

Dynamic computed tomography of the pituitary gland using a single slice scanner in dogs with pituitary-dependent hypercortisolism

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ARTICLE INFO

Article history:

Received 14 July 2015

Received in revised form 25 January 2016

Accepted 25 April 2016

Keywords:

Dog

Dynamic computed tomography

Hypercortisolism

Hypophysectomy

Pituitary gland

ABSTRACT

Selective removal of the pituitary adenoma has not been advocated in dogs with pituitary-dependent hypercortisolism because the pituitary adenoma is usually not visualized on routine computed tomography (CT). Dynamic pituitary CT scanning is aimed at the detection of the pituitary flush and, indirectly, at the presence and position of the adenoma. The first aim of this retrospective study was to compare findings of a multiple slice dynamic scanning protocol with those of a single slice dynamic protocol using a single slice CT scanner. The second aim was to compare the CT findings with surgical findings, and surgical findings with histopathological findings. Computed tomography with single and multiple slice dynamic scanning protocols was performed in 86 dogs with pituitary-dependent hypercortisolism. Thirty dogs underwent transsphenoidal hypophysectomy and pituitary specimens were collected as tumor, normal, mixed and neurohypophyseal samples and processed for histology.

The pituitary flush was not detected more frequent in multiple slice dynamic scanning series than in single slice dynamic scanning series. However, in non-enlarged pituitaries, the flush was seen significantly more frequently than in enlarged pituitaries.

Prediction of the nature of the tissue during hypophysectomy by the surgeon was inconclusive.

In conclusion, when using a single slice CT scanner, both single or multiple slice dynamic scanning protocols can be used for localization of the neurohypophyseal flush, and, indirectly, the adenoma. However, based on this study, the aim of surgery in dogs with pituitary-dependent hypercortisolism remains total adenohypophysectomy, and when the neurohypophysis is recognized, it may be left in situ.

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1. Introduction

Hypercortisolism is a common endocrinopathy in dogs and is caused by a pituitary corticotroph adenoma in 80% of the cases (Galac et al., 2010). The diagnosis of pituitary-dependent hypercortisolism (PDH or Cushing's disease) is based primarily on clinical signs, biochemistry and endocrinological tests (Kooistra and Galac, 2012), but pituitary imaging using computed tomography (CT) or magnetic resonance imaging (MRI) is important to determine the pituitary dimensions and to confirm the presence of an adenoma (Meij et al., 1997a; Auriemma et al., 2009; Kooistra and Galac, 2012). Pituitary imaging using contrast-enhanced CT is also a prerequisite for hypophysectomy since it enables the determination of the bony surgical landmarks in relation to the pituitary gland (Meij et al., 1997a, 1998). Pituitary imaging with CT allows the distinction between enlarged and non-enlarged pituitary

glands (Kooistra et al., 1997). In dogs with pituitary-dependent hypercortisolism and non-enlarged pituitary glands, an isolated microadenoma was rarely directly visible on contrast-enhanced conventional CT (van der Vlugt-Meijer et al., 2003). In healthy dogs, dynamic contrast-enhanced CT allows the visualization of the neurohypophysis due to its early arterial enhancement, called the neurohypophyseal "flush" (van der Vlugt-Meijer et al., 2004). In dogs with pituitary-dependent hypercortisolism, the pituitary flush may be absent, displaced, or distorted, and this is considered indirect proof of a pituitary adenoma affecting the neurohypophyseal integrity (van der Vlugt-Meijer et al., 2003). Dynamic CT scanning of the pituitary gland in dogs with pituitary-dependent hypercortisolism in one slice, called single slice dynamic scanning (SSDS), may indirectly reveal the adenoma when the flush is contained in the slice. However, when the flush is outside the field of the single slice that is scanned, the position of the adenoma cannot be determined. In multiple slice dynamic scanning (MSDS), performed with a single slice helical CT scanner, the complete pituitary gland is scanned during and after maximum contrast

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enhancement. Thus, it is hypothesized that using a single slice CT scanner, the pituitary flush, and indirectly, the adenoma, will be detected more frequently using multiple slice dynamic CT series than with single slice dynamic series.

