Origin of abdominal or thoracic effusions in cats with wet FIP and reasons for their persistence during treatment

Niels C. Pedersen, DVM, PhD
Center for Companion Animal Health
University of California, Davis
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Origin of FIP effusions. Effusions of wet FIP originate from small, inflamed blood vessels (venules) within the tissue that lines the surfaces of abdominal and thoracic organs, mesenteries/mediastinum, and omentum. This thin tissue or membrane is called the serosa. The serosa lining the abdomen and abdominal organs is also called the peritoneum and the serosa of the chest cavity is called the pleura. The serosa (or pleura) lining the walls of the body cavity is referred to as "parietal" while the tissue lining the organs is referred to as "visceral". The mesentery is a membrane lined by serosa that supports the abdominal viscera, while the mediastinum performs a similar function in the chest cavity. The omentum is a thick curtain-like organ that hangs behind the stomach and is also lined by the peritoneum. It is rich in blood vessels and fat and is thought to play a key biological role in immune-regulation and tissue regeneration. It also acts as a plug and healing blanket for penetrating wounds of the abdomen or internal organs. These various abdominal and thoracic tissues and structures are involved to varying degrees in cats with the abdominal or thoracic effusive forms of FIP. The specific target tissue in wet FIP is the serosa. The term peritonitis refers specifically to inflammation of the peritoneum.

The spaces around venules in the serosa contain a specific type of macrophage that is derived from monocyte progenitors that are in continuous recirculation between bloodstream, interstitial spaces around venules, afferent lymph, regional lymph nodes, and back to the bloodstream. Additional sites for this recirculation are small venules found in the meninges, ependyma of the brain, and uveal tract and retina of the eyes. A small proportion of these monocytes evolve into immature macrophages (monocyte/macrophages) and eventually resident macrophages. Macrophages function continuously survey for infections.

FIPV originates by mutation from feline enteric coronavirus (FECV) present in lymphoid tissues and lymph nodes in the lower bowel. The mutation changes the cellular tropism of FECV from enterocytes and ultimately to peritoneal type macrophages. Monocyte/macrophages appear to be the first cell type to be infected. This infection causes more monocytes to leave the bloodstream and begin their transformation into macrophages, which continue the cycle of infection [2]. Monocyte/macrophages do not undergo programmed cell death as normally expected but continue their maturation into large virus laden macrophages. These large macrophages ultimately undergo programmed cell death (apoptosis) and release large amounts of virus, which then infect new monocyte/macrophages [1]. Infected monocyte/macrophages and macrophages produce several substances (cytokines) that mediate both the intensity of inflammation (disease) and immunity (resistance) [1,2].

The inflammation associated with FIP leads to three types of changes in venules. The first is loss of vessel wall integrity, microhemorrhage and leakage of a plasma protein rich in activated clotting and complement activation factors and other inflammatory proteins. A second type of damage involves thrombosis and blockage of blood flow. The third insult occurs in more chronic cases and involves fibrosis (scarring) around the vessels. Variations in these three events determine the amount and makeup of the effusions according to the four Starling's forces which determine the movement of fluids between the bloodstream and interstitial spaces [3].

The classical effusion of wet FIP results from mainly from acute damage to the vessel walls and leakage of plasma into the interstitial spaces and eventually into body cavities. Protein leaked into the interstitial spaces attracts more fluids, which can be exacerbated by blockage of venular blood flow and increased capillary pressure. This type of effusion, known as an exudate, also contains high levels of proteins involved in inflammation, immune responses, and blood clotting. This fluid also contains large numbers of neutrophils, macrophage/monocytes, macrophages, eosinophils, and lower numbers of lymphocytes and red blood cells. This classical type of fluid has an egg-white consistency and forms weak clots contains high levels of bilirubin. The bilirubin is not from liver disease, but rather from the destruction of red cells leaked into the interstitial tissue's cells and engulfed by monocyte/macrophages and macrophages. The red cells are broken down and the hemoglobin cleaved to heme and globin. The globin is further metabolized to biliverdin (greenish color) and ultimately to bilirubin (yellowish color), which is then excreted by the liver. However, cats are deficient in the enzymes used for conjugation and are therefore inefficient in clearing bilirubin from the body [4]. This leads to a buildup of bilirubin in the bloodstream and gives the effusion a yellow tinge. The darker the yellow tinge, the more bilirubin is in the effusion, the more severe the initiating inflammatory response, and the more severe the resultant bilirubinemia, bilirubinuria and jaundice.

At the opposite extreme to the classical and more acute FIP effusion are effusions generated predominately from more chronic infections and blockage of venular blood flow and resultant increase in capillary pressure. High capillary pressure leads to effusion that more loosely resemble interstitial fluid than plasma, with lower protein content, watery rather than sticky, clear or slightly yellow-tinged, not prone to clotting, and lower number of acute inflammatory cells such as neutrophils. There are also FIP effusions that are intermediate between these extremes, depending on the relative degree of acute inflammation and chronic fibrosis. These intermediate types of fluid have been commonly referred to as a modified transudate in veterinary literature, but this is a misnomer. A modified transudate begins as a transudate and is altered when it persists and elicits mild inflammation. Low protein/cell effusions of FIP originate as exudates and not transudates and do not fit this description. A more correct term is "modified exudate" or "variant exudative effusion."

