Intranasal mass in a dog

Contributors

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Specimen

- Intranasal mass cytology-squash preparation from endoscopic biopsy

Signalment

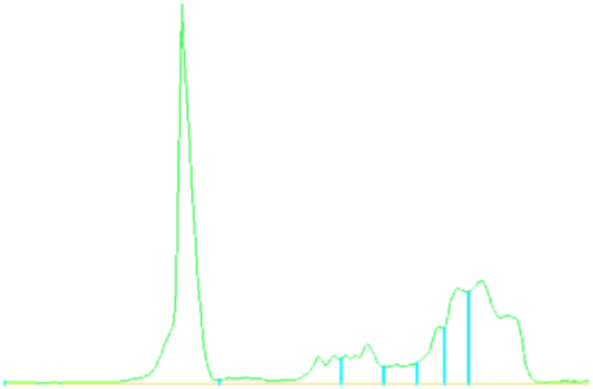
4 years old neutered female mixed breed dog.

History

Dea is a 4-year-old female dog adopted 2 years ago from South Italy. When adopted, leishmaniosis had been diagnosed by the referring veterinarian based on the presence of compatible clinical signs, a serological positivity to a quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against *Leishmania infantum*, and a compatible electrophoresis.
The dog had been placed under treatment various time for the persistence of symptoms despite of the therapy. The last treatment was withdrawn a few weeks prior to the presentation to San Francesco veterinary hospital, apparently leading to resolution of clinical signs. Vaccinations and anti-parasitic prophylaxis were up to date.
The referring veterinarian send the dog to San Francesco veterinary hospital for the investigation of a nasal discharge causing inspiratory dyspnoea and stertor. Clinical signs appeared during the previous month and led to difficulty in resting and sleeping.

Clinical findings

Serology for *Leishmania infantum* was performed some weeks before the onset of respiratory signs, immediately after withdrawal of the last treatment. At the same time, urinalysis and serum protein electrophoresis (SPE) were performed.
Complete urinalysis was unremarkable, while the quantitative ELISA used for serology still revealed a low positivity; also, a polyclonal peak in beta-gamma regions was evident on SPE (**figure 1**).



	intervallo di riferimento	
	min	max
Albumina (%): 39.80	51	65
Globuline ALFA (%): 12.90	10	18
Globuline ALFA1 (%): 6.10	2	6
Globuline ALFA2 (%): 6.80	7	15
Globuline BETA (%): 21.60	15	22
Globuline BETA1 (%): 3.40	2	5
Globuline BETA2 (%): 6.30	2	9
Globuline BETA3 (%): 11.90	5,8	12
Globuline GAMMA (%): 25.70	5	15
Rapporto ALB/GLOB: 0.66		

Figure 1. *Capillary zone SPE.*

TEST	RESULT	REFERENCE INTERVAL
Quantitative ELISA	**4,5**	<0,7: negative 0,7-1,5: ambiguous >6: high positive

Hematological and biochemical exams were performed a few days before the presentation at San Francesco veterinary hospital by the referring veterinarian. Hematology showed a moderate leukocytosis with neutrophilia, eosinophilia and monocytosis. No morphological alterations were reported on the blood smear evaluation. On biochemistry profile, only mild hyperglobulinemia was recorded. Data are shown in the table below.

TEST	RESULT	REFERENCE INTERVAL	UNITS
Hematology			
HCT	38,4	37,3-61,7	%
RBC	5,75	5,65-8,87	M/uL
Hb	13,5	13,1-20,5	g/dL
MCV	66,8	61,6-73,5	fL
MCHC	35,2	32-37,9	g/dL
WBC	**32,43**	5.6-14	K/uL
Segmented neutrophils	**22,31**	3,8-8,9	K/uL
Band neutrophils	0	0-0.3	K/uL
Lymphocytes	4,1	1,2-4,1	K/uL
Monocytes	**3,01**	0,2-0,75	K/uL
Eosinophils	**2,07**	0,15-1,1	K/uL
Platelets	216	103-395	K/uL
Biochemistry			
ALT	27	15-64	IU/L
ALP	53	20-120	IU/L
Urea	18	11-43	mg/dL
Creatinine	0,8	0,7-1,3	mg/dL
Total Protein	7,33	5,5-7,6	g/dL
Albumin	2,56	2,4-3,8	g/dL
Globulin	**4,77**	2,5-4,3	g/dL

The physical examination at the first presentation to San Francesco veterinary hospital revealed severe respiratory distress; the dog showed mouth breathing. The airflow seemed to be obstructed in both of the nostrils. The other organic functions were normal. The same day it was decided to submit the dog to diagnostic imaging to investigate the nasal discharge. In particular, computed tomography (CT) scan of the whole body and rhinoscopy were performed . CT scan of the head revealed the presence of a mass in the nasopharynx, associated with mild osteolysis of the hard palate region and rostral endo-nasal mild invasiveness.
Rhinoscopy revealed bilateral catarrhal rhinopathy, hyperaemic nasal-pharyngopathy, and an irregular, smooth, soft nasal mass which completely obstructed the choanae.

CYTOLOGY
During rhinoscopy, several biopsies were performed to submit to histopathology. One of the biopsies was used to prepare two cytological smears using the squash preparation's technique (figures **2**-**7**).

