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Evaluation of leukocyte ratios as survival prognostic markers in feline retrovirus infections

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ABSTRACT

The utility of neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR) as prognostic markers in Feline Leukemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV) infections has not yet been investigated. The aim of this study was to investigate these leukocyte ratios in retrovirus-positive cats and to evaluate their prognostic value for survival. This retrospective case-control study included 142 cats, 75 FIV-Antibodies (Ab)-positive, 52 FeLV-Antigen (Ag)-positive, and 15 FIV-Ab+FeLV-Ag-positive, and a control population of 142 retrovirus-negative age-, sex-, and lifestyle-matched cats. Signalment, complete blood count at the time of serological testing, and outcome were recorded. Leukocyte ratios were compared within the same case-control population, among the three retrovirus-seropositive populations, and were related to survival time. No significant difference was found in NLR, MLR, or PLR between FIV-Ab-positive and FIV-Ab+FeLV-Ag-positive cats and their cross-matched controls. In the FeLV-Ag-positive population, MLR was significantly lower than in the control population (0.05 and 0.14, respectively, P=0.0008). No ratio discriminated among the three infectious states. No ratio was significantly different between survivors and non-survivors in the population of FIV-Ab-positive cats. MLR at diagnosis was significantly higher in FeLV-Ag-positive cats that died 1–3 years after diagnosis than in FeLV-Ag-positive cats still alive at 3 years (P=0.0284). None of the three ratios could predict retroviruses-positive cats that would survive to the end of the study. Overall the results indicate that NLR, MLR, and PLR are not significantly different among retrovirus statuses evaluated and had a very limited prognostic value for the survival time in retrovirus-positive cats.

Introduction

Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) are the two most common infectious diseases of cats worldwide (Little et al., 2020). FIV is a Lentivirus that, in most cats, causes mild hematologic changes, such as anemia, lymphopenia, and neutropenia (Fujino et al., 2009; Rungsuriyawiboon et al., 2022; Spada et al., 2018). These changes only become marked in the symptomatic stages of infection (Linenberger and Abkowitz, 1995). The long latency of FIV infection may allow an infected cat to live as long as a healthy cat (Gleich et al., 2009; Kent et al., 2022; Luckman and Gates, 2017). FeLV is a Gammaretrovirus that can cause immunosuppression and lead to the development of fatal neoplasms and nonregenerative anemia in infected cats (Addie et al., 2000; Robert et al., 2023). If the cat does not have an adequate immune response, FeLV spreads to the bone marrow infecting hematopoietic precursors (Little et al., 2020), leading to anemia, thrombocytopenia, neutropenia, and lymphopenia (Biezus et al., 2019; Spada et al., 2018). Generally, FeLV-infected cats survive between 1 and 3 years after infection but this depends mainly on the stage of infection, host immunity, and subtype of FeLV involved (Hofmann-Lehmann and

Abbreviations: FeLV, Feline Leukemia Virus; FIV, Feline Immunodeficiency Virus; Ab, Antibody; Ag, Antigen; Hb, Hemoglobin; Hct, Hematocrit; MLR, Monocyte-Lymphocyte Ratio; NLR, Neutrophil-Lymphocyte Ratio; PLR, Platelet-Lymphocyte Ratio; RBC, Red Blood Count.

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Hartmann, 2020). Co-infection with both retroviruses results in reduced life expectancy due to the worsening of FIV-associated signs by FeLV infection (Courchamp et al., 1997).

In human medicine, several studies have demonstrated the diagnostic and prognostic value of leukocyte ratios, such as neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR), in infectious diseases (Naranbhai et al., 2014; Russell et al., 2019). In companion animal medicine, NLR is a marker of prognosis and disease severity in dogs with acute pancreatitis (Johnson et al., 2023; Neumann, 2021), babesiosis (Kučer et al., 2008), sepsis (Gori et al., 2021; Pierini et al., 2020), and parvovirosis (González-Domínguez et al., 2023), although it is not able to distinguish septic from non-septic peritonitis (Hodgson et al., 2018). Fewer studies have been performed on leukocyte ratios in cats, where an increase in NLR is associated with increased mortality in cats with hypertrophic cardiomyopathy (Fries et al., 2022), acute pancreatitis (Neumann, 2021), and sepsis (Kopilovic et al., 2023). An increase in NLR and a decrease in MLR is reported in cats with a plethora of infections, neoplasms, and in chronic renal failure (Tsouloufi et al., 2021). Pretreatment NLR may be of value in identifying cats at higher risk of local recurrence after curative-intent surgery for feline injection site sarcomas (Chiti et al., 2020). A recent study showed that NLR and MLR correlated positively with inflammatory markers typically used in cats such as serum amyloid A, and with levels of albumin, globulins, and albumin-globulin ratio (Donato et al., 2023).

This study aimed to investigate the NLR, MLR, and PLR ratios in cats with different retrovirus statuses and the ability of these ratios to provide a prognostic indication of the survival time of retroviruses-positive cats.

Materials and methods

Serum samples

This retrospective case-control study included data of cats FIV-Ab-positive and/or FeLV-Ag-positive collected between January 2002 and June 2016 from a previously published study (Spada et al., 2018) and of cats followed by the authors (DP, RP, ES) from the database of the Small Animal Veterinary Hospital and the database of the Veterinary Transfusion Research Laboratory (REVLab), Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Lodi, Italy from July 2016 and until March 2023.

Databases were reviewed to identify all cats that had been tested for FIV antibodies and FeLV antigen, using a commercial SNAP COMBO PLUS® rapid enzyme-linked immunosorbent assay (ELISA) kit (IDEXX Laboratories Srl, Italy) that detects, in serum, plasma or whole blood samples, FIV-specific anti-p24 and anti-gp40 antibodies (sensitivity: 100%, specificity: 99.6%) and FeLV antigen p27 (sensitivity: 92.3%, specificity: 97.3%) (Hartmann et al., 2007). For each retrovirus-positive cat included in the study, a healthy cat negative for both viruses and with matched sex, age, and lifestyle was assigned as a cross-matched control cat. Healthy cats included blood donors or cats presented for neutering, with no clinical or clinicopathologic abnormalities.

