Pertanika J. Trop. Agric. Sci. 45 (3): 711 - 729 (2022)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Case Study

Radiographic, Computed Tomographic, and Cellular Phenotypic Features of Primary Nasal Transmissible Venereal Tumors in Four Dogs

Muhammad Waseem Aslam, Seng Fong Lau*, Puteri Azaziah Megat Abdul Rani and Ikhwan Saufi Ahamad Azahari

Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), 43400 UPM, Serdang, Selangor, Malaysia

ABSTRACT

The primary nasal canine transmissible venereal tumor (CTVT) is a rare disease that develops by the allografted transmission of neoplastic cells in the nasal cavity. The disease is uncommonly reported in free-roaming dogs, with the social behavior of excessive licking and vigorously sniffing the affected parts of the other dogs in an endemic population. Post-chemotherapeutic computed tomography (CT) scan features and correlation of vincristine sulfate with cellular phenotypes have been scarcely reported in previously available primary nasal CTVT studies. This study describes the radiographic, computed tomographic, and cellular phenotypic features in four dogs affected with stage-4 intranasal CTVTs. The post-chemotherapeutic features of the nasal cavity in fully recovered cases are also highlighted. All data were analyzed retrospectively. All four dogs had stage 4 modified Adam's staging for nasal tumors due to the complete or partial lysis of the cribriform plate and lymphocytoid plasmacytoid (mixed) phenotype of the neoplastic cells based on the cellularity of cytological samples. All four dogs responded well to five cycles of vincristine sulfate and recovered completely. Two out of four dogs have follow-up scanning after chemotherapy. Based on the present study results, vincristine sulfate is still an effective monotherapy to achieve full recovery, although the number of cycles can vary, possibly depending on the expressed phenotype. Permanent loss of nasal turbinates is the sequelae of therapeutic chemotherapy. Prognosis is not correlated to the staging system but seems good with vincristine sulfate in mixed phenotype cases.

Keywords: Canine transmissible venereal tumor, cellular phenotype, computed tomography, modified Adam's staging, vincristine sulfate

ARTICLE INFO

Article history:
Received: 23 January 2022
Accepted: 20 April 2022
Published: 28 July 2022

DOI: https://doi.org/10.47836/pjtas.45.3.11

E-mail addresses:
dr.waseemaslam@gmail.com (Muhammad Waseem Aslam)
lausengfong@upm.edu.my (Seng Fong Lau)
azaziah@upm.edu.my (Puteri Azaziah Megat Abdul Rani)
ikhwans.azahari@upm.edu.my (Ikhwan Saufi Ahamad Azahari)
*Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

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INTRODUCTION

The canine transmissible venereal tumor (CTVT) is also known as sticker's sarcoma, transvenereal sarcoma, infectious sarcoma, canine condyloma, transmissible lymphosarcoma, venereal granuloma, and contagious venereal tumor (Das & Das, 2000; Nemzek et al., 2015; Thamm, 2007). Despite immense mutation, CTVT adapted, survived, and spread across multiple continents, making it the oldest known continuously passaged somatic cell line (Nemzek et al., 2015). CTVTs are distributed globally, predominantly in tropical and subtropical regions. It is a naturally occurring, horizontally transmitted, parasitic-like, infectious neoplasia of dogs (Ganguly et al., 2013; Strakova & Murchison, 2014; Thamm, 2007; Ujvari et al., 2017). Due to the uncertainty of its origin, previous studies have described it as lymphatic, reticuloendothelial, macrophage or myeloid, and histiocytic origin tumors. Other than this ambiguity, it is considered one of the dogs' most common round cell tumors (Albanese et al., 2002; Gimeno et al., 1995; Mukaratirwa & Gruys, 2003). The most common transmission mode is coitus, although it can spread through biting, licking, and sniffing the neoplastic sites of the dog's body (Conte et al., 2022; Das & Das, 2000; Ganguly et al., 2013; Thamm, 2007).

Exfoliation of living neoplastic cells and allogenic transplantation across abraded mucosa are mandatory for the development of genital or extragenital TVT (Nemzek et al., 2015; Strakova & Murchison, 2014). Studies on experimental transplantation stated three distinct phases of CTVT growth: (1) progressive, (2) static, and (3) regressive phases (Mukaratirwa & Gruys, 2003). CTVT cells avoid detection by the immune system due to the downregulation of the major histocompatibility complex I (MHC-I) via secretion of inhibitory cytokines such as transforming growth factor-beta 1 (TGFβ-1) and interleukin 6 (IL-6) molecules, and no MHC-II activity (Foster, 2016). Little MHC class I or II expression, was detected in an experimental model during the initial proliferative phase of tumor growth. However, at 12 weeks, MHC expression increased remarkably, further stimulated by lymphocytes, resulting in tumor regression (Foster, 2016). In previous studies, spontaneous regression due to the immune response (immunoglobulin G formation, lymphocyte-mediated cytotoxicity) has been documented at the tumor age of 2 to 9 months (Das & Das, 2000; Foster, 2017a; Nemzek et al., 2015; Thamm, 2007; Ujvari et al., 2017).