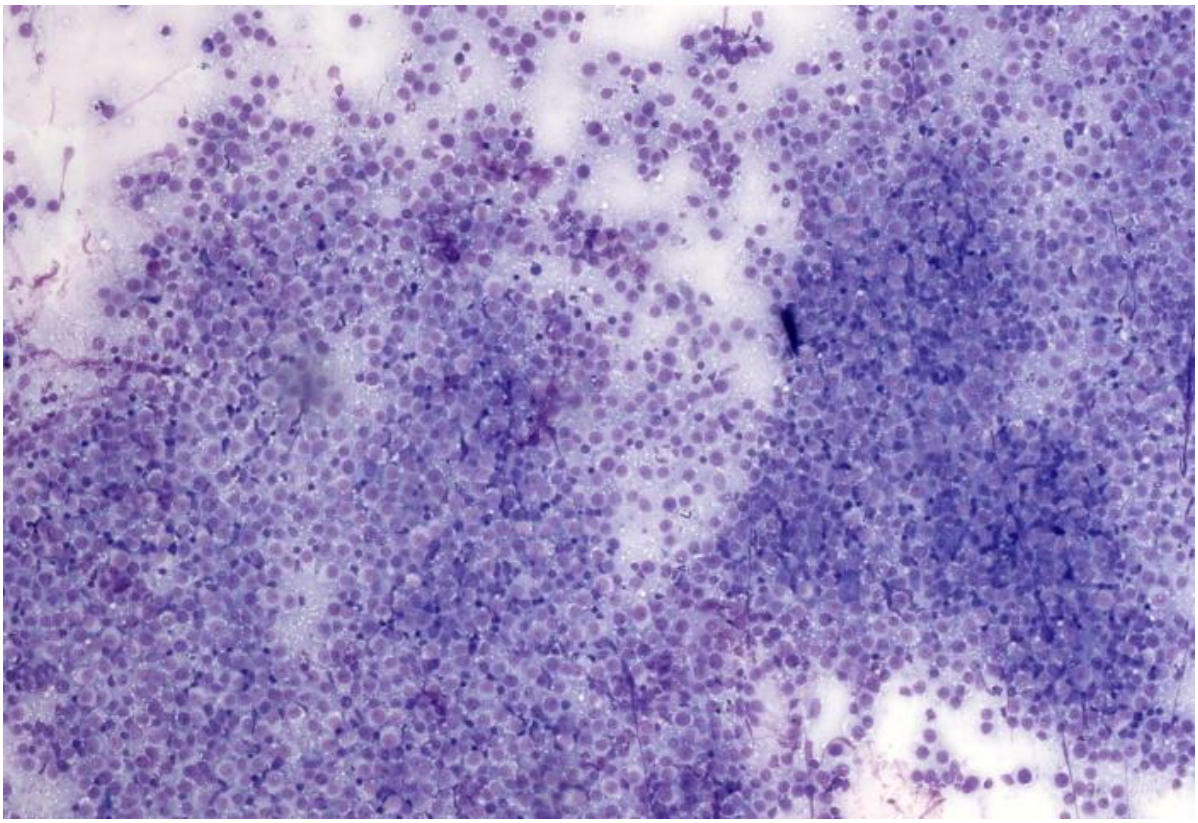


Figure 2. *Squash preparation of the intra-nasal mass, May-Grunwald Giemsa stain, 10x objective.*

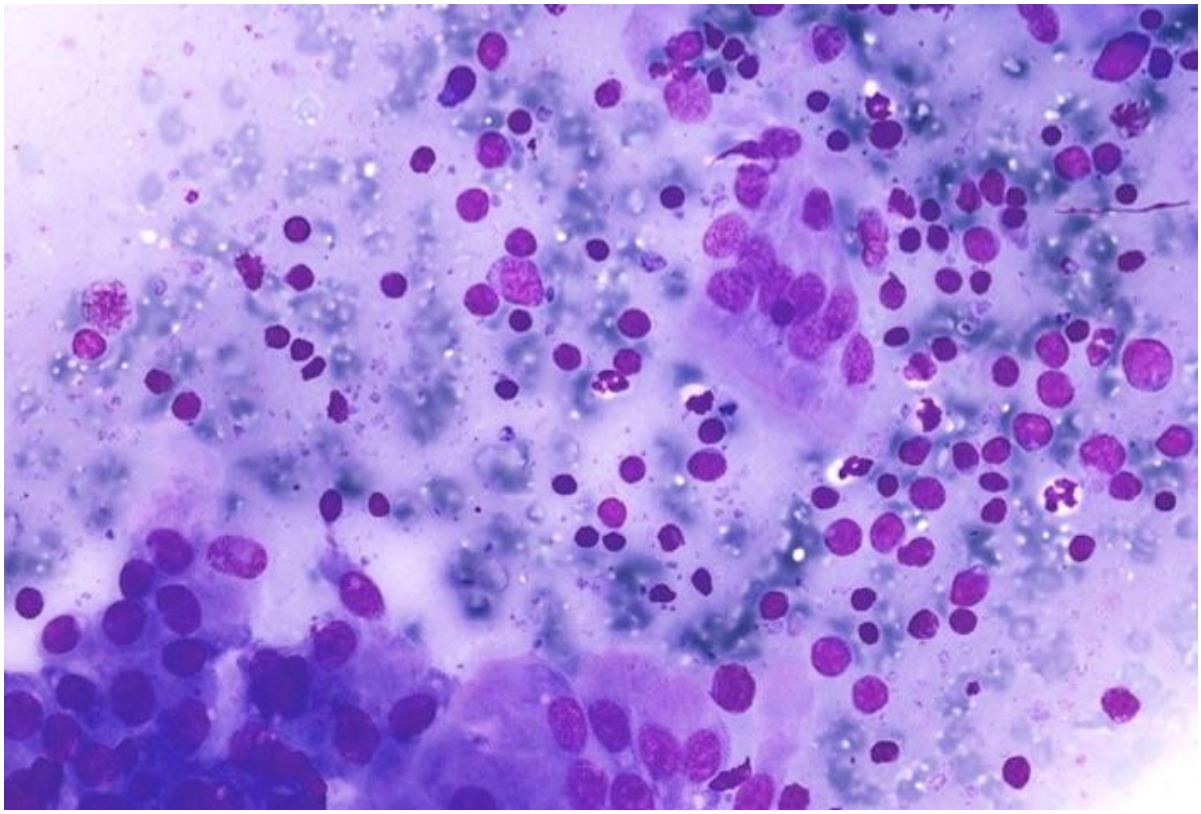


Figure 3. *Squash preparation of the intra-nasal mass, May-Grunwald Giemsa stain, 40x objective.*

