Laboratory and clinical findings and their association with viral and proviral loads in cats naturally infected with feline leukemia virus

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\textbf{A R T I C L E   I N F O}

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Feline retrovirus
Laboratory abnormalities
Hematopoietic disorders
Bone marrow
Virus quantification

\section{A B S T R A C T}

This study was conducted to correlate clinical, laboratory, and bone marrow (BM) changes in cats naturally infected with feline leukemia virus and their association with viral loads in blood and BM and proviral loads in BM. Cats were classified into five groups based on antigenemia, clinical and/or laboratory findings and viral/ proviral loads, according to a prospective study: symptomatic progressive (GI); asymptomatic progressive (GII); unclassified (GIII); unclassified (GIIV); or healthy (GV). |Correlations between these five groups and viral/proviral loads were evaluated. High viral and proviral loads were detected in GI and GIIV and viral loads were significantly associated with laboratory signs. Proviral loads detected in BM were significantly lower in GIIV and GIIV. GI cats were more likely to develop hematopoietic disorders than those from the other groups. Hematological and clinical disorders and disease severity are related to higher viral blood and proviral BM loads.

\section{1. Introduction}

Feline leukemia virus (FeLV) infections occur worldwide and still account for the majority of deaths among cats \cite{1} in endemic regions. In Brazil, the prevalence of FeLV infections in cats is high, ranging in some studies between 11.5 \% and 38.5 \% when ELISA and indirect immunofluorescence are used \cite{2,3,4} and approximately 47.5 \% in cases in which blood proviral DNA was detected \cite{5}.

The stages of FeLV infection have been classified according to antigenemia and to blood viral/proviral loads as abortive, focal or atypical, progressive, or regressive \cite{1,6}. In progressive infection, antigenemia and high viral and proviral loads are associated with clinical disease, leading to death in a few years. Immunocompetent cats produce abortive infection since they produce high levels of neutralizing antibodies and do not have antigenemia or viral/proviral loads in their blood. Focal or atypical infection is characterized by localized viral replication with low or intermittent antigen production. In regressive infection, cats present a transient viremia (within weeks or months) which leads a negative result in antigen and viral load tests; however, proviral loads can be identified in blood and hematopoietic tissues. Regressive cats may reactivate infection following immunosuppressive events that culminate in antigenemia and virus-associated diseases. Initially, progressive and regressive infections are characterized by the persistence of proviral DNA in the blood, detected through PCR, but they are later associated with different viral loads when qPCR is performed \cite{7,8}.

As a consequence of bone marrow (BM) infection, FeLV induces several alterations in peripheral blood, such as anemia, neutropenia, leukopenia, platelet disorders, thrombocytopenia, lymphocytosis, pancytopenia, hematopoietic neoplasms, and myeloproliferative disorders.

\textbf{Abbreviations:} ALL, acute lymphocytic leukemia; ALP, alkaline phosphatase; AML, acute myeloid leukemia; AML-M6, erythroleukemia; BM, bone marrow; CBC, complete blood count; ELISA, enzyme-linked immunosorbent assay; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; HB, hemoglobin; HT, hematocrit; IgG, immunoglobulin G; IgM, immunoglobulin M; IMHA, immune-mediated hemolytic anemia; LTR, long terminal repeat; MCHC, mean corpuscular hemoglobin concentration; MDS, myelodysplastic syndrome; MDS-Er, myelodysplastic syndrome with erythroid predominance; MCV, mean corpuscular volume; M:E, myeloid to erythroid ratio; PCR, polymerase chain reaction; qPCR, quantitative real-time PCR; RBC, red blood cell; WBC, white blood cell

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Cats with progressive or regressive infections are more commonly affected by BM disorders, since virus-induced BM suppression and integration of proviral DNA into hematopoietic cells may occur [8]. Cats with latent FeLV infection and negative antigen blood test results are strongly suspected of viral infection when unexplained cytopenia is detected in blood examinations [10]. Progressive and regressive infections have not been completely understood and characterized. In endemic areas, studies relating hematopoietic disorders, clinical manifestations associated with molecular tests must be conducted to elucidate the role of viral/proviral loads in blood and BM in naturally infected cats.

