

Case Report

Nasopharyngeal malignant amelanotic melanoma in a gelding age 9 years

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Introduction

Tumours involving the pharynx and guttural pouches are rare in equidae. Most reports on pharyngeal tumours are squamous cell carcinomas (Sundberg *et al.* 1977; Hance and Bertone 1993; Jones 1994; Tuckey *et al.* 1995). There have also been reports of lymphosarcoma and mast cell tumour (Adams *et al.* 1988; Hance and Bertone 1993; Richardson *et al.* 1994). Melanomas of the retropharyngeal and adjacent parotid tissues are well recognised in grey horses (Hance and Bertone 1993; Valentine 1995; Fintl and Dixon 2001; May and Howard 2001). These horses usually have obvious, discrete, subcutaneous swelling(s). It is common for these animals to live with the condition for years with no effect on their lifespan. Reports on guttural pouch neoplasia include haemangiosarcoma (Hance and Bertone 1993; Baptiste *et al.* 1996), haemangioma (Greene and O'Connor 1986), squamous cell carcinoma (Trigo and Nickels 1981; Hance and Bertone 1993), fibroma (Merriam 1972) and melanoma (Hance and Bertone 1993; Baptiste *et al.* 1996; Fintl and Dixon 2001; May and Howard 2001).

Here, we report a case with an amelanotic malignant melanoma arising from an unusual site and in which some of the presenting signs and clinical investigations inferred other disease processes.

Case details

History

A 9-year-old dark brown gelding was presented with severe halitosis of one month duration. The horse also had an intermittent bilateral mucopurulent nasal discharge and occasional cough.

Clinical examination

The horse was bright and in good bodily condition. Pulse rate,

respiratory rate and body temperature were within normal limits. Chest auscultation was unremarkable. Scant bilateral nasal mucoid discharge was present (primarily left-sided) with marked halitosis. Palpation of the submandibular and parotid regions did not reveal any abnormalities. The gelding was exercised for a few minutes before endoscopy and the taking of a tracheal wash. The horse moved well and there was no untoward respiratory noise.

Endoscopy

Endoscopic examination of the upper respiratory tract revealed a 2 cm fissure within the roof of the nasopharynx, left of midline, at the level of the guttural pouch ostia (**Fig 1**). Exploration of this site with an endoscopic biopsy instrument yielded scant malodorous, necrotic debris. Samples were taken for bacterial culture. A large (approximately 35 mm diameter) nodular swelling in the roof of the pharynx (mainly left of midline) was present, immediately rostral to the larynx, and partially obscured the left arytenoid cartilage. A moderate amount of mucus was present within the right guttural pouch. Within the left pouch, a large smooth round nodular (50 mm diameter) mass protruded through the ventral aspect of the medial compartment. A 3.8 cm (1.5 inch) 16 gauge needle (attached to a length of polyethylene tubing placed down the endoscope biopsy channel) was inserted into the mass to a depth of approximately 10 mm. Attempts at aspiration were unsuccessful.

Radiography

A 30° oblique projection was taken of left and right sinuses. These were unremarkable. A series of standing lateral radiographs was taken of the pharynx, larynx and guttural pouches. A large, soft tissue density, spherical swelling within the floor of one guttural pouch (left from endoscopy) and, rostrally, a more diffuse swelling occupying the rostroventral wall of the guttural pouches were present. There was marked thickening of the soft tissues between the guttural pouches and

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Fig 1: Endoscopic appearance of the defect in dorsal nasopharynx (x-x) situated at the level of the guttural pouch ostia.

