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New developments in the diagnosis of canine pancreatitis: utilization of in-house testing with FUJI DRI-CHEM

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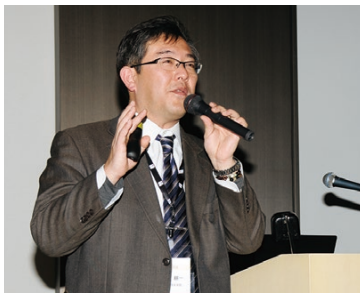

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(as of 2014)

The development of pancreatic lipase immunoreactivity (PLI) has become a major turning point in the diagnosis of canine pancreatitis.However, many aspects of canine pancreatitis are still not fully understood, and issues such as severity and prognosis prediction remain as future investigative challenges.Recently, FUJI DRI-CHEM parameter was developed in Japan. It can measure novel lipase activity, which is highly specific to pancreatic lipase and canine C-reactive protein. Thus, in this article, I discuss the future outlook regarding the diagnosis of canine pancreatitis.

Review of the current status of the diagnosis of canine pancreatitis

■ Should we diagnose canine pancreatitis even if there are no symptoms?

First, I would like to review the diagnosis of canine pancreatitis. Since the launch of PLI, a major question regarding the diagnosis of pancreatitis remains: "Even if a dog is symptom free, should the dog undergo diagnostic testing to ascertain if it has pancreatitis?" Whether PLI assay or other assays should be performed for screening depends on future research. At present, however, pancreatitis is often diagnosed only after symptoms are observed. Thus, first, I will explain the risk factors and symptoms of pancreatitis.

—Risk factors and symptoms of pancreatitis (Table 1)

Regarding breeds, there is a higher risk for pancreatitis in Yorkshire Terriers and Miniature Schnauzers.As has been said previously, a high-fat diet is also a risk factor in dogs. However, whether obesity is truly a risk factor is disputable. There have been multiple reports of studies on hyperlipidemia in recent years, and indeed, hyperlipidemia is considered to be an important risk factor in dogs. Diabetes, hyperadrenocorticism, hypothyroidism and other diseases are also known to be risk factors and these diseases are also known to predispose to hyperlipidemia. IBD, GDV, cholangitis, posttraumatic/postoperative state, and hypercalcemia have also been suggested as risk factors.Regarding symptoms, dogs tend to exhibit symptoms relatively easily, and in particular,

Table 1 Risk factors for pancreatitis and symptoms of pancreatitis

Cited/adapted from reference 1

Risk factors for pancreatitis	Symptoms of Pancreatitis
Breed: Yorkshire Terrier, Miniature Schnauzer	Dogs (n = 70)
High-fat diet/(obesity?)	· Loss of appetite 91%
Hyperlipidemia (dog) Diabetes, hyperadrenocorticism, and hypothyroidism	· Vomiting 90%
Gastrointestinal/biliary tract diseases IBD, GDV, and cholecystitis	· Debilitation 79% · Abdominal pain 58% · Dehydration 97% · Diarrhea 33% · Pyrexia 32% · Jaundice 26%
Posttraumatic/postoperative state	
Drugs and hypercalcemia (?).	

Table 2 Blood tests and biochemical tests in pancreatitis

You can see that many of the findings of blood tests are nonspecific. Regarding biochemical tests, it is important to distinguish pancreatitis from liver diseases Cited/adapted from reference 1

Blood tests			
Dogs (n = 70)			
· Thrombocytopenia	59%		
· Neutrophilia and left shift	55%		
· Anemia	29%		
Biochemical tests			
Cl ↓	81%	TBil ↑	53%
ALP ↑	79%	Alb ↓	50%
P ↓	68%	TCho ↑	48%
ALT ↑	61%	Glu ↓	39%
BUN ↑	59%	Glu ↑	30%

vomiting is much more frequent in dogs than in cats. Other well-known symptoms include abdominal pain, loss of appetite, and diarrhea. In Table 1, the only symptom that requires attention is jaundice. In dogs, biliary obstruction accompanying pancreatitis is not uncommon, and in cats, concurrent development of cholangitis and pancreatitis is not uncommon. Therefore, when jaundice is observed, pancreatitis must be included in any differential diagnosis.

