



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



Eva Spada*, Roberta Perego, Elena Assunta Sgamma, Daniela Proverbio

Department of Veterinary Medicine (DIMEVET), University of Milan, Via G. Celoria 10, 20133 Milan, Italy

ARTICLE INFO

Keywords:

Feline
FIV
FeLV
Seropositivity
Survival time
Predictor variable

ABSTRACT

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are among the most important feline infectious diseases worldwide.

This retrospective study investigated survival times and effects of selected predictor factors on survival time in a population of owned pet cats in Northern Italy testing positive for the presence of FIV antibodies and FeLV antigen.

One hundred and three retrovirus-seropositive cats, 53 FIV-seropositive cats, 40 FeLV-seropositive cats, and 10 FIV + FeLV-seropositive cats were included in the study. A population of 103 retrovirus-seronegative age and sex-matched cats was selected. Survival time was calculated and compared between retrovirus-seronegative, FIV, FeLV and FIV + FeLV-seropositive cats using Kaplan-Meier survival analysis. Cox proportional-hazards regression analysis was used to study the effect of selected predictor factors (male gender, peripheral blood cytopenia as reduced red blood cells – RBC- count, leukopenia, neutropenia and lymphopenia, hypercreatininemia and reduced albumin to globulin ratio) on survival time in retrovirus-seropositive populations.

Median survival times for seronegative cats, FIV, FeLV and FIV + FeLV-seropositive cats were 3960, 2040, 714 and 77 days, respectively. Compared to retrovirus-seronegative cats median survival time was significantly lower ($P < 0.000$) in FeLV and FIV + FeLV-seropositive cats. Median survival time in FeLV and FIV + FeLV-seropositive cats was also significant lower ($P < 0.000$) when compared to FIV-seropositive cats. Hazard ratio of death in FeLV and FIV + FeLV-seropositive cats being respectively 3.4 and 7.4 times higher, in comparison to seronegative cats and 2.3 and 4.8 times higher in FeLV and FIV + FeLV-seropositive cats as compared to FIV-seropositive cats.

A Cox proportional-hazards regression analysis showed that FIV and FeLV-seropositive cats with reduced RBC counts at time of diagnosis of seropositivity had significantly shorter survival times when compared to FIV and FeLV-seropositive cats with normal RBC counts at diagnosis.

In summary, FIV-seropositive status did not significantly affect longevity of cats in this study, unlike FeLV and FIV + FeLV-seropositivity. Reduced RBC counts at time of FIV and FeLV diagnosis could impact negatively on the longevity of seropositive cats and therefore blood counts should always be evaluated at diagnosis and follow-up of retrovirus-seropositive cats.

1. Introduction

The two feline retroviruses, feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV), are global and widespread, but differ in their potential to cause disease.

FIV, a retrovirus of the genus Lentivirus, can cause an acquired immune deficiency syndrome, which predisposes cats to other infections. Clinical signs are most often a reflection of opportunistic infections, neoplasia and/or myelosuppression. However, in most naturally

infected cats, FIV does not cause a severe clinical syndrome; with appropriate care, FIV-infected cats can live many years before succumbing to conditions unrelated to their FIV infection. Thus, overall survival time is not necessarily shorter than in uninfected cats, and quality of life is usually high over many years or lifelong (Addie et al., 2000; Hofmann-Lehmann et al., 1997; Levy et al., 2008).

FeLV, a γ -retrovirus member of the Oncornavirus subfamily of retroviruses, is more pathogenic than FIV. Progressive FeLV infection can cause tumors, bone marrow suppression and immunosuppression, as

* Corresponding author.

E-mail address: eva.spada@unimi.it (E. Spada).

well as neurological and other disorders, resulting in decreased life expectancy (Lutz et al., 2009). In contrast to FIV, persistent FeLV infection has a much greater effect on life expectancy of infected cats (Addie et al., 2000; Hofmann-Lehmann et al., 1997). However, with appropriate care, many FeLV-infected cats can also live for several years after diagnosis with a good quality of life (Helfer-Hungerbuehler et al., 2015; Hofmann-Lehmann et al., 1995; Levy et al., 2008).

Many studies have explored the risk factors for seropositivity and clinic-pathological features for retrovirus infected cats. Amongst these risk factors and features, male sex (Arjona et al., 2000; Burling et al., 2017; Chhetri et al., 2015; Garigliany et al., 2016; Gates et al., 2017; Gleich et al., 2009a,b; Levy et al., 2006; Liem et al., 2013; Murray et al., 2009; Peri et al., 1994; Ravi et al., 2010; Spada et al., 2012; Westman et al., 2016) and peripheral blood cytopenias such as anemia, leukopenia with neutropenia and lymphopenia are a common and well documented findings for both FIV and FeLV infections (Ackley et al., 1990; Arjona et al., 2000; Fujino et al., 2009; Gleich and Hartmann, 2009; Hofmann-Lehmann et al., 1997; Kohmoto et al., 1998; Shelton et al., 1995; Spada et al., 2012; Sparkes et al., 1993). In addition FIV-infected cats can develop an excessive immune response leading to hypergammaglobulinemia, reduced albumin to globulin ratio (A/G) and hyperproteinemia (Gleich and Hartmann, 2009; Hofmann-Lehmann et al., 1997; Liem et al., 2013; Mirò et al., 2007) which reflects polyclonal B-cell stimulation (Ackley et al., 1990; Flynn et al., 1994; Gleich and Hartmann, 2009; Shelton et al., 1995; Sparkes et al., 1993). Finally renal involvement can occur in a proportion of naturally and experimentally FIV-infected cats (Baxter et al., 2012; Poli et al., 2012).

