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Squash-preparation cytology from nasopharyngeal masses in the cat: cytological results and histological correlations in 30 cases☆

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Upper airway obstruction in cats can be a life-threatening condition. Early recognition of clinical signs and an appropriate diagnostic approach increases the possibility of appropriate therapeutic choices. The purpose of this study was to assess the efficacy and diagnostic value of squash-preparation cytology in providing an accurate diagnosis of masses growing in the nasopharynx of cats. Cytological specimens prepared by a squash technique from nasopharyngeal masses in 30 cats were collected under direct endoscopic guidance and classified into four groups: benign inflammatory/hyperplastic mass, lymphoma, carcinoma and sarcoma. The cytopathological diagnosis was compared with the final histopathological diagnosis and indices of diagnostic test accuracy were calculated. The results showed good agreement between the cytological and histological diagnosis with a sensitivity of 0.94, a specificity of 0.81, a positive likelihood ratio of 0.9, a negative likelihood ratio of 0.9 and an overall accuracy of 0.9. Squash-preparation cytology is considered an accurate diagnostic tool for distinguishing benign from malignant nasopharyngeal masses in cats. For differentiation of lymphoma and lymphoid reactions histopathological confirmation is recommended.

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Upper airway obstruction in cats is common and can be life-threatening because of cats inability or unwillingness to breathe through the mouth (Hunt et al 2002). While clinical presentation can help to localise disease, signs of upper airway obstruction are variable and non-specific to any disease process. Investigation of nasopharyngeal diseases in particular, because of its relatively inaccessible location dorsal to the hard and soft palate, has in the past often been limited to digital palpation and dental mirror observation (Hunt et al 2002). More recent papers (Allen et al 1999, Willard and Radlinsky 1999, Griffon 2000a, 2000b, Hunt et al 2002) highlight the usefulness of endoscopic evaluation of the nasopharynx in cats when respiratory signs such as stertor, nasal discharge, sneezing, phonation change and dysphagia are present. Flexible endoscope examination permits the recognition of a wide range of nasopharyngeal abnormalities such as foreign bodies (Riley 1993), stenosis, choanal deformation (Willard and Radlinsky 1999), abscesses and benign or malignant masses (Malik et al 1997, Allen et al 1999, Hunt et al 2002).

Masses that grow in the nasopharyngeal region can arise from any of the cells found in that region and may originate in any structure comprising its walls or boundaries, including the base of the skull, eustachian tubes, soft and hard palate, caudal nasal turbinates and pharyngeal mucosa (Hunt et al 2002).

Definitive diagnosis of a nasopharyngeal mass must be achieved by microscopic examination of cytological and/or histological representative samples as gross and endoscopic features of these lesions are non-specific. Fine-needle aspiration

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biopsy, tissue dislodging by nasal flushing, blind biopsy by specialised nasopharyngeal forceps, biopsy taken by cranial retraction of the soft palate and endoscopic biopsies under direct observation have all been described (Hunt et al 2002). Blind biopsies or nasal flushing could lead to an inaccurate diagnosis, especially in masses located in the hard palate region (Allen et al 1999).

Tissue samples retrieved from endoscopic biopsies can be used both for cytology and histology. This small tissue sample can be gently dabbed against a microscope slide to produce an impression smear (Willard and Radlinsky 1999). A 'squash technique' for endoscopic-guided nasal biopsies has been described in the cytological diagnosis of nasal aspergillosis of the dog (De Lorenzi et al 2006).

The purpose of this study was to assess the efficacy of squash-preparation cytology in providing an accurate diagnosis in cats with endoscopically detected nasopharyngeal masses, with special reference to making a distinction between benign and malignant growths. We calculated diagnostic accuracy estimates and a good general agreement was found between cytological findings and histological diagnosis.

Materials and methods

Medical records of cats that had endoscopic examination of nasal cavities and nasopharynx at the Clinica Veterinaria San Marco between January 2003 and December 2006 were reviewed. Inclusion criteria were the detection of one or more nasopharyngeal masses during nasopharyngoscopy, the presence of adequate squash-preparation cytological samples from endoscopic biopsies and of a definitive histopathological diagnosis from endoscopic biopsies. In addition, in the presence of a co-existing nasal mass adequate squash-preparation cytological samples and a conclusive histopathological diagnosis were also considered as inclusion criteria.

