

Pharmacological Therapy of 141 Client-Owned Cats with Feline Infectious Peritonitis with Mutian[®] Xraphconn Adenosine Nucleoside Analogue and Prognostic Prediction of Their Clinical Outcomes

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Research Article

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Abstract

Background: Feline infectious peritonitis (FIP) is a fatal disease caused by feline coronavirus or its mutated pathogen designated as FIP virus. The most common form of FIP is wet or effusive, with non-regenerative anemia and clinical signs of mainly non-specific, such as recurrent fever, anorexia and weight loss. Recently, promising results using new anti-viral drug for treating cats with FIP were observed, but identification of rescuable FIP has been still challenging. It is highly worth to identify infected cats possible to be saved by such an anti-viral agent.

Methods: At the initial veterinarian's examination, owner inquiry-based signalments, viral gene detection by PCR and representative laboratory tests for diagnosis of FIP including hematocrit, A to G ratio, total bilirubin, serum amyloid-A and α 1-acid globulin of 141 cats with effusive FIP were compared with those of 28 non-FIP disease cats. Consequently, 116 of them were rescued by administration of anti-viral drug Mutian X and the residual 25 were deceased unfortunately under treatments. Clinical and laboratory indicators observed prior to initial medication were also evaluated statistically between survived and non-survived groups.

Results: Expectedly, levels for a few items of signalments (appetitive and activity scores), hematocrit, A to G ratio, total bilirubin, serum amyloid-A, α 1-acid globulin and viral gene were found to be distributed distinctively between 141 FIP and 28 non-FIP cats. In the comparison between survived and non-survived FIP cats, most of their parameters including levels for hematocrit, A to G ratio, serum amyloid-A, α 1-acid globulin and viral gene were not statistically different. Interestingly, total bilirubin concentrations of survived FIP cats were declined significantly than those of non-survived, and similarly, body temperatures, appetitive and activity scores appeared to be higher probably in accordance with their physical condition.

Conclusions: Several clinical and laboratory indicators were informative in diagnosis of effusive FIP. We have investigated that one of the quantitative markers, total bilirubin levels, tend to be distributed characteristically in rescuable cats with effusive FIP. Elevated levels of total bilirubin may be a prognostic risk factor for severe FIP, predicting no clinical benefit obtained by using Mutian X as a therapeutic agent.

Background

Feline infectious peritonitis (FIP) is a fatal disease that occurs in felids caused by a mutated feline coronavirus, sometimes referred to FIP virus (FIPV).^{1,2} FIP-infected cats at its early phase are known to show non-specific symptoms, including recurrent fever, vomiting, diarrhea, and progressively worsen-weight loss, and characteristic signs of overt FIP are expressed according to the disease progression. Disease types of FIP are classified into effusive type (wet-type), non-effusive type (dry-type) or mixture of effusive and non-effusive types (wet & dry-type). Wet-type of the disease is characterized by fibrous thoracic peritonitis with accumulation of ascitic or pleural fluids, and the dry-type is characterized by granulomatous involvement of several organs, central nervous system symptoms and ocular manifestation. Wet & dry-type of FIP exhibits both of those features, including granulomatous, uveitis and

nervous symptoms, also with accumulation of ascites or pleural fluid.¹ Diagnosis of the disease has been performed comprehensively by analyzing FIP-specific clinical features as described above, physical examination, clinicopathological findings, antibody-titers for feline coronavirus, and viral gene determination by PCR (polymerase chain reaction) using biological specimens including blood, ascites or pleural effusions.³

Currently, there is no effective therapy established for FIP and only symptomatic treatment has been performed including drainage of ascites or pleural effusions, administration of steroids or antibiotics, and supplement nutrition, just in order to prolong life.⁴ Although several studies were reported utilizing interferon- ω and polyprenyl immune-stimulant as anti-viral agents, their clinical advantages have never been verified.^{5,6} In addition, itraconazole or cyclosporin A were also tried in the treatments, but their side effects and low outcomes are unignorable.^{7,8} Consequently, as there is no prophylactic agent investigated, avoiding group hoarding or reducing cat's stress are the residual measures just to relieve their symptoms.^{1,4}

Present study described about our experience that drastic symptomatic improvement and apparent life prolonging were observed by Mutian® Xraphconn (designated as Mutian X, in the following section) administration to the cats after their onset of wet-type FIP.

