FIP – ADVANCES IN DIAGNOSTIC TESTING & TREATMENT

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WHERE WE ARE WITH UNDERSTANDING THE CAUSE OF FELINE INFECTIOUS PERITONITIS?
Feline coronavirus (FCoV) infection is very common in cats, and it is FCoV infection which can sometimes result in FIP. Infections with FCoV are usually asymptomatic but result in FIP in around 5-10% of cats. Asymptomatic FCoV infection was previously believed to be confined to the intestinal tract, but we now know that healthy FCoV-infected cats can have systemic FCoV infection, albeit with lower FCoV viral loads than cats with FIP. Why FCoVs result in FIP in some cats and not in the majority of FCoV-infected cats is the subject of much investigation. Viral factors are important. FCoVs have a spike (S) protein that binds to the host (feline) receptor, mediating host cell entry, and S gene mutations can result in amino acid substitutions in the transcribed S protein that influence the tropism of FCoV, and these are believed to be associated with the ability of FCoV replication to occur outside of the intestinal tract (i.e. in monocytes/macrophages) as systemic FCoV infection, which is a prerequisite to the development of FIP. Other viral factors are also likely to be important for the subsequent development of FIP following systemic FCoV infection. Host factors are also very likely to play an important part in FIP development; these include the immune response (e.g. T-lymphocyte depletion occurs in cats that develop FIP), the ability of monocytes to sustain FCoV replication, breed and genetics.

CAN I / SHOULD I MAKE A DEFINITIVE DIAGNOSIS OF FIP IN A SUSPECTED CASE?
A definitive diagnosis of FIP relies on consistent histopathological changes in tissues with detection of FCoV antigen within lesions by immunohistochemistry (IHC). Immunostaining of FCoV antigen in effusion (also CSF & aqueous humour) samples showing biochemical and cytological features consistent with FIP is also likely to be adequate to definitively diagnose FIP, although positive results are not obtained in all cats with FIP. Although recent reports have suggested that some false positive results can occur with effusion immunostaining, this may have been due to methodology and the effusion characteristics were unlikely to have been consistent with FIP. Some have suggested that using cell pellets prepared from centrifuged effusion samples, to prepare formalin-fixed, paraffin embedded samples that can then be treated like a tissue specimen for IHC, can improve the reliability of detection of FCoV antigen.

In the absence of a definitive diagnosis, a high index of suspicion of FIP may be obtained from background information, clinical signs and routine clinicopathological results. With experience, this can be used as a basis to discuss with the owner whether additional, more invasive, diagnostic tests, such as collection of biopsies, are warranted. In such cases it may be that euthanasia is discussed as an alternative to pursuing a definitive diagnosis ante-mortem, and this may be preferable in, for example, shelter cats, where there are financial limitations, or when cats are very sick and concerns exist over a patient’s ability to tolerate diagnostic procedures (e.g. surgical biopsy). If euthanasia is performed without a definitive diagnosis, a simple post-mortem examination should be performed to look for gross changes consistent with FIP, and ideally sampling for histopathological examination. It is possible that fine needle aspirates (FNAs) could be used as samples for FCoV antigen immunostaining; further studies are necessary to evaluate their utility in the diagnosis of FIP.

EVALUATING ANY BACKGROUND EVIDENCE FOR FIP
FIP is most common in young cats, especially < 2 years, that have lived previously in multi-cat households. Male cats are also at a slightly higher risk. Some breeds in some countries may be predisposed to FIP, but this is likely due to the presence of unknown specific genetic risk factors in those breeding lines in those countries and worldwide breed predispositions may not exist. A recent history of stress (e.g., adoption, being in a shelter) may be apparent.
ARE THE CLINICAL SIGNS CONSISTENT WITH FIP?

Disease manifestations of FIP typically comprise a vasculopathy resulting in ('wet') effusions (most common), granuloma formation resulting in ('dry') mass lesions, or a combination of the two and indeed most FIP cases with effusions also have granulomatous lesions visible at post-mortem examination.