In the past, some studies over-represented the male or female populations. Nonetheless, in recent case reports and a survey study conducted on a larger scale, gender bias was not observed (Kabuusu et al., 2010; Strakova & Murchison, 2014). In addition, adult and intact dogs of reproductive age (2 to 8 years) are more prone to this disease, although there are some reports on neutered and 11 years old dogs (Ganguly et al., 2013; Kabuusu et al., 2010; Rogers et al., 1998; Thamm, 2007).

Extragenital primary sites have been documented for CTVT in previous studies. The nasal cavity is the second most common site (5% to 13% of all CTVT cases), followed by the skin, oral cavity, eyes, and rectum. Additionally, it is over-represented in adult male dogs (Brandão et al., 2002; Ganguly et al., 2013; Ojeda et al., 2018; Rogers et al., 1998; Thamm, 2007). The most common historical findings associated with the nasal form of CTVT are sneezing, snoring, inspiratory dyspnea, bilateral epistaxis, sanguinopurulent nasal discharge, submandibular/mandibular lymphadenopathy, nasal deformation, and soft fleshy swelling at the level of the nasal bone area (Levy et al., 2006; Ojeda et al., 2018; Rezaei et al., 2016; Sukhbir & Sood, 2016; Veloso et al., 2018).

Grossly, the tumor can be soft to firm in consistency and single or multinodular, sessile, or pedunculated, with or without ulcerative surface in both genital and extragenital form. Tumor size can be up to 15 cm in diameter in the genital form of the disease (Foster, 2017a, 2017b). Microscopically, plasmacytoid, lymphocytoid, and mixed phenotypes have been described in detail in previous studies (do Amaral et al., 2007; Setthawongsin et al., 2017). Generally, it is a large but uniform and round to ovoid or polyhedral-shaped cell tumor resembling lymphocytes, and its cytoplasm is pale blue with distinct peripheral cytoplasmic vacuolization. Binucleation, mitotic figures, and single or multiple nucleoli are often observed in cytology samples (Foster, 2016, 2017a; Thamm, 2007). A definitive diagnosis can be made based on the cytological findings of sterile cotton swab samples, impression smear, fine-needle aspirated samples, and histopathology of the biopsy tissue. Polymerase chain reaction (PCR) of the rearranged long interspersed nuclear element (LINE)-1/c-myc gene can be a suitable option in cases where the definitive diagnosis is difficult based on cytology or histopathology results (Ojeda et al., 2018; Thamm, 2007).

Cytological features of the tumor are uniform discrete round to polyhedral-shaped cells with moderately abundant pale blue cytoplasm and an eccentrically located nucleus, with occasional binucleation and mitotic figures. The most characteristic feature is the presence of numerous discrete clear cytoplasmic vacuoles.

Histopathology of TVT reveals compact masses of round or polyhedral cells with slightly granular, vacuolated, and eosinophilic cytoplasm. The neoplastic cells are arranged in a diffused pattern and supported by a thin trabecula of fibrovascular tissue (Thamm, 2007).

Radiographs are helpful for the observation of nasal deformities, including soft tissue or fluid opacity (radiopaque) of the nasal cavity and frontal sinuses (Levy et al., 2006; Rezaei et al., 2016; Sukhbir & Sood, 2016). A computed tomography scan is currently the best available supportive modality to see the aggressiveness of the nasal cavity and frontal sinuses changes, including bone lysis and metastasis in neighboring areas, before

and after treatment (Patsikas et al., 2018). Additionally, it is quite useful to perform CTVT staging by a modified Adam's staging system (Ojeda et al., 2018). Chemotherapy with vincristine or doxorubicin is the most common treatment used and suggested in previous studies of dogs diagnosed with the nasal form of the disease (Levy et al., 2006; Ojeda et al., 2018; Rezaei et al., 2016; Sukhbir & Sood, 2016). A fatal outcome and high metastasis risk (up to 5% in routine cases of CTVT) are documented in puppies and immunosuppressed adult dogs (Foster, 2016; Mukaratirwa & Gruys, 2003).

The present report describes the radiological, particularly computed tomographic, features of the disease in the canine nasal cavity during pre-and post-treatment phases. These features are rarely described in detail with the staging system, and information started emerging in recent publications with a limited number of cases from other parts of the world.

MATERIAL AND METHODS

The retrospective data of four dogs definitively diagnosed with primary intranasal CTVT at the University Veterinary Hospital (UVH) of Universiti Putra Malaysia were analyzed for history, diagnostics, clinical findings, and treatments related to the outcome. In addition, telephonic interviews were conducted with the owners regarding the current status of the dogs and any further history of tumor recurrence. All dogs were tested serologically for *Ehrlichia canis* with an enzyme-linked immunosorbent assay (ImmunoComb® Canine Ehrlichia Antibody Test Kit, Israel) and microscopically for other common blood parasites, such as *Babesia canis vogeli* and *Babesia gibsoni*, from peripheral blood smears. In addition, complete blood count (CBC) and selected parameters from the serum biochemistry such as protein level, renal, and hepatic panels, including electrolytes, were tested in all four cases.