The retrovirus-positive cats were divided into three groups: FIV-Ab-positive, FeLV-Ag-positive and FIV-Ab+FeLV-Ag-positive cats. A retrovirus-negative control cat was enrolled for each retrovirus-positive group. For each retrovirus-positive and negative cat, information collected from the medical records included: demographic data (origin: stray, privately owned or shelter cats, age, breed: purebred or mixed breed, sex: male or female, reproductive status: intact or neutered, and lifestyle: only indoor, only outdoor or indoor/outdoor cats), time and outcome of the serologic testing, complete blood count at diagnosis of retrovirus infection, outcome, and survival time (in days). Cats were classified as survivors if they were alive at the end of the study (set at 06/27/2016 for cases of the previous study and 03/15/2023 for new cats selected from the databases), or non-survivors if they died during either study period. In the case of missing data on survival at the end of the study, the owners were contacted by e-mail. Cats whose survival status at the end of the study was unknown were considered lost to follow-up. Hematological data collected at the time of the FIV/FeLV test were red blood cells (RBCs) count, hemoglobin (Hb), hematocrit (Hct), white blood cells (WBCs), leukocyte differential count, and platelets (PLTs) count. Blood counts were performed using different automated hematology analyzers, reflecting the change in instruments in the two university laboratories of the same Department (DIVAS) during the study period: at the REVLab - ADVIA 120 (Siemens Healthcare S.r.l, Italy), from January 2002 until the end of May 2010 and Cell-Dyn 3500 (Abbott S.r.l, Italy) from June 2010 to June 2022 while at the University Hospital Laboratory - ADVIA 120 analyzer (Siemens Healthcare S.r.l, Italy) used from January 2002 until December 2018; XT2000i (Sysmex Partec S.r.l, Italy) during the years 2019–2020 and XN-1000 (Sysmex Partec S.r.l, Italy) from 2021 until the end of the study. The leukocyte differential provided by the instruments was checked microscopically at 1000X magnification on blood smears stained with a modified Romanowsky rapid stain (Dif-stain kit, Titolchimica S.P.A., Rovigo, Italy or May Grunwald Giemsa quick stain - Bio Optica Milano SpA, Italy) by counting at least 100 nucleated cells. The absolute number of each leukocyte population was then calculated based on the total number of WBC and on the percentage of each cell population as provided by the manual differential. The platelet estimate was verified by counting the mean number of platelets in 10 high-power fields. If there was a discrepancy then the visual count was used.

The NLR was calculated by dividing the total neutrophil count by the total lymphocyte count, the MLR was calculated by dividing the total monocyte count by the total lymphocyte count, and the PLR was calculated by dividing the total platelet count by the total lymphocyte count.

Statistical methods

All the collected data were captured in Microsoft Excel and analyzed using the statistical software MedCalc® (version 22.007, Ostend, Belgium). Descriptive statistics were presented to define the characteristics of the three populations (FIV-Ab-positive, FeLV-Ag-positive, and FIV-Ab+FeLV-Ag-positive cats), and to compare demographic variables such as age, sex, breed, and lifestyle between control and case groups. Continuous variables were tested for normality using a D'Agostino Pearson test. All values were not normally distributed and were described as medians, 25th and 75th percentiles, and ranges. Values of hematologic parameters and cellular ratios were compared within the same case-control population using a Mann-Whitney test and between the three retrovirus-positive populations using a Kruskal-Wallis test. The ROC (Receiver Operating Characteristics) curve was constructed, and the cut-off value of the leukocyte ratios was calculated, with relative sensitivity and specificity to distinguish retrovirus-positive from negative control cats. Kaplan Meier curves were constructed to calculate and compare survival times between retrovirus-positive cats and controls and between the three different retrovirus-positive populations. A Mann-Whitney test was performed to analyze the associations between NLR, MLR, and PLR, and outcomes for retrovirus-positive cats. The evaluation of leukocyte ratios as indicators of survival was also conducted considering different ranges of survival times for FIV-Ab- and FeLV-Ag positive cats. Survival of less than 1 year, between 1 and 4 years, and greater than 4 years from the diagnosis of infection for FIV-Ab-positive cats; while less than 6 months, between 6 months and 1 year, between 1 and 3 years, and greater than 3 years were used for FeLV-Ag-positive cats. Finally, a ROC curve analysis was used to identify cut-off levels of sensitivity and specificity for the leukocyte ratios that showed a statistically significant difference between survivor- and nonsurvivor-retrovirus-positive cats. A P-value <0.05 was considered statistically significant.

Results

From the previous study (Spada et al., 2018), consisting of a population of 103 retrovirus-positive cats (53 FIV-Ab-positive cats, 40 FeLV-Ag-positive cats, and 10 FIV-Ab+FeLV-Ag-positive cats) and their 103 retrovirus-negative cross-matched cats (controls), 9 retrovirus-positive cats (and 9 controls) that did not have a complete blood count were excluded. Forty-eight retrovirus-positive cats and their 48 retrovirus-negative cross-matched controls were selected from the databases and added to the study population. Therefore, the total number of cats examined in this study was 284, 142 retrovirus-positive cats (75 FIV-Ab-positive cats, 52 FeLV-Ag-positive cats, and 15 FIV-Ab+FeLV-Ag-positive cats) and 142 retrovirus-negative cross--matched cats.

