Chronic lameness caused by vascular compression of the iliac artery and vein by a malignant melanoma in the pelvis of a grey Spanish gelding

Running title: Malignant melanoma causing chronic lameness

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Summary

An 11-year-old grey Spanish gelding was presented with chronic lameness of the right hind limb, with abduction of the limb during the swing phase. Based on a comprehensive lameness examination, the problem was localised in the pelvic region. Rectal and ultrasonographic examination revealed a mass in the pelvic region in close association with the iliac artery and vein, and ultrasonographic indications of vascular compression. Considering the clinical presentation of a grey middle-aged horse with multifocal melanoma-like nodules at the level of the perineum, tale base, preputium and penis, a malignant melanoma with secondary vascular or neurological compression was suspected. Post-mortem and histological examination confirmed the diagnosis of malignant melanoma with vascular compression of the iliac artery and vein and infiltrative growth in the iliac vein.

Keywords: melanoma; lameness; neoplasia; horse; orthopaedics

Introduction

Melanomas are typically observed in grey or white-coated horses, with a prevalence of approximately 80% in aged populations (>15 years), but with an age range from 2 to more than 15 years (Reed *et al.* 2010; Curik *et al.* 2013; Moore *et al.* 2013). They can develop by a neoplastic transformation of dermal melanocytes or melanoblasts, caused by genetic mutations in the melanin metabolic molecular pathway (Pielberg *et al.* 2000; Reed *et al.* 2010). Recent studies described that both the STX17 (syntaxin 17) and the neighbouring NR4A3 genes are overexpressed in melanomas from grey horses (Pielberg *et al.* 2000). Grey horses are more at risk to develop melanomas, possibly because the grey coat phenotype is associated with a duplication in intron 6 from the STX17 gene (Pielberg *et al.* 2000). Curik *et al.* (2013) described three pathologically defined clinical patterns: (1) the majority of cases

present as slowly growing melanomas over many years without evidence of regional or distant metastases; (2) malignant transformation of a benign melanoma (melanocytoma); and (3) the rare cases of melanomas that are malignant from onset. A recent review by Moore *et al.* (2013) described melanomas as a variably pigmented neoplasia with the capacity of local invasion and metastasis, and Cavalleri *et al.* (2014) reported that this condition may present itself in a more advanced stage as a multicentric malignancy (equine malignant melanoma).

Clinically, the majority of melanomas are diagnosed as dark pigmented, single, small, solid dermal nodules or as solitary or multiple infiltrating plaques with a slow growth (Moore *et al.* 2013). The most common external predilection sites are the ventral part of the tale, the perianal region, the commissures of the lips, the external genitalia, the eyelids, the parotid gland and the guttural pouches (Fintl and Dixon 2001; Jeffrey and Lembcke 2013).

In case of expansive growth, melanomas localised on the former locations can cause secondary problems like interference with bridle, saddle or halter, problems with bending the head in case of a melanoma in the parotid gland, problems with defecation in case of a large melanoma in the perianal region or problems with eating and drinking in case of a large melanoma on the lips (de Blaauw *et al.* 2003; Groom and Sullins 2018). From these primary predilection places, melanomas can spread by a haematogenic or lymphogenic route to other places on the skin, lymph nodes, visceral organs like spleen, liver and lungs, or the spinal cord (Patterson-Kane *et al.* 2001; MacGillivray *et al.* 2002; Phillips and Lembcke 2013). It has been described that expansive growth and metastasis of melanomas can cause compression of blood vessels, peripheral nerves or the spinal cord resulting in ischemia, necrosis or neurological symptoms (Crawford 1982; Korthals *et al.* 1985; Rodriguez *et al.* 1998; Patterson-Kane *et al.* 2001). In rare cases, melanomas may primarily develop in the organs of

the gastro-intestinal tract, the spinal cord, the skeletal muscles, the guttural pouches or the parotid glands of grey horses (Moore *et al.* 2013).

This case report describes a rare case of chronic lameness due to vascular compression caused by a melanoma in the pelvis of an 11-year-old grey Spanish gelding, detailing the clinical and ultrasonographic examination and the pathological findings of this particular case.

Case history

An 11-year-old grey Spanish gelding was presented with chronic right hind lameness which gradually developed over several months. During the swing phase, right hind limb abduction was evident. Prior to referral, the horse had been treated by local injection of corticosteroids and hyaluronic acid in the stifle, fetlock and distal interphalangeal joint without any effect. Non-steroidal inflammatory drugs had also been administered for a few days.

