Hct of the anemic dogs was 22.2 ± 7.7%, whereas the mean Hct of dogs with regenerative anemia and nonregenerative anemia was 21.1 ± 6.1% and 22.9 ± 8.6%, respectively. The median HbA1c value in anemic dogs was 4.3% (range, 2.6% to 7.5%), which differed significantly ($P < 0.001$) from the median value in nonanemic dogs (2.6%; range, 2.0% to 4.7%; **Figure 1**). The median HbA1c value in dogs with regenerative anemia was 4.0% (range, 2.6% to 4.7%), whereas the median value in dogs with nonregenerative anemia was 4.5% (range, 3.1% to 7.5%); these values did not differ significantly ($P = 0.058$). There was not a significant correlation detected between the Hct and HbA1c value in the overall population ($r = –0.02$; $P = 0.9$), in dogs with regenerative anemia ($r = 0.14$; $P = 0.7$), and in dogs with nonregenerative anemia ($r = –0.13$; $P = 0.7$).

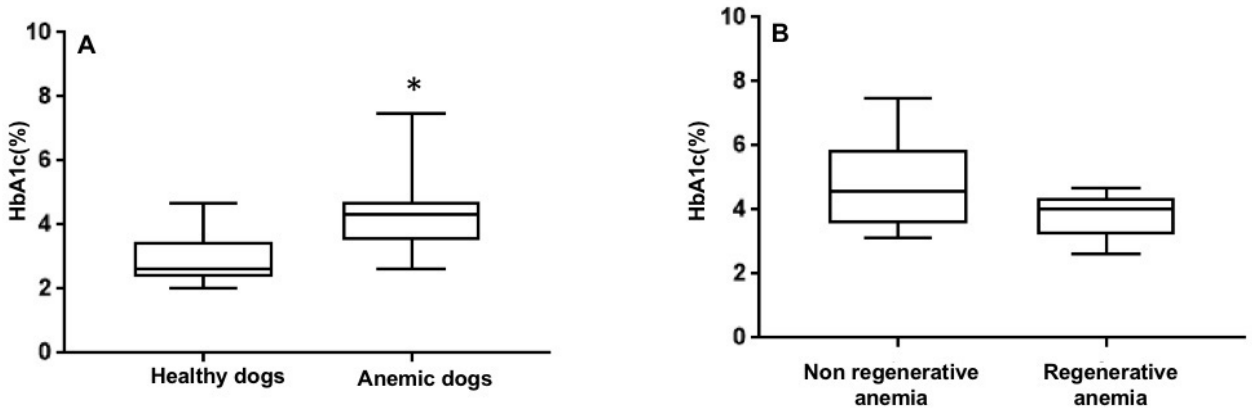

Figure 1 Box-and-whisker plots of HbA1c values in 40 healthy dogs and 23 anemic dogs (A) and in those 23 anemic dogs (14 dogs with nonregenerative anemia and 9 dogs with regenerative anemia; B). The box represents the interquartile (25th to 75th percentile) range, the horizontal line in each box represents the median, and the whiskers represent the range. In panel B, the values did not differ significantly ($P = 0.058$) between the 2 groups of anemic dogs. *Value differs significantly ($P < 0.001$) from the value for the healthy dogs.

Evaluation for HbA1c and SF values for use in classifying glycemic control

A total of 200 reevaluations for 46 diabetic dogs were performed. There were 24 (52%) spayed females, 12 (26%) sexually intact males, and 10 (22%) neutered males, consisting of 21 mix-breed dogs, 6 English Setters, 4 Poodles, 3 Labrador Retrievers, 2 Pomeranians, 2 Yorkshire Terriers, 2 Samoyeds, 2 Maltese, 1 Cocker Spaniel, 1 Yugoslavian Shepherd Dog, 1 Cavalier King Charles

Spaniel, and 1 Doberman Pinscher. Mean ± SD age was 10 ± 2.08 years, and median body weight was 10.7 kg (range, 2.8 to 50 kg).

Twenty-eight dogs were treated with lente insulin (Caninsulin, MSD, Boxmeer, Netherlands), and 18 were treated with NPH insulin (Humulin I, Eli Lilly Italia S.p.A., Sesto Fiorentino, Italy). Nine of 46 dogs had concurrent diseases (6 dogs had hyperadrenocorticism and received trilostane (Vetoryl, Dechra Pharmaceuticals, Northwich, England), and 3 dogs had hypothyroidism and received levothyroxine (Canitroid, Dechra Pharmaceuticals, Northwich, England.)). Slightly more than half (n = 106) of the reevaluations were for dogs with newly diagnosed DM that had been receiving insulin treatment for < 3 months, and 94 were for dogs with DM that had been receiving insulin treatment for > 3 months.

More than 1 reevaluation was performed for 39 dogs; 1 dog had 13 reevaluations, 1 dog had 11 reevaluations, 1 dog had 8 reevaluations, 6 dogs had 7 reevaluations, 6 dogs had 6 reevaluations, 6 dogs had 5 reevaluations, 5 dogs had 4 reevaluations, 7 dogs had 3 reevaluations, and 6 dogs had 2 reevaluations. Glycemic control, as evaluated by use of the clinical score, was classified as good in 90 of 200 (45%) reevaluations, moderate in 58 of 200 (29%) reevaluations, and poor in 52 of 200 (26%) reevaluations. None of the reevaluations revealed the Somogyi effect (hypoglycemia or rapid decrease of glycemia followed by marked hyperglycemia induced by excessive dose of insulin).

Total clinical score was significantly ($P < 0.001$) and inversely correlated with the SF concentration ($r = –0.40$) and the HbA1c value ($r = –0.33$). In addition, the SF concentration and HbA1c value were significantly correlated ($r = 0.48$; $P < 0.001$).

Assessment of differences among the 3 groups of clinical control (good glycemic control, moderate glycemic control, and poor glycemic control groups) revealed that both SF concentrations and HbA1c values were significantly higher in dogs with poor glycemic control, compared with results for dogs with moderate glycemic control, and they were also significantly higher in dogs with moderate glycemic control than in dogs with good glycemic control **(Figure 2)**.

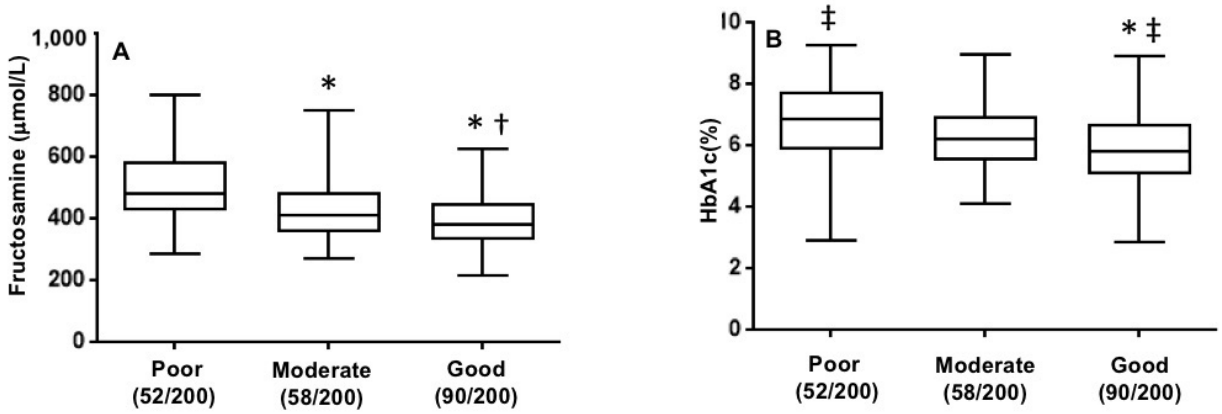

Figure 2 Box-and-whisker plots of SF concentrations (A) and HbA1c values (B) for 200 reevaluations of 46 dogs in 3 groups of clinical control. The definition of clinical control of DM was based on the results of a clinical history obtained from the owner, body weight, and assessment of BG curves. *Value differs significantly ($P < 0.001$) from the value for the poor glycemic control group. †Value differs significantly ($P = 0.03$) from the value for the moderate glycemic control group. ‡ Value differs significantly ($P = 0.01$) from the value for the moderate glycemic control group.

Because there were significant differences between the SF concentration and HbA1c value for the dogs with good glycemic control, moderate glycemic control, and poor glycemic control, 2 ROC curve analyses were performed for each glycated protein, first by combining the moderate and good glycemic control groups versus the poor glycemic control group and then by combining the moderate and poor glycemic control groups versus the good glycemic control group.

The ROC curve analyses for the use of SF and HbA1c values to differentiate good glycemic control from moderate or poor glycemic control revealed a significant ($P < 0.001$) AUC of 0.69 and 0.66, respectively **(Figure 3)**. Use of an SF concentration of < 400 µmol/L to differentiate dogs with good glycemic control from dogs with moderate or poor glycemic control yielded a specificity of 71% and sensitivity of 61%. Use of an HbA1c value of < 5.5% to differentiate dogs with good glycemic control from dogs with moderate or poor glycemic control yielded a specificity of 79% and sensitivity of 41%. The ROC curve analyses for the use of SF concentrations and HbA1c values to differentiate poor glycemic control from moderate or good glycemic control revealed a significant ($P < 0.001$) AUC of 0.75 and 0.69, respectively. Use of an SF concentration of > 500 µmol/L to differentiate dogs with poor glycemic control from dogs with moderate or good glycemic control yielded a specificity of 85% and sensitivity of 45%. Use of an HbA1c value > 6.8% to differentiate dogs with poor

glycemic control from dogs with moderate or good glycemic control yielded a specificity of 79% and sensitivity of 52%.