Radiographs of the skull, mainly the nasal cavity, with left and right lateral and ventrodorsal views were taken for all dogs. Contrast-enhanced computed tomography (CT) images were acquired for all four dogs using a cone beam CT scanner (Fidex, Animage, USA). Moreover, the results of the "modified Adam's staging" criteria were used to stage the nasal tumor. The criteria were adapted from Adam et al. (2009), where stage 1 is confined to the unilateral nasal passage, paranasal sinus, or frontal sinus without any bony involvement beyond nasal turbinates. Stage 2 is confined to any bony involvement beyond nasal turbinates without evidence of orbital, subcutaneous, or submucosal mass. Stage 3 is confined to the orbit or nasopharyngeal or submucosal mass involvement, and stage 4 is indicated when the tumor causes lysis of the cribriform plate. Rigid rhinoscopy was performed in Dog-2. Follow-up CT scans were performed in Dog-3 and Dog-4 for three months after completing five intravenous (IV) chemotherapy cycles.

Tissue biopsy samples were collected by punch biopsy from Dog-1 only at the left maxillary gum caudal to the canine teeth. Impression smears and sterile cotton swab

samples of nasal secretions were collected for cytology and culturing of pathogenic bacteria and fungi, respectively, in all four dogs. Blood agar and MacConkey agar (HiMedia Laboratories Private Limited, India) were used for primary bacterial culture. In contrast, Sabouraud dextrose agar (HiMedia Laboratories Private Limited, India) was used to detect fungi in the samples. In addition, fine needle aspiration (FNA) samples were collected from the left side of the hard palate in Dog-1, soft swelling at the nasal bridge in Dog-2, intranasal soft tissue mass in Dog-3, and soft swollen mass at the right side of the hard palate in Dog-4.

RESULTS

Signalment

Two of the dogs were mixed breeds (Dog-2 and Dog-3), one Spitz (Dog-1), and another Siberian Husky (Dog-4). All dogs were young adult males. One was neutered, and the remaining three were intact. The mean ± standard error (range) age and body weight of these dogs are 2.5 ± 0.46 (1.5 to 3.5 years) and 18.4 ± 2.14 (12.4 to 22.4 kg), respectively. All four dogs were raised in a free-roaming lifestyle (Table 1).

Table 1

Age, sex, breed, body weight, remarkable clinical findings, and duration of clinical signs before the first presentation to UVH in four dogs diagnosed with primary nasal CTVT

Case no.	Breed	Sex lifestyle	Age (Years)	B. Wt (kg)	Primary complaint/s and remarkable clinical findings on first and subsequent visits	Duration of clinical signs
Dog-1	Spitz	M FR	2Y 3Mo	12.4	Occasional profuse bleeding or blood clots from nares. Serosanguinous discharge from left nares. 2-cm mass present at left maxillary gum with an ulcerative mark on the surface. Slight bulging at the level of the left lateral nasal bone surface. Left-sided epiphora	5-months
Dog-2	Mixed	MN FR	3Y 5Mo	22.4	Eleven (11) months before the current presentation recovered from genital-organ TVT with three vincristine cycles. Presented with bilateral epistaxis. Asymmetrical face because of the distorted nasal bone with a small soft lump on the dorsal surface. Right-sided epiphora with conjunctivitis. Small growth at the level of the right upper premolar. Discoloration of nasal planum. Left-sided sub-mandibular lymphadenomegaly	4-months

Table 1 *(Continue)*

Case no.	Breed	Sex lifestyle	Age (Years)	B. Wt (kg)	Primary complaint/s and remarkable clinical findings on first and subsequent visits	Duration of clinical signs
Dog-3	Mixed	M FR	1Y 8Mo	20	It started as unilateral epistaxis and turned into bilateral just one week before the presentation. Continuous blood mixed mucoid nasal discharge from left nares and occasional bleeding from right nares when the patient is subjected to physical stress. Prolapsed third eyelid of the left eye	5.5-months
Dog-4	Siberian Husky	M FR	3Y 3Mo	18.8	Epistaxis from both nares. Serosanguinous discharge from medial canthus of right eye without any sign/history of injury started one day before the presentation. Slight bulging-out of the right eye globe. Another bulging was noticed at the level of the right lateral nasal bone surface. A 1.5 × 1.5 cm mass observed on the right side of the hard palate	2-months

Note. B. Wt = Body weight; TVT = Transmissible venereal tumor; M = Male; MN = Male neutered; FR = Free roamer

Clinical Findings

The summary and duration of the detailed clinical signs before the first visit to UVH for individual dogs are summarized in Table 1. The most remarkable presenting complaint was chronic bilateral epistaxis in all four dogs, which started unilaterally in Dog-1 and Dog-3 and was reported bilaterally at the time of presentation. In addition to epistaxis, the second most remarkable finding was concurrent ocular manifestations in all four cases. Dog-1 showed left-sided mild epiphora, while Dog-2 and Dog-3 manifested right-sided epiphora, conjunctivitis, and left-sided prolapsed third eyelid. Dog-4 manifested serosanguinous discharge from the medial canthus, as shown in Figures 1 (a and b), and a popped-out right eye. A soft swelling-type bulging surface was noticed at the level of nasal bone in Dog-1, Dog-2 (Figure 1 [d and e]), and Dog-4 (Figure 1 [b]). Facial asymmetry was reported in Dog-2 due to distorted nasal bone surface. The same dog also noticed discoloration of the nasal planum and left-sided submandibular lymph node enlargement. Oral lesions were observed in three dogs. A 2-cm mass

Figure 1. (a, b) Dog-4 presented with epistaxis and serosanguinous discharge from the medial canthus of the right eye and bulging-out of the right dorsolateral nasal bone surface, diagnosed with primary nasal CTVT; (c) The same dog on follow-up, after successful treatment; (d, e) Dog-2 presented with epistaxis and soft swelling at the level of the dorsal nasal surface, causing asymmetry of the face, diagnosed with primary nasal CTVT; (f) The same dog on follow-up, after successful treatment

on the left maxillary gum, a small growth at the level of the right upper premolar, and a 1.5 × 1.5 cm mass on the right side of the hard palate were documented in Dog-1, Dog-2, and Dog-4. There were no other abnormalities or evidence of genitalia growth in the dogs during the initial presentation.