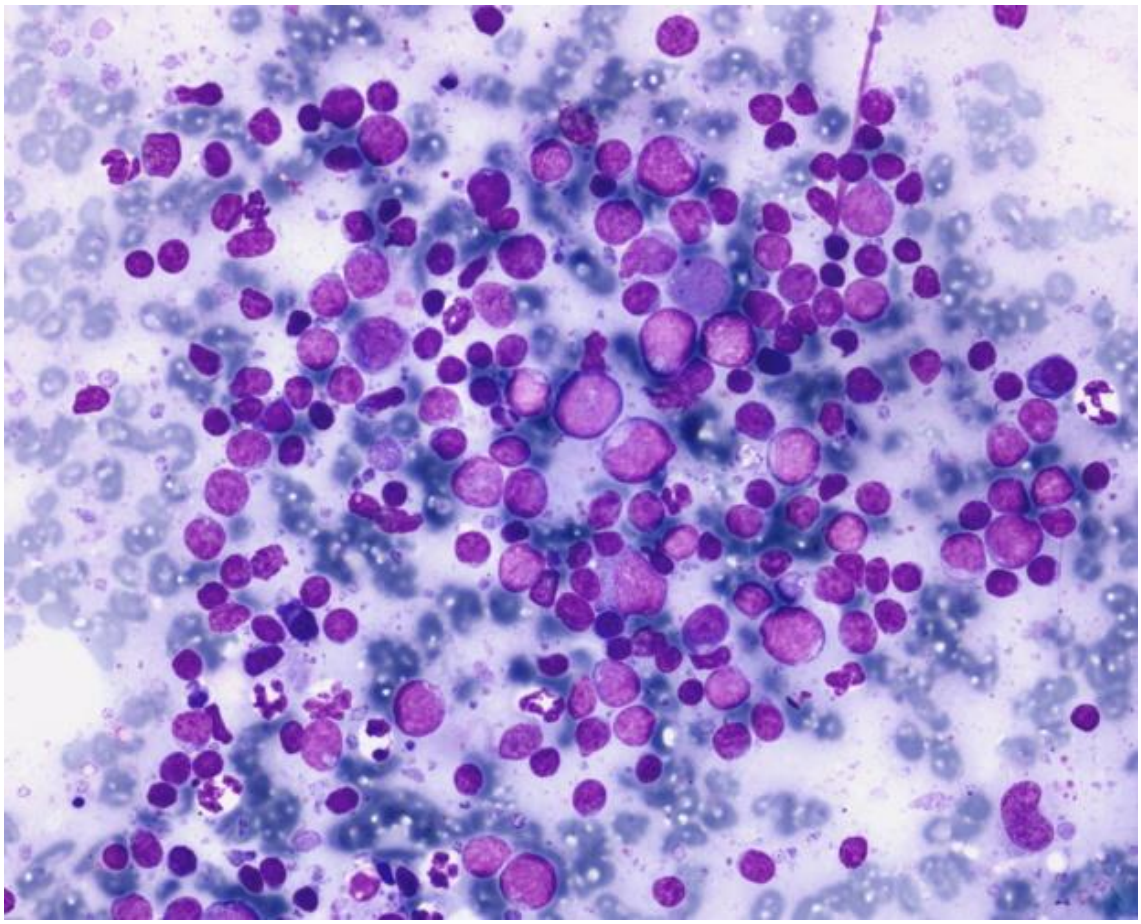


Figure 4. *Squash preparation of the intra-nasal mass, May-Grunwald Giemsa stain, 40x objective.*