—Blood tests and biochemical tests (Table 2)

In blood tests, decreased platelet count, neutrophilia with left shift, and anemia are often seen, but these are nonspecific findings. In biochemical tests, elevated hepatic enzyme levels (ALT and ALP), bilirubin, and BUN are often seen. Therefore, it is important to distinguish pancreatitis from liver diseases. In general, blood glucose level often goes up. With some complications, however, it may go down. Lipase is central to the diagnosis of pancreatitis and is described later.

—Imaging tests

There are few salient findings that can be obtained by X-ray examination. Therefore, it is extremely difficult to diagnose pancreatitis with this modality.On the other hand, abdominal ultrasonography is considered to have a certain degree of diagnostic utility. The specificity of ultrasonography is relatively high. However, the sensitivity varies among reports because it is highly dependent on the equipment used and the operator's skill. Moreover, it has been reported that the more histopathologically severe pancreatitis is, the more likely it is that abnormal findings will be obtained using ultrasonography [2]. In other words, mild pancreatitis is difficult to detect using this method. Characteristic ultrasonographic findings include an enlarged pancreas, decreased echogenicity, increased peripancreatic echogenicity, fluid accumulation, and dilatation of the common bile duct. In dogs and cats, the current consensus is that there is no significant benefit in routinely performing CT.

—Exploratory laparotomy and biopsy

Histopathological examination is the gold standard for diagnosis in most diseases. At present, however, I do not recommend exploratory laparotomy or biopsy for the diagnosis of pancreatitis. Some of the reasons for this stance are as follows: macroscopic findings obtained by exploratory laparotomy are not consistent with histological findings, and lesions of pancreatitis are often localized. According to a report on the distribution of lesions in dogs with pancreatitis [3], in 20% of the dogs, pancreatitis was actually observed in the entire pancreas, and dogs whose lesions were localized within 40% or less of the whole pancreas accounted for half of the dogs. The deterioration of perfusion if anesthesia is used for dogs with acute pancreatitis also causes a problem.

■ Then, how about measuring pancreatic enzymes?

—Conventional amylase activity and lipase activity

Regarding the measurement of conventional serum amylase and lipase activity, it was previously known that these enzyme measurements are of little significance, especially in cats, due to their extremely low sensitivity and specificity. Although canine lipase activity has a certain degree of utility, it is not possible to measure only pancreatic-specific enzymes due to substrate limitations. Therefore, the diagnostic significance of measuring canine lipase activity has been considered to be low (however, lipase in ascites is believed to be highly specific for pancreatic conditions [4]).

—Trypsin-like immunoreactivity (TLI)

A method for measuring trypsin/trypsinogen using antibodies was developed and has been reported to be highly specific for pancreatic exocrine [5]. However, a subsequent investigation pointed out that its utility in diagnosing pancreatitis is not particularly high. However, TLI is extremely useful for diagnosing exocrine pancreatic insufficiency.

—Pancreatic lipase immunoreactivity (PLI)

Then, the measurement of pancreatic lipase immunoreactivity (PLI) developed in response to this situation. PLI is a method for measuring pancreatic lipase immunologically by generating antibodies that bind only to pancreatic lipase on the basis of the amino acid sequence specific to the lipase derived from the pancreas. Various studies have proved that PLI is currently the most sensitive and specific among serological markers and test methods for canine pancreatitis [6–8].

Current diagnosis of pancreatitis

■ Novel methods for measuring lipase activity

As described above, conventional measurement systems not only measure pancreatic lipase activity but also lipases from other organs (gastric and hepatic lipases and lipoprotein lipase). This makes it difficult to use lipase activity as an indicator for diagnosing pancreatitis. In recent years, however, enzyme measurement systems using new substrates that are highly specific to pancreatic lipase have been developed. One of them is the measurement of lipase activity using DGGR (1,2-o-dilauryl-rac-glycero-3-glutaric acid-[6'-methylresorufin] ester) developed by Roche as a substrate. While it has been adopted to some degree, the necessity of having to always outsource measurement testing has limited its use.