Hematology and selected biochemistry parameters were tested in all four dogs (Table 2). Cross verification of all hematology reports and examination of routine blood parasites were performed through blood smears. The most remarkable findings in hematology mean ± SE [reference range]) leukocytosis 24.14 ± 2.89 (6–17), with band neutrophilia 0.53 ± 0.21 (<0.3), segmented neutrophilia 16.57 ± 1.90 (3–11.5), and monocytosis 2.46 ± 0.71 (0.2–1.4) in all four dogs. Major changes in the biochemistry parameters are seen in total proteins 78.08 ± 4.96 (55–75), with hypoalbuminemia 24.83 ± 0.59 (25–40) and hyperglobulinemia 56.6 ± 5.74 (25–45), consequently reducing the albumin to globulin ratio 0.47 ± 0.07 (0.5–1.2).

Table 2

Hematological and biochemistry parameters of four dogs diagnosed with primary nasal CTVT

Parameters (units)	Dog-1	Dog-2	Dog-3	Dog-4	Reference range
Erythrocytes $x10^{12}$/L	6.84	6.59	7.34	6.54	5.5 – 8.5
Hemoglobin g/L	156	144	164	138	120 – 180
PCV L/L	0.35	0.38	0.45	0.35	0.35 – 0.55
MCV fL	51	58	61.6	54	60 – 77
MCHC g/L	446	379	363	394	320 – 360
CWCC $x10^9$/L	26.8	29.3	24.48	15.99	6 – 17
Band-neutrophils $x10^9$/L	0.54	0.88	--	0.16	< 0.3
Segmented-neutrophils $x10^9$/L	16.62	21.68	15.36	12.63	3 – 11.5
Lymphocytes $x10^9$/L	3.75	2.34	5.13	1.60	1.5 – 4.8
Monocytes $x10^9$/L	2.68	4.10	2.40	0.64	0.2 – 1.4
Eosinophils $x10^9$/L	3.22	0.29	1.55	0.96	0.1 – 1.3
Basophils $x10^9$/L	0	0	0.04	0	Rare
Thrombocytes $x10^9$/L	316	243	485	318	200 – 500
Sodium mmol/L	--	141	152	143	140 – 155
Potassium mmol/L	--	6.7	5.5	5.4	3.7 – 5.5
Chloride mmol/L	--	108	117	107	96 – 122
Calcium mmol/L	2.75	2.5	2.6	--	2 – 2.8
Inorganic phosphate mmol/L	--	1.3	1.63	--	0.8 – 2.5
Urea mmol/L	3.7	7.3	2.9	8.6	3 – 7.5
Creatinine µmol/L	103	92	79	85	88 – 176
Total bilirubin µmol/L	--	3	3	--	1.7 – 17
ALT U/L	35.4	25	41	22	5 – 90
ALP U/L	--	70	72	--	40 – 100
GGT U/L	--	--	9	--	0 – 11
Total Protein g/L	68	82.5	72	89.8	55 – 75
Albumin g/L	--	24.4	26	24.1	25 – 40
Globulin g/L	--	58.1	46	65.7	25 – 45
A:G Unit	--	0.4	0.6	0.4	0.5 – 1.2

Note. PCV = Packed cell volume; MCV = Mean corpuscular volume; MCHC = Mean corpuscular haemoglobin concentration; CWCC = Complete white cell count; ALT = Alanine transaminase; ALP = Alkaline phosphatase; GGT = Gamma-glutamyl transferase; A:G = Albumin: globulin ratio

Serologically, all dogs were tested for *E. canis*. Dog-1 was treated with doxycycline before the presentation and reported negative upon testing. Dog-2 showed a serologically high antibody titer (scale 5/6) and was treated with doxycycline in the initial management of epistaxis with poor outcomes. Dog-3 and 4 tested negatives for *E. canis* but managed with doxycycline with poor outcome in the initial course of the disease diagnosis while dealing with epistaxis.

Radiology and Rhinoscopy

A detailed summary of radiographic and CT findings with the staging of nasal tumors has been outlined in Table 3. All four dogs fell into stage 4 of the modified Adam's staging for canine nasal tumors due to the involvement of the cribriform plate. The radiographic appearance of neoplasia in Dog-2 is shown in Figure 2. The right lateral and ventrodorsal (VD) radiographs of the skull, including the nasal cavity, revealed an abnormally radiopaque (especially on the right side compared to the left in VD view) nasal cavity with loss of the nasal conchae and turbinates detail. In addition, the bony lysis of the cribriform plate is noticed in both lateral and VD views. The pre-and post-treatment CT scan appearances of the nasal passages of Dog-3 and Dog-4 are presented in Figures 3 and 4. Meanwhile, rhinoscopy examination of the nasal cavity in Dog-2 did not reveal the involvement of any foreign body in the nasal disease.

