

Performances of different diagnostic tests for feline infectious peritonitis in challenging clinical cases

L. GIORI, A. GIORDANO, C. GIUDICE, V. GRIECO AND S. PALTRINIERI

Department of Veterinary Pathology, Hygiene and Public Health, Section of Veterinary General Pathology and Parasitology, Università degli Studi di Milano, Milan, Italy

OBJECTIVES: Feline infectious peritonitis (FIP) can be difficult to diagnose. Histopathology is considered the gold standard test but immunohistochemistry (IHC) is mandatory to confirm/exclude the disease. This study aimed to assess the performances of tests carried out in vivo or at postmortem examination in challenging cases in which FIP was confirmed or excluded based on IHC or on adequate follow-up.

METHODS: Twelve cases (four without FIP, eight with FIP) were retrospectively studied. Clinical findings, serum protein electrophoresis (SPE), analysis of the effusions (AE), antifeline coronavirus serology, serum concentration of α_1 -acid glycoprotein (AGP) and histopathology were classified as consistent, doubtful or non-consistent with FIP. Sensitivity, specificity and concordance (κ) with the final diagnosis were calculated.

RESULTS: Concordance was absent for serology ($\kappa=-0.08$) and AE ($\kappa=-0.52$), poor for histopathology ($\kappa=0.09$), fair for SPE ($\kappa=0.25$) and perfect for AGP ($\kappa=1.00$). Sensitivity was high for AGP (100%) and low for AE (50%), SPE (37.5%) and histopathology (37.5%). Specificity was high for AGP or histopathology (100%) and low for SPE (50%) and AE (0%).

CLINICAL SIGNIFICANCE: IHC must always be performed to confirm FIP. If this is not possible, when histopathology is controversial, elevated AGP concentrations may support the diagnosis of FIP.

Journal of Small Animal Practice (2011) **52**, 152–157
DOI: 10.1111/j.1748-5827.2011.01042.x

Accepted: 17 January 2011

INTRODUCTION

Feline coronavirus (FCoV) causes feline infectious peritonitis (FIP), a lethal disease characterised by vasculitis and/or pyogranulomatous lesions in different organs (Pedersen 2009). The diagnosis of FIP is difficult, especially in non-effusive (dry) cases (Kipar and others 1999, Hartmann 2005, Pedersen 2009). A diagnosis cannot be based solely on serology or polymerase chain reaction (PCR), which detects the antibody response or the virus but does not provide information about the relationship between infection and clinical disease (Pedersen 1976, Herrewegh and others 1995, Pedersen 1995, Meli and others 2004). A diagnosis should be based on a combination of history (cats younger than 3 years or older than 10 years, especially living in crowded conditions), clinical signs and clinico-pathological or pathological changes (Sparkes and others 1991, 1994, Foley and others 1998, Andrew 2000, Addie and others 2004, Hartmann 2005, Pedersen 2009).

Common abnormalities include lymphopenia and changes in serum protein electrophoresis (SPE) such as hyperproteinaemia with inverted albumin/globulin (A/G) ratio and increased serum α_2 - and γ -globulin concentrations (Sparkes and others 1991, 1994, Paltrinieri and others 1998, Addie and others 2004). This electrophoretic pattern is also evident in effusions when present (Shelly and others 1988, Paltrinieri and others 1998). The macroscopic, physico-chemical and cytological pattern of the effusion is also typical and has a high positive predictive value for FIP (Paltrinieri and others 1999). It is described as a yellowish, transparent to cloudy, sticky fluid, often with fibrin clots and characterised by high protein content, high specific gravity and variable amounts of cells, mostly composed of non-degenerated neutrophils, macrophages and lymphocytes in a granular proteinaceous eosinophilic background.

Recently, it has been proposed that the serum concentration of α_1 -acid glycoprotein (AGP) is used as a diagnostic tool for FIP (Paltrinieri and others 2007, Pedersen 2009). AGP is the major

acute phase protein in cats and it increases in several inflammatory and non-inflammatory conditions (Ceron and others 2005, Paltrinieri 2008). Nevertheless, the most prominent increases in serum AGP concentration have been recorded in cats with FIP (Duthie and others 1997, Giordano and others 2004) and AGP could also be measured in effusions (Bence and others 2005). However, even moderate increases in serum concentration of AGP can be used to support a diagnosis in cats with a strong clinical suspicion of FIP (Paltrinieri and others 2007).

Histopathology is considered the only method for a conclusive diagnosis (Sparkes and others 1991, 1994, Pedersen 2009). However, the low yield of histopathological lesions in tru-cut sections limits the potential application of this approach for ante-mortem diagnosis of FIP (Giordano and others 2005).