In all cases the relevant clinical data (signalment, vaccination status, history and clinical signs, results of blood count, chemical profile, urinalysis and coagulation screening) and radiological findings (radiography and/or computed tomography) were available.

All endoscopic procedures were performed under inhalatory general anaesthesia. For anterograde rhinoscopy a rigid endoscope (K Storz—diameter 2.7 mm, length 18 cm, forward oblique 30°, 64018BS) was used while nasopharyngoscopy was accomplished with a flexible bronchoscope retroflexed over the soft palate (K Storz—diameter 5.2 mm, length 85 cm, 60001VL) during the same anaesthetic period.

When there was a mass in the nasal cavity and/or rhinopharynx, multiple biopsies were taken under direct endoscopic examination using a rigid endoscopic biopsy forceps for anterograde rhinoscopy (K Storz, 723036) and a flexible biopsy forceps inserted in the working channel of the fibroscope (K Storz, 60001 KL) for nasopharyngoscopy (Fig 1); cytological samples were prepared by a 'squash technique', firmly squashing the small biopsy fragment between two microscope slides until it becomes a thin film. The two slides were then separated with a direct, rapid movement avoiding smearing the cells. Cytological samples were air dried then stained with May−⊜ Grünwald Giemsa (MCG) stain in an automatic slide stainer (7100 Aerospray Slide Stainer; Wescor-Logan, UT); all the cytological samples were examined by the same cytopathologist (D,D,L,).

All histological specimens obtained from abnormal tissue during endoscopy were placed on biopsy sponges, fixed in 10% neutral-buffered formalin, processed and embedded in paraffin wax; 4-μm sections were stained with haematoxylin and eosin, and then evaluated by a pathologist who was unaware of the cytological diagnosis.

Cytopathological specimens were classifieds, on the basis of cytomorphological criteria (Andreasen et al 1999, Baker and Lumdsen 2000a, Burkhard et al 2001), into four groups: (1) benign mass



Fig 1. Endoscopic guided forceps biopsy from a small nasopharyngeal mass.

(including inflammation, hyperplasia and benign neoplasia), (2) lymphoma, (3) malignant epithelial proliferation (carcinoma) and (4) malignant mesenchymal proliferation (sarcoma).

The diagnostic accuracy of cytopathology in discriminating malignant tumours from benign inflammatory/hyperplastic masses was calculated by considering the histopathological diagnosis as the 'gold standard' cytopathological diagnoses were compared with final histopathological diagnoses and indices of diagnostic test accuracy (sensitivity, specificity, positive predictive value, negative predictive value, overall accuracy) were calculated. For all the indices the 95% confidence interval (95% CI) was calculated according to the following formula (Cockcroft and Homes 2003):

$$\mathrm{CI} = p \pm z \times \sqrt{\frac{p \times (1-p)}{n}}$$

where p = proportion (eg, number in sample group/number of samples examined); z = standardised normal deviate (when $z = 1.96$ the formula determines a 95% confidence interval); n = number of samples examined.

The formula was implemented as a function of the software R (R Development Core Team 2003).

Results

Only 30 out of 38 cases with an endoscopically detected nasopharyngeal mass were included in this study (retrieval rate 79%); for the eight remaining cases three were excluded because cytological samples were considered inadequate (two cases with only blood cells and one case with only necrotic debris and blood), two cases were excluded because of an inconclusive histological diagnosis (one case with only necrosis and one case with only a few superficial ciliated respiratory cells and blood clots), one case was excluded for the lack of cytological samples and the last two cases were excluded for the lack of both cytological samples and a definitive histological diagnosis.

In 12 out of 30 cases (40% of total cases) the presence of a coexistent nasal mass was endoscopically detected.

The 30 cats included in this study were 17 males and 13 females, with a mean age of 8.5 years (range 10 months−16 years); breeds included mixed breed (21), Siamese (three), Persian (three), Ragdoll (one), Abyssinian (one) and Birman (one).