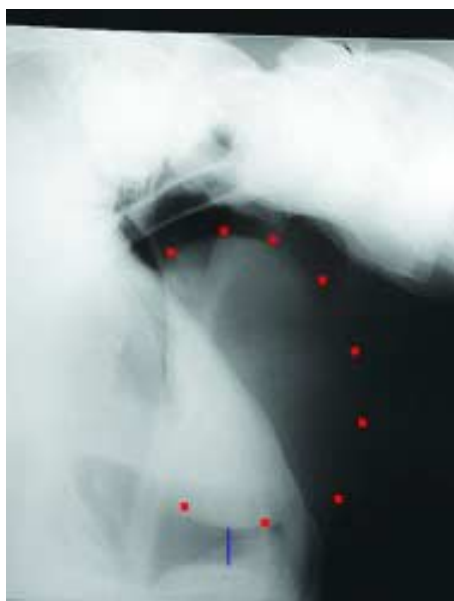


Fig 2: Standing lateral radiograph of pharynx, larynx and guttural pouches showing a large soft tissue mass within a guttural pouch (red markers). There is marked soft tissue thickening between the guttural pouches and pharynx and considerable ventral deviation of the dorsal pharynx (blue line).

dorsal pharynx causing pronounced narrowing of the pharyngeal airway and ventral compression of the larynx (Fig 2).

Clinical pathology

Blood analysis

Samples recovered at initial examination revealed a white blood cell count $9.28 \times 10^9/l$ (laboratory range $6-10 \times 10^9/l$). Differential white cell counts were within normal ranges, 54% neutrophils, 39% lymphocytes and 7% monocytes. Serum fibrinogen was 3.58 g/l (normal range 1.5–4.0 g/l).



Fig 3: Ultrasonogram of the right parotid region of the neck showing a single large echolucent mass starting at a depth of 50 mm from the skin surface (x markers).



Fig 4: Ultrasonogram of the left parotid region showing a conglomerate of 3 echolucent masses (x markers).

Tracheal wash results

Cytological analysis was unremarkable and bacterial culture negative.

Samples from pharyngeal fissure

Bacteriology yielded moderate mixed growths of β haemolytic *Streptococcus* spp. and *E. coli*. These were regarded as nonsignificant.

Differential diagnoses

Three candidates were considered: 1) retropharyngeal lymph node abscessation (i.e. *Streptococcus equi* or a similar organism); 2) foreign body penetration through the roof of the pharynx and 3) neoplasia.

Retropharyngeal abscessation was considered to be the probable diagnosis. A pharyngeal foreign body penetration was thought unlikely, as the fissure was present in the nasopharynx rostral to the caudal margin of the soft palate.

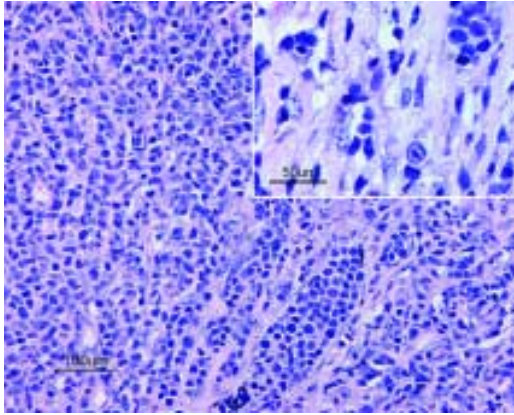


Fig 5: Nasopharyngeal mass histopathology. Densely packed plump polygonal nonpigmented neoplastic cells. In other areas cells are loosely packed and have a round to elongated profile (*inset*). H&E.

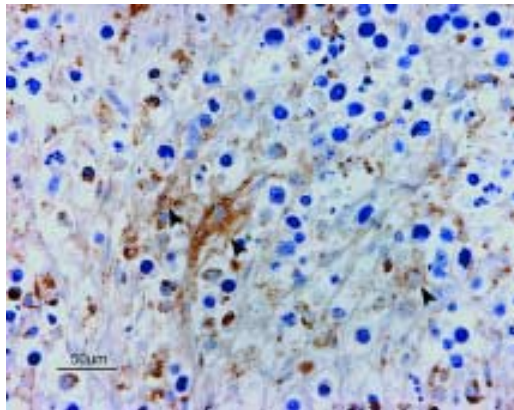


Fig 6: Nasopharyngeal mass histopathology. Numerous round and fusiform neoplastic cells stain positive for melanin (*arrowheads*). EnVision+ peroxidase stain with Mayer's haematoxylin counterstain.