Age at diagnosis of retrovirus-positive status recorded for FIV-Ab-positive, FeLV-Ag-positive cats, and for FIV-Ab+FeLV-Ag-positive cats was a median of 5 years (range 0.4–16 years), 4 (range 0.1–13 years), and 6.5 (range 0.6–11 years) respectively. Age at diagnosis for retrovirus-negative cross-matched cats was a median age of 5 years (range 0.5–16 years) for FIV-Ab-negative cats, 2 years (range 0.1–12 years) for FeLV-Ag-negative cross-matched cats, and 6.5 years (range 0.5–15 years) for FIV-Ab+FeLV-Ag-negative cross-matched cats. No statistical differences were found between the median age of FIV-Ab-positive cats and FIV-Ab-negative cross-matched cats, FeLV-Ag-positive cats and FeLV-Ag-negative cats, and FIV-Ab+FeLV-Ag-positive cats and FIV-Ab+FeLV-Ag-negative cats (P=0.9793, P=0.4257, and P=0.8371, respectively).

The signalment of the cats included in the study is shown in Supplementary Table 1. In the population of FIV-Ab-positive cats, no demographic variable was significantly associated with seropositive status compared with FIV cross-matched controls (P>0.05). The FeLV-Ag-positive population showed a significantly higher presence of mixed breed cats (P=0.0145) and a higher number of cats with an indoor/outdoor lifestyle (P=0.0002) relative to FeLV cross-matched control cats. The FIV-Ab+FeLV-Ag-positive population had more cats with an indoor/outdoor lifestyle (P=0.0079) with respect to FIV+FeLV cross-matched control cats.

Summary statistics relating to hematological parameters and leukocyte ratios evaluated at the time of retrovirus test are reported in Tables 2, 3, and 4 for FIV-Ab, FeLV-Ag, and FIV-Ab+FeLV-Ag-positive and cross-matched control cat populations, respectively. At the Mann-Whitney test, FIV-Ab-positive cats showed a significant decrease in RBCs count (P=0.0129), Hb (P=0.0111), and Hct (P=0.0029) than FIV cross-matched controls, while the value of eosinophils and basophils was significantly higher (P<0.0001 for both). FeLV-Ag-positive cats showed a significantly lower value of RBCs count (P=0.006), Hb (P=0.0175), and monocytes (P<0.0001) than FeLV cross-matched controls. FIV-Ab+FeLV-Ag-positive cats had significantly lower RBCs count, Hb, and Hct (P=0.0066, P=0.0144, and P=0.0107, respectively) than FIV+FeLV cross-matched controls.

Relative to leukocyte ratios, only MLR showed a significantly lower value in FeLV-Ag-positive cats than in FeLV cross-matched controls (P=0.0008) at the time of diagnosis, while for NLR and PLR there were no significant differences for any of the three case-control populations. An MLR $\leq$ 0.05 showed a 56% sensitivity and 83% specificity in differentiating FeLV-Ag-positive cats from FeLV cross-matched control cats. The area under the ROC curve to distinguish FeLV-Ag-positive cats from FeLV cross-matched controls using the MLR was 0.690 (95% CI=0.592–0.777, P=0.0004).

At the Kruskal-Wallis test, NLR, MLR, and PLR showed no statistically significant differences between the three retroviral positive groups (P=0.1265, P=0.1351, and P=0.2284, respectively), ie none of the ratios could distinguish one retroviral positive status from another.

Through Kaplan-Meier curves, survival times between cases and controls within the three retroviral groups, and between FIV-Ab-positive cats, FeLV-Ag-positive cats, and FIV-Ab+FeLV-Ag-positive cats and their cross-matched control population were analyzed and compared (Table 5). The median survival time of FIV-Ab-positive cats showed no significant difference from FIV cross-matched controls (P=0.3394). The mean survival time of FeLV-Ag-positive cats was statistically lower than FeLV cross-matched controls (P=0.0001). The FIV-Ab+FeLV-Ag-positive cats showed significantly lower mean survival times when compared to the control cats (P=0.0013). The different retroviral infections had also a significant influence on survival time. The survival time of FIV-Ab+FeLV-Ag-positive cats was significantly lower than both FIV-Ab-positive or FeLV-Ag-positive cats (P=0.0002, and P=0.0305, respectively), and survival time in FeLV-Ag-positive cats was significantly lower than FIV-Ab-positive cats (P=0.0485).

At the Mann-Whitney test, there were no statistically significant associations between the outcome of retrovirus status and either the NLR, the MLR, or the PLR (Table 6). The prognostic role of leukocyte ratios was not evaluated in the FIV-Ab+FeLV-Ag-positive group, because of the small number of surviving cats.

When different ranges of survival times were considered, none of the three ratios were found to be significantly different among the different ranges of survival times for FIV-Ab-positive cats, while there was a significantly higher value of MLR in FeLV-Ag-positive cats that died between 1 and 3 years after the diagnosis compared with the

Table 2
Summary statistics relating to selected hematologic parameters and leukocyte ratios evaluated in 75 FIV-Ab-positive cats and 75 FIV-Ab-negative cross-matched control cats to investigate the survival prognostic ability of neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR).

Parameter	FIV-Ab-positive cats (n = 75)			FIV-Ab-negative cats (n = 75)			*P*-value
	Median (25th and 75th percentiles)	Min	Max	Median (25th and 75th percentiles	Min	Max	
RBC x 10^6/μL	7.65 (5.94–8.46)	1.82	10.60	8.09 (6.78–8.88)	3.10	13.20	**0.0129**
Hb g/dL	10.90 (8.50–11.88)	2.52	15.50	11.60 (9.93–12.90)	4.10	16.10	**0.0111**
Hct %	31.00 (27.23–35.60)	8.34	49.60	34.40 (30.55–37.63)	11.70	49.30	**0.0029**
Leukocyte x 10^3/μL	10.00 (7.18–15.00)	2.09	43.21	10.50 (8.14–15.15)	3.73	43.04	0.4705
Neutrophil x 10^3/μL	6.40 (4.07–9.77)	0.70	31.37	7.20 (3.59–10.00)	1.20	38.30	0.4590
Lymphocyte x 10^3/μL	2.48 (1.68–3.79)	0.22	12.57	2.98 (1.59–4.34)	0.68	8.60	0.6506
Monocyte x 10^3/μL	0.21 (0.13–0.49)	0.00	10.25	0.25 (0.12–0.36)	0.00	2.57	0.9266
Eosinophil x 10^3/μL	0.24 (0.07–0.49)	0.00	2.60	0.00 (0.00–0.18)	0.00	8.96	**<0.0001**
Basophil x 10^3/μL	0.00 (0.00–0.01)	0.00	0.22	0.00 (0.00–0.00)	0.00	0.11	**<0.0001**
Platelet x 10^3/μL	228.00 (155.25–340.00)	29.00	970.00	265.00 (171.00–345.75)	28.00	579.00	0.3445
NLR	2.52 (1.30–4.64)	0.09	95.00	2.49 (1.24–4.29)	0.29	34.20	0.9266
MLR	0.08 (0.04–0.22)	0.00	3.00	0.14 (0.04–0.18)	0.00	1.50	0.6231
PLR	82.99 (54.02–180.98)	8.76	648.65	89.31 (51.89–156.24)	15.38	544.67	0.9416