Results

The cats aged 1 to 11 years were entered into our study, but about 80% of the cats with wet-type FIP were younger than 2 years of age. Wet-type FIP occurred in cats consisted of approximately 30% of crossbreed and 70% of purebred, mainly including Scottish fold, Bengal and Norwegian forest cat as breeds.

We compared the results of our examination, body temperatures, weights and blood tests measured of the cats with wet-type FIP ($n = 141$) and non-FIP ($n = 28$), at the first veterinarian's consultation within our study period described in the Methods (Table 1). None of significant differences has been observed in ages, body temperatures or weights between wet-type FIP and non-FIP, but appetite scores, activity scores, hematocrit (HCT) levels and albumin to globulin (A/G) ratios in the wet-types were found to be significantly reduced as compare with those in non-FIP ($P < 0.003$, < 0.002 , < 0.0001 and < 0.0001 , respectively). Levels for total bilirubin (T-bilirubin) and serum amyloid-A (SAA) in the wet-types were significantly elevated more than three times the averages of those in non-FIP subjects ($P < 0.002$ and < 0.0001 , respectively).

Table 1
Comparison of physical and clinical parameters between wet-type FIP and non-FIP cats

	wet-type FIP			non-FIP			<i>P</i> -value ^a
	<i>n</i>	average	SE	<i>n</i>	average	SE	
age (months)	141	15.72	1.92	28	19.36	3.81	NS
appetite score	141	3.94	0.28	28	6.29	0.69	< 0.003
activity score	141	4.55	0.21	28	6.61	0.68	< 0.002
body temperature (°C)	105	38.80	0.09	23	38.48	0.19	NS
body weight (kg)	141	2.68	0.10	28	2.68	0.19	NS
HCT (%)	138	25.18	0.54	27	34.45	1.50	< 0.0001
A/G ratio	132	0.46	0.01	26	0.70	0.04	< 0.0001
T-bilirubin (mg/dL)	101	1.46	0.17	13	0.45	0.16	< 0.002
SAA (µg/mL)	130	109.96	6.36	27	33.46	11.44	< 0.0001
<i>FIP</i> feline infectious peritonitis, <i>SE</i> standard error of the mean, <i>NS</i> not significant, <i>HCT</i> hematocrit, <i>A/G</i> albumin to globulin, <i>T-bilirubin</i> total bilirubin, <i>SAA</i> serum amyloid-A							
^a Mann-Whitney-U test (non-parametric) was used as statistical analysis and defined to be significant as <i>P</i> -value < 0.05.							

According to our expectation, positive rates for feline coronavirus genes detected by PCR in blood, ascites or pleural effusion samples were apparently higher in the wet-type FIP group than those in non-FIP group ($P < 0.0001$), and we have also found that number of the cats with elevated circulating $\alpha 1$ -acid globulin ($\alpha 1AG$) levels were significantly larger in the wet-type FIP as compared with non-FIP ($P < 0.0001$), although prevalence of diarrhea or vomiting were not significantly different between these 2 groups (Table 2).