Such an injury would usually be found more caudally in the nasopharynx. A neoplastic process was considered unlikely.

Further course and investigation

The horse was discharged and the owners instructed to monitor the horse for 2 weeks. No treatment was given as it was hoped that, with time, any retropharyngeal abscesses would rupture. The owners were instructed to check daily the horse's general demeanour and temperature, and for signs of dyspnoea.

The owners reported that there had been no change in the horse's demeanour over this 2 week period. The gelding coughed at times, an intermittent bilateral nasal discharge was present and the halitosis had worsened. When brought in from the field on one day there was some grass-stained nasal discharge.

The horse was re-examined after the 2 week period. Radiography and endoscopy revealed that the nasopharyngeal and guttural pouch swellings had increased in size. Haematology remained normal. With the horse under sedation, it was possible to palpate some firm swellings of the dorsal laryngeal and pharyngeal regions. A discrete nodular



Fig 7: View of the medial compartment of the left guttural pouch at necropsy. The left mandible has been removed. Two tumours are present. The more rostral has a necrotic core with a communication into the nasopharynx.

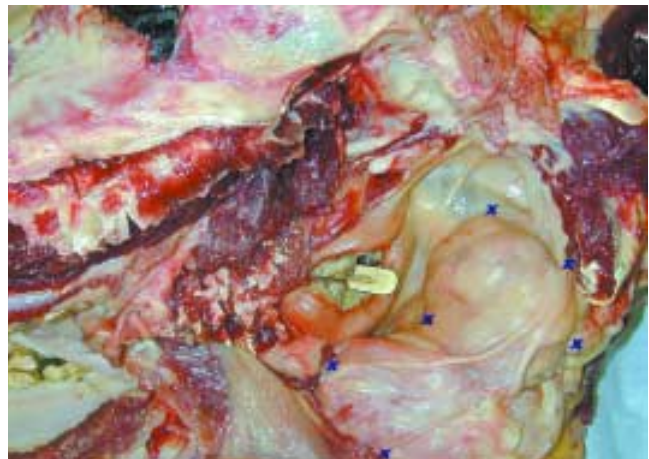


Fig 8: View of left pharyngeal region at necropsy. Left mandible and left stylohyoid bone have been removed. The periphery of the large tumour mass, the dorsal part of which lies within the left guttural pouch, is marked by *x*. The scalpel handle in the rostral guttural pouch mass demonstrates the communication into the nasopharynx.

mass was palpable on the right side and a larger, more irregular, firm swelling was palpable on the left.

Ultrasonography

An ultrasonic sector scanner (Ausonics Impact) with a 3.5 MHz transducer was used to examine the above-mentioned region from both right and left sides. The right side (**Fig 3**) showed a well-circumscribed 80 x 60 mm echolucent mass at a tissue depth of 50 mm. The left side (**Fig 4**) showed a conglomerate of 3 smaller swellings at a similar depth from the skin surface. These swellings had identical ultrasonographic appearances. A 7.6 cm (3 inch) 19 gauge spinal needle was unsuccessfully used in an attempt to obtain an aspirate from the right mass.

The appearance of the ultrasonograms suggested fluid-filled masses, perhaps abscesses, and a decision was made to investigate further with exploratory surgery.

Surgery

A standing tracheostomy was performed prior to induction. Anaesthesia was induced, the horse placed in dorsal recumbency and anaesthesia maintained with an endotracheal tube via the tracheostomy. With the use of a Hausmann's gag, a hand was placed through the mouth and into the pharynx. The swelling depressing the nasopharyngeal roof felt very firm. The fissure just rostral to the pharyngeal recess could be palpated, with difficulty, by passing fingers around the caudal margin of the soft palate and rostrally into the nasopharynx. A precision-cut biopsy needle¹ was passed into the larynx via an incision through the cricothyroid membrane and then guided into the mass, with a hand placed through the mouth. Assistance was needed to take a series of biopsies. The biopsy tissue was pale yellow, of even consistency and firm.

