D-dimer is a sensitive tool for early diagnosis of thromboembolic disease such as pulmonary thromboembolism (PTE) and disseminated intravascular coagulation (DIC) both in human and veterinary practice. The objective of this study was to evaluate the accuracy and clinical utility of SelexOn™ canine D-dimer device (Infopia, Korea) in comparison to the NycoCard™ D-dimer (Nycomed Pharma AS, Norway). Blood samples were collected from normal (n = 90), clinically ill (n = 33), PTE (n = 40) and DIC (n = 37) diagnosed dogs. The each collected samples were tested with above mentioned two devices and compared to identify any differences. There were no statistically significant differences in D-dimer levels in between two devices either from normal or all diseased dogs. Moreover, the correlation co-efficient was r = + 0.9725. The calculated correlation was significant (p < 0.001) which proves its accuracy. Therefore, SelexOn™ canine D-dimer device is reliable and suitable device to use for the clinical test for measuring of blood D-dimer concentration in dogs.

Introduction

D-dimer is a protein fragment and degradation product of fibrin which is a sensitive tool for early diagnosis of thromboembolic disease such as pulmonary thromboembolism (PTE) and disseminated intravascular coagulation (DIC) both in human and veterinary practice. The objective of this study was to evaluate the accuracy and clinical utility of SelexOn™ canine D-dimer device (Infopia, Korea) in comparison to the NycoCard™ D-dimer (Nycomed Pharma AS, Norway). Blood samples were collected from normal (n = 90), clinically ill (n = 33), PTE (n = 40) and DIC (n = 37) diagnosed dogs. The each collected samples were tested with above mentioned two devices and compared to identify any differences. There were no statistically significant differences in D-dimer levels in between two devices either from normal or all diseased dogs. Moreover, the correlation co-efficient was r = + 0.9725. The calculated correlation was significant (p < 0.001) which proves its accuracy. Therefore, SelexOn™ canine D-dimer device is reliable and suitable device to use for the clinical test for measuring of blood D-dimer concentration in dogs.

Accuracy and Clinical Utility of Selexon™ Canine D-Dimer Device in Measuring of Blood D-Dimer Level in Healthy and Diseased Dogs

Md. Mahbubur Rahman¹, Myung-Jin Kim¹, Hyeon-Kyu Go¹, Hee-Young Ko², Won-Dong Kim², Ji-Hun Shin², Do-Hyung Kim¹*

¹KNOTUS Co., Ltd. Research Center, 189, Donggureung-Ro, Guri-Si, Gyeonggi-Do, Republic of Korea
²Infopia Co., Ltd. 132, Anyang, Cheondong-ro, Dongan-gu, Anyang-si, Gyeonggi-do, Republic of Korea

*Corresponding author: Do-Hyung Kim, Chief of laboratory, KNOTUS Co., Ltd. 189 Donggureung-Ro, Guri-Si, Gyeonggi-Do, Republic of Korea, Tel: +82-31-572-8899; Fax: +82-31-572-6699; E-mail: lab@knotus.co.kr

Abstract

D-dimer is a protein fragment and degradation product of fibrin which is a sensitive tool for early diagnosis of thromboembolic disease such as pulmonary thromboembolism (PTE) and disseminated intravascular coagulation (DIC) both in human and veterinary practice. The objective of this study was to evaluate the accuracy and clinical utility of SelexOn™ canine D-dimer device (Infopia, Korea) in comparison to the NycoCard™ D-dimer (Nycomed Pharma AS, Norway). Blood samples were collected from normal (n = 90), clinically ill (n = 33), PTE (n = 40) and DIC (n = 37) diagnosed dogs. The each collected samples were tested with above mentioned two devices and compared to identify any differences. There were no statistically significant differences in D-dimer levels in between two devices either from normal or all diseased dogs. Moreover, the correlation co-efficient was r = + 0.9725. The calculated correlation was significant (p < 0.001) which proves its accuracy. Therefore, SelexOn™ canine D-dimer device is reliable and suitable device to use for the clinical test for measuring of blood D-dimer concentration in dogs.

improve the mortality in these patients[17,18]. So the scientist focused on developing the new laboratory tests for early diagnosis of hemostasis and prothrombotic states[17]. The measurement of D-dimer is highly sensitive and specific for the rapid diagnosis of early stage active coagulation and fibrinolysis related systemic diseases in dogs[6,12,13].

