

The Current and Future of Acute Phase Protein Assays in Dogs and Cats: A Dialog from the Experts



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Veterinarian and Doctor of Veterinary Science, Division Chief of Gastrointestinal Medicine, Veterinary Internal Medicine, Veterinary Medical Center of The University of Tokyo. After completing his doctorate at the University of Tokyo, he went to United States and engaged in research, primarily on gene therapy, at the New York University School of Medicine. Then, after working as a Teaching Assistant at the Yamaguchi University, he was appointed Assistant Professor of the Laboratory of Veterinary Internal Medicine in the Graduate School of Agricultural and Life Sciences of the University of Tokyo. His areas of clinical specialization and research include diseases of the gastrointestinal diseases including liver and pancreas, and inflammatory and immune-mediated diseases in dogs and cats.



Acute phase proteins are a class of proteins that fluctuate in concentration and are released into the blood serum by the liver, consequent upon an acute phase reaction when an infection or inflammatory disease has occurred. There is a strong need in the clinical care of small animals to obtain an accurate grasp of the presence and level of these inflammation markers and utilize them to evaluate the animal's condition. In veterinary clinics in Japan, it has become an established practice to carry out routine in-house tests to measure C-reactive protein (CRP). However, insufficient knowledge about other acute phase proteins, such as serum amyloid A (SAA), and the issue of user-friendliness mean that Japanese veterinary diagnostics cannot be regarded as advanced in this aspect. Accordingly, in this article we present a dialog between José Cerón, the head of the Interdisciplinary Laboratory of Clinical Analysis at the University of Murcia, who is making advances in biomarker research with a particular focus on acute phase proteins and Koichi Ohno, an Assistant Professor at the University of Tokyo, who has long been engaged in research into acute phase proteins from the perspective of a clinical researcher. Here they talk about the characteristics of acute phase proteins, the significance of measuring them, the issues involved, and the prospects for the future.

About acute phase proteins

Ohno I'd like to start by asking about 'positive' acute phase proteins and 'negative' acute phase proteins. What are your thoughts about the classification and characteristics of acute phase proteins?

Cerón Acute phase proteins are basically proteins whose concentrations fluctuate when an inflammatory stimulus occurs (Fig.1). Therefore, when an inflammatory stimulus is triggered within the body by various physical traumas, tumors or infectious diseases, a local reaction within a tissue occurs and interleukins and cytokines are produced. Acute phase proteins are mainly produced in the liver, and interleukins and cytokines work like inducers to produce acute phase proteins in the liver. Acute phase proteins whose concentration increases are referred to as 'positive' and those whose concentration decreases are referred to as 'negative'. Positive acute phase proteins can be further classified into moderate acute phase proteins and major acute phase proteins. Basically, major acute phase proteins

demonstrate large fluctuations in concentration, and these fluctuations occur rapidly. The fluctuations are not so large in the case of moderate acute phase proteins, and the fluctuations take longer.

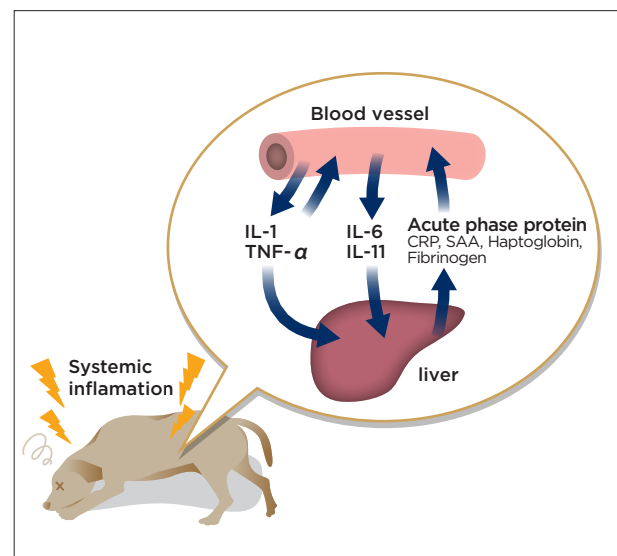


Fig. 1 Acute phase proteins



Generally speaking, acute phase protein reactions occur rapidly. Since the reaction is extremely swift, fluctuations in the concentration of acute phase proteins can be detected before clinical signs appear. This is one of the main advantages of acute phase protein as a marker.

Ohno What are your thoughts regarding interleukins and cytokines? By definition, they are not acute phase proteins, aren't they?