In the authors' experience, postmortem test results and routine histopathology are occasionally inconclusive, especially in cases with an atypical clinical presentation. This difficulty has also been highlighted elsewhere, with reports of FIP lesions misdiagnosed as tumours or vice versa (Kipar and others 1999, Pedersen 2009). Anti-FCoV immunohistochemistry (IHC) is mandatory to confirm/exclude the disease in doubtful cases (Addie and others 2004, Hartmann 2005, Pedersen 2009).

The purpose of this study was to retrospectively assess the results of tests recorded in vivo and at postmortem examination in doubtful cases where FIP was clinically suspected and definitely confirmed or excluded by IHC or based on complete recovery and to evaluate which test had the best sensitivity, specificity and concordance for FIP.

MATERIAL AND METHODS

Selection of cases

The database in our Department was retrospectively analysed to select cases with the following criteria: (1) presence of complete information about in vivo and postmortem tests and/or a minimum follow-up of approximately 2 years; (2) inclusion of FIP among the differential diagnoses based on the presence of clinical or laboratory abnormalities supportive of FIP and also the presence of one or more clinical, laboratory, gross or histopathological features not considered consistent with FIP or suggestive of a disease other than FIP; (3) final confirmation/exclusion of FIP based on the following criteria: cats were classified as not affected by FIP (without FIP) based either on gross or histopathological findings inconsistent with FIP with negative IHC for intralésional FCoVs or on the in vivo identification of diseases other than FIP and/or based on complete remission of clinical signs after appropriate therapy. Conversely, cats were classified as affected by FIP based on positive IHC despite gross or histopathological lesions not completely consistent with FIP.

Clinicopathological and pathological tests

At admission, all the selected cats underwent a complete clinicopathological screening, except for PCR that, when performed, was requested by the referring veterinarians at their own respective service laboratories. In the case of death, cats were immediately

taken for postmortem examination and histopathology and IHC were performed as described below. The details of the tests included in this study are as follows.

Analysis of the effusions: Approximately 2 mL of fluid was placed in an EDTA-coated tube. Cells were counted using an impedance counter (Hemat 8, SEAC, Calenzano, Firenze, Italy). Then 50 to 100 µL of fluid was cytocentrifuged (Cytospin 2, Shandon Scientific, Runcorn, Cheshire, UK) at 130g for 10 minutes, and the remaining effusion was centrifuged (500g for 8 minutes), to obtain the cell-free supernatant used to evaluate the total protein by the biuret method with an automated analyser (Cobas Mira Classic, Roche Diagnostics Basel, Switzerland), to perform electrophoresis using the same method described below for serum and to estimate the specific gravity using a portable refractometer (Clinical Refractometer 5711-2020, Schüco, Tokyo, Japan). Cytocentrifuged slides were stained with May Grünwald-Giemsa, cover slipped and examined under a light microscope.

Serum protein electrophoresis: This was performed using an automated system (Genio, Interlab Srl, Rome, Italy). Cellulose acetate electrophoresis was run at room temperature in a buffer solution (Tris buffer >5%, sodium 5,5-diethylbarbiturate <1%, pH ≥8) for 16 minutes at 140 V. Strips were stained with Poncau red, destained, diaphanised in appropriate solutions provided by the manufacturer and tested by densitometry to obtain electrophoretograms and the percentage area under each peak.

AGP: AGP concentrations were measured using a radial immunodiffusion (SRID) kit (Tridelta Development Ltd, Maynooth, Kildare, Ireland). Five microlitres of each serum sample or of standard solutions containing 0.5 and 2.0 mg/mL of feline AGP, respectively, were put in each well of a multi-well SRID plate. After incubation (48 hours at room temperature), the diameter of precipitation rings was measured. Values of the two standard solutions were used to design a standard curve. Values from the case samples were then plotted to extrapolate absolute AGP levels, expressed as mg/mL.

Serology: The presence of anti-FCoV antibodies was assessed using an indirect immunofluorescence test performed on 10 multi-well slides produced at the University of Zurich according to Osterhaus and others (1977), by coating each well with 4.5×10^3 PD-5 cells, half of which were infected with swine transmissible gastroenteritis virus (serologically cross-reacting with FCoVs). Twofold dilutions (1:25 to 1:400) of each serum sample were prepared and 20 µL of each dilution was applied to the wells. After incubation (30 minutes, 37°C in a moist chamber), slides were washed with phosphate-buffered saline (PBS), dried and 15 µL of fluorescein isothiocyanate-conjugated rabbit-anticat immunoglobulin (Nordic Immunological Laboratories, Tilburg, The Netherlands) was added to each well. After incubation (30 minutes, 37°C in a moist chamber), slides were washed, dried, cover-slipped with PBS and Kaiser's glycerin (1:3 v/v) and observed on a fluorescence microscope. When the dilutions showed a clear fluorescent signal in about half of the cells, they were judged as positive. Samples that were still positive at a 1:400 dilution were further diluted on a twofold basis until they were negative.

