Thesis for the degree of Master of Veterinary Medicine

Evaluation of Positive Rate of Canine Pancreatic Lipase Immunoreactivity (SNAP cPL®) in Relationship to the Severity of Clinical Signs and Concurrent Diseases

Jongjin Park

Department of Veterinary Medicine

The Graduate School

Jeju National University

February 2024

Evaluation of Positive Rate of Canine Pancreatic Lipase Immunoreactivity (SNAP cPL®) in Relationship to the Severity of Clinical Signs and Concurrent Diseases

A Thesis submitted to the graduate school of
Jeju National University in partial fulfillment of
the requirements for the degree of Master of veterinary medicine
under the **Woo-Jin Song**

The thesis for the degree of Master of veterinary medicine
by **Jongjin Park**
has been approved by the dissertation committee.

December 2023

Chair	Yun, YoungMin	_____
Member	Kim, Myung-Chul	_____
Member	Song, Woo-Jin	_____

Contents

Evaluation of Positive Rate of Canine Pancreatic Lipase Immunoreactivity (SNAP cPL®) in Relationship to the Severity of Clinical Signs and Concurrent Diseases

Jongjin Park

Department of Veterinary Medicine
The Graduate School
Jeju National University

Abstract

Acute pancreatitis (AP) is a common disorder characterized by the inflammation of the exocrine pancreas in dogs. Clinical signs of acute pancreatitis are usually gastrointestinal (GI) and nonspecific. In addition, the severity of the clinical signs of pancreatitis varies greatly. Therefore, the diagnosis of pancreatitis is challenging. The canine pancreatic lipase immunoreactivity (SNAP cPL®) test is rapid and relatively accurate in diagnosing pancreatitis. The purpose of this study was to evaluate the positivity rate of the SNAP cPL test in dogs with GI signs of pancreatitis at the Veterinary Medical Teaching Hospital of Jeju National University (JejuNU VMTH) and to suggest more specific indications for the SNAP cPL test. Medical records of dogs that presented to the internal medicine department of JejuNU VMTH between November 2022 and June 2023 were reviewed. Data were collected from the electronic medical records. For retrospective classification, dogs that underwent the SNAP cPL test (IDEXX Laboratories Inc., Westbrook, ME, USA) with GI signs (lethargy,

anorexia, diarrhea, and vomiting) were enrolled (n = 80). Additionally, concurrent diseases on the day of the SNAP cPL test were reviewed. A positive SNAP cPL test result was observed in 26 of the 80 dogs (32%) with GI signs. Despite the small sample size, dogs with concurrent chronic kidney disease had a significantly higher positivity rate than those with other diseases. Furthermore, the severity of GI signs using the scoring system and the positivity rate are statistically related; however, a large number of samples are needed to suggest a cut-off value. The SNAP cPL test has a relatively high sensitivity, which may help rule out AP in dogs. Moreover, a scoring system for the severity of GI signs and information on concurrent diseases might help clinicians decide whether a SNAP cPL test is needed.

Keywords: Acute pancreatitis, Gastrointestinal sign, Concurrent disease, SNAP cPL, Dog

Ⅰ. Introduction

Acute pancreatitis is common disorder which is inflammation of exocrine pancreas [1,10]. Etiology and pathogenesis of acute pancreatitis is poorly understood and clinical sign is non-specific [9]. Although Hisological examination is golden standard for diagnosis of acute pancreatitis, but not performed well because it is invasive and may not alter treatment and outcome [1,6]. Therefore, conjunction with history, clinical signs, and diagnostic imaging and serum pancreatic lipase concentration is needed to establish a clinical diagnosis of acute pancreatitis [1,6]. SNAP cPL test was developed to permit more rapid return of result than Spec cPL test. Because of high sensitivity the SNAP cPL test is used to rapidly rule out pancreatitis, and it is recommended that a positive result be followed by quantitative immunoassay, such as SPec cPL. [6]

However, there is no specific indication of perform SNAP cPL test (IDEXX Laboratories Inc., Westbrook, ME, USA). Thus, purpose of this study is to refine indication for SNAP cPL test by evaluating the positive rate of SNAP cPL and the relationship between signalment, age, severity of clinical symptoms, and concurrent diseases.