The aim of the present study was to correlate regressive and progressive FeLV infections with clinical and laboratory findings, including hematological, biochemical, and BM evaluations. In addition, an attempt was made to associate viral and proviral loads with the clinical stages of infection.

2. Materials and methods

2.1. Animals and inclusion criteria

A total of 51 male and female cats older than six months admitted to the Feline Medicine Outpatient Clinic of the Veterinary Teaching Hospital affiliated with the Federal University of Rio Grande do Sul were included in this prospective study. To initial evaluation for inclusion of study, the clinical signs considered were unspecific and related with immune suppression (neutropenia, lymphopenia, opportunistic infections, stomatitis and chronic rhinitis), anemia (regenerative or non-regenerative) and other cytopenia, hematopoietic malignances or other diseases previously related of FeLV infection [1,11].

Thirty-three of these cats were clinically healthy; 18 showed FeLV-related disease as defined by clinical and laboratory findings. From all cats, FeLV vaccinal status was missing. The inclusion criteria were: hematological alterations in peripheral blood (anemia, leukopenia, lymphopenia, thrombocytopenia, and leukocytosis); no prior therapy with immunosuppressive drugs, erythropoiesis- or granulopoiesis-stimulating agents, or antivirals; and sufficient BM specimen for cytological analysis. The study protocol was approved by the institutional Committee on Animal Care and Experimental Usage (process no. 28928). All cat owners agreed to participate in the study.

2.2. Blood sample collection and laboratory parameters

All selected cats were subjected to clinical evaluations and blood sampling after clinical examination. EDTA blood samples were immediately analyzed in approximately 20–30 min after sampling at Veterinary Clinical Pathology Laboratory (LACVET-UFRGS). A complete blood count (CBC) was performed using an automated hematology analyzer (ProCyte DX Hematology Analyzer, Idexx Laboratories, USA). The CBC included red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hematoglobin concentration (MCHC), WBC total and differential counts, platelet count, and manual reticulocyte count [12]. The complete WBC differential count was manually determinate by the same trained veterinary clinical pathologist and consisted of all nucleated cells, including immature mononuclear cells. Cytochemical stains such as myeloperoxidase and Sudan Black B were used to differentiate immature mononuclear cells in blood smears.

Serum blood chemistry included albumin, alanine aminotransferase, creatinine, urea, alkaline phosphatase (ALP), glucose, and total proteins, obtained by the colorimetric and enzymatic method performed on an automated spectrophotometer (CM 200, Wiener Lab Group, Argentina). Globulin levels were quantified.

FeLV antigenemia was determined in all serum samples by a rapid immunoassay for feline immunodeficiency virus (FIV) and FeLV (Snap FIV/FeLV Combo Test, Idexx Laboratories, USA), which simultaneously detects FeLV antigen p27 and FIV antibodies. After the analyses, the remaining sera were stored at −70 °C for later RNA extraction and qPCR.

2.3. Bone marrow cytology

BM aspiration was performed in all cats under general anesthesia and analgesia following the previously described methodology [13]. The BM aspirate was immediately placed on a Petri dish for the selection of spicules and squash preparation. The residual BM specimen was stored in microtubes and kept at −70 °C for a maximum of 24 months for RNA and DNA extraction. All BM smears were stained with Wright-Giemsa and the microscopic analysis followed previously described guidelines [13]. Cytological examination consisted of the evaluation of general BM population and bone spicules under a low-power objective (10x), followed by a differential count from 500 to 1000 nucleated cells and morphological analysis under high-power objectives (100x in immersion oil). The overall degree of cellularity, content of particles (iron stores, megakaryocytes, stromal cells, hematopoietic cells, and other cells), myeloid to erythroid ratio (M:E ratio), sequence and completeness of maturation order, followed by morphological evaluation of hematopoietic lineages, were determined for all BM specimens.

Counts of all nucleated cells and non-erythroid cells and of other cell types (lymphocytes, plasma cells, mononuclear phagocytes, mast cells, and neoplastic cells) were obtained. BM cytological findings were interpreted with a recent BM obtained up to 24 h prior to sampling. Complementary cytochemical staining, with either myeloperoxidase or Sudan Black, was performed, whenever necessary, to identify the origin of undifferentiated nucleated cells [13].