Table 2
Comparison of categorical parameters between wet-type FIP and non-FIP cats

	wet-type FIP			non-FIP			<i>P</i> -value ^a
	<i>n</i>	positive	negative	<i>n</i>	positive	negative	
diarrhea	141	22	119	28	7	21	NS
vomiting	140	10	130	28	5	23	NS
PCR testing (blood)	125	114	11	26	0	26	< 0.0001
PCR testing (ascites or pleural effusions) ^b	141	139	2	2	0	2	< 0.0001
α1AG ^c	126	125	1	25	15	10	< 0.0001
<i>FIP</i> feline infectious peritonitis, <i>NS</i> not significant, <i>PCR</i> polymerase chain reaction, <i>α1AG</i> α1-acid globulin							
^a <i>Fisher's exact test was used as statistical analysis and defined to be significant as P-value < 0.05.</i>							
^b Feline coronavirus was judged as positive if target gene could be detected in both or either of ascites or pleural effusions at least. ^c Defined to be positive if higher than 736μg/mL, upper limit of measurable range.							

In addition, we have tried to investigate whether any significant differences could be detected in ages, body temperatures, weights, appetite scores, activity scores, and blood test parameters (HCT, A/G ratio, T-bilirubin and SAA) observed at the first examination, between the survived group after receiving standard course of Mutian X treatment for 84 days ($n = 116$) and another group of the wet-type subjects deceased unfortunately prior to drug therapy completed ($n = 25$), as shown in Table 3. As a result, there was no statistically significant difference found in ages, body weights, HCT levels, A/G ratios, SAA concentrations, between these 2 groups. However, body temperatures, appetite and activity scores prior to initial drug administration were detected to be significantly reduced in the non-survived group as compared with those in the survived group ($P < 0.0005$, < 0.0001 and < 0.0001 , respectively). Interestingly, we have investigated significantly elevated levels for T-bilirubin in the non-survived cats, approximately 3 times as compared with those in the survived ($P < 0.0001$), as shown in Table 3. These results indicated the first possibility that any of apparent clinical benefit cannot be obtained by Mutian X therapy in the case of significant reduction for appetite, activity scores or body temperatures, and the second possibility that the medicine cannot work enough also in the case of elevated T-bilirubin levels, in the wet-type FIP subjects.

Table 3

Comparison of physical and clinical parameters between survived and non-survived cats with wet-type FIP

	survived			non-survived			<i>P</i> -value ^a
	<i>n</i>	average	SE	<i>n</i>	average	SE	
age (months)	116	15.18	2.10	25	17.80	4.76	NS
appetite score	116	4.42	3.22	25	1.72	2.51	< 0.0001
activity score	116	4.94	2.39	25	2.72	2.48	< 0.0001
body temperature (°C)	86	38.99	0.79	19	37.94	1.21	< 0.0005
body weight (kg)	116	2.70	1.21	25	2.57	1.02	NS
HCT (%)	113	25.11	6.30	25	25.51	6.33	NS
A/G ratio	108	0.46	0.09	24	0.45	0.09	NS
T-bilirubin (mg/dL)	77	0.94	0.95	24	3.11	2.35	< 0.0001
SAA (µg/mL)	107	112.19	72.67	23	99.79	72.76	NS
<i>FIP</i> feline infectious peritonitis, <i>SE</i> standard error of the mean, <i>NS</i> not significant, <i>HCT</i> hematocrit, <i>A/G</i> albumin to globulin, <i>T-bilirubin</i> total bilirubin, <i>SAA</i> serum amyloid-A							
^a Mann-Whitney-U test (non-parametric) was used as statistical analysis and defined to be significant as <i>P</i> -value < 0.05.							

Additionally, we have found that there was no significant difference in frequency of diarrhea prior to initial drug administration between the survived and non-survived cats, but observed the tendency that vomiting occurred significantly more times in the non-survived ($P < 0.003$). We have detected no statistical difference in the number of the cats with elevated $\alpha 1AG$ levels, positive for feline coronavirus genes in plasma, ascites or pleural effusions by qualitative PCR testing, between the survived and non-survived groups (Table 4).