Cerón I regard interleukins and cytokines not as acute phase proteins but as proteins that regulate acute phase reactions. While there are interleukins that facilitate the synthesis of acute phase proteins, there are also interleukins that inhibit acute phase protein synthesis. I therefore consider interleukins to be something more akin to a regulatory factor than to acute phase proteins. However, if acute phase proteins are perceived as proteins that are consequent to the inflammation process, interleukins can be considered to be the same as acute phase proteins.

Ohno What about negative acute phase proteins like albumin? Acute phase proteins usually increase when an inflammation occurs. However, albumin decreases slightly, so it can also be regarded as a negative acute phase protein (**Table 1**).

Cerón In my opinion, albumin can be regarded as an acute phase protein. Veterinarians already have more knowledge about albumin than CRP and for them albumin changes are easier to interpret, particularly when they are being trained in acute phase proteins interpretation. However, albumin basically displays only small fluctuations in concentration, and those fluctuations take time to occur. Usually, when inflammation occurs, multiple acute phase proteins and interleukins are produced, so many proteins are required. It is assumed that albumin production decreases in order to produce other acute phase proteins and maintain the required concentration. Thus, albumin can be considered as a negative acute

Table 1 Differences in the reactivity of acute phase proteins in humans, dogs and cats

Cited from reference 1 with modifications

	Humans	Dogs	Cats
CRP	↑↑↑	↑↑↑	→
SAA	↑↑↑	↑↑↑	↑↑↑
α1AG	↑↑	↑↑	↑↑
Haptoglobin	↑↑	↑↑	↑~↑↑
Fibrinogen	↑↑	↑↑	?
Albumin	↓	↓	↓?

↑↑↑ : 10 times or more increase, ↑↑ : 2 to 10 times increase, ↑ : Double or less increase, ↓ : Decrease, ? : Unclear

phase protein with a different mechanism.

Ohno I mainly see gastrointestinal disorders. With dogs in particular, albumin sometimes decreases in cases of severe malnutrition caused by anorexia, so albumin could be a nutritional marker.

Cerón In cases of decreased albumin, it is interesting to assess the liver by markers of liver damage and function to rule out liver failure. Then I assess whether or not there is protein loss by the kidneys. Thirdly, I assess the gastrointestinal tract for issues causing protein loss and then rule out hemorrhages and burns. When I can rule out all those conditions, if decreased albumin is still observed and CRP and other acute phase proteins are still increasing, I think we can say that albumin is decreasing by inflammation.

Ohno Albumin is affected by many factors, isn't it?

Cerón Yes, it is. Since there are many factors which could potentially have an effect, I personally do not place a great deal of importance on albumin as an acute phase protein.

Ohno By the way, we veterinarians have traditionally used white blood cells (WBC) count as a parameter of inflammation, but which would be better to use to determine and assess inflammation, acute phase proteins or WBC count?

Cerón It's important to get a look at the whole picture from all data that can be obtained including the case history, clinical signs, WBC count, and acute phase proteins. This is because there are cases where there's a divergence between the WBC count and acute phase proteins. For example, it's not unusual in cases of chronic inflammation to observe a change in acute phase proteins even though there is no change in the WBC count. Also, when monitoring the treatment of cases where there is inflammation, I often feel that the speed of the decrease, particularly for CRP in dogs and serum amyloid A (SAA) in cats, is faster than the decrease in WBC count.

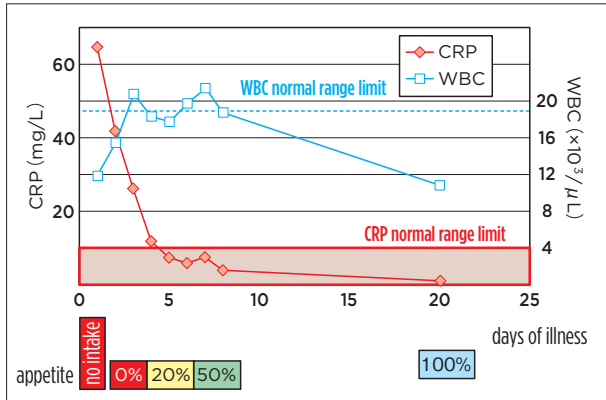


Fig. 2 CRP/WBC transitions in a case of pancreatitis, and their relation to symptoms

In the case of a four-year old mixed breed dog brought to the clinic with the main complaints of acute vomiting and loss of appetite, pancreatitis was diagnosed from various tests. **Transitions in CRP and white blood cell count (WBC) in this case are shown in the graph.** The symptoms are at their worst at the time of the initial examination, at which point CRP has increased to more than 6 mg/dL, however this later decreases during the course of medical treatment as the symptoms improve. Meanwhile, WBC is within the normal range at the time of the initial examination, when the symptoms are at their worst, and we can see that the reaction is sluggish with the level going only slightly beyond the normal range for the first time three days later.

