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Short Communication

Role of paraoxonase-1 as a diagnostic marker for feline infectious peritonitis

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Highlights

- Paraoxonase-1 (PON-1) activity was evaluated as a biomarker in cats
- Lowest PON-1 activities were observed with feline infectious peritonitis (FIP)
- Low paraoxonase-1 activities were also observed with septic effusions
- PON-1 activity accurately discriminated FIP from similar clinical conditions
- The utility of PON-1 activity to diagnose non-effusive FIP needs further study

Abstract

Feline infectious peritonitis (FIP) is characterised by the presence of systemic inflammation accompanied by oxidative stress. Paraoxonase-1 (PON-1) is a negative acute phase reactant produced by the liver. A paraoxon-based method has been validated to measure PON-1 activity in feline serum. The aim of this study was to investigate the usefulness of PON-1 activity as a biomarker to discriminate FIP from other diseases with similar clinical signs. Of 159 cats enrolled, 71 were healthy, 34 had FIP and 54 had another disease but presented with clinical signs that could be consistent with FIP. PON-1 activity was lower (P <0.0001) in cats with FIP (median, 26.55 U/L; range, 5.40-78.20 U/L) compared to healthy (median, 87.5 U/L; range, 46.60-215.50 U/L) and Non-FIP Sick group cats (median, 57.90 U/L; range, 3.80-122.60 U/L). Two receiver operating characteristic curves were used to determine the thresholds that maximised the performance of PON-1 activity in predicting FIP

both from a screening and diagnostic point of view. A threshold of 78.30 U/L yielded a sensitivity of 100%, a specificity of 50.4%, and a negative likelihood ratio of 0.00 (screening curve). While a threshold of 24.90 U/L maximised specificity (94.4%), had a sensitivity of 44.1%, and increased the likelihood ratio to 7.94, making PON-1 activity a good confirmatory test for FIP (diagnostic curve). Using these thresholds, serum PON-1 activity showed good diagnostic performance in discriminating FIP affected cats from cats with other inflammatory conditions.

Keywords: Acute phase protein; Biomarker; FIP; PON-1

Feline infectious peritonitis (FIP) is a frequently fatal, systemic inflammatory disease caused by a virulent biotype of the feline coronavirus (FCoV). Dependent upon the host immune response, clinical presentation can be effusive or non-effusive, the latter characterised by the presence of granulomatous lesions (Pedersen, 2014). The ante-mortem confirmation of FIP can be challenging, especially if non-effusive, despite the availability of several diagnostic modalities including measurement of serum acute phase proteins (Tasker, 2018). Paraoxonase-1 (PON-1) is a glycoprotein enzyme whose hepatic synthesis is inhibited by systemic inflammation (Feingold et al., 1998). It has been determined that PON-1 is a negative acute phase reactant in several animal species, including cattle, dogs and cats (Giordano et al., 2013; Rossi et al., 2014; Giordano et al., 2020). A paraoxon-based method of measuring PON-1 activity has been recently validated in cats (Rossi et al., 2020).

The aim of this study was to evaluate the potential of PON-1 activity in discriminating FIP from other diseases with a similar clinical presentation.

Healthy cats and sick cats, with clinical signs consistent with FIP, were enrolled (Table 1). Inclusion criteria included the availability of clinicopathological results of haematology, serum biochemistry (including creatinine, urea, total protein, glucose concentrations and alanine aminotransferase and alkaline phosphatase activities) and effusion analysis (when present) for review, as well as residual serum (minimum 150 µL; stored at -20 °C). According to the regulations of our institution, a formal approval of the Institutional Ethical Committee was not required (EC decision 29 Oct 2012, renewed with Protocol Number 02-2016), as samples were collected for diagnostic purposes under informed owner consent. Healthy cats (n = 71) with no clinical or clinicopathological abnormalities were enrolled as controls. Sick cats (n = 88) that exhibited one or more clinical finding commonly associated with FIP (i.e. presence of effusion, pyrexia, anorexia, jaundice, neurological or ocular signs; Table 2; Tasker, 2018) were identified and divided into FIP (n = 34) and Non-FIP Sick (n = 54) groups. A diagnosis of FIP was either Confirmed (n = 19) by positive immunostaining for FCoV antigen on tissue using a previously described protocol (Stranieri et al., 2020) or Presumptive (n = 15; Table 3) based upon signalment, clinical signs (effusive FIP only) and multiple clinicopathological changes consistent with FIP. The FIP group was also divided according to the clinical presentation (Effusive FIP, n = 27; or Non-effusive FIP, n = 7; Table 2). In the Non-FIP group, diagnostic imaging and clinicopathological results confirmed an underlying disease other than FIP (Table 4). PON-1 activity was measured as previously described (Rossi et al., 2020). Statistical analyses were performed using Analyse-it software for Microsoft Excel (Analyse-it Software).