Ⅱ. Materials and Methods

1. Animals

Medical records of dogs that presented to the internal medicine department of Jeju National Teaching Hospital between November 2022 and June 2023 were reviewed. Electronic medical records (EMR) were used to obtain data, including breed, age, sex, neuter status, weight, body temperature, chief complaint, laboratory tests, and final diagnosis.

2. Inclusion criteria

Dogs that underwent the SNAP cPL test in at least one gastrointestinal session, including those with lethargy, anorexia, diarrhea, and vomiting, were enrolled. All patients had a medical history and underwent a physical examination and SNAP cPL test. Some patients underwent laboratory tests, including complete blood count (CBC) and serum chemistry panels, on the day of the SNAP cPL test. Patients with concurrent diseases were also classified.

3. Clinical sign scoring system

Clinical signs included lethargy, appetite, diarrhea, vomiting, and hyperthermia. Except for anorexia, when evaluating clinical signs, if clinical signs were absent, they were assigned a score of 0; if clinical signs were present, a score of 1 was assigned. Moreover, appetite was classified as non-anorexia, eating little food or snacks, or anorexia (Table 1).

Table 1. Scoring system of clinical sign

	Vomiting	Diarrhea	Lethargy	Hyperthermia
Nonexistence	0	0	0	0
existence	1	1	1	1

	Appetite
Non-anorexia	0
Eat little bit of food	1
Only eat snack	2
Anorexia	3

4. Laboratory findings

CBC and serum chemistry panels were performed using routine methods at the diagnostic laboratories of Jeju National Teaching Hospital. The SNAP cPL test was performed at Jeju National Teaching Hospital according to the manufacturer's instructions using the SNAP Pro Analyzer, and only trained technicians performed the test.

5. Concurrent disease

Concurrent diseases were classified as chronic kidney disease (CKD), myxomatous mitral valve disease (MMVD), and endocrinopathy, including hyperadrenocorticism (HAC), hypothyroidism, and diabetes mellitus (DM). CKD was diagnosed based on the guidelines of the International Renal Interest Society (IRIS). MMVD was diagnosed based on the 2019 American College of Veterinary Internal Medicine (ACVIM) consensus. HAC was diagnosed based on the 2012 ACVIM consensus guidelines. Hypothyroidism was diagnosed according to the 2001 Veterinary Clinics of North America guidelines. The diagnosis of DM was based on the 2018 American Animal Hospital Association Diabetes management guidelines [2,3,12,13].

6. Statistical analysis

Age and clinical sign was compared by two-tailed unpaired student's *t*-test. Concurrent diseases were compared by chi square test. Statistical significance was set at $P < 0.05$

Ⅲ. Results

The final study population comprised 80 dogs with 43 males, including 38 castrated males, and 37 females, including 33 spayed females. The mean age of the dogs at the time of the SNAP cPL test was 9 years (range 8 month-17 years), and the standard deviation age of the dog was 3.7 years. Mean weight of the dogs at time of SNAP cPL test performed was 9.37 kg (range 1.5 kg-44.4 kg), and standard deviation weight of the dog was 9.1 kg. There were 22 Maltese, 8 poodles, 7 mixed breeds, 7 Shih Tzus, and 6 Pomeranians. The remaining 30 dogs represented a variety of breeds, including Italian Greyhound, Jindo dog, Chihuahua, Labrador retriever, Bernese Mountain, Welsh Corgis, Golden Retriever, French bulldog, Bichon Frise, Alaskan Malamute, Sptiz, Minipin, Cocker Spaniel, Malinois, and Dachshund (Table 2).

Table 2. Signalment of patients that performed SNAP cPL test

Sex		**Age**	
Male	5	Average	9 years
Castrated male	38	(range)	(8 month-17 years)
Female	4	Standard deviation	3.7 years
Spayed female	33		
Breed		**Weight**	
Maltese	22	Average	9 years
Poodle	8	(range)	(1.5 kg-44.4 kg)
Mixed	7		
Shih tzu	7		
Pomeranian	6	Standard deviation	3.7 years
Others	30		

Dogs in the SNAP-cPL test-positive group (n = 26) were significantly older than those in the SNAP-cPL test-negative group (n = 54) (10.46 versus 8.32, p = 0.0157) (Figure 1).

