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Efficacy of chemotherapy and palliative hypofractionated radiotherapy for cats with nasal lymphoma

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ABSTRACT. Nasal lymphoma (NL) is the most common nasal tumor in cats, and radiotherapy, chemotherapy, or a combination of these treatments have been described as the treatment for this disease. However, the previous studies included various machines and protocols of radiotherapy. Therefore, we aimed to retrospectively compare the prognosis among cases treated with palliative hypofractionated radiotherapy, chemotherapy, and a combination of them with united machine and protocol of radiotherapy. When compared overall survival and progression free survival, there was no significant difference among these three groups. The data of this study suggested that similar efficacy could be achieved by palliative hypofractionated radiotherapy, chemotherapy, or a combination of them.

KEY WORDS: cat, chemotherapy, nasal lymphoma, prognosis, radiotherapy

Nasal lymphoma (NL) is the most common nasal tumor in cats [1, 15]. The cats with NL present respiratory clinical signs including nasal discharge, sneezing, epistaxis and dyspnea [4, 9] as well as facial deformities, anorexia and buphthalmos [7, 17]. Histologically, NL in cats is usually high-grade, and 61–71% of feline NL are B cell lymphoma [8, 15]. Prognosis of feline NL is better than lymphomas of other anatomical locations [8, 16] and it has good chance of long-time remission [14]. In a previous report, anorexia, anemia, and destruction of cribriform plate before treatment were identified as negative prognostic factors in cats with NL [7, 17].

There are three options of treatment for feline NL; radiotherapy, chemotherapy, or a combination of these treatments. Lymphoma is sensitive to chemotherapy, and response rate of feline NL to chemotherapy is reported to be 67–73% [7, 19] and median survival time is 116–358 days when treated with chemotherapy alone [7, 19, 20]. Feline lymphoma is also sensitive to radiotherapy [2, 3]. Radiotherapy is suitable to treat feline NL with localized lesion. In fact, cats with NL treated with radiotherapy seem to have favorable prognosis and median survival time was reported to be 456–922 days [7, 10, 18]. In cats with NL that received a combination of radiotherapy and chemotherapy, median survival time was reported to be 174–955 days [7, 17]. Although a previous study has reported that cats with NL treated with radiotherapy seemed to have longer survival time, no significant difference in survival has been found when efficacies were compared among the three treatments [7]. However, the machines and protocol of radiotherapy varied among cases in the previous study.

Hypofractionated radiotherapy is used as palliative treatment, while multifractionated one is used as definitive treatment. Multifractionated radiation is considered to be the standard protocol in veterinary medicine [6]. However, some owners select hypofractionated radiotherapy because of cost and burden of visit to the veterinary hospitals. In dogs, hypofractionated radiotherapy is reported to be a viable option for the treatment of nasal tumors that are not candidates for conventional multifractionated radiotherapy [5].

The aim of this study was to compare the prognosis among cats with NL treated by palliative hypofractionated radiotherapy with

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united machine and protocol, chemotherapy, and combination of them.

Medical records of cats diagnosed with NL and treated in the Veterinary Medical Center of University of Tokyo or Veterinary Medical Teaching Hospital of Nippon Veterinary and Life Science University from 2006 to 2017 were retrospectively reviewed. The diagnosis of lymphoma was based on histological evaluation or cytologic evaluation. Cats were excluded if they received radiotherapy with protocols other than hypofractionated protocol using mega-voltage radiation machine as describe below.

Treatment planning of radiotherapy was constructed based on X-ray CT images using 3D treatment planning software (XiO, CMS Japan, Tokyo, Japan). Systemic X-ray CT was performed in all cases to evaluate thoracic and abdominal metastasis. Radiotherapy was weekly schedule using a 4 MV X-ray linear accelerator (Primus, Canon Medical Systems, Otawara, Japan) on cats with NL. The planning target volume (PTV) was defined as the region 0.3–0.5 cm outside the gross tumor volume (GTV). Dose–volume histograms were calculated to confirm coverage of over 90% of the GTV and over 80% of the PTV. Throughout all processes, cats were anaesthetized and positioned prone.