A limited number of studies have reported survival times for retrovirus infected cats, compared survival time to uninfected cats and studied the effect of predictor factors on survival times both for FeLV (Addie et al., 2000; Chhetri et al., 2015; Gleich et al., 2009b; Levy et al., 2008) and for FIV-infected cats (Addie et al., 2000; Chhetri et al., 2015; Gleich et al., 2009b; Levy et al., 2008; Liem et al., 2013; Ravi et al., 2010). In addition, results of studies on survival time may differ geographically as the clinical course of retrovirus infection is determined by a combination of viral and host factors. Some of these differences can be traced to properties of the virus itself, such as the subgroup that determines differences in the clinical picture. For example FeLV-B is primarily associated with tumors, FeLV-C is primarily associated with non-regenerative anemia (Dean et al., 1992). Different FIV subtypes exist, and subtype B is a less virulent strain of FIV than subtype A (Bachmann et al., 1997; Kohmoto et al., 1998). Therefore, there may be geographical/strain-related variations in clinical signs, diseases, and opportunistic infections associated with retrovirus infections.

The aims of this study were to estimate the survival times and to explore the effect of selected predictor factors on survival times in owned pet cats that were tested for FIV antibodies and FeLV antigen for a variety of medical reasons at a referral clinic in northern Italy over a 14 years period. Our hypothesis was that the type of retrovirus might alter the effect on survival time and that some risk factors for retrovirus seropositivity could also have a prognostic value for survival.

2. Material and methods

A retrospective cohort study was designed. Medical records of cats referred for different medical reasons to the Veterinary Clinic of the University of Milan were examined retrospectively to identify cats that had been tested for FIV antibodies and FeLV antigen, between January 2002 and the first semester of 2016 (30 June 2016). Presence of antibody to FIV target antigens p24 and gp40 and of FeLV p27 antigen were simultaneously checked on plasma, serum, or whole blood samples using a commercial rapid enzyme-linked immunosorbent assay (ELISA) kit (SNAP® Combo Plus FeLV Ag/Fiv Ab, IDEXX Laboratories, Europe). This ELISA test kit showed excellent performance in diagnosis of these retroviruses with a sensitivity and specificity for FeLV diagnosis of 100%

and a sensitivity and specificity for FIV diagnosis of 97.9% and 99.0%, respectively (Levy et al., 2017).

FIV and FeLV-seropositive cats formed the study population. A comparison group of retrovirus-seronegative cats with similar sex and age characteristics was created. For each retrovirus-seropositive cat, one retrovirus-seronegative was enrolled in the study. This retrovirus-seronegative cat was selected as the first cat of the same sex and of similar age tested in the same day or month or year (within 1 year). If age and sex matching were not simultaneously available matching was performed based only on one of the two parameters, depending on which was available.

For each retrovirus-seronegative and seropositive cat, data retrieved from medical records were: data of the first FIV/FeLV test, age at test, sex, reason for FIV-FeLV test (pre-FeLV vaccination, pre-neuter check, pre-blood donor check, unhealthy cat), hematological and biochemical data, details of death/euthanasia, clinical condition in alive cats.

Laboratory data collected at time of test at admission were: complete blood count (CBC) using ADVIA 120 analyser (Bayer) until end of may 2010 and Cell-Dyn 3500 haematology analyser (Abbott Diagnostic Laboratories) from July 2010 to the end of the study comprising red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), mean cell volume (MCV), mean cell Hb (MCH), mean cell Hb concentration (MCHC), and white blood cells (WBCs). Differential leukocyte count was made by blood smear evaluation (microscopic magnification 100 x immersion objective) to make a quantitative evaluation of the percentage in 10 fields.

Biochemical data collected comprised: serum creatinine (by cinetic modified Jaffè method) and total serum protein (by biuret colorimetric method) evaluated using Cobas Mira Classics analyser (Roche). Alfa, beta and gammaglobulins, albumin to globulin (A/G) ratio were evaluated by agarose gel serum electrophoresis (Hydrasys-Sebia). Laboratory reference ranges used were taken from the literature (Giordano and Paltrinieri, 2010; Moritz et al., 2004; Silverstein and Hopper, 2015).

2.1. Statistical analysis

All the collected data were captured in Microsoft Excel 2007 and analyzed using statistical software (MedCalc statistical software version 16.4.3 and SPSS statistics software, version 18.0).

Descriptive statistics were presented to define the population and survival predictor variables for cats at entry into the study.

Survival times were calculated from the date of diagnosis of retroviral seropositivity to the date of death or euthanasia. If these data were not available, the owner was contacted by telephone to request follow-up information. If the cat had died and the exact date of death was unknown, the month of death was recorded and it was assumed that the cat died on the 15th of that month for the purposes of the survival analysis. Cats were censored in the survival analysis if they were still alive at the end of the follow-up period (June 30, 2016) or if they were lost to follow-up. Cats were categorized as lost to follow up if they had not attended the clinic for 6 months and their owners were not contactable by telephone (after three calls made on three different days at three different times of day).

Differences between the median age of the groups were tested for significance using the non-parametric Mann–Whitney *U* test for unpaired samples.

Kaplan–Meier survival analyses were performed to estimate survival of retrovirus-seronegative and seropositive cats groups (ie FIV, FeLV or FIV + FeLV-seropositive cats). Pairwise comparison of survival curves was performed using the Mantel–Cox logrank test. The hazard ratios with 95%CI (ie how rapidly death occurred in the groups) were also calculated (Klein and Moeschberger, 2003).

Patients lost to follow-up or still alive at the time of follow-up were censored by the date on which they were last seen by the diagnosing veterinarian or the last date known to be alive.

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