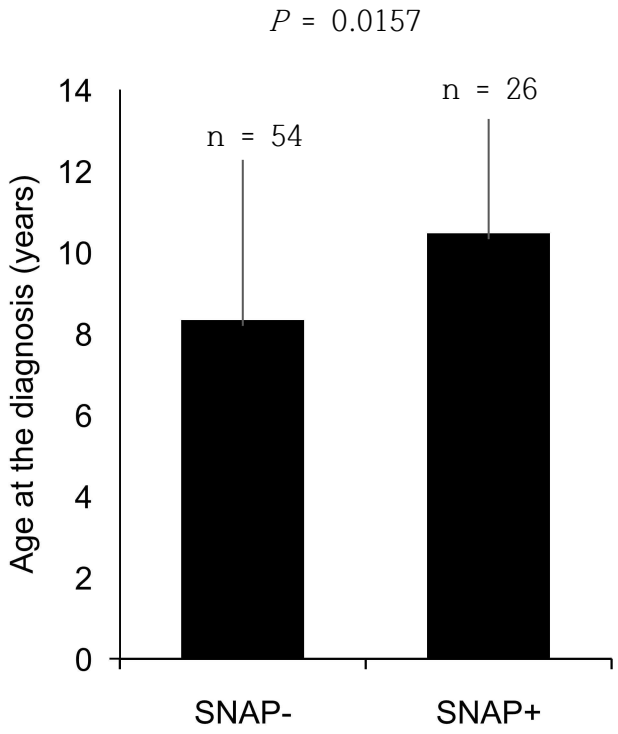

Figure 1. Age associated with SNAP cPL test. SNAP-cPL test-positive group were older than SNAP-cPL test-negative group, and there were significantly different.

Dogs in the SNAP-cPL test-positive group (n = 26) had a significantly greater number of clinical signs, including lethargy, appetite, diarrhea, vomiting, and hyperthermia, than those in the SNAP-cPL test-negative group (n = 54) (2.52 versus 1.32, $p < 0.01$) (Figure 2). Moreover, the SNAP-cPL test-positive group (n = 26) had significantly higher clinical sign scores using the scoring system than the SNAP-cPL test-negative group (n = 54) (3.38 versus 2.64, $p = 0.021$) (Figure 3).

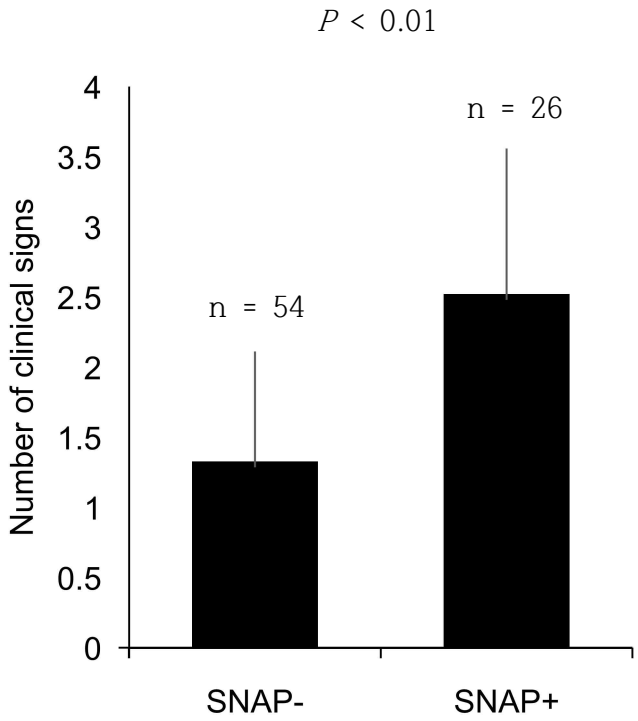

Figure 2. Number of clinical signs associated. SNAP cPL test-positive group had greater number of clinical signs than those in the SNAP cPL test-negative group, and there were significantly different.