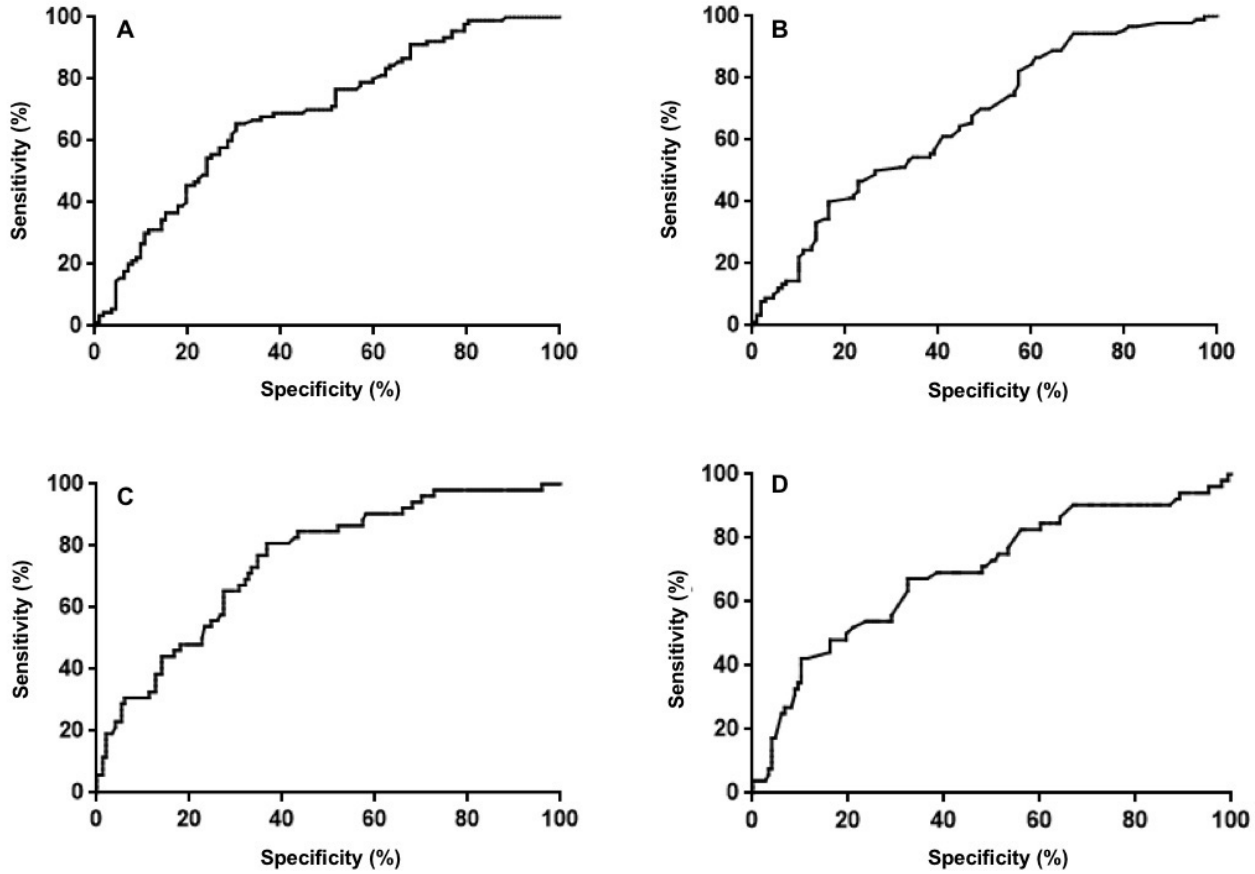

Figure 3 The ROC curves for assessment of SF concentration (A and C) and HbA1c value (B and D) as predictors of clinical control for good versus moderate or poor (A and B) and poor versus good or moderated (C and D). The AUC was 0.69 (95% CI, 0.61 to 0.76), 0.66 (95% CI, 0.59 to 0.74), 0.75 (95% CI, 0.68 to 0.82), and 0.69 (95% CI 0.60 to 0.78) for panels A through D, respectively.

The classification of each reevaluation was determined on the basis of clinical score and by use of established cut-off values for glycated proteins **(Table 1)**. The number of misclassified reevaluations was determined on the basis of the clinical score and use of established cut-off values for glycated proteins. There were 36 of 200 (18%) reevaluations misclassified by use of cut-off values for SF concentrations, 41 of 200 (21%) reevaluations misclassified by use of cut-off values for HbA1c values, and 65 of 200 (33%) reevaluations misclassified by use of cut-off values for both SF concentrations and HbA1c values.

6. Glycated hemoglobin (HbA1c) and serum fructosamine: comparison of the two glycated proteins for the assessment of glycemic control in dogs with diabetes mellitus

Table 1—Results for 200 reevaluations for 46 dogs classified on the basis of the clinical score in 3 groups of clinical control and by use of established cut-off values for glycated protein values.

Glycated protein	Good	Moderate	Poor
SF (μmol/L)			
< 400	55	24	8
400 to 500	25	21	21
> 500	10	13	23
HbA1c (%)			
< 5.5	38	16	8
5.5 to 6.8	40	25	19
> 6.8	12	17	25

Classification of the reevaluations when considering the duration of insulin treatment (< 3 months or > 3 months) as well as considering the time from the last adjustment of the insulin dose was also determined **(Table 2)**.

Table 2—Number (%) of 200 reevaluations for 46 dogs categorized by the use of SF concentration and HbA1c as well classified or misclassified on the basis of duration of insulin treatment and adjustment of insulin dose.

	SF		**HbA1c**	
Insulin treatment	**Well-classified**	**Misclassified**	**Well-classified**	**Misclassified**
Receiving < 3 months	50 (47%)	56 (53%)	47 (44%)	59 (56%)
Receiving > 3 months	49 (52%)	45 (48%)	42 (44%)	52 (56%)
Dose adjusted within past 3 months	81 (48%)	87 (52%)	78 (46%)	90 (54%)
Dose stable > 3 months	18 (56%)	14 (44%)	16 (50%)	16 (50%)

Finally, SF concentration could be used to correctly classify glycemic control in 99 of 200 (50%) reevaluations and HbA1c value could be used to correctly classify glycemic control in 88 of 200 (44%) reevaluations; these percentages did not differ significantly ($P = 0.22$).

Discussion

The colorimetric and immunoturbidimetric assays used in the present study for the quantification of SF and HbA1c, respectively, in dogs were precise and accurate. The colorimetric nitroblue tetrazolium reduction method is extensively used in veterinary laboratories for the measurement of SF concentrations; however, the analytic performance of this assay for samples from dogs has been evaluated in only a few studies.[1,5] In the present study, the overall intra-assay CV was slightly higher than that reported in 1 study[1] and similar to that reported in another study.[5] In contrast to results for those studies, higher, but still acceptable, variability for SF concentrations was found in the study reported here. The interassay precision for the present study compared favorably with the data reported in the literature.[1,5] Linearity was deemed good, although there was a tendency to overestimate the expected SF concentration in all diluted samples, which was similar to results reported in another study.[1] The reference interval of SF concentrations obtained in the study reported here was comparable to that reported previously when the same analytical method was used.[3,5,31]

The human and canine β-chains of hemoglobin are identical in the sequences of the last 5 amino acids[32]; the immunoturbidimetric method used in the present study was designed for humans and used monoclonal antibodies directed against this region of the molecule; therefore, it could also be used for dogs. In comparison with other studies[11–13] in which the same analytic method was used, lower intra-assay and interassay CVs were obtained in the present study. Linearity was good, although there was a tendency to underestimate the actual HbA1c value in diluted samples, especially for values of HbA1c < 0.3 mg/dL. However, such low concentrations are not expected in canine samples, especially in diabetic patients, so the clinical importance of this underestimation would be negligible.

Compared with data reported in another study,[13] results for the present study provided slightly better linearity.

The reference interval for HbA1c obtained in the present study (1.6% to 4.5%) partially overlaps with results obtained in another study[11] by use of the same method, even though the upper limit was higher. However, the inclusion criteria for healthy dogs in that other study[11] are not known, and a different immunoturbidimetric assay was used. In 2 other studies[12,13] in which the immunoturbidimetric assay was used, the reference interval was narrower and the lower limit was higher, compared with results obtained for the study reported here. The reference interval of HbA1c obtained in other studies by use of different analytic assays was higher[33] or similar[8,21,34] to results obtained for the present study. Because of this high variability, a reference interval should be determined at each laboratory.