Information extracted from medical records included signalment, clinical signs at presentation, complete blood count (CBC), infection status of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV), dose of fraction, fraction times, and medication. Immunophenotype of tumor cells were determined based on the result of immunohistochemistry and PCR amplification test for antigen receptor gene rearrangement (PARR) when available. PARR was performed as previously described [11, 12]. Immunohistochemistry was performed using antibodies against CD20 (polyclonal rabbit anti-human RB-9013-P; Thermo Fisher Scientific, Waltham, MA, USA) and CD3 (polyclonal rabbit anti-human A0452; DAKO, Glostrup, Denmark). Clinical stage was determined based on systemic CT examination or thoracic X-ray and abdominal ultrasound examinations according to clinical staging system [13, 14]. Response to the treatment was subjectively determined by the veterinarians based on the clinical examination and clinical signs. Progression of the disease was determined by the information in the medical record including physical examination, recurrence of clinical signs and diagnostic imaging. Progression free survival (PFS) was defined as the duration from the documentation of response to the date of progression. Date of death was extracted from the medical record or reported by the primary care veterinarians, and overall survival (OS) was defined as the duration from the date of diagnosis to the date of death from any cause.

For the comparisons of the efficacies among treatments, the cats with NL were divided into three groups; cats treated with radiotherapy alone (Group R), those treated with chemotherapy alone (Group C), and those received both radiotherapy and chemotherapy simultaneously or separately (Group RC). In addition, cats were also divided into three groups based on the treatment that they received until the recurrence of NL; cats who recurred after initial treatment with radiotherapy alone (Group rR), those recurred after initial treatment with chemotherapy alone (Group rC), and those that received both radiotherapy and chemotherapy before recurrence (Group rRC), and PFS was compared among Group rR, rC and rRC.

Survival probabilities were estimated using Kaplan–Meier product limit method. Log-rank test was used for comparison of OS and PFS among the three groups. In addition, univariate analysis by Log-rank test was conducted to determine whether the presence of anemia (PCV <35%), clinical stages, not receiving radiotherapy, and not receiving chemotherapy influenced overall survival, and a forced entry Cox proportional hazards model was developed to assess the independent contributions of these variables. In cats treated with radiotherapy, univariate analysis by Log-rank test was conducted to investigate whether cats receiving radiotherapy with total dose of >32 Gy had longer OS. Fisher's exact test was used to compare the proportion of the cases that died of lymphoma among the groups. A value of $P<0.05$ was regarded to be significant in all statistical tests. Data were analyzed using commercially available statistics software (JMP, version 4, The Statistical Discovery Software, SAS Campus Drive, Cary, NC, USA).

Fifty-five cats were included in this study. The median age and body weight were 9.3 years (range, 1.9–17.8 years) and 4.0 kg (range, 2.0–8.8 kg), respectively. Twenty-one cats were castrated males, 3 were intact females, and 31 were spayed females. There were 47 mixed breed cats, 5 Persian, and 1 each of American Shorthair, Russian Blue, and Abyssinian. FeLV antigen was positive for 2 of 38 tested cats (5%), and FIV antibody was positive for none of the 38 tested cats. Fifty cats (91%) presented one or more respiratory clinical signs including nasal discharge (*n*=48, 87%), sneezing (*n*=27, 49%), dyspnea (*n*=24, 44%), epistaxis (*n*=23, 42%), snoring (*n*=7, 13%) and coughing (*n*=1, 1.8%). Cats also showed other clinical signs: anorexia (*n*=36, 65%), facial deformities (*n*=29, 53%) and eye mucus or lacrimation (*n*=24, 44%). CT was performed in 47 (85%) cats. Of these, 5 cats also underwent MRI. In those cats who underwent CT or MRI, 30 (64%) cats showed bony lysis. The information of CBC was obtained from 54 cats, and anemia was identified in 18 cats (33%). Clinical stage and substage were Ib in 49 cats (89%), IIIb in 3 cats (5%), IVb in 1 cat (2%), and Vb in 2 cats (4%).