Table 4

Comparison of categorical parameters between survived and non-survived cats with wet-type FIP

	survived			non-survived			<i>P</i> -value ^a
	<i>n</i>	positive	negative	<i>n</i>	positive	negative	
diarrhea	116	17	99	25	5	20	NS
vomiting	115	4	111	25	6	19	< 0.003
PCR testing (blood)	103	92	11	22	22	0	NS
PCR testing (ascites or pleural effusions) ^b	116	114	2	25	25	0	NS
α 1AG ^c	104	103	1	22	22	0	NS
<i>FIP</i> feline infectious peritonitis, <i>NS</i> not significant, <i>PCR</i> polymerase chain reaction, <i>α1AG</i> α 1-acid globulin							
^a Fisher's exact test was used as statistical analysis and defined to be significant as <i>P</i> -value < 0.05.							
^b Feline coronavirus was judged as positive if target gene could be detected in both or either of ascites or pleural effusions at least. ^c Defined to be positive if higher than 736 μ g/mL, upper limit of measurable range.							

Regarding the survived cats, changes of the parameters including body weights, HCT, A/G ratios and SAA levels, were statistically analyzed, before and after standard Mutian X therapy (Fig. 1). We have observed apparent increase of body weights, HCT levels or A/G ratios in the survived cats after drug treatments, as compared with each of those measured at the initial examination, respectively ($P < 0.0001$). In contrast, SAA levels were drastically decreased after therapy ($P < 0.0001$), as shown in Fig. 1. [Insert Fig. 1 here]

Discussion

Currently, there is no effective treatment or vaccine for FIP and its mortality is recognized as extremely high. Several approaches have been used to treat cats with FIP, including steroidal and interferon therapies to stimulate the immune system non-specifically in the hope that it will be able to overcome the infection.^{4,5} Recently, some of *in vitro* experiments has revealed that itraconazole, classified as an azole anti-fungal agent, has a potential anti-viral therapeutic effect on the cats with early phase dry-type FIP.⁷ Another study has shown that extremely higher dosage of cyclosporine known as immunosuppressive drug can inhibit FIPV replication, but it may be more important to minimize its side effects including gastrointestinal disorders.⁸ These drugs have never shown any significant clinical benefits for FIP cats.⁴

In 2018, it was reported that the nucleoside analog designated as GS-441524 can exhibit a suppressive effect on FIPV.⁹ Phosphoramidate prodrug of GS-441524, designated as GS-5734 (Remdesivir; Gilead Sciences) has been previously shown to inhibit the replication of several taxonomically diverse RNA viruses such as Ebola. Exciting efficacy of GS-441524 against naturally occurring FIP has been proved by over 80% of recovery from disease onsets, although mortality rates of FIP is known to be almost 100% with the previous therapies.¹⁰

There is no information about anti-viral mechanism for main active component of Mutian X, with molecular structure similar to the adenosine nucleoside analogues including GS-5734, and the drug product possible to be administered orally has been reported as drastically effective on FIPV infections in particular experimental conditions.¹¹ However, therapeutic effects of Mutian X on naturally occurring FIPs in the client-owned cats under variable dietary and environmental conditions have not been investigated yet. In our present study, the drug was administered to 141 client-owned cats onset with wet-type FIP in Japan according to the standard orally-administration schedule for 84 days, and consequently, about 82% of all (116/141 cats with wet-type) have survived and their quality of life could be obviously improved, confirming its apparent clinical benefits (Tables 3 and 4). These results demonstrated that most of the cats with wet-type FIP can be treated efficiently, even though its mortality rate had been known to be almost 100% at disease onset. Wet-type is well-known to account for majority of FIPs.¹⁻³ Here, we have considered it is valuable to demonstrate Mutian X's therapeutic efficacy on the wet-types, occupying majority of onset FIP cases. Only the cats with wet-type FIP were entered into our present study for the reason why a certain number of the subjects could be collected ($n = 141$). We also would like to obtain a larger number of the cats with dry or wet & dry-types in the future, enough to enable reliable statistical analysis, and hopefully, their results can be described in another report.