The HbA1c value was significantly higher in anemic dogs, compared with the HbA1c value in healthy dogs, and no correlation was found between the Hct and HbA1c value. These results are consistent with those in another study,[21] in which HbA1c values in anemic dogs were significantly higher than those of the control group and there was no correlation between the Hct and HbA1c value. In contrast, investigators of another study[8] found significantly lower HbA1c values in anemic dogs than in healthy control dogs and a positive correlation between the Hct and HbA1c values. Because glycation of hemoglobin occurs only as the RBCs circulate in the plasma, hemoglobin in older erythrocytes is more glycosylated, whereas in younger RBCs such as reticulocytes, hemoglobin is less glycosylated.[27] In humans, any condition that shortens erythrocyte survival or decreases mean erythrocyte age (eg, acute blood loss or hemolytic anemia) falsely lowers HbA1c test results regardless of the assay method.[35] In contrast, iron-deficiency anemia reportedly increases test values,[36] probably because the mean age of circulating erythrocytes is higher. In the present study, the median HbA1c value of dogs with regenerative anemia was lower than that of dogs with nonregenerative anemia, although the values did not differ significantly. The lack of a significant difference could have been attributable to the low number of dogs included in these 2 groups. However, it seems that dogs with nonregenerative anemia tends to have higher values of HbA1c.

Hence, interpretation of HbA1c values should always take into consideration a dog's Hct and, when the Hct is low, the type of anemia.

Clinical score was significantly and inversely correlated with the SF concentration and HbA1c value; however, the correlation was weak to moderate for both, but higher for the SF concentration than for the HbA1c value. This result suggested that SF concentration might reflect glycemic control better than is reflected by the HbA1c value. In the study reported here, the clinical score was used as a reference method to classify glycemic control; however, most variables used in the clinical score reflected glycemic control for that particular day (BG curve) or a few days before measurement of the glycated protein concentrations (clinical signs observed by the owner). For these reasons, a variable that is more indicative of recent glycemic control, such as the SF concentration, was more likely to be correlated with criteria used to classify glycemic control in this study than were glycosylated protein concentrations that reflected glycemic control over a longer period (HbA1c values). Moreover, the dose of insulin was often changed a few weeks before the reevaluation of the dogs. Therefore, this short-time period could have altered the glycemic control but not allowed changes in HbA1c values because it requires 2 weeks for HbA1c to respond to increases in glucose concentrations.[37] Potentially, HbA1c is a better indicator of glycemic status in dogs that are not receiving changes in insulin treatment. In humans, self-monitoring and the daily adjustment of insulin dose makes the use of a variable that reflects long-term glucose regulation more reliable. Some authors[24] claim that a variable that reflects short-term glucose control (eg, SF concentration) would be better for monitoring the long-term diabetic stability of dogs with DM. In the present study, there was a poor correlation between the SF concentrations and HbA1c values. Another study[38] revealed a higher degree of correlation between the SF concentration and HbA1c value in dogs. The reason for the poor correlation in the present study is unknown but may be have been attributable to the increased sensitivity of SF, compared with that of HbA1c, to temporary fluctuations in BG concentrations.[39]

Although the median SF concentration and HbA1c value differed significantly between the 3 groups of clinical control, there was marked overlap in values of the 2 glycated proteins among groups. Overlapping may have been the result of differences in the duration of good, moderate, or poor glycemic control before SF concentrations and HbA1c values were measured. Previous studies[3,8] conducted to investigate the use of glycated proteins to monitor metabolic control in dogs with DM have found that SF and HbA1c values differ significantly according to clinical control; however, for both glycated proteins, there is substantial overlap among groups. In addition, in one of those studies,[3] metabolic control was defined only on the basis of the fasting glucose concentration, whereas in the other study,[8] the clinical condition was also considered. Detecting glycated protein values within the reference interval in dogs with poorly controlled DM and high glycated protein values in dogs with well-controlled DM may suggest a delay between the change in mean BG concentration and the corresponding change in glycated protein values. Moreover, a recent study[40] conducted to evaluate the in vitro effect of hyperglycemia on plasma proteins in dogs revealed a lower predisposition to glycation in samples from canines than to human plasma proteins. This might lead to an underestimation of the severity of hyperglycemia in dogs with DM that could explain the low accuracy of SF concentrations for the classification of glycemic control. On the other hand, any condition (ie, hypothyroidism) that leads to a decrease in protein turnover might cause prolonged exposure between glucose and plasma proteins that results in an increase in the SF concentration and misclassification of well-controlled patients. Furthermore, dogs with recently adjusted insulin doses may have had an improvement in glycemic control and clinical signs that was not yet reflected by a decrease in the SF concentration.

Some authors[8,24] have suggested the use of glycated proteins for the monitoring of diabetic dogs to clarify discrepancies between the medical history, physical examination findings, and serial BG concentrations, and they propose a HbA1c value < 6.5%[8] or < 6.0%[24] and an SF concentration < 450 μmol/L[24] to identify dogs receiving insulin treatment that are well controlled. Results of the study

reported here indicated that both glycated proteins had low ability for differentiating between the 3 groups of clinical control as defined on the basis of the clinical score. In 99 of 200 (50%) reevaluations, glycemic control was misclassified by use of SF concentrations. In particular, 77 (37%) reevaluations were misclassified by use of at least 1 of the glycated proteins, whereas 65 (33%) of the reevaluations were misclassified by use of both the glycated proteins. Hence, the cut-off values had limited clinical usefulness, and use of the glycated proteins alone to determine glycemic control cannot be recommended.

For the same reasons, in dogs with DM in which repeated BG testing is not a practical option, assessment of glycemic stability by use of the glycated proteins over a period of weeks to months might not be appropriate. Because HbA1c has a long half-life (2 to 3 months), and 94 reevaluations were for diabetic dogs receiving insulin treatment for < 3 months, each reevaluation was further classified by separating the dogs receiving insulin treatment for > 3 months from dogs receiving insulin treatment for < 3 months as well as by separating dogs that had the insulin dose adjusted within the past 3 months from dogs that had received a stable insulin dose for at least 3 months. However, the percentage of dogs well-classified by use of the 2 glycated proteins was similar in these subgroups.

Taken together, analysis of the results for the present study confirmed that glycated protein concentrations should not be used alone to assess the control of DM in dogs and raised questions about whether they have any clinical utility in the monitoring of DM. Although trends in HbA1c and SF values in individual patients during ongoing treatment were not evaluated in the present study, several studies[8,28,41,42] have found that SF and HbA1c values decrease substantially in dogs with DM during insulin treatment. Moreover, human patients have differing abilities to glycate hemoglobin.[43] Therefore, monitoring individual canine patients on the basis of their previous values rather than by use of population-derived reference intervals might be more appropriate. Establishing a baseline glycated protein concentration for an individual patient and then monitoring patterns rather than

absolute changes associated with alterations in control of DM might be the most appropriate method for use of glycated protein measurements in diabetic dogs.

The present study had some limitations. The definition of clinical control was based on the results of a clinical history obtained from the owner, body weight, and assessment of BG curves. Although owners of the diabetic dogs had been instructed to evaluate the clinical signs of their dogs, they could have underestimated or overestimated some signs or failed to recognize the signs of DM. Furthermore, all the BG curves in this study were performed in a hospital setting, which might have affected accuracy of the curves. Another limitation was that some of the dogs included in the study may have had concurrent diseases (eg, hypothyroidism, hyperlipidemia, hypoalbuminemia, anemia, or hyperadrenocorticism) that could have influenced the SF and HbA1c values as well as the interpretation of the clinical signs (eg, presence of polyuria and polydipsia in dogs with hyperadrenocorticism). However, these dogs were not excluded from the study to mirror the population of dogs with DM, despite evidence of concurrent disease that may have affected the SF and HbA1c results. Moreover, as mentioned previously, because the dose of insulin had often been changed shortly before the reevaluation, the dogs with a dose adjustment could have had a modification of the clinical score that was not yet reflected by the glycated protein values.

The assays used in the present study were reliable methods with good analytic performance for measuring SF concentrations and HbA1c values in dogs. In particular, HbA1c appeared to be extremely stable when stored under various storage conditions and could represent an alternative to measurement of SF concentrations in diabetic dogs. Use of SF concentrations and HbA1c values had a similar and poor ability to classify glycemic control; thus, they should not be used alone to assess glycemic control and for adjustments of the insulin dose.

6. Glycated hemoglobin (HbA1c) and serum fructosamine: comparison of the two glycated proteins for the assessment of glycemic control in dogs with diabetes mellitus

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Capitolo 7

SURVIVAL ESTIMATES AND OUTCOME PREDICTORS IN DOGS WITH NEWLY DIAGNOSED DIABETES MELLITUS TREATED IN A VETERINARY TEACHING HOSPITAL

A.M. Tardo, F. Del Baldo, F. Dondi, M. Pietra, R. Chiocchetti, F. Fracassi

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Dipartimento di Scienze Mediche Veterinarie,

Scuola di Agraria e Medicina Veterinaria,

Bologna

7. Survival estimates and outcome predictors in dogs with newly diagnosed diabetes mellitus treated in a veterinary teaching hospital

Abstract

Diabetes mellitus (DM) is one of the most common endocrine disorders in dogs, but prognostic factors are still largely unknown. The aim of this retrospective, single centre, case series study was to determine overall survival time and identify the prognostic value of several clinical and clinicopathological variables in dogs with newly diagnosed DM. Cases of DM were identified within the electronic medical records of one referral centre. Sixty-eight dogs with DM were included. The median survival time was 964 days (range 22–3140). Cox proportional hazards models were used to analyse variables associated with survival. In multivariable model analysis length of survival was significantly shorter for dogs with higher haematocrit value (HR 1.06, 95% CI 1.00–1.13) and higher serum phosphate concentrations (HR 1.83, 95% CI 1.13–2.97). Serum phosphate concentrations were above the reference interval in 24/65 (37 per cent) of dogs. The presence of pancreatitis might not be associated with an unfavourable outcome.