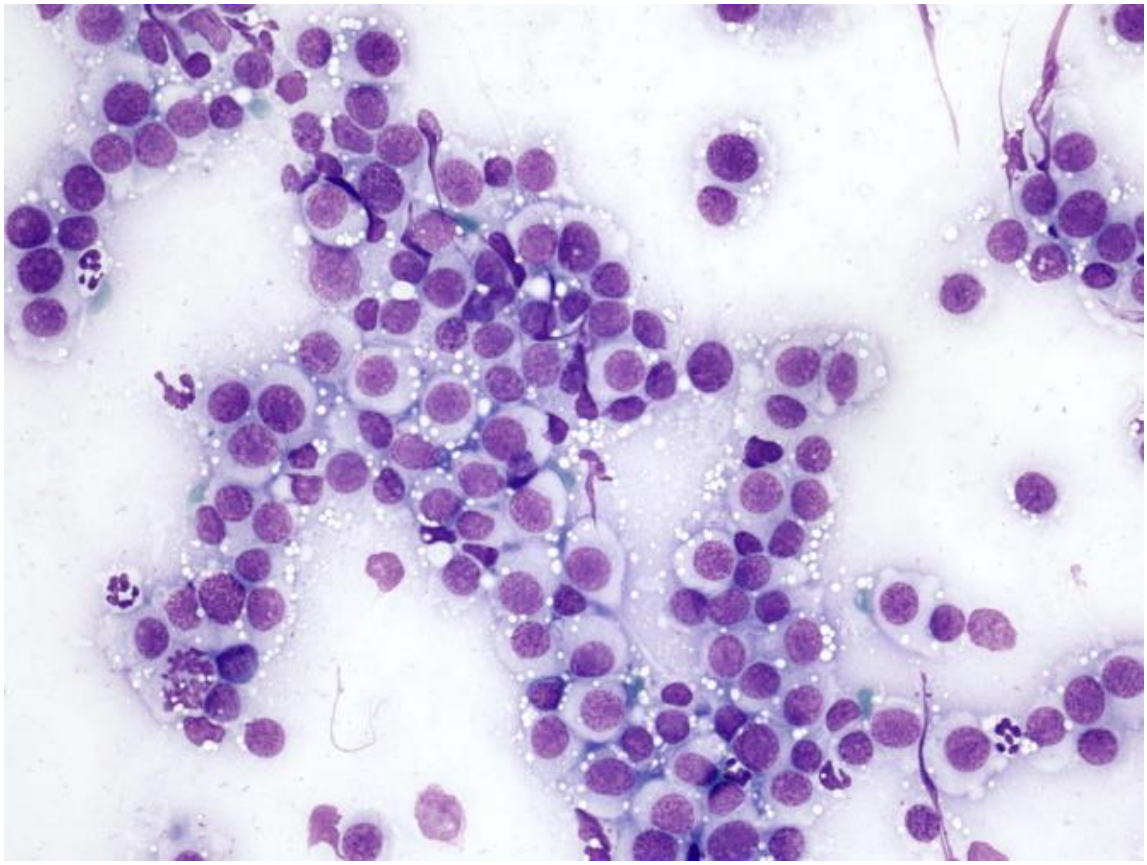


Figure 5. *Squash preparation of the intra-nasal mass, May-Grunwald Giemsa stain, 40x objective*.

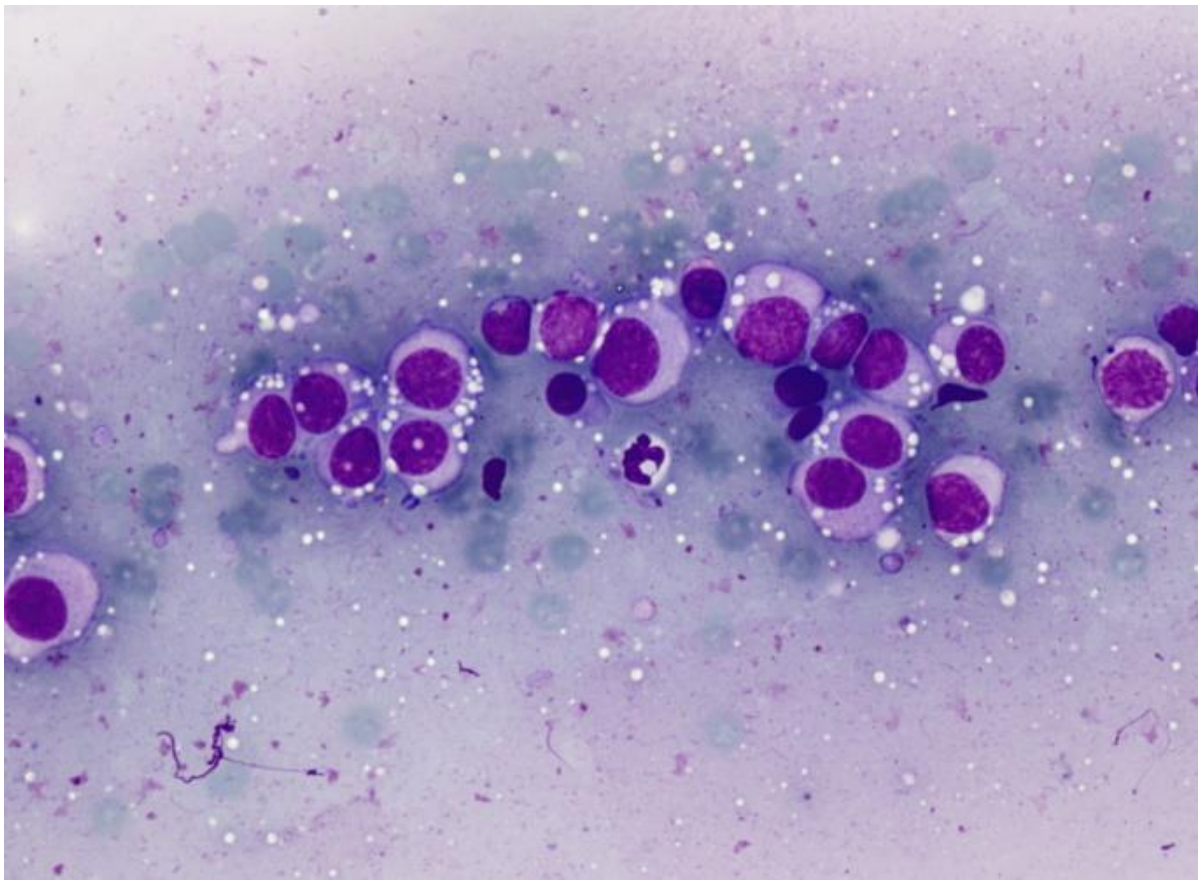


Figure 6. *Squash preparation of the intra-nasal mass, May-Grunwald Giemsa stain, 40x objective.*

