Patient-Side Assay of Lipase Activity Correlating with Pancreatic Lipase Immunoreactivity in the Dog

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ABSTRACT. Pancreatitis is a common exocrine pancreatic disease in dogs, and the pancreatic lipase immunoreactivity (PLI) test is used for diagnosis. Enzyme catalytic assay is thought to have low specificity, but a lipase activity assay with increased specificity has been developed in human clinical chemistry. We measured serum lipase activity of 65 client-owned dogs using the newly developed FUJI DRI-CHEM slide and compared the results with their PLI concentrations. The results showed a good correlation ($r=0.91$), and the normal and pancreatitis dogs identified based on the PLI values were correctly separated based on lipase activity. The present study suggests that FUJI DRI-CHEM lipase activity would be helpful for diagnosis of pancreatitis in dogs and, in particular, that it can be used as a patient-side assay and contributes to immediate treatment.

KEY WORDS: dry chemistry test, lipase, pancreatitis.

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Pancreatitis is the most common exocrine pancreatic disease in both dogs and cats. Historically, amylase and lipase activities have been utilized as diagnostic indices for pancreatitis. But these enzyme activities are of limited clinical values for the diagnosis of pancreatitis in the dog and are of no clinical value in the cat because of their low specificity [10]. Many different organs produce lipase, and the catalytic assay for the lipase activity is thought to measure these isozymes together. Recently, Steiner *et al.* developed an assay for the pancreatic lipase immunoreactivity (PLI) of the dog [9] and cat. The use of immunoassays does allow for the specific measurement of lipase produced by the exocrine pancreas. Currently, PLI is regarded as the best diagnostic index of pancreatitis in both dogs and cats because it has high sensitivity and specificity. The weak point of PLI is that it requires a few days for measurement. When we measure PLI, we have to send the serum to a laboratory, and it is impossible to get the measurement results on the same day. Nevertheless, pancreatitis is often an "acute" severe condition that requires immediate diagnosis and treatment. Recently, an immunochromatography test for PLI (SNAP cPLTM) was developed to solve this problem, but this method is semiquantitative and does not measure the concentration. An enzyme activity test has an advantage in promptness and quantifiability, so if we can increase the specificity, the test would be a beneficial diagnostic index for pancreatitis. In human medicine, the lipase activity test has been continuously improved to target higher specificity for pancreatic lipase. In human clinical chemistry, it is

known that specificity depends on reactive conditions, such as type of substrate, electrochemical attributes of detergents and other additive agents. These factors modulate the contact ratio between lipase and substrates, and they can differ among the isozymes because of possible differences in their electrochemical nature. Recently, we developed a new lipase test slide for a dry chemistry analyzer, FUJI DRI-CHEM, and made it commercially available. This slide was designed to detect pancreatic lipase specifically using triolein as the reaction substrate and negatively charged detergent as an auxiliary agent.

In the present study, we measured the lipase activity of canine serum samples using FUJI DRI-CHEM lipase slides and compared the results with PLI concentrations to evaluate the utility of this lipase activity test for diagnosis of pancreatitis in the dog.

Serum samples were collected from client-owned dogs visiting the Veterinary Medical Teaching Hospital at Nippon Veterinary and Life Science University during 2009 and 2010. They were collected without regard to age, gender, breed and symptoms. Hemolytic, icteric and lipemic serum were eliminated in this study. Generally, clinical samples in dogs suspected of having pancreatitis are sometimes affected by these factors, but in the present study, we wished to verify the correlation between lipase activity and PLI without complex factors as the initial step. Ultimately, we obtained 65 samples for use in this study.

We sent portions of the serum samples to a commercial laboratory and requested that they measure the PLI concentrations (SpecTM cPL). Lipase activity was measured using a dry chemistry analyzer (FUJI DRI-CHEM 7000V, FUJIFILM corporation, Tokyo, Japan) with dedicated lipase slides. For the reference, we ordered a laboratory to measure lipase activity using another lipase activity test simulta-

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Fig. 1. Correlation between the serum PLI concentration and lipase activity in dogs (n=53). Lipase activity was measured by dry chemistry using FUJI DRI-CHEM (A) and Mauck's enzyme method (B).

neously. The laboratory performed Mauck's enzyme method [7], and the reaction substrate was 1-oleoyl 2,3-diacetyl glycerol.

When we measured the PLI concentrations of the 65 serum samples, the concentrations of 53 samples ranged from 30 to 1,000 $\mu\text{g/l}$ (effective measuring range), but the concentrations of 11 samples were less than 30 $\mu\text{g/l}$, and the concentration of 1 sample was more than 1,000 $\mu\text{g/l}$. The lipase activity in the 53 samples (PLI at 30–1,000 $\mu\text{g/l}$) ranged from 49.5 to 738.5 U/l. When we compared the PLI concentration and lipase activity using the 53 samples (12 samples were eliminated because their PLI values were outside the range), the values showed a good correlation ($r=0.91$), as shown in Fig. 1-A. The lipase activity in the 11 samples (PLI < 30 $\mu\text{g/l}$) ranged from 42.0 to 67.5 U/l and was mostly below those in the above 53 samples. The lipase activity in the 1 remaining sample (PLI > 1,000 $\mu\text{g/l}$) was 657.1 U/l. The lipase activity measured by the commercial laboratory and PLI showed lower correlation ($r=0.63$), as shown in Fig. 1-B.