Clinical signs include lethargy, anorexia, weight loss (or failure to gain weight/stunted growth in younger cats), non-responsive pyrexia, jaundice (more common in effusive FIP), lymphadenomegaly, renomegaly and ocular signs (anterior and/or posterior uveitis). Effusive 'wet' FIP is associated with abdominal, pleural and/or pericardial effusions, and is often quite acute in nature, progressing within a few days or weeks and severely limiting survival. These cats can present with dyspnoea, tachypnoea and/or abdominal distension. Non-effusive 'dry' FIP can be associated with neurological signs (can be focal, multifocal or diffuse in nature, often with central vestibular signs, occasionally as a T3-L3 myelopathy) and is more chronic, progressing over a few weeks to months. Occasionally a diffuse pyogranulomatous pneumonia is reported. Non-effusive FIP occasionally presents as a palpable abdominal (lymph node or intestinal) mass.

ARE HAEMATOLOGY AND BIOCHEMISTRY CONSISTENT WITH FIP?

Haematological changes are non-specific in FIP but lymphopenia is particularly common (55-77% of cases; although a recent study found only 49.5% of FIP cases to be lymphopenic); additionally a neutrophilia (39-57% of cases), a left shift, microcytosis, and mild-moderate normocytic, normochromic anaemia (37-54%) may occur.

Hyperglobulinaemia is reported in 89% of cases, often with hypoalbuninaemia or low-normal serum albumin, total protein concentrations may be normal. This combination means that the albumin:globulin (A:G) ratio is low. It has been suggested that an A:G ratio of <0.4 makes FIP very likely, whilst an A:G ratio of >0.8 makes FIP very unlikely, but the lower the value, the bigger the suspicion for FIP becomes, especially if other findings are consistent with FIP. Hyperbilirubinaemia occurs in 21-63% of FIP cases, especially in effusive FIP, often without marked elevations in alanine aminotransferase, alkaline phosphatase or gamma-glutamyltransferase enzyme activity (although these can be moderately elevated in FIP cases). Serum α1-acid glycoprotein (AGP) elevations (>0.48 mg/ml) per se are not specific for FIP, but markedly elevated AGP levels (>1.5 mg/ml) often occur with FIP, so the magnitude of the AGP increase may be helpful, with higher concentrations being more useful in raising the index of suspicion for FIP. A study found that when history and clinical findings were supportive of FIP, moderate serum AGP levels (1.5-2 mg/ml) could discriminate cats with FIP from cats without FIP, but only higher serum AGP levels (>3 mg/ml) could support a diagnosis of FIP in cats with less supportive evidence for FIP.

A positive FCoV antibody test indicates that the cat has been infected with FCoV and has seroconverted (this takes 2-3 weeks from initial infection). Although FIP cats tend to have higher FCoV antibody titres than non-FIP cats, there is much overlap, with no difference between median FCoV antibody titres in healthy and suspected FIP cats, so the value in an individual cat to distinguish cats with FIP is very limited.

IS AN EFFUSION PRESENT TO SAMPLE?

Ultrasonography is generally regarded as being more sensitive than radiography for the detection of small volumes of fluid in the thorax and abdomen, but this may depend on where pockets of fluid reside. Repeated ultrasonography to identify any small volume effusion is recommended and, similarly, ultrasonography can be used to guide fluid sampling. FIP effusions are usually clear, viscous/sticky, straw-yellow and protein-rich (thick eosinophilic proteinaceous backgrounds are often described on cytology), with a total protein concentration of >35 g/l (>50% globulins). They have similar low A:G ratios and raised AGP concentrations as serum. FIP effusions are poorly cellular (usually <5 x10⁷/l cells), and are typically pyogranulomatous in nature with macrophages, non-degenerate neutrophils and very few lymphocytes. Immunostaining for FCoV antigen and reverse-transcriptase polymerase chain reaction (RT-PCR) for FCoV RNA on effusion samples can also be performed (see below).
FCOV RT-PCR