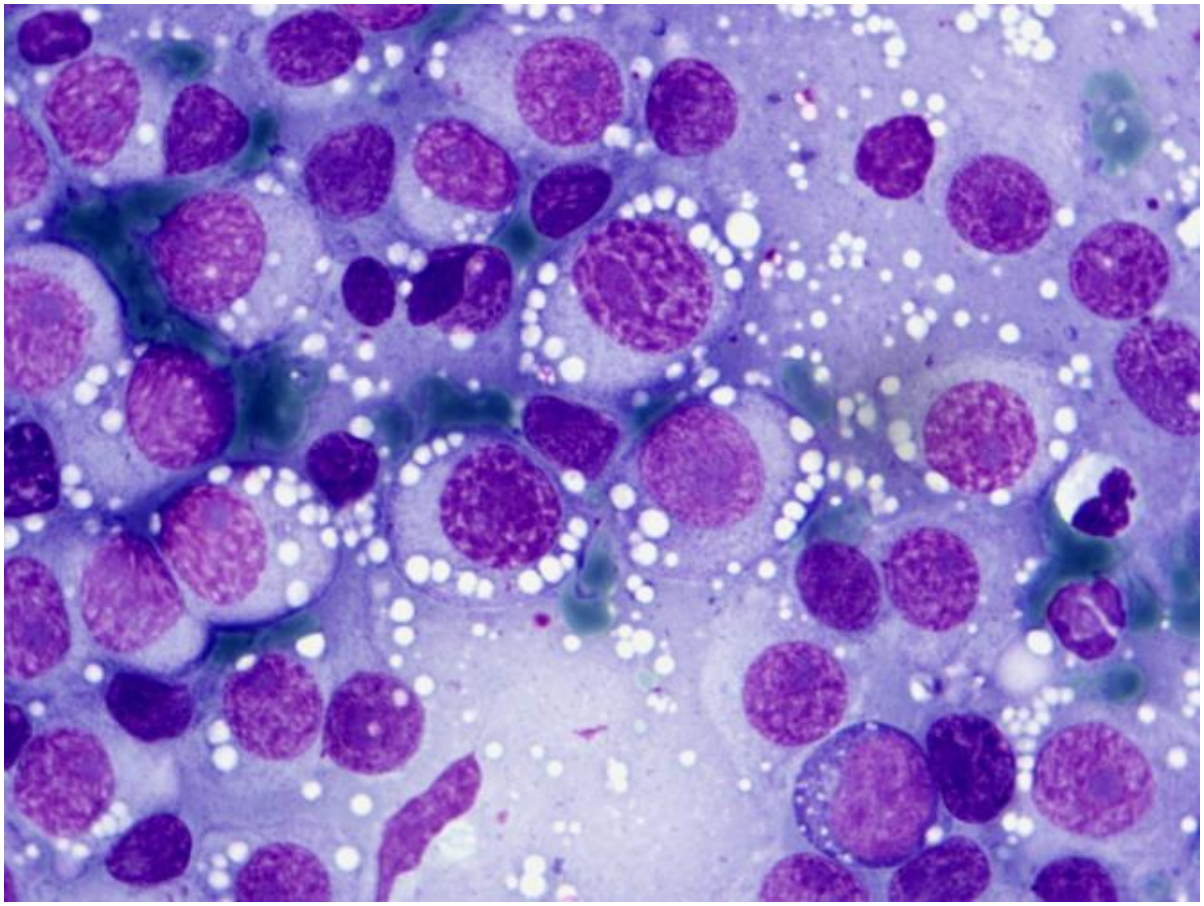


Figure 7. *Squash preparation of the intra-nasal mass, May-Grunwald Giemsa stain, 100x objective.*

Questions

1. What is your cytological description?
2. What is your interpretation of the cytological findings?
3. Which ancillary tests do you suggest to reach a definitive diagnosis?

Interpretation and diagnosis

CYTOLOGICAL DESCRIPTION

The preparations had high nucleated cellularity, well preserved, with only few broken cells and smudged nuclei. The background was granular and bluish, with mild blood contamination and few cytoplasmic fragments.

A mixed population was seen, unevenly distributed on the smear. In several fields there was a clear prevalence of isolated round cells, rarely bi-nucleated, characterized by having a moderate amount of slightly basophilic cytoplasm, with sharp to blurred borders, containing small, round, clear vacuoles. The nucleus was roundish, 2-3 RBC in diameter, centrally to paracentrally located, with finely granular to coarse chromatin and 1-2 evident nucleoli. Rare mitoses were present.
In other fields the prevalent population was represented by small lymphocytes; several medium and large lymphocytes, few plasma-cells, rare mast-cells, macrophages, and Mott cells were also present. Finally, occasional groups of ciliated columnar respiratory epithelial cells were seen.

CYTOLOGICAL INTERPRETATION

The cytological findings are consistent with round cell neoplasia, most likely with canine transmissible venereal tumor (TVT), associated with a mixed lymphocytic inflammation.
Possible differential diagnoses such as histiocytic disorder and lymphoma, although unlikely, cannot be ruled out.

Additional informations

To reach a definitive diagnosis histopathology and immunohistochemistry were performed.

Furthermore, to assess the possible presence of amastigotes within the tumoral cells, molecular biology for the search of *Leishmania infantum* was executed.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY OF THE MASS

The biopsies taken during the rhinoscopy were submitted for histological evaluation, and afterwards for immunohistochemical study.
Histology showed a neoplastic mass, characterized by the proliferation of round cells and partially covered with columnar ciliated respiratory epithelium (**figures 8** and **9**). These cells had moderate amount of slightly eosinophilic finely granular cytoplasm, frequently containing little vacuolisations. The nucleus was roundish with irregular chromatin and a single nucleolus. Moderate atypia was present (anisocytosis and anisokaryosis), and 8 mitoses/HPF were detected (**figure 10**).

Immunohistochemistry revealed moderate positivity of neoplastic cells to vimentin, and negativity to CD3 and CD20. IBA 1 and CD18 showed positivity in few cells, considered to be macrophagic/histiocytic inflammatory infiltrates between the neoplastic cells. Neoplasia of lymphoid or histiocytic origin were considered unlikely, while the positivity only to vimentin supported the hypothesis of canine TVT.

