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MULTI MODALITY IMAGING APPROACHES TO INJECTION-SITE SARCOMA IN CATS

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Abstract

The aim of this study was to investigate different diagnostic imaging approaches for Injection-Site Sarcomas in cats (ISS) and their mutual relationships with the surrounding tissues between December 2014 and October 2016. In the literature there is a lack of anatomical references about the interscapular region of the cat, with very few publications displaying the musculoskeletal anatomy in transverse sections. Computed Tomography (CT) has been proven to be useful in the staging process of feline patients affected by ISS, not only for correct estimation of the volumetric balance of the tumour but also for thorough staging of distant and local metastatic spread. ISS are mesenchymal soft tissue tumours characterized by a typical interscapular location and highly infiltrative behaviour. CT can be considered the modality of choice for ISS staging and a double positioning (dynamic approach) was reported to successfully detect the exact amount of infiltration of the muscles. A correct estimation of the spatial relationship between neoplastic, inflammatory and normal tissue is crucial, since the indicated treatment is mainly characterized by wide surgical excisions of the neoplasm, including 3-5 cm of perilesional tissues. Our study consists of an anatomic atlas of the interscapular region of the cat in a clinically normal population, using magnetic resonance imaging (MRI), computed tomography (CT) and gross sectional anatomy. In addition we compared both clinical and CT findings for pre-surgical assessment of the tumour. We performed a final volumetric estimation utilising dynamic double positioning in a cohort of 84 patients.

Results from our study provide a new and dynamic way to investigate the interscapular region of the cat, with anatomical references for in vivo CT and MRI, considering muscular changes according to forelimb positioning. Analysing the discrepancy between clinical and CT tumour measurements there was a tendency for CT measurements to be greater than clinical dimensions, and this difference increased with increasing tumour size. Based on our results, further studies focusing on ISS in cats, should specify the kind of assessment used to define tumour dimensions (CT versus clinical examination) in order to interpret surgical results and prognostic impact of this variable.

Finally, results from the investigation of the use of the dynamic approach in patients referred for pre-surgical staging of interscapular ISS demonstrated good agreement between observers, with higher tumour volumes detected via the ellipsoid method. Lower tumour volumes showed slightly decreased muscular infiltration. In conclusion, the dynamic approach should be performed for a complete evaluation of the invasiveness of the ISS along with an appropriate selection of tumour volume methodology, which could potentially affect the pre-surgical assessment of ISS.

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1. Literature review

1.1 THE ANATOMY OF THE INTERSCAPULAR REGION

The interscapular region has been extensively described in dogs with books and atlases displaying the relationship between the skeletal structures and the adipose tissue lying between them, as reported by Evans HE and De Lahunta A in "Miller's anatomy of the dog" (3rd ed., 2013) or in Done SH, Goody PC, Evans SA and Stickland NC in "Color atlas of Veterinary Anatomy" (2nd ed., 2009). These books act nowadays as a reference for the correct assessment of different anatomical regions, such as the interscapular one.

Though extensively described in dogs, this region was considered mainly with a comparative approach in cats, as particularly shown in the aforementioned "Color atlas of Veterinary Anatomy" by Done et al. (1st ed., 1996), or in other illustrious texts like Barone R "Anatomie comparée des mammifères domestiques" (1976), König HE and Liebich HG "Veterinary Anatomy of Domestic Mammals" (6th ed., 2014) or Sisson S, Grossman DJ and Getty R "The anatomy of the domestic animal" (5th ed., 1975).

The first papers published in literature in which the anatomical reference has been coupled with slices obtained through advanced imaging techniques (CT) are those published between 1992-1993 by George TF and Smallwood JE on Veterinary Radiology and Ultrasound. The Authors described for the first time the conception of coupling gross sectional anatomy with the correspondent CT slices previously obtained on the same patient, in order to give a direct reference for the Imaging description and improving the ability of Clinicians in the study of this region. The Authors produced in these papers a complete collection of reference atlases focused on head and neck, thorax and cranial abdomen, caudal abdomen and pelvis. The main limitation of these papers is that the anatomical and tomographic slices were not obtained with the same slice thickness, originating equivocal reference when small anatomical structures were studied.

Nevertheless, there is an important lack of information regarding feline patients because the only paper describing the "Normal cross-sectional anatomy of the feline thorax and abdomen", comparing CT and cadaveric anatomy in cats, was published in 1998 on Veterinary Radiology and Ultrasound by Sami VF, Biller DS and Koblik PD. The same Authors one year later published a second anatomical reference on the Magnetic Resonance Imaging of the feline normal abdomen (Rousset N et al., 2013).

The only two studies describing specifically the dorsal muscles in cat, including the interscapular region, are those published by Sami et al. (1998, 1999): currently these works represent the only guidelines for the *in toto* study of the anatomy on the feline thorax and abdomen.

1.2 INTERSCAPULAR FELINE INJECTION-SITE SARCOMA

The interscapular region is a common location where feline patients usually receive subcutaneous injections, mainly vaccines. [1,2,3] It has been reported that this condition along with other traumatic event triggering an uncontrolled panniculitis, such as foreign bodies, the presence of suture material, microchip or antibiotic injections, could contribute to the growth of mesenchymal tumors referred to the group of Injection-Site Sarcomas (ISS).[2-6]

Firstly reported in 1991, Feline ISS are mesenchymal malignant tumours of different histotypes, with fribrosarcoma being the most common.[4] These tumours have been linked to rabies vaccination and the presence of adjuvants, such as aluminium; however large epidemiological studies failed to prove a higher evidence of the increased risk of tumour development in these conditions.[5]

Feline ISSs are characterized by an aggressive behaviour towards the perilesional tissues, a low distant metastatic potential (10-25% of lung involvement) but considerably high grade of local recurrence rate (up to 50% of cases with histopathological clean margins), even with a correct therapeutical approach.[5] The reported incidence of Feline ISS varies from 1.3/1000 vaccinations to 1/10000 vaccinations[20-21] and a recent study identified a higher incidence of tumour development in case of long-acting corticosteroid injections and inactivated rabies vaccines.[5]

Even though chemotherapy and radiotherapy have been considered as pre/post-surgical therapies also for this type of tumor, their role as adjuvant treatments still has to be clarified.[5] Radical and extensive surgery is still considered the treatment of choice, with 3-5cm of perilesional margins included in the excised tissues.[4-5,7]

Being the surgical excision highly extensive and invasive, CT and MRI have been recently introduced into clinical planning for a better definition of the relationship between neoplastic lesions and surrounding soft and hard tissues, improving the pre-surgical assessment of ISS.[5,8-9]

Despite the advances in the Imaging evaluation of ISS a specific anatomical reference is still lacking, considering the different papers and publications available in literature. In fact, although books and papers describe carefully the anatomy of this region, only few of them focus thoroughly on the feline patient.[10-11] The main concern is that feline patients have been always considered with a comparative approach in all the published literature, pointing out what is different from the dog.[12-14]

In 1992-1993 three studies describing the normal anatomy of the head, neck, thorax, abdomen and pelvis in the dog have been published.[15-17] The novelty of these papers is the coupling of gross sectional anatomy with the correspondent CT slices, previously obtained from the same patient. This is one of the first examples of comparative approach improving the ability of Clinicians in the study of this region. However, the main limitation of these papers is the different slice thickness between the anatomical and tomographic slices, originating equivocal reference when small anatomical structures are studied. Only few years later the first comparative anatomical studies in cats have been published on the same journal, representing the actual guidelines for the *in toto* study of the anatomy on the feline thorax and abdomen.[1,18]

Another important issue is that anatomic tomographic images from published literature generally refer to animals in sternal or dorsal recumbency, even though sternal recumbency with forelimbs pulled cranially is indicated as the standard protocol by the CT reference book.[19]

Moreover, common literature considers only a static approach describing gross and/or sectional anatomy and/or tomographic anatomy only in a fixed position, not considering the changes in the appearance of muscles the high mobility of the interscapular region can induce, depending on the position assumed by the forelimbs.

As a consequence of the high mobility of the interscapular region, it has been postulated that the mass could change its relationship with surrounding tissues depending on the forelimbs position during the CT scan.[3]

Effectively, in the interscapular region of both dogs and cats, the lack of an articulated clavicle to the skeleton make the role of the muscles essential to hold the forelimbs to the chest and allow the mutual movement of the myofascial components.

1.3 The significance of “double-positioning”

The usefulness of this positioning has been previously suggested during CT investigations for ISS, based on the results collected from 10 feline patients.[3] In these patients the tumour was interscapular and after the conventional pre and post contrast study with the forelimbs pulled cranially an additional post contrast exam with the forelimbs flexed along the body was performed. The Authors highlighted the slight reduction in the muscles infiltrated by the tumour in two cases, when the flexed positioning was performed. Therefore double positioning was suggested and later recommended in a recent review on the Journal of Feline Medicine and Surgery.[5]

Nowadays, the utility of this novel technique is not widely considered in the standard staging of ISS in most Institutions, mainly because of a lack of more extensive case-series highlighting the real benefit of double positioning in the estimation of the number of structures infiltrated by the Feline ISS.

1.4 THE CONCEPT OF MULTI-MODALITY AND MULTIDISCIPLINARY APPROACH

In 2011 the inevitable need of sophisticated Imaging techniques to improve diagnosis and help the surgical planning of ISS was suggested.[4] Following the recent advances reached, the Authors suggested the use of a multi-modal treatment, including also chemotherapy and radiotherapy.[4] This was a novel way of considering the potential of Imaging as a helpful modality for an effective therapeutical treatment, since MRI and CT have not always been considered in this sense before.

Despite the peculiar presence of asymmetric and infiltrating long tumor extensions departing from the main mass making the estimation of the dimensions of feline ISS difficult to achieve, many studies have used clinical measurements for prognostic and surgical purposes.[4,7]

The current literature discusses the amount of lateral margins that should be excised around and beyond the FISS. In recent studies, discrepancies between CT measurement of the neoplasms and clinical measurements have been identified. These discrepancies could potentially represent an issue for the surgeon during the excision of the neoplasm because the same wide lateral margins could not be equally wide if clinical or tomographic measurements were previously considered.

Moreover a recently published study specifically compared different methods of assessment of tumour volume in patients affected by Feline ISS suggesting a to use the 3-D software method when tumour volume is of particular concern. The Authors also identified mild shrinkage of the neoplasms after excision and formalin fixation.[22]

In this sense there is an important lack of prospective studies focused on the investigation of the agreement between different modalities and methods of volume estimation, in order to statistically quantify these differences and help both clinicians and surgeons in understanding the limits of their measurements and choosing the appropriate method of tumour estimation.

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2. Project overview

According to our time schedule the PhD project was designed with four main aims:

1. Fill the gap in the common literature concerning the cross-sectional representation of the interscapular region of the cat between different advanced Imaging modalities.
2. Investigate the agreement/disagreement between clinical and Computed Tomography tumour's volume estimation in feline patients affected by ISS.
3. Evaluate the utility of the dynamic approach in the CT study of patient affected by ISS.
4. Test the level of the agreement between different computational methods in the study of ISS.

Time Schedule	Jan-Feb 2014	Mar-Apr 2014	May-Jun 2014	Jul 2014 - Jul 2016	Aug – Sep 2016	Oct- Dec 2016
Scientific literature						
Anatomic study						
Prospective - retrospective clinical trial						
Data analysis and Conclusion						

3. Scientific publications

1. Advances in the anatomic study of the interscapular region of the cat.

2. Clinical and computed tomography tumour dimension assessments for planning wide excision of injection site sarcomas in cats: how strong is the agreement?

3. Dynamic tomographic studies on Injection Site Sarcoma in cats: essential or useless practice?

3.1 Advances in the anatomic study of the interscapular region of the cat

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Abstract

Background: New clinical oriented approaches are supported by the integration of advanced imaging techniques, e.g. computed tomography and magnetic resonance, with gross anatomy imaging. The interscapular region of the cat is a typical site of a highly invasive infiltrative pathology, i.e. Feline Injection-Site Sarcoma. Even if chemotherapy and radiotherapy have been considered as pre-surgical therapies, extensive surgery is still the recommended treatment. Evidence suggested that the relationships between muscles, infiltrative mass and adjacent musculoskeletal structures could change according to the forelimb positions: a fact to be duly considered while planning the surgical approach. Anatomic and tomographic atlases provide only images of the interscapular region from cats positioned with their forelimbs extended cranially, which means that, they do not record musculoskeletal modifications due to the forelimb movements. Aim of this study was to provide detailed images of the changes occurring in the musculoskeletal structures of the interscapular region of cats according to their forelimb position by comparing cross-sectional gross anatomy, computed tomography and magnetic resonance imaging.
Results: We provide an atlas of normal cross-sectional anatomy, computed tomography and magnetic resonance imaging of the interscapular region of the cat, from the fifth cervical vertebra to the fifth thoracic vertebra. We compare and couple the slices obtained both in flexed and extended forelimb positioning with the animal maintained in sternal recumbency.
Conclusion: This study shows a new and dynamic way to investigate the interscapular region of the cat and provides anatomical references for in vivo computed tomography and magnetic resonance imaging, considering changes in the muscular form according to the forelimb positioning. We believe that an in-depth anatomical knowledge of the interscapular region may be helpful to approach the study of any pathology located there and, in particular, to set up an appropriate therapy for the feline injection-site sarcoma.

Keywords: Cat, Cadaver, Interscapular region, Cross sectional anatomy, Gross anatomy, Computed tomography, Magnetic resonance imaging, Double forelimb positioning, Feline injection-site sarcoma, Fibrosarcoma.