FeLV, Feline Leukemia Virus; FIV, Feline Immunodeficiency Virus; Ab, antibody; Ag, antigen; RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; Min, minimum; Max, maximum. P-values for significant variables (P<0.05) are highlighted in bold font.

Table 3
Summary statistics relating to selected hematologic parameters and hematologic ratios evaluated in 52 FeLV-Ag-positive cats and 52 FeLV-Ag-negative cross-matched control cats to investigate the survival prognostic ability of neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR).

Parameter	FeLV-Ag-positive cats (n = 52)			FeLV-Ag-negative cats (n = 52)			*P*-value
	Median (25th and 75th percentiles	Min	Max	Median (25th and 75th percentiles	Min	Max	
RBC x 10^6/μL	7.10 (5.40–8.48)	1.17	11.60	8.56 (7.6.-10.02)	2.79	11.80	**0.0006**
Hb g/dL	10.80 (8.60–12.60)	3.00	16.20	11.85 (10.70–13.25)	4.30	15.30	**0.0175**
Hct %	30.50 (24.75–37.55)	8.50	49.00	35.30 (29.55–38.55)	12.10	45.10	0.0743
Leukocyte x 10^3/μL	8.44 (7.22–11.25)	2.72	85.90	9.62 (7.19–11.69)	4.54	31.08	0.2084
Neutrophil x 10^3/μL	4.63 (3.18–6.71)	1.14	31.30	5.04 (3.46–7.71)	1.68	22.79	0.4411
Lymphocyte x 10^3/μL	2.71 (1.96–4.18)	0.24	54.98	3.18 (1.90–4.31)	086	12.77	0.3425
Monocyte x 10^3/μL	0.13 (0.06–0.29)	0.00	3.49	0.40 (0.23–0.72)	0.00	3.64	**<0.0001**
Eosinophil x 10^3/μL	0.36 (0.09–0.85)	0.00	1.28	0.31 (0.12–0.53)	0.00	1.33	0.6583
Basophil x 10^3/μL	0.00 (0.00–0.00)	0.00	0.18	0.00 (0.00–0.01)	0.00	0.35	0.2460
Platelet x 10^3/μL	164.50 (82.50–348.50)	6.00	518.00	238.00 (140.00–377.25)	55.00	772.00	0.0501
NLR	1.62 (0.96–3.49)	0.20	15.67	1.66 (0.89–3.14)	0.26	14.50	0.8734
MLR	0.05 (0.02–0.13)	0.00	0.73	0.14 (0.07–0.21)	0.00	0.83	**0.0008**
PLR	67.19 (27.14–140.67)	0.73	654.17	77.50 (39.62–125.99)	0.00	400.76	0.6941

FeLV, Feline Leukemia Virus; FIV, Feline Immunodeficiency Virus; Ab, antibody; Ag, antigen; RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; Min, minimum; Max, maximum. P-values for significant variables (P<0.05) are highlighted in bold font.

Table 4
Summary statistics relating to selected hematologic parameters and leukocyte ratios evaluated in 15 FIV-Ab+FeLV-Ag-positive cats and 15 FIV-Ab+FeLV-Ag-negative cats to investigate the survival prognostic ability of neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR).

Parameter	FIV-Ab+FeLV-Ag-positive cats (n = 15)			FIV-Ab+FeLV-Ag-negative cats (n = 15)			*P*-value
	Median (25th and 75th percentiles)	Min	Max	Median (25th and 75th percentiles)	Min	Max	
RBC x 10^6/μL	5.40 (2.71–7.35)	1.73	8.72	7.79 (6.34–9.34)	2.70	10.60	**0.0066**
Hb g/dL	6.74 (4.63–9.95)	3.30	15.30	11.30 (8.93–13.00)	4.00	14.80	**0.0144**
Hct %	24.00 (13.63–29.10)	10.30	38.00	30.70 (26.53–38.25)	13.20	41.10	**0.0107**
Leukocyte x 10^3/μL	10.60 (4.59–18.00)	2.41	68.40	9.89 (8.71–14.51)	2.23	27.38	0.9504
Neutrophil x 10^3/μL	4.78 (2.77–14.01)	0.27	31.19	6.20 (3.75–11.72)	1.20	17.80	0.8381
Lymphocyte x 10^3/μL	2.80 (1.32–5.15)	0.09	43.09	3.12 (2.01–4.77)	0.37	7.08	0.9349
Monocyte x 10^3/μL	0.18 (0.05–0.79)	0.00	1.30	0.09 (0.05–0.37)	0.00	1.18	0.6177
Eosinophil x 10^3/μL	0.20 (0.04–0.86)	0.00	1.99	0.00 (0.00–0.35)	0.00	1.49	0.0904
Basophil x 10^3/μL	0.00 (0.00–0.03)	0.00	0.11	0.00 (0.00–0.10)	0.00	0.40	0.1026
Platelet x 10^3/μL	231.00 (104.75–381.25)	23.00	510.00	285.00 (237.75–393.00)	44.00	560.00	0.2997
NLR	1.71 (0.79–4.67)	0.12	46.99	2.11 (1.13–3.39)	0.60	34.59	0.7244
MLR	0.04 (0.02–0.15)	0.00	2.00	0.05 (0.02–0.19)	0.00	0.33	0.8840
PLR	68.25 (37.81–191.58)	4.13	665.80	80.90 (67.13–117.03)	19.04	770.27	0.5949

FeLV, Feline Leukemia Virus; FIV, Feline Immunodeficiency Virus; Ab, antibody; Ag, antigen; RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; Min, minimum; Max, maximum. P-values for significant variables (P<0.05) are highlighted in bold font.