Diabetic dogs have a good life expectancy. Hyperphosphataemia is a relatively common finding in dogs with newly diagnosed DM and represents a negative prognostic factor.

Introduction

The term diabetes mellitus (DM) describes a group of metabolic diseases characterized by chronic hyperglycaemia resulting from defects in insulin production, action or both.[1] DM is one of the most frequent endocrinopathies in dogs. The prevalence of canine DM has been estimated, from first opinion practices and insurance database populations, at about 0.3 per cent.[2,3]

The most common form of DM in dogs resembles the human type 1 condition, characterized by permanent hypoinsulinaemia that requires exogenous insulin to maintain control of glycaemia, avoid ketoacidosis, and survive.[4] Transient or reversible DM is a rare event in dogs.[5] The aetiology of type 1 DM has not been completely elucidated in dogs but is undoubtedly multifactorial, involving both genetic and environmental factors.[6] DM generally occurs in middle-aged and older dogs;[2,7,8] some studies indicate that females are at greater risk,[7,8] and breed predispositions have been suggested.[7-10] Moreover, different risk factors, related to lifestyle[11] and the presence of concurrent diseases,[12-14] are believed to play a potential role in the development of DM in dogs.[15]

Although the pathophysiological mechanisms, clinical aspects, diagnostic methods, treatment and monitoring options for dogs with DM have been investigated in a number of studies, only a few[3,8] have mentioned life expectancy and prognostic factors of the disease. Furthermore, the predictive value of clinicopathological variables at the time of diagnosis has never been analysed in any study. This might be explained by the fact that the diagnosis of DM is often carried out in first-opinion veterinary practices, whereas the referral centres see the case when insulin treatment has already started; therefore, it is difficult to obtain laboratory data at the time of diagnosis, before treatment, for a large number of dogs from a single referral institution that uses a single internal laboratory.

Detailed data about the outcome and prognostic factors of DM in dogs would help to characterize the disease better and, conceivably, make the owners more inclined to accept lifetime treatment for their dogs and maintain excellent compliance. Hence, the purpose of the present study was to assess the survival time and the prognostic significance of different clinical and clinicopathological variables evaluated in dogs newly diagnosed with DM.

Materials and Methods

Inclusion criteria

The medical records of all diabetic dogs admitted to the Department of Veterinary Medical Sciences, University of Bologna, Italy, between January 2005 and December 2017 were reviewed. Dogs were included in the study if they had newly diagnosed DM, had not been treated for diabetes, and had follow-up examinations at the same institution until death or until the last re-evaluation for which records were available. Dogs were excluded if, at diagnosis, a thorough diagnostic evaluation (i.e., complete blood count – CBC –, chemistry profile and urinalysis) was not available or if the dogs had previously been treated by referring veterinarians.

Diagnostic procedures

DM was diagnosed on the basis of appropriate clinical signs (i.e., polyuria, polydipsia, polyphagia, weight loss), persistent fasting hyperglycaemia and concomitant glycosuria. The concurrent presence of ketonuria established the diagnosis of diabetic ketosis (DK), while ketonuria with an increased anion gap metabolic acidosis (venous blood pH < 7.35 and bicarbonate concentrations < 17.5 mmol/l) established the diagnosis of diabetic ketoacidosis (DKA). Complete blood count, chemistry profile (which included measurement of serum fructosamine and/or blood glycated haemoglobin – Gly Hb – concentrations), and complete urinalysis were performed to identify clinicopathological abnormalities consistent with DM or concurrent disorders. Additional diagnostic procedures were carried out when clinically indicated.

CBC (CELL-DYN 3500R, Abbott Laboratories, Abbott Park, IL, USA [from year 2005 to year 2009, 15 dogs]; Advia 2120 Hematology System, Siemens Healthcare Diagnostics, Erlangen, Germany [from year 2010 until the end of the study, 53 dogs]), chemistry profiles (AU 400 and AU480, Beckman-Coulter/Olympus, Brea, California, USA) and urinalyses were performed by standard laboratory methods at the medical laboratory of the referral institution. Serum fructosamine analysis was performed using a colorimetric nitroblue tetrazolium reduction method (17350H, Sentinel Diagnostic, Milano, Italy). Gly Hb was assessed by an immunoturbidimetric method while total

haemoglobin was measured using a colorimetric method (HbA1c, B00389, Beckman Coulter Inc., Brea, California, USA); the Gly Hb/total haemoglobin ratio was expressed as a percentage. The methods for measuring glycated proteins were subjected to internal validation.

Treatment protocol and monitoring

Dogs were managed using the therapeutic and monitoring protocol implemented at our institution. Insulin therapy was started at an initial dose of approximately 0.1–0.25 U/kg bodyweight twice daily, according to the insulin preparation administered. Dietary therapy was initiated simultaneously. As a standard procedure at our clinic, all diabetic dogs were reassessed at 1, 2 to 3, 6 to 8, 10 to 12 weeks after diagnosis, and every 4 months thereafter, or as needed. Each re-evaluation included an assessment of history, physical examination and body weight. Furthermore, glycated proteins (i.e. serum fructosamine and/or glycated haemoglobin) were measured and a blood glucose curve (BGC) was performed. The decision on additional diagnostics (i.e., routine laboratory evaluation, tests for concurrent diseases) was the responsibility of the clinician managing the case. Adjustments of insulin dosage, in the range of 10–25%, were made on the basis of the owner's perception of clinical signs in response to treatment, BGC and glycated protein concentrations.

Medical records review

Data obtained at the time of diagnosis from medical records included signalment, history (including administration of glucocorticoids and progestogens in the previous 6 months), physical examination findings, and laboratory test results that comprised CBC, serum chemistry profile and urinalysis. DK, DKA and any concurrent disease diagnosed at initial evaluation were recorded. Information concerning insulin therapy, including type of insulin, starting dosage and regimen of administration was retrieved. The occurrence of diabetic remission (i.e., insulin treatment was no longer required to maintain normal blood glucose level) was recorded. Date of death or survival of all cases was recorded and entered into the database, which was closed on December 31, 2017 before analysis. When necessary owners were contacted.

Data analysis

Descriptive statistics were generated to characterize the study population. Continuous variables were presented as mean and standard deviation (± SD) or median and range (minimum and maximum value), depending on whether the data were normally or not normally distributed, respectively. Categorical variables were described with frequencies, proportions or percentages.

The median survival time was estimated using the Kaplan-Meier product limit method. The survival time was defined as the time between the diagnosis and the date on which the dog was last known to be alive, or the date of its death due to any cause. Dogs had censored survival time if alive at the end of the study or lost to follow-up.

The following variables were investigated to determine their association with overall survival time: age, sex (male or female), reproductive status (entire or neutered), breed (crossbred or purebred), bodyweight, diet (petfood, homemade food or mixed), previous administration of corticosteroids and progestogens, clinical signs (polyuria, polydipsia, polyphagia, weight loss, cataracts, weakness, anorexia, vomiting), haematocrit value (Hct), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), white blood cell count (WBC); neutrophils, lymphocytes, monocytes, eosinophils and platelet count; concentrations of glucose, fructosamine, Gly Hb, total bilirubin, total protein, albumin, cholesterol, triglycerides, creatinine, urea, total calcium, phosphate, sodium, potassium and chloride; serum activity of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (GGT); urinary specific gravity, urinary protein to creatinine ratio (UPC), urinary glucose and urinary ketones concentrations. The presence of ketosis/ketoacidosis, pancreatitis, Cushing's syndrome, mitral and/or tricuspid valve disease, and any other concurrent disorders was considered. The type and the starting dose of insulin were also included in the analysis.

Univariate Cox proportional hazards regression analysis was used to screen potential predictors for subsequent inclusion in a multivariate model. Variables with a value <0.05 via univariate analysis

were included in the final model-building process. Variables were then gradually removed until the model with the best fit was identified. In the model-building process, the selection of variables that were strongly collinear (i.e., creatinine and urea concentrations) was also considered. Hazard ratios and 95% CIs were calculated.

Continuous variables associated with survival in the multivariate analysis were assessed by the receiver-operating characteristic (ROC) curve analysis to select the optimum cut-off value, with the highest sensitivity and specificity, for prediction of the outcome. Survival of diagnostic groups was estimated by Kaplan-Meier analyses and compared by log-rank test. All statistical analyses were performed using a commercially available software program (MedCalc®). The significance level was set at $P<0.05$.