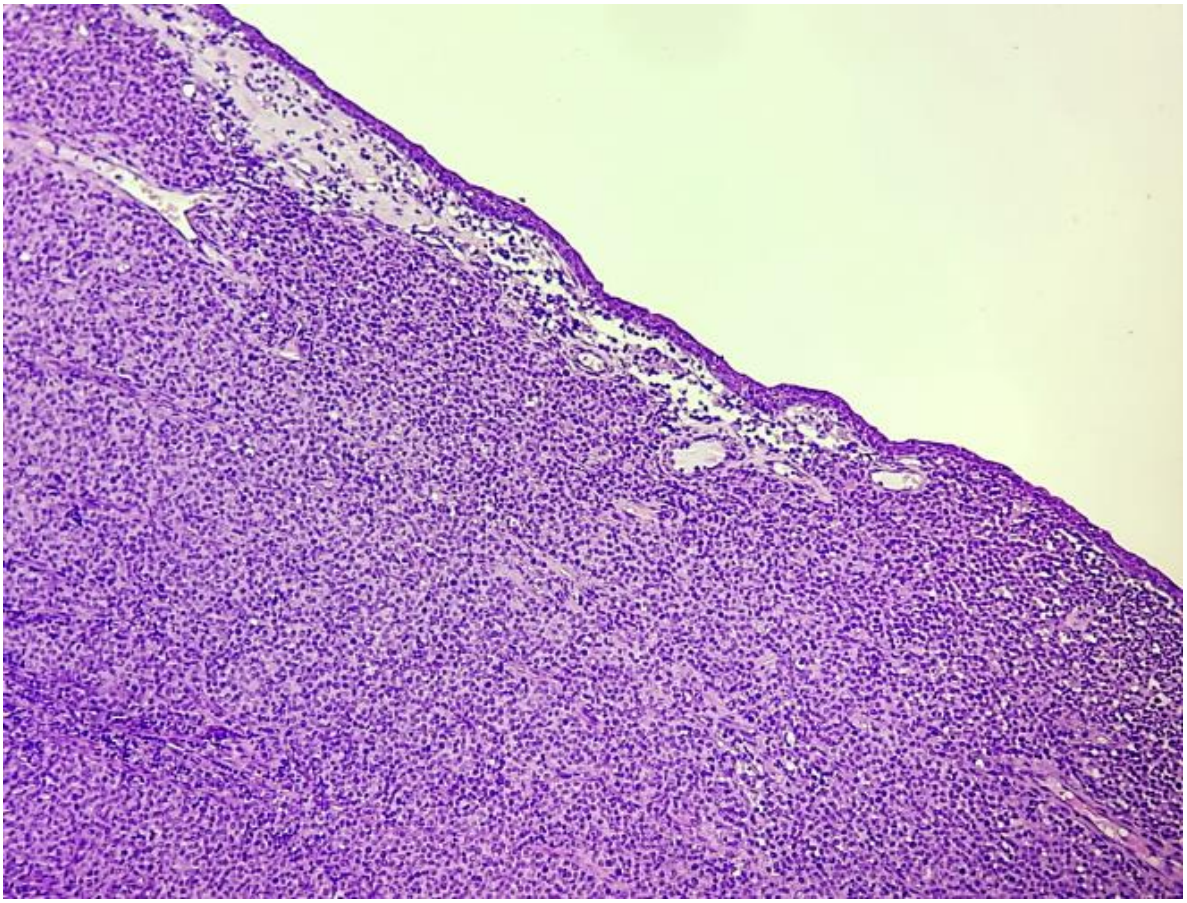


Figure 8. *Histology of the intra-nasal mass biopsy. Hematoxylin-eosin stain, 4x objective.*