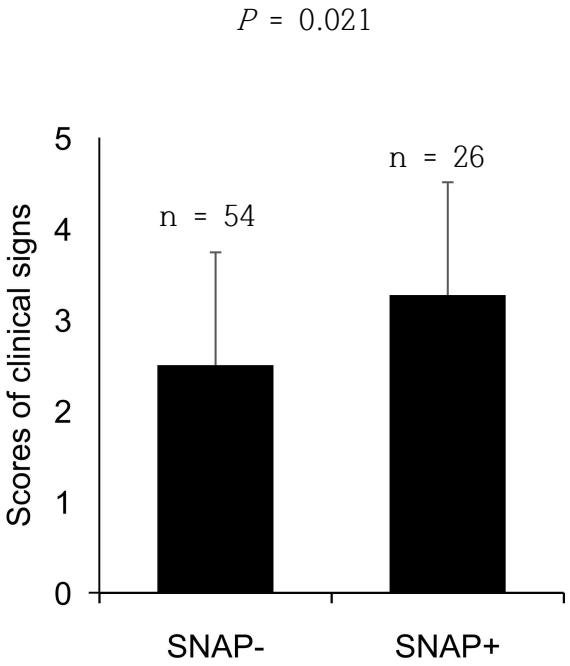

Figure 3. Scores of clinical signs that performed SNAP cPL test. SNAP-cPL test-positive group had higher clinical sign scores using the scoring system than the SNAP-cPL test-negative group, and there were significantly different.

Dogs in the CKD group (n = 10) had a significantly higher SNAP cPL test-positive rate than those in the non-CKD group (n = 70) (60% vs. 27.53, p = 0.0391) (Figure 4). However, the MMVD group (n = 17) showed a difference from the non-MMVD group (n = 63) in the SNAP cPL test-positive rate, but the difference was not statistically significant (41.17 versus 30.15, p = 0.3894) (Figure 5). Dogs in the endocrinopathy group (n = 8) had a higher SNAP cPL test-positivity rate than those in the non-endocrinopathy group; however, these differences were not statistically significant. (n = 72) (62.5 versus 29.16, p = 0.0561) (Figure 6).

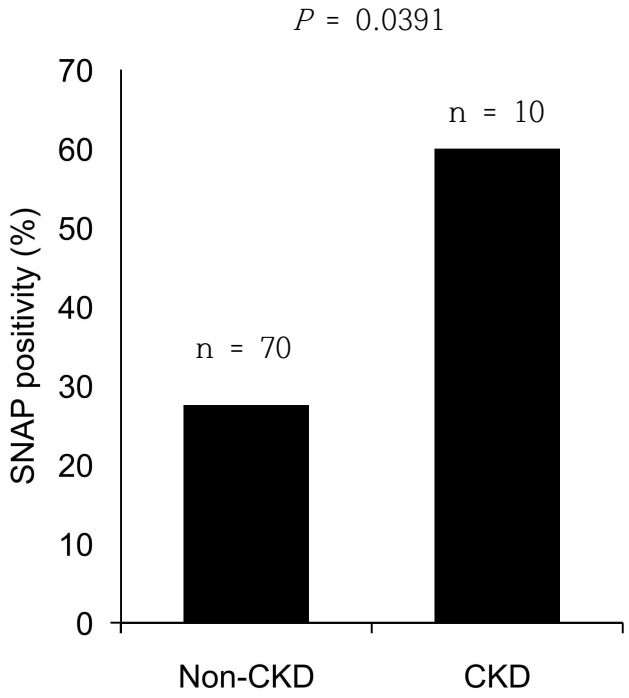

Figure 4. Chronic kidney disease (CKD) associated with SNAP cPL test. Dogs in the CKD group had higher SNAP cPL test-positive rate than those in the non-CKD group, and there were significantly different.