Histopathology/IHC: Tissue samples were fixed in buffered 10% iso-osmotic formalin and embedded in paraffin. Microthomic

sections (5 µm) were stained by haematoxylin-eosin and by IHC using a monoclonal antibody (mAb) against the FCoV (kindly provided by Prof. N.C. Pedersen, Davis, USA). The avidin biotin complex (ABC) method with a commercially available kit (Vectastain Elite, Vector laboratories Inc., Burlingame, CA, USA) was used to detect the positive reaction (Paltrinieri and others 2003), after inhibition of the endogenous peroxidase (H₂O₂ 1% in methanol) and antigen unmasking using microwave pretreatment (two cycles of 5 minutes each in citrate-buffered solution, 0.01 M, pH 6.2). 3-Amino-9-ethyl-carbazole or diaminobenzidine served as chromogen for the reaction and then the slides were counterstained with Mayer's haematoxylin. Some sections of each sample were used as negative controls, with the primary antibody replaced by normal mouse serum (DAKO A/S, Glostrup, Denmark).

Statistical analysis

Data regarding clinical, clinicopathological and postmortem examination results recorded in each case were classified as consistent, doubtful or non-consistent with FIP as described in Table 1.

Based on the final diagnosis, data were used to classify test results as true positive (TP), false positive (FP), true negative (TN) and false negative (FN). Based on these values, sensitivity [$\text{sens}=(\text{TP}/\text{TP}+\text{FN})\times 100$] and specificity [$\text{spec}=(\text{TN}/\text{TN}+\text{FP})\times 100$] were calculated (Stockham and Scott 2008).

The concordance between each single test interpretation and the final diagnosis was assessed by determining the Cohen's κ coefficient and graded as suggested by Landis and Koch (1977) as poor (κ: <0), slight (κ: 0.0 to 0.20), fair (κ: 0.21 to 0.40), moderate (κ: 0.41 to 0.60), substantial (κ: 0.61 to 0.80) and almost perfect (κ: 0.81 to 1.00).

RESULTS

Final diagnosis in the four cats without FIP

These four cats had symptoms consistent with FIP such as uveitis (cat 1) or effusions (cats 2, 3 and 4), but FIP was excluded for the

following reasons: cat 1 recovered after local therapies and cat 2 had a diaphragmatic hernia. Lesions consistent with FIP or intralesional FCoVs were not found in tissues from the remaining two cats and in cat 3, the "effusion" actually was the fluid content of a hepatic cyst. The cat recovered completely after surgical removal of the cyst, cat 4 had a focally extensive hepatic nodular lesion, histologically diagnosed as hepatocarcinoma and negative for FCoV on IHC.

Final diagnosis in the eight cats with FIP

Three of the cats with FIP had atypical clinical presentations, including a mediastinal mass cytologically consistent with lymphoma (cat 6), a haemorrhagic syndrome with abdominal effusion classified as "transudate" (cells and proteins were virtually absent; cat 7) and absence of other signs except stunted growth (cat 9). No effusions were detected in vivo in this latter cat but a small amount of abdominal fluid was collected at postmortem examination (see electrophoretic analysis in Fig 1).

Five cats with FIP had in vivo tests partially consistent with FIP, including the presence of effusions, but postmortem examination findings were atypical due to the presence of the following: mesenteric fibrotising lymphadenopathy without additional lesions (cats 5 and 12), intestinal pyogranulomatous lesions containing Ziehl-Neelsen positive rods and Periodic acid-schiff (PAS)-positive fungal hyphae (cat 8), segmental thickening of the intestinal wall histologically characterised by severe fibrosis (cat 10, Fig 2) or a mediastinal cyst-like lesion (detected by computed tomography scan) filled with a fluid content cytologically consistent with FIP (cat 11; Fig 3; Viganì and others 2009).

Intralesional FCoVs were detected by IHC in all the eight cats belonging to this group (Fig 4).