—Triglyceride method

In addition to the DGGR method, the triglyceride method is also a novel method for measuring lipase activity.
FUJIFILM Corporation has developed a dry form of emulsified triolein, a substrate for the triglyceride method, (FUJI DRI-CHEM SLIDE v-LIP-P) and this has enabled the measurement of lipase activity in the blood using an in-house automatic biochemical analyzer (Figure 1).

—Is v-LIP a marker that can be used for the diagnosis of pancreatitis?

Since this is a relatively new measurement system, there has not been sufficient investigation into its clinical usefulness compared with PLI. Thus, we investigated its usefulness at our hospital, and here I provide a summary of the investigation (Dr. Mariko Suzuki at our hospital presented the findings of this investigation at the Japanese College of Veterinary Internal Medicine, the 10th Anniversary Scientific Meeting).

Investigation of correlation between v-LIP and cPLI [9]

v-LIP, cPLI (Spec cPL™, IDEXX), and CRP were measured in 102 dogs suspected of having pancreatitis on the basis of symptoms, blood tests, abdominal ultrasonography, and other findings, and a strong correlation between v-LIP and cPLI was observed (r_S = 0.912) (**Figure 2**). If the results of cPLI were used as the gold standard for the diagnosis of pancreatitis (if 200 μg/L was used as the reference value), v-LIP had a positive predictive value of 98.1% and a negative predictive value of 74.0% (**Figure 3**).

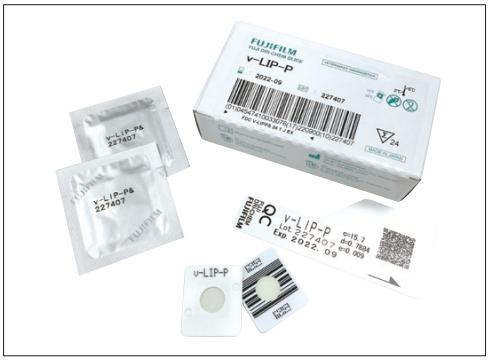

Figure 1 **FUJI DRI-CHEM and v-LIP-P SLIDE**

FUJIFILM Co., Ltd. developed a novel substrate (emulsified triolein) for a dry-based pancreas-specific lipase activity assay. When measuring v-LIP in plasma samples, jaundice does not affect measurement provided it does not exceed 20 mg/dL, and measurement is not affected by chyle. However, it is preferable to avoid hemolyzed samples.
FUJI DRI-CHEM can also measure CRP, and jaundice and chyle do not have a marked effect on the measurement of CRP in plasma samples

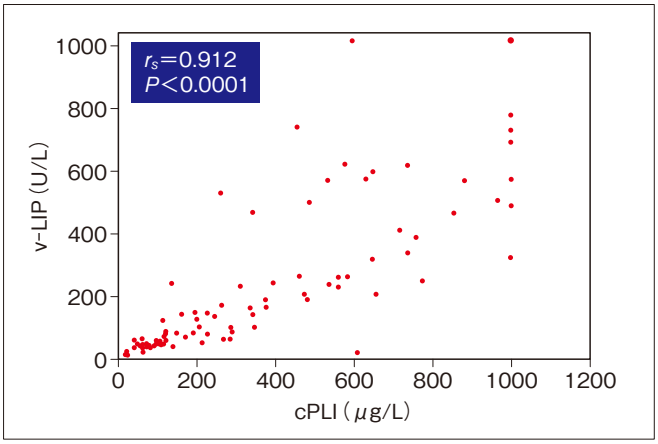

Figure 2 **Correlation between v-LIP and cPLI in dogs suspected of having pancreatitis**

A strong correlation between v-LIP and cPLI was observed (rs = 0.912)

- **v-LIP correlates well with cPLI**
  - Elevated v-LIP → may be pancreatitis (cPLI, > 200 μg/L)
  - v-LIP falls within the reference range → not definitively indicative of pancreatitis (cPLI, < 400 μg/L)
  - ··Both include a gray area
  - ··Must be evaluated in conjunction with symptoms and other test results
- **Its clinical utility is high!**
  - Rapidly measured in-house
  - Low cost