RT-PCR assays detect FCoV; however, they are not specific for FIP-associated FCoVs so can never be used to definitively diagnose FIP as both cats with and without FIP can show positive results, although cats with FIP are far more likely to be FCoV RT-PCR positive than cats without FIP, and cats with FIP also have significantly higher FCoV loads in samples than cats without FIP. Thus, the presence of particularly high levels of FCoV RNA in samples associated with consistent FIP pathology can be highly supportive of a diagnosis of FIP. The FCoV RT-PCR used must be quantitative in order that FCoV loads are reported. FCoV RT-PCR can be used to detect FCoV RNA in effusion, tissue (biopsy or maybe ultrasound-guided FNAs), CSF, or aqueous humour samples from suspected cases of FIP. Selection of appropriate samples to submit for RT-PCR can be guided by clinical signs such as presence of effusions, ocular or neurological signs, imaging results, cytological findings (e.g. pyogranulomatous inflammation), and non-invasive sampling methods are generally preferred, particularly in sick cats. FCoV RT-PCR can also be performed on faecal samples, but this is primarily used to identify cats that are shedding FCoV for the management of infection in multi-cat households, not for a diagnosis of FIP.

FCOV RT-PCR FOLLOWED BY S GENE MUTATION ANALYSIS

Following the detection of FCoV RNA in a sample by RT-PCR, it may be possible to then characterise targeted sequences of the FCoV genome, especially the S gene, using molecular techniques. Such techniques are not always successful in samples positive by RT-PCR because if only low levels of FCoV are present, sequencing may not be possible, or if the target FCoV sequence does not match those being looked for by the sequencing method.

Such sequence characterisation would be extremely useful if FIP-specific mutations existed. Although recent research documented so-called FIP-specific S gene mutations; these were identified by comparing the sequences of FCoVs found in the tissues of FIP cats with those found in the faeces of healthy non-FIP cats. Researchers in our group hypothesized that these sequence mutations could reflect systemic FCoV (i.e. monocyte/macrophage-associated FCoV compared to intestinal epithelium-associated FCoV) rather than being specific for FIP, knowing that non-FIP cats can have systemic FCoV infection. We therefore compared the S gene sequences of FCoV detected in the tissues of FIP cats with those detected in the tissues of non-FIP cats. This allowed us to evaluate the S gene sequences of FCoVs associated with systemic FCoV infection in both non-FIP and FIP cases. We found that the S gene mutations present in most of the FIP tissues were also present in most of the tissues of non-FIP cats that had systemic FCoV infection. A recent more extensive study confirmed the same findings, and calculated that if the identification of S gene mutated FCoVs was included as an additional confirmatory step to the detection of FCoV by RT-PCR alone, this only slightly increased specificity for the diagnosis of FIP in tissue samples (from 92.6% for FCoV RT-PCR alone to 94.6% with the addition of S gene mutation analysis) but moderately decreased sensitivity (from 89.8% to 80.9%, respectively, mainly because mutation analysis was not possible in all tissue samples). Similar results were obtained for fluid samples (primarily effusions but also CSF and aqueous humour; specificity stayed at 97.9% but sensitivity was markedly decreased from 78.4% to 60% when S gene mutation analysis was performed following FCoV RT-PCR).

Other studies on mutation analysis have reported lower sensitivities but higher specificities compared to FCoV RT-PCR alone than those obtained in our studies. When interpreting studies, it is worth remembering that if a mutation assay is heavily reliant on having a significant FCoV load in the sample to enable sequencing, its sensitivity can appear to be quite poor as some samples from FIP cats may not have adequate FCoV loads on which to perform successful sequencing. Conversely, such assays may appear to have good specificity as they are unable to sequence mutated sequences in cats without FIP, and thus don’t generate false positives. Hence significant increases in specificity for mutation assays over FCoV RT-PCR alone may be due to their inability to identify mutated FCoV in cats without FIP; indeed some studies reporting 100% specificity for mutation analysis have failed to sequence any FCoVs in cats without FIP.