Results of clinicopathological or postmortem tests

Clinical findings and results of clinicopathological or postmortem tests are summarised in Table 2, where the results have been categorised according to the criteria reported in Table 1. To

Table 1. Categorisation of diagnostic parameters as consistent, doubtful and not consistent with FIP

	Consistent (C)	Doubtful (D)	Not consistent (NC)
History	Cattery with previous cases of FIP or high prevalence of positive serology	—	Households or pet cats without contacts with potential shedders of FCoV
Clinical signs	Fever, effusion, ocular lesions, jaundice, masses, neurological signs	Masses, fever or neurological signs	None of the findings reported for group C or D
Effusion	Macroscopic and cytological pattern consistent with FIP, high protein content, low A/G	Only some of the changes reported for group C	Macroscopic and cytological pattern not consistent with FIP, low protein content, normal A/G
SPE	Hyperproteinemia, increased α ₂ - and γ-globulin, low A/G ratio	One or few electrophoretic abnormalities	Normal electrophoretic pattern
AGP	>1.5 (mg/mL)	0.5 to 1.5 (mg/mL)	<0.5 (mg/mL)
Serology and/or PCR	Positive serology and/or PCR in blood and/or effusions	Positive serology and negative PCR (or vice versa)	Negative serology and/or PCR in blood and/or effusions
Postmortem examination and/or histology	Fibrinous serositis and/or nodular lesions in one or more organs, histology consistent with fibrinous serositis and/or pyogranulomatous foci	Lesions as in group C but atypical localisation or presence of additional potential pathogens (e.g. fungi and bacteria)	Absence of lesions described in group C; presence of lesions consistent with diseases other than FIP (e.g. fibrosis)

SPE serum protein electrophoresis, AGP α₁-acid glycoprotein

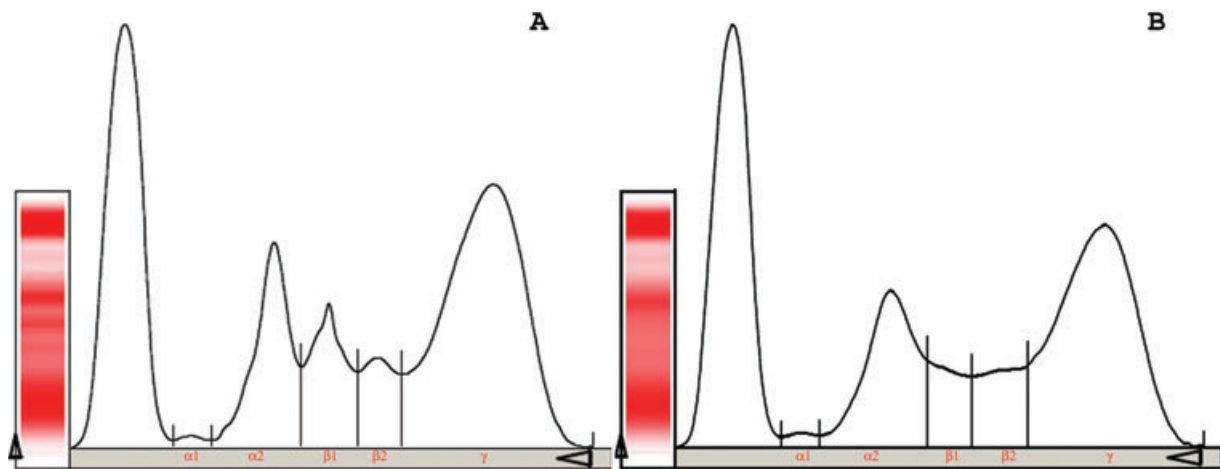


FIG 1. Comparison of electrophoretograms obtained using cellulose acetate electrophoresis from serum (A) and abdominal effusion (B) of a cat with FIP (cat 9). Both the electrophoretograms have a similar profile and are characterised by increased α_2 - and γ -globulins

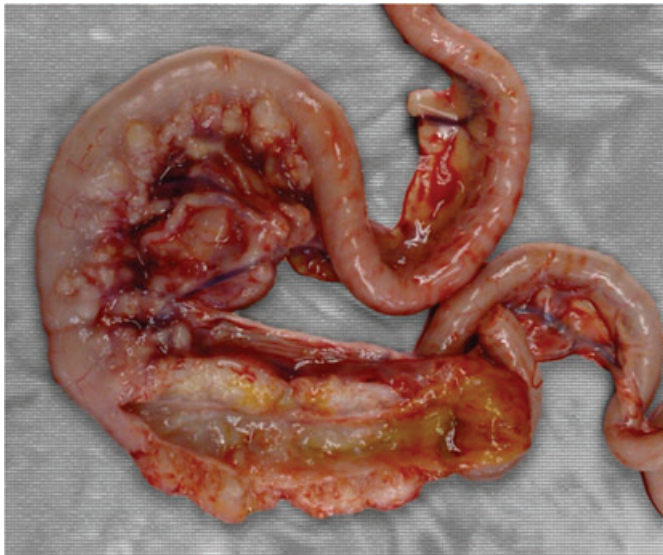


FIG 2. Small intestine (cat 10). Locally extensive, marked thickening of intestinal wall by dense, white, firm irregular proliferating tissue extending through the wall of the intestine