Background

The interscapular region is characterized by several layers of muscles held together by a complex myofascial system, often infiltrated and covered by adipose tissue, which allows their mutual movement, contributing to stabilize the scapula against external forces and preventing its displacement or rotation [1, 2]. In cats, the region, though extensively described in dogs [1], has been considered mainly with a comparative approach [2–5]. Traditional anatomical atlases and textbooks typically describe the musculature of the interscapular region in standing animals with forelimbs in neutral position, considering the muscles in their superficial and deep layers [1–7]. Conversely, anatomic tomographic images from scientific articles generally refer to animals in sternal [6–8] or dorsal recumbency [9, 10], even though sternal recumbency with forelimbs pulled cranially is indicated as the standard protocol [11]. Moreover, common literature considers only a static approach describing gross and/or sectional anatomy and/or tomographic anatomy only in a fixed position; not considering the high level of mobility of the interscapular region can induce significant change in the appearance of muscles during dissection procedures and/or image acquisition, depending on the position assumed by the forelimbs. The only two studies describing specifically the dorsal muscles in cat, including the interscapular region, were published by Sami et al. [9, 10]. They compare cross sectional gross anatomy and computed tomography (CT) [9], cross sectional gross anatomy and magnetic resonance imaging (MRI) [10]. Currently, these works represent the only guidelines for the in toto study of the anatomy on the feline thorax and abdomen. Cats commonly receive subcutaneous injections in their interscapular region, which has been widely described as a condition that may contribute to the growth of mesenchymal tumors referred to the group of Feline Injection-Site Sarcomas (FISS) [12–19]. FISS are characterized by an aggressive behaviour towards perilesional tissues, a low distant metastatic potential and a high grade of local recurrence [12–14, 17]. Though chemotherapy and radiotherapy have been considered as pre-surgical therapies also for this type of tumor [13, 14], radical and extensive surgery is still the treatment of choice [12–14, 17]. Being the surgical excision highly invasive and seriously compromising the patient’s health [12], CT and MRI have been recently introduced into clinical planning for a better definition of the relationship between neoplastic lesions and surrounding soft and hard tissues. As a consequence of the high mobility of the interscapular region, it has been postulated that the mass could change its relationship with surrounding tissues depending on the forelimb position during CT scan [12]. A fact to duly consider while planning the indicated surgical approach: in fact, an improper description of the neoplastic lesion could compromise both treatment and follow-up [12, 14] for the patients. The aim of this work is to provide an atlas of normal cross-sectional gross anatomy, CT and MRI anatomy of the interscapular region of the cat with the forelimbs extended cranially and caudally along the body, in sternal recumbency. In order to obtain a more accurate description of the region, all the images were correlated by comparing the different features assumed by soft and hard tissues, in the two different positioning.

Methods

Three adult domestic shorthaired cats (subjects A, B, C) supplied by owners prior written informed consent and died for causes unrelated to the present study (i.e. on natural or euthanized according to good clinical practice guidelines) were enrolled in our Veterinary Teaching Hospital for educational purpose. No specimen showed any musculoskeletal lesion in the interscapular region.

CT and MRI images acquisition

Cadavers were stored at 4 °C for 24 h, then a complete CT and MRI scan were performed in subject A (5 kg body weight) and in subject B (5 kg body weight), respectively. CT images were obtained employing a single slice CT unit model Picker PQ2000S (Philips Healthcare, Monza, Italy) in helical mode by 3 mm thickness contiguous slices, with a pitch of 1, 120 kV, 150 mA, 1 s of tube rotation and an average scan time of 35 s. Images were assessed using certified software OsirixPro 64-bit (Aycan Medical Systems, LLC, Rochester, NY). MRI scans were performed using a low field MRI unit (0,2 T) model Vet-MR (Esaote S.p.A, Genova, Italy). On MRI, sections of 3 mm thickness were acquired with an average total acquisition time of 50 min. In all specimens T1-weighted, T2-weighted and High Resolution Gradient Echo sequences were employed.

Both CT and MRI scans were performed with the following protocols: 1) sternal recumbency and volumetric acquisition with the forelimbs pulled cranially; 2) sternal recumbency and volumetric acquisition with the forelimbs flexed caudally along the body, obtained immediately after the first acquisition.

Subject C (4 kg body weight) was submitted to a CT scan and, immediately after, to an MRI scan, with the following protocol: CT images were obtained by 1.25 mm thickness contiguous slices employing a 16- slice CT unit model Brightspeed-16 (General Electric Healthcare, Milano, Italy). MRI images were obtained by 4 mm thickness employing the above-mentioned low- filed MRI unit.

For both CT and MRI, the field of view was extended from the fifth cervical vertebra (C5) to the fifth thoracic vertebra (T5). An unmovable marker placed on the body of the animal identified the top and the bottom of the study. All the images were evaluated in all three axes. On CT study, dorsal and sagittal planes were obtained by multiplanar reconstructions, while on MRI study they were directly acquired.

Anatomic images acquisition

In order to collect gross anatomical sections at the same level of the tomographic slices, at the very end of every tomographic study, subjects A and B were placed in a plastic case of polystirene (dimensions of the case: 55 × 36 × 19 cm) and kept in a−18 °C freezer until solid, strictly maintained in the same position assumed in CT or MRI study by wooden sticks and synthetic resin sponges.

The frozen cadavers obtained in a rectangular shape in the plastic case were sectioned along the transverse plane with an electric band saw. Six mm thick sections, extended from C5 to T5, previously identified by the markers, were obtained. Consequently, each section included two adjacent CT or MRI slices, in order to obtain the best correspondence between cross-sectional cuts and imaging slices.

Frozen slices were numbered and gently cleaned of debris with cold water and light brushing. The two surfaces of each section were photographed instantaneously (i.e., before thawing) with a digital Nikon D90 photo- graphic camera, with a 300 dpi resolution.

Organisation of the tables

Photographs were examined and identifiable anatomic structures were labelled with the available anatomical references [1–5, 9, 10]. The terminology adopted was chosen in accordance to the Nomina Anatomica Veterinaria (2012). For each anatomical slice, a corresponding CT and MRI slice was chosen on the basis of their comparable appearance and presented in flexed and extended positioning, considering as reference pivot the vertebral bodies.

Identified structures were subsequently located on the anatomical sections and on CT and MRI slices. Afterward, the list of the identified structures was evaluated against CT and MRI images from cadaver C.

Results

Atlas of matched cranio-caudal sections of the interscapular region of a normal cat obtained by CT, MRI and cross- sectional anatomy, in double positioning of the forelimbs

Eight representative transverse combinations of CT, cross sectional anatomy and MRI slices with the forelimbs in cranial position and caudal position were obtained and then coupled. Figure 1a and b show lateral scanograms of the animal acquired immediately before the beginning of the scan in extended and flexed position respectively. The lines represent the levels of each CT, MRI and anatomic slice of the cadaver. Figures 2, 3, 4, 5, 6, 7, 8 and 9 show the cranio-caudal CT-slice (A), cross sectional anatomy (B) and MRI-slice (C) coupled in the flexed (on the left) and the extended (on the right) forelimbs position from C5 to T5.

All the muscles of the interscapular region were identified. Most of them were visible in both positioning; on the contrary, some muscles appeared only in flexed or extended positioning. As expected, the muscles *supraspinatus, infraspinatus, subscapularis, rhomboideus, serratus ventralis, semispinalis cervic*i modified their aspect and their relationship to each other and to the scapulae, depending on the position assumed by the forelimbs. Substantial differences showed mainly remarkable between T1 and T5 as a direct consequence of the highest mobility of this tract (Figs. 5, 6, 7, 8 and 9).

When the forelimbs were maintained extended, in fact, the scapulae were aligned to the body, in a horizontal position (Fig. 1a). Consequently, the muscles *supraspinatus* and *infraspinatus* followed the position of the scapulae, lying horizontally at the same distance. When the forelimbs were maintained flexed, the scapulae acquired a vertical position (Fig. 1b). In this case four events occur: 1) the muscles *supraspinatus, infraspinatus and subscapularis* followed the scapulae and assumed a vertical position in all images; therefore, scans were obtained on the major axis of every muscle; 2) a minor scapulae surface in the interscapular region was included; 3) the distance that divides the scapulae was not the same: bones showed closer to each other cranially than caudally; 4) a slight raising of the dorsal aspect of the scapulae resulted in a consequent raising of the muscles *romboideus, semispinalis capitus* and *serratus ventralis*; they appeared more defined, thinner and horizontally oriented if compared to the images obtained with extended forelimbs. In addition, with flexed forelimbs the adipose tissue and the myofascial organization were generally better visualised, appearing more opened and less compressed. These features were especially more identifiable on MRI and anatomical cross-sections.

Discussion

The interscapular region is complex to investigate, with layers of overlaid muscles whose spatial relationships are difficult to evaluate from conventional imaging and anatomic references. Moreover, this region is characterized by high mobility that in some instances can over or underestimate the relationships among muscles and between muscles and scapulae. A relevant aspect not considered in traditional literature together with a rapid identification of muscles themselves and of their spatial relationship with surrounding structures.

To our knowledge, this is the first atlas that provides an exhaustive description of the interscapular region of the cat and considers its dynamic behaviour. Data were obtained by comparing cross-sectional imaging of gross, CT and MRI anatomy, which permitted to better identify the musculoskeletal and myofascial components of the region while taking full advantage of the potential of the three methods.

From a technical point of view, this multiple approach represents a novelty in its considering both techniques simultaneously while previous studies in rabbits [20–23] dogs [6–8] and cats [9, 10] compared either cross-sectional anatomy and CT or cross-sectional anatomy and MRI. Further, unlike other studies [6–8, 10, 20–23], in our work the sections were 3 mm-thick on CT, 3 mm-thick on MRI, and approximately 6 mm-thick on cross-sectional anatomy, allowing improved comparison between gross anatomy slices and advanced imaging techniques. In this contest, the choice to maintain vertebral bodies as the reference pivot proved to be a useful tool in detecting and describing the anatomical structures.

All images obtained in this study were of excellent quality and no abnormalities due to sample preservation were observed.

As expected, soft tissue details were superior on MRI than on CT, and the sequence identified as High Resolution Gradient Echo showed better rendering of the morphology of the musculoskeletal structures. Low field MRI required a long acquisition time when 3 mm thickness was applied (total average acquisition time: 50 min); an aspect that might represent a problem only in clinical activities requiring a prolonged anaesthesia time, but not in our investigation carried on dead animals. Conversely, CT acquisition required a short resolution time; depending on the tomography used, it ranged from 35 to 15 s for single or multi-detector, respectively. In subject C we decreased thickness of the CT-slices down to 1.25 mm and no significant improvement in image quality was observed in a transverse plane. Opposite to CT, in the same subject we increased thickness of MRI slices up to 4 mm. Data demonstrated that the quality of images kept unchanged, but time taken was significantly shorter (total average acquisition time: 35 min), which makes clinical application more feasible.

The corpses of the cats were positioned in sternal recumbency with their forelimbs extended cranially or caudally along their bodies. As a consequence, the scapula and its related muscles moved against the chest wall in two different ways even thanks to the characteristic loose connective tissue presents between the limb and the trunk, where the axilla is located.

We observed that changes in the position of the scapula can modify the shape of the muscles, as clearly appreciable by CT and MRI and confirmed by gross anatomy images. Although Travetti et al. [12] provided exhaustive images of the interscapular region, it is important to underline that those images derived from cats affected by Feline Injection-Site Sarcoma (FISS), with manifest tumours in between their muscles. A situation where not only the movements of the limbs, but also the volume of the neoplastic masses may change the shape of the muscles implied, thus complicating the interpretation of the images. One of the key strengths of our work is that its images were derived from cats with no lesion, and therefore their consultation should avoid any misinterpretation.

In veterinary medicine, sternal recumbency is the standard positioning commonly recognized for CT studies of the thoracic region [11]; however, in the cat, tomographic references available in literature are all obtained with animals positioned in dorsal recumbency [9, 10].

In animals, sternal recumbency should be preferred to dorsal recumbency to reduce possible lung congestion occurring in prolonged dorsal recumbency, with the forelimbs extended cranially to avoid including them in the visual field. In our study, sternal positioning associated to forelimbs either flexed along the body or pulled cranially was aimed at better visualizing the musculo-skeletal structures by enhancing their mobility. Moreover, since double positioning made the myofascial system and tissue adipose infiltrations visible, the images acquired can support a more accurate estimation of the relationship between both infiltrating and neoplastic lesions and surrounding tissues.

In particular, in cats affected by FISS, a deep knowledge of the sectional anatomy of the region is mandatory in order to reduce post-surgical complications. It has recently been demonstrated that surgery time is the best predictor of wound healing complications and that they are influenced by excision pattern and mass size [24].

Currently, establishing the best relationship possible between a mass and its surrounding tissues while considering the mobility of the region is critical as extensive surgery, including ostectomy if necessary, is still the treatment of choice [12–14, 17, 24].

Cost implication and availability of CT and MRI scanners are the most important limitations in the integrating cross-sectional anatomy. Moreover these techniques require a training and assessment in the interpretation of post-mortem imaging. In fact, by understanding the benefit and limit of each imaging technique we can employ CT and MRI to their maximal advantages.

Conclusion

This work indicates a new approach to the interpretation of the interscapular anatomy of the cat in that it considers the dynamic behaviour of the region, a highly necessary component in the study of all pathologies, neoplastic and non-neoplastic, affecting it. Our claim was to provide radiologists and clinicians with an anatomical and tomographic map, integrating available literature, rather than to define the best positioning for the estimation of the interscapular region of the cat, in order to guide them toward a correct interpretation before submitting the animal to surgery, especially in case of FISS.

Finally, this issue was addressed ethically since, unlikely most of previous publications with comparable technical approaches, no animals were sacrificed to perform the investigation.

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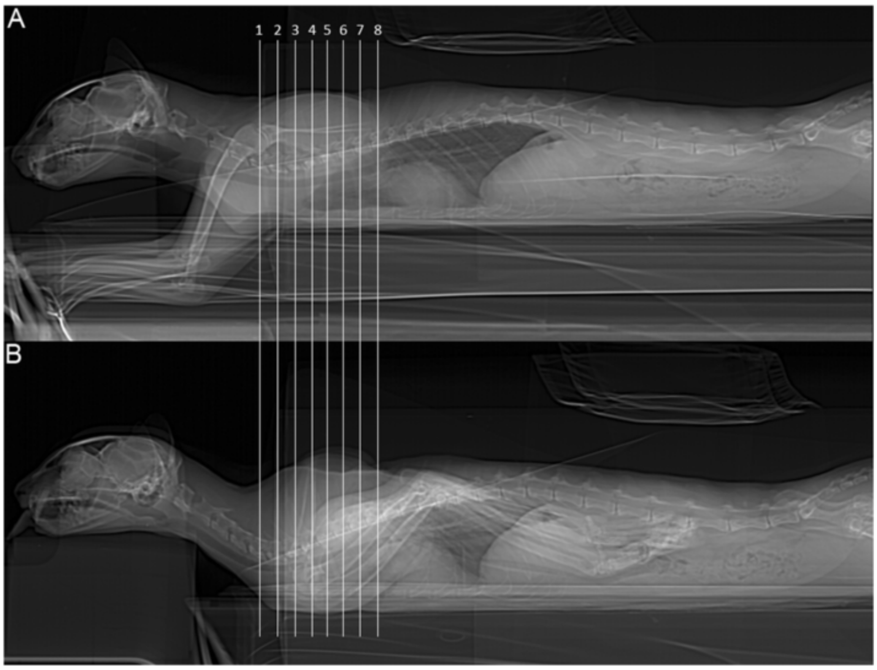

Fig. 1 Whole body scanogram with extended (a) and flexed (b) forelimbs obtained with a 16 slice CT. Numbered lines indicate the approximate levels of each anatomic slice of the frozen cadaver and the two corresponding contiguous CT and MRI sections