PON-1 activity was lower (P <0.0001; Fig. 1) in the FIP group (median, 26.55 U/L; range, 5.40-78.20 U/L), than both the Non-FIP Sick group (median, 57.90 U/L; range, 3.80-122.60 U/L) and the Healthy group median, 87.50 U/L; range, 46.60-215.50 U/L). This was

considered likely due to the robust inflammatory response and the oxidative stress that characterises FIP (Regan et al., 2009; Tecles et al., 2015). PON-1 activity was also lower (P <0.0001) in the Non-FIP Sick group than the Healthy group, likely because some of the sick cats in this group had septic effusions, which cause a marked inflammatory response often associated with oxidative damage and are predicted to induce a decrease in PON-1 synthesis (Bojic et al., 2014). PON-1 activities in the Presumptive FIP (median, 30.60 U/L; range, 5.40-68.10 U/L) and Confirmed FIP (median, 25.70 U/L; range, 6.30-78.20 U/L) subgroups were equivalent to each other (P = 0.61); whereas both diagnostic subgroups were lower (P <0.0001) than both Non-FIP Sick and Healthy groups. This supports a correct diagnosis in the Presumptive FIP group cats. The Effusive FIP subgroup had a lower (P = 0.035) PON-1 activity (median, 23.20 U/L; range, 5.40-71.50 U/L) than the Non-effusive FIP subgroup (median, 48.00 U/L; range, 15.10-78.20 U/L), possibly because lesions in the cats with the effusive form were generally more extensive than in the non-effusive form (Paltrinieri et al., 2020; Fig. 2). PON-1 activity in the Effusive FIP subgroup was lower than in Non-FIP Sick and Healthy groups (P <0.0001). The Non-effusive FIP subgroup had a lower PON-1 activity than the Healthy group (P = 0.0003), but not the Non-FIP Sick group (P = 0.44), likely due to the presence of cats with septic effusions in the Non-FIP Sick group.

Two receiver operating characteristic (ROC) curves were generated: the first with a screening purpose (Fig. 3) that included all the cats enrolled in the study (FIP vs. Healthy + Non-FIP Sick), the second with a diagnostic purpose (Fig. 4) that included only sick cats with clinical signs that could be consistent with FIP (FIP vs. Non-FIP Sick). In both curves a difference from the no-discrimination line was highlighted (P <0.0001), with an AUC of 88.7% (95% confidence intervals [CI 95%], 83.1-94.4%) and 77.2% (CI 95%, 67.0-87.4%) for screening and diagnostic purposes respectively, thus indicating that PON-1 activity may