Table 5
Comparison of the survival times between FIV-Ab-positive cats, FeLV-Ag-positive cats, and FIV-Ab+FeLV-Ag-positive cats and their cross-matched control population to investigate the survival prognostic ability of neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR).

	Survivors n (%)	Nonsurvivors n (%)	Lost to follow-up n (%)	Median survival time in days (95% CI)	*P*-value
FIV-Ab-positive cats (n = 75)	31 (41%)	24 (32%)	20 (27%)	2040 (990–3221)	*P*=0.3394
FIV-Ab-negative cats (n = 75)	31 (41%)	16 (21%)	28 (37%)	2160 (947–3960)	
FeLV-Ag-positive cats (n = 52)	15 (29%)	23 (44%)	14 (27%)	1231 (769–1692)	***P*=0.0001**
FeLV-Ag-negative cats (n = 52)	33 (63%)	5 (10%)	14 (27%)	2707 (2428–2985)	
FIV-Ab+FeLV-Ag -positive cats (n=15)	1 (7%)	10 (66%)	4 (27%)	443 (11−875)	***P*=0.0013**
FIV-Ab+FeLV-Ag -negative cat (n=15)	8 (53%)	2 (13%)	5 (33%)	2318 (1645–2992)	

95% CI, 95% confidence interval. FeLV, Feline Leukemia Virus; FIV, Feline Immunodeficiency Virus; Ab, antibody; Ag, antigen. P-values for significant variables (P<0.05) are highlighted in bold font.

corresponding FeLV-Ag-positive cats still alive at the end of the same period (0.11 and 0.02 respectively, *P*=0.0284). Again, due to the small number of FIV-Ab-+FeLV-Ag-positive cats considered and the limited number of FIV-Ab+FeLV-Ag-positive surviving cats, this evaluation was not performed in this group.

Table 6
Neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and platelet-lymphocyte ratio (PLR) comparison between surviving and non-surviving cats in FIV-Ab-positive and FeLV-Ag-positive feline populations evaluated for the survival prognostic ability of selected leukocyte ratios.

	NLR median (95% CI)	MLR median (95% CI)	PLR median (95% CI)
FIV-Ab-positive survivors	1.70 (0.97–3.66)	0.05 (0.02–0.12)	77.33 (67.52–111.86)
FIV-Ab-positive nonsurvivors	2.86 (1.96–4.92)	0.11 (0.07–0.18)	86.30 (56.80–188.52)
P-value	0.0997	0.0595	0.7343
FeLV-Ag-positive survivors	1.25 (0.97–2.06)	0.02 (0.00–0.06)	57.24 (20.06–131.15)
FeLV-Ag-positive nonsurvivors	1.44 (0.99–1.89)	0.05 (0.02–0.10)	84.13 (37.89–120.84)
P-value	0.6868	0.0944	0.7013

95% CI, 95% confidence interval; FeLV, Feline Leukemia Virus; FIV, Feline Immunodeficiency Virus; Ab, antibody; Ag, antigen.

Discussion

This study evaluated selected leukocyte ratios in different retrovirus statuses and their prognostic values for survival in FIV-Ab-positive and FeLV-Ag-positive cats. To our knowledge, this is the first study to investigate the cellular ratio in retrovirus-positive cats. No leukocyte ratio evaluated in this study was able to distinguish retrovirus-positive from negative cats. Furthermore, none of the assessed leukocyte ratios could predict the survival outcome within or among the different groups of retrovirus-positive cats.

The lack of significant differences in NLR, MLR, and PLR ratios between FIV-Ab-positive cats and FIV cross-matched control cats can be explained by the fact that no significant hematologic alteration of leukocytes used for the calculation of leukocyte ratios was detected in FIV-Ab-positive versus control cats. This contrasts with data reported in the literature, in which FIV-Ab-positive cats tended to have neutrophilia, monocytosis, leukocytosis, neutropenia and lymphopenia, thrombocytopenia, and monocytopenia (Gleich and Hartmann, 2009; Liem et al., 2013; Priolo et al., 2022; Rudan et al., 2017; Rungsuriyawiboon et al., 2022). However, our study did not consider the stage of FIV infection and hematologic alterations are only evident in the symptomatic and terminal phase (Shelton et al., 1990).

In the FeLV-Ag-positive population, MLR was found to be significantly lower than in the control population. Although an MLR $\leq$ 0.05 could differentiate FeLV-Ag-positive from FeLV-Ag-negative cats, it only had a sensitivity of 56% and specificity of 83%. This performance is significantly lower than several point-of-care tests, including the one used in this study to identify FeLV-Ag-positive cats. Therefore, even though this was a statistically significant result, it is of little practical use in diagnosing FeLV-Ag-positive cats.

The lower MLR in FeLV-Ag-positive cats may be a direct consequence of the increase in the number of lymphocytes, which is common in cats with progressive infection characterized by leukemias, or because of chronic stimulation of the immune system secondary to inflammation (Duda et al., 2020). The lower MLR may be also related to the decrease in monocytes in FeLV-infected cats, although monocytopenia has been reported in some studies related to FIV-seropositive cats (Liem et al., 2013), but not as significant alteration in FeLV-infected cats.