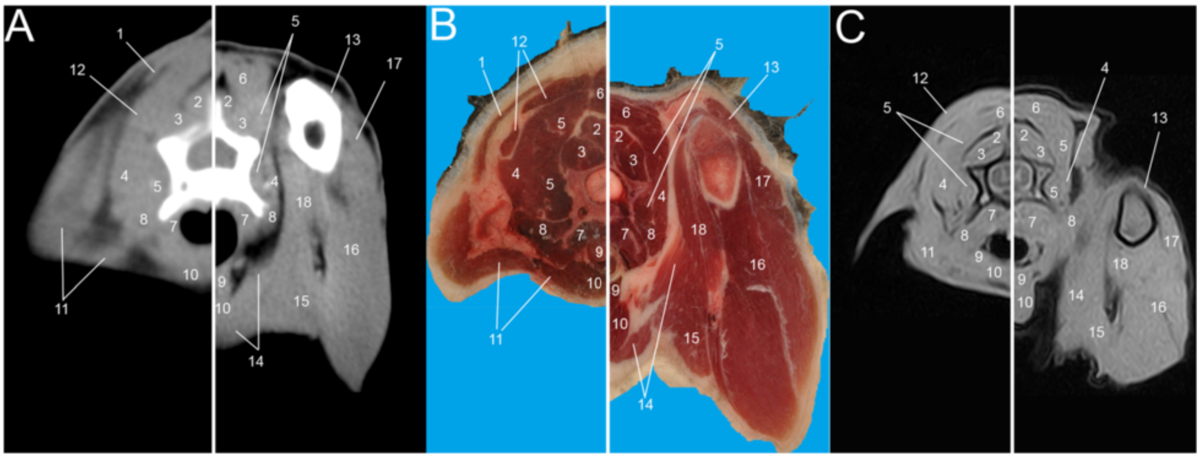

Fig. 2 Single slice CT with 400 WW and 40 WL (a), Anatomical (b) and low field T1-weighted MRI (c) slices obtained at the level of C5. Images show flexed forelimb positioning on the left and extended forelimbs positioning on the right: *1. M. trapezius, 2. M. spinalis, 3. M. multifidus, 4. M. serratus ventralis, 5. M. longissimus cervicis et capitis, 6. M. splenius, 7. M. longus colli, 8. M. scalenus, 9. M. sternohyoideus, 10. M. sternocephalicus, 11. M. brachiocephalicus, 12. M. rhomboideus, 13. M. brachialis, 14. M. ectorals superficialis, 15. M. ectorals profundus, 16–17. M. triceps brachii, 18 M. biceps brachii*

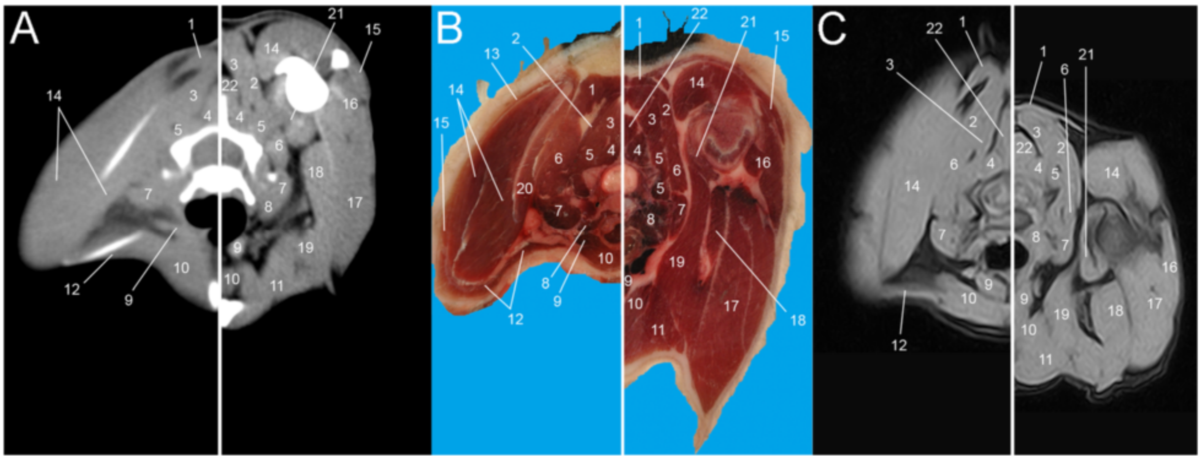

Fig. 3 Single slice CT with 400 WW and 40 WL (a), Anatomical (b) and low field T1-weighted MRI (c) slices obtained at the level of C6. Images show flexed forelimb positioning on the left and extended forelimbs positioning on the right: *1. M. rhomboideus, 2. M. splenius, 3. M. longissimus capitis, 4. M. multifidus, 5. M. longissimus cervicis, 6. M. serratus ventralis, 7. M. scalenus, 8. M. longus capitis, 9. M. sternohyoideus, 10. M. sternocephalicus, 11. M. ectorals superficialis, 12. M. brachiocephalicus, 13. M. trapezius, 14. M. supraspinatus, 15. M. omotransversarius, 16. M. deltoideus, 17. M. triceps brachii, 18. M. teres major, 19. M. ectorals profundus, 20. M. subscapularis, 21. M. biceps brachii, 22. M. spinalis*

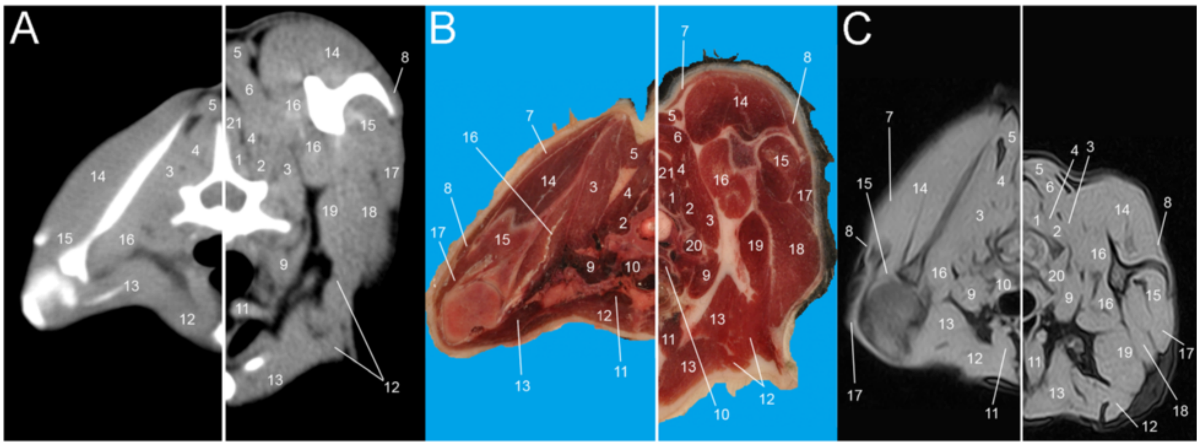

Fig. 4 Single slice CT with 400 WW and 40 WL (a), Anatomical (b) and low field T1-weighted MRI (c) slices obtained at the level of C7. Images show flexed forelimb positioning on the left and extended forelimbs positioning on the right: 1*. M. multifidus, 2. M. longissimus cervicis, 3. M. serratus ventralis, 4. M. semispinalis, 5. M. rhomboideus, 6. M. splenius, 7. M. trapezius, 8. M. omotransversarius, 9. M. scalenus, 10. M. longus colli, 11. M. sternocephalicus, 12. M. ectorals superficialis, 13. M. ectorals profundus, 14. M. supraspinatus, 15. M. infraspinatus, 16. M. subscapularis, 17. M. deltoideus, 18. M. triceps brachii, 19. M. teres major, 20. M. longissimus capitis, 21. M. spinalis cervicis*

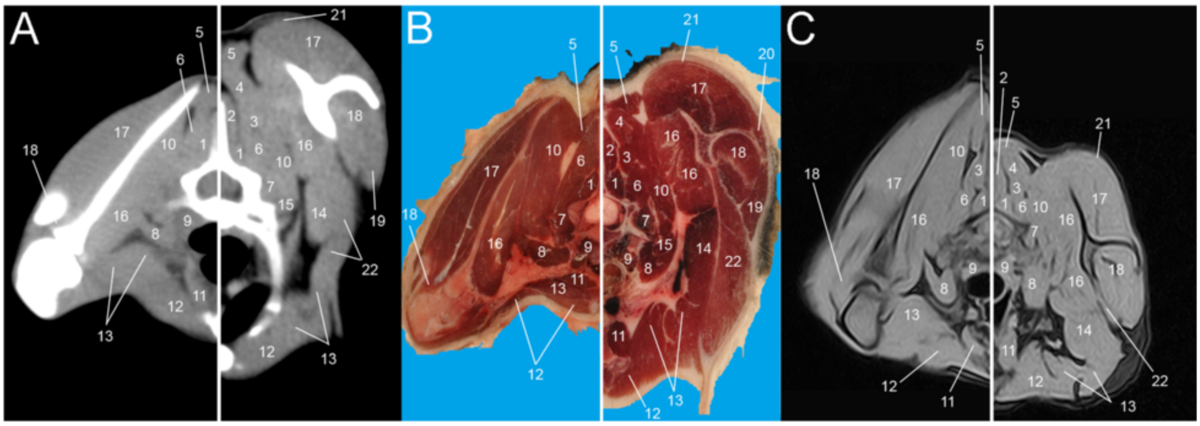

Fig. 5 Single slice CT with 400 WW and 40 WL (a), Anatomical (b) and low field T1-weighted MRI (c) slices obtained at the level of T1. Images show flexed forelimb positioning on the left and extended forelimbs positioning on the right: *1. M. multifidus, 2. M. spinalis cervicis, 3. M. longissimus capitis, 4. M. splenius, 5. M. rhomboideus, 6. M. longissimus cervicis, 7. M. complexus, 8. M. scalenus, 9. M. longus colli, 10. M. serratus ventralis, 11. M. sternocephalicus, 12. M. ectorals superficialis, 13. M. ectorals profundus, 14. M. teres major, 15. M. iliocostalis, 16. M. subscapularis, 17. M. supraspinatus, 18. M. infraspinatus, 19. M. deltoideus, 20. M. omotransversarius,. M. trapezius, 22. M. triceps brachii*

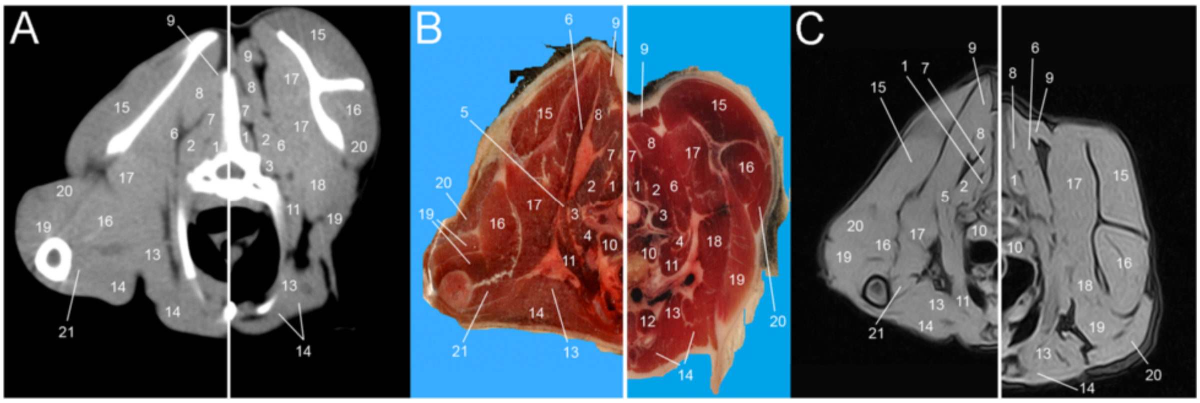

Fig. 6 Single slice CT with 400 WW and 40 WL (a), Anatomical (b) and low field T1-weighted MRI (c) slices obtained at the level of T2. Images show flexed forelimb positioning on the left and extended forelimbs positioning on the right: *1. M. multifidus, 2. M. longissimus cervicis, 3. M. longissimus thoracis, 4. M. iliocostalis, 5. M. serratus dorsalis, 6. M. serratus ventralis, 7. M. spinalis thoracis, 8. M. rhomboideus, 9. M. trapezius, 10. M. longus colli, 11. M. scalenus, 12. M. sternothyroideus, 13. M. ectorals profundus, 14. M. ectorals superficialis, 15. M. supraspinatus, 16. M. infraspinatus, 17. M. subscapularis, 18. M. teres major, 19. M. triceps brachii, 20. M. deltoideus, 21. M. biceps brachii*

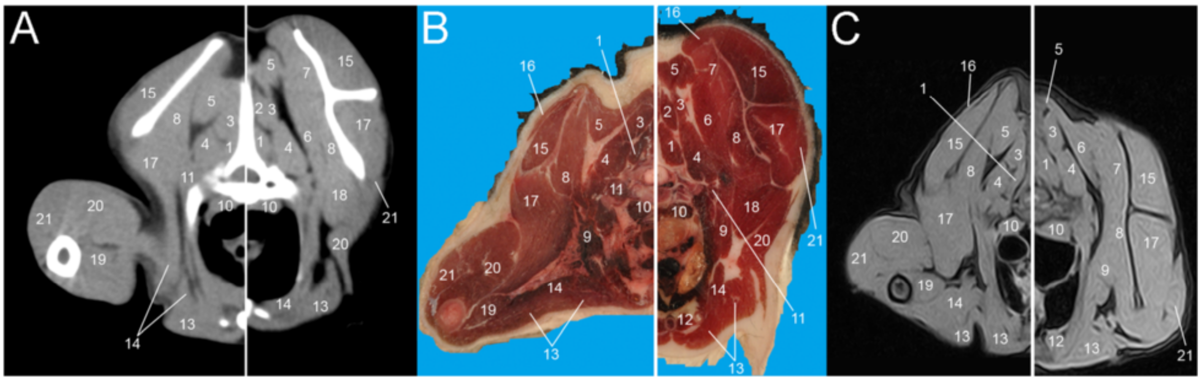

Fig. 7 Single slice CT with 400 WW and 40 WL (a), Anatomical (b) and low field T1-weighted MRI (c) slices obtained at the level of T3. Images show flexed forelimb positioning on the left and extended forelimbs positioning on the right: *1. M. multifidus, 2. M. spinalis thoracis, 3. M. longissimus cervicis, 4. M. longissimus thoracis, 5. M. rhomboideus, 6. M. serratus dorsalis, 7. M. serratus ventralis, 8. M. subscapularis, 9. M. scalenus, 10. M. longus colli, 11. M. iliocostalis, 12. M. sternothyroideus, 13. M. pectorales superficialis, 14. M. pectorales profundus, 15. M. supraspinatus, 16. M. trapezius, 17. M. infraspinatus, 18. M. teres major, 19. M. biceps brachii, 20. M. triceps brachii, 21. M. deltoideus*