2.4. Molecular analysis

Total RNA from serum and BM samples was extracted using TRIzol (Invitrogen), following the manufacturer’s instructions. In addition, genomic DNA was extracted from BM samples using PureLink DNA Mini Kit (Invitrogen). DNA quantification was performed with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific). The extracted RNA and DNA were immediately stored at −80 °C and −20 °C, respectively.

First, to qPCR and RT-qPCR, a standard curve was constructed by cloning a FeLV amplicon into a pCR2.1 vector from TOPO TA Cloning vector (Invitrogen), according to the manufacturer’s instructions. The amplicon was obtained by conventional PCR using the following primers: FeLV_U3-F (2): 5'-GCTCCCCAGTGACACAGAT-3' [14] and FeLV_U3-exo_r: 5'-TTTATACGAAATA GCGCCGC-3' [15]. The target region is a 93-base pair (bp) fragment in the U3 region of FeLV’s LTR [14]. Competent E. coli (NEB 10-beta) were used for transformation and plasmid DNA was extracted according to standard methods [16]. In order to confirm the FeLV clone insertion, the clones were sequenced and quantified using Qubit Fluomer (Thermo Fisher Scientific). Tenfold serial dilutions (10^0 - 10^2) were used for plotting the standard curves of amplification.

Viral and proviral genome loads were evaluated by qPCR. For viral load evaluation, RNA extracted from serum and BM samples was subjected to cDNA synthesis. Reactions were performed with 10 μL of RNA, 200 μM of dNTPs, 0.2 μM of random primers, 1 U of Invitrogen SuperScript reverse transcriptase, and water q.s.p. 20 μL. Cyclings were carried out in an Eppendorf Mastercycler Personal following the manufacturer’s instructions.

The viral genome loads were determined by qPCR as follows: fifteen microliters containing 3 μL of standard curve or 2 μL of cDNA, 1X Fast EvaGreen qPCR Master Mix (Biotium, USA), 0.2 μM of each primer (FeLV_U3-F (2) and FeLV_U3-exo_r), 1X ROX, and water up to the final volume. The reactions were performed in a StepOnePlus Real-Time PCR system (Applied Biosystems, Life Technologies) under the following conditions: initial denaturation at 95 °C for 2 min, followed by 45 cycles...
of amplification (5 s at 95 °C and 30 s at 60 °C) and melting curve analysis range extending from 60 °C to 95 °C. All reactions were performed in triplicate and the results were reported as means (copies/mL). Fluorescent measurements were carried out during the elongation stage and a threshold cycle (Ct) value was calculated by comparison with the standard curve.

The proviral load of the DNA extracted from BM samples (100 ng) was evaluated by qPCR under the same conditions described above.

2.5. Groups and statistical analysis

Based on antigenemia, serum and BM viral loads and BM proviral loads, and presence or absence of FeLV related clinical signs [1,11], the cats were distributed into five groups: group I (GI) corresponded to cats with symptomatic progressive infection (antigenemia, high viral and proviral loads, and clinical signs); GII refers to samples from cats with asymptomatic progressive infection (antigenemia, high viral and proviral loads, and absence of clinical signs); GIII refers to cats with regressive infection (absence of antigenemia and viral load, presence of proviral load, with or without clinical signs); GIV corresponded to cats with regressive infection (absence of antigenemia and viral load, presence of proviral load, with or without clinical signs); and GV, which included samples from cats with no signs of infection (absence of antigenemia and viral and proviral loads and no clinical signs).

To statistics analysis, Kolmogorov-Smirnov test was used to determine the normality of data. Asymmetrically distributed variables were described as median, minimum and maximum, and the Kruskal-Wallis test was utilized for between-group comparison. Those variables with a symmetric distribution were described as mean and standard deviation and the comparison between groups was made by ANOVA. Tukey’s post-hoc test was used whenever significant differences were found. A logarithmic transformation was performed for asymmetric variables before the post-hoc test. Spearman’s correlation coefficient was used for quantitative variables. SPSS v20.0 was used for the analysis and the level of significance was set at 5%.