The results of cytological and histological findings are shown in Tables 1 and 2.

Based on 30 nasopharyngeal masses with both adequate cytological and histological diagnosis, our results showed an overall 90% (27/30) accuracy between the diagnosis of benign inflammatory/hyperplastic diseases versus neoplasia obtained by squash preparation and histology, with a sensitivity of 94% (95% CI 0.47−1.40), a specificity of 81% (95% CI 0.78−0.83), a predictive value of a positive test of 90% (95% CI 0.76−1.03) and a predictive value of a negative test of 90% (95% CI 0.88−0.91).

In general, considering all the cases with an adequate sample for cytology, for histology, or for both, the biopsy of nasopharyngeal masses under direct endoscopic evaluation lead to an accurate sampling in 36 out of 38 cases (94.7%).

All the five cases of malignant epithelial tumours, the two cases of malignant mesenchymal tumours and the five cases of nasopharyngeal polyps, as diagnosed by histology, were correctly diagnosed by cytology. Cytology was in disagreement with histology in three cases: a lymphoma was diagnosed as benign lymphoid hyperplasia and two lymphoid hyperplasia were erroneously diagnosed as lymphoma.

Table 1. Diagnoses of 30 nasopharyngeal masses from cats according to cytological and histological findings

Cytological diagnosis	Histological diagnosis				
	Benign inflammatory/ hyperplasia	Lymphoma	Carcinoma	Sarcoma	Total
Benign inflammatory/hyperplasia	9	1	–	–	10
Lymphoma	2	11	–	–	13
Carcinoma	–	–	5	–	5
Sarcoma	–	–	–	2	2
Total	11	12	5	2	30

Table 2. Comparison of benign and malignant masses made by cytology and histology in a series of nasopharyngeal masses in 30 cats

Cytological diagnosis	Histological diagnosis		
	Malignant neoplasia	Benign neoplasia	Total
Malignant neoplasia	18 true positive	Two false positive	20
Benign neoplasia	One false negative	Nine true negative	10
Total	19	11	30

In seven out of 12 cases (58.3%) in which the coexistence of a mass was endoscopically detected both in the nasopharynx and nasal cavity there was a corresponding cytological diagnosis from squash preparation of the two different masses and histological diagnosis (five cases of lymphoma and two cases of malignant epithelial tumours); in the remaining five cases (one lymphoma, two malignant epithelial tumours and two malignant mesenchymal tumours) biopsy sampling from nasal masses was considered inadequate because of the presence of necrotic tissue and mixed inflammatory cells. In all those five cases biopsy samples from the coexistent nasopharyngeal mass were considered diagnostic and in concordance with the histological diagnosis.

Discussion

This study comprises the largest case series of feline nasopharyngeal masses to be characterised both cytomorphologically and histomorphologically by endoscopic biopsies.

Nasopharyngeal masses can be broadly classified into benign (inflammatory/hyperplastic) or malignant but this distinction cannot be made with direct observation or by using diagnostic aids such as imaging and endoscopy. As previously stated for nasal masses (Caniatti et al 1998), a definitive diagnosis requires cytological and/or histological evaluation from adequate biopsy samples. While different surgical and non-surgical techniques have been described and compared for the collection of cytological samples from nasal masses both in dogs and cats (Clercx et al 1996, Caniatti et al 1998), to the best of authors' knowledge no similar studies of nasopharyngeal masses have been reported.

Nasal cytological specimens may be collected by several procedures (nasal flushing, swabbing, brushing, fine-needle aspiration, impression and squash preparation from blind or endoscopic-guided biopsies) but many of these techniques produce unpredictable results and have an unacceptably low yield of diagnostic material (McCarthy 2005). One of the main disadvantages of many among the above-mentioned collection techniques is that the biopsy is taken blindly. For this reason, only in the presence of large masses or diffuse inflammation will this approach produce diagnostic samples.

Small tumours can be missed and inflammatory cytological findings cannot differentiated between an accurate diagnosis and a missed lesion.