In our hospital, follow-up period for 3 months has been given to each of the cats after standard drug administration course for 84 days, and any of the stable subjects without apparent quality of life impairment could be judged as remissions. On our observation, most of rescuable FIP cats could exhibit significant improvements for their appetite and activity within 5 days after Mutian X administration started, but on the contrary, most of the residual subjects without disease improvement by the initial drug therapy were prone to decease (data not shown).

Levels for appetite and activity of the cats, strongly suspected or evidently diagnosed as wet-type FIP in our hospital, were transferred into our arbitrary scores and statistically analyzed in order to evaluate their physical conditions at owner's home, simultaneously with our routine examination (body temperatures, weights, echography, auscultation and palpation). We have also measured whole blood cell counts, total protein levels and hepatic function parameters including glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), in all of the cats entered in the study, but these clinical indicators were excluded from our statistical study because any significant characteristics could not be newly investigated and no previous reports described their relationship with FIP. Elevated T-bilirubin levels are not always correlated with higher GOT or GPT, as hyperbilirubinemia in cats with FIP is known to be caused by parenchymal liver disease but can be due to excessive erythrocyte fragility leading to

hemolysis with decreased clearance of hemoglobin-derived products.^{4,12} Levels of T-bilirubin, A/G ratio, SAS and α 1AG were analyzed in our study, as each of them has been already described as correlated with onset or severity of FIP.^{3,4,13-15} In our present study, almost all of the cats with wet-type FIP showed extremely elevated levels of α 1AG, which exceeds the upper limit of measurable concentration (2200 μ g/mL) and could not be accurately determined. Therefore, we have conducted to utilize categorical analysis of Fisher's exact test in order to investigate differences between the groups, regarding cats as positive whose α 1AG levels were higher than the upper limit of its normal range (736 μ g/mL). The positive rate of α 1AG level in the cats with wet-type FIP was found to be approximately 99% (125/126 cats), apparently higher than those in non-FIP cats (60%; 15/25 cats), and thus, this marker has been confirmed as a subsidiary indicator for FIP diagnosis according to the previous data.⁴ Our present study revealed that multiple pre-dilution of plasma specimens will be necessary to quantify α 1AG levels accurately because of their drastic elevations in the wet-type FIP.

In order to assess therapeutic effectiveness of Mutian X on the cats with wet-type FIP and predict their outcomes in the future, determination for several clinical parameters of 141 cats with wet-type FIP was conducted at first veterinarian's examination, and then, statistical comparison of those numeral or categorical measures between survived subjects benefiting from Mutian X treatment and non-survived unresponsive to it consequently (Table 3). Here, in our present assessment, we have investigated for the first time that T-bilirubin levels were significantly elevated in the wet-type FIP cats to result in incurable condition, as well as body temperatures, appetite and activity scores were suspected to be indicators in our statistical analysis (Table 3). As described in the previous study, in serial blood examinations of the cats with wet-type FIP, anemia and increases in T-bilirubin were observed from 2 weeks to 0-3 days before death.¹⁷ Clinical indicators including the packed cell volume and bilirubin were established to predict disease staging and survival time, providing useful information for the ante-mortem diagnosis of FIP. In contrast, we have suggested here a distinctive scheme to discriminate promising wet-type FIP potential to be rescued by Mutian X treatment, just only at initial examination. These results demonstrated that classification of disease status for wet-type FIP by initial T-bilirubin concentration may be a valuable surrogate marker to contribute possible reduction of owner's medical expenses on Mutian X therapy considered unnecessary in such a case.