Results

Study population

Of the 202 cases of canine DM that were retrieved from the records, 68 dogs met the inclusion criteria and were used in the analysis. One hundred and two dogs were excluded because the diagnosis had been made previously and they had been treated by private practitioners, 25 dogs were excluded because they had no follow-up examinations after the diagnosis, and seven dogs were excluded because the owners denied permission for a comprehensive diagnostic evaluation.

The characteristics of the study population were summarized (**Table 1**). The median age at diagnosis was 10 years (range 5–14 years). There were 21 (31%) entire females, 21 (31%) spayed females, 11 (16%) entire males, and 15 (22%) neutered males. All entire females (21 dogs) were spayed within 4 weeks after the diagnosis of DM. The median body weight was 11.5 kg (range 2.8–50.0 kg). Twenty different breeds were counted. The most commonly represented breeds were mixed breed (22), English Setter (16) and Yorkshire Terrier (6). At the time of diagnosis, 38.5% of the dogs were fed with petfood, 23% with homemade diet, and 38.5% with a mixed diet. Seven (10%) dogs had been treated with corticosteroids or progestogens up to 6 months prior to admission.

7. Survival estimates and outcome predictors in dogs with newly diagnosed diabetes mellitus treated in a veterinary teaching hospital

The clinical signs reported at diagnosis by the owners or observed at the physical examination were – in order of frequency – polydipsia (93%), polyuria (91%), weakness (73%), weight loss (48%), vomiting (41%), anorexia (35%), polyphagia (28%), and cataracts (25%).

Table 1. Descriptive statistics of continuous variables in 68 dogs with newly diagnosed diabetes mellitus. Data are presented as median and range (min-max value) or as mean ± SD based on their distribution.

Variable (Unit)	Tested, n	Result	Above RI, n (%)	Within RI, n (%)	Below RI, n (%)	Reference Interval (RI)
Haematocrit (%)	68	43.1 ± 7.2	2 (3)	52 (76)	14 (21)	37.0–55.0
RBC (x10^3/µl)	68	6.47 ± 1.15	1 (1)	53 (78)	14 (21)	5.50–8.50
MCV (fL)	68	68.7 (51.0–76.8)	0	62 (91)	6 (9)	60–77
MCHC (g%)	68	33.9 (18.9–48.1)	3 (4)	57 (84)	8 (12)	32.0–38.0
RDW (%)	65	13.4 (2.0–20.3)	12 (18)	28 (43)	25 (39)	13–15.7
WBC (x10^3/µl)	68	14.04 (2.09–47.00)	21 (31)	46 (68)	1 (1)	6.00–17.00
Neutrophils (x10^3/µl)	67	10.74 (5.07–40.29)	28 (42)	39 (58)	0	3.00–12.00
Lymphocytes (x10^3/µl)	67	1.49 (0.30–4.20)	0	51 (76)	16 (24)	1.00–4.80
Monocytes (x10^3/µl)	65	0.98 (0.26–3.87)	22 (34)	43 (66)	0	0.10–1.40
Eosinophils (x10^3/µl)	58	0.17 (0.00–1.20)	3 (5)	55 (95)	0	0.00–0.75
Platelets (x10^3/µl)	68	416 (6–3147)	25 (37)	41 (60)	2 (3)	160–500
Glucose (mmol/l)	68	24.5 (11.5–65.5)	68 (100)	0	0	3.8–6.9
Fructosamine (µmol/L)	32	537 ± 149	28 (90)	3 (10)	0	222–382
Gly Hb (%)	11	6.9 ± 1.3	11 (100)	0	0	1.56–4.46
ALT (U/L)	67	114 (27–1399)	56 (84)	11 (16)	0	20–55
AST (U/L)	65	66 (19–1434)	47 (72)	18 (28)	0	20–42
ALP (U/L)	66	551 (103–20368)	61 (92)	5 (8)	0	42–180
GGT (U/L)	63	7.9 (0.1–365)	40 (63)	23 (37)	0	0–5.8
Total bilirubin	66	4.3 (1.7–20.5)	12 (18)	54 (82)	0	1.2–5.8
Total proteins (g/L)	68	66 ± 8	3 (5)	60 (88)	5 (7)	56–79
Albumin (g/L)	68	32 (12–47)	6 (9)	45 (66)	17 (25)	28–37
Cholesterol (mmol/l)	68	8.8 (3.4–22.0)	32 (47)	36 (53)	0	3.6–9.1
Triglycerides (mmol/l)	21	3.6 (0.9–26.3)	19 (90)	2 (10)	0	0.3–1.4
Urea (mmol/l)	65	16.4 (2.4–157.7)	24 (37)	34 (52)	7 (11)	6.4–19.6
Creatinine (µmol/L)	68	70.7 (35.3–477.3)	12 (18)	50 (73)	6 (9)	57.4–119.3
Calcium (mmol/l)	65	2.4 ± 0.2	2 (3)	47 (72)	16 (25)	2.2–2.9
Phosphate (mmol/l)	65	1.4 (0.8–2.4)	24 (37)	36 (55)	5 (8)	0.8–1.5
Sodium (mmol/l)	66	139 (115–148)	0	16 (24)	50 (76)	143–154
Potassium (mmol/l)	66	4.5 (2.6–7.4)	4 (6)	51 (77)	11 (17)	3.9–5.3
Chloride (mmol/l)	53	99.5 ± 7.5	0	8 (15)	45 (85)	108–118
Urinary specific	61	1028 (1009–1080)	--	--	--	--
UPC	35	1.7 (0.2–7.2)	29 (83)	6 (17)	0	0.0–0.4

At the time of admission to the clinic, the median glucose concentration was 24.5 mmol/l (range 11.5–65.5), mean fructosamine concentration was 537 μmol/L (SD, ± 149), and mean blood Gly Hb concentration was 6.9% (SD, ± 1.2). In comparison with the laboratory reference interval, other common alterations (present in more than 60% of cases) in the chemistry profile were increased concentrations of serum ALP, ALT, AST, GGT, triglycerides and decreased concentrations of sodium and chloride. Frequent abnormalities in urinalysis included increased UPC in 29/35 (83%) dogs, and the presence of glucose and ketones in urine. Ketosis and ketoacidosis were diagnosed in 2 (3%) and 26 (38%) dogs, respectively. One or more concurrent diseases were documented in 34 (50%) dogs, including 13 (19%) with pancreatitis; eight (12%) with mitral and/or tricuspid valve disease; seven (10%) with Cushing's syndrome; four with mammary neoplasia; three with hepatic disease; two each with hypothyroidism, urolithiasis, or disseminated intravascular coagulation (DIC); and one each with inflammatory bowel disease, acute kidney injury (AKI) or cutaneous mastocytoma.

With regard to treatment, 36 (53%) dogs received lente insulin (Caninsulin, MSD, Boxmeer, The Netherlands), 12 (17.5%) received NPH insulin (Humulin I, Eli Lilly Italia S.p.A., Sesto Fiorentino - FI, Italy), 12 (17.5%) received insulin glargine (Lantus, Sanofi S.p.A., Anagni - FR, Italy), and insulin detemir (Levemir, Novo Nordisk A/S, Bagsværd, Denmark) was administered to eight (12%) dogs. The median starting dose of insulin was 0.3 U/kg (range 0.02–1 U/kg) twice daily.

Survival analysis

Of the 68 diabetic dogs, at the time of censorship, 39 were dead, 24 alive, and five had been lost to follow-up. In the former group, 15 dogs had undergone euthanasia, and 24 had died spontaneously. Of the 39 dogs that had died by the end of the study, the cause of death or reason for euthanasia was recorded when possible (**Table 2**). However, the cause of death was not supported by postmortem examination in any of the cases.

Table 2. Reason for euthanasia/death in 39 of 68 dogs with diabetes mellitus at the time of censorship.

Reason for euthanasia/death	**Number recorded**
• DM deterioration	7
• Neoplasia	4
• Severe hypoglycaemia	3
• Respiratory diseases (dyspnoea)	3
• Anorexia/asthenia	3
• Neurological signs	2
• Aortic thromboembolism	1
• Intussusception	1
• Heart diseases	1
• Car accident injuries	1
• Drowning	1
• Old age/physical deterioration	4
No cause recorded	8

The median survival time of the 68 dogs was 964 days (range 22–3140 days). Fifty-four of the 68 dogs (79%) lived more than 6 months, 43/68 (63%) more than 1 year, 26/68 (38%) more than 2 years, and 15/68 (22%) more than 3 years (**Figure 1**). Eleven of the 26 dogs (42%) with DKA survived more than 2 years, and 12 dogs (46%) with DKA were still alive by the end of the study.

Different variables were potentially associated with a poor outcome in the univariate analysis, including age, breed, RBC, Hct, glucose, ALP, urea, creatinine, phosphate and sodium concentrations, concurrent diseases, and Cushing's syndrome (**Table 3**).