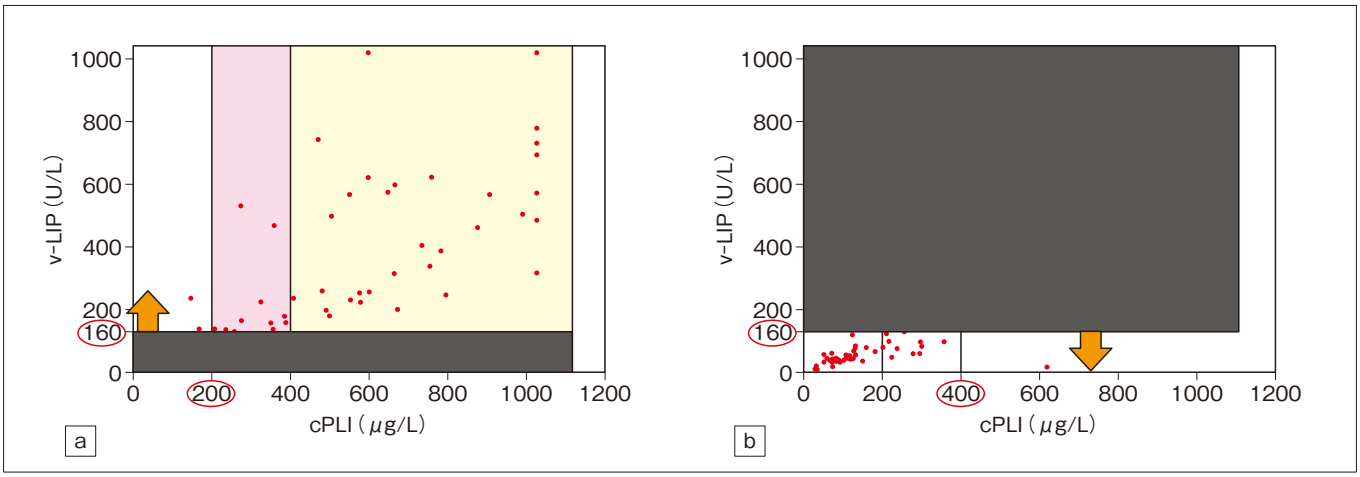

Figure 3 **Association between v-LIP and cPLI in dogs suspected of having pancreatitis**

Spec cPL™ of IDEXX has the following reference values: ≤ 200 μg/L: within the reference range; unlikely to have pancreatitis. 200–399 μg/L: slightly elevated; retesting is needed. ≥ 400μg/L: elevated, likely to have pancreatitis. v-LIP of FUJIFILM Co., Ltd. has a reference value of 160 U/L
a: When v-LIP was elevated (> 160 U/L), the probability of a cPLI being 200 μg/L or higher was 98%.
b: On the other hand, when v-LIP was within the reference range (≤ 160 U/L), the probability of a cPLI being below 400 μg/L was 98%.

■ Problems with the diagnosis of pancreatitis

—Discrepancy between clinical assessment and cPLI values

According to a presentation at a scientific meeting, there are many cases in which cPLI was elevated while pancreatitis was clinically ruled out on the basis of results from a range of other clinical laboratory tests (Figure 4) [10]. This is the "discrepancy" veterinarians in the field feel exists between measured values and clinical assessment.

Such a problem may also occur with v-LIP. One cause of this discrepancy is that there are many abnormalities at the tissue level in a macroscopically normal pancreas [11] (histologically common lesions include nodular hyperplasia, lymphocytic inflammation, fibrosis, and atrophy). That is, an elevated cPLI in dogs in which pancreatitis is not suspected might reflect abnormalities at the tissue level in the pancreas.

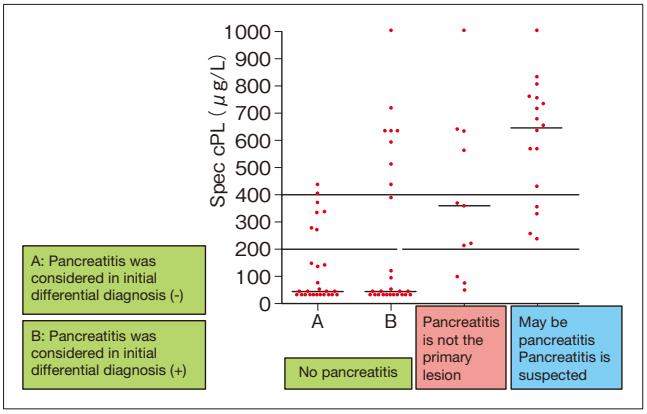

Figure 4 **Clinical assessment of pancreatitis and its correlation with cPLI values**