Table 3

Summary of radiographic and CT scan findings in all four dogs and staging of primary nasal CTVT

Case	Radiographic findings	Computed tomography findings at different regions of the nasal cavity			Modified Adam's staging
		Rostral region	Maxillary 4th premolar region	Frontal sinuses region	
Dog-1	Abnormally radiopaque nasal cavity in both lateral and VD views, partial loss of nasal turbinates, and cribriform plate detail	Soft tissue density filled the cavity with nasal bone and septum lysis	Lysis of septum, turbinates, and palatine bone. Soft tissue density was invading from right to left nasal passage	Soft tissue density filled (partial) right rostral frontal sinus, partial cribriform lysis	Stage 4
Dog-2	Abnormally radiopaque nasal cavity in both lateral and VD views, loss of nasal turbinates, and cribriform plate detail	Soft tissue density filled the cavity with nasal bone and septum lysis	Bilateral space-occupying soft tissue density involving right orbit also. Lysis of septum and turbinates	Soft tissue density in both frontal sinuses, prominent cribriform lysis	Stage 4

Table 3 *(Continue)*

Case	Radiographic findings	Computed tomography findings at different regions of the nasal cavity			Modified Adam's staging
		Rostral region	Maxillary 4th premolar region	Frontal sinuses region	
Dog-3	Abnormally radiopaque nasal cavity in both lateral and VD views, loss of nasal turbinates, and cribriform plate detail	Soft tissue density filled the cavity with nasal bone and septum lysis	Right orbit involved lysis of septum, turbinates, and palatine bone (orbital process). Soft tissue density invading from right to left nasal passage	Soft tissue density filled right frontal sinus, prominent cribriform lysis	Stage 4
Dog-4	Abnormally radiopaque nasal cavity in both lateral and VD views, loss of nasal turbinates, and cribriform plate detail	Soft tissue density filled the cavity with nasal bone and septum lysis	Both orbits involved lysis of the septum, turbinates, perpendicular plates (palatine), and the palatine bone. Soft tissue density filled both nasal passages	Soft tissue density in both frontal sinuses. Massive lysis of the orbital process, perpendicular plate (palatine), and cribriform	Stage 4

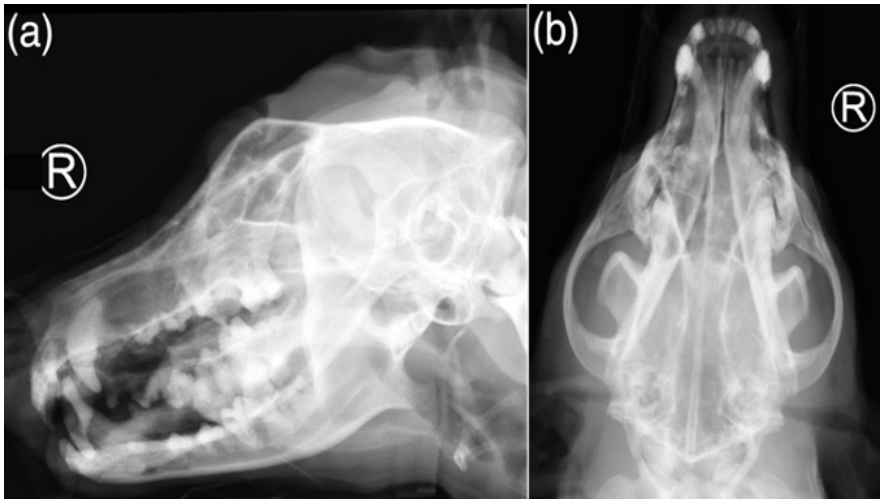

Figure 2. (a, b) Right lateral and ventrodorsal (VD) radiographs of the skull, including the nasal cavity, in Dog-2, diagnosed with primary nasal CTVT. An abnormally radiopaque (especially on the right side compared to left in VD view) nasal cavity with loss of the nasal conchae and turbinates detail and bony lysis of cribriform plate noticed in both lateral and VD views

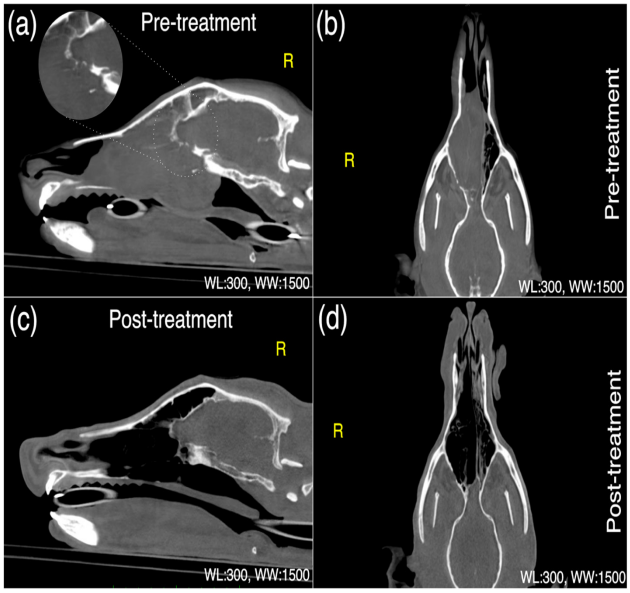

Figure 3. (a, b, c, d) Sagittal and dorsal pre-contrast bone reconstruction images of Dog-3 before and three months after chemotherapy. The right side is denoted by "R," and window level and width are labeled. (a, b) A large isoattenuating density occupies a major portion of the right nasal passage; (b) causing left-side deviation of the nasal septum and lysis of the cribriform plate [highlighted in (a)] including surrounding bony structures; (c, d) The nasal passage is clear post-chemotherapy. However, permanent loss of the nasal turbinates is observed, predominantly in the right nasal passage