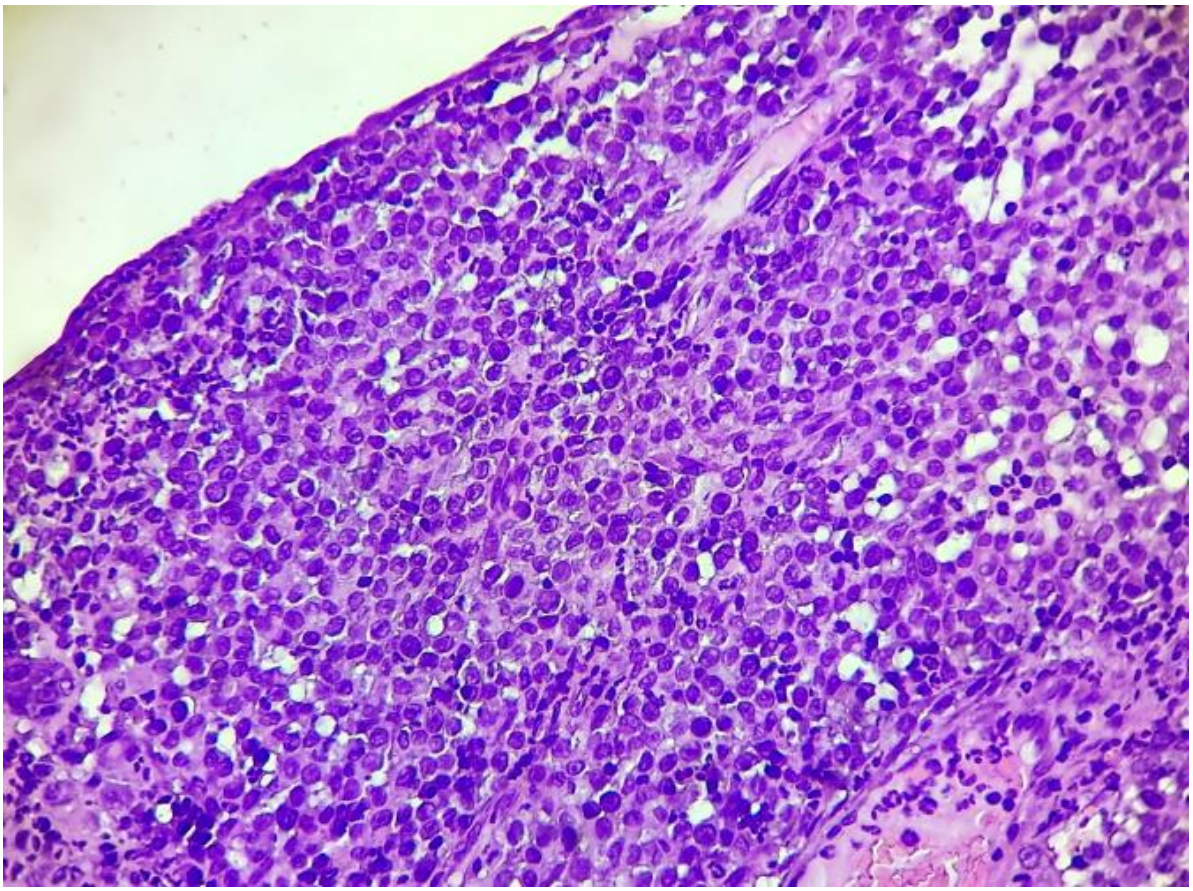


Figure 9. *Histology of the intra-nasal mass biopsy. Hematoxylin-eosin stain, 10x objective.*

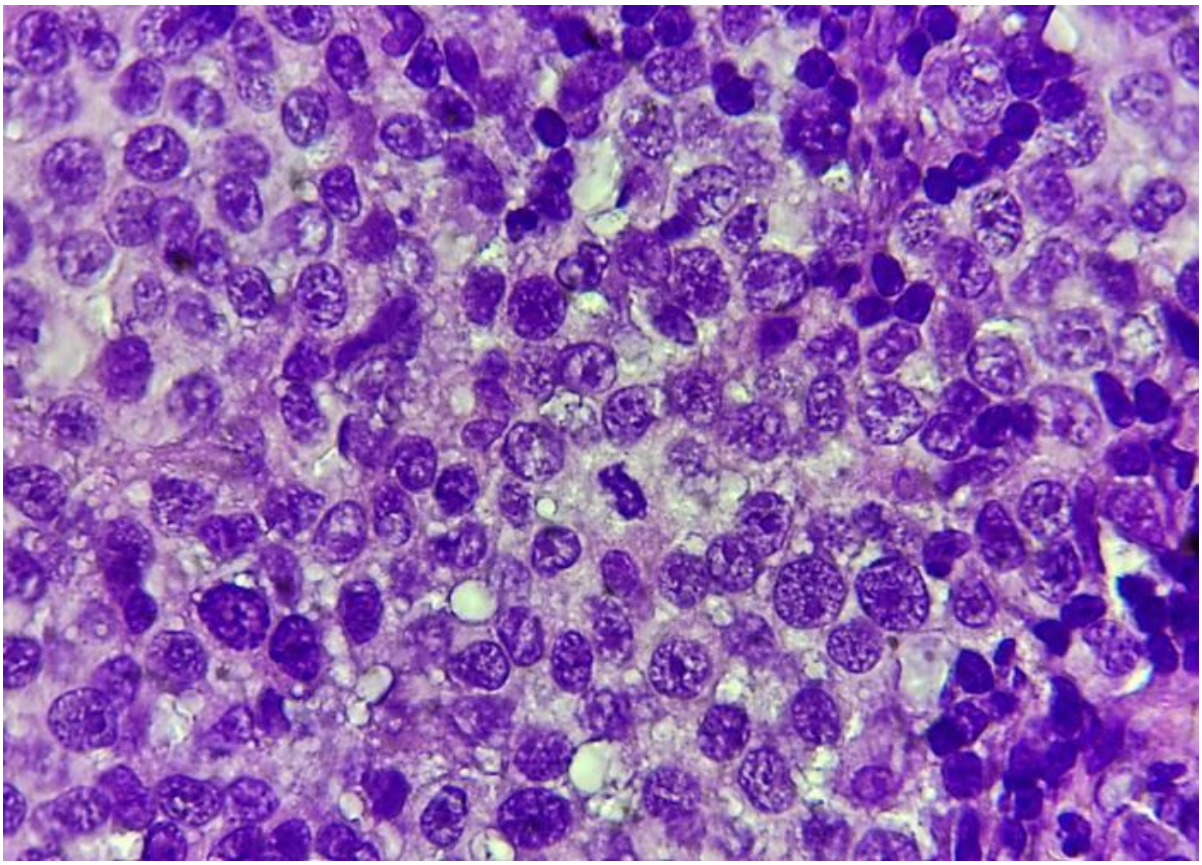


Figure 10. *Histology of the intra-nasal mass biopsy. Hematoxylin-eosin stain, 40x objective.*

MOLECULAR BIOLOGY
Qualitative nested polymerase chain reaction (PCR) for the detection of *Leishmania infantum* was performed on a paraffin-embedded section of the biopsy submitted for histopathology. The result was negative.

Follow up and clinical outcome

At the time of writing the case, the clinical condition of the dog is stable. No therapy has been started yet. The dog should carry out a check-up with the referring veterinarian and start the election therapy (represented by vincristine chemotherapy).