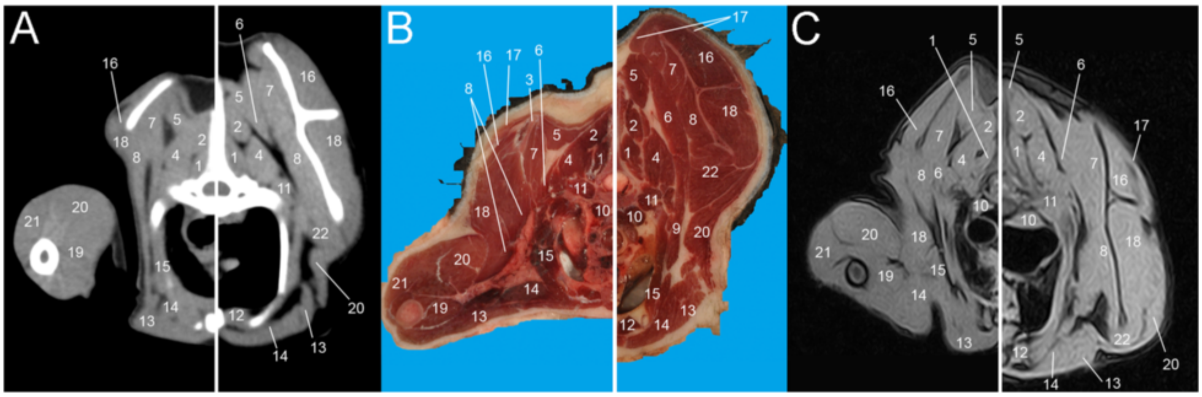

Fig. 8 Single slice CT with 400 WW and 40 WL (a), Anatomical (b) and low field T1-weighted MRI (c) slices obtained at the level of T4. Images show flexed forelimb positioning on the left and extended forelimbs positioning on the right: *1. M. multifidus, 2. M. spinalis thoracis, 3. Scapula, 4. M. longissimus thoracis, 5. M. rhomboideus, 6. M. serratus dorsalis, 7. M. serratus ventralis, 8. M. subscapularis, 9. M. scalenus, 10. M. longus colli, 11. M. Iliocostalis, 12. M. sternothyroideus, 13. M. pectorales superficialis, 14. M. pectorales profundus, 15. M. intercostalis, 16. M. supraspinatus, 17. M. trapezius, 18. M. infraspinatus, 19. M. biceps brachii, 20. M. triceps brachii, 21. M. deltoideus, 22. M. teres major.*

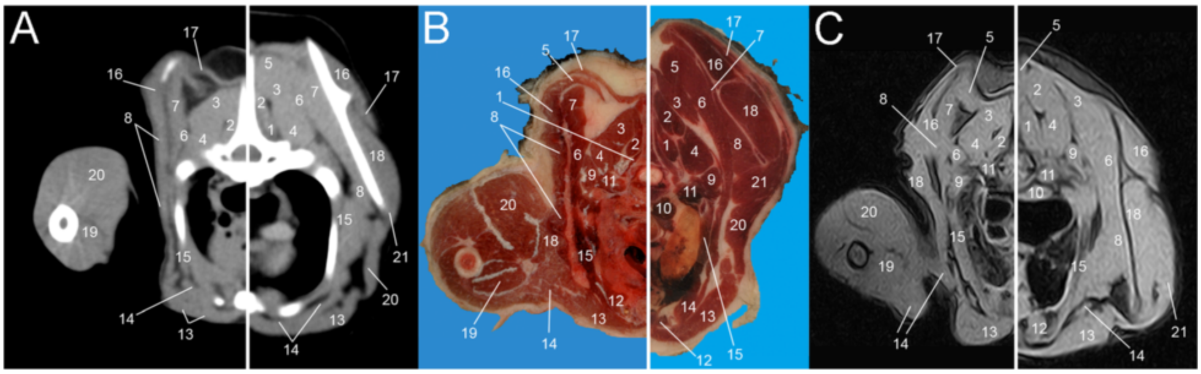

Fig. 9 Single slice CT with 400 WW and 40 WL (a), Anatomical (b) and low field T1-weighted MRI (c) slices obtained at the level of T5. Images show flexed forelimb positioning on the left and extended forelimbs positioning on the right: *1. M. multifidus, 2. M. spinalis thoracis, 3. M. longissimus cervicis, 4. M. longissimus thoracis, 5. M. rhomboideus, 6. M. serratus dorsalis, 7. M. serratus ventralis, 8. M. subscapularis, 9. M. scalenus, 10. M. longus colli, 11. M. iliocostalis, 12. M. sternothyroideus, 13. M. pectorales superficialis, 14. M. pectorales profundus, 15. M. intercostalis, 16 M. supraspinatus, 17. M. trapezius, 18. M. infraspinatus, 19. M. biceps brachii, 20. M. triceps brachii, 21. M. teres major*

3.2 Clinical and computed tomography tumour dimension assessments for planning wide excision of injection site sarcomas in cats: how strong is the agreement?

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Abstract

In injection site sarcoma (ISS) in cats lateral as well as deep margins should be correctly planned for a successful surgical outcome. The discrepancy between clinical and computed tomography (CT) measurements of dimension in resectable tumour has led to possible bias that affects the subsequent surgical dose. The aim of this study was to prospectively investigate the agreement between clinical and CT measurements of dimension in newly diagnosed ISS in cats. Fifty-three client-owned cats that underwent both clinical and CT measurements of the length and width of ISS were included. CT measurements showed a tendency towards being larger than clinical dimensions, and this difference increased with increasing tumour size. Based on our results, in further studies focusing on ISS in cats, the kind of assessment used to define tumour dimensions (CT versus clinic) should be declared and specified to properly consider surgical results and prognostic impact of this variable.

Introduction

Injection site sarcoma (ISS) in cats is a well-recognized soft tissue sarcoma characterized by very aggressive local behaviour with a high probability of local recurrence and a relatively low probability of distant dissemination.[1-7] Although multimodal therapeutic approaches have been proposed, wide margins or radical surgical excision based on tumour extent remains the primary therapeutic procedure.[6-8] Despite the peculiar presence of asymmetric and infiltrating long tumour extensions departing from the main mass making the estimation of the dimensions of ISS objectively difficult to achieve,[9-11] many studies have used clinical measurements for prognostic and surgical purposes.[4,5,7,9] The usefulness of advanced diagnostic imaging, such as magnetic resonance imaging (MRI) and/or computed tomography (CT), to plan preoperatively the surgical excision of ISS has been previously discussed and suggested,[8,9,11-15] but it has not always been applied for surgical planning.[4,5,7,9] The available veterinary literature discuss on the amount of lateral margins that should be excised around ISS in cats, whereas regarding the deep margins it has been suggested to consider one or (more recently) two not infiltrated underlying muscles layers.[6,7,9] In those studies in which both clinical and advanced imaging measurements have been reported, the tumour dimensions obtained with CT have generally been larger than those obtained with clinical measurements.[13,16,17] Recently different amount of lateral margins was hypothesized for surgical excision of ISS in cats based on the possibility that the same wide lateral margins could not be equally wide if a surgeon consider the clinical or the computed tomographic measurements of the same tumour.[16]

Size discrepancies between clinical and CT measurements of the same tumour size can impact the surgical dose applied. The aim of the study was to prospectively investigate the agreement between the clinical and CT measurements of tumour dimension in newly diagnosed ISS in cats.

Materials and methods

Client-owned cats affected by histologically confirmed, newly diagnosed ISS[18] and referred to our clinic from January 2002 to December 2013 were included. Before surgery, all the cats underwent a whole body CT examination for staging of oncologic disease, and clinical and CT evaluations of tumour dimensions were performed on the same date by the same clinician and the same radiologist, respectively. The surgeon and the radiologist were blinded to the CT and clinical measurements, respectively. All the owners provided written consent before the clinical and diagnostic procedures.

The clinical measurements were obtained before a whole-body CT examination of the anaesthetised cats. The cats were placed in sternal recumbency when the tumour was localized on the dorsal thorax and dorsal abdomen or in lateral recumbency when it was localized on the lateral thorax or abdomen. The clinical dimensions (CD) obtained by the surgeon were the longest length (CD-l) and the longest width (CD-w) of the palpable tumour, measured with digital callipers. All the CT measurements were performed with the patients in sternal recumbency with the forelimbs extended cranially, even when the ISS was localized in the interscapular region, and double positioning was performed with the forelimbs flexed/extended.[11] The dimensions obtained by CT (CTDs) were measured by the radiologist (Aycan Workstation OsiriXPRO Manager, Aycan Digitalsysteme GmbH, Würzburg, Germany) and consisted of the longest length (CTD-l) and the longest width (CTD-w) of the tumour with a so tissue window (Window Width 350, Window Level 40), based on post-contrast CT images (PQ2000S, Philips, Amsterdam, the Nether- lands; single slice fourth generation CT, with slice thickness of 1–3 mm, pitch = 1, 200–250 mA) after an intravenous injection via the cephalic vein of non-ionic contrast medium (Iohexol 350 mg I mL−1 , Omnipaque GE Healthcare, Milan, Italy) at a dose of 600 mg I kg−1.[19] For both the clinical and CT dimensions, we defined ‘length’ as the cranio-caudal axis of the tumour and ‘width’ as the transversal axis for an ISS located on the dorsal thorax and dorsal abdomen or as the dorsum – ventral axis for an ISS located on the lateral side of the body.

The shape of each tumour was also considered and was categorized as ‘regular-shaped’ when the tumour had a spheroidal (the two axes beings approximately equal) or oval (when one axis was longer than the other) shape and ‘irregular-shaped’ when a geometric shape was not recognizable. This variable was obtained both by clinical evaluation (CD-shape) and by CT imaging (CTD-shape).

For each cat, the following data were also recorded: breed, age, sex, weight, body condition score (BCS – from 1 to 5)[20] and site of the tumour.

Statistical analysis

In the absence of a ‘gold standard’ method for defining the tumour size of ISS, the agreement between dimensions retrieved by clinical and CT measurements was evaluated according to the Bland and Altman approach,[21] representing the relationship between the differences between the two methods (CTD − CD) versus the average [1/2(CTD + CD)]. The limits of agreement were than obtained as the values containing 95% of the differences between the two measurements. If, on the basis of clinical consideration, the limits of agreement were considered too wide, it can be concluded that the two measurements disagree, and they cannot be considered ‘interchangeable’. In the simplest situation, differences and their variability do not depend on the tumour size being measured, and the lack of agreement can be simply summarized by the bias (estimated by the mean of the differences) and the standard deviation of the differences. In the absence of a gold standard method, neither of the two measurements can be assumed to be ‘true’, and the average of the two measurements is considered an estimate of the tumour size being measured. The assumption of constant difference between the two measurements was evaluated by a regression model of the differences as a function of the averages and by assessing whether the estimated slope was equal to 0. The assumption of constant variability was evaluated by a regression model of the absolute value of the residuals of the above-cited regression model as a function of the averages and by assessing whether the estimated slope was equal to 0.22. In the case of non-constant differences and non-constant variances, the bias and approximate limits of agreement were obtained by considering a linear relationship between differences and averages and between standard deviations and averages.[22,23] A measurement of overall concordance, using the concordance correlation coefficient (CCC), was obtained according to Lin et al.[24] The value of CCC ranges between −1 (perfect discordance) to 1 (perfect concordance). A value of 0 corresponds to the lack of a relationship between the two measurements. The CCC is subdivided into its components: precision and accuracy. The 95% confidence interval (95% CI) of the CCC was obtained by the bootstrap method. Because of the limited number of cases, only an overall analysis was performed for all of the cases, and for a subsample of cases in which the tumours were retained as ‘regular-shaped’ by both measurement methods, without considering other clinical characteristics of the subjects. The 95% CI for the proportions of the disagreement between the classification of the tumour shape as regular-shaped or irregular-shaped on the basis of clinical and CT evaluations was obtained by the ‘exact method’ procedure suggested by Clopper and Pearson.[25] To evaluate the risk of failure in eliminating overall tumour mass, using a safety margins of 3 and 5 cm starting from each side of the clinical measurement, length and width of clinical measurements were firstly added by 6 and 10 cm, respectively and then the percentages of clinical measures exceeding CT measurement were calculated with the corresponding exact 95% CI. The statistical analysis was performed using the R and MethComp software packages (www.r-project.org). A *P* value ≤0.05 was considered statistically significant.

Results

Fifty-three cats were prospectively included in the study. Forty-eight were domestic short-hair cats, two were Persian, two were Norwegian forest cats, and one was a Chartreux. Thirty-two cats were female (of which 31 were spayed), and 21 cats were male (of which 19 were castrated). The median age at presentation was 10 years old (range: 4 – 16 years). The median body weight was 4.5 kg (range, 3 – 8.5 kg). The body weight was not available for two cats. Eight cats had a BCS of 2 of 5, 31 had a BCS of 3 of 5, 9 had a BCS of 4 of 5 and 5 had a BCS of 5 of 5. Thirty-one tumours were located on the interscapular region, 13 were on the lateral thorax, 8 were on the lateral abdomen and 1 was on the lumbar region. Forty-eight tumours were histologically diagnosed as fibrosarcomas (2/48 with areas of chondroid metaplasia) and five as malignant fibrous histiocytomas. Moderate to abundant inflammation was seen in all tumours. Inflammatory cells were mainly represented by lymphocytes and fewer macrophages. The median CD measured was 3cm (range: 0.5 – 10 cm). Both the median CD-l and median CD-w were 3 cm (range: 0.5–10 cm and 0.7–10 cm, respectively). The CD-shape evaluation considered 46 cases as regular-shaped tumours (spheroidal in 34 cases, oval in 12 cases) and seven cases as irregular-shaped tumours. The median CTD measured was 5cm (range: 0.6 – 13 cm). The median CTD-l was 5 cm (range: 0.9 – 12.7 cm), and the median CTD-w was 4.4 cm (range: 0.6 – 13 cm). The CTD-shape evaluation considered 41 cases as regular-shaped tumours (spheroidal in 18 cases, oval in 23 cases) and 12 cases as irregular-shaped tumours. Concerning the shapes of the tumours, 36 cases were classified as regular-shaped, and 3 tumours were classified as irregular-shaped by both measurement methods. Five of 53 cases (9.4%; 95% CI: 3.13–20.66%) were classified as irregular-shaped according to clinical measurements and as regular-shaped according to CT measurements, whereas 9 of 53 cases (17%; 95% CI: 8.07 – 29.80%) were classified as regular-shaped according to clinical measurements and as irregular-shaped according to CT measurements.

Length

The relationships between the CT and clinical measurements are reported in Figure 1A. In the majority of tumours, the CT measurement was larger than the clinical measurement (Figure 1B).