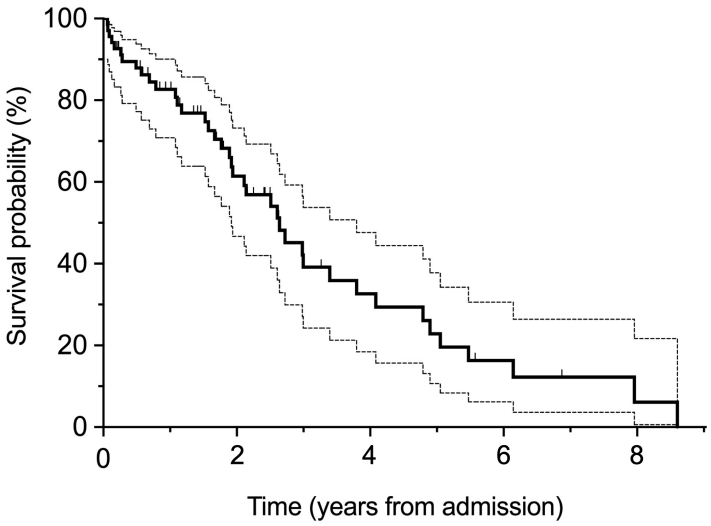

Figure 1. Kaplan-Meier survival curve for 68 dogs with newly diagnosed diabetes mellitus. Solid line represents median survival time and dashed lines 95% CI.

Table 3. Results of univariate and multivariate analysis. Factors potentially associated with survival time (P<0.05) in dogs with newly diagnosed diabetes mellitus. Survival time of diagnostic groups was estimated by Kaplan-Meier analysis and compared by log-rank test.

Variable	**Median survival time, days (P value, log-rank test)**	**Hazard Ratio (95% CI)**	**P value, Cox regression**
Univariate Cox regression analysis			
Age	--	1.01 (1.00–1.02)	0.042
Breed (purebred vs crossbred)	1089 vs. 916 (0.025)	2.17 (1.08–4.37)	0.028
Red blood cells count	--	1.00 (1.00–1.00)	0.007
Haematocrit value	--	1.08 (1.02–1.15)	0.008
Glucose	--	1.04 (1.01–1.07)	0.008
Alkaline phosphatase	--	1.00 (1.00–1.00)	0.012
Urea	--	1.01 (1.00–1.02)	0.008
Creatinine	--	1.00 (1.00–1.00)	0.012
Phosphate	--	1.81 (1.11–2.94)	0.015
Sodium	--	0.94 (0.90–0.99)	0.037
Concurrent diseases (0=absence vs. 1=presence)*	1089 vs.781 (0.045)	1.94 (1.00–3.78)	0.049
Cushing's syndrome (0=absence vs. 1=presence)	993 vs. 645 (0.038)	2.46 (1.01–5.97)	0.045
Multivariate Cox regression analysis			
Haematocrit value	--	1.06 (1.00–1.13)	0.032
Phosphate	--	1.83 (1.13–2.97)	0.013
***Concurrent diseases also include Cushing's syndrome**			

In the multivariate analysis only two variables were retained in the model; in particular higher Hct (HR 1.06, 95% CI 1.00–1.13) and higher serum phosphate concentrations (HR 1.83, 95% CI 1.13–

2.97) at diagnosis were significantly associated with decreased survival time. Of the 65 dogs with available laboratory data concerning serum phosphate at diagnosis, concentrations of serum phosphate were above the reference interval in 24/65 (37%) cases. Moreover, four of the seven dogs (57%) with concurrent Cushing's syndrome had hyperphosphataemia at the time of diagnosis. The ROC curve analysis showed that a serum phosphate concentration of 1.35 mmol/l and an Hct of 46% were the optimal cut-offs to discriminate dogs with short-term survival from dogs with long-term survival. The median survival time was 1748 days (range 22–3140 days) in dogs with serum phosphate concentrations < 1.35 mmol/l and 770 days (range 24–2905 days) in dogs with serum phosphate concentrations ≥ 1.35 mmol/l (**Figure 2**); however, the difference was not significant (P=0.10, log-rank test). A significant difference was reached in the Kaplan-Meier analysis of the Hct (P=0.04, log-rank test); the median survival time was 1089 days (range 96–3140 days) in dogs with Hct < 46% and 708 days (range 22–2242 days) in dogs with Hct ≥ 46%. The categorical variables that yielded a significant value in the Kaplan-Meier analysis (P<0.05, log-rank test) were reported in **Table 3**. Factors such as serum fructosamine, blood Gly Hb, ketoacidosis, and pancreatitis were not associated with survival time.

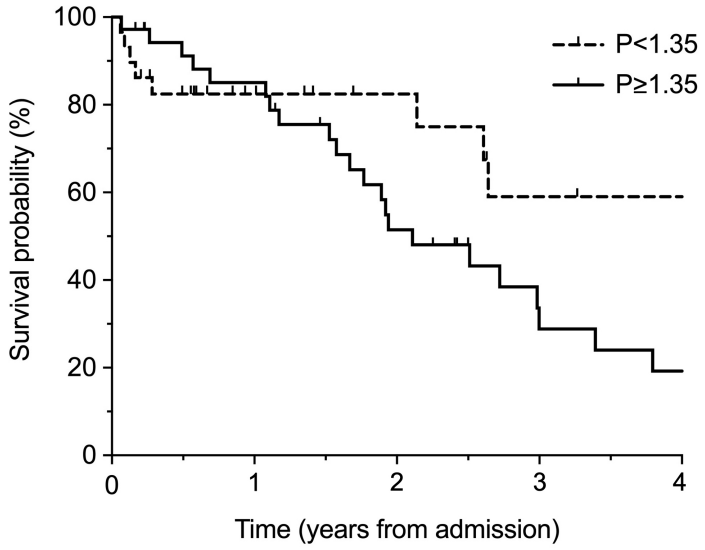

Figure 2. Overall survival in Kaplan-Meier survival curves differentiating two groups of dogs with newly diagnosed diabetes mellitus according to initial serum phosphate (P) concentrations (mmol/l). Survival time has been truncated at 4 years.

Discussion

The dogs in the current study had a median survival time of 964 days (32 months). This is longer than the median survival time of 2 months and 17.3 months reported for a population of insured diabetic dogs in Sweden,[8] and for a population of diabetic dogs attending first opinion practice in England,[3] respectively. This discrepancy may be attributable to the fact that survival times vary between countries and between socioeconomic regions within a country. Furthermore, dogs in the present study were handled in a referral clinic, which implies optimal case management and, possibly, attracts owners with greater motivation than first opinion practices. In the two studies mentioned above, the fact that most of the deaths occurred shortly after DM diagnosis probably reflects a greater rate of elective euthanasia than in the current study. Mattin and others[3] showed that insured diabetic dogs had an increased survival time. This may indicate that DM is a low-cost disease to diagnose but its long-term management requires an important emotional and financial commitment, and therefore not all owners are willing to accept the lifetime treatment option. The results of the present study indicate that diabetic dogs, if well controlled, have a median survival time that can be over 2 years. The cause

of death in diabetic dogs can often be related to diseases other than DM. Nevertheless, in the current study, considering the 31 dogs for which the cause of death/euthanasia was recorded, in at least 10 dogs the cause was diabetes-related.

The haematocrit value and serum phosphate concentrations were significantly associated with survival; therefore, at the time of diagnosis, dogs with higher Hct or serum phosphate concentrations had an increased risk of death. High Hct in diabetic dogs may be caused by dehydration/haemoconcentration resulting from osmotic diuresis; the latter is caused by the presence of glucose and ketone bodies in the urine that results in polyuria. Likewise, the presence of concomitant disorders that induce vomiting (e.g. pancreatitis) or that exacerbate polyuria (e.g. hypercortisolism) may result in a further deficiency of body fluids. Therefore, the finding of severe dehydration and secondary relative erythrocytosis, at the time of diagnosis, may indicate a severe and prolonged diabetic condition, or may suggest the presence of concomitant disorders. Unfortunately, the hydration status of the dogs included in the study was not precisely documented in the medical records, and thus it was not included in the analysis. Furthermore, it is possible that the effect of the haematocrit on survival is minimal, as indicated by the results of the statistical analysis; for this reason future investigations can be useful to confirm the prognostic potential of this variable.

An interesting finding of the current study was that a higher serum phosphate concentration at diagnosis was significantly associated with reduced survival time. The prognostic value of inorganic phosphorus has already been highlighted in other diseases. Fracassi and others[16] found that increased serum phosphate concentrations were associated with a shorter life expectancy in a population of dogs with newly diagnosed pituitary dependent hypercortisolism. Although it was not possible to figure out the cause of hyperphosphataemia in dogs of the aforementioned study, the authors argued that it might be a consequence of reduced renal excretion of phosphate, increased intestinal absorption of phosphate and mobilization of phosphate from bone tissue. In the present study, Cushing's syndrome was detected in seven dogs (10 per cent), among which four (57 per cent) had serum phosphate values above the reference range; in addition, hypercortisolism was found to be associated

with a shorter survival time, therefore it may represent a plausible explanation of the prognostic value of serum phosphate.