summarise, in cats without FIP, the clinical features and intracavitary effusions, when present, were always consistent with FIP. Serology and SPE were occasionally consistent with FIP. Postmortem examination or histopathology, when performed, and serum AGP concentration were never consistent with FIP (i.e. AGP > 1.5 mg/mL), although in two cases, AGP values [cat 2 (0.74 mg/mL) and cat 4 (1.33 mg/mL)] were higher than the upper reference limit of the laboratory (0.56 mg/mL).

In cats with FIP, the tests that were not consistent with or doubtful of FIP included clinical signs (3 of 8 cases), analysis of effusion (AE; 3 of 6), SPE (5 of 8), serology or PCR (4 of 5) and postmortem examination/histology (5 of 8). Conversely, all these cats had AGP values higher than the cut-off established as consistent with FIP (1.5 mg/mL). The serum concentration recorded in these cats ranged from 2.04 to 14 mg/mL (median value of 2.89 mg/mL).

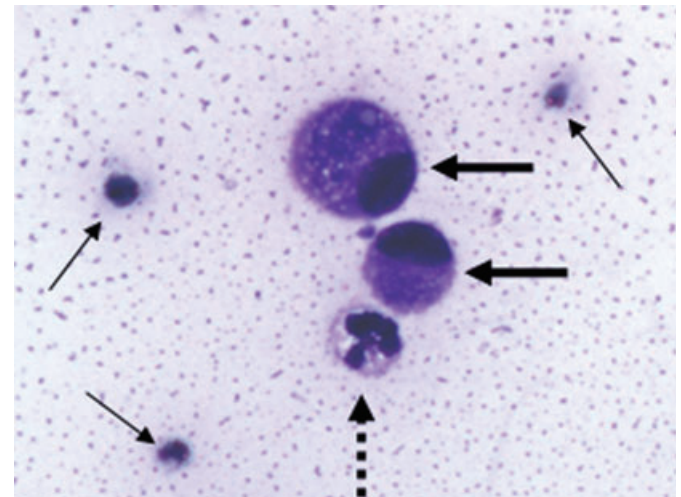


FIG 3. Cytocentrifuged abdominal effusion from a cat with FIP (cat 11). A non-degenerated neutrophil with vacuolated hyperbasophilic cytoplasm (dashed arrow), two mesothelial cells (thick arrows) and scattered erythrocytes (thin arrows) embedded in a granular proteinaceous eosinophilic background. May Grünwald-Giemsa, $\times 100$

Diagnostic performances of the tests

Data regarding sensitivity, specificity and concordance with IHC are reported in Table 3. The diagnostic concordance was maximal only for AGP, which had 100% specificity and sensitivity, and low for histopathology, which had high specificity but low sensitivity, and poor or slight for the remaining tests. The AE was not specific but had a moderate sensitivity.

DISCUSSION

These results confirm that the diagnosis of FIP can be difficult in vivo. In this study, both the history and clinical presentation could be misleading even if they were highly consistent or inconsistent with FIP. Likewise, macroscopic and cytological aspects of the effusion were often not considered pathognomonic, as

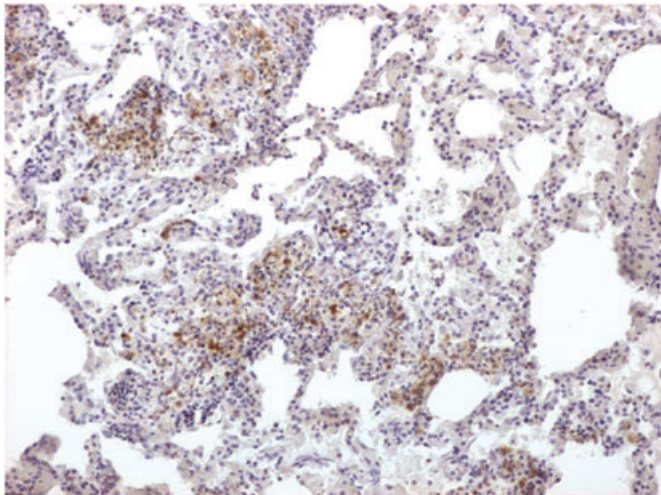


FIG 4. Section of lung (cat 11). Feline coronavirus antigen (brown staining) was detected within alveolar walls in areas of severe interstitial pneumonia. Immunohistochemical staining, ABC method, DAB (3, 3'-diaminobenzidine) chromogen, $\times 100$