A plot of the differences between the two measurements (CTD − CD) against their averages suggested a tendency of the differences to increase with increasing of the estimated tumour length (slope of the estimated regression coefficient is 0.1403, *P* = 0.27) and a tendency of the variability of the differences to increase with increasing of the estimated tumour length (slope of the estimated regression coefficient is 0.15194, *P* = 0.06). Because of the small size of the case series, although the estimated regression coefficients were not significantly different from 0, a conservative approach was applied, accounting for possible increases in difference and variability.

The corresponding estimated bias and limits of agreement are reported in Figure 1B. The estimated bias ranged from 1.2 cm for an estimated tumour size of 1 – 2.18 cm for an estimated tumour size of 8 cm. The estimated limits of agreement became wider with increasing estimated tumour length, starting with values of bias of ±2.24 for an estimated tumour length of 1 cm to bias of approximately ±4.9 for an estimated tumour size of 8 cm (Figure 1B). The CCC between the two measurements was 0.52 (95% CI: 0.27 – 0.69) with corresponding precision of 0.66 and accuracy of 0.78. The value of this CCC also suggested unsatisfactory concordance between the clinical and CT dimensions. Considering the analysis of the subsample of 36 tumours classified as 'regular-shaped' using both clinical and CT evaluations, the results were similar (Figure 1C). The estimated regression slopes for the difference between measurements as a function of the estimated length and for the variability of the difference as a function of estimated length were 0.20 (P = 0.12) and 0.09 (P = 0.21), respectively. The estimated bias and limits of agreement started at 0.74 ± 2.44 for an estimated tumour length of 1 cm and ranged to 2.14 ± 4.12 for an estimated tumour length of 8 cm (Figure 1C). The estimated CCC was 0.63 (95% CI: 0.36–0.81).

Width

The relationships between CT and clinical measurements of width are reported in Figure 2A. The tendency of a CT measurement to be larger than the clinical measurement was less evident than the observed pattern for length (Figure 2A). A plot of the difference between the two measurements (CTD − CD) against their average suggested a tendency of the differences to increase with increasing estimated tumour width (slope of the estimated regression coefficient was 0.1453; P = 0.32). The increase in the variability of the differences with increasing estimated tumour width was evident and was confirmed by the regression analysis (slope of the estimated regression coefficient was 0.25055; P = 0.0062). Although the estimated regression coefficients were not both significantly different from 0, a conservative approach was applied, accounting for the possible increase in difference and variability as a function of the estimated tumour width. The corresponding bias and limits of agreement are reported in Figure 2B. The estimated bias ranged from 0.5 for an estimated tumour width of 1cm to 1.55 for an estimated tumour width of 8cm. The limits of agreement became wider with increasing estimated tumour width, ranged from values for bias of approximately ± 2.1 for an estimated tumour size of 1 cm to bias of approximately ± 6.44 for an estimated tumour size of 8 cm (Figure 2B). The CCC between the two measurements was 0.52 (95% CI: 0.27 – 0.68) with corresponding precision of 0.57 and accuracy of 0.92. The value of this CCC also suggested an unsatisfactory concordance between the clinical and CT measurements. Considering the analysis of the subsample of 36 cases classified as 'regular-shaped' tumours using both clinical and CT evaluations, the trend towards an increase in the difference between measurements with the increase in estimated tumour width is very weak (slope of the estimated regression coefficient was 0.05; P = 0.68), as was the trend towards an increase in the difference in variability with the increase in estimated tumour width (slope of the estimated regression coefficient was 0.04, P = 0.57). The average bias between the two measurements was approximately 0.56 cm, and the average limits of agreement were approximately −2.67 and 3.80, respectively (Figure 2C). The estimated CCC was 0.73 (95% CI: 0.50

– 0.86). Considering the addition of a lateral margins to each side of the clinical measurement, only in two cases CTD-l was greater than CD-l added by 6 cm (3.78%: 95% confidence interval 0.46 – 12.98%) and only in one case CTD-w was greater than CD-w added by 6cm (1.89%: 95% confidence interval 0.048–10.07%). Overall, if 3cm for each side are added to CD, the risk of failure to eliminate the corresponded tumour highlights by CT should be 3/53 (5.7%, 95% confidence interval 1.18–15.66%). If 5 cm for each side were added to CD, no CTD was greater than CD added by 10 cm. The risk was estimated to be 0/53 = 0%, nevertheless, after computing 95% CI, the upper limit was 6.72%.

Discussion

Tumour dimension is one of the first aspects evaluated in the pre-operative setting, to outline the prognostic consultation, as well as to calibrate the surgical dose. These considerations are particularly relevant for ISS in cats because the correct surgical approach is crucial for ensuring clean surgical margins and increasing the probability of a cure.[6,10,15] In addition, the dimensions of ISS in cats influenced the surgical time and therefore indirectly impacted the risk of wound healing complications, which could induce postoperative morbidity and postpone other adjuvant therapies.[16] Despite the relevance of such aspects, a standardized approach for measuring the dimensions of ISS in cats is not currently available. In small animal oncology, especially when dealing with feline ISS, the use of advanced imaging techniques in the pre-surgical setting has recently increased, but their use is still not a rule, and it is mostly left to surgeon choice.[4,7,9,15,16] In this study, tumour dimensions, both length and width, evaluated by CT showed a tendency towards being larger than the dimensions measured with callipers, consistent with previous reports.[13,16,17] In addition, in a proportion of cats, the shapes retrieved by the two methods demonstrated discrepancies. These findings could be related to the specific characteristics of ISS and specifically to the presence of non-palpable tumour extensions departing from the clinically palpable tumour. These thin tumour peripheral projections are mostly detectable only with CT contrast medium or histologically.[10,11,17] Actually, these extensions are not always composed of neoplastic cells because inflammatory infiltration (small lymphocytes and rarer macrophages) and the rich neovascularization that often histologically characterizes ISS can also be highlighted by CT contrast medium.[11,12,18] A distinction between neoplastic and inflammatory tissue can be achieved only histologically; however, it has been suggested that inflammatory tissue around an ISS should also be excised.[11] Therefore, the tumour burden in this study included all the tissue enhanced by the CT contrast medium. The difference between tumour dimensions obtained with the two techniques was also corroborated by the statistical analysis, which revealed a weak concordance between clinical and CT evaluations of tumour dimensions. This weak concordance was confirmed even when considering the subsample of 'regular-shaped' tumours, in which greater agreement was expected. Moreover, other results also showed wide limits of agreement, particularly with increasing tumour size, indicating that, when the surgeon clinically measures the tumour, a wide range of possible CT measurements is possible. In addition, this range of possible values became wider if the tumour increased in size. This could be explained by the hypothesis that a tumour that has the ability and/or the time to grow also has a greater likelihood of infiltrating the surrounding tissues, forming longer tumour extensions and thus increasing the discrepancy between the two methods of measurement by increasing the tumour dimensions. Another explanation could be that, while CT images allow for the precise evaluation of only the tumour edges, when the tumour was clinically measured with callipers, the thickness of the skin and so tissues that covered the mass could not be subtracted from the value obtained, and other variables related to the single cat, such as BCS and the anatomical site of the tumour, could contribute to that variability. These findings suggested that it is not correct to estimate the tumour size only using one method of assessment but rather than both methods should be applied. The gold standard for measuring tumour dimensions should be the method that provides a value closer to the real dimensions of the tumour. In human breast cancer, another tumour category in which surgery is the mainstay of treatment, the gold standard is to obtain the tumour size measured by pathology, and with calibration, it is possible to record which technique of measurement applicable in the pre-surgery setting was closer to that gold standard.[26] Unfortunately, in the specific case of ISS in cats, the gold standard method for tumour measurement remains unknown. Some studies have emphasized the utility of advanced imaging techniques for better clarifying tumour edges and planning excision,[8,9,11-15] but the real impact of this procedure on surgical and oncologic outcomes, rather than being applied for clinical evaluation only, has not been well documented.[8] In the absence of a gold standard, it is not possible to determine the perfect pre-surgical approach. The wide margins proposed for ISS excision have ranged from 3 to 5cm,[6,8] as previously hypothesized,[16] the results of the study emphasized the necessity for the surgeon to approach the same ISS in a different manner, depending on whether the tumour dimensions were obtained with CT or callipers. In cases of clinical measurement the widest margin should be required, whereas in cases of tumour dimensions obtained with CT this margin could be reduced. Recently, in fact, a wider margin of excision of 5 cm of healthy tissue around clinically palpable margins of ISS in cats that did not undergo a pre-surgical CT examination was proposed.[7] In the case series of this study the addition of 10 cm (5 cm of margins for each side of the linear measurement) to the value obtained clinically led to include all the measures retrieved by CT. Simplistically this result seemed to suggest that using a safety margin of 5 cm around palpable mass almost all tumour detectable tomographically would be excised. At the same time it is also probable that for some of these tumours a 5 cm margins in clinics correspond to a marginal excision of the tumour highlighted by the CT contrast medium. Based on this consideration, a perspective randomized study comparing the two methods of size assessment (and consequent margin of excision) in relation to oncological outcome should be ideal in further studies. The dimensions of the tumours in cats are often reported as maximum diameters,[7,9,15] but in this study, two perpendicular axes were considered because the major axis between length and width on clinical evaluation is not necessarily the major axis even on CT evaluation, thus making the comparison between the two methods susceptible to bias. The third axis was not calculated because of presence of anatomical limits, such as underlying bone or cavities, making it impossible to measure clinically with callipers the thickness of the tumour. These data are therefore not available for comparison with CT measurements. In addition, the surgeon calibrates the depth of the excision based on the infiltration of fascial planes and not based on linear measurement of the thickness. Regarding this practice, the use of CT evaluation could facilitate the identification of deep so and bone tissue infiltration,[11,15] the detection of which is not always easy to perform in a clinical fashion. The use of other measurements, e.g. area, was not applied because these data could only be retrieved using a mathematical formula that approximates the real extension of the tumour, particularly in 'irregular-shaped' tumours, so a comparison of linear measurements was considered more appropriate.

This study emphasized that dimension of ISS in cats obtained with CT are often larger than those measured with callipers. This finding suggested that the margin of excision in cases of clinical measurements should be larger than in cases of CT evaluation. These differences highlighted the necessity to declare and standardize the method of tumour dimension assessment in studies focusing on ISS in cats in order to properly consider surgical results and prognostic impact of this variable. As it has already been reported in the literature, the usefulness of contrast-enhanced whole-body CT for ISS in cats is not only linked to the role of planning lateral excision margins, but it also has the ability to estimate deep margins and to detect distant metastasis.[11,27] These aspects are crucial points to discuss before surgery with surgical staff and the owner. Further studies are necessary to evaluate other advanced imaging techniques, such as MRI, and to introduce a gold standard method to create guidelines for the pre-surgical evaluation of the dimension of ISS in cats.

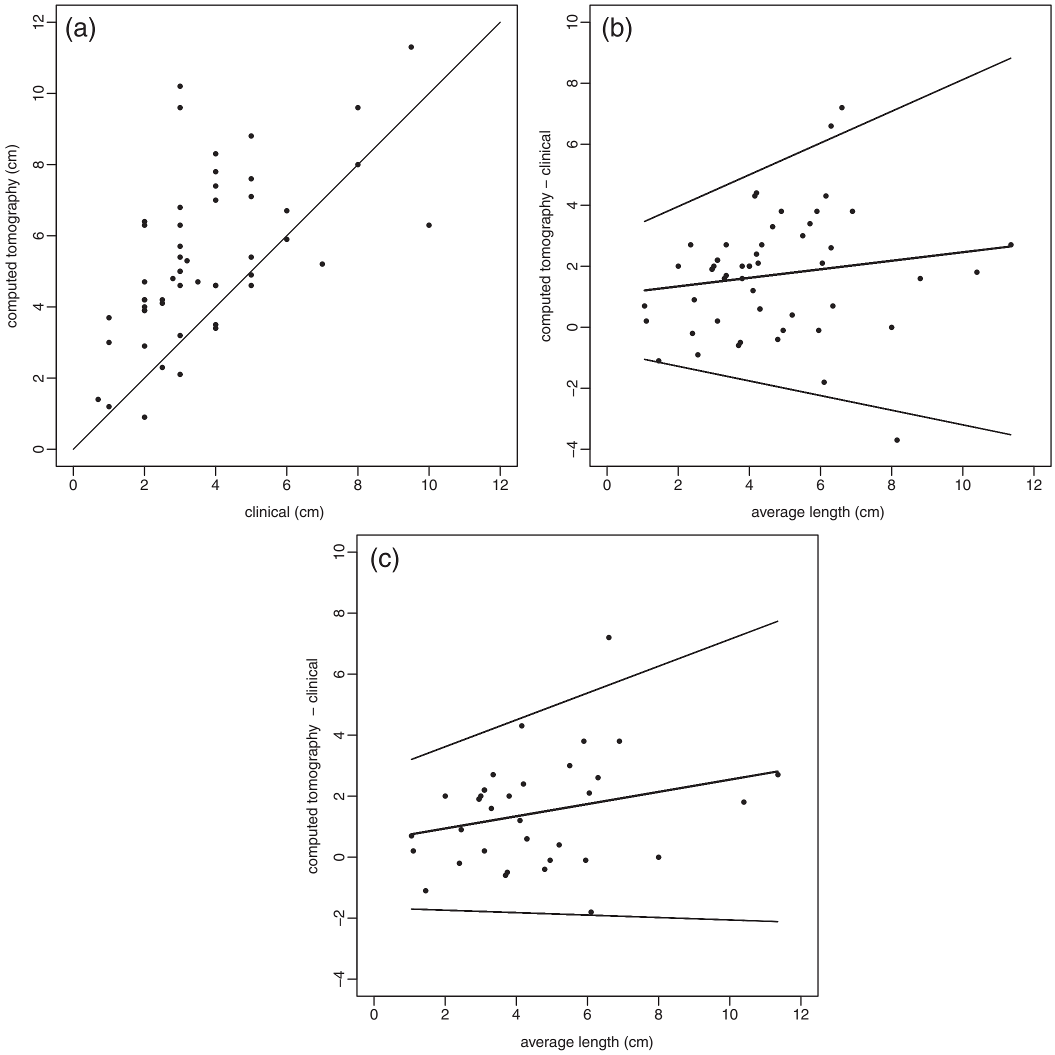

Figure 1. (A) Tumour length on the whole tumour series: clinical measure against computed tomography measure. The couple of measurements for each tumour are represented by points and the line represents the putative perfect agreement between the two measures. (B) Bland–Altman plot describing the agreement between the computed tomography measure of tumour length and the clinical measure of tumour length on the whole tumour series. The couple of measurements for each tumour are represented by points. The central line is the estimated bias and lower and upper lines are the estimated limits of agreement. (C) Bland–Altman plot describing the agreement between the computed tomography measure of tumour length and the clinical measure of tumour length on the subsample of regular-shaped tumours. The couple of measurements for each tumour are represented by points. The central line is the estimated bias and lower and upper lines are the estimated limits of agreement.