Immunohistochemical staining using antibodies against CD20 and CD3 was performed in 25 and 7 cats, respectively. Consequently, CD20 was positive in 25 cats (100%) and CD3 was positive in 1 cat (14%). The cat positive for CD3 was also positive for CD20. In the PARR analysis, clonal rearrangement of *IgH* (*immunoglobulin heavy chain*) gene was detected in 15 of 19 tested cats (79%), and clonal rearrangement of *TCRγ* (*T-cell receptor gamma chain*) gene was detected in none of 11 tested cats.

Based on treatments, 55 cats were divided into Group R (*n*=13), Group C (*n*=18), and Group RC (*n*=24). In Group RC, two cats were treated with chemotherapy and radiotherapy simultaneously and 22 cats received both treatments separately. In cats that received radiotherapy, the median dose of radiation was 8 Gy (range, 4–10 Gy), and median total dose was 32 Gy (range, 6–50 Gy) with 4 (range, 2–6) fractions. In cats that received chemotherapy, L-CHOP-based or COP-based protocol was used in 35 of 42 cats (83%) and lomustine was used in 1 cat (2%). Among 35 cats that received L-CHOP-based or COP-based protocol, 3 cats completed the planned protocol, but other 32 cats could not. Detailed information of chemotherapeutic agents could not be obtained in 6 cats.

Of the 55 cats, 20 were censored from the investigation of prognosis because it was difficult to follow-up, and the median OS for all 55 cats was 154 days (range, 6–3,730 days). The median OS was 1,013 days (range, 12–3,080 days) in Group R, 80 days

(range, 6–3,730 days) in Group C, and 160 days (range, 21–1,939 days) in Group RC. There was no significant difference in OS among these three groups (Fig. 1A, P=0.09). There was also no significant difference in OS among these groups when cats with stage II–V NL were excluded (Fig. 1B, P=0.23). In Group R, 4 of 13 cats died of lymphoma and 4 cats died of other or unknown causes. In Group C, 9 of 18 cats died of lymphoma and the causes of death were unclear in 3 cats. In Group RC, 10 of 24 cats died of lymphoma and 6 cats died of other or unknown causes. There was no significant difference in the proportion of the cases that died of lymphoma among the three groups (P=0.68). In the univariable analysis, it was suggested that OS of cats that received radiotherapy was significantly longer than those of cats that did not received radiotherapy (Table 1, P=0.045). In cats treated with radiotherapy, OS of cats treated with total dose of >32 Gy tended to be longer than those of ≤32 Gy (375 and 157.5 days, respectively), although it is not statistically significant (P=0.10). In the multivariate analysis with the Cox's proportional hazards model, no factor was determined as a significant prognostic factor (Table 2).