Immunoenzymatic assay (ELISA), the immuno-turbidimetric assay, and latex agglutination tests are available techniques to measure D-dimer in dogs[9]. The ELISA technique is highly sensitive, but it is not suitable for the emergency case as it is time-consuming as well as it is expertise people and high equipment dependent[21]. Latex agglutination assays have lower sensitivity[22]. Currently, immuno-turbidimetric based sensitive rapid tests are commercially available permitting the optimization of cut-off level for different conditions. SelexOn™ (Infopia, Anyang, Korea) is POCT analyzer utilizing chromatographic immunoassay to quantitatively or qualitatively detect the concentration of more than 30 cardiac, cancer, thyroid and infection markers in human blood such as cardiac troponin I (cTnI), alpha-fetoprotein (AFP), C-reactive protein (CRP), thyroid-stimulating hormone (TSH)[23] and has been working authentically for a long time in the field. Recently, SelexOn™ canine D-dimer is developed and it is a quantitative device capable of measuring D-dimer in dogs. The objective of this study was to evaluate the clinical utility of SelexOn™ canine D-dimer in dogs and compared with a comparison to the NycoCard™ D-dimer (Nycomed Pharma AS, Norway).

Materials and Methods

Animals and sample collection

The dogs (n = 200) which were brought to Seoul local veterinary hospitals by the client for different diseases or for the health assessments were used in this study. 110 dogs had been diagnosed with various diseases (PTE 40, DIC 37 and clinically ill were 33) while 90 of the dogs were healthy. The clinically ill were traumatic injury, neoplasia, hepatic disease and renal disease. The animals were grouped based on clinical findings, imaging tests (ultrasonography, radiography), laboratory results (prolonged PT and aPTT, decreased platelet count, and high D-dimer concentration), blood gas analysis (hypoxygen), echocardiographic criteria for pulmonary hypertension, by the expertise veterinarian in the respective hospitals and further carefully examined by the authors in this study. Primarily, blood samples were collected in the test tube containing EDTA for diagnosis or assessments purpose. Secondarily, the remaining blood was used for this study. The clients were informed about that. SelexOn™ canine D-dimer device (Infopia, Korea) was used to evaluate D-dimer by using blood. Then the remaining blood was centrifuged (3000 RPM for 5 minutes). Then prepared serum was used for D-dimer analysis by NycoCard™ D-dimer (Nycomed Pharma AS, Norway). Measurement of D-dimer concentration was performed within 0-3 hours after sampling. The samples were stored either at 4°C or room temperature for the short-term study. The objective of this study was the evaluation of accuracy and clinical utility of SelexOn™ canine D-dimer device in D-dimer concentration measurement.

Test devices and protocols

The following two POCT analyzers were selected for this study: by NycoCard™ D-dimer (Nycomed Pharma AS, Norway) and SelexOn™ canine D-dimer (Infopia, Anyang, Korea). NycoCard™ D-dimer is quantitative test based upon an immunometric flow-through principle by NycoCard™ Reader II. According to the NycoCard™ manual, the measuring range of NycoCard™ D-dimer is 0.1 – 20.0 mg/L and the cut-off level is 0.3 mg/L. SelexOn™ canine D-dimer is a sandwich assay using two monoclonal antibodies specific to canine D-dimer and is quantified by the thickness of the test line depending on the color of gold nano particle. EDTA-treated venous whole blood is injected into the blood inlet area of test strip and then the erythrocyte is separated and only plasma is transferred. Target analyte in plasma binds to gold-nanoparticle labelled antibody and then when gold-nanoparticle labelled antibody-target analyte complexes pass through the test line that is immobilized antibody specific to analyte, these produce visible red lines on nitrocellulose membrane. The optical system of the SelexOn™ meter measures the intensity of red lines and the integrated software converts the signal intensity to a quantitative result and shows it on the display. According to the SelexOn™ manual, the measuring range of canine D-dimer is 100 – 3000 ng/mL and the cut-off level is 500 ng/mL.

Statistical analysis

Prism 5.03 software (Graph Pad Software Inc., San Diego, CA, USA) were used for statistical analysis of the data. Results are expressed as mean ± standard deviation of the mean (SD). The paired Student’s t-test was used and correlated with the Spearman’s rank correlation coefficient. The level of significance was set at p < 0.05.

Results and Discussion

A total of 200 dogs were included in this study after informing the owners, of these 128 dogs was purebred, including 19 different breeds, and 72 were mixed-breed. 103 of the dogs were male (77 castrated and 26 intact) and 97 of the dogs were female (43 spayed and 54 intact). The age of the 37 dogs were more than 10 years, 121 were over 5 years and 42 dogs were 2 or less than 2 years age. 110 dogs had been diagnosed with various diseases (PTE 40, DIC 37 and 33 were clinically ill) while 90 of the dogs were healthy. All types of dogs were used for sample collection such as different breeds, sex, disease, or healthy were tested and the d-dimer concentration results were compared with NycoCard™ D-dimer (Nycomed Pharma AS, Norway) which was almost near to this popular devices.