Concerning the survival time of retrovirus-positive cats in our study, FIV-Ab-positive cats showed a median survival not significantly different from their control cats (2040 versus 2160 days, respectively). FIV-infected cats may remain clinically healthy for many years due to the long incubation period of the virus, as reported in many previous studies (Gleich et al., 2009; Kent et al., 2022; Ravi et al., 2010). The results of this study are also similar to findings in previous literature reports (Addie et al., 2000; Beall et al., 2021; Spada et al., 2018) regarding the lower survival of FeLV seropositive cats (mean 1231 days) compared with FeLV seronegative cats (mean 2707 days). FIV-Ab+FeLV-Ag-positive cats showed significantly shorter survival times than the uninfected controls, with a mean of 443 days compared with 2318 days for seronegative cats. FIV+FeLV-coinfection leads to worsening immunodeficiency in seropositive individuals, a finding confirmed by the reduced survival of the latter compared with only FIV-infected or FeLV-infected cats (Courchamp et al., 1997; Spada et al., 2018).

The main objective of this study was to evaluate the possible prognostic value of leukocyte ratios for the survival time of retrovirus-positive cats. To our knowledge, there is no literature about NLR, MLR, and PLR as survival markers in FIV- or FeLV-positive cats. Our study was unable to demonstrate an association between any of the three ratios and FIV-Ab-positive and FeLV-positive patients concerning overall survival. However, when the survival time was divided into different time frames, the MLR was significantly higher in the FeLV-Ag-positive cats that died between 1 and 3 years after serological testing compared with the FeLV-Ag-positive cats still alive after 3 years (0.11 and 0.02 respectively, P=0.0284). The higher MLR in FeLV-Ag-positive cats that died between 1 and 3 years after the serological testing may be caused by lymphopenia resulting from the state of immunodepression in the terminal stage of infection (Duda et al., 2020). It should be considered, however, that alterations in the lymphocyte value may also be related to para-physiological conditions, such as states of stress and fear, or to the presence of pathologies not necessarily FeLV-related that were not investigated in our study (Fam et al., 2010).

This study has some limitations. The first and most important limitation is that some of the included FeLV-Ag-positive cats could be cats with transient viremia and their status of progressive infection was not confirmed by PCR testing searching for FeLV provirus. For the same reason, some FeLV-Ag-negative cats may have been latently FeLV infected (regressive cats) (Little et al., 2020). In addition, the stage of FIV and FeLV infection was not established. FIV-Ab-positive cats tend to have mild or absent hematologic changes in the prodromal and generalized lymphadenopathy stages, as well as no detectable pathologic changes during the asymptomatic stage, whereas during the FAIDS stage infected cats become symptomatic and show severe hematologic changes (Linenberger and Abkowitz, 1995). In abortive or atypical FeLV infection cats are clinically healthy (Little et al., 2020). FeLV-regressor cats show transient and mild hematologic changes, while progressive cats develop clinical signs and hematologic changes related to the state of chronic inflammation, immunodepression, and FeLV-related neoplasms (Duda et al., 2020; Little et al., 2020). Furthermore, our study did not consider any concurrent diseases that could have affected the retrovirus seropositive cats. Another limitation could be the use of different hematology analyzers in the two university laboratories of the same department. However, well-trained personnel who work under the supervision of specialized clinical pathologists and evaluation of differential leukocyte and platelet counts by microscopic examination in all samples should limit the bias relative to this aspect. The presence of cats lost to follow-up and the low number of cats included in the study, especially FIV-Ab+FeLV-Ag-positive cats, limited the analysis regarding the use of leukocyte ratios as biomarkers of survival in this population. Finally, although the platelet count was manually counted since PLT aggregation interferes with manual counting, the PLR may be inaccurate in the feline population evaluated.

Conclusions

Although leukocyte ratios are easily calculated and are inexpensive biomarkers shown to be useful in clinical feline practice (Chiti et al., 2020; Donato et al., 2023; Fries et al., 2022; Gori et al., 2021; Tsouloufi et al., 2021), they did not prove to be an accurate prognostic survival marker in retrovirus-positive cats. Only MLR was significantly higher in FeLV-Ag-positive cats that died between 1 and 3 years after diagnosis

than in FeLV-Ag-positive cats that were still alive at the end of this period.

CRediT authorship contribution statement

A. Rossi: Writing – original draft, Software, Formal analysis, Data curation. **D. Proverbio:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation. **R. Perego:** Writing – review & editing, Visualization, Validation, Methodology, Investigation. **L. Baggiani:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Data curation. **E. Spada:** Writing – review & editing, Supervision, Project administration, Methodology, Data curation, Conceptualization.

Off-label antimicrobial declaration

Authors declare no off-label use of antimicrobials.

Institutional animal care and use committee (IACUC) or other approval declaration

Authors declare no IACUC or other approval was needed.

Human ethics approval declaration

Authors declare human ethics approval was not needed for this study.

Conflict of interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2024.106128.

References

Addie, D.D., Toth, S., Reid, S., Jarrett, O., Dennis, J.M., Callanan, J.J., 2000. Long-term impact on a closed household of pet cats of natural infection with feline coronavirus, feline leukaemia virus and feline immunodeficiency virus. Veterinary Record 146, 419–424. https://doi.org/10.1136/vr.146.15.419.

Beall, M.J., Buch, J., Clark, G., Estrada, M., Rakitin, A., Hamman, N.T., Frenden, M.K., Jefferson, E.P., Amirian, E.S., Levy, J.K., 2021. Feline leukemia virus p27 antigen concentration and proviral DNA load are associated with survival in naturally infected cats. Viruses 13, 302. https://doi.org/10.3390/v13020302.