differentiate cats with FIP from those in the Non-FIP Sick and Healthy groups. In the ROC curve that included all the cats enrolled, the threshold that maximised the diagnostic power of the test was 51.40 U/L (sensitivity and specificity both 82%; positive likelihood ratio, 4.65), while the threshold that maximised the sensitivity of the test, preferable in a screening scenario to identify all the cats that do not have FIP, was 78.30 U/L (sensitivity, 100%; specificity, 50.4%; negative likelihood ratio 0.0). In the ROC curve that included only sick cats the threshold that maximised the diagnostic power of the test was 42.20 U/L (sensitivity 70.6% and specificity 68.5%; positive likelihood ratio 2.24). In the absence of commercially available antiviral agents with proven efficacy for the treatment of FIP and given the potential impact of a terminal diagnosis on management options, it is important to minimise false-positive results. In this latter curve, a threshold of 24.90 U/L maximises specificity (specificity, 94.4%; sensitivity, 44.1%); a PON-1 activity lower than this threshold increases the odds of a diagnosis of FIP almost 8-fold (positive likelihood ratio, 7.94). In this study, only 3/125 cats without FIP had PON-1 activities below this threshold. These three cats had septic effusions, which can often be readily differentiated from FIP effusions by visualising bacteria within cytological preparations.

The outcome of a single test is insufficient to confirm FIP ante-mortem (Tasker, 2018), especially if non-effusive, and PON-1 activity measurement is no exception. Nevertheless, this study indicates that very low PON-1 activities could support the suspicion of FIP. PON-1 activity measurement through a paraoxon-based method was considered easy to set up and perform. The low number of sick cats without effusions in both FIP and Non-FIP Sick groups prevented their comparison. This is a limitation as the minimally invasive diagnosis of non-effusive FIP can be particularly frustrating, and a reliable biomarker could prove important. Therefore, future studies using PON-1 activity measurement in cats should

focus on its ability to differentiate those with FIP from those without, in the absence of detectable effusions.

Conflict of interest statement

None of the authors has any financial or personal interest that could influence or bias the content of the paper.

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Fig. 1. Paraoxonase (PON-1) activity recorded in the Feline infectious peritonitis (FIP), Non-FIP Sick and Healthy groups of cats. The boxes indicate the first to third interquartile range (IQR), the horizontal lines indicate the median value, whiskers extend to further observation within the first quartile minus 1.5*IQR or to further observation within the third quartile plus 1.5*IQR. The shaded area indicates the reference interval of PON-1 activity in healthy cats (Rossi et al., 2020).

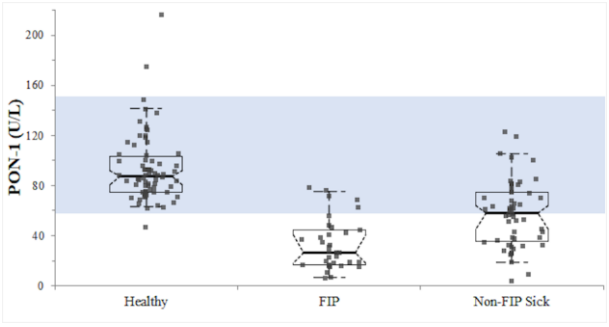

Fig. 2. Paraoxonase (PON-1) activity recorded in the Non-effusive feline infectious peritonitis (FIP), Effusive FIP, Non-FIP Sick and Healthy groups of cats. The boxes indicate the first to third interquartile range (IQR), the horizontal lines indicate the median value, whiskers extend to further observation within the first quartile minus 1.5*IQR or to further observation within the third quartile plus 1.5*IQR. The shaded area indicates the reference interval of PON-1 activity in healthy cats (Rossi et al., 2020).

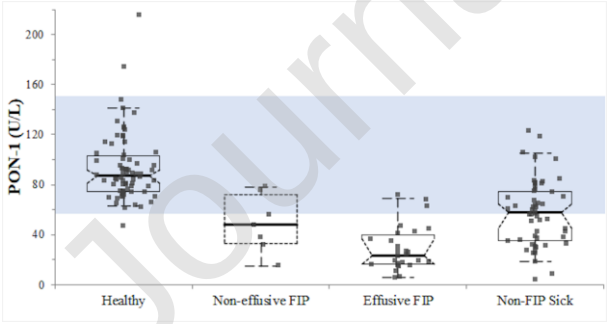

Fig. 3. Receiver operating characteristic curve built, with screening purposes, using the values of paraoxonase 1 activity measured in all the cats enrolled in the study (Feline infectious peritonitis [FIP] vs. Non-FIP Sick + Healthy). The central line represents the no-discrimination line.

