Transitional cell carcinoma in the urinary bladder of a common dolphin (Delphinus delphis) with emphasis on imaging diagnosis

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Abstract
An adult male common dolphin Delphinus delphis cadaver was studied using ultrasound, computed tomography and magnetic resonance imaging and subsequently frozen and cross-sectioned for anatomical description purposes. Imaging of an abnormal mass in the urinary bladder was seen using ultrasound and magnetic resonance. Anatomical sections and gross dissection allowed macroscopic confirmation of the mass, and histological analysis characterized it as a transitional cell carcinoma. A purulent abscess was also detected in the abdominal wall of the same dolphin, by the three used imaging techniques. In cetacean species, tumours are not reported frequently and have been considered as rare events, but realistic cancer incidence rates are most likely largely unknown. In this context, every case reported, as well as the description of techniques which increase the accuracy of diagnostics, should be considered essential.

Keywords: Magnetic resonance imaging, ultrasound, computed tomography, neoplasia, marine mammals

On the other hand, the diagnostic imaging technology applied to veterinary medicine is considered an excellent tool for neoplasia diagnosis, allowing accurate definition of tumor margins and greater sensitive for detecting metastasis (28). In the last years, ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) are increasingly being used for clinical, pathological and anatomical purposes in marine mammals (5,26). Despite the increased use of modern technology, only one diagnostic imaging study of a neoplasm has been described in cetaceans to date: a poorly differentiated brain carcinoma in a beluga by MRI (24).

Realistic neoplastic tumour incidence rates in cetaceans could be considered largely unknown due to the lack of epidemiological studies directly oriented to cancer lesions in these marine species (19), except in the case of St. Lawrence belugas. Contrary to the case of companion animals, in which the discipline of oncology has widely grown, in marine mammals we are in the process of discovering the type and range of neoplastic lesions as well as potential aetiologies. In this context, every single case reported, as well as the description of the techniques used for an accurate diagnosis, should be considered essential. We present here the detection of a carcinoma in the urinary bladder of an adult common dolphin Delphinus delphis by means of US and MRI, emphasizing the value of imaging techniques in marine mammal diagnostic procedures.
Material and Methods

An adult male common dolphin cadaver with a total body length of 195 cm was fully examined by US, CT and MRI at the Veterinary School of León (Spain). The dolphin was found stranded on a beach in northwestern Spain with evidence of a recent death less than 48 hours before (13). Imaging equipment used included an ultrasound device RT 3600 (General Electrics Healthcare, Buckinghamshire, UK) with a 4.5 MHz probe, a single slice CT scanner Tomoscan M-EG (Philips Medical Systems, Andover, USA), and a 0.2 Tesla Signa Profile HD (General Electrics Healthcare, Buckinghamshire, UK) MRI. For MRI scan acquisition, a whole body coil was used to receive the signal, using Fast Recovery Spin Echo sequences in T1 and T2-weighted modes (T1W and T2W) with a field of view (FOV) of 28, as well as pulse repetition time (TR) and echo time (TE). Slice thickness and spacing were 4.0 mm and 1.2 mm respectively, and the image acquisition matrix was 512x512. MRI scans in transverse, sagittal and dorsal planes were obtained. As a general protocol, CT scans were acquired in transverse slices of 1mm thickness at 120 kV. Sagittal and dorsal planes could also be examined thanks to the software image analysis. Two algorithms (soft tissues and bony tissues) were used during CT scanning.

After imaging, the animal was frozen at -24ºC in ventral recumbency and then cross-sectioned with a band saw in its transverse plane at 1 cm intervals, to carry out a cross-sectional anatomy study (2). Although sampling from cross-sections was possibly bound to offer low quality samples for histological analysis due to the freezing/thawing and cross-sectioning processes, apparently abnormal tissues noted on images were taken from cross-sections and preserved in 10% buffered neutral formalin. A haematoxylin and eosin staining protocol was used to stain histological sections of selected tissues.

Results

During the ultrasound examination of the caudal quarter of the abdominal cavity, some abnormal structures were identified. A noticeable, invasive, and irregular tumour emerging from the walls of the urinary bladder into the lumen was clearly observed. The irregular margins of the tumour were very well defined due to the contrast between the anechoic urine and the surrounding soft tissues (Figures 1a and 1b). The tumour was seen at the cranial portion of the bladder occupying the ventral and lateral walls, affecting also the caudal dorsal wall. The tumour occupied one third of the cranial half and two thirds of the caudal half. Dorsal rectal lymph nodes were enlarged compared to normal size nodes in healthy common dolphins (Figure 1a). An abnormal mass located in the abdominal wall at the right side of the dolphin was also observed at bladder level. Maximal dimensions were approximately 10x14x10 cm (left-right, dorsoventral and craniocaudal), and it was located deep to the blubber layer. Its ultrasonographic pattern was diffusely hyperechoic in relation to the adjacent muscles (Hypaxialis lumborum and Pubocaudalis), providing a sharper identification of its margins (Figure 1c).