Clinical findings

On presentation, the gelding was alert and in a good body condition (4-5/9, using a 1-9 score system adapted from Henneke *et al.* 1983). There were no abnormalities on auscultation of the heart, lungs and abdomen. The respiratory rate and rectal temperature were within normal limits. On inspection and palpation, there was a slight distention of the fetlock, digital flexor tendon sheath and medial femorotibial joint of the right hind limb. Several multifocal, black nodules of approximately 1 cm diameter were observed at the base of the tale, at the preputium and the penis.

Lameness examination

At the walk, the horse presented a shortened cranial phase of the stride of the right hind limb with abduction of the limb during the swing phase. A moderate to severe (4/5 according to the American Association of Equine Practitioners grading system) right hind lameness was also evident at the trot on a straight line and while lunging on a circle to the right on hard and soft surfaces. A distal digital plantar nerve block, a 6-point nerve block at the distal aspect of the metatarsus, intra-articular anaesthesia of the medial femorotibial joint, intra-articular anaesthesia of the tarsometatarsal joint, anaesthesia of the deep branch of the lateral plantar nerve, and a tibial and fibular (peroneal) nerve block were all negative. Based on these results, the source of pain causing the lameness was suspected to be in the pelvic area.

Rectal and ultrasonographic examination

Rectal palpation revealed a mass at the level of the lumbosacral region, which could be followed in the direction of the right iliosacral region and cranially to the body of the ilium. Ultrasonography was performed using an Esaote MyLab Class C with a rectal probe of 5.5-7.5 MHz. For the application of Doppler, a frequency of 5.6 MHz was used. The mass measured approximately 2-3 cm in thickness, was well delineated from the bone surface and presented a heterogeneous, hyperechoic echogenicity. However, there was no clear delineation between the mass and the wall of the iliac vein. The bone surfaces appeared intact. The mass displaced the aorta and the external and internal iliac arteries. Moreover, the ultrasonographic images suggested that the internal iliac artery was interrupted or at least deviating where it came in contact with the mass at the level of the mid-ventral aspect of the sixth lumbar vertebra. Doppler imaging showed turbulences in the internal iliac artery just cranially of the mass (Fig. 1).

Based on this information, intra-arterial infiltration of the mass and/or intra-arterial thrombosis and secondary vascular compression were suspected.

Post-mortem findings

Due to the poor prognosis, the horse was euthanised and submitted for post-mortem examination. Macroscopically, multifocal subcutaneous nodules of approximately 1 cm diameter were observed at the tail base, the preputium and the penis. Furthermore, black discoloration of several lymph nodes as well as black nodules at the level of the parotid gland were seen

At the cranial part of the right ilium, a poorly demarcated black mass of approximately 20 x 15 x 10 cm was present. This soft black mass, weighing approximately 1 kg was situated close to the iliac vein and artery. Additionally, there were multinodular infiltrative, non-encapsulated intramural black masses of approximately 4 by 3 cm, bulging out in the lumen of the iliac vein (Fig. 2). The surrounding lymph nodes were swollen and contained black pigmentation.

Histologic examination of the mass close to the iliac vein confirmed the presumptive diagnosis of a melanoma, as there were large masses of polymorphous cells with large amounts of black pigment accumulation within their cytoplasm, infiltrating the wall of the iliac vein and bulging out into the vascular lumen (Fig. 3a, b). The endothelial layer was absent and could not be examined adequately (Fig 3b). Immunohistochemical staining for Von Willebrand factor to highlight any remaining endothelial cells did not provide additional information as to whether the mass was covered by endothelium or not. An infiltrative mass bulged out into the lumen. The masses within the parotid gland were composed of groups of

similar polymorphous cells with black pigmentation in the interlobular connective tissue .

In conclusion, the post-mortem and histological examination confirmed the diagnosis of a multicentric malignant melanomas with a large melanoma near the iliac vein and artery resulting in secondary vascular compression.

Discussion

This report details the clinical, ultrasonographic and histological findings in a rare case of lameness caused by vascular compression by a malignant melanoma. In contrast with the fact that melanomas are frequently diagnosed in daily veterinary practice, lameness associated with this neoplasia has been reported infrequently. De Blaauw *et al.* (2003) reported two cases of melanomas and concurrent lameness due to their proximity to the blood vessels and nerves of the hind limbs. However, postmortem examination was only performed in one case, and in the other case, only histology of a biopsy taken during laparoscopy was performed, and these cases presented perivascular growth without any intramural infiltration. Moreover, in contrast with the clinical signs of the case presented here, the affected limb of one of the cases described by de Blaauw *et al.* (2003) was diffusely swollen and oedematous, and the other case presented vascular distension of the distal limb.