3. Results

3.1. Cats and clinical signs

Forty-four out of 51 cats were selected for this study. Seven of them were excluded due to lack of sufficient material for complete BM cytology (n = 2), death before BM sampling (n = 2), hemolytic anemia unrelated to FeLV infection (n = 2), and positivity for FIV (n = 1). Thirty-one cats were female (70.5 %) and 13 were male (29.6 %). Mean age was 2.5 years (6 months to 11 years).

Considering clinical signs, cats with symptomatic progressive infection (GI) displayed nonspecific clinical signs, such as apathy, dyspnea, coughing, hyporexia, anorexia, loss of appetite, progressive weight loss, or oral lesions. Clinical signs such as apathy, hyporexia, and progressive weight loss were found in one cat with regressive infection (GIII) and in one cat from GIV (unclassified infection).

3.2. Blood and bone marrow parameters

Symptomatic cats from GI presented nonregenerative anemia, among other individual alterations in peripheral blood in CBC and WBC count (Table 1). Individual biochemical changes varied slightly, with groups presenting differences only in creatinine and glucose levels (Table 2).

BM cytology interpretation, based on previous studies [13] and classifications [17,18], revealed that cats from GI, GIII, and GIV presented myelodysplastic syndrome with erythroid predominance (MDS-Er; n = 3), immune-mediated hemolytic anemia (IMHA; n = 3), acute lymphocytic leukemia or leukemic lymphoma (ALL; n = 2), erythroleukemia (AML-M6; n = 1), and hypereosinophilic syndrome (n = 1). Considering mean and median of cellular lineages (Table 3), a difference was observed between GI and GV in the percentage of immature myeloid cells (P = 0.027). Antigenemia was detected in 21/44 cats (47.7 %) and one cat presented a weakly positive result.

3.3. Molecular analysis

According to molecular analysis, FeLV RNA was detected in the serum of 22/44 cats (50 %) and in the BM of 24/44 (54.5 %) cats, whereas FeLV DNA was found in the BM of 34/44 (77.3 %) cats. The viral and proviral loads differed between the groups (Table 4), progressively symptomatic and asymptomatic infected cats showing significantly higher loads. Serum viral loads were higher in GI cats (P = 0.009). Differences were also observed for BM viral loads between GI and GIV and between GIIV and GIV (P = 0.007). BM proviral loads were higher in GI and GIV than in GIIV and GIV (P < 0.001).

GI and GII cats presented antigenemia associated with high serum and BM viral loads and high BM proviral loads. Four cats from GIV were negative for antigenemia with a low viral in serum or BM and with a low BM proviral load. One cat from GIV was weakly positive in the rapid immunoassay test, with low serum and BM viral loads (105.92 and 40.8 copies/mL, respectively) and low BM proviral load (79.15 copies/mL).

Considering all studied cats, a moderate correlation was observed between viral loads in serum and BM (R = 0.574; P = 0.006) and between viral and proviral loads in BM (R = 0.568; P = 0.004). Blood parameters showed a moderately positive correlation between serum and BM viral loads with the WBC count (R = 0.625, P = 0.002 and R = 0.420, P = 0.041, respectively). In biochemical parameters, a moderately negative correlation was found between BM viral and proviral loads and glucose (R = -0.442; P = 0.030 and R = -0.539; P = 0.001, respectively) and between BM proviral load and ALP (R = -0.423; P = 0.013) and albumin (R = -0.466; P = 0.006). Correlation was found between BM proviral load and globulins (R = 0.342; P = 0.047). BM parameters showed a moderately negative correlation between serum viral load and plasma cells (R = -0.468; P = 0.028) and a moderately positive correlation between proviral load and percentage of immature myeloid cells (R = 0.402; P = 0.018).

4. Discussion

Most laboratory and clinical findings on FeLV natural progressive infection are correlated with higher viral and proviral loads in serum and BM. Our study was the first to correlate hematological disorders and BM cytology with FeLV loads. Regressive and progressive infections are a frequent consequence after natural infection of cats with FeLV. We attributed all abnormal hematological findings in peripheral blood to a FeLV natural infection, particularly to a progressive stage. Although not significantly related with infection, nonregenerative anemia and thrombocytopenia, as well as other hematopoietic disorders, are mainly caused by the suppressive effect of the virus on the BM, resulting from infected hematopoietic stem cells and BM stromal cells [8].