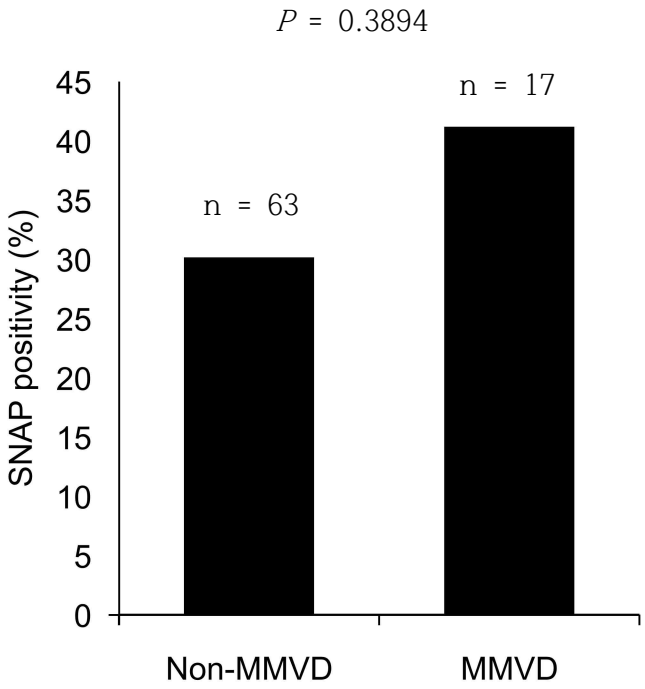

Figure 5. Myxomatus mitral valve disease (MMVD) associated with SNAP cPL test. The MMVD group were not significantly different than the non-MMVD group in the SNAP cPL test-positive rate.

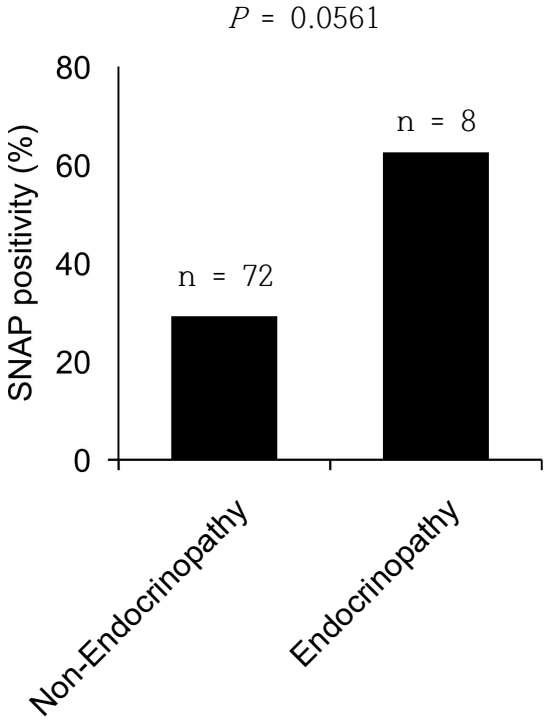

Figure 6. Endocrinopathy associated with SNAP cPL test. Dogs in the endocrinopathy group had a higher SNAP cPL test-positivity rate than those in the non-endocrinopathy group, but there were not significantly different.

Ⅳ. Discussion

This study showed that the mean age of the SNAP-cPL test-positive group was significantly higher than that of the SNAP-cPL test-negative group, which may have been because of the incidence of chronic pancreatitis. The prevalence of chronic pancreatitis increases with age. Watson *et al.* suggested that the mean age of patients with chronic pancreatitis was 9.1 years [15,16]. Moreover, chronic pancreatitis may have high pancreatic lipase immunoreactivity; therefore, chronic pancreatitis itself can increase pancreatic lipase levels, resulting in a positive SNAP cPL test [15]. Furthermore, chronic pancreatitis can cause acute pancreatitis [15]. The prevalence of chronic pancreatitis increases with the increasing age of dogs. As cases of chronic pancreatitis may also have acute pancreatitis, it may result in a positive SNAP cPL test. Thus, based on the previous study, it can be assumed that the higher average age of the SNAP cPL test-positive group than that of the SNAP cPL test-negative group in this study was related to chronic pancreatitis.