Ohno You're absolutely right. I frequently encounter such cases in the clinical field. There are many cases where the WBC count remains high even though the CRP level has decreased and the symptoms have been brought under control (**Fig. 2**). However, although major acute phase proteins fluctuate rapidly, the fluctuations of moderate acute phase proteins such as haptoglobin are slow. How do you think we could utilize moderate acute phase proteins?

Cerón Moderate acute phase proteins are not suited to the monitoring of treatment. We do encounter cases where the haptoglobin has still not decreased even though the animal's condition has recovered. My personal opinion is that moderate acute phase proteins should basically be used as a supplementary to the major acute phase proteins such as CRP, with the aim of acquiring data in the event of a divergence being observed. There are many divergences between major acute phase proteins and moderate acute phase proteins. For example, when there is intravascular hemolysis due to a cause such as babesia or filaria, an increase in CRP (major acute phase protein) is observed but the haptoglobin (moderate acute phase protein) value remains normal or may even be low. Also, divergences may occur even when there is an increase in glucocorticoid. In this case, the CRP is low and the haptoglobin is high. From the perspective of a comparison with other parameters, such as CRP and white blood cells, it's very interesting that a significant correlation usually exists between white blood cell changes and CRP when a serious acute inflammation is exhibited. However, when I say white blood cell change I am referring not only to an increase in the WBC count but to changes

connected with inflammation and so on. For example, things such as a left shift in neutrophilic leukocytes and changes in toxicity. There is usually a correlation in these changes and CRP in cases of pancreatitis or pyometra. The problem is that veterinarians do not have much time available during their daily diagnosis and treatment, to observe blood smears for those changes. In some cases, they simply have to rely on numerical values.

Ohno Just the WBC count.

Cerón With pyometra and pancreatitis, for example, the WBC count does increase but there are also cases where the level remains normal or even can decrease.

Ohno There is the possibility of changes of leukocytes appearance and the differential leukocyte count on blood smear. However, it takes time to confirm such changes and the clinician needs to be familiar with the work process. That is why CRP and other acute phase proteins are easier for veterinarians to understand.

Cerón I agree. However, I personally think that it's also essential to scrutinize cell toxicity via blood smears. For that reason, veterinarians need to be trained to observe blood smears through a microscope.



The significance of point of care testing

Ohno Going back a little bit, acute phase proteins became one of the topics at a conference in Copenhagen around 10 years ago. At that time many clinical pathologists in the field of veterinary medicine were already aware of the importance of acute phase proteins. But I remember that there were not so many veterinarians in Europe at that time who measured acute phase proteins. Did that change later?

Cerón It does depend on the country, but things are changing. As far as I'm aware, the utilization of acute phase proteins is increasing in Europe.

Ohno It means that there are companies which provide services to measure acute phase proteins, isn't it?

Cerón Yes. And thanks to that, the number of veterinarians and researchers utilizing acute phase proteins continues to increase.

Ohno Currently most of the research concerning acute phase proteins is being done in Europe. But for some reason, European veterinarians still do not routinely measure acute phase proteins, do they?

Cerón Experts need to explain clearly to veterinarians about the advantages of acute phase proteins. There also needs to be ongoing training so that veterinarians can build up experience in measuring acute phase proteins.

Ohno Do European veterinarians use Point of Care Testing (POCT) equipment (**Fig. 3**)?

Cerón Large-scale testing organizations provide acute phase protein assay services, so in most cases the veterinarians send samples to them. But POCT equipment can now also be used, and I think that the opportunities to use such equipment will increase over the next few years. Europe is influenced by American researchers, and acute phase protein assays were not being widely performed in United States. I think these are some of the reasons why acute phase protein assays were not widespread in Europe.

Ohno Around eight years ago, Dr. Mads Kjølgaard-Hansen gave a lecture about acute phase proteins at the American College of Veterinary Internal Medicine (ACVIM) forum, and I remember that the attendance was rather low. But 3 or 4 years ago when he gave another lecture, the atmosphere was different. There were even people standing at the back to listen. I can say that the situation is changing in United States as well, and it seems that many veterinarians are now aware of the significance of acute phase protein assays.