How long to effusions normally persist in GS-441524 or GC376 treated cats? The presence of abdominal effusions often leads to gross distension of the abdomen, and confirmed by palpation,

blind needle aspiration, radiograph, or ultrasound. Cats with thoracic effusions are most likely to present with severe dyspnea and confirmed by radiology and aspiration. Thoracic effusions are almost always removed to relieve the dyspnea and are slow to recur compared to abdominal effusions. Therefore, abdominal effusions are not normally removed unless massive and interfering with breathing, as they are rapidly replaced. Repeated drainage of abdominal effusions can also deplete proteins and cause harmful shifts in fluid and electrolyte balances in severely ill cats.

Thoracic effusions clear more rapidly with GS-441524 treatment, with improvement in breathing within 24-72 hours and disappearance usually within less than 7 days. Abdominal effusions are usually grossly diminished by 7-14 days and gone by 21-28 days. Detection of effusions that persist after this time depend on their amount and the method of detection. Small amounts of persistent fluid are only detectable by ultrasound.

Persistence of effusions during or after antiviral treatment. There are three basic reasons for effusions to persist. The first is persistence of the infection and resultant inflammation at some level, which can be due to an inadequate treatment, poor drug, or drug resistance. Inadequate treatment may result from dosage miscalculation to bad drug, or to the virus acquiring drug resistance. The second reason for fluid persistence is chronic damage to venules and increased capillary pressures. This can be due to a low-grade infection or residual fibrosis from an infection that has been eliminated. The third reason for persistence is the existence of other diseases that may also manifest by effusions. These include congenital heart disease, in particular cardiomyopathy, chronic liver disease (acquired or congenital), hypoproteinemia (acquired or congenital), and cancer. Congenital diseases causing effusions are more apt to occur in young cats, while acquired causes and cancer are more likely to be diagnosed in older cats.

Diagnosis and treatment of persistent effusions. A thorough examination of the fluid as described above in pre-requisite to diagnosis and treatment. If the fluid has an inflammatory or semi-inflammatory nature and a cell pellet is positive by PCR or IHC, a reason for persistence of the infection must be determined. Was the antiviral treatment correctly managed, was the antiviral drug active and the concentration correct, was there evidence for acquired drug resistance? If the fluid has an inflammatory nature and the PCR and IHC are negative, what other diseases are possible? Fluids that are low in protein and cells, and do not indicate the presence of inflammation and test negative by PCR and IHC, point the diagnosis towards residual small vessel fibrosis and/or other contributing causes such as heart disease, chronic liver disease, hypoproteinemia (intestinal or renal disease). Some of the disorders causing this type of effusion may require an exploratory laparotomy with careful inspection of the abdominal organs and selective biopsies to determine the origin of the fluid. The treatment of the persistent effusions will vary greatly depending on the ultimate cause. Persistent effusions due to residual small vessel fibrosis in cats cured of infection will often resolve over many weeks or months. Persistent

effusions due wholly or in part to other conditions will require treatment targeted at those disorders.

Identifying and characterizing persistent effusions- The presence of fluid after 4 weeks of GS treatment is troublesome and is usually detected in several manners, depending on the amount of the fluid and its localization. Large amounts of fluids are usually detected by abdominal distension, palpation, radiographs, and abdominal aspiration, while smaller amounts of fluid are best detected by ultrasound. Persistent pleural effusion is usually detected by radiographs or ultrasound. Overall, ultrasound is the most accurate means to detect and semi-quantitate effusions in the thoracic and abdominal cavities. Ultrasound can also be used in combination with fine needle aspiration to collect small and localized amounts of fluid.

The second step in studying persistent effusions is to analyze them for their color, protein content, white and red cell numbers, and types of white cells that are present. Fluids generated primarily by inflammation will have protein levels near or equal to plasma and large numbers of white cells (neutrophils, lymphocytes, monocyte/macrophages, and large vacuolated macrophages). Fluids generated by increased capillary pressure more closely resemble interstitial fluid with proteins nearer to 2.0 g/dl and cell counts <200. The Rivalta test is often used to diagnose FIP associated effusions. However, it is not a specific test for FIP, but rather for effusions of an inflammatory nature. It is usually positive in FIP effusions that are high in proteins and cellularity but often negative in effusions that are very low in protein and cells. Fluids that are intermediate between these two types of effusions will test either positive or negative, depending on where they lie in the spectrum.

The third step is to analyze the effusions for the presence of the FIP virus. This usually requires 5 to 25 or more ml of fluid. Lesser amounts may suffice for fluids with higher protein and cell counts, while larger amounts are required for fluids with low protein and cells. A freshly collected sample should be centrifuged and the cell pellet analyzed for viral RNA by PCR or cytocentrifuged for immunohistochemistry (IHC). The PCR test should be for the FIPV 7b RNA and not for the FIPV specific mutations, as the mutation test lacks sensitivity and offers no advantages for diagnosis [5]. Samples that test positive by either PCR or IHC provide definitive evidence of FIP. However, up to 30% of samples from known cases of FIP can test falsely negative because of either inadequate sample and sample preparation, or because the levels of FIP virus RNA is below the level of detection. Also, the less inflammatory the fluid, the lower the virus levels. Therefore, effusions with lower protein and white cell levels are more likely to test negative as viral RNA is below the detection limit of the assay.

References

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