Hypophysectomy is an effective-long term treatment for pituitary-dependent hypercortisolism in dogs (Meij et al., 2002; Hanson et al., 2005) and the aim has been complete removal of the pituitary gland because of the low correlation between the dynamic CT findings on single slice dynamic CT series and the surgical and/or histological findings (van der Vlugt-Meijer et al., 2003). However, in humans selective adenomectomy is the first choice in Cushing's disease and removal of the microadenoma leaves the normal pituitary gland unaffected (Biller et al., 2008; Juszczak and Grossman, 2013). The question arises therefore whether selective removal of part of the pituitary gland based on results of multiple slice dynamic CT scanning (van der Vlugt-Meijer et al., 2007) is also an option in dogs with pituitary-dependent hypercortisolism. The hypothesis was therefore as follows: the pituitary flush, and indirectly, the adenoma, will be detected more frequently using multiple slice dynamic CT series then with a single slice dynamic series in dogs with confirmed pituitary-dependent hypercortisolism. Results of MSDS may guide the surgeon to a better distinction between normal and neoplastic tissue and therefore would allow for a more selective removal of the adenoma in dogs with PDH.

The aims of the present study using a single slice helical CT scanner were: 1) to compare the findings of multiple slice dynamic CT with those of single slice dynamic CT in a cohort of dogs with pituitary-dependent hypercortisolism, and 2) to compare the CT findings with surgical findings and to compare surgical findings with histopathological findings in a cohort of dogs with pituitary-dependent hypercortisolism that underwent single slice dynamic scanning, multiple slice dynamic scanning and hypophysectomy.

2. Materials and methods

2.1. Animals

Inclusion criteria for this retrospective, descriptive study were dogs diagnosed with pituitary-dependent hypercortisolism, referred to the Department of Clinical Sciences of Companion Animals of Utrecht University between January 2007 and November 2009. These dogs underwent pituitary imaging with CT including a contrast-enhanced spatial scanning series, and a single and multiple dynamic scanning series. A cohort of these dogs subsequently underwent hypophysectomy as primary treatment for PDH. Dogs with concurrent pituitary and adrenal neoplasia were excluded from the study.

2.2. Diagnosis

Diagnosis of PDH was based on the history, clinical signs, and results of hematology, clinical biochemistry, and the high dose dexamethasone suppression test measured by urinary corticoid/creatinine ratios (UCCRs) (Stolp et al., 1983; Rijnberk et al., 1988; Smiley and Peterson, 1993; Galac et al., 1997). In the dogs in which the suppression to dexamethasone was <50%, dexamethasone-resistant pituitary-dependent hypercortisolism was diagnosed by measurement of elevated plasma adrenocorticotropin (ACTH) concentrations (Galac et al., 2010; Feldman, 1983).

2.3. Computed tomography

CT was performed in all the dogs. Following intravenous (IV) premedication with 10–20 µg dexmedetomidine/kg BW (Dexdomitor, Orion Corporation, Espoo, Finland) and 0.1 mg butorphanol/kg BW (Dolorex, Intervet Nederland BV, Boxmeer, The Netherlands), anesthesia was induced by IV administration of 1–2 mg propofol/kg BW (Rapinovel, Mallinckrodt Veterinary, Mundelein, Illinois). The trachea

was intubated and inhalation anesthesia was maintained in a semi-closed system with a mixture of isoflurane, air and oxygen. Intravenous fluids (Sterofundin ISO/Ringerfundin; Braun Melsungen AG, Germany) were administered at a rate of 10 ml/kg/h during the procedure.