The horse was then placed in left lateral recumbency and the right pharyngeal region prepared for aseptic surgery (chosen as the swelling was most palpable on this side). A 5 cm incision was made through Viborg's triangle followed by careful blunt dissection. An 8 mm punch biopsy instrument² was used to take a core of tissue which was similar in appearance to the other biopsies. Wound closure was routine, recovery from anaesthesia uneventful, and a tracheostomy tube was left in place for one day following surgery. All samples were fixed in 10% formal saline and sent for histopathological analysis.

Histopathology

A neoplasm was identified consisting of densely packed polygonal (epithelioid) to fusiform cells (**Fig 5**). Cells had variable amounts of finely granular cytoplasm, indistinct cell borders and variably sized round to oval and frequently indented nuclei (10–15 µm diameter). Nuclear chromatin was coarse with an occasional single nucleolus. Mitoses averaged 3/high power field. Tumour cell apoptoses were common and scattered randomly. Neoplastic cells often formed streams and bundles separated by dense collagenous connective tissue. In some areas, cells were loosely packed and were round to ovoid (**Fig 5, inset**). Moderate numbers of randomly scattered lymphocytes and neutrophilic leucocytes were present. The histological appearance was of a malignant anaplastic tumour.

Immunohistopathology

Immunohistology for melan A (Clone A103; H7196)³, cytokeratins (Clone AE1/AE2; H3515)³ and vimentin (Clone V9; H0725)³ was performed on representative sections (EnVision+ peroxidase, Mayer's haematoxylin counterstain)³. Variable numbers of tumour cells were positive for melan A

(**Fig 6**) and vimentin. Neoplastic cells were negative for cytokeratins. Immunohistology led to a diagnosis of a malignant amelanotic melanoma.

Various treatment options were discussed with the owner, including the use of cimetidine and radiation therapy. Due to the location of the neoplastic masses, surgical removal was not considered to be an option. The prognosis was poor and the owner consented to euthanasia.

Post mortem

Gross changes were confined to the head and neck. There was a large (50 mm diameter) tumour protruding through the floor of the medial compartment of the left guttural pouch. A second, centrally necrotic mass was present in the rostral wall of the same pouch (**Fig 7**). A communication between the second mass and nasopharynx (fissure in dorsal roof) was also present. This would have accounted for the halitosis. The mass in the floor of the left guttural pouch medial to the stylohyoid bone represented the dorsal portion of a large (90 x 100 mm) multilobulated, firm, pale tan-yellow neoplasm between the roof of the pharynx and left guttural pouch (**Fig 8**). This tumour compressed the roof of the pharynx, larynx and oesophageal orifice. Two smaller nodular masses were present in the right pharyngeal region, the larger of which had been biopsied during surgery. There was no evidence of metastases within local lymph nodes or other organs. There were no other significant lesions.

Discussion

In this case, the main presenting sign was halitosis with a history of an intermittent nasal discharge. In most instances this would indicate a sinus or tooth root infection. Radiography eliminated such a diagnosis. A draining retropharyngeal abscess was then considered to be a probable cause of the halitosis. Some of the history and clinical investigation results supported such a diagnosis.

The horse had recently been treated for a respiratory infection. The peripheral white cell count and fibrinogen analyses were within our normal laboratory ranges. The lateral pharyngeal radiographs showed considerable soft tissue swelling, dorsal to the larynx, and guttural pouch involvement. These could have been interpreted as abscesses. Endoscopy revealed ventral deviation of the roof of the nasopharynx just rostral to the larynx. This was initially considered to be the result of an extrapharyngeal abscess and the large mass protruding up through the floor of the medial compartment of the left guttural pouch was also thought to be an abscess. The second, centrally necrotic, guttural pouch mass and its communication into the nasopharynx was missed during this examination. The fissure in the pharyngeal roof was considered to be the site where an abscess had ruptured. Ultrasonography of tissues dorsal to the pharynx demonstrated well-demarcated echolucent structures. Attempts at aspiration failed and therefore did not provide information to exclude differential diagnoses.