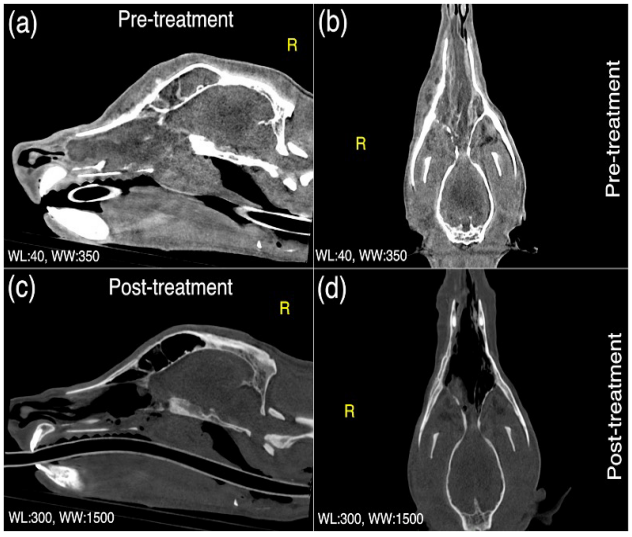

Figure 4. (a, b, c, d) Sagittal and dorsal pre-and post-contrast, soft tissue (a, b), and bone (c, d) reconstruction images of Dog-4 before and three months after chemotherapy. The right side is denoted by "R," and the window level and width are labeled. (a, b) A large mixed (iso, hyper, and hypo) attenuating density occupies the entirety of both nasal passages and (b) further infiltrates into right lateral nasal soft tissue; (b) causing significant left-side deviation of the nasal septum and lysis of the cribriform plate, including surrounding bony structures; (c, d) Nasal passages are clear post-chemotherapy. However, permanent loss of the nasal turbinates is noticed in both the left and right nasal passages

Cytology and Histopathology

Cytology was performed in all four dogs, and phenotypically, all samples exhibited identical findings. A mixed plasmacytoid and lymphocytoid cell population is noticed in impression smears and FNA samples of intranasal, paranasal, and invasive soft tissue swelling of the oral cavity. Characteristically, round to ovoid or polyhedral cells with distinct boundaries was noticed in all four dogs. Round nuclei containing prominent angular nucleoli and slight basophilic cytoplasm with distinct vacuolation were also observed. Mitotic figures were noticed in all four cytological samples, as shown in Figure 5.

Only Dog-1 had a histopathology report of a few pieces of whitish tissue measuring 1.8 × 1.5 × 0.2 cm in aggregate. Neoplastic cells identical to cytology samples were identified in the nasal mucosa arranged in sheets with fibrous septa, separating the tumor into vague nodules. Occasional mitotic figures and subepithelial lymphocytic infiltration were also observed in the histopathology samples.