In a group of dogs suspected of having pancreatitis on the basis of symptoms or results of various tests other than cPLI (blue in the figure), cPLI exceeded 200 μg/L. However, even in a group of dogs not suspected of having pancreatitis (green in the figure), cPLI exceeded 400 μg/L in a considerable number of cases. Cited/adapted from reference 10

Table 3 **Underlying/comorbid diseases in dogs with elevated cPLI (n = 84)[12]**

Of the 84 dogs, 74 (88.0%) had comorbid diseases. More than half of neoplastic diseases were not diagnosed when cPLI was measured. It is very risky to assume it is pancreatitis based solely on elevated cPLI.

Non-neoplastic disease	53 (71.6%)	Neoplastic disease	21 (28.4%)
Digestive/hepatobiliary system	20	Lymphoma	10
Immune-mediated	13	Mammary gland tumor	3
Endocrine	5	Histiocytic sarcoma	2
Renal/urological	5	Chronic lymphocytic leukemia	2
Circulatory	3	Acute lymphoblastic leukemia	1
Brain/nervous system	2	Ileal adenocarcinoma	1
Respiratory	1	Hepatocellular carcinoma	1
Reproductive system	1	Malignant tumor affecting the entire liver	1
Unknown	3		

· Already diagnosed when cPLI was measured: 9 dogs
· Undiagnosed: 12 dogs

—Pitfalls of the diagnosis of pancreatitis

It must also be remembered that relying solely on elevated values of markers such as cPLI and v-LIP for diagnosing pancreatitis can be risky. In our study, dogs with elevated cPLI (probably, those with elevated v-LIP as well) had various underlying/comorbid diseases including tumors, and these diseases were often not yet diagnosed when cPLI and v-LIP were measured. Therefore, a comprehensive evaluation is required (Table 3) [12].

Summary of the presentation so far:

At present, there is no single gold standard for the diagnosis of pancreatitis!

- Symptoms that suggest pancreatitis
- Ruling out differential diagnoses considered for various tests
- Abnormal PLI values
- Abnormal v-LIP values
- Abnormal ultrasonographic findings of the pancreas

A comprehensive diagnosis should be made on the basis of these factors

Japan-specific evolution of the diagnosis of pancreatitis

■ CRP in canine pancreatitis

The blood concentration of a group of proteins called acute phase proteins (APPs) changes rapidly when the organism is subjected to inflammatory stress. They are utilized as markers for inflammation. Among them, C-reactive protein (CRP) has been extensively researched and developed in both human and veterinary medicine in Japan. Therefore, it is widely used in Japan as general inflammatory marker. Canine CRP cannot be measured at testing centers for humans or using reagents designed for humans. It must be measured using antibodies against canine CRP. Regarding in-house testing equipment for CRP, since the launch of the Laser CRP-2 (reference value: < 1 mg/dL) by Arrows Co., Ltd., it is thought that its adoption has advanced in veterinary medicine. Recently, it became possible to measure canine CRP with FUJI DRI-CHEM (reference value: < 0.7 mg/dL) manufactured by FUJIFILM Co., Ltd.

■ Measuring CRP many times can provide insight into the progression of a condition over time.

CRP is a marker for inflammation, not a diagnostic marker to identify a certain disease. Thus, instead of waiting for many days for test results from a CRP test, the significance lies in conducting CRP measurements "repeatedly over time" to