In the case that ascites or pleural effusions could not be collected from the cats, we have routinely conducted qualitative PCR testing using blood samples for diagnosis of FIP, and resultantly 139 cats were defined as feline corona virus-positive at the rate for 98.6% (139/141 of all the cats with wet-type FIP). Corona virus gene detected by PCR-based technology were apparently decreased in 116 cats with wet-type FIP, consequently survived after standard Mutian X treatments, including 112 cats as virus-positive to negative conversion and 4 other case. We have observe, in addition, levels for body weights, HCT and A/G ratio of them were significantly elevated after drug treatments, and circulating levels for SAA, known as an inflammatory indicator, were drastically reduced, confirming apparent improvement of their quality of life (Fig. 1). It can be easily presumed that more prospective benefits of Mutian X on wet-

type FIP cats can be obtained by utilizing T-bilirubin level as a predictive marker for their outcome, through veterinarian's recommendation on aggressive therapy with Mutian X for their clients.

We also observed a few cases with recurrence, showing multiple symptoms including appetite loss, less activity, fever, neurologic manifestation, deposition of ascites or pleural effusions, within 4 weeks after standard drug administration completed. In such cases, we should perform the additional administration of Mutian X at increased dose (200mg/kg) to them for 42 days. Number of the recurrent cats were found to be small (2.1%, only 3 of 141 cats with wet-type FIP entered into our present study). We hope to further investigate these recurrent subjects in our next study.

In another study reported in 2020, minimum and short-term dose of Mutian X (4mg/kg, q24h for 4 days) ensure viral clearance from the faces of asymptomatic virus-shedding cats, resultantly lead to establish feline corona virus-free households of cats.¹⁷ Some previous studies have already revealed that widespread use of preemptive therapy with anti-viral drugs could be generally suspected as inefficient, because certainly increase risk factors of multi-drug resistance and epidemic infections.¹⁸⁻²⁰ As far as considering severity and mortality rates for FIP at onset, potential epidemic of multi-drug resistant strains must be a great threat for us. Therefore, we are highly confident that Mutian X should be administered selectively to the FIP subjects possible to get certain therapeutic effects, leading to a variety of benefits on the cats and their owners.

Conclusion

Our present study has demonstrated a valid effectiveness of Mutian X therapy on the client-owned household cats with naturally occurring wet-type FIPs. In addition, the cats with elevated T-bilirubin observed prior to initial drug administration could not be treated satisfactorily and always rescued, reasonably providing us some subsidiary measures such as discontinuation of the drug dosing or alternative therapies recommended to be performed at earlier phase. We hope to conduct similar prospective studies for Mutian X therapeutic efficacy on another groups of FIP cats classified as dry-type or wet & dry-type, after collecting a sufficient number of the subjects in the future.

Methods

Therapeutic agent and administration

We have used Mutian® Xraphconn (Mutian X) as a therapeutic agent for FIP, which has been developed by MUTIAN Life Sciences (Nantong, China) in 2019. Two types of the drug products as capsulized for oral administration and filled in vials for subcutaneous injection are now commercially available.²¹ Anti-FIPV mechanism of the adenosine nucleoside analogue, main active ingredient of Mutian X, has not been elucidated yet (informally obtained from MUTIAN Life Science). Digestive organ abnormality (mainly diarrhea) and liver dysfunction were observed to be mild after the drug administration, but there is no serious adverse reaction (unpublished data personally obtained from MUTIAN Life Science). We

performed orally or subcutaneously administration of Mutian X to 141 cats, all of them had been diagnosed to be wet-type FIP in the Bloom Animal Hospital (Tsurumi, Yokohama, Japan) between June 2019 and December 2020, according to the manufacturer's recommendation described as follows: immediately after the first diagnosis of FIP, Mutian X was given orally or injected subcutaneously to the cats at 100mg/kg q24h and the drug administration continued between day 0 and 84. Basically, the capsulized form should be administered orally to the cats. Alternatively, subcutaneous injection can be available at the same dosage, if we find difficulty in its oral administration because of gastrointestinal dysfunction and inability to absorb nutrients caused by onset of FIP. Both of the drug administration must be performed on time every day and at an empty stomach.