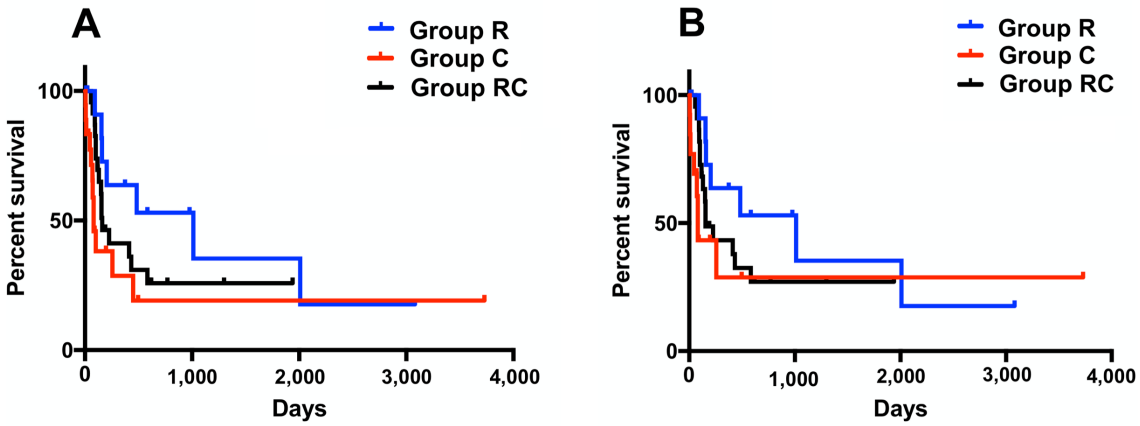

Fig. 1. (A) Kaplan-Meier curves showing the difference in overall survival (OS) among Group R (Blue line), Group C (Red line), and Group RC (Black line). There was no significant difference among these groups (P=0.09). (B) Kaplan-Meier curves showing the difference in OS among these groups excluding cats with nasal lymphoma (NL) of stage II–V. There was no significant difference among these groups (P=0.23).

Table 1. Variabes included in the univariate analysis with Log-rank test.

	Risk factor (downside)	Median OS (days)	HR	95% CI	P value
Radiotherapy	Radiotherapy	160	0.42	2.32–3.31	0.046
	No radiotherapy	76.5			
Chemotherapy	Chmotherapy	154	1.81	–0.28–0.59	0.106
	No chemotherapy	1,013			
Clinical stage	Ib	155	0.30	1.61–2.39	0.173
	Others	86			
Anemia	PCV ≥35	155	1.07	0.19–1.17	0.854
	PCV <35	115.5			

OS, overall survival; HR, hazard ratio; CI, confidence interval.

Table 2. Variabes included in the multivariate analysis with the Cox propotional hazard model

	Risk factor (downside)	Median OS (days)	HR	95% CI	P value
Radiotherapy	Radiotherapy	160	1.49	0.61–3.63	0.37
	No radiotherapy	76.5			
Chemotherapy	Chemotherapy	154	0.61	0.22–1.51	0.29
	No chemotherapy	1,013			
Clinical stage	Ib	155	1.62	0.51–4.30	0.39
	Others	86			
Anemia	PCV ≥35	155	1.12	0.49–2.46	0.78
	PCV <35	115.5			

OS, overall survival; HR, hazard ratio; CI, confidence interval.

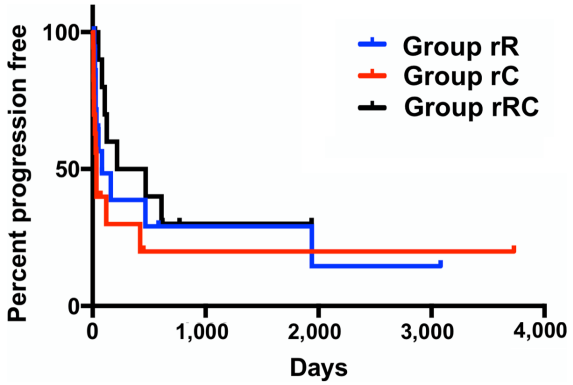

Fig. 2. Kaplan-Meier curves showing the difference in progression free survival (PFS) among Group rR (Blue line), Group rC (Red line), and Group rRC (Black line). There was no significant difference among these groups (P=0.17).

PFS could be calculated in 45 of 55 cats in this study. Based on treatment until the recurrence of NL, cats were divided into Group rR (n=16), Group rC (n=18), and Group rRC (n=11). In Group rRC, all cats received radiotherapy at first and achieved remission, and then received chemotherapy before recurrence. The median PFS was 84 days (range, 12–3,080 days) in Group rR, 34 days (range, 6–3,730 days) in Group rC, and 342.5 days (range, 14–1,939 days) in Group rRC. There was no significant difference in PFS among these groups (Fig. 2, P=0.17).

In this study, we compared efficacy among palliative hypofractionated radiotherapy with united machine and protocol, chemotherapy, and a combination of them.