Biezus, G., Ferian, P.E., Da Silva Pereira, L.H.H., Withoeft, J.A., Antunes, M.M., Nunes Xavier, M.G., Volpato, J., De Cristo, T.G., Fonteque, J.H., Casagrande, R.A., 2019. Clinical and haematological disorders in cats with natural and progressive infection by feline leukemia virus (FeLV). Acta Scientiae Veterinariae 47, 101491. https://doi.org/10.22456/1679-9216.90027.

Chiti, L.E., Martano, M., Ferrari, R., Boracchi, P., Giordano, A., Grieco, V., Buracco, P., Iussich, S., Giudice, C., Miniscalco, B., Zani, D.D., Proverbio, D., Morello, E., Stefanello, D., 2020. Evaluation of leukocyte counts and neutrophil-to-lymphocyte ratio as predictors of local recurrence of feline injection site sarcoma after curative intent surgery. Veterinary and Comparative Oncology 18, 105–116. https://doi.org/10.1111/vco.12534.

Courchamp, F., Suppo, C., Fromont, E., Bouloux, C., 1997. Dynamics of two feline retroviruses (FIV and FeLV) within one population of cats. Proceedings of the Royal Society of London Series B, Biological Sciences 264, 785–794. https://doi.org/10.1098/rspb.1997.0111.

Donato, G., Pennisi, M.G., Persichetti, M.F., Archer, J., Masucci, M., 2023. A Retrospective comparative evaluation of selected blood cell ratios, acute phase proteins, and leukocyte changes suggestive of inflammation in cats. Animals 13, 2579. https://doi.org/10.3390/ani13162579.

Duda, N.C.B., Ortiz, L.C., Valle, S.F., da Costa, F.V.A., Varela, A.P.M., Nunes, N.J.S., Okano, F.Y., Franco, A.C., Roehe, P.M., González, F.H.D., 2020. Laboratory and clinical findings and their association with viral and proviral loads in cats naturally infected with feline leukemia virus. Comparative Immunology, Microbiology & Infectious Diseases 71, 101491. https://doi.org/10.1016/j.cimid.2020.101491.

Fam, A.L.P.D., Rocha, R.M.V.M., Pimpão, C.T., Cruz, M., de, A., 2010. Alterations on leukogram of domestic felines (Felis catus) due to acute and chronic stress. Revista Acadêmica: Ciências Agrotikosárias e Ambientais 8, 299–306.

Fries, R.C., Kadotani, S., Stack, J.P., Kruckman, L., Wallace, G., 2022. Prognostic value of neutrophil-to-lymphocyte ratio in cats with hypertrophic cardiomyopathy, 813524–813524 Frontiers in Veterinary Science 9. https://doi.org/10.3389/fvets.2022.813524.

Fujino, Y., Horiuchi, H., Mizukoshi, F., Baba, K., Goto-Koshino, Y., Ohno, K., Tsujimoto, H., 2009. Prevalence of hematological abnormalities and detection of infected bone marrow cells in asymptomatic cats with feline immunodeficiency virus infection. Veterinary Microbiology 136, 217–225. https://doi.org/10.1016/j.vetmic.2008.11.007.

Gleich, S., Hartmann, K., 2009. Hematology and serum biochemistry of feline immunodeficiency virus-infected and feline leukemia virus-infected cats. Journal of Veterinary Internal Medicine 23, 552–558. https://doi.org/10.1111/j.1939-1676.2009.0303.x.

Gleich, S.E., Krieger, S., Hartmann, K., 2009. Prevalence of feline immunodeficiency virus and feline leukaemia virus among client-owned cats and risk factors for infection in Germany. Journal of Feline Medicine and Surgery 11, 985–992. https://doi.org/10.1016/j.jfms.2009.05.019.

González-Domínguez, A., Cristobal-Verdejo, J.I., Fontela-González, S., Vázquez, S., Justo-Domínguez, J., González-Caramazana, J., Alvarez-Punzano, A., Herrería-Bustillo, V.J., 2023. Retrospective evaluation of hematological ratios in canine parvovirosis: 401 cases. Journal of Veterinary Internal Medicine 1–6. https://doi.org/10.1111/jvim.16972.

Gori, E., Pierini, A., Lippi, I., Lubas, G., Marchetti, V., 2021. Leukocytes ratios in feline systemic inflammatory response syndrome and sepsis: a retrospective analysis of 209 cases. Animals 11, 1644. https://doi.org/10.3390/ani11061644.

Hartmann, K., Griessmayr, P., Schulz, B., Greene, C.E., Vidyashankar, A.N., Jarrett, O., Egberink, H.F., 2007. Quality of different in-clinic test systems for feline immunodeficiency virus and feline leukaemia virus infection. Journal of Feline Medicine and Surgery 9, 439–445. https://doi.org/10.1016/j.jfms.2007.04.003.

Hodgson, N., Llewellyn, E.A., Schaeffer, D.J., 2018. Utility and prognostic significance of neutrophil-to-lymphocyte ratio in dogs with septic peritonitis. Journal of the American Animal Hospital Association 54, 351–359. https://doi.org/10.5326/JAAHA-MS-6808.

Hofmann-Lehmann, R., Hartmann, K., 2020. Feline leukaemia virus infection: a practical approach to diagnosis. Journal of Feline Medicine and Surgery 22, 831–846. https://doi.org/10.1177/1098612×20941785.

Johnson, M.M., Gicking, J.C., Keys, D.A., 2023. Evaluation of red blood cell distribution width, neutrophil-to-lymphocyte ratio, and other hematologic parameters in canine acute pancreatitis. Journal of Veterinary Emergency and Critical Care 33, 587–597. https://doi.org/10.1111/vec.13325.

Kent, M.S., Karchemskiy, S., Culp, W.T.N., Lejeune, A.T., Pesavento, P.A., Toedebusch, C., Brady, R., Rebhun, R., 2022. Longevity and mortality in cats: a single institution necropsy study of 3108 cases (1989–2019). PLoS One 17, e0278199. https://doi.org/10.1371/journal.pone.0278199.