First, we suggest that all findings associated with erythroid lineage observed in FeLV-positive cats are multifactorial and related to IMHA, inflammation, and hematopoietic neoplasms [1]. Even though the Coombs test was not performed, four cats from GI, GIII, and GIV, presented hematological manifestations suggestive of IMHA [19]. Non-regenerative to poorly regenerative macrocytic anemia, ineffective erythroid hyperplasia, icteric plasma, dyserythropoiesis, erythropagocytosis, mild plasmacytosis, and iron stores present in BM cytology could be associated with IMHA. According the consensus, the evidence that FeLV infection induces IMHA is low and should prompt further investigation [19]. Nonspecific increase of immunoglobulins, loss of T-cell activity, and antigen antibody complexes can lead to overactive or dysregulated immune response in FeLV infection. These
mechanisms leading to immune-mediated disorders include IMHA [8].

Considering all selected cats, lymphopenia concomitant with lymphoid hypoplasia was not associated with FeLV loads. Most cats from Wallis or ANOVA to parametric values. *Reference values from laboratory.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference*</th>
<th>G1 (symptomatic progressive, n = 10)</th>
<th>G11 (asymptomatic progressive, n = 10)</th>
<th>GII (regressive, n = 9)</th>
<th>GIV (unclassified; n = 5)</th>
<th>GV (control, n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (10^12/L)</td>
<td>6–10</td>
<td>3.8 ± 2.5*b</td>
<td>9.1 ± 1.2*b</td>
<td>8.2 ± 2.3*b</td>
<td>6.5 ± 3.4*b</td>
<td>8 ± 1.39*b</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>8–15</td>
<td>6.1 ± 3.4*b</td>
<td>12.7 ± 1.6*b</td>
<td>11.4 ± 2.7*b</td>
<td>9.6 ± 4.7*b</td>
<td>10.6 ± 1.5*b</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>24–45</td>
<td>19.6 ± 10.3*a</td>
<td>38.6 ± 4.0*a</td>
<td>35.8 ± 7.8*b</td>
<td>30.2 ± 14.5*b</td>
<td>33.8 ± 4.6*b</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>39–55</td>
<td>54.7 (43.1–78.6)*b</td>
<td>43.4 (41.1–48.9)*b</td>
<td>44 (40–60.9)*b</td>
<td>46.1 (44.3–65.4)*b</td>
<td>42.6 (37–48.8)*b</td>
<td>0.002</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>31–35</td>
<td>30.4 ± 1.8</td>
<td>31.9 ± 1.4</td>
<td>31.6 ± 1.1</td>
<td>31.7 ± 1.1</td>
<td>31.4 ± 0.8</td>
<td>0.106</td>
</tr>
<tr>
<td>TPs (g/L)</td>
<td>60–80</td>
<td>70.6 ± 8.1</td>
<td>76.8 ± 10.4</td>
<td>74.7 ± 5.3</td>
<td>70.4 ± 16.3</td>
<td>68.2 ± 4.8</td>
<td>0.221</td>
</tr>
<tr>
<td>Aggregate reticulocytes (10^12/L)</td>
<td>0–0.4</td>
<td>0.35 (0–7.2)</td>
<td>0.2 (0–0.2)</td>
<td>0.2 (0–0.4)</td>
<td>0.1 (0–0.1)</td>
<td>0.2 (0–0.5)</td>
<td>0.169</td>
</tr>
</tbody>
</table>

RBCs - red blood cells; Hb - hemoglobin; Ht - hematocrit; MCV - mean cell volume; MCHC - mean cell hemoglobin concentration; TPs - total plasma proteins; nRBCs - nucleated red blood cells; WBCs - white blood cells. Different letters indicate significantly different between groups, using to Kruskal-Wallis or ANOVA to parametric values. *Reference values from laboratory.