they were also observed in inflammatory conditions other than FIP. Cytology of effusions is therefore not specific and cannot be used to support a diagnosis of FIP when histopathology is doubtful. Nonetheless, the cytological evaluation of effusions is always mandatory in the investigation as it can identify diseases other than FIP (e.g. tumours or septic effusions) that were not included in this study. Direct staining of FCoV within macrophages by immunofluorescence in cytocentrifuged effusions is considered the most specific tool to confirm FIP (Hartmann 2005). However, it could not be applied in non-effusive forms or in poorly cellular samples as those detected in some cats in this study. In addition, although this test was first established by our group years ago (Cammarata Parodi and others 1993), it is not routinely performed in our laboratory, as it is time consuming and other tests, such as SPE or AGP, can provide information that strongly support the diagnosis of FIP in cats with a high pretest probability of disease (Paltrinieri and others 2007).

Table 3. Concordance between test results and IHC results and sensitivity and specificity of the different tests

	Coefficient k (95% CI)	Sens (%)	Spec (%)
History and symptoms	-0.4 (-0.61/0.53)	62.5	0
Effusion	-0.52 (-0.99/-0.05)	50	0
SPE	0.25 (-0.07/0.55)	37.5	50
AGP	1.00 (1.00/1.00)	100	100
Serology or PCR	-0.08 (-0.71/0.55)	nc	nc
Postmortem examination or histology	0.09 (-0.11/0.29)	37.5	100

SPE serum protein electrophoresis, AGP α 1-acid glycoprotein, CI confidence interval, Sens sensitivity, Spec specificity, nc not calculable

It is commonly accepted that the diagnosis of FIP should be based on histopathological features (fibrinous polyserositis, infiltration of mononuclear cells and/or pyogranulomatous parenchymal foci; Kipar and others 1998, Addie and others 2004, Pedersen 2009). In this study, however, histopathology proved highly specific for FIP but was not a sensitive diagnostic test as it failed to confirm FIP in most affected cases and it had a poor concordance with IHC, which is considered to have the highest specificity for diagnosing FIP (Pedersen 2009). As such, the detection of lesions clearly consistent with FIP is intrinsically conclusive, but when the interpretation of lesions is not clear, FIP cannot be excluded. There is limited information available on how to approach these cases when IHC is not available. Although our study is limited by the small number of cases, the measurement of serum AGP concentration appeared to be most helpful as it was the only diagnostic test in complete concordance with IHC. However, it must be pointed out that AGP is an acute phase protein and, by definition, its serum concentration increases in any inflammatory/infectious condition (Kajikawa and others 1999, Ceron and others 2005, Paltrinieri 2008) or even in non-inflammatory disease such as lymphoma (Correa and others 2001) or other tumours (Setling and others 2000). The excellent performance of AGP in this study could thus be biased because of the lack of cases with other severe inflammatory diseases. Nevertheless, two factors must be

Table 2. Classification of cases according to the results obtained

Group	Cat number	History and symptoms	Effusion	SPE	AGP	Serology and/or PCR	Postmortem examination and/or histology
Without FIP	1	C	—	NC	NC	—	—
	2	C	C	C	NC	NC	NC
	3	C	C	NC	NC	C	—
With FIP	4	C	C	C	NC	NC	NC
	5	C	NC	NC	C	—	D
	6	NC	—	C	C	—	D
	7	C	NC	D	C	NC	D
	8	D	C	NC	C	C	D
	9	NC	C	C	C	NC	C
	10	C	NC	NC	C	—	C
	11	C	C	C	C	NC	D
	12	C	—	NC	C	NC	C

SPE serum protein electrophoresis, AGP α 1-acid glycoprotein, C consistent, D doubtful, NC not consistent

taken into account in evaluating AGP concentrations to support a clinical diagnosis of FIP: the magnitude of the increase and the pretest probability of FIP. The cut-off employed in this study was as suggested by Duthie and others (1997) who noted that, although AGP can increase in any inflammatory condition, the increase is more pronounced in FIP than in other diseases. This has been confirmed by other studies (Giordano and others 2004, Paltrinieri and others 2007) and ultimately also by the results of this study. In a recent study (Paltrinieri and others 2007), it was demonstrated that AGP can be used to support a clinical diagnosis of FIP according to the Bayesian approach. When the pretest probability of FIP is high (when most of the in vivo or postmortem tests are consistent with but not absolutely conclusive of FIP), a "positive test" (a high AGP value) can increase the posttest probability of disease. In other words, although AGP alone cannot be used to confirm a clinical suspicion of FIP, it could be confirmatory when FIP is probable but not definitely diagnosed based on other clinicopathological findings or on postmortem examination results.