This study also showed a relationship between concurrent diseases, including CKD and endocrinopathy, and SNAP cPL test positivity rate. In humans, several concurrent conditions, such as heart disease and CKD are recognized as risk factors for acute pancreatitis [4,8]. Additionally, Cridge *et al.* identified the risk factors for pancreatitis in dogs, including endocrinopathy [5]. While Cridge *et al.* did not specifically propose CKD as a potential risk factor for pancreatitis in dogs, our study revealed an association between CKD and SNAP cPL test positivity [5]. Furthermore, existing research has explored the relationship between acute pancreatitis and various concurrent diseases, including hepatobiliary abnormalities, kidney disease, hypothyroidism, hyperadrenocorticism, and diabetes mellitus [7]. However, it is important

to note that the identified risk factors may not consistently indicate a causative relationship, and may instead be incidental or reflect shared risk factors for disease [7]. Consequently, comprehensive research is required to elucidate the association between multiple concurrent diseases across diverse populations. Previous studies have confirmed that age and many concurrent diseases can act as risk factors for acute pancreatitis [5,7,9]. The findings of this study that the average age of the SNAP cPL test-positive group was higher than that of the negative group, and that CKD and endocrinopathy might be related to the SNAP cPL test positivity rate are consistent with those of previous studies. Thus, the SNAP cPL test should be performed more actively in patients with gastrointestinal (GI) symptoms who are over 10 years old and have CKD and endocrine diseases.

Although histopathology of the pancreas is the gold standard for diagnosing acute pancreatitis, its progression is difficult [1]. Therefore, the diagnosis of acute pancreatitis relies on clinical signs, serological tests, and ultrasonographic findings [1,9]. Similarly, for the diagnosis of acute pancreatitis in humans, at least two of the following symptoms must be present: abdominal pain; serum lipase or amylase activity; and detection by ultrasonography, computed tomography, or magnetic resonance imaging [14]. Notably, abdominal pain is a crucial factor in diagnosing acute pancreatitis [14]. However, diagnosing abdominal pain in dogs remains challenging [7]. Cridge *et al.* observed that abdominal pain occurred at a relatively low rate (32%) in patients with acute pancreatitis [7]. Anorexia was the most common symptom, followed by diarrhea and vomiting [7]. This may be because of the difficulty in recognizing pain. Moreover, ultrasonography is subjective and difficult to perform in veterinary medicine depending on the patient's condition. Thus, the SNAP cPL test and clinical signs are commonly used for diagnosing

acute pancreatitis [7].

Additionally, according to a recent study, the symptoms of lethargy and anorexia, which were identified at a high rate in the cridge's study, can also be indicative of pain [11]. Therefore, dividing the assessment into more objective clinical signs, including anorexia, lethargy, vomiting, diarrhea, and hyperthermia, rather than relying solely on abdominal pain, may aid in identifying the indications for the SNAP cPL test based on human diagnostic standards. Therefore, in this study, a scoring system based on clinical signs was employed. It was suggested that when more than two of the five clinical signs or a score of more than 3 on the scoring system is present, the SNAP cPL test should be considered.

This study has several limitations. First, the sample size was relatively small. Additionally, acute pancreatitis was suspected based on the SNAP cPL test and clinical signs; however, not all patients underwent the quantitative immunoassay, such as the Spec cPL test. Furthermore, CKD was not categorized according to the IRIS stage, and MMVD was not classified according to the ACVIM stage. Additionally, chronic pancreatitis was not specifically classified. Finally, variations among clinicians may have introduced subjective factors into the analysis.

Ⅴ. Conclusion

Dogs in the SNAP cPL test-positive group had a significantly older than SNAP cPL test-negative group. Dogs in the SNAP cPL test-positive group had a significantly greater number of clinical signs and higher clinical sign scores using the scoring system than the SNAP cPL test-negative group. Moreover, dogs in the CKD group had a significantly higher SNAp cPL test-positive rate than those in the non-CKD group.

Therefore, this study suggests that the SNAP cPL test should be more actively considered when the patient age is 10 years or older, the number of GI clinical signs is three or more, the clinical scoring system is 2.5 points or higher, or concurrent diseases, such as CKD and endocrine disorders are present.