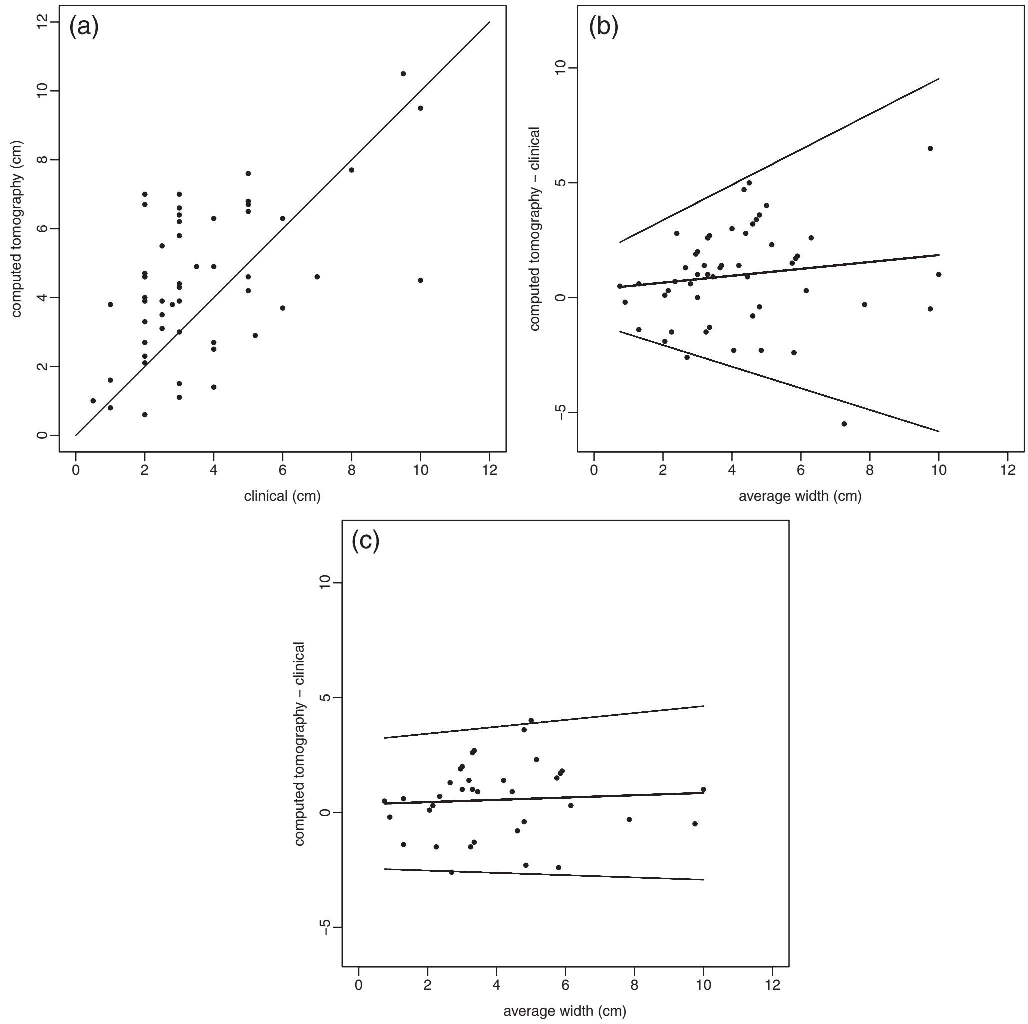

Figure 2. (A) Tumour width on the whole tumour series: clinical measure against computed tomography measure. The couple of measurements for each tumour are represented by points and the line represents the putative perfect agreement between the two measures. (B) Bland–Altman plot describing the agreement between the computed tomography measure of tumour width and the clinical measure of tumour width on the whole tumour series. The couple of measurements for each tumour are represented by points. The central line is the estimated bias and lower and upper lines are the estimated limits of agreement. (C) Bland–Altman plot describing the agreement between the computed tomography measure of tumour width and the clinical measure of tumour width on the subsample of regular-shaped tumours. The couple of measurements for each tumour are represented by points. The central line is the estimated bias and lower and upper lines are the estimated limits of agreement.

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3.3 Dynamic tomographic studies on Injection Site Sarcoma in cats: essential or useless practice?

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Abstract

Objectives: Feline Injection Site Sarcomas (ISS) are soft tissue tumours characterized by a typical interscapular location and highly infiltrative behaviour. Computed Tomography can be considered the modality of choice for ISS staging and a double positioning (dynamic approach) was reported to successfully detect the exact amount of infiltrated muscles. The aim of the study was to investigate the utility of the dynamic approach in patients referred for presurgical staging of interscapular ISS.
Methods: tumour volume assessment between the ellipsoid/semi-automated segmentation methods, muscular infiltration between different observers/ISS and positioning were assessed by two radiologists blinded to patient coding.
Results: Results demonstrate good agreement between observers, with higher tumour volumes detected via the ellipsoid method. Moreover, lower tumour volumes showed slightly decreased muscular infiltration.
Conclusions and relevance: In conclusion dynamic approach should be performed for a complete evaluation of the invasiveness of the ISS along with an appropriate selection of tumour volume methodology, which could potentially affect the presurgical assessment of ISS.

Key words: injection site sarcoma, double positioning, dynamic approach, interscapular, volume, CT

Introduction

Feline Injection Site Sarcomas (ISS) are highly infiltrative mesenchymal tumours exhibiting aggressive local behaviour in the adjacent musculoskeletal structures, such as the interscapular muscles, the spinous processes and the scapulae. The high local recurrence rate is one of the major concerns of this subcutaneous sarcoma, which is frequently located in the interscapular region. Even following the current surgical indications of excising up to 3-5 cm of perilesional tissue and up to 2 muscles layers, the local recurrence rate is up to 14-22%, in case of non-infiltrated margins at the histopathological examination and up to 69% with infiltrated margins.[1-5]

Despite different publications focused on the strategies for reducing the high local recurrence rate, including chemotherapy and radiotherapy as useful complementary tool to reduce tumour regrowth[2-3,6] and margins' infiltration, this peculiar feature of the tumour is still representing a relevant debate topic, since surgery is often the unique and mainstem therapy.[2] Computed Tomography (CT) and Magnetic Resonance (MRI) have been introduced into clinical planning for a better definition of the relationship between neoplastic lesions and the surrounding soft and hard tissues, considering the high degree of surgical excision invasiveness.[7-9] Although no specific gold standard diagnostic imaging technique has been standardised for the surgical planning of ISS and even the only clinical evaluation of the neoplasm has been proposed in the past,[1] CT is commonly applied for a better understanding of the relationship with the adjacent musculoskeletal structures and assessment of deep margins.[8,10] Few studies have been published concerning new imaging strategies for a specific definition of the anatomy of the region[11] and consequently its margins, or to compute the real volume of the neoplasia increasing the agreement with the histopathological measurements.[10,12] Moreover the effects of formalin fixation on the assessment of tumour volume have been investigated, comparing CT measurements with histopathology.[12] Results showed small shrinkage after ISSs excisions and formalin fixation. The tumour volume estimated by the 3-D software was described to be more accurate compared to the ellipsoid formula, suggesting the use of this method when accuracy of the tumour's volume is of particular concern on CT.[12]

In 2013 it has been postulated that as a consequence of the high mobility of the interscapular region, the common location for ISS,[2] the mass may change its relationship with the surrounding tissues depending on the forelimbs' position during the CT exam.[8] An additional post-contrast scan with the forelimbs flexed along the body was suggested, after the conventional study with the forelimbs extended cranially (Figure 1), to correctly estimate the extent of the local invasion and avoid unnecessary invasive surgical resection of structures non infiltrated by the tumour, such as the scapulae or the spinous processes.[8] Results of dynamic positioning in 10 patients affected by ISS showed a slightly reduced number in the muscles infiltrated by the tumour in flexed positioning. Therefore double positioning (Figure 1) was suggested as a standard protocol for the evaluation of the ISS when the common interscapular location is displayed.[8]

Nevertheless, there is a lack of more extensive case-series highlighting pros and cons of double positioning in the estimation of the number of structures infiltrated by the ISS, with convincing statistical evidence. Our first aim was to collect a high number of patients affected by interscapular ISS to investigate the utility of double positioning in the presurgical evaluation of the neoplasms and its feasibility between different observers.

Moreover our hypothesis was that larger volumes of the neoplasms could have a proportional major impact in the muscoloskeletal infiltration detected by CT, with small neoplasms showing poor infiltration within the surrounding anatomical structures.

Materials and methods

Client-owned cats that underwent CT exam for pre-surgical staging between December 2006 – January 2016 were included in the study, if histological diagnosis of injection site sarcomas was available. Only ISS at first presentation presented within the interscapular region (between C5-T5) were included. All the owners provided written consent before diagnostic procedures.

CT studies have been performed with two different scanners: a single slice Picker PQ 2000S (Philips, Monza Italy) and a 16 slice GE Brightspeed Elite (General Electric, Milano Italy). Images have been acquired in helical mode with thin slice thickness ranging between 1-3mm and a pitch=1. Three thoracic and abdominal scans have been performed with the patient in sternal recumbency: a pre and post contrast scan with the forelimbs extended cranially followed by a final post contrast scan with the forelimbs flexed caudally, along the body (Figure 1). Contrast medium (350mg/I Iohexol, Omnipaque, General Electric, Milano Italy) has been injected with a concentration of 600mgI/Kg through the cephalic vein, by manual injection or a power injector system (Medrad® Mark V Plus, Bayer, Milano Italy), at a rate of 2-3ml/s according to the patient's weight.

Images were sent to PACS (Picture Archive and Communication System) and processed with a certified medical software (OsirixPRO® 64 Bit – Aycan Medical Systems, Genève Switzerland). Two radiologists who were blinded to patient coding assessed the number of muscles infiltrated by the tumour on the post contrast scan in extended and flexed position, as previously described.[8] Additional measurements consisted in tumour volume by means of the ellipsoid formula[10] (4/3π(1/2X*1/2Y*1/2Z)), with X= width (latero-lateral dimension), Y= height (dorso-ventral dimension), Z= length (cranio-caudal dimension) and 3D semi-automated segmentation, similar to what previously reported in literature.[12] These measurement were performed by adjusting the window levels and computing the volume on the post contrast scan by the use of the "ROI segmentation" tool provided by the certified medical software: a ROI (region of interest) was drawn around the tumour in every slice, then the software computed a volume rendered model of the neoplasm. One radiologist recorded the distance between the neoplasm and the adjacent skeletal structures in all the patients.

Results have been recorded in a spreadsheet file. The R statistical system (The R Foundation 2016 – www.r-project.org) was used to plot and analyse the data. The relationship between ellipsoid volume and segmentation volume was assessed using a linear model with no intercept term, as a tumour with zero volume would be expected to have zero volume by either method. The difference in counts of muscle infiltration between the extended and flexed positions were assessed by means of a two sample permutation test with the null hypothesis that the distribution of counts was the same for both methods. The observer discrepancy was assessed compared to the positioning method by means of an exact signed rank test. The correlation between tumour volume and number of muscles infiltrated was assessed by means of Spearman's rank correlation.

Results

From 248 cats referred for ISS staging eighty-four cats met the inclusion criteria: 78 Domestic Short Hair, 2 Chartreux, 1 Maine Coon, 1 Siamese, 1 Persian, 1 Norwegian. Age ranged between 1-15 years (mean age 10.03 years, median=10). The weight was not available in 12 patients. For 72 cats, mean and median weight was 4.4 kg and 4 kg, respectively (range 2.8-7 kg). The computed volumes ranged between 0.26-323.87cm^3 (average volume 45.9 cm^3; median volume 16.41 cm^3) with the ellipsoid formula and between 0.14-238.21 cm^3 with the 3D-software formula (average volume 32.24 cm^3; median volume 10.18 cm^3).

The relationships between ellipsoid and segmentation estimates of tumour volume are shown in Figure 2. A linear model of segmentation volume on ellipsoid volume explained almost most of the variation in segmented volume (adjusted r squared = 97.5%). Segmentation volume was estimated to be 70% of the ellipsoid volume (95% confidence interval 67.5% to 72.3%, $p < 0.001$).

Twenty-five neoplasms did not show any relationship with the surrounding musculoskeletal structures with increased adipose tissue lying in between. The rest of the neoplasms (N=59) invaded the adjacent muscular structures with up to 15 muscles infiltrated. The most represented muscles were in descending order of representation: *trapezius, rhomboid, latissimus dorsi, supraspinatus, longissimus colli, longissimus thoracis, dentatus dorsalis, dentatus ventralis, subscapularis, infraspinatus, scalenus.*

Between the two positioning (extended and flexed) the average number of muscles infiltrated was 1.9 (extended) and 1.84 (flexed) for the observer A, respectively 1.89 (extended) and 1.85 (flexed) for the observer (B).
In six cases for the observer A and eight cases for the observer B a discrepancy between the extended and flexed positioning was detected. In particular, between the discrepancies for the observer A in five cases the extended positioning showed higher muscles' infiltration, while in only one case the flexed positioning recorded a higher number of infiltrated muscles. For the observer B eight discrepancies were identified between the different positioning with five cases recording a higher number of muscles infiltrated in extended and three cases in flexed positioning. There was no evidence of a statistically significant difference between the extended and flexed counts of muscle infiltration ($p = 0.8708$, two-sample permutation test). Furthermore, there was no evidence of a difference in counts between observers, being associated with extended versus flexed positions ($p = 0.6875$, exact signed rank test) (Figure 3).
The observers disagreed on five extended measurements and three flexed measurements. Overall disagreement between observers consisted only of a difference of 1 in the counts of the muscles' infiltration (Figure 3).
There was a weak positive correlation between tumour volume and the number of muscles infiltrated by the tumour for both extended (correlation = 0.52, $p < 0.001$) and flexed (correlation = 0.51, $p < 0.001$) positions. No large tumours showed absent muscle infiltration however some small tumours showed large amount of infiltration (Figure 4).
In 38 cases the ISS appeared adjacent to at least one spinous process, with only one case showing mixed osteolytic and osteoproliferative changes. Between 112 spinous processes displayed adjacent to the ISS, 84 showed similar adhesions in both positioning, 19 were adjacent to the ISS in flexed but not in extended, 9 were adjacent in extended but not in flexed.
Contiguity of to at least one scapula was visible in 24 cases, with overall 32 scapulae close to the ISS: 15 were adjacent in both positioning, 12 only in flexion and 5 only in extension. No bone changes have been detected at the level of the scapulae.