Discussion

The canine transmissible venereal tumor (TVT) is one of the few naturally occurring neoplastic processes in mammals in which implantation of neoplastic cells from host to recipient take place. The transfer of living cancer cells is mostly seen during the coitus, and it usually affects young, sexually active, free-roaming dogs[1]. Some Authors report no sex difference in rate of infection[2], while others claim a higher risk for females (assuming that a male infected dog can mate with different females and spread the disease)[3]. It is considered endemic in 90 countries, with highest prevalence in temperate climate countries. In northern Europe only imported cases are reported, while in south and eastern Europe the prevalence can rise to 10%[2]. The external genitalia are the most represented primary site of tumor growth. Metastases are reported to occur in ~5% of cases, with the most involved sites being the regional lymph nodes, the skin, the oral and nasal cavity, and the eye. Rarely, eyes, skin and nasal cavity are also reported to be primary site of TVT growth, most likely secondary to licking, biting and sniffing beaviour[2,3].

The dog presented in this case report had a primary TVT in an extra-genital site, given the absence of apparent other masses in the reproductive organs both at the physical examination and on the CT. Few reports are present in the literature describing primary intra-nasal canine TVT[4-7]: in all cases, the

dogs were presented for nasal discharge, epistaxis or sneezing, and a nasal mass was found with the aid of diagnostic imaging (CT, rhinoscopy, magnetic resonance, radiography). Cytology was crucial in giving a strong suspect of canine TVT in all cases: cytologically, neoplastic cells have a very peculiar appearance, mainly caused by the presence of clear round vacuoles. Nevertheless, in case of suspected primary extra-genital TVT ancillary tests are strongly recommended to reach a definitive diagnosis[3].

The ancillary tests available rely on the use of immunohistochemistry and molecular biology. A specific immunohistochemistry panel for TVT is lacking, partially because the origin of the tumoral cells is currently unknown. A histiocytic origin is suspected, for the positivity the tumor shows to vimentin, lysozyme, alpha-1-antripsin antibodies[8,9]. As a general rule, the immunohistochemistry panel should be broad enough to exclude with sufficient evidence the other differential diagnoses[9] and should include: **vimentin** and/or **lysozyme** to confirm the suspect of TVT, considering that all other round cell tumors are negative to vimentin; **CD3** or **CD79** to exclude lymphoma; **IBA1** to exclude histiocytic disorders; **S100** or **melan A** to exclude amelanotic melanoma; **CD117** to exclude anaplastic mast-cell tumor, only in case the specific stain (**toluidine blue stain**) gives ambiguous results. In our case, the panel was chosen primarily to rule out lymphoma and histiocytic disorders, which were the main differential diagnoses according to cytologic and histopathological evaluation.

Molecular biology might be useful in the definitive diagnosis: the TVT cells have a particular *c-myc* oncogene rearrangement absent in the normal somatic cells, gametes and other neoplastic cells of dog[10]. The rearrangement is due to the insertion of a LINE (long interspersed nuclear element) near the *c-myc.* Among studies present in literature, no positivity to this PCR was found when tested on normal cells of dog, and on cells derived from other canine tumors[10]. Thus, some authors suggest that the finding of this LINE element near the oncogene might be used as a diagnostic tool to make a definitive diagnosis of canine TVT, using the *in-situ* PCR[11]. However, currently the most frequent use is for research purpose only, and further studies are needed to better understand the accuracy of this method.
To the authors' knowledge, this test is currently not available in commercial laboratories in Europe.

Interestingly, in our case the dog was concurrently infected with *Leishmania infantum*.
The clinical-pathological alterations were considered most likely to be secondary to a possible recurrence of leishmaniasis. Nevertheless, neutrophilia, eosinophilia and monocytosis had also been reported in dogs with intra-nasal primary TVT[4], so it is not possible to exclude that the tumor caused these hematological alterations.
In the literature, there are few studies reporting the co-existence of canine TVT and leishmaniasis, of which just one referred to a primary nasal TVT[12]. The presence of *Leishmania* spp. amastigotes was reported only in the macrophages present as inflammatory population within the tumor[13], or both in the macrophages and in the neoplastic cells[12,14-16]. One study[17] evaluated dogs having co-existence of both TVT and leishmaniasis, a quite common occurrence in areas where both of the diseases are endemic, like in the south of Italy. *Leishmania* spp. amastigotes were seen interspersed within the TVT tumoral cells on the cytological examination in 26% of cases. Amastigotes were seen more frequently when the clinical stage of leishmaniasis was considered moderate to severe, while in most mild clinical stages they were not detectable.
Some authors suggested that the parasitisation of the neoplastic cells could support the suspected histiocytic origin of the TVT cells, as the presence of *Leishmania* spp. amastigotes in histiocytic disorders in dogs is also reported [18]. Others suggest also that the parasitised neoplastic cells could be an alternative route of infection for *Leishmania infantum*[12], even if this has never been demonstrated.
In our case, *Leishmania* spp. amastigotes were not evident on cytological or histological examination. We chose to also perform PCR because its analytical sensitivity is higher than the sole slides review. Our result is consistent with the literature, which states that in most mild clinical stages of leishmaniasis amastigotes are not found within the tumor[17]. Though, we can't exclude that their absence was secondary to the recent parasiticide treatment.

The prognosis for primary nasal TVT was reported to be good, with complete remission after the election therapy with vincristine[3-7]. The presence of concurrent leishmaniosis was never investigated as possible factor affecting the prognosis. When considering 19 cases of concurrent TVT and leishmaniosis, 43% of tumors were considered to have an aggressive behaviour (indicated as: size of the tumor greater than 3 cm; local invasiveness; distant metastases). The authors anyway didn't perform a follow up, so it's not known if the aggressive behaviour eventually led to worsen prognosis[17].

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