The bladder tumour was seen on MRI scans at the cranial half of the bladder due to the contrast of signal intensities obtained from urine, clearly hypointense in T1W (Figure 2a) and hyperintense in T2W (Figure 2b), compared to soft tissues (including the mass). Caudally, the tumour produced a very similar signal intensity to urine and could not be well defined, except for an internal rounded portion of 1 cm in diameter, producing a very hypointense signal compared to the surrounding mass (Figure 3a). The rectal lymph nodes and rectum could be identified in T2W MRI scans due to the lower intensity of the signal compared with the adjacent tissues (Figure 3a). Additionally, a craniocaudal and dorsoventral extension of the tumour could be assessed by means of sagittal MRI images (Figure 2c). The lateral mass was clearly observed in T2W MRI transverse series given that the lesion was hyperintense in comparison to the adjacent abdominal wall muscles (Figure 3a).

The bladder mural tumour and other intra-abdominal organs could not be identified on CT scans due to similar CT attenuation (Figure 3b). However the lateral abdominal defect could be seen and measured because of contrast in attenuation relative to the adjacent musculature (Figure 3b).

Anatomical transverse cross-sections of the dolphin corresponding to the urinary bladder confirmed the existence of a pale-brown tumour of soft tissue...
emerging from the urinary bladder wall with a firm (mineralized) rounded central portion 1 cm in diameter (Figure 3c), as well as the enlargement of the rectal lymph nodes and a purulent abscess on the lateral abdominal wall. Several parasitic larval cysts (0.5-1 cm) were also seen embedded in the blubber and subcutaneously around the genital area. No other lesions were observed in any other organ of the dolphin on imaging or on gross dissection.

Histologically, several areas of autolysis and disorganized tissues were observed in all the samples studied. This was due to freezing, cross-sectioning, thawing and collection from anatomical sections. Despite this, bladder samples showed an enlarged mucosa with the transitional epithelium of the smooth muscle with more layers than normal and formed by neoplastic cell sheets. These neoplastic cells were characterized by dark nuclei and acidophilic cytoplasm, and occasional abnormal mitosis was observed. No areas of neoplastic extension were identified beyond the muscular wall of the bladder. All of these findings support the histological diagnosis of a transitional cell carcinoma. Tumour lesions were not seen in the lymph nodes which presented reactive hyperplasia. The samples of the lateral abscess only showed necrotic debris. The tumour/node/metastasis or TNM classification of the tumour was established as T2/N0/M0 (Tumour invading the bladder wall with induration/Regional lymph nodes not involved/No evidence of distant metastasis), according to the scheme for canine urinary bladder cancer defined by the World Health Organization (22).

Figure 1a: Transverse sonogram (4.5 MHz) of the caudal abdominal cavity at the middle urinary bladder level. Probe location: left flank (see upper dolphin icon for references). B: blubber, HL: Hypaxialis lumborum muscle, LN: lymph nodes, R: rectum, T: tumourous mass, U: urine (not frozen).

Figure 1b: Sagittal sonogram (4.5 MHz) of the caudal abdominal cavity at the urinary bladder level. Probe location: ventral midline (see upper dolphin icon). White arrows: tumourous mass, B: blubber, RA: Rectus abdominis muscle, U: urine (not frozen).

Figure 1c: Transverse sonogram (4.5 MHz) of the caudal abdominal cavity at the urinary bladder level. Probe location: right flank (see upper dolphin icon). LA: Lateral abscess, HL: Hypaxialis lumborum muscle, PC: Pubocaudalis muscle.
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Figure 2a: Transverse T1W MRI of the caudal abdominal cavity at the cranial urinary bladder level (see upper dolphin icon). B: blubber, BO: small intestine, HL: Hypaxialis lumborum muscle, LV: lumbar vertebrae, T: tumourous mass, U: urine (not frozen).

Figure 2b: Transverse T2W MRI of the caudal abdominal cavity at the cranial urinary bladder level (see upper dolphin icon). B: blubber, BO: small intestine, HL: Hypaxialis lumborum muscle, LV: lumbar vertebrae, T: tumourous mass, U: urine (not frozen).

Figure 2c: Sagittal T1W MRI of the abdominal cavity, approximately through the midline (see upper dolphin icon). AC: abdominal cavity, B: blubber, RA: Rectus abdominis muscle, T: tumourous mass, U: urine (not frozen), VC: vertebral column.
Figure 3a: Transverse T2W MRI of the abdominal cavity at the caudal urinary bladder level (see upper dolphin icon). B: blubber, C: central portion of hard tissue, HL: Hypaxialis lumborum muscle, LA: lateral abscess, LN: lymph nodes, LV: lumbar vertebrae, R: rectum, RA: Rectus abdominis muscle.

Figure 3b: Transverse CT scan of the abdominal cavity at the caudal urinary bladder level (see upper dolphin icon). AC: abdominal cavity, B: blubber, HL: Hypaxialis lumborum muscle, LA: lateral abscess.