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference*</th>
<th>G1 (symptomatic progressive, n = 10)</th>
<th>G11 (asymptomatic progressive, n = 10)</th>
<th>GII (regressive, n = 9)</th>
<th>GIV (unclassified; n = 5)</th>
<th>GV (control, n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/L)</td>
<td>21–33</td>
<td>28.7 ± 3.3</td>
<td>29 ± 1.7</td>
<td>31 ± 2.1</td>
<td>29.4 ± 7.64</td>
<td>31.3 ± 1.9</td>
<td>0.313</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>&lt; 83</td>
<td>95 (5–2372)</td>
<td>58 (3–78)</td>
<td>26 (0–77)</td>
<td>18 (1–1752)</td>
<td>66 (27–101)</td>
<td>0.333</td>
</tr>
<tr>
<td>GGT (mg/dL)</td>
<td>0.8–1.8</td>
<td>0.9 ± 0.23*a</td>
<td>1.0 ± 0.3*a</td>
<td>0.9 ± 0.25*a</td>
<td>1.4 ± 0.6b</td>
<td>0.9 ± 0.1*a</td>
<td>0.014</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>&lt; 93</td>
<td>34 (0–1262)</td>
<td>35 (0–187)</td>
<td>48.2 (24–244)</td>
<td>42.2 (0–43.6)</td>
<td>57.9 (27.3–141)</td>
<td>0.229</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>73–134</td>
<td>93.8 ± 42.1ab</td>
<td>86.4 ± 12.7a</td>
<td>123.7 ± 27.3ab</td>
<td>140.6 ± 20.9ab</td>
<td>103.4 ± 42.5ab</td>
<td>0.019</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>32–54</td>
<td>52.3 ± 28.9</td>
<td>57.9 ± 11.3</td>
<td>52.3 ± 10.1</td>
<td>52.3 ± 10.1</td>
<td>56.3 ± 11.8</td>
<td>0.943</td>
</tr>
<tr>
<td>TP (g/L)</td>
<td>54–78</td>
<td>71.9 ± 5</td>
<td>77.1 ± 9.1</td>
<td>72.9 ± 5.0</td>
<td>71.4 ± 15.1</td>
<td>74.6 ± 8</td>
<td>0.605</td>
</tr>
<tr>
<td>Globulins (g/L)</td>
<td>27–40</td>
<td>43.2 ± 5</td>
<td>48.1 ± 9.9</td>
<td>41.9 ± 5.8</td>
<td>42 ± 9.6</td>
<td>43.3 ± 8.7</td>
<td>0.404</td>
</tr>
</tbody>
</table>

ALT = alanine aminotransferase; ALP = alkaline phosphatase; TP = total protein. Different letters indicate significantly difference between groups, using to Kruskal-Wallis or ANOVA to parametric values. *Reference values from laboratory.
the presence of the virus in BM, in addition to active integration of the DNA hematopoietic cells.

So far, few studies have assessed the relationship between BM proviral loads, related to FeLV infection and hematological outcome. One study on FeLV naturally infected cats did not identify the presence of regressive infection [25]. Two studies conducted in England and Switzerland reported that 9.3% and 10%, respectively, were positive for DNA proviral load in blood [26,27], but there was no attempt of regressive infection [25]. Two studies conducted in England and Switzerland reported that 9.3% and 10%, respectively, were positive for DNA proviral load in blood [26,27], but there was no attempt of regressive infection [25].