Patients And Diagnosis

A hundred and forty-one cats were diagnosed as wet-type FIPs at initial veterinarian's examinations in our hospital between June 2019 and December 2020. Another group of 28 cats, possibly suspected as FIP and transferred from the other institutions, were finally diagnosed not to be FIP by us within the same period (non-FIP). These subjects ($n = 169$, at total) were entered in our present study. All of the owner agreed to the use of data and sample material for our study. Diagnosis of the disease was confirmed by comprehensively considering apparent clinical symptoms (anorexia, underactivity, vomiting, diarrhea, seizures, tremors, stagger, or the others), qualitative PCR to detect feline coronavirus using blood, ascites or pleural effusions as specimens and blood tests including levels for HCT, whole cell count, total protein, GOT, GPT, T-bilirubin, A/G ratio, SAA, and α 1AG. Ages, body temperatures and weights of all cats were recorded with signalments at the initial veterinarian's consultation.

When we could collect both of ascites and pleural effusion or either of them, detection of feline coronavirus gene in those specimens were performed by Canine Lab Co., Ltd (Tokyo, Japan), according to the standard PCR technology. Feline coronavirus was judged as positive if target gene could be detected in both or either of them at least. PCR tests using blood specimens were performed at the lack of ascites or pleural effusion obtained, and resultantly, viral gene-detected cats were diagnosed to be FIPV-positive.

At the initial interview with animal owners, we have measured each of the parameters for appetite (volume, frequency and speed of feed intake) and activity (momentum, walking speed and agility). Then, each of their parameters were converted into the arbitrary value as an appetite or activity score, on the scale of 10. In addition, all of SAA levels over the upper limit of measurable range were regarded to be 225 μ g/mL in statistical calculation. We have also qualitatively determine the cats as positive, whose circulating α 1AG levels were detected to be higher than 736 μ g/mL, upper limit of measurable range for α 1AG.

Non-FIP group ($n = 28$, as a total) included 5 cats with cold, 3 with gastroenteritis, 2 each with pyogenic granuloma, idiopathic uveitis, brain disorder, lymphoma or estrus, 1 each with gastrointestinal eosinophilic sclerosing fibroplasia, hepatic lipidosis, epidermal tumor, idiopathic hyperammonemia,

portasystemic shunt, Trichomonas, cryptogenic epidermal lymphadenoma, cryptogenic ascites, neurological dysfunction or inflammatory disease.

Clinical specimens (EDTA added whole bloods or plasma samples isolated by centrifugation from heparinized whole blood) were collected from all of the cats at the initial medication in our hospital. Furthermore, similar samples were obtained again from the subjects treated with Mutian X, after standard drug administration completed (84 days later than the first medication). T-bilirubin levels and A/G ratios in plasmas were measured in our hospital by DRI-CHEM4000V system (FUJIFILM Corporation, Tokyo, Japan), and SAA levels in plasmas were measured by DRI-CHEM IMMUNO AU10V (FUJIFILM Corporation). Determination of HCT levels in EDTA added whole blood samples was performed by MEK-6550 Celltac- α (NIHON KOHDEN Corporation, Tokyo, Japan). Levels of α 1AG in plasma samples was measured by FUJIFILM VET Systems Co.,Ltd. (Tokyo, Japan), and PCR testing using EDTA added whole blood, ascites or pleural effusion were performed by Canine Lab Co., Ltd.

Statistical Methods

Numerical indicators including age, appetite score, activity score, body temperature, body weight, HCT, A/G ratio, T-bilirubin or SAA levels between the different groups were compared statistically by Mann-Whitney non-parametric U-test. Presence of diarrhea, vomiting, feline coronavirus genes detected by PCR and significant levels of α 1AG could be classified into categorical data, and Fisher's exact tests were used in 2x2 contingency tables with any expected cell values below 5. Additionally, we measured 4 numerical parameters including body weights, HCT levels, A/G ratios and SAA levels of the survived cats in the wet-types treated with Mutian X. Differences of these values observed before and after drug administration were checked by Wilcoxon signed-rank test. Any of P value < 0.05 defined the level of statistical significance. All of statistical calculation in our present study was performed by using StatView 5.0 software (SAS institute, NC).