CT of skull was performed with a single slice helical CT-scanner (Secura CT Scanner, Philips, Best, The Netherlands). With the dog in sternal recumbency, transverse scans of the skull base were made from the rostral clinoid processes to the dorsum sellae, using 0.45 s scanning time with 120 kV, 200 mA and 2-mm-thick consecutive slices. At a position just rostral to the dorsum sellae, a single slice dynamic scanning protocol was performed consisting of 2-mm-thick scans using 0.45 s scanning time with 1 image/s, with 120 kV and 300 mA for 80 s. The single slice dynamic scanning protocol started 10 s following the start of the IV administration of 2 ml iobitridol (Xenetix 350, Guerbet Nederland BV, Gorinchem, The Netherlands, containing 350 mg iodine/ml)/kg BW with an angiographic injector (Medrad Mark V plus, Medrad Europe BV, The Netherlands), with 4 ml/s and a pressure limit of 300 psi, through a 20 or 18 gauge vasofix certo needle (Braun Melsungen AG, Melsungen, Germany), depending on the size of the dog positioned in the antebrachium. Single slice dynamic scanning was followed by a series of scans (2-mm-thick consecutive slices) from the rostral clinoid processes to the dorsum sellae, using 0.45 s scanning time with 120 kV, 200 mA (spatial series). Ten to 12 min following single slice dynamic scanning, a multiple slice dynamic scanning protocol was performed, scanning the whole pituitary gland 8 to 10 times over a length of 13 mm with a collimation of 1 mm, 120 kV, 260 mA, a table feed of 1 mm/rotation (pitch 1), scanning time of 0.45 s, 1 rotation/s, and 11 s delay between the individual scans in this series. Multiple slice dynamic scanning started 10 s following the second IV administration of contrast medium similar to single slice dynamic scanning.

Measurements were made from the display monitor using CT computer software (Philips, Best, The Netherlands) with all images displayed at the same window setting (window width 250, window level + 80). Pituitary dimensions (height and width) were measured from the contrast-enhanced image of the spatial series that contained the largest cross-section of the pituitary gland. On the same image the edges of the brain were traced and the enclosed area was calculated by the computer. The pituitary height (mm)/brain area (mm²) × 100 (P/B) value was calculated. Pituitaries with P/B value > 0.31 were considered enlarged and those with P/B value ≤ 0.31 were considered not enlarged (Kooistra et al., 1997). The maximum length of the pituitary gland was measured on the midsagittal reconstruction image.

The contrast enhancement pattern of the pituitary gland was assessed visually on the images of the single slice dynamic scanning series and the multiple slice dynamic scanning series by two observers (SDM and GV). The enhancement pattern of the pituitary gland was assessed in the single and in the multiple slice dynamic scanning series with special attention to the detection of the pituitary flush (early and strong enhancement of the neurohypophysis) (van der Vlugt-Meijer et al., 2004). The presence or absence of the flush sign was recorded. When the flush sign was present it was classified as: normal (central), dorsolateral displacement to the right side, dorsolateral displacement to the left side, rostral displacement or caudal displacement.

2.4. Hypophysectomy

To all owners of the dogs included in the study, medical treatment with trilostane and hypophysectomy were presented as the treatments of pituitary-dependent hypercortisolism. For various reasons, like personal preference of the owner and financial willingness, 30 of 86 dogs underwent transsphenoidal hypophysectomy as described previously (Meij et al., 1997a, 1998). All the surgeries were performed by the same boarded surgeon (BPM), using only the transverse contrast-enhanced and sagittal reconstruction CT series before surgery for orientation of the surgical landmarks. From this series, the sizes of the

pituitary were measured and the P/B ratio was calculated. Also, the relation of the position of the pituitary and the surgical landmarks were identified in preparation of the surgery. On the transverse slices the surgeon could assess the shape of the sphenoid bone and the presence or absence of the hamular processes from rostral to caudal in relation to the appearance and disappearance of the pituitary contrast enhancement. The single slice and multiple slice dynamic scanning series were not evaluated by the surgeon prior to the surgery. The surgeon used the contrast-enhanced spatial series that was performed between the SSDS and MSDS protocols for pituitary assessment in relation to the bony surgical landmarks (Meij et al., 1997a). Also, the goal of the pituitary surgery was always to perform a total hypophysectomy. During the surgery, the surgeon recorded the position of normal and affected pituitary tissue and, whenever possible, the normal and affected tissues were collected in separate formalin vials. Normal adenohypophyseal tissue was recognized by its white color and more firm consistency. Adenomatous adenohypophyseal tissue was recognized by its abnormal color (grey, glassy-grey, brown, dark blue, green) and abnormal consistency (soft, mucous, fluid-like, necrotic). The neurohypophysis was recognized as a firm small (2 to 3 mm in diameter) round nodule attached to the dorsum sellae. The surgeon classified the samples as normal adenohypophyseal tissue, adenomatous adenohypophyseal tissue, mixed (normal and adenomatous adenohypophyseal tissue), and neurohypophyseal tissue. The position of the tumor was recorded as central, dorsal or ventral and if the neoplasia was lateralized it was classified as right or left. When visualized, also the position of the neurohypophysis was registered, especially if it was dorsal to the dorsum sellae.