Ⅵ.References

1. Aupperle-Lellbach H, Törner K, Staudacher M, Stadler C, Tress U, Grassinger JM et al. Histopathological findings and canine pancreatic lipase immunoreactivity in normal dogs and dogs with inflammatory and neoplastic diseases of the pancreas. J Vet Intern Med 2020;34(3):1127-1134.
2. Behrend E, Holford A, Lathan P, Rucinsky R, Schulman R. 2018 AAHA Diabetes Management Guidelines for Dogs and Cats. J Am Anim Hosp Assoc 2018;54(1):1-21.
3. Behrend EN, Kooistra HS, Nelson R, Reusch CE, Scott-Moncrieff JC. Diagnosis of spontaneous canine hyperadrenocorticism: 2012 ACVIM consensus statement (small animal). J Vet Intern Med 2013:1292-304.
4. Bexelius TS, Ljung R, Mattsson F, Lagergren J. Cardiovascular disease and risk of acute pancreatitis in a population-based study. Pancreas 2013;42(6):1011-5.
5. Cridge H, Lim SY, Algül H, Steiner JM. New insights into the etiology, risk factors, and pathogenesis of pancreatitis in dogs: Potential impacts on clinical practice. J Vet Intern Med 2022;36(3):847-864.
6. Cridge H, MacLeod AG, Pachtinger GE, Mackin AJ, Sullivant AM, Thomason JM et al. Evaluation of SNAP cPL, Spec cPL, VetScan cPL Rapid Test, and Precision PSL Assays for the Diagnosis of Clinical Pancreatitis in Dogs. J Vet Intern Med 2018;32(2):658-664.
7. Cridge H, Scott N, Steiner JM. Risk Factors and Clinical Presentation in Dogs with Increased Serum Pancreatic Lipase Concentrations-A Descriptive Analysis. Animals (Basel) 2022;12(12):1581.
8. de Tersant M, Kwon T, Macher MA, Maisin A, Deschênes G, Niel O. Hypernatremia and acute pancreatitis in chronic kidney disease: back to the salt mines. Questions. Pediatr Nephrol 2018;33(7):1155-1156.

9. Dias-Pereira, P. Morbidity and mortality in elderly dogs – a model for human aging. BMC Vet Res 2022;18,457.
10. Forman MA, Steiner JM, Armstrong PJ, et al. ACVIM consensus statement on pancreatitis in cats. J Vet Intern Med. 2021;35:703-723.
11. Gruen ME, Lascelles BDX, Colleran E, Gottlieb A, Johnson J, Lotsikas P, Marcellin-Little D, Wright B. 2022 AAHA Pain Management Guidelines for Dogs and Cats. J Am Anim Hosp Assoc 2022;58(2):55-76.
12. Keene BW, Atkins CE, Bonagura JD, Fox PR, Häggström J, Fuentes VL, et al. ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. J Vet Intern Med 2019;33(3):1127-1140.
13. Kemppainen RJ, Behrend EN. Diagnosis of canine hypothyroidism. Perspectives from a testing laboratory. Vet Clin North Am Small Anim Pract 2001;31(5):951-62, vii.
14. Walkowska J, Zielinska N, Tubbs RS, Podgórski M, Dłubek-Ruxer J, Olewnik Ł. Diagnosis and Treatment of Acute Pancreatitis. Diagnostics (Basel) 2022;12(8):1974.
15. Watson PJ, Archer J, Roulois AJ, Scase TJ, Herrtage ME. Observational study of 14 cases of chronic pancreatitis in dogs. Vet Rec 2010;167(25):968-76.
16. Watson PJ, Roulois AJ, Scase T, Johnston PE, Thompson H, Herrtage ME. Prevalence and breed distribution of chronic pancreatitis at post-mortem examination in first-opinion dogs. J Small Anim Pract 2007;48(11):609-18.