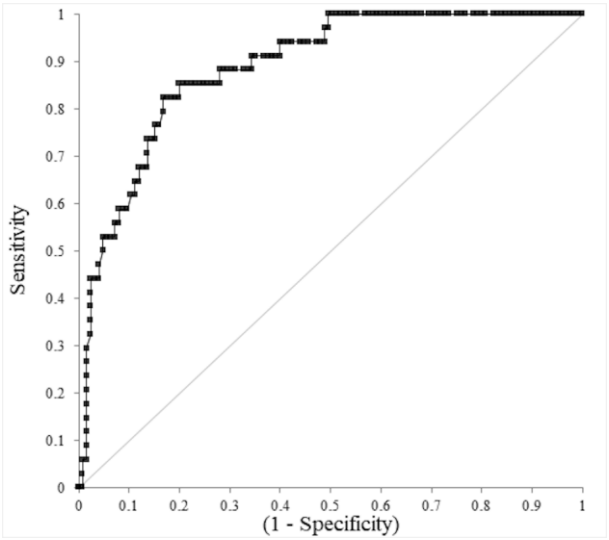

Fig. 4. Receiver operating characteristic curve built, with diagnostic purposes, using the values of paraoxonase 1 activity measured in cats presented with similar clinical signs (Feline infectious peritonitis [FIP] vs. Non-FIP Sick). The central line represents the no-discrimination line.

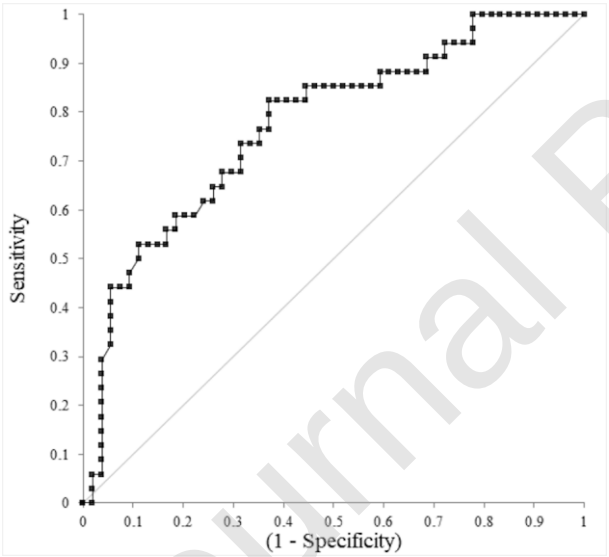

Table 1

Breed, sex, and median age at time of sampling of cats enrolled in the three group: cats that were clinically healthy at time of presentation (Healthy); cats that had one or more clinical signs that could be consistent with feline infectious peritonitis, but were ultimately diagnosed with another disease process (Non-FIP Sick); and cats diagnosed with feline infectious peritonitis (FIP).

Group	Breed	Sex	Median age (Min - Max)
Healthy (*n* = 71)	Domestic shorthair (*n* = 32), Ragdoll (*n* = 27), Maine Coon (*n* = 3), Sphynx (*n* = 3), British shorthair (*n* = 1), Persian (*n* = 1), Siberian (*n* = 1), NR (*n* = 3)	F (*n* = 26), M (*n* = 43), NR (*n* = 3)	2 y (4 mo – 13 y)
Non-FIP Sick (*n* = 54)	Domestic shorthair (*n* = 36), Maine Coon (*n* = 3), Ragdoll (*n* = 3), Bengal (*n* = 1), British shorthair (*n* = 1), Exotic shorthair (*n* = 1), Russian blue (*n* = 1), Sphynx (*n* = 1), NR (*n* = 8)	F (*n* = 21), M (*n* = 28), NR (*n* = 5)	8.5 y (4 mo – 19 y)
FIP (*n* = 34)	Domestic shorthair (*n* = 23), British shorthair (*n* = 2), Siberian (*n* = 2), Bengal (*n* = 1), Exotic shorthair (*n* = 1), Persian (*n* = 1), Scottish fold (*n* = 1), Sphynx (*n* = 1), NR (*n* = 3)	F (*n* = 12), M (*n* = 19), NR (*n* = 4)	1 y (4 mo – 10 y)

FIP, Feline infectious peritonitis; F, female; M, male; mo, months; NR, not reported; y, years

Table 2

Frequency of clinical signs of sick cats enrolled in the FIP or Non-FIP Sick groups.