Despite the good correlation coefficient, the correlation between lipase activity and PLI decreased as the values approached the upper end of the range. We then investigated the clinical utility of the lipase activity test by comparison with the diagnostic criteria of PLI. In the PLI criteria, a PLI concentration less than or equal to 200 $\mu\text{g/l}$ is eliminative for pancreatitis (normal), a concentration between 201 and 399 $\mu\text{g/l}$ is suggestive of pancreatitis (boundary) and a concentration greater than or equal to 400 $\mu\text{g/l}$ is indicative of pancreatitis. We classified the present results into the 3 categories based on their PLI values. The lipase activity of the normal group ranged from 42 to 197 U/l (n=33), that of the boundary group ranged from 91.5 to 307 U/l (n=15) and

that of the pancreatitis group ranged from 225 to 738.5 U/l (n=17), as shown in Fig. 2.

FUJI DRI-CHEM lipase activity showed a good correlation with PLI, suggesting it is helpful for diagnosis of canine pancreatitis. It is generally documented that the catalytic assay for lipase activity has a lack of specificity because it detects isozymes from different organs together. But in previous studies [5], the specificities of the assays were reported to be different among the methods. Actually, we requested that a commercial laboratory measure the lipase activity of the present samples, and they were measured by a different method (using different substrate and reaction reagents); this produced poor correlation with PLI (Fig. 1-B). These results indicate that we should not lump all enzyme assay methods together and that we should discuss the methods individually. We were not able to absolutely determine why FUJI DRI-CHEM lipase activity correlated well with PLI, but the following points are possible reasons. First, we used triolein as a substrate, and triolein is reported to be more specific for pancreatic lipase than other types of lipase [4]. Secondly, we used negatively charged detergent. The interface of triolein and water is necessary as the reaction site for lipase activity. Thus, formation of an emulsion is important [1, 3, 8], and detergent is required as an auxiliary agent. In human clinical chemistry, negatively charged detergent suppresses lipase activity, but pancreatic lipase forms a complex with colipase [6], which eliminates this suppression [2]. Thus, it was presumed that negatively charged detergent maintained the specificity of the pancreatic lipase and formed an optimal emulsion reaction site for the lipase. These hypotheses may be parts of the mechanism producing pancreatic lipase specificity, but more unknown factors may be involved. Further studies are necessary to

Fig. 2. Distribution of lipase activity measured by dry chemistry in dogs of three different PLI categories. The dogs were sorted to 3 groups based on their PLI values, i.e., normal ($\leq 200 \mu\text{g/l}$, $n=33$), boundary ($201\text{--}399 \mu\text{g/l}$, $n=15$) and pancreatitis ($\geq 400 \mu\text{g/l}$, $n=17$). The bars indicate the range between the maximum and minimum values, and the boxes indicate the central 50% of the data.

reveal these factors. In the present study, we focused on the clinical utility of this lipase slide.

As shown in Fig. 1-A, lipase activity seemed to lose touch with PLI as the values approached the upper end of the range. But when we classified the present results into the 3 categories based on their PLI values, the lipase activity of the normal and pancreatitis groups separated completely, although those of the boundary group overlapped with both groups (Fig. 2). This means we can separate normal animals and pancreatitis patients based on FUJI DRI-CHEM lipase activity to some degree.

The limitations of the present study are as follows. We did not use hemolytic, icteric and lipemic samples in this study because we wished to eliminate complex factors and make interpretation of the results simple. But clinically, some pancreatitis patients show icterus and/or hyperlipemia. Therefore, we have to investigate the effects of these factors on the lipase activity measurements as the next step. In the present study, we collected the samples without collecting information about the subjects' clinical signs and other examination results, so we cannot compare the present results with their pathological states. Nevertheless, consid-

ering that PLI has previously been reported to be a good pancreatitis marker and that the FUJI DRI-CHEM lipase activity showed a good correlation with PLI values, it is likely reasonable to view FUJI DRI-CHEM lipase activity as a decent pancreatitis marker indirectly. For now, we should measure PLI to diagnose pancreatitis in the dog, but pancreatitis is often an acute severe condition that requires immediate treatment. FUJI DRI-CHEM lipase activity is a new supportive index for provisional diagnosis of pancreatitis in dogs. Another merit of the lipase slide is that lipase activity can be measured together with other biochemical parameters, such as BUN, creatinine, AST, ALT and so on. In other words, we can perform "rough screening" of pancreatitis together with liver and kidney diseases in routine blood biochemistry testing. More case studies are required to validate this test in more detail.

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