임상증상의 정도와 병발질환에 따른 개 췌장유래 리파제 검사(SNAP cPL)의 양성율 평가

박 종 진

제주대학교 대학원 수의학과

요약

급성 췌장염은 개에서 흔히 나타나는 외분비 췌장의 장애로 외분비 췌장의 염증을 특징으로 한다. 이러한 급성 췌장염의 임상 증상은 주로 위장관 증상으로 비특이적으로 나타난다. 또한, 급성 췌장염에서 임상 증상의 정도는 환자에 따라 크게 다르므로 췌장염을 진단하는 것은 어렵다. 이때, 개 췌장유래 리파제 (SNAP cPL®) 테스트는 급성 췌장염을 진단하는 데 빠르고 비교적 정확한 테스트이다. 본 연구의 목적은 제주대학교 수의과대학 부설동물병원에서 위장관 증상을 보이는 개들의 SNAP cPL 테스트 양성률을 평가하고 더 구체적인 SNAP cPL 테스트의 적용 지침을 제안하는 것이다. 데이터는 전자 차트를 기반으로 하여 2022년 11월부터 2023년 6월까지 제주대학교 수의과대학 부설동물병원 내과에 내원한 개들을 대상으로 후향적으로 모집되었다. 전자 차트를 통해 소화기 증상(기력저하, 식욕저하, 설사 및 구토)을 보인 개들 (n = 80)이 포함되었다. 또한, SNAP cPL 테스트 당일의 동반 질환에 대한 정보도 검토되었다. 80마리의 개 중 26마리 (32%)에서 SNAP cPL 테스트 양성 결과를 확인하였다. 샘플 크키가 작음에도 불구하고, 만성 신장 질환을 가진 개들은 다른 질환을 가진 개들보다 유의미하게 높은 양성률을 보였다. 또한, 점수 체계를 사용하였을 때 위장관 증상의 심각도와 양성률 간에 통계적인 관련성이 있었으나, 경계값을 제안하기 위해서는 큰 샘플 수가 필요하였다. SNAP cPL 테스트는 개의 급성췌장염을 배제하는데 상대적으로 높은 민감도를 가지고 있으며 위장관 증상의 심각도에 대한 점수 체계 및 동반 질환에 대한 정보는

수의사가 이러한 SNAP cPL 테스트의 필요성을 결정하는 데 도움이 될 수 있다.

주요어: 급성 췌장염, 소화기 증상, 동반 질환, SNAP cPL, 개

감사의 글

정말 많은 분들의 도움으로 지금의 논문을 작성할 수 있었습니다. 말로써 다 표현할 수는 없겠지만, 그래도 정말 감사하는 마음을 담았습니다.
저를 제주대학교 수의과대학 석사과정으로 받아주시고 논문 작성을 비롯한 임상수의사로서의 길을 잘 지도해주신 것 뿐 아니라, 앞으로의 방향성도 세세하게 알려주시는 송우진 교수님께 존경과 감사의 말씀을 드립니다. 학부생 때부터 내과학교실로 절 받아주시고 졸업과 공부에 많은 도움을 주신 것 뿐 아니라, 졸업 이후에도 내과학교실을 따뜻하게 해주시는 윤영민 교수님께 감사의 말씀을 드립니다. 논문 작성에 정말 많은 도움을 주시고 논문 뿐만 아니라 지식적인 면에서도 제가 모르는 부분에 있어 항상 친절하게 대해주시면 자세하게 알려주시려고 하는 김명철 교수님께 감사의 말씀을 드립니다. 다시 한번, 제주대학교 동물병원의 발전에 많이 신경 써주시고 정말 많은 기회를 가질 수 있도록 해주신 윤영민 교수님, 송우진 교수님, 김명철 교수님께 감사드립니다.
함께 2년을 달려 의지할 수 있게 도와준 제주대학교 석사과정 동기 이새영 선생님, 김민건 선생님께 정말 감사합니다. 그리고, 정말 많이 알려주시고 도움 주신 작년 2년 차 선생님인 안혜린 선생님, 조희수 선생님, 구지영 선생님께 감사드리며, 작년에 졸업하고 제주대학교 부설동물병원 팀장으로 있으며 옆에서 많은 도움을 주신 최솔지 팀장님께 감사드립니다. 또한, 지금 1년 차 선생님들의 많은 지지로 졸업할 수 있었고 논문을 작성할 수 있었습니다. 감사합니다.
제주대학교 부설동물병원에서 진료에 정말 많은 도움을 주시고 부족한 저를 잘 가르쳐 준 수의사 선생님들, 수의테크니션 선생님들, 영상기사 선생님께 감사드립니다.
29년 동안 항상 응원해주시고, 옆에서 버팀목이 되어주시며 조언과 응원을 아끼시지 않은 부모님 너무나 감사드리고 존경하고 사랑합니다.
끝으로 8년이라는 긴 시간동안 제주대학교에서 공부할 수 있게 도움을 주신 교수님들, 동기들, 선배와 후배들에게 정말 감사의 말씀을 전합니다. 앞으로도 계속해서 정진해나가는 수의사가 되겠습니다.

2024년 2월
박종진