	Effusive	Non-effusive
FIP group (*n* = 34)	*n* = 27 (peritoneal *n* = 21, pleural *n* = 8, and pericardial *n* = 2; bi- or tri-cavitary *n* = 4)	*n* = 7 (pyrexia, jaundice, and lethargy *n* = 3; neurological or ocular signs *n* = 4)
Non-FIP Sick group (*n* = 54)	*n* = 47 (peritoneal *n* = 19, pleural *n* = 30, and pericardial *n* = 6; bi-cavitary *n* = 8)	*n* = 7 (pyrexia and lethargy *n* = 4; neurological or ocular signs *n* = 3)

FIP, Feline infectious peritonitis

Table 3

Laboratory results for cats strongly suspected of having feline infectious peritonitis, but for whom tissue samples were not available for confirmative feline coronavirus antigen immunostaining (Presumptive FIP subgroup, *n* = 19). An X indicates that this result was consistent with FIP. All cats had a cavitary effusion and severe clinical signs that progressed to spontaneous death or necessitated euthanasia on welfare grounds.

ID	LY [a]	TP [b]	ALB [c]	A:G [d]	SPE [e]	AGP [f]	PCR [g]	ΔTNC [h]	CYTO [i]	OTHER [j]
126	X		X	X	X	X	X	X	X	J
127	X	X	X	X	X	X		X	X	
128	X		X	X	X			X	X	ICC [k]
129	X		X	X	X			X		
130		X	X	X	X	X		X	X	J, P
131		X	X	X			X	X	X	
132		X	X	X				X	X	J
134		X	X	X	X		X		X	
135			X	X	X		X	X	X	P
136			X	X			X	X	X	P
137			X	X	X			X	X	P
138	X		X	X				X	X	
139	X		X	X	X			X	X	J, P

140	X		X	X	X			X	X
141	X	X	X	X	X				

FIP, Feline infectious peritonitis; LY, absolute lymphocyte count measured on peripheral blood; TP, serum total protein; ALB, serum albumin; A:G, albumin to globulin ratio; SPE, serum protein electrophoresis; AGP, serum alpha-1-acid glycoprotein; CYTO, effusion cytology; J, jaundice; P, post-mortem examination.

[a] Lymphocyte count $<1.5 \times 10^9$/L

[b] Total protein concentration >80 g/L

[c] Albumin concentration <21 g/L

[d] A:G ratio <0.8;

[e] Polyclonal gammopathy with increase in the alpha2-globulin fraction (Tasker, 2018)

[f] A-GP concentration >1.5 mg/mL (Paltrinieri et al., 2007)

[g] Positive RT-PCR targeting the 3' UTR region of feline coronavirus, using previously described protocols (Stranieri et al., 2020), performed on effusion samples, except for case 134 where cerebrospinal fluid was used

[h] ΔTNC values >2.5 (Giordano et al., 2015)

[i] Presence, on effusion samples, of a nonspecific inflammatory process within a proteinaceous background (Stranieri et al., 2018)

[j] Other findings consistent with FIP (e.g. jaundice)

[k] Immunocytochemistry for feline coronavirus antigen was performed on an effusion sample

Table 4

Definitive diagnosis of cats belonging to Non-FIP Sick group (*n* = 54)

Diagnosis	*n*
Neoplasia	22
Cardiomyopathy	7
Chylothorax	6
Septic effusion	7
Infectious disease (non-FIP)	4
Immune-mediated haemolytic anaemia	2
Intestinal obstruction due to stenosis	1
Trauma	3
Hepatic lipidosis	2

FIP, Feline infectious peritonitis