The same problems have been proposed for nasopharyngeal masses (Allen et al 1999). Many of the described collection techniques (fine-needle biopsy through the soft palate in palpable lesions, anterograde flushing, specialised nasopharyngeal forceps) are undertaken without direct evaluation of the nasopharyngeal area and this can lead to an inaccurate or missed diagnosis especially when the mass is located in the hard palate region (Allen et al 1999).

Endoscopy allows direct visualisation of the nasopharyngeal and choanal area and provides for gross diagnosis and selection of an appropriate sample site before the collection (Fig 1). It can be difficult to advance a biopsy tool through the retroflexed endoscope; however, this problem can be minimised by passing the forceps before insertion and retroflexion of the instrument (Hunt et al 2002). Biopsy forceps can be placed under direct visualisation allowing multiple sampling from an adequate site or from different sites of the same mass. In addition, intermittent irrigation can be used to clean the fibrescope lens and remove blood or exudates, and maintain a clear visual field during the different biopsy procedures.

Inadequate samples were obtained in three cytological and two histological samples but in all the cases a definitive diagnosis was possible because of the good quality of both histological and cytological samples. In another of the excluded cases (absence of cytological samples) the histological samples lead to a definitive diagnosis of a hyperplastic/inflammatory lesion while in the last two excluded cases (absence of cytological samples) the histological samples were considered inadequate because of the presence of necrotic debris, scattered neutrophils and blood.

Biopsy samples have been used for cytological evaluation by gently dabbing the small tissue

fragment against a microscope slide to produce an impression smear (Clercx et al 1996, Willard and Radlinsky 1999). While the obvious advantage of this technique is that the same sample can be used both for cytology and histology, in authors' opinion the impression smear has several disadvantages: first, often the samples are extremely soft and the impression can cause their complete disruption producing samples that are no longer suitable for histology. Second, in hard biopsy samples, such as fragments from mesenchymal tumours, the cells adhere to the slide in very small numbers, often leading to an inadequate, hypocellular sample. In addition, samples collected by endoscopic forceps are usually small (only a few mm^3) and the apparently simple manoeuvre to dab the biopsy while holding it with dissecting forceps can be difficult if not impossible.

With the squash technique both the cytopathologist and the pathologist can study the same amount of cells having the same likelihood of examining an adequate sample, because cell cohesiveness does not influence the adequacy of the sample.

A comparison between imprint and brush cytology from nasal tumours of the dog was reported by Clercx et al (1996). In this work malignancy was significantly more frequently diagnosed by imprint cytology (81% of the cases) than by brush cytology (56%). In particular, imprint cytology determined a correct diagnosis of epithelial tumour in 88% of cases and a correct diagnosis of mesenchymal tumour in only 50% of cases.

In this study our results showed an overall 90% (27/30) agreement between cytology obtained by squash preparation and histology; considering neoplastic lesions only (19 out of 30 total cases) a correct cytological diagnosis was made in 18 out of 19 cases (94.7%) with all epithelial and mesenchymal tumours correctly diagnosed by squash-preparation cytology.

A possible disadvantage of the squash-preparation technique is that it cannot be easily used when biopsy fragments are too tough or fibrotic to spread on a slide. However, this happens only rarely as nasal and nasopharyngeal tumours usually have a soft consistency and an inadequate preparation is the exception rather than the rule.

Another possible problem linked to squash-preparation samples can be the increased rupture of cells caused by an unnecessary excessive vertical pressure between the two slides (Baker and Lumdsen 2000b). In our series of cytological samples no slide was considered inadequate because of too many ruptured cells; in particular all the squash preparations from nasopharyngeal lymphomas (traditionally neoplastic lymphoid cells are considered to be very fragile) were considered diagnostic (Fig 2).

Even if nasopharyngeal disease is considered a common event in cats with upper respiratory disease (Allen et al 1999), the true incidence of nasopharyngeal masses is actually unknown in this species.