Cerón In countries such as Spain, too, appropriate training is now being provided with regard to acute phase proteins. If people start utilizing acute phase



Fig. 3 Example of POCT equipment: Fuji DRI-CHEM
Left: FUJITSU DRI-CHEM IMMUNO AU10V,
Right: FUJITSU DRI-CHEM NX700

proteins for certain specific symptoms (illnesses) to begin with, they will gradually come to understand their importance. If they recognize the usefulness of acute phase proteins and have the opportunity to get results by external labs and/or try out POCT equipment, I think that veterinarians will come to use acute phase proteins. You could say that they will get into the habit of doing so.

Ohno There was a similar situation before in Japan. When POCT equipment capable of measuring canine CRP came onto the market, veterinarians didn't really understand its importance and I have to say that there wasn't much interest in it. Even so, the equipment was not very expensive so some veterinary clinics did purchase it. Word gradually got around and it became the norm amongst veterinarians, and now they cannot manage daily clinics without in-house CRP assays using POCT equipment. The important thing is to "give it a try". I personally held seminars all over Japan about acute phase proteins and CRP at that time.

Cerón Spanish veterinarians who utilize acute phase proteins sometimes complain that acute phase proteins are not covered as a clinical topic at international and domestic conferences. This is a training issue. Would I be right in thinking that in Japan CRP assays are incorporated into routine tests?

Ohno Many veterinary clinics in Japan are installed with POCT equipment, so I think that many veterinarians now regard CRP assays as routine tests. Veterinarians usually want to get swift readings from tests for inflammation markers, so I imagine that it will be the same in Spain and that many veterinarians will carry out in-house CRP assays.

Cerón I think that veterinarians want to obtain swift measurement values to boost the added value of their clinics. Speed may not be all that important in the monitoring of chronic disease. However, it is very important to get fast results in the case of an acute disease. For example, in the case of parvovirus, we do not want CRP to exceed 100 mg/L and there will be a need to intensify the treatment. The same holds true for pancreatitis.



Ohno Another important point is that we can grasp the progress and severity of an illness and the response to treatment from acute phase proteins fluctuations over time.

Cerón In humans, for example, in cases where there is a fever or acute inflammation, if CRP has not decreased within 24 to 48 hours, there is the possibility that the wrong treatment is being given so it is recommended to carry out a reassessment or to use a different antibacterial drug. In other words, there is a need to obtain adequate information about CRP fluctuations within a 24-hour period and decide on the treatment that needs to be given.

Ohno I do the same thing myself. If there is no decrease in the CRP readings, I conclude that there is a possibility that the diagnosis or treatment is wrong and consider making revisions to it.

Cerón It is sometimes difficult to suspect some diseases such as endocarditis, for example, so if CRP does not decrease within 24 hours we need to consider that it might be a case of endocarditis or some hidden infection.

Utilizing acute phase proteins in dogs

Ohno We touched upon this topic earlier, but I sometimes encounter cases where there is no increase in WBC count even though the CRP value is markedly elevated. If there seems to be no major problem with the dog's condition, I decide to just keep an eye on it. However, CRP sometimes still remains high even at the next visit.

Cerón I also find these cases, usually when the CRP range becomes 20 to 60 or 70 mg/L. I do my best to monitor the dog's condition, but I agree that there are cases where no clinical signs appear even though CRP remains high and it is impossible to get an accurate grasp of why.

Ohno There must be a valid reason.

Cerón We should probably consider that something is happening to the dog even though no clinical signs appear.

Ohno On the contrary, CRP usually decreases when steroids are used. As a result, there is a possibility to overlook the severe inflammation and make the wrong decision. In such cases, I sometimes think that it might be better to utilize other acute-phase proteins. For example, it is said that SAA isn't affected by steroids in human. What are your thoughts on that point?

Cerón The relationship between SAA and the administration of glucocorticoid is not totally understood at the moment.

Ohno I have carried out several research projects regarding SAA, and SAA concentrations fluctuate according to the severity and progress of the inflammatory diseases. I think that SAA can be utilized as an acute phase protein in dogs as well. However, CRP is favorable because the speed of the fluctuations in concentration makes it more desirable as an inflammatory marker. Although I have not attempted a comparison of CRP and SAA yet, measuring both CRP and SAA could probably give us more detailed information.