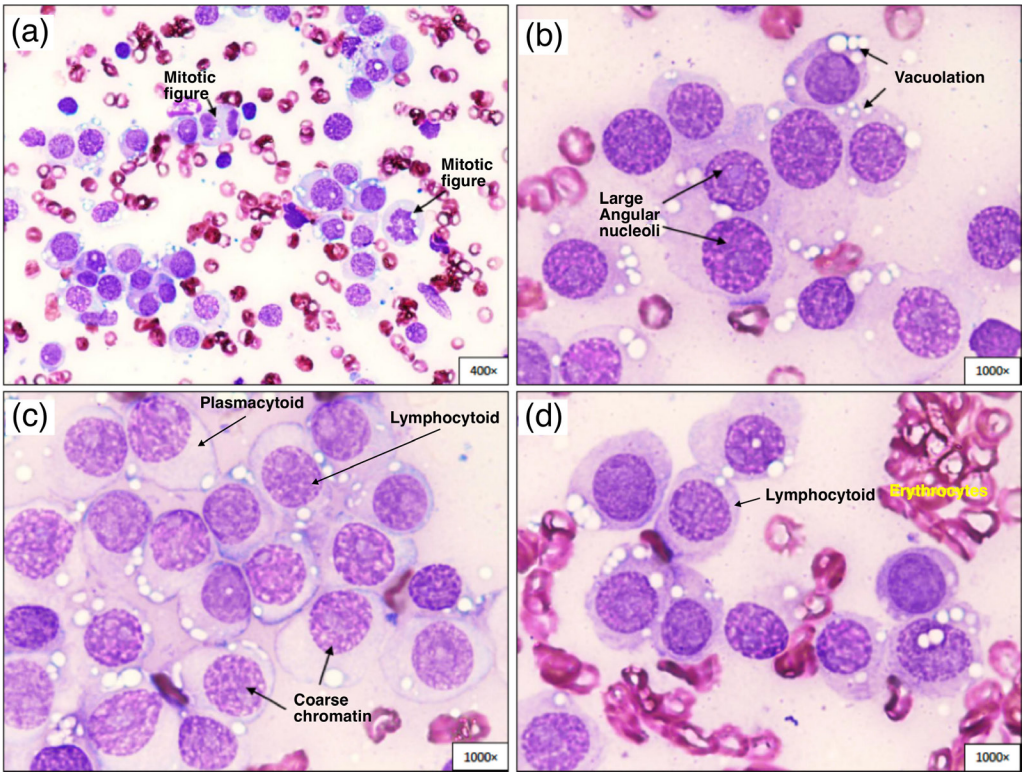

Figure 5. (a, b, c, d) FNA of the intranasal mass of Dog-3 was diagnosed with primary nasal CTVT. The phenotype is mixed type by classification characterized by plasmacytoid and lymphocytoid cell population (labeled). Several rounds to ovoid cells are observed with distinct cellular margins, cytoplasmic vacuolation, round nuclei with large angular nucleoli, and coarse chromatin. A mitotic figure is also noticed in this smear, as shown in section (a) 400 × (Modified Giemsa stain)

Treatment and Outcome

A detailed summary of treatment and outcome is presented in Table 4. All four dogs achieved complete resolution of clinical signs shown in Figures 1 (c and f) with vincristine sulfate (Vincristine Sulfate®, 1 mg/mL, Korea United Pharm. Inc., Korea) at a dose rate of 0.025 mg/kg q7days intravenously in five cycles without any obvious adverse reactions or side effects. Hematology was repeated at every follow-up for each case before administration of the chemotherapeutic agent. The most common finding was thrombocytosis in all cases. Furthermore, all dogs responded well to this therapy and are still alive without any recurrence of clinical signs, confirmed during subsequent follow-up post-treatment protocols, by detailed telephone interviews with the clients, and CT scans for Dog-3 and Dog-4. Follow-up scans were done after three months of the fifth cycle of vincristine. Though CT findings for Dog-4 at 3-month follow-up post fifth vincristine cycle are slightly questionable in remission scan (Figure 3), the density of the questionable tissue is homogenous, and it is quite caudal in the nasal cavity. On the other hand, owners were satisfied with the current remission state as the quality of life had improved significantly without any obvious clinical signs. Hence, cytology was not repeated on follow-up visits as constrained by clients. The last case in the present study was treated approximately seven months ago.

Table 4

Diagnostic confirmation, drug of the choice, outcome, and follow-up data of four dogs diagnosed with primary nasal CTVT

Case	Diagnostic confirmation	Treatment	Follow-up	Final outcome
Dog-1	Cytology (mixed) and histopathology	Vincristine chemotherapy, q7days for 5 weeks	Still alive after 37 months of last treatment without recurrence	Recovered
Dog-2	Cytology (mixed)	Vincristine chemotherapy, q7days for 5 weeks	Still alive after 19 months of last treatment without recurrence	Recovered
Dog-3	Cytology (mixed)	Vincristine chemotherapy, q7days for 5 weeks	Still alive after 10 months of last treatment without recurrence	Recovered
Dog-4	Cytology (mixed)	Vincristine chemotherapy, q7days for 5 weeks	Still alive after 08 months of last treatment without recurrence	Recovered

Note. Mixed = Phenotypically plasmacytoid and lymphocytoid cell population of cytological sample; q7 days = Quaque 7 days = Every 7 days

DISCUSSION

The most remarkable historical findings in the present study are the presentation of young adult male dogs with chronic (2–5.5 months duration) epistaxis and/or serosanguinous nasal discharge, which is quite consistent with previous case studies of primary nasal CTVT, where nasal discharge was reported from one month (Ojeda et al., 2018) to one year (Sukhbir & Sood, 2016). Although some concurrent oral and ocular manifestations were also noticed in all dogs during physical examination, these findings are also quite consistent with previously reported cases where allografted transmission causes an invasive nature of the disease spreading to surrounding areas of the nasal cavity (Komnenou et al., 2015; Veloso et al., 2018). In dogs, the top listed differential of epistaxis in tropical countries like Malaysia is canine ehrlichosis (Neer et al., 2002). In the present study, all dogs tested and went through standard therapy of doxycycline for at least four weeks, regardless of the serological results, and did not respond well.

Mild, microcytic, and non-regenerative anemia (packed cell volume [PCV] at low normal) was noticed in Dog-1, 2, and 4, likely due to prolonged bleeding resulting from chronic epistaxis, which agrees with the previous study of primary nasal CTVT (Papazoglou et al., 2001). Nevertheless, a similar finding was absent in Dog-3, where the PCV was at mid-range, possibly due to compensatory polycythemia induced by increased erythropoietin, endogenously produced by the tumor (Papazoglou et al., 2001; Rogers et al., 1998). All four dogs had mild-to-moderate leukocytosis, which could occasionally be present due to possible inflammation of the tumor surface (Ganguly et al., 2013).