Thus, only a slight increase in specificity is seen if the mutation assay is reliable. Although specificity should be maximised for tests used in the diagnosis of FIP, because we know that FCoVs in cats without FIP can have these mutations too, a positive mutation result should never be used to confirm a diagnosis of FIP.
FURTHER INFORMATION ON DIAGNOSTIC TESTS FOR FIP
More information on the sensitivity and specificity of the diagnostic tests described above can be found in a recently published review article\(^{23}\).

NEW FIP TREATMENT STUDIES
FIP is generally regarded as being incurable, although descriptions of long-term FIP survivors with a confirmed diagnosis (including FCoV antigen immunostaining) are reported, such as a non-effusive FIP case that survived 787 days on varied treatments including prednisolone, meloxicam, tramadol and antibiotics\(^{24}\). Supportive treatment is usually aimed at modification of the host immune response to infection, with immunomodulators or corticosteroids. However, there are no controlled studies to prove any beneficial effect of corticosteroids, although they are frequently used especially in cats with effusions. Recently, some important FIP treatment studies have been published. None have included untreated control cats for ethical reasons.

Polyprenyl immunostimulant (PPI) treatment (3 mg/kg orally three times per week), which upregulates Th-1 cytokines as an immunostimulant, has recently been evaluated in 60 non-effusive FIP cases\(^{25}\). The diagnosis of dry FIP was largely made by the attending veterinarian and only 13 of the 36 cats that had so-called specialised laboratory testing had FCoV immunostaining performed, although acquiring samples for the diagnosis of non-effusive FIP cases is notoriously difficult. Of the 60 cats, 8 survived for over ~6½ months, including 1 cat for 2½ years and 1 cat for just over 5 years; the survival times of cats that were given corticosteroids concurrently with PPI were significantly shorter than those given PPI without corticosteroids.

Anti-viral agents have also been evaluated. One 3C-like protease inhibitor compound, GC376, showed great promise in an experimental study in young cats with effusive FIP\(^{26}\) where 6 of 8 cats with clinical FIP recovered from disease following 2-3 weeks of GC376 treatment. A follow up study evaluating GC376 treatment in 20 cats with naturally occurring effusive or non-effusive FIP\(^{27}\) was then performed. Cats were confirmed as having FIP based on signalment, history, prior test results, clinical examination, repeat of blood and effusion testing and ultrasonography and ophthalmological examinations when necessary. Of the 20 treated cats in the study, 6 cats (these were mostly young acute effusive FIP cases) showed long-term remission of at least 18 months (this duration was stipulated in a subsequently published study\(^{28}\)) following GC376 treatment (usually for 12 weeks). A sustained remission was not seen in all, and some treatment side effects were reported (injection reactions and abnormal eruption of permanent teeth). The cats that showed relapses of FIP often showed signs of neurological FIP.

The most recent study\(^{28}\) has described treatment using the nucleoside analogue GS-441524 in an experimental study in young cats with effusive FIP. In this study 10 cats that developed effusive FIP were treated with GS-441524 subcutaneously once daily for 2 weeks and showed a rapid reversal of clinical signs (pyrexia and lymphopenia). Two of the 10 treated cats required a 2nd treatment course following a relapse at 4 and 6 weeks post-treatment, respectively, and both improved again. All 10 treated cats remained clinically healthy until the time of publication, at least 8 months post-infection. No signs of toxicity were noted besides a transient "stinging" injection reaction in some cats\(^{28}\). This treatment now requires evaluation in a field study of cats with FIP.

REFERENCES