Cerón You pointed out something interesting just now. We know, for example, that CRP decreases when glucocorticoid is used. If no decrease in CRP is observed in such cases, it means that some kind of problem has occurred. In other words, we can see it as a source of information that the treatment is not going well. For example, glucocorticoid is used in the treatment of immune-mediated hemolytic anemia (IMHA) but for cases where CRP remains high, the hematocrit does not increase.

Ohno In such cases we also need to suspect thrombosis, which complicates things even more. And there may be tissue damage and so on...

Cerón Or stronger immunosuppressive therapy may also be used, for example, mycophenolate mofetil in addition to the glucocorticoid.

Ohno We sometimes use mycophenolate mofetil to immune-mediated diseases such as IMHA.

Cerón Because it's more powerful, isn't it?

Ohno I guess so. Are there any other acute phase proteins that we might be able to utilize for dogs in the future? Or is CRP sufficient? I'd like to hear your current thoughts.

Cerón In my opinion, it might be beneficial to measure moderate acute phase proteins such as haptoglobin with the aim of combining the assay results.

Ohno I believe that we will be able to measure the values of two kinds of acute phase protein, such as CRP and haptoglobin, or CRP and SAA, on a single device. I don't know whether or not that would give us more detailed information, but I do think that a series of studies are required.

Cerón I personally would like to utilize various acute phase proteins but from a practical point of view, we should probably focus on CRP at this stage.

Utilizing acute phase proteins in cats

Ohno I'd like to change the topic now and talk about utilizing acute phase proteins in cats. I've been doing research on SAA for quite a long time. However, because the clinic doesn't have the appropriate in-house equipment, I send feline samples to a reference laboratory and don't measure it as a routine test. Some Japanese companies manufacture SAA assay reagents, but the reagents use antibodies for human SAA so there is a slight problem with the sensitivity. Do you routinely measure SAA in cats?

Cerón I routinely measure SAA using an automated analyzer. I think that an increase in SAA can be observed when a cat has an inflammation (Table 2). As you reported in your papers, pancreatitis and feline infectious peritonitis (FIP) are possible causes of a very high level of SAA. Also alpha-1-acid glycoprotein (AGP) is another inflammation marker. There have been a number of reports about the usefulness of AGP in particular in the diagnosis of FIP. I am interested to see whether AGP will be used as a complement to SAA for cats in the future. AGP is generally considered to be a moderate acute phase protein.

Ohno Do you also routinely measure AGP in cats?

Cerón We don't measure it, as currently there are no

Table 2 Main ailments in which an SAA increase has been reported in cats

Cited from reference work 2, with modifications

Ailment	Ratio of cats exceeding normal range (%)
Acute pancreatitis	100
Malignant mesothelioma	83
Feline infectious peritonitis (FIP)	75
Lymphoma	57
Hyperthyroidism	50
Polycystic kidney disease	50
Chronic kidney disease	45
Immune-mediated hemolytic anemia	40
Squamous cell carcinoma	38
Diabetes	38
Cholangitis	36

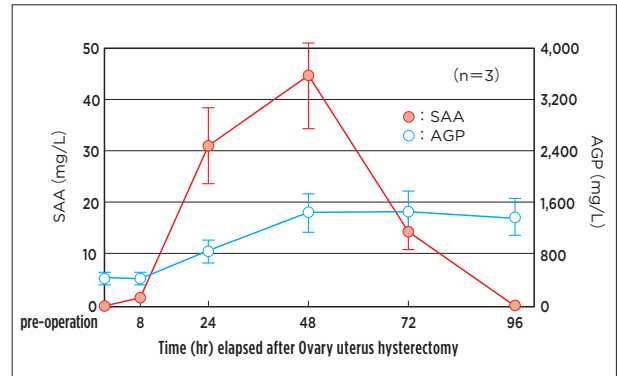


Fig. 4 Fluctuations in SAA and AGP in a cat prior to and after an ovariohysterectomy

SAA was measured with an SAA-TIA kit, and AGP was measured with a feline-specific single radial immunodiffusion assay.

Cited from reference 2 with modifications

commercial assays available. There were assays before but they were not appropriate for routine use.

Ohno I used to measure it by using commercial simple kit as well. Even now, I consider AGP to be a promising acute phase protein for cats. One approach for feline acute phase proteins would be to shift to AGP if SAA is very hard to measure. It is of course a much better option than having nothing at all to use. However, I am not convinced that an adequate method of measuring AGP exists. SAA fluctuations are big and fast so I think it is more useful than AGP (Fig. 4).