Discussion

Feline ISSs are fast growing tumours characterized by local infiltration towards the musculoskeletal structures and high local recurrence rate (19-69%)[1,3,4], while the distant local metastatic spread is reported to be considerably low, between 12-28%.[1,3,13] These peculiar features of the tumour make the surgical planning an essential step to reach a wide and or radical excision of the neoplasm, that may provide a longer disease-free period (901 days) compared to the excision of neoplasms with infiltrated margins (79 days).[1,3] CT detection of the peripheral digitations including tumour's extension, skip metastasis or perilesional panniculitis is of critical importance for scar tissue healing and reduction of tumour's regrowth.
The surgical goal is the removal of as much perilesional tissue as possible and CT detection of the peripheral digitations including tumour's extension, skip metastasis or perilesional panniculitis could direct the surgeons' preoperative evaluation.[1-2,4,8] However, because of the highly dynamic interscapular location of the tumour, the adjacent skeletal structures could appear involved based on the conventional CT images acquired with the forelimbs extended cranially (Figure 1), resulting in a highly aggressive surgery for the resection of the desired amount of perilesional margins.[8]
In 2013 Travetti et al. firstly described the dynamic approach,[8] with results from 10 patients affected by interscapular ISS, showing preliminary results of slight reduction of muscles' infiltration by the tumour between flexed and extended positioning of the forelimbs. In fact, the large amount of fat lying in between the scapula and the fascial planes makes this region

extremely movable as shown in a previous publication[11] describing the changes of the relationship of the musculoskeletal structures in a dynamic approach in non-pathological patients, between different imaging modalities (CT, MRI and gross-sectional anatomy). Although in the majority of our cases the number of infiltrated muscles was the same, slight discrepancies between the two positioning and observers have been detected (6 for the observer A, 8 for the observer B). Between the discrepancies a tendency of the flexed positioning to record a reduced number of muscles infiltrated by the tumour is observed in 5 cases, in both observers.
The discrepancies of the measurements between the observers could be considered unrelated to the two different positioning (Figure 3). Our results partially agree with the study of Travetti et al. 2013,[8] strongly suggesting to consider a double positioning for a correct estimation of ISS, although it is difficult to elect the most accurate positioning for the study of ISS. We believe that a thorough estimation of tumour's margin and infiltration could potentially decrease unnecessary inclusion of structures not involved in the pathological process, within the perilesional margins. Moreover the difference of the measurement of the distance between the ISS and the adjacent skeletal structures (vertebral spinous processes, scapulae) resulted markedly different between the two positioning. This result also supports to include an additional dynamic evaluation with the forelimb flexed caudally along the body (Figure 1) when the neoplasm is adjacent to the skeletal structures, to underline possible movement of the neoplasia away from these structures. In fact, a movement of these structures away from the neoplasm could enhance different amount of tissues included in the perilesional margins based on the CT evaluation, especially in cases where the reduced amount of subcutaneous adipose tissue makes the interscapular structures lying in close proximity (Figure 1).
The assessment of tumour dimension via clinical calliper has shown wide limits of agreement compared to CT, with increased variability when the neoplasms increased in size.[10] Both CT and MRI have been described as useful tool for the investigation of ISS[7-9]; however CT is commonly used because of the ability to investigate the tumour characteristic and perform tumour staging in the same scan.[8-10] Moreover multiplanar reconstructions represent an important advantage for the correct estimation of the volume of the tumour, reducing the gap between tomographic volumetric measurements and histopathology. We tested the difference in computing the volume with a semi-segmentation software tool compared to the ellipsoid formula. Our results show considerably different measurements between the 2D and 3D method (Figure 2). In particular, in contrast to what stated by previous Authors[16] the tumour volume estimated by the ellipsoid formula resulted consistently and significantly higher than that estimated by the 3-D CT software tool (Figure 2). These results could be attributed to different reasons: first some of the neoplasms included in the sample showed a very irregular shape that could be easily overestimated by the ellipsoid formula and less with the semi-automated computed volume, where the contour of the mass is exactly drawn manually and computed by the algorithmic conversion. Moreover the software used was different from the one previously described, which consisted in a fully automated segmentation. However, we consider this hypothesis less likely because in all the sample population no variation was recorded in the results and in general it is more likely that the assumption of an ellipsoid shape could cause a slight overestimation of the tumour volume. The reduced number of the sample in the previously reported paper could potentially has affected the results, if less irregular neoplasms were displayed. In humans, the application of accurate segmentation is crucial on MRI, CT and ultrasound in assessment of tumour RECIST (Response Evaluation Criteria in Solid Tumours)[14] and progression of disease, such as Benign Prostatic Hyperplasia (BPH).[15] Semi-automated and fully automated techniques produce reliable exact measurements of the pathologies and will be considered essential in the future when US-CT, PET-CT and PET-MRI fusion techniques will be widely applied also in veterinary medicine. The

agreement between the ability of the two radiologists to detected muscles' infiltration resulted in only 8 disagreements over 84 cases, which is not affected by the similar advanced degree of experience of both observers. A weak correlation has been detected between tumour volume and number of infiltrated muscles: as expected infiltration was higher with larger volumes; however caution should be taken into account because even few small tumours showed consistent muscles' infiltration (up to 15 muscles – Figure 4). These results are in agreement with previous postulation and confirm that even small neoplasms could show a highly infiltrative behaviour towards the surrounding musculoskeletal structures.[16] We believe that this statement could suggest to consider a complete diagnostic imaging work-up even in case of small palpable lesion, to avoid any improper assessment of the neoplastic infiltration.

From our results none of the two positioning appeared always more accurate than the other one, however the flexed positioning reached a lower infiltration rate in 5 patients, between both observers. One main limitation of this study is the lack of post surgical and oncologic outcome in the patients included in this study. Future perspective will be oriented in this direction to detect how the CT diagnostic performance could potentially affect the clinical outcome of patients affected by ISS. An additional limitation of this study is the undetected increased amount of radiation exposure due to the additional final scan, although we consider this aspect of less concern for veterinary oncologic patients.

Conclusion

Our study strongly suggests to consider a CT dynamic approach in the study of feline ISS, by including an additional post contrast scan with the forelimbs flexed caudally along the body when the tumour lies in the interscapular region, in proximity to the musculoskeletal structures. The semi-automated segmentation of the neoplasms should be used for an accurate pre-surgical planning of tumour volume, also in case of small neoplasm that could potentially show a highly infiltrative behaviour on CT.

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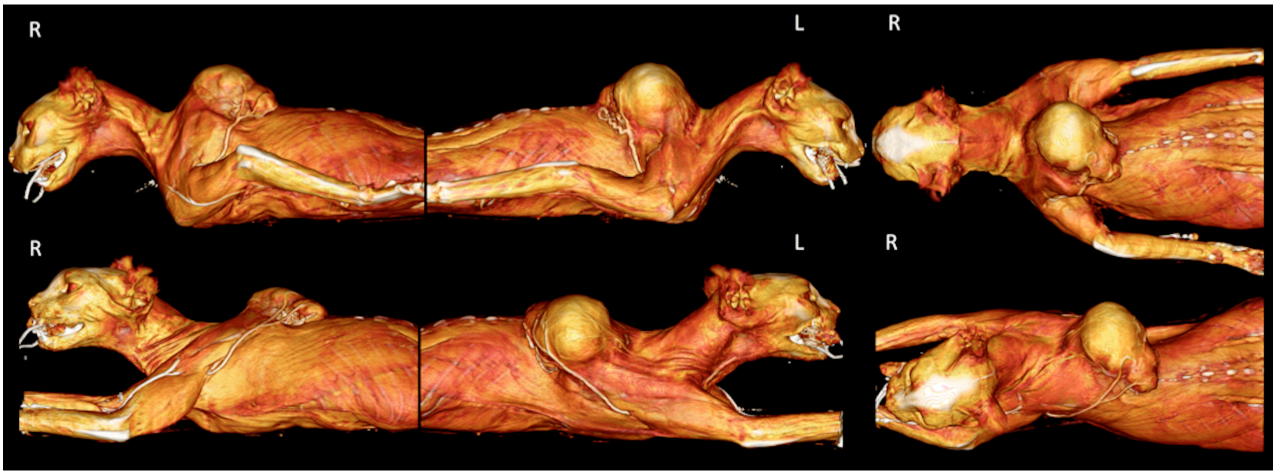

Figure 1. 3D CT MPR reconstruction of the double positioning of the patient with the forelimbs extended cranially and flexed caudally along the body. R means right and L means left.

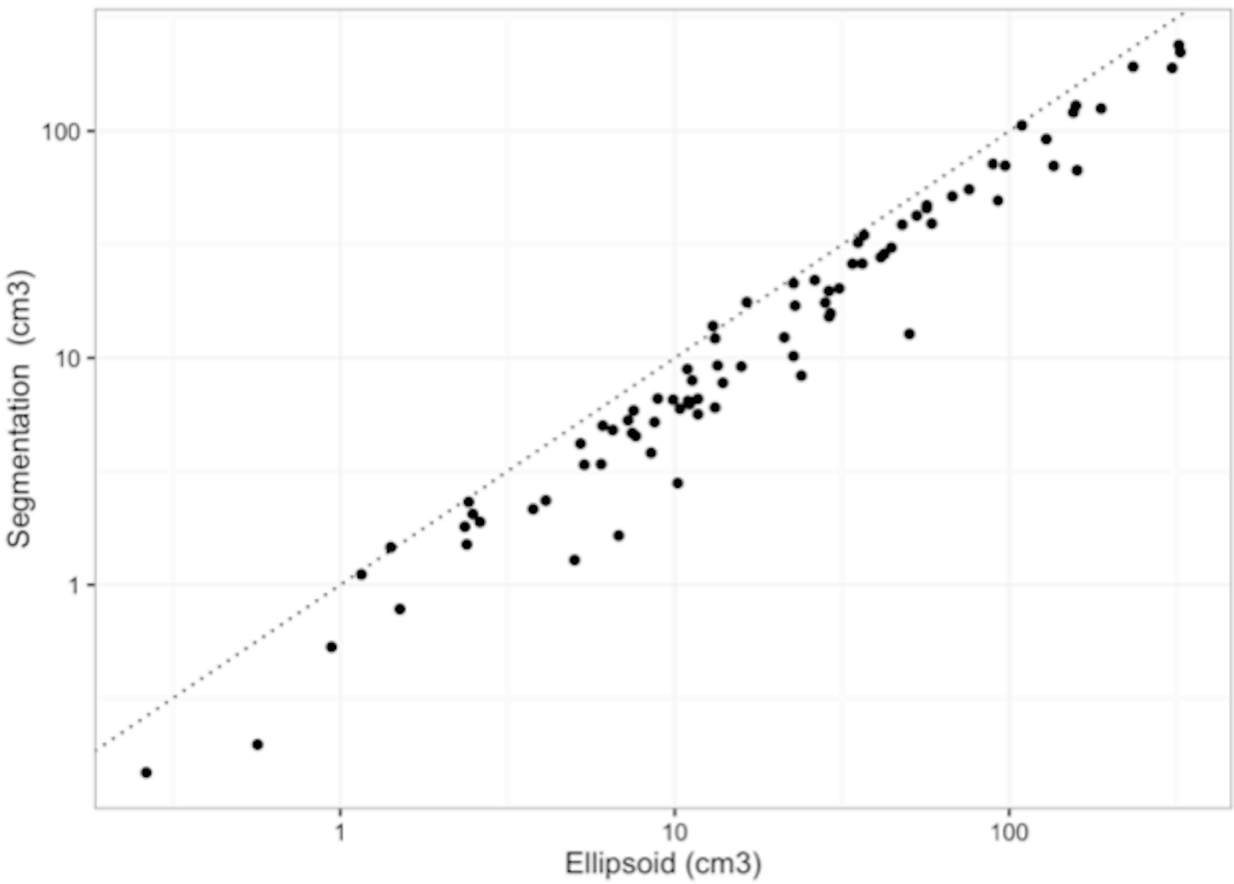

Figure 2. Scatter plot of ellipsoid estimate and segmentation estimate of tumour volume. The dotted line is an identity line. Logarithmic scales are used as there was a wide range of tumour volumes. The ellipsoid method generally estimated higher neoplasm volumes than the segmentation method.

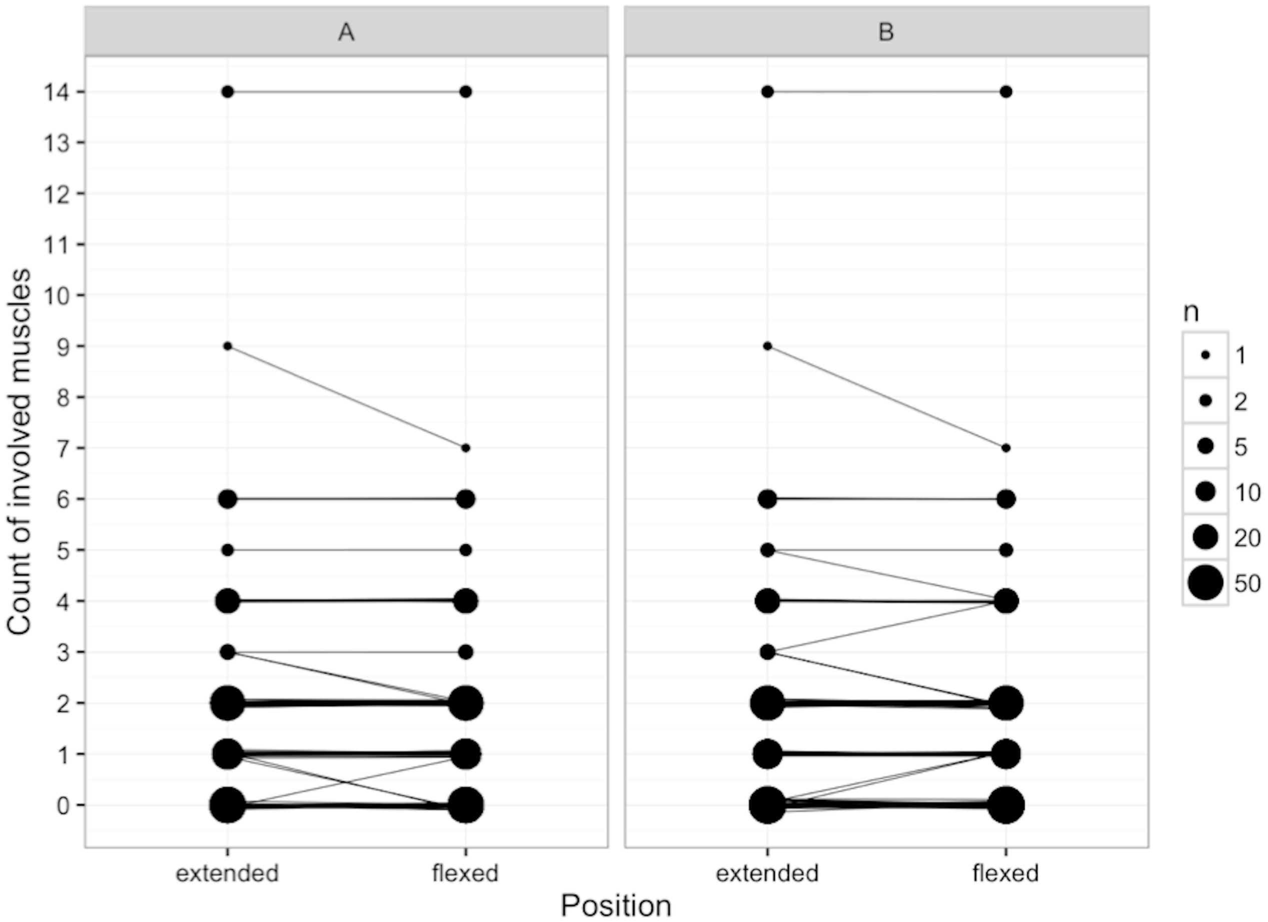

Figure 3. Observer assessment of muscle involvement by positioning methods. For each observer, assessments of the same tumour are joined by a solid line. The sizes of the points show the number of tumours assessed to have a particular count by each observer/positioning method. Horizontal lines represent assessments that were the same for each positioning mode; sloping lines represent differing counts.