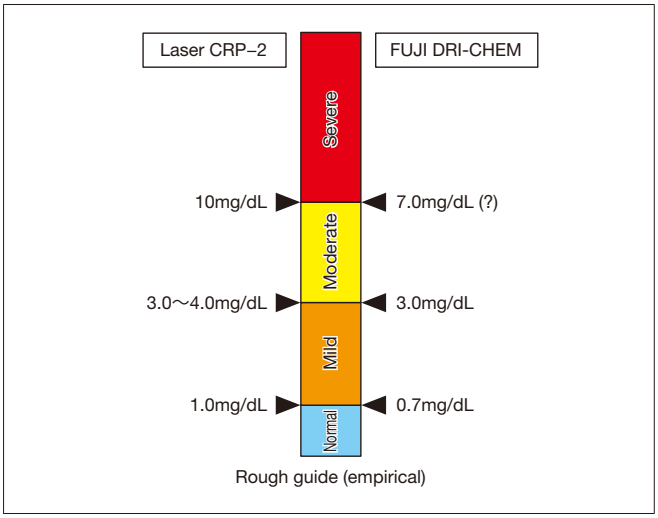

Figure 5 Reference values for "Laser CRP-2" and "FUJI DRI-CHEM"

Laser CRP-2 has a cut-off value of 1.0 mg/dL. FUJI DRI-CHEM has a cutoff value of 0.7 mg/dL, and its measurement limit is 7.0 mg/dL (a sample above the upper limit can be measured after dilution). It is important to gain a rough idea of whether CRP levels are normal or abnormal and by how much they have increased.

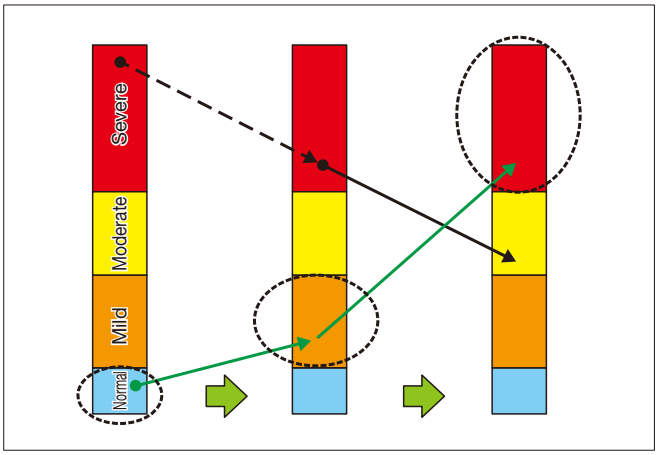

Figure 6 Schema showing how to interpret absolute values of CRP

After gaining a rough understanding of abnormal CRP levels, it is important to monitor changes over time

evaluate changes occurring on the same day. Therefore, it can be said that measuring CRP at the bedside is the best approach. Generally, as an indicator of inflammation, I think many of you measure white blood cell count; however, as an inflammation marker, CRP can be measured with a similar frequency. When interpreting measured values of CRP, especially in the context of a single data point, it is important to first roughly determine whether the value is abnormal or not (**Figure 5**). Then, by recording the rough changes in these absolute CRP values over time, importantly, it is possible to gain an understanding of the overall trend or flow (**Figure 6**).

■ CRP in (acute) canine pancreatitis

The clinical significance of measuring CRP in dogs includes the following: the determination of the presence/absence and degree of inflammation, the determination of treatment effect and recurrence through repeated measurement over time, and its utility as a potential prognostic factor. The determination of the presence/absence and degree of inflammation is also often useful in assisting diagnosis. In (acute) pancreatitis, CRP rises in almost all dogs, although to varying degrees [13–15]. Therefore, a dog whose symptoms suggest pancreatitis but whose CRP is not elevated is unlikely to have pancreatitis. In such a case, other diseases, for example, Addison's disease and gastrointestinal and hepatic diseases, should be considered first. You may think "Doesn't CRP rise in gastroenteritis because it is also an inflammatory condition?" Actually, however, in many cases, CRP does not rise markedly (**Table 4**). In general, CRP rises markedly when inflammation is systemic, for example, in cases of pancreatitis, but its rise is negligible when lesions are localized (except for abscess).

CRP is a marker that allows the presence/absence and degree of (systemic) inflammation to be ascertained and should not be used for diagnosis. However, "the presence/absence and degree of inflammation" are often useful in assisting diagnosis.

