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# Influence of chemotherapy for lymphoma in canine parvovirus DNA distribution and specific humoral immunity

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ABSTRACT

In man, the combination of cancer and its treatment increases patients' susceptibility to opportunistic infections, due to immune system impairment. In veterinary medicine little information is available concerning this issue. In order to evaluate if a similar dysfunction is induced in small animals undergoing chemotherapy, we assessed the complete blood count, leukocytic, plasma and fecal canine parvovirus (CPV) viral load, and anti-CPV protective antibody titers, in dogs with lymphoma treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) protocol, before and during chemotherapy.

There was no evidence of decreased immune response, either at admission or after two chemotherapy cycles, indicating that the previously established immunity against CPV was not significantly impaired, supporting the idea that immunosuppression as a result of hematopoietic neoplasms and their treatment in dogs requires further investigation and conclusions cannot be extrapolated from human literature.



## 1. Introduction

Improving nutrition and vaccination programs in veterinary medicine, as well as the development of new diagnostic and treatment techniques, have increased the life expectancy of our patients. In contrast, there is a greater probability of developing geriatric diseases, such as cancer.

Lymphoma is one of the most common cancers in dogs, representing the most frequent hematopoietic cancer in this species [1]. The incidence of canine non-Hodgkin lymphoma has been increasing over the years, and is now similar to human incidence [1,2].

Eighty percent of dogs with lymphoma develop the multicentric form, which is typically characterized by a generalized painless lymphadenopathy.

Chemotherapy is the treatment of choice for systemic malignancies [4]. In general, combination chemotherapy protocols have a higher efficacy than single agent protocols and, regarding lymphoma, the most frequently used in veterinary medicine are modifications of CHOP protocols, a combination of cyclophosphamide (C), doxorubicin (H – hydroxydaunorubicin), vincristine (O – Oncovin®) and prednisone/prednisolone (P), originally developed for the treatment of human lymphoma. The original protocol induces a complete remission in approximately 60–90% of dogs with median survival times of 6–12 months and is

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considered the standard treatment for canine lymphoma [5,6].

Most chemotherapeutic agents and protocols used in veterinary oncology are well tolerated by companion animals, resulting in less than a 5% hospitalization rate for chemotherapy toxicity and less than a 1% mortality rate directly caused by toxicity [7]. However, these drugs may cause chronic or acute side effects, especially in organs with actively dividing cells, like the bone marrow and the gastrointestinal (GI) tract [8].

Cancer and its treatments are well known immunosuppressive factors in human medicine [9,10]. This combination, along with the frequent exposure to risk environments, puts oncological patients at risk of developing severe opportunistic infections, as well as the reactivation of latent or vaccine-preventable agents and a decreased immune response after vaccination [11–14].

Contrary to human medicine, the existing knowledge regarding immunosuppression in tumor-bearing dogs remains scarce and controversial. Moreover, considering that most clinically relevant agents, such as canine parvovirus (CPV) are species specific, it would not be appropriate to extrapolate this information from human literature [15]. Thus, a better understanding of the effect of cancer and its treatment on veterinary patients' immune system is required.

Ultimately, it is important to assess whether these animals retain the ability to mount an adequate immune response against new pathogens or against the reactivation of vaccine-preventable diseases or whether, by contrast, these patients are at the same risk as unvaccinated animals. If properly vaccinated animals are not able to maintain protective antibody (Ab) titers after chemotherapy, it would also be important to prevent the spread of contagious agents and zoonotic infections.

CPV is a suitable viral model for this study as its infection can be prevented by vaccination, allowing protective anti-CPV Ab titers to be present at baseline, thus enabling the evaluation of titer variation as a result of chemotherapy. Also, parvoviruses may induce persistent and asymptomatic infections [16] and CPV is able to induce subclinical infections in immune competent adult dogs [17].

To assess the systemic effects of a potentially immunosuppressive cancer such as lymphoma, as well as the effects of chemotherapy on viral humoral immunity, we performed a prospective study in dogs with lymphoma, receiving CHOP-based chemotherapy. These dogs were evaluated regarding CPV DNA distribution and anti-CPV Ab titers. The initial viral load and Ab titers of dogs with lymphoma were compared with those of healthy dogs to determine whether the ongoing cancer would affect their immune status.

## 2. Materials and methods

### 2.1. Inclusion criteria

Dogs of any breed, gender or age with cytologically or histopathologically confirmed lymphoma were included in the study. Cancer patients should also have been vaccinated against canine parvovirus with a valid protocol. Dogs previously submitted to chemotherapy or any immunomodulatory treatments within 2 weeks of CHOP-chemotherapy initiation were excluded. Therefore, 8 client-owned dogs admitted to the Faculty of Veterinary Medicine Teaching Hospital, University of Lisbon and to Oncovet for initial chemotherapeutic treatment of lymphoma were included. All 8 dogs were treated with CHOP protocol [5,6] between April and July 2012. For the control group, 8 age/breed-matched healthy dogs, also correctly vaccinated against CPV, were included. None of the dogs from both groups were vaccinated during the study.

In order to profile CPV distribution, a third group of 8 naturally infected, symptomatic dogs were included.

The study was approved by the Faculty of Veterinary Medicine, University of Lisbon Ethics and Animal Welfare Committee, and informed owner consent was obtained prior to study enrollment.

### 2.2. Sample collection

Blood and rectal swabs were collected from the lymphoma group prior to CHOP chemotherapy initiation and during the protocol first two cycles (weeks 3, 6 and 9). Blood sampling was part of routine scheduled complete blood count (CBC) before each chemotherapy session. A total of 3 mL of whole blood were collected into EDTA-containing tubes at each time point; 1 mL was used for complete blood count (CBC) and 2 mL were used to leukocytic and plasma viral load, as well as specific anti-CPV protective Ab titers determination. Rectal swabs were used for fecal CPV viral load evaluation. In the control group, samples were obtained at the same time points. In the CPV group, samples were collected once. All samples were kept at 4 °C and processed within 24 h after collection.

### 2.3. CPV molecular and serological diagnosis

Plasma was obtained from whole blood by centrifugation at $5000 \times g/10$ min and stored at −80 °C until processing. The cellular portion was homogenized with an erythrocyte lysis buffer (Buffer EL, Qiagen®, Germany) and the white cell pellet was homogenized with 200 μL of PBS, according to the manufacturer's instructions. Rectal swabs were homogenized with 300 μL of PBS and stored at −80 °C until DNA extraction.

### 2.4. DNA extraction and quantification

The commercial kit DNeasy Blood & Tissue® (Qiagen®, Germany) was used for total and viral DNA extraction from the plasma and white blood cells, according to the manufacturer's instructions. After DNA extraction and quantification in a 2000c Nanodrop Spectrophotometer® (Thermo Fisher Scientific), nucleic acid samples were stored at −80 °C.

### 2.5. Viral load detection and quantification

Leukocytic, plasma and fecal CPV viral load (molecules/μL) determination was performed by real