It was surprising that this horse showed no signs of dyspnoea. The grass-stained nasal discharge, noted on one occasion, was the only clinical sign that indicated diseases of the pharynx and/or guttural pouches. This case shows that the slow onset in clinical signs and difficulty in visualising or recognising pharyngeal masses can allow for severe enlargement and extensive tissue invasion of tumours prior to presentation for examination and treatment.

Melanomas in the horse have been recognised for over 2 centuries, primarily in grey horses, and are often cutaneous in origin (Rooney and Robertson 1996). It has been suggested that 80% of grey horses greater than age 15 years are likely to have melanomas (Stannard 1972). Melanomas are less common in nongrey horses, although in one report 15 out of 53 horses with melanomas were coloured other than grey (Valentine 1995). The true amelanotic form is rare in the horse (Johnson 1998). Of the melanomas in the study of Valentine (1995), just 2 out of 53 were poorly pigmented. These were malignant melanomas and, interestingly, in nongrey horses considerably older than in our case. The tumour tissue submitted in this case represented an amelanotic malignant melanoma (without melanin pigmentation).

In horses, melanocytes and melanophages preferentially accumulate to form tumours in the skin of the perineum, under the tail and in the parotid region within the parotid lymphoid tissue. The presence of parotid lymphoid tissue is variable in horses and, when present, this tissue lies medial to the parotid salivary glands (Johnson 1998). Horses with melanomas of the parotid region almost invariably have obvious external swellings (Fintl and Dixon 2001; May and Howard 2001). This case was unusual, as there was no external evidence of the tumour and the primary mass (largest) was subepithelial in origin, being situated in the connective tissue between the nasopharynx and the left guttural pouch. The tumour did not present as an exophytic nodular mass of the parotid region.

The current World Health Organisation classification of melanocytic tumours in domestic species has a classification of melanocytoma, melanoacanthoma and malignant melanoma (Goldschmidt *et al.* 1998). In our case, there was no gross evidence of melanin pigmentation and so a definitive diagnosis was not possible until completion of both histopathology and immunohistology. Histopathology indicated that the tumour was poorly differentiated and it was classified as a malignant (anaplastic) tumour. It has been stated that such anaplastic, melanocytic tumours require immunohistology in order to reach a diagnosis (Hellquist 1989; Ramos-Vara *et al.* 2000). The immunohistology in this case revealed that the tumour cells were variably positive for melan A and vimentin. These findings, together with the histopathology, gave a diagnosis of a malignant (anaplastic), amelanotic melanoma.

Malignant melanomas are composed of variably pleomorphic cells with variable amounts of melanin pigmentation and numerous mitotic figures. In horses, this condition is usually seen in older individuals that are not always grey. There have been few studies published on malignant melanoma in horses and even those include

relatively low numbers of cases, and often do not include oral or pharyngeal forms. Amelanotic malignant melanomas are regarded as highly metastatic, leading to widespread dissemination within months of diagnosis (Valentine 1995). In our case, no tumours were found in association with the skin, no distant metastasis were found and the primary tumour was considered to be one of the nasopharyngeal masses.

The behaviour of equine malignant melanomas has been described previously (Scott 1988; Yager *et al.* 1993) and generally they show a rapid and invasive growth presenting as a dark pigmented soft tissue mass. Melanocytic tumours are common only in the dog and horse, uncommon in the cat, and rare in other species except the pig, in which melanocytic tumours have been seen in several breeds (Scott 1988). Oral melanomas in the dog, for example, usually have a predilection for the gums, palate and lips, whereas in the cat, they are uncommon but half are usually malignant (Pulley and Stannard 1990).

This case outlines the problem in diagnosing amelanotic malignant melanomas both clinically and histopathologically in domestic animals. Immunohistochemistry is often needed to obtain a definitive diagnosis.

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Manufacturers' addresses

¹Becton-Dickinson UK Ltd, Oxford, Oxfordshire, UK.