King and others[17] reported that higher serum phosphate concentrations were associated with a poor outcome in cats with chronic kidney disease (CKD). Hyperphosphataemia during CKD is caused by a progressive reduction in renal function and the development of secondary renal hyperparathyroidism. In the current study some findings led to the supposition that the occurrence of CKD may be related to increased phosphate concentrations and reduced life expectancy; in fact, the majority of the study population consists of middle-aged and older dogs (median age 10 years) and UPC showed values above the reference interval in 83 per cent of cases with available laboratory data. These results suggest the need for more investigations on diabetic nephropathy, which is a common chronic complication in diabetic humans, and has occasionally been reported in diabetic dogs. Indeed, diabetic nephropathy is initially manifested as proteinuria, primarily albuminuria, and only when the changes in the glomerulus progress does it result in the development of azotemia and clinical signs.[18] However, in the present study CKD was not reported as a cause of death. This may have been partly due to the fact that in many cases it was not possible to ascertain the cause of death, and in none of the cases was a postmortem examination performed.

Finally, an intriguing clue to the possible cause of hyperphosphataemia comes from research in human medicine, in which diabetes mellitus has been associated with a condition of 'functional hypoparathyroidism', which seems to be one of the factors leading to decreased bone mineral density in diabetic patients.[19] Some studies[19-22] have shown altered secretion of parathormone (PTH) in diabetic subjects, however in none of these has it been possible to determine the specific cause; it has been assumed that hyperglycaemia may directly suppress PTH secretion and/or that insulin may be required for the maintenance of parathyroid secreting cells.[23] Some authors also suggested that magnesium depletion, caused by osmotic diuresis, may be an explanation for the reduced secretion and action of PTH.[21,24] Several studies[19,20,22] observed increased renal calcium excretion, according to a lower PTH level, in diabetic subjects; moreover, one study[22] reported a higher serum phosphate

concentration and reduced renal phosphate excretion in diabetic humans with decreased PTH levels. In the present study, the median serum phosphate concentration (1.4 mmol/l) and the mean total serum calcium concentration (2.4 mmol/l) were within the reference ranges; however there was a tendency of the two values towards the upper and lower limits of the reference intervals, respectively. In addition, hyperphosphataemia and hypocalcaemia were detected in 37 per cent and 25 per cent of dogs, respectively. Similarly, in a large study involving 221 diabetic dogs,[25] 20 per cent of subjects showed hyperphosphataemia and 47 per cent had hypocalcaemia at the time of initial examination. These interesting results show that there is an apparent basis for a connection between impaired calcium/phosphate homeostasis and DM in dogs. The data of the current study support the proposal that serum phosphate, at the time of diagnosis, may be a good indicator of long-term outcome. However, further prospective investigations are necessary to determine the exact aetiopathogenesis of the detected clinical-pathological abnormalities, to determine the clinical importance of these findings and to confirm the prognostic value of serum phosphate.

In the current study, the cut-off values of serum phosphate concentrations and Hct, that were used in the Kaplan-Meier analysis, were selected to have the highest sensitivity and specificity in order to discriminate the length of survival between diagnostic groups. However, their clinical usefulness appears limited. This is due to the fact that the cut-off values used are within the reference interval of the respective variables.

The presence of concomitant diseases and Cushing's syndrome was associated with decreased survival time in univariate and Kaplan-Meier analyses, but not in multivariate analysis. This correlation might be explained by the insulin resistance induced by the presence of concomitant disorders, including hypercortisolism as one of the most common causes, which leads to the difficult management of DM; in turn, this results in a diminished propensity of the owners to pursue treatment and an unfavorable outcome. With regard to Cushing's syndrome, this result supports a recent study[26] showing that the occurrence of DM in dogs with hypercortisolism shortens life expectancy. However, univariate analysis does not take into account confounders, for this reason the association between

the presence of Cushing's syndrome, or concurrent diseases, and survival should be interpreted cautiously. Diagnosis of pancreatitis was not associated with survival, a finding that contrasts with a study performed in the UK, in which diabetic dogs with pancreatitis had an increased risk of death.[3] These discrepancies may have resulted from differences in the veterinary facilities (first opinion vs referral clinic) and geographical locations between the studies.

Diabetic ketoacidosis was diagnosed in 38 per cent of dogs, although it was not associated with length of survival. Hume and others[27] reported that, in a population of dogs with naturally occurring DKA, 30 per cent of cases died or were euthanized during hospitalization. However, because these studies had different study populations, methodologies, and geographical locations, they are not directly comparable. In the current study, it is also worth mentioning that 42 per cent of dogs with DKA at diagnosis survived more than 2 years, and 46 per cent of DKA cases were still alive at the time of censorship. These results indicate that ketoacidosis, considered by practitioners as a life-threating condition, is not necessarily associated with a negative prognosis. Therefore, treatment of DKA should always be pursued, consistent with the severity of underlying medical disorders.

Serum glucose was associated with survival in the univariate analysis, but there was no association between glycated proteins and life expectancy. In human medicine, Gly Hb has a strong predictive value for the complications of diabetes mellitus.[28] In addition, higher Gly Hb values have been associated with an increased mortality risk.[29] In the current study, the lack of association between glycated proteins and survival could be accounted for by the fact that laboratory data on serum fructosamine and glycated haemoglobin concentrations were available in 47 percent and 16 percent of dogs, respectively. This deficiency of data is partially due to the fact that many dogs were admitted by the emergency service and endocrinologists saw the case at a later time, when the diagnostic tests had already been performed. The results might have been significant if more laboratory data had been included. Hence, in light of the prognostic importance of Gly Hb in human medicine additional studies aimed at investigating the prognostic potential of glycated proteins are recommended.

The main limitation of the present study is the small number of cases included, which influenced the power of statistics. This derives from the very restrictive inclusion criteria. It is likely that some associations with survival were not detected because of this bias. Other limitations are largely related to the retrospective nature of the study and the incompleteness of some of the records. For instance, in some cases it was not possible to ascertain the cause of death, and the latter was not supported by postmortem examination in any of the cases. Furthermore, important data such as the body condition score (BCS) were not recorded. Thus, the absence of some data may have partially biased the analysis. One limitation is that the laboratory reference intervals were not gathered from an age-matched control population but were those provided by the laboratory for routine use. This could have influenced the number of the dogs with abnormal laboratory findings reported in this study. However, the fact that clinicopathological data were obtained from a single medical laboratory represents a strength of the present study. Further strengths of the study are related to the management of the cases; indeed, all dogs were diagnosed, treated and monitored using standard protocols implemented at a single referral institution.

In conclusion, dogs with newly diagnosed diabetes mellitus had a good prognosis. The survival time was shorter in dogs with higher haematocrit value and higher serum phosphate concentrations. At diagnosis, the presence of pancreatitis might not represent a negative prognostic factor.

7. Survival estimates and outcome predictors in dogs with newly diagnosed diabetes mellitus treated in a veterinary teaching hospital

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Capitolo 8

DISCUSSIONE E CONCLUSIONI

Il DM è la più comune patologia endocrina del cane nel quale tipicamente si manifesta con segni clinici quali poliuria, polidipsia, polifagia e perdita di peso. La diagnosi di tale endocrinopatia è molto semplice e si basa sul riscontro di iperglicemia a digiuno associata a glicosuria. Nel momento in cui viene eseguita la diagnosi di DM è indispensabile intraprendere immediatamente una terapia insulinica al fine di controllare la concentrazione di glucosio nel sangue e quindi la sintomatologia. Nel cane le insuline più utilizzate sono l'insulina lenta di origine suina e l'insulina NPH, entrambe ad azione intermedia. L'efficacia di queste due insuline è stata dimostrata in studi clinici (Lorenzen, 1992; Monroe et al 2005) ottenendo buoni risultati, ma non erano presenti studi che le comparassero in termini di efficacia e sicurezza. Nel **capitolo 3** è riportato lo studio effettuato allo scopo di comparare l'efficacia e la sicurezza di tali preparazioni insuliniche per il trattamento di cani con DM neo-diagnosticato. Dai risultati di questo studio è emerso che entrambe le insuline sono efficaci e sicure per il trattamento del DM non complicato. Sebbene al termine dello studio nel gruppo di cani sottoposti a terapia con insulina NPH vi era una maggiore percentuale di soggetti con migliore controllo glicemico, tale differenza non è risultata statisticamente significativa. Nessuno dei soggetti inclusi ha manifestato ipoglicemia sintomatica ed in generale si è registrata una bassa percentuale di episodi ipoglicemici.

L'obiettivo della terapia insulinica è quello di ottenere delle glicemie che consentano di controllare i segni clinici (tipicamente <250 mg/dL) ed evitare nel contempo l'ipoglicemia. Al fine di valutare l'efficacia della terapia insulinica, il monitoraggio del paziente diabetico è di fondamentale importanza. Quest'ultimo può essere molto difficoltoso poiché i metodi di monitoraggio di cui dispone il clinico quali curva glicemica, misurazione del glucosio interstiziale, quantificazione del glucosio urinario, misurazione delle proteine glicate e valutazione dei segni clinici e del peso corporeo, offrono spesso risultati conflittuali.