Table 4 CRP values and the extent of inflammation

CRP is more likely to rise markedly with extensive/systemic inflammation than with only localized inflammation. Reference values by disease, such as those shown below, have not been determined. However, if you measure CRP regularly, you will gain an intuitive understanding of the degree to which CRP is elevated by each disease

Localized	CRP value	Systemic	CRP value
Gastritis	→↑	Pancreatitis	↑↑～↑↑↑
Enteritis	→↑	Intestinal perforation and peritonitis	↑↑～↑↑↑
Upper respiratory tract inflammation	→↑	Pneumonia	↑↑
Cystitis	→	Pyelonephritis	↑～↑↑
Osteoarthrosis deformans	→	Polyarthritis	↑↑～↑↑↑
As an exception, CRP levels may rise even with localized inflammation if an abscess is formed			
Hepatitis	→↑	Hepatic abscess	↑↑～↑↑↑
Dermatitis	→↑	Subcutaneous abscess	↑↑～↑↑↑
Stomatitis	→↑	Apical abscess	↑↑

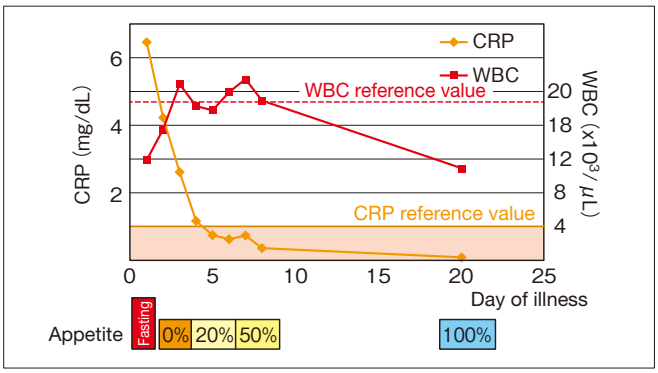

Figure 7 Changes in CRP and white blood cell count, and symptoms in a dog with pancreatitis

A rise in CRP is observed on day 0 of illness with a subsequent decrease in CRP with continued treatment. On the other hand, a rise in white blood cell count is not observed on day 0 of illness. Subsequently, a delayed rise is observed, and you can see a decrease around day 8

—Is the white blood cell count insufficient as an indicator of inflammation?

Why is using the white blood cell count on its own inadequate as an indicator of inflammation? The answer is lies in the fact that white blood cells are not inherently "a sensitive marker of inflammation."

For example, CRP is elevated in most dogs with pyometra, while white blood cell count is slow to respond and is not elevated in some animals. In a pathological condition requiring a quick judgment, such as pancreatitis, CRP should be measured along with the white blood cell count (Figure 7).

■ Correlation of v-LIP and cPLI with clinical symptoms, white blood cell count, and CRP

In our study, no correlation was observed between v-LIP or cPLI and clinical symptom score, total white blood cell count, or CRP (Table 5) [9]. That is, it is difficult to determine the degree of inflammation by v-LIP or cPLI alone, and a major increase in these does not necessarily mean that symptoms are severe. On the other hand, there is some degree of correlation between CRP and clinical symptoms (Figure 8) [9], implying the significance of measuring CRP in canine pancreatitis.

■ Relationship between CRP and the severity and prognosis of canine pancreatitis

In humans, CRP is recommended as the sole marker for the severity of pancreatitis. In dogs with pancreatitis, however, CRP values measured "at the first visit" were not associated with prognosis. In a previous study the author conducted on the relationship between CRP and prognosis in dogs with idiopathic polyarthritis [16], CRP values measured at the first visit were also not associated with prognosis. However, change in CRP concentration during the first 1–2 weeks of early treatment (responsiveness to early treatment) was associated with long-term prognosis (prognosis at 6 months after initiation of treatment). That is, if early treatment does not steadily decrease CRP, the prognosis is poor. Thus, in dogs with pancreatitis, although CRP values measured at the first visit were not associated with prognosis, change in CRP levels over time during early treatment may be associated with prognosis, and I am currently continuing research on this question.