Kopilovic, A., Gvozdic, D., Radakovic, M., Spariosu, K., Andric, N., Francuski-Andric, J., 2023. Selected hematology ratios in cats with non-septic effusions highly suspected of feline infectious peritonitis. Veterinarski Glasnik 77, 164–175. https://doi.org/10.2298/vetgl230710008k.

Kučer, N., Matijatko, V., Kiš, I., Grden, D., Brkljačič, M., Foršek, J., Žvorc, Ž., Rafaj, R.B., 2008. White blood cell count and neutrophil to lymphocyte ratio in uncomplicated and complicated canine babesiosis caused by Babesia canis canis. Veterinarski Arhiv 78, 321–330.

Liem, B.P., Dhand, N.K., Pepper, A.E., Barrs, V.R., Beatty, J.A., 2013. Clinical findings and survival in cats naturally infected with feline immunodeficiency virus. Journal of Veterinary Internal Medicine 27, 798–805. https://doi.org/10.1111/jvim.12120.

Linenberger, M.L., Abkowitz, J.L., 1995. Haematological disorders associated with feline retrovirus infections. Baillière's Clinical Haematology 8, 73–112. https://doi.org/10.1016/S0950-3536(05)80233-1.

Little, S., Levy, J., Hartmann, K., Hofmann-Lehmann, R., Hosie, M., Olah, G., Denis, K.S., 2020. 2020 AAFP feline retrovirus testing and management guidelines. Journal of Feline Medicine and Surgery 22, 5–30. https://doi.org/10.1177/1098612×19895940.

Luckman, C., Gates, M.C., 2017. Epidemiology and clinical outcomes of feline immunodeficiency virus and feline leukaemia virus in client-owned cats in New Zealand. Journal of Feline Medicine and Surgery Open Reports 3, 1–9. https://doi.org/10.1177/2055116917729311.

Naranbhai, V., Hill, A.V.S., Abdool Karim, S.S., Naidoo, K., Abdool Karim, Q., Warimwe, G.M., McShane, H., Fletcher, H., 2014. Ratio of monocytes to lymphocytes in peripheral blood identifies adults at risk of incident tuberculosis among HIV-infected adults initiating antiretroviral therapy. Journal of Infectious Diseases 209, 500–509. https://doi.org/10.1093/infdis/jit494.

Neumann, S., 2021. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in dogs and cats with acute pancreatitis. Veterinary Clinical Pathology 50, 45–51. https://doi.org/10.1111/vcp.12979.

Pierini, A., Gori, E., Lippi, I., Lubas, G., Marchetti, V., 2020. Are leukocyte and platelet abnormalities and complete blood count ratios potential prognostic markers in canine sepsis? Frontiers in Veterinary Science 7, 1–7. https://doi.org/10.3389/fvets.2020.578846.

Priolo, V., Masucci, M., Donato, G., Solano-Gallego, L., Martínez-Orellana, P., Persichetti, M.F., Raya-Bermúdez, A., Vitale, F., Pennisi, M.G., 2022. Association between feline immunodeficiency virus and Leishmania infantum infections in cats: a retrospective matched case-control study, 107–107 Parasites & Vectors 15. https://doi.org/10.1186/s13071-022-05230-w.

Ravi, M., Wobeser, G.A., Taylor, S.M., Jackson, M.L., 2010. Naturally acquired feline immunodeficiency virus (FIV) infection in cats from western Canada: prevalence, disease associations, and survival analysis. Canadian Veterinary Journal 51, 271–276.

Robert, L.I., Puig, J., Tumbarello, M., Farigola, M., Seth, M., Mesa, I., Bernabe, L.F., 2023. A retrospective review of cats with suspected false positive results in point-of-care feline leukemia virus tests and concurrent immune-mediated anemia. Journal of the American Veterinary Medical Association 261, 1–7. https://doi.org/10.2460/javma.23.02.0059.

Rudan, N., Marković, E., Kučer, N., 2017. Evaluation of clinical and haematological parameters in differentiation of feline immunodeficiency and feline leukemia virus infection. Veterinarski Arhiv 87, 731–743. https://doi.org/10.24099/vet.arhiv.160525.

Rungsuriyawiboon, O., Jarudecha, T., Hannongbua, S., Choowongkomon, K., Boonkaewwan, C., Rattanasrisomporn, J., 2022. Risk factors and clinical and laboratory findings associated with feline immunodeficiency virus and feline leukemia virus infections in Bangkok, Thailand. Veterinary World 15, 1601–1609. https://doi.org/10.14202/vetworld.2022.1601-1609.

Russell, C.D., Parajuli, A., Gale, H.J., Bulteel, N.S., Schuetz, P., de Jager, C.P.C., Loonen, A.J.M., Merekoulias, G.I., Baillie, J.K., 2019. The utility of peripheral blood leucocyte ratios as biomarkers in infectious diseases: a systematic review and meta-analysis. Journal of Infection 78, 339–348. https://doi.org/10.1016/j.jinf.2019.02.006.

Shelton, G., Linenberger, M., Grant, C., Abkowitz, J., 1990. Hematologic manifestations of feline immunodeficiency virus infection. Blood 76, 1104–1109. https://doi.org/10.1182/blood.V76.6.1104.1104.

Spada, E., Perego, R., Sgamma, E.A., Proverbio, D., 2018. Survival time and effect of selected predictor variables on survival in owned pet cats seropositive for feline immunodeficiency and leukemia virus attending a referral clinic in northern Italy. Preventive Veterinary Medicine 150, 38–46. https://doi.org/10.1016/j.prevetmed.2017.12.001.

Tsouloufi, T.K., Frezoulis, P.S., Soubasis, N., Kritsepi-Konstantinou, M., Oikonomidis, I. L., 2021. Diagnostic and prognostic value of peripheral blood leucocyte ratios in sick cats. Acta Veterinaria Hungarica 69, 308–314. https://doi.org/10.1556/004.2021.00042.