²Kruuse UK Ltd, Leeds, Yorkshire, UK.

³Dako Cytomation Ltd., Ely, Cambridgeshire, UK.

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Clinical Commentaries

The spectrum of equine melanocytic tumours

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This case illustrates the fact that equine **melanocytic tumours are not a single tumour type**, but rather exhibit a spectrum of clinical and histopathological features. Cutaneous and mucocutaneous melanomas occurring in aging grey horses are the most common, but are by no means the only type of melanocytic tumour that occurs in the horse.

Over the years, many sweeping generalisations have been made regarding 'equine melanoma'. Some have stood the test of time, and others have not. It is probably true that almost all grey horses develop melanoma, if they live long enough. It is not true, however, that melanocytic tumours are rare in horses less than age 6 years, or that all melanomas in nongrey horses are malignant.

Melanocytoma (melanocytic nevus) is a benign tumour that is most common in the skin of horses less than age 6 years and can even be present at birth. Melanocytoma affects nongrey and grey horses equally.

There is, however, an aggressive form of malignant melanoma that occurs in older horses, most of which are not grey. The most common equine melanocytic tumour, melanoma of ageing grey horses, is not a single entity either, but instead exhibits 2 distinct clinical syndromes. Some ageing grey horses develop one to several relatively discrete tumours

that can often be excised surgically, whereas others develop a melanomatosis syndrome in which multiple, often contiguous, tumours occur that defy surgical intervention.

Typical tumour location varies depending on the tumour type. Melanocytoma occurs most often on the neck, trunk and legs, whereas **grey horse melanomas** occur most frequently in the skin of the perineum, underside of the tail, prepuce and lips and in the parotid gland. **Anaplastic malignant melanoma**, of which this case represents a form, appears to have the most diverse range of locations. This case describes **anaplastic malignant melanoma** occurring within the nasopharyngeal region; this tumour can also occur in such unusual locations as bone and hoof wall.

Tumour behaviour depends on the tumour type. Melanocytoma is typically noninvasive and cured readily by surgical excision. Melanomas of ageing grey horses are unique and do not conform to the melanocytic tumour classifications applied to other species, especially as regards behaviour. **Melanomas occurring in ageing grey horses** are typically slow growing, but are locally invasive and will eventually metastasise. **Metastatic grey horse melanoma** may or may not, however, be clinically significant. This behaviour is of a **low grade malignant tumour**, which

makes use of the term 'malignant' when referring to equine melanocytic tumours somewhat confusing. **Use of the term anaplastic malignant melanoma** has been suggested as a way to avoid this confusion, as the melanomas of ageing grey horses do not display cellular anaplasia. Anaplastic malignant melanoma exhibits aggressive local invasion and is highly prone to early metastasis.

This case is unusual in that the tumour occurred in a relatively young horse (age 9 years) compared to previous reports of equine anaplastic malignant melanoma. This case also illustrates **the difficulty pathologists have in definitively diagnosing tumours known as amelanotic melanoma.**

The production of melanin defines a melanocytic tumour and, by definition, amelanotic melanoma does not produce melanin. Therefore, it is not possible to make a definitive diagnosis of amelanotic melanoma on routine slide preparations. A poorly differentiated tumour of a single or mixed population of ovoid, spindle or epithelioid cells could be an amelanotic melanoma. The index of suspicion for this tumour is raised when such tumours arise within what we recognise as typical locations, such as the oral cavity of dogs.

Congratulations are in order to the pathologist in this case, who recognised that additional studies, to include a melanocyte marker, were indicated.

In years gone by, the diagnosis of amelanotic melanoma relied on identification of premelanosomes in ultrastructural preparations. Such preparations were labour-intensive and time-consuming, and it is likely that many amelanotic melanomas in animals have gone undiagnosed. The advent of immunohistochemical procedures for identification of specific cell markers has greatly advanced both our knowledge of the origin of tumours in animals and our ability to diagnose accurately many undifferentiated tumours.