In the investigations of OS, no significant difference was observed among cats that received radiotherapy, chemotherapy, or a combination of these treatments. Although the investigations of OS were also performed in cats with stage I, there was no significant difference in OS among the three groups. These results are consistent with a previous report including cats treated with multifractionated radiotherapy [7]. However, this previous study and the present study showed that cats treated by radiotherapy tended to have longer OS compared with other treatments. It seems to be due to the long-term survival of a part of cats treated with radiotherapy. Actually, 9 of 13 cats in Group R lived longer than 1 year in the present study. It might be also one of the causes of this result that 16 of 20 cats with NL that relapsed after radiotherapy were treated with additional chemotherapy and included in Group RC in the present study. A previous study revealed that half of the cats with sinonasal lymphoma treated with radiotherapy and died of relapse/progression died within 6 months of treatment [10], suggesting that cases with relapse/progression after radiotherapy had poor prognosis and such cases were included in Group RC in the present study.

In the investigations of PFS, cats with NL were divided into three groups based on treatment until the recurrence of NL. In Group rRC, all cats received radiotherapy at first and achieved remission, and then received chemotherapy before recurrence. As a result, no significant difference was observed among Group rC, rR and rRC. This result suggested that additional treatment with chemotherapy for cases that were in remission induced by radiotherapy might not extend PFS. However, there was a tendency that PFS of cats in Group rRC was longer than those in the other two groups. It is possible that small number of the cases in the present study may have influenced the results, and further studies are needed.

A protocol of radiotherapy was limited to hypofractionated palliative one in the present study. The data of this study suggested that hypofractionated protocol could have similar efficacy to chemotherapy in terms of OS and PFS. A previous study also suggested the possible efficacy of hypofractionated palliative radiotherapy for feline nasal tumor including lymphoma [4]. However, it is possible that multifractionated radiotherapy [7, 17] might be more effective for feline NL than hypofractionated palliative radiotherapy and chemotherapy. Thus, prospective study is needed to compare the efficacy among multifractionated radiotherapy, hypofractionated radiotherapy, and chemotherapy.

Anemia, anorexia, destruction of cribriform plate were reported as negative prognostic factors [7, 17], and complete response to the treatment and total radiation dose >32Gy were reported as positive prognostic factors [7] in feline NL. In the present study, use of radiotherapy was significantly associated longer overall survival in univariate analysis. However, no significant prognostic factor was indicated in the multivariate analysis when examined clinical stage, presence of anemia, not receiving radiotherapy, and not receiving chemotherapy. This result suggests the existence of confounding factor in the results of univariate analysis, and this result might be also attributed to the problem that other clinical symptoms and response to treatment could not be examined in the present study due to the lack of enough information in medical records. In cats treated with radiotherapy, OS of cats treated with total dose of >32 Gy tended to be longer than those of ≤32 Gy in the present study, although it is not statistically significant. It is possible that the number of cases was not enough to detect statistically significant difference in the present study.

There were several limitations in this study. The number of cases was relatively small, which might affect the results of statistical analysis. It is also limitation that chemotherapy protocol chemotherapy varied among cases. The difference of the chemotherapy may have influenced the OS and PFS, and further investigations should be performed with united protocol of chemotherapy. In addition, lack of enough information in medical records made it difficult to examine some candidate prognostic factors and to

investigate adverse effects of chemotherapy and radiotherapy.

In conclusion, this study suggested that there might be no significant difference in OS and PFS among cats with NL that received hypofractionated palliative radiotherapy, chemotherapy, or a combination of these treatments. Although it was shown in univariate analysis that OS of cats that received radiotherapy was significantly longer than those of cats that did not, no significant prognostic factor was indicated in multivariate analysis. Therefore, it was unclear which treatment was appropriate in the present study, and further studies are needed to compare the efficacy among multifractionated radiotherapy, hypofractionated radiotherapy, and chemotherapy for feline NL.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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