In conclusion, the detection of lesions consistent with FIP is a confirmatory test in cases with atypical clinical presentation. In the absence of lesions clearly consistent with FIP or potentially consistent with other diseases, IHC should always be performed. If this is not possible, the presence of typical SPE patterns or of effusions cytologically consistent with FIP must not induce the pathologist to interpret ambiguous histological results as "consistent with FIP." Conversely, the presence of high concentrations of AGP may support the diagnosis of FIP in those cases in which results of other clinicopathological tests or histopathology are inconclusive.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

- ADDIE, D. D., PALTRINIERI, S. & PEDERSEN, N. C. (2004) Recommendations from workshops of the second international feline coronavirus/feline infectious peritonitis symposium. *Journal of Feline Medicine and Surgery* **6**, 125-130
- ANDREW, S. E. (2000) Feline infectious peritonitis. *Veterinary Clinics of North America: Small Animal Practice* **30**, 987-1000
- BENCE, L. M., ADDIE, D. D. & ECKERSALL, P. D. (2005) An immunoturbidimetric assay for rapid quantitative measurement of feline alpha-1-acid glycoprotein in serum and peritoneal fluid. *Veterinary Clinical Pathology* **34**, 335-341
- CAMMARATA PARODI, M., CAMMARATA, G., PALTRINIERI, S., LAVAZZA, A. & APE, F. (1993) Using direct immunofluorescence to detect coronaviruses in peritoneal and pleural effusions. *Journal of Small Animal Practice* **34**, 609-613
- CERON, J. J., ECKERSALL, P. D. & MARTINEZ-SUBIELA, S. (2005) Acute phase proteins in dogs and cats: current knowledge and future perspectives. *Veterinary Clinical Pathology* **34**, 85-99
- CORREA, S. S., MAULDIN, G. N., MAULDIN, G. E. & MOONEY, S. C. (2001) Serum alpha 1-acid glycoprotein concentration in cats with lymphoma. *Journal of the American Animal Hospital Association* **37**, 153-158
- DUTHIE, S., ECKERSALL, P. D., ADDIE, D. D., LAWRENCE, C. & JARRET, O. (1997) Value of alpha 1-acid glycoprotein in the diagnosis of feline infectious peritonitis. *Veterinary Record* **141**, 299-303
- FOLEY, J. E., LAPOINTE, J. M., KOBLIK, P., POLAND, A. & PEDERSEN, N. C. (1998) Diagnostic features of clinical neurologic feline infectious peritonitis. *Journal of Veterinary Internal Medicine* **12**, 415-423
- GIORDANO, A., PALTRINIERI, S., BERTAZZOLO, W., MILESI, E. & PARODI, M. (2005) Sensitivity of tru-cut and fine needle aspiration biopsies on liver and kidney for diagnosis of feline infectious peritonitis. *Veterinary Clinical Pathology* **34**, 368-374
- GIORDANO, A., SPAGNOLO, V., COLOMBO, A. & PALTRINIERI, S. (2004) Changes in some acute phase protein and immunoglobulin concentrations in cats affected by feline infectious peritonitis or exposed to feline coronavirus infection. *Veterinary Journal* **167**, 38-44
- HARTMANN, K. (2005) Feline infectious peritonitis. *Veterinary Clinics of North America: Small Animal Practice* **35**, 39-79
- HERREWEGH, A. A. P. M., DE GROOT, R. J., CEPICA, A., EGBERINK, H. F., HORZINEK, M. C. & ROTTIER, P. J. M. (1995) Detection of feline coronavirus RNA in feces, tissues, and body fluids of naturally infected cats by reverse transcriptase PCR. *Journal of Clinical Microbiology* **33**, 684-689
- KAJIKAWA, T., FURUTA, A., ONISHI, T., TAJIMA, T. & SUGII, S. (1999) Changes in concentrations of serum amyloid A protein, alpha-1-acid glycoprotein, haptoglobin, and C-reactive protein in feline sera due to induced inflammation and surgery. *Veterinary Immunology and Immunopathology* **68**, 91-98
- KIPAR, A., BELLMANN, S., KREMENDAHL, J., KÖHLER, K. & REINACHER, M. (1998) Cellular composition, coronavirus antigen expression and production of specific antibodies in lesions in feline infectious peritonitis. *Veterinary Immunology and Immunopathology* **65**, 243-257