In the healthy dogs the average D-dimer level was 140 ± 56 (mean ± standard deviation) in SelexOn™ canine D-dimer which is near to NycoCard™ D-dimer (Nycomed Pharma AS, Norway) (140 ± 62 ng/ml) (Figure 1) indicating its accuracy. The D-dimer concentration level in both devices was within the reference value (0 - 250 ng/ml). DD is the pure degradation products of cross-linked fibrin. Therefore, its concentration in blood is specific for the presence of coagulation, fibrinolysis and thrombosis. Although, thrombosis is thought to be pathological condition but under some circumstances it’s considered as an independent line of host defense in physiology that constitutes an intrinsic effect or mechanism of innate immunity. This called “immunothrombosis” which play an important role in intravascular immunity contributes a wide range of host strategies to
Accuracy and Clinical Utility of Selexon™ Canine D-Dimer Device

detect and protect against pathogens within the vasculature\[^{124}\]. So, it is not strange to present D-dimer in the blood of healthy dogs. Therefore, D-dimer evaluation could be important tools for regular health checkup of dogs and SelexOn™ canine D-dimer could be reliable device for that purpose.

Therefore, D-dimer evaluation could be important tools for regular health checkup of dogs and SelexOn™ canine D-dimer could be reliable device for that purpose.

**Figure 1:** Comparison of the results obtained by SelexOn™ canine D-dimer and NycoCard™ D-dimer tests in healthy and diseased dogs.

NC, normal healthy; CI, clinically ill; PTE, pulmonary thromboembolism; DIC, disseminated intravascular coagulation. Collected from normal (n = 90), clinically ill (n = 33), PTE (n = 40) and DIC (n = 37) diagnosed dogs. The data were analyzed by Bonferroni post hoc test following one-way ANOVA versus the normal control group. There were no significant difference between two groups.

However, the regulation of immunothrombosis or balance between fibrin production and lysis is a critical for determining thrombus size, it dysregulation can lead to pathologic condition like DIC, PTE, myocardial infarction, stroke etc. In these pathologic conditions blood D-dimer elevates. D-dimer concentration measurement is specific and important tool in dogs with DIC\[^{6,17,18}\]. In this study, the average D-dimer in DIC diagnosed dogs was 1891 ± 641 (mean ± standard deviation) in NycoCard™ and in SelexOn™ canine D-dimer was 1844 ± 649 (mean ± standard deviation) (Figure 1). The data of SelexOn™ canine D-dimer showed its accuracy and clinical utility in DIC dogs. PTE is another common disease for mortality in dogs\[^{18,19}\]. Recently, imaging modalities and elevated D-dimer as a laboratory marker are considered as a diagnostic tool for PTE. In this study, D-dimer also elevated level in PTE group. The average levels of D-dimer in the dogs with PTE were 1895 ± 668 (mean ± standard deviation) in NycoCard™ and in SelexOn™ canine D-dimer was 1871 ± 642 (mean ± standard deviation) (Figure 1). Statistically there was no significant difference between the results of two devices. So, the data of SelexOn™ canine D-dimer showing its accuracy of measurement of D-dimer level and clinical utility in PTE dogs. It was reported that D-dimer level also elevates in clinically ill dogs (traumatic injury, neoplasia, hepatic disease and renal disease)\[^{17}\]. Consistently, the average D-dimer level in clinically ill dogs was 532 ± 200 (mean ± standard deviation) in NycoCard™ and in SelexOn™ canine D-dimer was 532 ± 215 (mean ± standard deviation) (Figure 2) in this study which is comparatively higher than normal healthy dogs. Moreover, there were no statistically significant differences in D-dimer levels in between two devices either from normal or all diseased dogs. In addition, the correlation co-efficient was \(r = +0.9725\). The calculated correlation was significant (\(p < 0.001\)) indicating its accuracy and reliability for clinical use.


Accuracy and Clinical Utility of SelexOn™ Canine D-Dimer Device

In conclusion, accumulating data in this study from the samples of normal, DIC, PTE or clinically ill dogs prove that the SelexOn™ canine D-dimer device (Infopia, Korea) promises for optimum accuracy in comparison to the NycoCard™ D-dimer (Nycomed Pharma AS, Norway) for diagnosis or clinical purpose. Therefore, SelexOn™ canine D-dimer device is reliable and suitable to use for the clinical test as an alternative to traditional laboratory devices.

Competing interests: The authors declare no conflicts of interest.

References


Figure 2: Spearman’s rank correlation between SelexOn™ canine D-dimer device and NycoCard™ D-dimer (Alere Technologies AS, Norway) in dogs (n = 200).