Following the definitive diagnosis of these cases, CT findings (Table 3) revealed remarkable lysis of nasal turbinates, septum, and nasal bone in all four dogs. Palatine bone lysis is noticed in Dogs 1, 3, and 4, conclusive of oral cavity contamination with neoplastic tissue. In addition, the lysis of the orbital process of palatine bone is noticed in Dogs 2, 3, and 4, causing the manifestation of ocular signs. Since bony lysis is quite significant in all cases, the external and internal tables of the cribriform plate and frontal sinuses, separating sinuses from the nasal cavity, were also affected and allowed discharge and/or neoplastic tissue to spread into these areas. These findings indicated stage 4 of the modified Adam's staging used to stage the primary nasal CTVT in the present study. Staging with the same criteria has been done recently in a case study of four dogs diagnosed with primary nasal CTVT, where only one dog was categorized as stage 4 based on the CT scan findings. Interestingly, in the same case report, the aggressive nature of CT scan findings was noticed in the male population, whereas another two males were categorized as stage 3, and the only female was categorized as stage 1 (Ojeda et al., 2018).

The primary nature of the disease was considered based on the absence of genital form and/or metastasis of the primary tumor from another site, history, and clinical

findings related to nasal CTVT. Additionally, a free-roaming lifestyle and social behavior, such as excessive licking of genital organs of affected animals and vigorous sniffing habits, can be a possible mode of allografted transmission (Papazoglou et al., 2001). A definitive diagnosis can be cytologically made due to this study's typical characteristics of neoplastic cells. It can be further determined with PCR and histopathology analysis. In the present study, all four dogs were diagnosed mainly through cytology, while histopathology assessment was performed only for Dog-1. Contrary to a recent study on primary nasal CTVTs where the lymphocytoid phenotype was considered less aggressive than the plasmacytoid phenotype with aggressive lytic changes in the nasal cavity (do Amaral et al., 2007), the present study had a mixed phenotype (none of the samples demonstrated more than 60% single phenotype; Setthawongsin et al., 2017), with aggressive changes recorded from CT scans, as mentioned in the preceding paragraph (Table 3).

Although complete remission can be seen on follow-up CT images of Dog-3 (Figure 3 [c and d]), it is questionable in Dog-4. It is unknown whether the remaining soft tissue density lesion (Figure 4 [c and d]) comprises a residual biologically active tumor or the tumor is in a possible sterile or regressive state. It can be differentiated by performing positive emission tomography (PET) imaging (Lawrence et al., 2010); however, it is not regularly available in veterinary medicine. Alternatively, post-treatment sequential CT scans can be performed to evaluate disease progression, recurrence, and remission (Bowles et al., 2016). In addition, several studies have proven the evidence of nasal turbinate regrowth in cases of brachycephalic dogs undergoing laser-assisted turbinectomy (Schuenemann & Oechtering, 2014), pigs with atrophic rhinitis (Robertson et al., 1990), and cats and dogs following intranasal polyps and angioleiomyoma removal (Schuenemann & Oechtering, 2014). However, it remains uncertain, as a recent (Ojeda et al., 2018) and present study showed persistent lysis of turbinate and adjacent bones upon follow-up CT scans. Interestingly, partial restoration of the right horizontal plate and the right orbital process of the palatine bone can be seen in Dog-3 and Dog-4, respectively, but not the turbinate. These occurrences warranted further studies involving characterization of lysis and regrowth of the turbinate and adjacent bones affected by intranasal CTVT, which could be achieved via advanced sequential imaging such as CT.

All four dogs are free of clinical signs until now, with five cycles of vincristine sulfate. A positive response to therapy in this phenotype with various bony changes in all these cases indicates that staging would be more related to local tissue changes than the duration of treatment and outcome. Ojeda et al. (2018) also suggested similar results regarding the correlation of the staging system with treatment and outcome. Either radiotherapy or surgical excision can be an option, but not as monotherapy due to the poor remission (Raghunath et al., 2015).

Uncommonly, if it occurs, metastasis usually involves regional lymph nodes (Nemzek et al., 2015). In the present study, Dog-2 had unilateral left-sided submandibular lymphadenomegaly, but it was not tested for metastasis.

Vincristine sulfate is still an effective monotherapy to achieve full recovery upon the comparison of the previous and present primary nasal CTVT reports, although the number of cycles can vary, possibly depending on the expressed phenotype (Ganguly et al., 2013; Ojeda et al., 2018; Papazoglou et al., 2001; Veloso et al., 2018). In the past, resistance to this drug was reported in some cases, especially with the plasmacytoid phenotype. In such cases, alternatives such as doxorubicin or a combination of surgical strategies can be adopted to achieve complete remission (Papazoglou et al., 2001). Prognosis is not correlated to the staging system; it is quite variable and has been documented well to poor in previous studies (Ganguly et al., 2013; Ojeda et al., 2018; Tyagi et al., 2018). However, based on the present study results, the prognosis for vincristine sulfate usage in mixed phenotype cases seems good.

ACKNOWLEDGEMENTS

The authors want to thank the dog owners for answering the follow-up phone calls and providing the necessary information for this study. Muhammad Waseem Aslam and Lau Seng Fong have contributed equally to this article's major drafting.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to this case study, authorship, and/or publication of this article.

DECLARATIONS

This work involved the use of non-experimental animals only (owned) and followed established internationally recognized high standards ('best practice') of individual veterinary clinical patient care. Therefore, ethical approval from a committee was not necessarily required.

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