Table 5 **Correlation of v-LIP and cPLI with clinical symptoms, white blood cell count, and CRP** [9]

v-LIP and cPLI are not associated with symptoms or markers for inflammation

	v-LIP (dogs with elevated v-LIP)		cPLI (dogs with elevated cPLI)	
	r_s	*P* value	r_s	*P* value
Clinical symptom score	−0.0324	0.8195	0.0088	0.9447
Total white blood cell count	0.0815	0.5658	−0.0097	0.9394
CRP	0.0892	0.5294	0.0665	0.6018

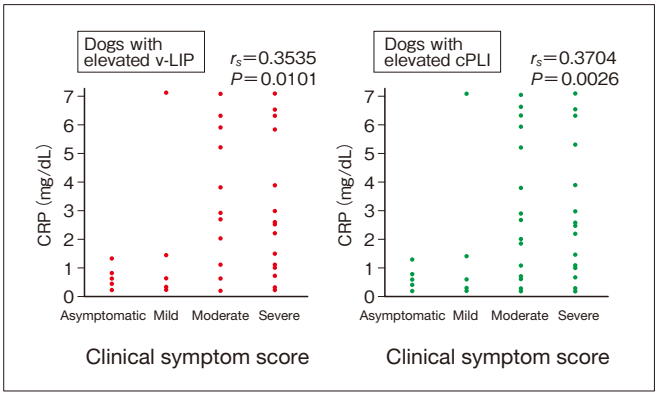

Figure 8 **Association between CRP and symptoms in dogs suspected of having pancreatitis (dogs with elevated v-LIP and dogs with elevated cPLI)** [9]

CRP is associated with clinical symptoms

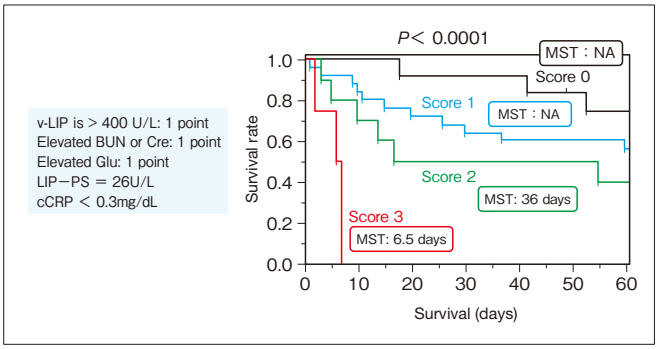

Figure 9 **Simple severity score and survival**

Prognosis prediction when v-LIP is elevated may become possible by measuring BUN, Cre, and Glu with FUJI DRI-CHEM and scoring them in a simplified manner, although more data needs to be accumulated going forward.

Table 6 **Report on criteria for assessing the severity of canine pancreatitis** [13]

Evaluation criteria (maximum score is 10 points, mortality rate is > 50% with a score of 4 or more)		points
Circulatory system	· Less than 60 VPCs/24 hours or heart rate of > 180/minute	1 point
	· Paroxysmal ventricular tachycardia	2 points
Respiratory system	· Dyspnea, and respiratory rate > 40/minute	1 point
	· Pneumonia or acute respiratory distress syndrome (ARDS)	2 points
Digestive system	· Loss of peristaltic sounds	1 point
	· Bloody stool, melena, or vomiting	2 points
	· Unable to eat for 3 days or longer	3 points
	· The above + bloody stool, melena, or vomiting for 2 days or longer	4 points
Blood pressure and osmotic pressure	· Systolic blood pressure: < 60 or > 180 mmHg, or ALB: < 1.8 g/dL	1 point
	· Systolic blood pressure: < 60 or > 180 mmHg, and ALB: < 1.8 g/dL	2 points

—Criteria for the severity of pancreatitis

As criteria for the severity of canine pancreatitis, methods for evaluating the circulatory, respiratory, and digestive systems, blood pressure, and osmotic pressure (Table 6) and evaluation methods involving the measurement of multiple biochemical test items [13] have been proposed. However, all these methods determine severity on the basis of the number of organs impaired. I have a strong impression that these methods involve many evaluation criteria and are overly complex. We are currently endeavoring to develop a simpler indicator. We are exploring the possibility of evaluating four items that can be measured with in-hospital measuring devices: v-LIP, BUN, Cre, and Glu (that is, if v-LIP is elevated, then the other three items are evaluated) (Figure 9). Moreover, at present, we think that tracking change in CRP is highly useful for determining the severity and prognosis of canine pancreatitis

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