Abbreviations

FIP

feline infectious peritonitis; PCR:polymerase chain reaction; Mutian X:Mutian® Xraphconn; FIPV:feline infectious peritonitis virus; wet-type:effusive type; dry-type:non-effusive type; wet & dry-type:mixture of effusive and non-effusive types; HCT:hematocrit; A/G:albumin to globulin; T-bilirubin:total bilirubin; SAA:serum amyloid-A; SE:standard error of the mean; NS:not significant; α 1AG: α 1-acid globulin

Declarations

Competing Interests

The authors declare that they have no competing interests. The work is an original paper and is not under consideration in other journals.

Authors' contributions

MK did most of the treatments, analyzed the data and prepared the manuscript and figures. YU contributed to data analysis and to discussion about the study. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

Ethical approval was not sought for the present study because all of the data had been obtained within the scope of usual veterinary care and properly anonymized. Informed consents were obtained by the owners in all cases enrolled in the study.

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Figures

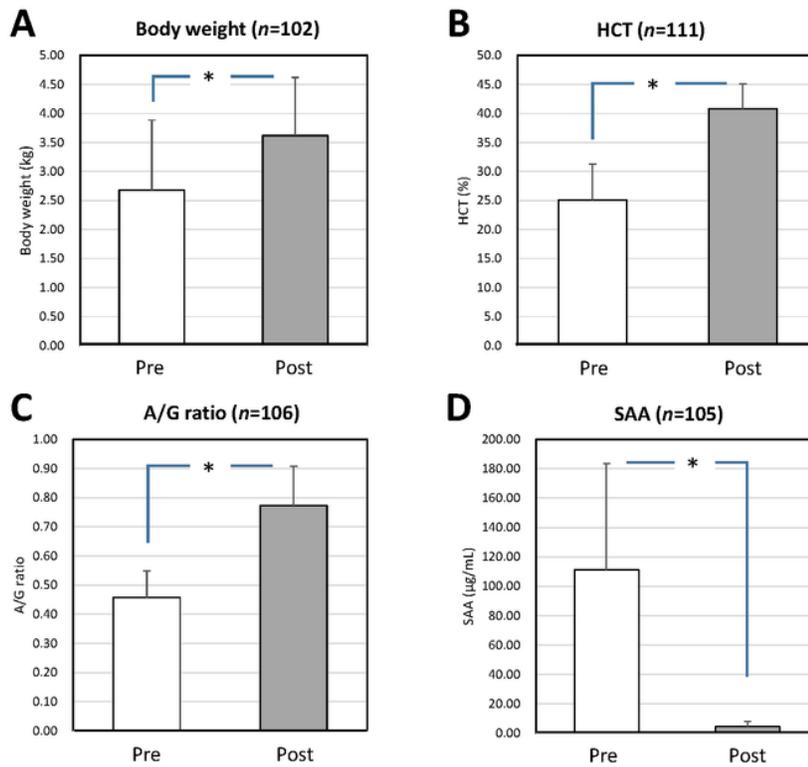


Figure 1

Changes of parameters with survived cats with wet-type FIP and statistical analysis before and after Mutian X treatment. Statistical significant increases of body weights (A), HCT levels (B) and A/G ratios (C) were observed in the survived cats after Mutian X therapy (Post), as compared with each of those measured at their initial examination prior to drug therapy (Pre), respectively (P < 0.0001, indicated by asterisks). In contrast, SAA levels (D) were drastically decreased after therapy (P < 0.0001, indicated by asterisk). Means and standard deviations are indicated by open/shaded bars and vertical lines, respectively. All of the statistical analysis were performed using Wilcoxon signed-rank test (non-parametric). HCT hematocrit, A/G albumin to globulin, SAA serum amyloid-A