- KIPAR, A., KOELHER, K., BELLMANN, S. & REINACHER, M. (1999) Feline infectious peritonitis presenting as a tumour in the abdominal cavity. *Veterinary Record* **144**, 118-122
- LANDIS, J. R. & KOCH, G. G. (1977) The measurement of observer agreement for categorical data. *Biometrics* **33**, 159-174
- MELLI, M., KIPAR, A., MÜLLER, C., JENAL, K., GÖNCZI, E., BOREL, N., GUNN-MOORE, D., CHALMERS, S., LIN, F., REINACHER, M. & LUTZ, H. (2004) High viral loads despite absence of clinical and pathological findings in cats experimentally infected with feline coronavirus (FCoV) type 1 and in naturally FCoV-infected cats. *Journal of Feline Medicine and Surgery* **6**, 69-81
- OSTERHAUS, A. D., HORZINEK, M. C. & REYNOLDS, D. J. (1977) Seroprevalence of feline infectious peritonitis virus infections using transmissible gastroenteritis virus as antigen. *Zentralblatt Veterinärmedizin B* **24**, 835-841
- PALTRINIERI, S. (2008) The feline acute phase reaction. *Veterinary Journal* **177**, 26-35
- PALTRINIERI, S., GIORDANO, A., TRANQUILLO, V. & GUAZZETTI, S. (2007) Critical assessment of the diagnostic value of feline alpha-1-acid glycoprotein for feline infectious peritonitis using likelihood ratios approach. *Journal of Veterinary Diagnostic Investigation* **19**, 266-272
- PALTRINIERI, S., PARODI CAMMARATA, M., CAMMARATA, G. & COMAZZI, S. (1998) Some aspects of humoral and cellular immunity in naturally occurring feline infectious peritonitis. *Veterinary Immunology and Immunopathology* **65**, 205-220
- PALTRINIERI, S., PARODI, M. C. & CAMMARATA, G. (1999) In vivo diagnosis of feline infectious peritonitis by comparison of protein content, cytology, and direct immunofluorescence test on peritoneal and pleural effusions. *Journal of Veterinary Diagnostic Investigation* **11**, 358-361
- PALTRINIERI, S., PONTI, W., COMAZZI, S., GIORDANO, A. & POLI, G. (2003) Shifts in circulating lymphocyte subsets in cats with feline infectious peritonitis (FIP): pathogenic role and diagnostic relevance. *Veterinary Immunology and Immunopathology* **96**, 141-148
- PEDERSEN, N. C. (1976) Serologic studies of naturally occurring feline infectious peritonitis. *American Journal of Veterinary Research* **37**, 1449-1453
- PEDERSEN, N. C. (1995) The history and interpretation of feline coronavirus serology. *Feline Practice* **23**, 47-51
- PEDERSEN, N. C. (2009) A review of feline infectious peritonitis virus infection: 1963-2008. *Journal of Feline Medicine and Surgery* **11**, 225-258
- SELTING, K. A., OGILVIE, G. K., LANA, S. E., FETTMAN, M. J., MITCHENER, K. L., HANSEN, R. A., RICHARDSON, K. L., WALTON, J. A. & SCHERK, M. A. (2000) Serum alpha 1-acid glycoprotein concentrations in healthy and tumor bearing cats. *Journal of Veterinary Internal Medicine* **14**, 503-506
- SHELLY, S. M., SCARLETT-KRANZ, J. & BLUE, J. T. (1988) Protein electrophoresis on effusions from cats as a diagnostic test for feline infectious peritonitis. *Journal of the American Animal Hospital Association* **24**, 495-500
- SPARKES, A. H., GRUFFYDD-JONES, T. J. & HARBOUR, D. A. (1991) Feline infectious peritonitis: a review of clinico-pathological changes in 65 cases, and a critical assessment of their diagnostic value. *Veterinary Record* **129**, 209-212
- SPARKES, A. H., GRUFFYDD-JONES, T. J., HOWARD, P. E. & HARBOUR, D. A. (1992) Coronavirus serology in healthy pedigree cats. *Veterinary Record* **131**, 35-36
- STOCKHAM, S. L. & SCOTT, M. A. (2008) Introductory concepts. In: *Fundamentals of Veterinary Clinical Pathology*, 2nd edn. Blackwell Publishing, Ames, IA, USA, p43
- VIGANI, A., GIORDANO, A. & TRAVETTI, O. (2009) Uncommon mediastinal cyst-like manifestation of feline infectious peritonitis. *Veterinary Record* **165**, 239-241