In a previous study (Allen et al 1999), in a series of 53 cats with nasopharyngeal disease the most frequently diagnosed tumour was lymphoma, accounting for 49% of the nasopharyngeal masses, while in a more recent work (Hunt et al 2002) neoplastic masses accounted for only 29% of nasopharyngeal diseases but lymphoma was the most frequent malignant tumour (six of seven malignant tumours).

In the present study 19 out of 30 nasopharyngeal masses were malignant tumours and the most frequent tumour was lymphoma, accounting for 63.1% (12 out of 19) of all malignant masses, in agreement with the studies of Allen and Hunt (Allen et al 1999, Hunt et al 2002).

Cytopathological interpretation disagreed with histopathological diagnosis in three cases: two cases of benign reactive lymphoid hyperplasia were cytologically diagnosed as lymphoma and one lymphoma was cytologically diagnosed as reactive lymphoid hyperplasia. Even if the morphology of respiratory tract lymphomas resembles that seen in other sites, to distinguish lymphoma from reactive lymphoid hyperplasia (Fig 3) can be problematic (Burkhard et al 2001). In some cases malignant lymphoproliferative

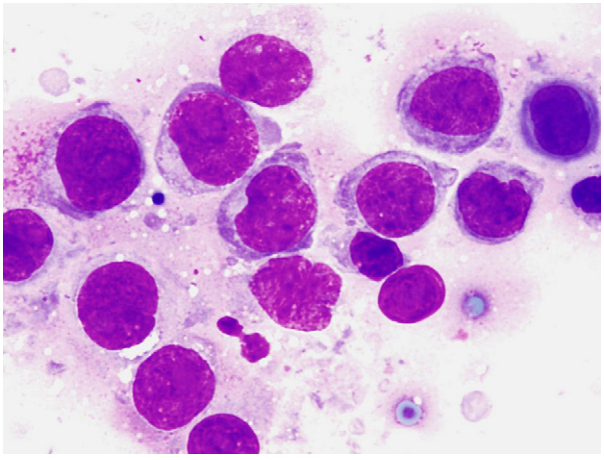


Fig 2. Squash-preparation from nasopharyngeal lymphoma: all the lymphoid, atypical blasts in the photomicrograph are well preserved and perfectly recognisable (MGG 100×).

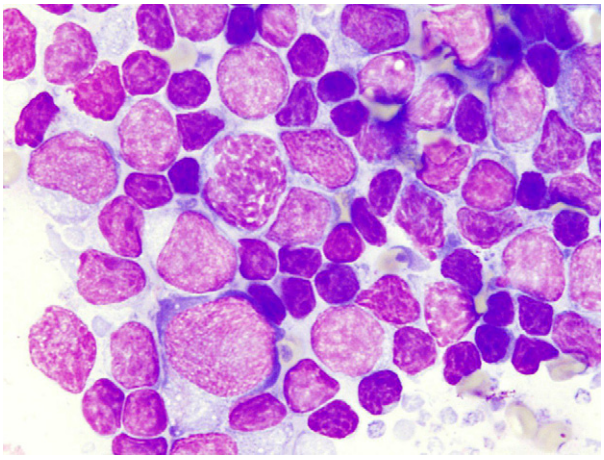


Fig 3. Squash-preparation from nasopharyngeal lymphoid hyperplasia: many small lymphocytes are present along with increased medium-sized lymphocytes and an immature lymphoid cell; a false positive diagnosis of lymphoma can be caused by overestimation of the medium-sized and large immature lymphoid cells (MGG 100×).

diseases are cytologically characterised by a predominance of medium-sized lymphocytes with smooth chromatin nuclei and the absence of nucleoli and some case have been described in which the neoplastic population consists of well differentiated, small lymphocytes (Caniatti et al 1998). On the other hand, false positive results can be caused by overestimation of the medium-sized or large immature lymphoid cells that are a normal component in lymphoid hyperplasia.

For this reason, as a general rule, we suggest routine storage of one or more of the biopsies in formalin while squash-preparation cytology is undertaken, in order that histology may be performed subsequently should squash-preparation cytology be inconclusive; furthermore, histology can help to eventually better define the tumour grade, as this cannot always be undertaken by cytological evaluation.

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