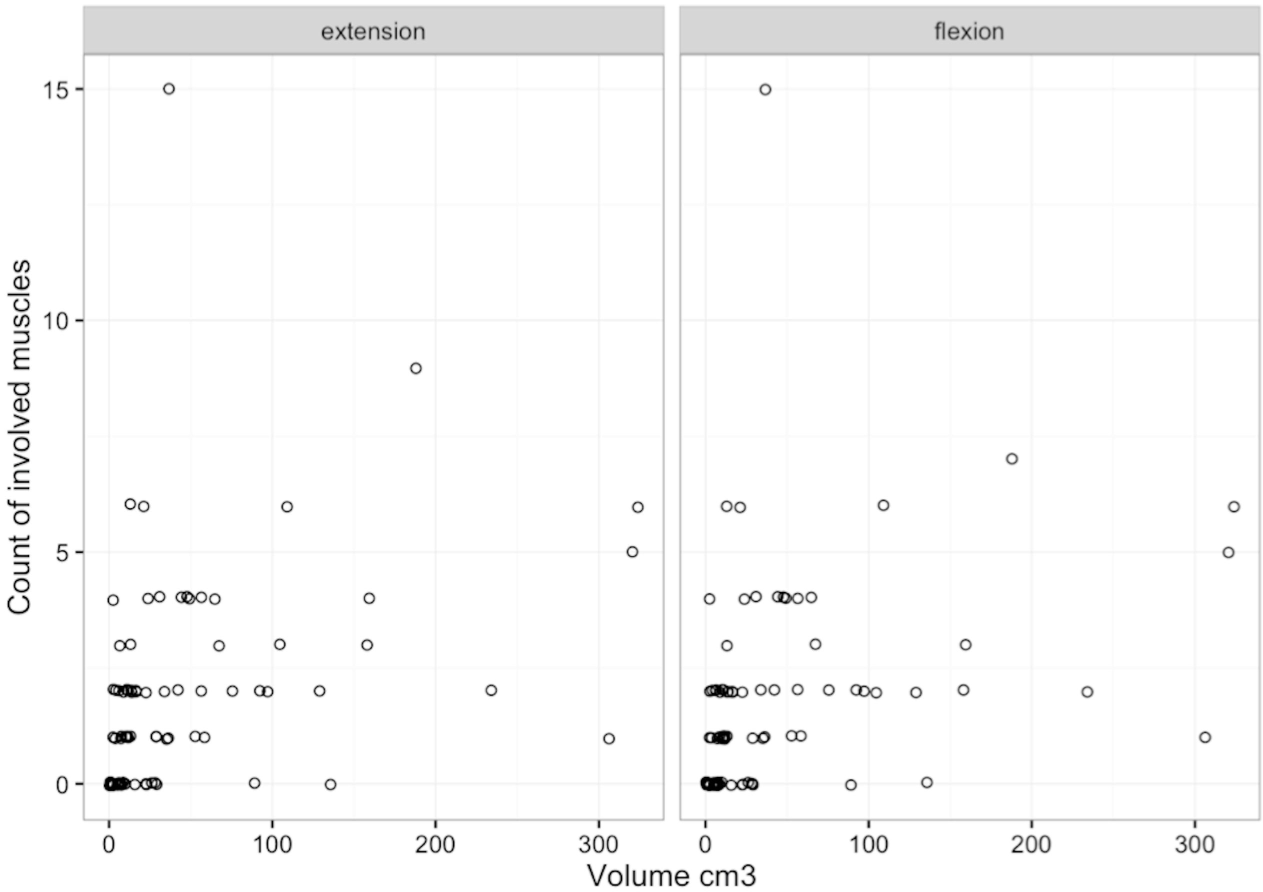

Figure 4. Scatter plot showing count of muscle involvement versus tumor volume for both positioning methods. Points were adjusted vertically to separate them for visibility. Higher muscle infiltration is visible at higher volumes; however, a few small neoplasms show consistent infiltration.

4. Discussion

The study of the interscapular region of the feline patient is challenging, because of different layers of muscles and adipose tissue lying in close proximity and adjacent to different skeletal structures. Moreover the absence of a clavicle connecting the forelimbs to the axial skeleton makes this region extremely movable and dynamic, especially during flexion and extension of the forelimbs. The lack of specific references highlighting the variability of the relationship between the different musculoskeletal structures is mainly due to the complexity in displaying these changes.

A rapid and accurate identification of muscles themselves and of their spatial relationship with the surrounding structures could improve the study of the margins of the lesions, calibrating the therapeutical interventions, based on this estimation.

An atlas providing an exhaustive description of the interscapular region of the cat considering its dynamic behaviour, by comparing cross-sectional imaging of gross, CT and MRI anatomy, was not available in the literature.

The dynamic multi-modality approach represents a novelty considering both techniques simultaneously in cats, comparing either cross-sectional anatomy and CT or cross-sectional anatomy and MRI. The use of a 3/6mm slice thickness provided an accurate comparison between gross anatomy slices and advanced imaging techniques. In this context, the choice to maintain vertebral bodies as the reference pivot proved to be a useful tool in detecting and describing the anatomical structures.

In veterinary medicine, sternal recumbency is the standard positioning commonly recognized for CT studies of the thoracic region; however, in the cat, tomographic references available in the literature were all obtained with animals positioned in dorsal recumbency.

In animals, sternal recumbency should be preferred to dorsal recumbency in cases of advanced imaging examinations, such as CT or MRI, to reduce possible lung congestion occurring in prolonged dorsal recumbency. In our study, sternal positioning with the forelimbs either being flexed along the body or pulled cranially, was aimed at better visualizing the musculoskeletal structures by enhancing their mobility. Furthermore, since double positioning made the myofascial system and adipose tissue infiltrations visible, the images acquired can support a more accurate estimation of the relationship between both infiltrating and neoplastic lesions and surrounding tissues.

Currently, establishing the best relationship between a mass and its surrounding tissues whilst considering the mobility of the region is critical and surgery still represents the treatment of choice, often including ostectomies or osteotomies, if necessary.

Tumour dimension is one of the first aspects evaluated in the pre-operative setting, to outline the prognostic consultation, as well as to calibrate the surgical dose. These considerations are particularly relevant for ISS in cats because the correct surgical approach is crucial for ensuring clean surgical margins and increasing the probability of a cure. In addition, the dimensions of ISS in cats have been reported to influence the surgical time and therefore indirectly impacting the risk of wound healing complications, which could induce postoperative morbidity and postpone other adjuvant therapies. Despite the relevance of such aspects, a standardized approach for measuring the dimensions of ISS in cats is not available in the literature.

In our study, tumour dimensions, both length and width, evaluated by CT showed a tendency towards being larger than the dimensions measured with callipers, consistent with previous reports. In addition, in a proportion of cats, the shapes retrieved by the two methods demonstrated discrepancies. These findings could be related to the specific characteristics of ISS and specifically to the presence of non-palpable tumour extensions departing from the clinically palpable tumour. The use of intravenous contrast media helps the radiologist to better define the margins of the neoplasms in CT or MRI, although in these margins both neoplastic and perilesional inflammatory tissue is included and cannot be differentiated without histopathology.

Statistical analysis revealed a weak concordance between clinical and CT evaluations of tumour dimensions. This weak concordance was confirmed even when considering the subsample of ‘regular-shaped’ tumours, in which greater agreement was expected. In addition, other results also showed wide limits of agreement, particularly with increasing tumour size, indicating that, when the surgeon clinically measures the tumour, a wide range of possible CT measurements is possible. Moreover, this range of possible values becomes wider if the tumour increases in size. This could be explained by the hypothesis that a tumour that has the ability and/or the time to grow also has a greater likelihood of infiltrating the surrounding tissues, forming longer tumour extensions and thus increasing the discrepancy between the two methods of measurement by increasing the tumour dimensions. Another explanation could be that, while CT images allow for the precise evaluation of only the tumour edges, when the tumour is clinically measured with callipers, the thickness of the skin and tissues that cover the mass can not be subtracted from the value obtained, and other variables related to the individual cat, such as body condition score (BCS) and the anatomical site of the tumour, could contribute to this variability. These findings suggest that it is not correct to estimate the tumour size using only one method of assessment but rather both methods should be applied.

In the specific case of ISS in cats, the gold standard method for tumour measurement remains unknown, therefore it is not possible to determine the perfect pre-surgical approach for ISS. The dimensions of these tumours in cats are often reported as maximum diameters, but in this study, two perpendicular axes were considered because the major axis between length and width on clinical evaluation is not necessarily the major axis, even on CT evaluation, thus making the comparison between the two methods susceptible to bias. The third axis was not calculated because of presence of anatomical limits, such as underlying bone or cavities, making it impossible to measure the thickness of the tumour clinically with callipers. This data is therefore not available for comparison with CT measurements. In addition, the surgeon calibrates the depth of the excision based on the infiltration of fascial planes and not based on linear measurement of the thickness.

Regarding this practice, the use of CT evaluation could facilitate the identification of deep soft and bone tissue infiltration, the detection of which is not always easy to perform in a clinical fashion. Moreover this modality is useful for staging the neoplasms, by evaluating the potential distant metastatic spread to the thoracic structures and abdominal organs.

In 2013 the results of this dynamic approach was described in 10 patients affected by interscapular ISS, with preliminary data showing slight reduction of muscle infiltration by the tumour between flexed and extended positioning of the forelimbs.

Although in the majority of our cases the number of infiltrated muscles was the same, slight discrepancies between the two positions and observers were detected. A tendency for the flexed positioning to record a reduced number of muscles infiltrated by the tumour was observed in 5 cases, with both observers, although unrelated to the two different positions. Our results strongly suggest considering a double positioning for the correct estimation of ISS, although it is difficult to elect the most accurate positioning for the study of ISS. We believe that a thorough estimation of tumour margins and infiltration could potentially decrease unnecessary inclusion of structures not involved in the pathological process, within the perilesional margins. Furthermore the measurement of the distance between the ISS and the adjacent skeletal structures (vertebral spinous processes, scapulae) was markedly different between the two positions. This result also supports the inclusion of an additional dynamic evaluation with the forelimb flexed caudally along the body when the neoplasm is adjacent to the skeletal structures, to underline possible movement of the neoplasia away from these structures.

We tested the difference in computing the volume with a semi-segmentation software tool compared to the ellipsoid formula. Our results show considerably different measurements between the 2D and 3D method. In particular the tumour volume estimated by the ellipsoid formula was consistently and significantly higher than that estimated by the 3-D CT software tool. These results could be attributed to different reasons: first some of the neoplasms included in the sample showed a very irregular shape that could be easily overestimated by the ellipsoid formula and less with the semi-automated computed volume, where the contour of the mass is drawn manually and computed by the algorithmic conversion. In addition the software used was different from the one previously described, which consisted in a fully automated segmentation. However, we consider this hypothesis less likely because in all the sample population no variation was recorded in the results and in general it is more likely that the assumption of an ellipsoid shape could cause a slight overestimation of the tumour volume.

Finally, a weak correlation has been detected between tumour volume and the number of infiltrated muscles, although few small tumours showed consistent muscle infiltration (up to 15 muscles). These results are in agreement with previous postulation and confirm that even small neoplasms could show a highly infiltrative behaviour towards the surrounding musculoskeletal structures. A complete diagnostic imaging work-up should be performed even in cases of small palpable lesions, to avoid any improper assessment of the neoplastic margins.

5. Conclusion

We started with an anatomical study whose aim was to bring together advanced imaging techniques and gross sectional anatomy.

An innovative approach to the interpretation of the interscapular anatomy of the cat has been provided, with an atlas considering the highly dynamic behaviour of this region for the first time. The authors believe that this tool could be useful and widely applied for understanding all the pathologies affecting this region, thanks to an anatomical and tomographic map that could guide both clinicians and surgeons, especially in cases of feline Injection-Site Sarcomas (ISS), in the estimation of the perilesional margins and tumour's infiltration.

The second part of the PhD project was focused on the clinical aspects of feline ISS related to staging through advanced imaging techniques, such as Computed Tomography (CT).

We stated that dimension of feline ISS obtained with CT are often greater than those measured clinically, with callipers. These differences highlight the necessity to declare and standardize the method of tumour dimension assessment in studies focusing on ISS in cats, in order to properly consider surgical results and prognostic impact of this variable. The usefulness of contrast-enhanced whole-body CT for ISS in cats is not only linked to the role of planning lateral excision margins, but it also has the ability to estimate deep margins and to detect distant metastasis.
These aspects are crucial points to discuss before surgery with surgical staff and the owner and strongly support the use of advanced imaging techniques in the staging of ISS.

The aim of the final study was to evaluate the usefulness of the double positioning in a large cohort of cats clinically affected by ISS, trying to highlight which is the best position (flexed vs extended) for tumour staging.

Neither of the two positions appeared better than the other, therefore our results strongly suggest to consider a CT dynamic approach in the study of feline ISS, by including an additional post contrast scan with the forelimbs flexed caudally along the body when the tumour lies in the interscapular region in proximity to the musculoskeletal structures. We finally compared different computational methods of tumour volumes and found that the semi-automated segmentation of the neoplasms should be used for accurate pre-surgical planning of tumour volume, also in cases of small neoplasms that could potentially show a highly infiltrative behaviour on CT.

Further studies focusing on feline ISS will be oriented to evaluate the impact and role of the tumour volume method selected on the surgical outcome and potential neoplastic recurrence. Moreover, current studies integrating non-invasive techniques such as B-mode and Contrast Enhanced Ultrasound (CEUS) will provide additional evidence for a gold standard method to create guidelines for the pre-surgical evaluation of the dimensions of ISS in cats.

Finally the role of CEUS in the post-surgical follow-up focusing on the surgical margins should be performed, as a method of early assessment of neoplastic recurrence.