After a comprehensive clinical examination including diagnostic anaesthesia localising the pain to the pelvic area, rectal palpation and ultrasonography revealed a pelvic mass and secondary vascular compression. Based on the presence of multifocal black subcutaneous nodules at the level of the perineum, tale base, preputium and penis in this 11-year-old grey horse, the presumptive diagnosis of a melanoma in the pelvis as cause of the lameness was

made. This diagnosis was confirmed through post-mortem and histologic examination of the mass. The melanomas markedly infiltrated the vessel walls. On histological examination, it could not be confirmed whether the masses were intravascular or only intramural, due to the artefactual loss of endothelium. Due to the smooth appearance of these masses on gross examination (Fig. 2), the intramural neoplasms were most likely covered with an endothelial layer. Disruption of the endothelial layer would have triggered the coagulation cascade resulting in the formation of fibrin and thrombi adhered to the vessel wall.

The specific localisation of the mass, extending from the lumbosacral region into the right iliosacral region and reaching cranially of the body of the right ilium can be explained by the presence of the sacral and medial iliac lymph nodes. Reportedly, melanomas can metastasise through the lymphatic system to the lymph nodes, with the sacral and medial iliac lymph nodes being most frequently involved in case of melanomas in the area of the perineum, tale and vulva (MacGillivray *et al.* 2002; de Blaauw *et al.* 2003).

The vascular compression and infiltrative growth of the melanoma in the iliac vessels may have resulted in decreased muscular perfusion of the right hind limb, with secondary ischemia, pain and lameness (Crawford 1982; Korthals *et al.* 1985; Moore *et al.* 2013). It cannot be excluded that the mass also resulted in compression of nerves (e.g. femoral nerve), or periosteum, which could also have caused lameness. In the absence of further histological examination of nerves and periosteum, this remains speculative.

Based on the ultrasonographic examination, the main differential diagnoses were intra-vascular infiltrative growth of a melanoma or another neoplastic mass, or an intra-vascular thrombosis. Although malignant neoplasia of vascular origin is uncommon in the horse,

haemangiosarcoma associated with lameness has been described (Kiupel *et al.* 2000; Pille *et al.* 2004). Other differential diagnoses included hematoma formation or abscessation, and aortic-iliac thrombosis, as described by Crawford (1982) in the area of the bifurcation of the iliac vessels, resulting in abdominal pain and chronic lameness. The chronic, progressive lameness in the absence of fever or weight loss, and the presence of multifocal melanomas at various other locations in the present case, resulted in a melanoma being considered most likely.

Rectal palpation revealed a mass of approximately 2 by 3 cm at the level of the lumbosacral region, while the post-mortem examination revealed a mass of approximately 20 x 15 x 10 cm. This illustrates that rectal palpation of a mass can easily underestimate the extent of a mass, which could increase the likelihood of false negative results.

Surgical excision is the treatment of choice when melanomas are small (< 3 cm diameter) and easily accessible (Philips and Lembcke 2013). In contrast with Moore et al. (2013) who stated that surgical excision of large tumours is unrewarding, Groom and Sullins (2018) recently demonstrated that even large (>4 cm) tumours can be successfully excised as long as the tumours are accessible, with no cases of tumour regrowth following excision and minimal complications. Groom and Sullins (2018) concluded that it is advisable to remove tumours in potentially problematic areas before they become large enough to cause a clinical problem. In the present case, surgical excision was not possible due to the localisation and the highly infiltrative character of the mass. Other therapies, with or without surgical excision, include intratumoural injection with cisplatin, cimetidine or cryotherapy (Goetz *et al.* 1990; Moore *et al.* 2013), and local or systemic immunotherapy (Moore *et al.* 2013; Phillips and Lembcke 2013).

Authors' declaration of interests

No conflicts of interest have been declared.

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Figure legends

Fig. 1.: Ultrasonographic view of the mass in the pelvis (white arrow) with interruption or at least deviation of the internal iliac artery at the level of the mass (A) and turbulences in the internal iliac artery cranially of the mass (asterisk) using colour Doppler (B). (Left of the picture is cranial, bottom is ventral, top is dorsal and right is caudal).

Fig. 2. Macroscopic view of the multinodular, infiltrative, non-encapsulated, black-pigmented mass surrounding the iliac vein and artery, with multinodular masses bulging out in the lumen of the vein (asterisk).

Fig. 3a : Histological image (HE; 50x, scale bar = 1 mm) of the mass in close proximity of the iliac vein. There is extensive infiltration of polymorphous cells containing high amounts of black pigment within the vessel wall (green asterisk). An infiltrative mass bulges out into the

lumen (yellow asterisk). The red asterisk indicates the wall of the iliac vein. The arrow indicates the endothelium.

Fig 3b: Immunohistochemical staining (50x, scale bar = 500 μm) of the mass in close proximity of the iliac vein. This colouration of the endothelic cells did not confirm disruption of the endothelium (black arrow) and infiltration of the melanocytic cells in the lumen of the vein (red arrow).