Table 3
Median (range) of proviral loads, viral loads in blood (RNA – SR loads) and bone marrow (RNA – BM loads) per mL of blood or bone marrow and presence of antigenemia and clinical signs of the five groups of cats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference*</th>
<th>GI (symptomatic progressive, n = 10)</th>
<th>GI (asymptomatic progressive, n = 10)</th>
<th>GII (regressive, n = 9)</th>
<th>GIIV (unclassified; n = 5)</th>
<th>GV (control, n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity (%)</td>
<td>25 – 75</td>
<td>87.5 (50 – 95)</td>
<td>75 (0 – 75)</td>
<td>75 (25 – 95)</td>
<td>50 (0 – 85)</td>
<td>50 (50 – 80)</td>
<td>0.052</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>25 – 75</td>
<td>12.5 (5 – 50)</td>
<td>25 (0 – 50)</td>
<td>25 (5 – 75)</td>
<td>50 (0 – 56)</td>
<td>50 (20 – 50)</td>
<td>0.090</td>
</tr>
<tr>
<td>Megakaryocytes (field/10x)</td>
<td>&gt; 5</td>
<td>2.2 ± 2.2</td>
<td>2.8 ± 1.6</td>
<td>2.6 ± 2.5</td>
<td>1.9 ± 1.4</td>
<td>2.9 ± 1.8</td>
<td>0.844</td>
</tr>
<tr>
<td>M&amp;E ratio</td>
<td>1.21 – 2.16</td>
<td>0.91 ± 0.8</td>
<td>1.1 ± 0.5</td>
<td>0.9 ± 0.6</td>
<td>0.7 ± 0.4</td>
<td>0.8 ± 0.5</td>
<td>0.642</td>
</tr>
<tr>
<td>Immature erythroid cells (%)</td>
<td>0 – 2.4</td>
<td>3.9 (0 – 14)</td>
<td>2.7 (0 – 5.7)</td>
<td>2 (0.7 – 4.7)</td>
<td>2 (0 – 11.7)</td>
<td>1.8 (0.3 – 7.6)</td>
<td>0.274</td>
</tr>
<tr>
<td>Mature erythroid cells (%)</td>
<td>11.2 – 39.8</td>
<td>35.3 ± 21.5</td>
<td>34.7 ± 14.2</td>
<td>47.4 ± 16.9</td>
<td>35 ± 20.6</td>
<td>51.7 ± 14.4</td>
<td>0.117</td>
</tr>
<tr>
<td>Immature myeloid cells (%)</td>
<td>0 – 3.4</td>
<td>3.2 ± 2.2b</td>
<td>3.8 ± 2.9a</td>
<td>1.7 ± 0.9b</td>
<td>2 ± 1.2b</td>
<td>1.4 ± 0.4b</td>
<td>0.027</td>
</tr>
<tr>
<td>Mature myeloid cells (%)</td>
<td>24.6 – 59.8</td>
<td>28.4 ± 19.0</td>
<td>40.9 ± 15.8</td>
<td>37.4 ± 16.5</td>
<td>29.6 ± 17.5</td>
<td>34.6 ± 12.3</td>
<td>0.459</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>11.6 – 21.6</td>
<td>8.5 (2.5 – 90)</td>
<td>4.2 (0 – 10.4)</td>
<td>8 (3.1 – 21.2)</td>
<td>7.3 (0 – 10)</td>
<td>5.4 (1.7 – 22.6)</td>
<td>0.312</td>
</tr>
<tr>
<td>Plasma cells (%)</td>
<td>0.2 – 1.8</td>
<td>0.4 (0 – 5.4)</td>
<td>1.1 (0 – 4.9)</td>
<td>0.2 (0 – 3.5)</td>
<td>1.6 (0 – 2.7)</td>
<td>1 (0.2 – 2)</td>
<td>0.239</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>0 – 0.2</td>
<td>0.1 (0 – 0.5)</td>
<td>0.3 (0 – 0.8)</td>
<td>0.2 (0 – 0.4)</td>
<td>0.6 (0 – 0.8)</td>
<td>0.2 (0 – 1)</td>
<td>0.226</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.2 – 1.6</td>
<td>0.9 ± 0.6</td>
<td>0.9 ± 0.6</td>
<td>0.9 ± 0.6</td>
<td>1.1 ± 0.9</td>
<td>0.6 ± 0.3</td>
<td>0.413</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.8</td>
<td>0.239</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>0.8</td>
<td>0.239</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


The quantitative results of viral loads found in blood were similar to those reported by previous studies for FIV-positive cats [31,32]. Clinically sick animals older than 5 years with hematopoietic disorders...
presented plasma viral loads significantly higher than those observed in healthy cats with no clinical signs [31], while viral loads increased as the disease progressed [32]. Which means that the immune system was unable to contain proviral replication. In one study that monitored viral and proviral loads in the tissues of cats experimentally infected with FeLV, the highest values were observed in cats with viremia, followed by healthy cats, and the lowest proviral loads were observed in regressive cats [33]. But no studies on the viral and proviral loads of cats naturally infected with FeLV have assessed the progress of the disease or of its symptoms. It has been recently reported that antigenemia is correlated with proviral loads in blood [34], but the relationship with clinical and hematological symptoms is still unknown.

A limitation of this study was the low adherence of cat owners to BM cytology, because of the risk of anesthesia among cats with poor clinical and hematological symptoms is still unknown.

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