La curva glicemica è uno degli strumenti più frequentemente utilizzati dai clinici e, per ottenerla, è necessario misurare la glicemia ogni 2 ore a partire da una goccia di sangue capillare avvalendosi di glucometri portatili (Portable Blood Gluocose Meter, PBGM). Affinché un PBGM possa essere

definito accurato e preciso per l'utilizzo clinico deve possedere determinate caratteristiche in termini di precisione ed accuratezza, valutate attraverso le norme ISO prodotte dall'organizzazione mondiale della sanità. Nel **Capitolo 4** è descritto uno studio che valuta l'accuratezza e la precisione di un glucometro (Gluco Calea, WellionVet; GC) e di un glucometro/chetometro (Belua, WellionVet; BE) ad uso veterinario nel cane, valutandone anche l'interferenza esercitata dal packed cell volume (PCV). Sono stati utilizzati campioni appartenenti a soggetti non anemici (PCV 37-54%) e anemici (PCV< 37%), classificati in 3 range glicemici: alto (>140 mg/dL), medio (90-139 mg/dL) e basso (<90 mg/dL). I valori di glicemia e di 3-β-idrossibutirrato (3-HB) ottenuti tramite l'utilizzo dei due dispositivi, sia da sangue capillare che venoso, sono stati comparati con il corrispondente valore ottenuto con la metodica di riferimento. La precisione è stata valutata esaminando la ripetibilità del risultato *within-run* e *between-day*. È stata individuata una correlazione significativa tra i valori di glicemia ottenuti con ciascuno dei due glucometri e la metodica di riferimento (R^2 >0,89); inoltre è stato dimostrato che il PCV esercita un'influenza significativa sull'accuratezza dei dispositivi, misurando valori di glicemia più elevati quando il PCV è più basso; infine, la precisione è risultata adeguata per entrambi i dispositivi. Tuttavia, nessuno dei due dispositivi soddisfaceva pienamente i requisiti richiesti dalla norma ISO, essendo la percentuale di valori che cadevano all'interno delle zone A+B della Parkes Error Grid Analysis variabile dall'82,2 al 97,8%. La comparazione tra i valori di 3-HB ottenuti con il chetometro BE, da sangue capillare e periferico, e quelli ottenuti con la metodica di riferimento ha dato luogo a una scarsa correlazione (rispettivamente di R^2=0,48 e R^2=0,59). In conclusione, nessuno dei due dispositivi è risultato sufficientemente accurato da consentirne l'utilizzo clinico nel cane.

Il **capitolo 5** riporta lo studio che ha valutato le performance cliniche del Flash Glucose Monitoring System (FGMS), un innovativo sistema di monitoraggio continuo del glucosio interstiziale che, rispetto gli altri CGMS, non necessita di calibrazioni e ha una durata massima di 14 giorni. Tale dispositivo si è rivelato accurato per l'uso nel cane diabetico, tuttavia non erano presenti studi che ne

valutassero l'utilizzo nel monitoraggio a lungo termine dei cani con DM. Dai risultati ottenuti è emerso che vi è una buona concordanza tra la dose insulinica dedotta utilizzando le scansioni del FGMS e quella ricavata valutando le curve glicemiche ottenute tramite l'utilizzo del PBGM. Inoltre, il FGMS si è rivelato più accurato nell'identificare gli episodi ipoglicemici e i nadir del glucosio rispetto l'uso del PBGM il quale, infatti, consente la misurazione della glicemia a intervalli di una o due ore. Dai medesimi risultati è emerso come la dose insulinica ipotizzata da profili glicemici ottenuti in giorni consecutivi possa essere differente e sottolinea quindi l'importanza di tenere in considerazione questo aspetto nel momento in cui si prende una decisione terapeutica valutando una singola curva glicemica. Infatti, come già dimostrato in medicina umana e veterinaria, esiste una variabilità glicemica tra giorni consecutivi che è ascrivibile a diversi fattori. Il monitoraggio del paziente diabetico tramite la singola curva glicemica non consente di tenere in considerazione questo aspetto. Pertanto, i nostri risultati suggeriscono che i CGMS dovrebbero essere maggiormente utilizzati nella pratica clinica quotidiana o, qualora non fosse possibile, si dovrebbero eseguire più curve glicemiche prima di intraprendere una determinata decisione terapeutica riguardo la dose insulinica.

Il **capitolo 6** riporta lo studio che ha valutato le performance di due metodiche per la misurazione di emoglobina glicata (HbA1c) e fruttosamine sieriche ed ha comparato l'abilità delle due proteine glicate nel classificare il controllo glicemico, utilizzando come gold standard uno score clinico. Dai risultati di tale studio è emerso che sia il metodo colorimetrico che quello immunoturbidimetrico, utilizzati rispettivamente per la misurazione di fruttosamine sieriche ed emoglobina glicata, sono precisi (CVs<5%) e lineari (R^2>0.99). Nonostante entrambe le proteine glicate fossero significativamente correlate con lo score clinico, il controllo glicemico veniva correttamente classificato solo nel 50% e 44% dei casi, rispettivamente, da fruttosamine sieriche ed emoglobina glicata. Alcuni autori consigliano l'utilizzo delle proteine glicate per chiarire discrepanze tra i segni clinici e il risultato della curva glicemica o per monitorare quei soggetti in cui, a causa della loro

indole, non è possibile eseguire la curva glicemica. A tale proposito, sono stati proposti dei cut-off che consentano di discriminare i soggetti ben controllati da quelli mal controllati. Dai risultati di questo studio è emerso che la concentrazione delle proteine glicate non dovrebbe essere mai utilizzata come singolo parametro per monitorare i cani con DM e fa sorgere il dubbio della loro utilità nel monitoraggio di questi pazienti. Considerando le caratteristiche di variabilità individuale nel processo di glicazione e i risultati di altri studi in cui il valore di proteine glicate sembra ridursi in modo significativo nei soggetti diabetici in corso di terapia, probabilmente è più appropriato monitorare il singolo paziente sulla base dei suoi precedenti valori di proteine glicate piuttosto che sulla base dei cut-off riportati in letteratura.

Nonostante il DM sia l'endocrinopatia più comune e una delle più studiate del cane, solo pochi studi hanno valutato i fattori prognostici e l'aspettativa di vita dei cani affetti da questa patologia. Tale mancanza in letteratura è probabilmente conseguente al fatto che la diagnosi di DM è spesso eseguita in cliniche di prima opinione, mentre nei centri di referenza vengono riferiti solo casi di difficile gestione una volta che la diagnosi è stata ottenuta e il trattamento già iniziato. Ne consegue che è complesso ottenere dati di laboratorio al momento della diagnosi (prima del trattamento) usando un singolo laboratorio di riferimento. Il **capitolo 7** riporta lo studio il cui obiettivo è stato quello di valutare la sopravvivenza e il significato prognostico di diverse variabili cliniche e clinico-patologiche di cani con DM neo-diagnosticato. Dai risultati è emerso che il tempo di sopravvivenza mediano è di 964 giorni (32 mesi) e che il valore ematocrito e la concentrazione di fosforo sierico sono significativamente associati con la sopravvivenza; quindi, al momento della diagnosi, i soggetti con più alto valore ematocrito e concentrazione di fosfati avevano un maggiore rischio di morte. Il significato di tali riscontri deve ancora essere chiarito ma possibili spiegazioni sono relative al fatto che l'elevato ematocrito nei soggetti con DM potrebbe essere conseguente alla disidratazione/emoconcentrazione indotti dalla diuresi osmotica, spesso aggravata da ulteriori perdite di liquidi indotte da altre patologie concomitanti quali pancreatite e sindrome di Cushing, che causano

rispettivamente vomito e poliuria. Pertanto, i soggetti più disidratati al momento della diagnosi potrebbero riflettere una più prolungata condizione di DM o la presenza di patologie concomitanti che possono influenzare negativamente la prognosi.

L'iperfosfatemia è stata associata a ridotta sopravvivenza anche in altre patologie quali l'ipercortisolismo nel cane e la malattia renale cronica nel gatto. La causa di iperfosfatemia nei soggetti con sindrome di Cushing è ancora da chiarire mentre, in corso di malattia renale cronica, è noto come l'elevata concentrazione sierica di fosforo sia conseguente a ridotta escrezione renale di fosfati e lo sviluppo di iperparatiroidismo secondario renale. È possibile che la concomitante presenza di tali patologie possa aggravare la condizione di DM e ridurre la sopravvivenza di questi soggetti. Inoltre, in medicina umana, il DM è stato associato ad una condizione di ipoparatiroidismo funzionale, che determina ridotta densità minerale ossea nei pazienti diabetici umani. Le cause di tale condizione sono ancora da definire ma è stato ipotizzato che l'iperglicemia possa sopprimere la secrezione di PTH e/o l'insulina possa essere necessaria per il mantenimento dell'attività secretoria delle cellule della paratiroide. Altra ipotesi è che la deplezione di Mg conseguente a diuresi osmotica possa essere causa di ridotta secrezione e azione del PTH. Tale risultato, associato ai riscontri di altri studi in cui approssimativamente il 30% di soggetti diabetici mostrano ipocalcemia ed iperfosfatemia, sottolinea come nei pazienti diabetici probabilmente ci siano alterazioni del metabolismo calcio-fosforo e pongono le basi per ulteriori studi finalizzati a determinare l'esatta patogenesi di questa alterazione clinico-patologica e la sua importanza clinica e prognostica.