Figure 3c: Transverse anatomical cross-section of the caudal abdominal cavity at the caudal urinary bladder level (see upper dolphin icon). White arrows: tumourous mass, black arrow: central portion of hard tissue, B: blubber, HL: Hypaxialis lumborum muscle, LA: lateral abscess, LN: lymph nodes, LV: lumbar vertebrae, P: parasite larval cyst, R: rectum, RA: Rectus abdominis muscle, U: urine (frozen).
Discussion

Transitional cell carcinoma of the urinary bladder has been documented as the most common malignant tumour of the urinary tract in other mammal species such as the dog (18,21) or humans (25). The only case of a transitional cell carcinoma reported in a cetacean species occurred in one population of belugas inhabiting the St. Lawrence estuary, Canada (15). In this area the incidence of cancer in belugas approaches or perhaps even exceeds that of humans (8,17), being related to the high level of agricultural and industrial pollution (8,9). Furthermore, urogenital carcinomas have been reported as endemic in California sea lions, and are considered a common cause of mortality in free-ranging adults (11). The etiology of this neoplasia in this species is considered likely multifactorial with several contributing factors: viral infections (6,12), alterations in endogenous hormone expression (7), genetic factors (3) and exposure to pollutants (29). Although high levels of some pollutants have been described in tissues from common dolphins of NW Spain (23), the case presented here is the first diagnosed cetacean neoplasia in this geographical area.

Abscesses embedded in the blubber and subcutaneous tissue around the genital area are frequently observed in dolphins stranded in NW Spain, most of the times associated with cestode *Phyllobothrium delphini* cysts degeneration (1). These processes commonly form smaller lesions than those observed in our case, but in some cases they may cause large purulent abscesses, especially in deeply weakened and immunosupressed dolphins. On the other hand, metastases of urogenital carcinomas in California sea lions are found throughout pelvic and abdominal lymph nodes, as well as abdominal and thoracic viscera (7). These metastatic lesions could also degenerate producing necrotic lesions and abscesses. In our case, definitive characterization of the lateral abscess origin was not possible due to the insufficient quality of samples obtained after the anatomical procedures.

Diagnostic imaging techniques such as US, CT and MRI applied to animal medicine are considered excellent tools for the diagnosis of neoplasia, allowing an accurate definition of tumour margins and effective detection of metastasis (28). The US and MRI images obtained in our case clearly revealed the invasive tumour, occupying half of lumen of the urinary bladder. Even though the definition of the lesions nature is not permitted by any imaging technique, the imaging patterns of the tumour presented here are coincident with those of TCC in dogs (14) or humans (27). Both techniques allowed transverse and sagittal imaging planes which were found to be essential for an accurate assessment of the tumour extension.

As in terrestrial mammals, urinary bladder proliferative lesions in cetacean species can be asymptomatic or provoke obstructive and/or irritative symptoms, hematuria, and flank pain. As these symptoms are considered non-specific, imaging techniques are valuable diagnostic tools to achieve more accurate diagnostics. Traditional diagnostic imaging of the urinary bladder included contrast cystography or intravenous urography (IVU). As described here, US and MRI could be alternative or complementary methodologies, with the additional value that they do not produce ionizing radiation exposure. Furthermore, CT-IVU has been successfully used to visualize the urinary bladder, but tissue could not be reliably distinguished from fluid without IV contrast which is not possible in post-mortem CT scans. US has been described as an ideal imaging tool for male dolphin’s genital-urinary tract examination, allowing a clear image of the urinary bladder (4). It is a non-ionizing, low cost, fast and easily repeatable imaging technique in which no tranquilization or general anaesthesia of the patient is required. In the last two decades, US portable devices have been developed and nowadays, they can be easily transported and used very close to marine mammal pools, permitting comfortable handling and reducing the stress level of the cetacean. The use of color Doppler to distinguish mural masses from avascular luminal sediment or hematomas is valid for living animals but not an option when dealing with cadavers. On the other hand, the use of CT and MRI in cetaceans is currently still limited because scanners are often not available and transport of cetaceans to the location of such equipment is not without risks. Nevertheless, the interest in moving forward in its use is considerable, especially when dealing with endangered species.

Finally, the use of imaging techniques in dead dolphins considerably improves post-mortem information acquisition and allows us to gather
pathological, anatomical and morphological data from museum specimens or dolphins that cannot be fully necropsied. This may be a key factor for increasing the low number of reported tumours and for widening the current knowledge of the real incidence of neoplasia in cetacean species.

Acknowledgements
The authors want to thank stranding networks staff of Galicia (CEMMA) for technical support to this research. We want also thank Fiona Read for her constructive comments on the manuscript. First author is currently funded under post-doctoral fellowship SFRH/BPD/47251/2008 of the Fundação para a Ciência e a Tecnologia (Portugal).

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