Melan A, employed in the current case, is a specific melanocyte marker that has been validated for use in tumours in a variety of species. Another cell marker that could be employed in the diagnosis of equine melanoma, which is less specific for melanocytes but also more commonly available to most veterinary pathologists, is **S-100**.

Given the spectrum of melanocytic neoplasia that occurs in the horse, it is clear that any generalisations regarding equine melanocytic tumours must take into account the type of tumour in question, as must any future studies of tumour behaviour and response to treatment.

Diagnostic problems in nasopharyngeal malignant amelanotic melanomas

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This case report is useful in describing diagnostic problems encountered in investigation of chronic halitosis and nasal discharge. Endoscopy and ultrasonography revealed a nodular mass involving pharynx and guttural pouch. Critical laboratory investigation was confined to histopathology of needle and punch biopsies of the upper respiratory mass; this revealed a poorly differentiated malignant tumour, of uncertain specific derivation.

This report is also useful in indicating the value of immunohistology in identifying more confidently the tissue origin of this anaplastic tumour. Identification of the tissue origin of tumours has, in the past, depended on considerations of anatomical site and histological patterns that may more or less recapitulate the microscopic features of the tissue from which the tumour is derived. With well-differentiated benign and malignant tumours, such considerations often support a diagnosis that is reliable or suggestive, at least for clinical purposes. With **poorly differentiated malignant tumours**, however, such clues may not be present, so pathologists and clinicians may be left with only a provisional diagnosis of anaplastic malignant tumour. In the face of such

academic uncertainty, clinicians have to decide on treatment options on the basis of the anatomical site(s) involved, effect(s) of the lesions and clinical feasibility of surgical removal.

In recent years, more refined tissue diagnosis of tumours has been assisted greatly by the use of immunohistology to identify more restricted cellular features. The principle of this is the use of antibodies raised against cellular components that are differentially expressed by some, but not all, cells. Common examples of such 'cell markers' include intermediate filaments (desmin in muscle cells; vimentin in mesenchymal cells; cytokeratins in epithelial cells; neurone-specific-enolase in nerve cells). In this report, the presence of **positive staining for melan A** in anaplastic tumour cells supported the notion that the neoplasm was of melanocytic derivation. Such diagnostically pleasing observations should not absolve pathologists and clinicians from critical evaluation of immunostaining, which may be far from providing definitive sensitive and specific diagnoses in all cases. We need to remember that 'cell markers' are gene products that are expressed normally in normal tissues. However, since all normal diploid cells possess the complete genome, all cells can,

potentially, express all the gene products of the species. In both normal and neoplastic cells, gene expression may be variably up- or downregulated, so gene products in tumour cells may be present or absent in ways that are not always predictable or explicable. Furthermore, there are technical problems with immunostaining, associated with autolytic change, preservation or masking of antigens, variations in effect of fixatives and different tissue processing schedules.

Therefore, **in a practical clinical context**, immunostaining may be helpful in supporting a final tissue diagnosis if a large proportion of unequivocal tumour cells express a gene product (e.g. melanin). It is important that 'cell marker' positivity be confidently associated with intrinsic tumour cells and not be misinterpreted when positive cells are those of normal adjacent tissue (e.g. muscle) that is being infiltrated by neoplastic cells. In the present report, cells also expressed **vimentin**, normally

considered a marker of mesenchymal cells, yet melanocytes are of neuroepithelial (neural crest) derivation. This raises the question of whether such vimentin-positive cells are intrinsic to the tumour, or whether they represent pre-existing connective tissue that is being invaded by the malignant melanoma cells. The variable quality of immunostained sections, and high nonspecific background staining, may combine to make difficulties in critically deciding between such possibilities.

At the present time, immunostaining can therefore play a useful part in veterinary histopathology practice, but **as an adjunct to classical skills** of careful and critical assessment of all the relevant clinical and pathological observations. As with special histological stains, immunostaining can be diagnostically helpful for confirmatory and 'rule-out' purposes, but we should remain critical about its potential for providing specific diagnosis in all clinical situations.