Cerón I agree. SAA will probably become an important indicator in cats, like CRP is in dogs.

Ohno There are however some concerns, since diseases are different in cats and dogs. In the case of dogs, CRP increases significantly when there is a bacterial infection or immune-mediated disease. I think that probably most acute phase proteins increase with these two kinds of causes. However, there are almost no immune-mediated diseases in cats but very many bacterial infections. My concern is that there are many hidden infections in cats. I don't know how much impact hidden infections have on the level of acute phase protein, but this might be a very big problem in the interpretation of SAA in cats.

Cerón I agree. There are many viral diseases that cats contract, such as feline immunodeficiency virus infection (FIV) and feline leukemia virus infection (FeLV). In my experience, increases in SAA can be observed in a number of these diseases when there is a concomitant inflammatory situation.

Ohno Are you saying that there is the possibility of SAA increasing in FIV and FeLV cases?

Cerón Generally speaking there's no increase. However, SAA increases are being seen in cases where there are concomitant infectious diseases such as cat scratch fever (Bartonellosis) or other complication.

Ohno I see. That's really interesting.

Cerón If an SAA increase is seen in FIV- or FeLV-positive cats, there is the possibility that the increase is caused by a secondary infection of Bartonellosis or some other complication.

Ohno It is extremely difficult to detect all the complications in cats. There is also the problem of dual infections. For example, the cause of SAA increase cannot be distinguished in a cat that is simultaneously infected with something like coronavirus and trichomonas. There's no problem if we already know which infectious diseases the cat has contracted. But we usually don't know why acute phase proteins are increasing until we have identified the infectious diseases. The problem is that there is a wide range of infectious diseases which cats contract. In addition to that, many elderly cats have chronic kidney disease, which is another of my concern. Some acute phase proteins such as SAA, in my experience, may be affected by chronic kidney disease. What are your thoughts on the connection between chronic kidney disease and the levels of acute phase proteins?

Cerón I think that normally acute phase proteins do not show major increase with chronic kidney disease. In my opinion, when it comes to SAA in cats, the primary inference we can make is pancreatitis. If lipase and SAA increase are linked, this becomes grounds for exploring the possibility that the cat is suffering from pancreatitis.

Ohno I use pancreatic lipase and CRP concurrently in dogs and it raises doubts when there is only an increase in lipase and not in CRP.

Cerón Yes. This kind of thing is also seen with gastrointestinal diseases, renal disorders or glucocorticoids .

Ohno It's interesting. Particularly in the case of cats, it is very difficult to diagnose chronic pancreatitis, even if we measure pancreas-specific lipase. It would be a tremendous improvement if SAA and lipase could be utilized together. It would surely be a huge advance for veterinarians as well.

Cerón You pointed out that the need for detailed research on acute phase proteins in chronic pancreatitis. This is due to the lack of information regarding acute phase protein fluctuations in chronic pancreatitis, and to the fact that the diagnosis itself is a difficult process. That is why even more information is required. The current problem with regard to acute phase proteins in cats is that there's not much information about acute phase protein fluctuations for each disease.

Ohno We need more data. There is insufficient data, particularly about cats. The problem is that we still cannot say that SAA is the best feline inflammation marker. We need to accumulate research on feline

acute phase proteins that is carried out from a variety of angles, such as whether SAA is the best marker, and explore its relation with AGP. If an excellent marker does exist, for sure veterinarians will actively make use of it. I hope that by conducting many research, a substantial amount of information can be acquired about feline acute phase proteins and SAA.

Cerón I hold seminars throughout Spain that are attended by veterinarians from all over the country. Some of them travel two or three hundred kilometers to attend. When I ask them why they have to travel such long distance just to attend the seminar, it is because they have purchased an equipment to measure SAA but they don't know how to interpret the assay results. This is a very important issue.

Ohno I've been holding CRP seminars throughout Japan for more than 10 years now. You will probably find yourself in the same situation as me and you will be much busier.

Bibliography

1. Petersen HH, Nielsen JP, Heegaard PM. Application of acute phase protein measurements in veterinary clinical chemistry. *Vet Res.* 2004 ; 35(2) : 163-187.
2. Tamamoto T, Ohno K, Ohmi A, et al. Verification of measurement of the feline serum amyloid A (SAA) concentration by human SAA turbidimetric immunoassay and its clinical application. *J Vet Med Sci.* 2008; 70(11): 1247-1252.