2.5. Histology and immunohistochemistry

The surgical tissue samples were placed in 10% neutral buffered formalin for 48 h. The specimens were dehydrated, embedded in paraffin and cut in sections of 5 μ m, and stained with hematoxylin and eosin for histological examination. For immunohistochemistry, the sections were stained for ACTH, alpha-melanocyte stimulating hormone (α -MSH) and growth hormone (GH), using the avidine-biotine technique with a monoclonal mouse antibody to synthetic ACTH_{1–24} (Middleton et al., 1987) (Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands), polyclonal rabbit antibodies to synthetic α -MSH (Spencer et al., 1983) (PU060-UP; Biogenex Laboratories, Duiven, The Netherlands), and rabbit antibodies to porcine GH (source 4750–3959; Biogenesis, Serotec, Oxford Biomedical Research, Oxford, UK) as previously described (Meij et al., 1997b, 1997c).

2.6. Relationship between surgical findings and histological findings

The surgical and histological findings were compared and the accuracy of the surgeon's assessment of the surgical pituitary tissue samples was assessed by comparing the surgical findings with the histological diagnosis of the separate samples.

2.7. Statistical analysis

Data were compared by the χ^2 -test. Data were analyzed using a commercially available software program (GraphPad Prism). $P < 0.05$ was considered significant.

3. Results

3.1. Animals and diagnosis

Eighty-six dogs were included in the study. Forty dogs were female (33 spayed), and 46 were male (18 castrated). The median age of the dogs was nine years (ranging from two to 14 years) and the

median body weight (BW) was 18.1 kg (ranging from 3.7 to 56.5 kg). The dogs included 35 different breeds and the most common breeds were Maltese (nine dogs), Dachshund (six dogs), Beagle (six dogs), Cavalier King Charles Spaniel (five dogs), and Jack Russell Terrier (four dogs). There were 31 other breeds (44 dogs) and 12 mixed breed dogs.

The median UCCR in 81 dogs was 106×10^{-6} (range 9 to 900×10^{-6} ; reference range $< 8.3 \times 10^{-6}$), which confirmed hypercortisolism. In 59 dogs suppression to dexamethasone was $> 50\%$ and PDH was diagnosed. In 22 dogs suppression to dexamethasone was $< 50\%$, and dexamethasone-resistant PDH was diagnosed by measurement of elevated plasma ACTH concentrations. In four dogs UCCRs were not available and in one dog with clear clinical signs consistent with hypercortisolism the UCCRs were in the upper range of normal but showed no suppression after administration of dexamethasone. In these five dogs PDH was also confirmed by measurement of elevated plasma ACTH concentrations.

3.2. CT findings

The pituitary gland was enlarged in 46 of 86 (53.5%) dogs and the P/B values ranged from 0.32 to 1.16 (median 0.54); in the other 40 (46.5%) dogs the pituitary gland was not enlarged and the P/B values ranged from 0.14 to 0.31 (median 0.24) (Fig. 1).

The height of the pituitary gland varied between 2.6 mm to 19.8 mm (median 6.8 mm), the width ranged between 4.1 mm and 20.1 (median 5 mm), and the length was between 2.3 mm and 18.3 mm (median 6.9 mm).

Analyzing the CT images of both series (SSDS and MSDS) in all 86 dogs, the pituitary flush was visible in single slice dynamic series in 36 of 86 (41.9%) dogs and in multiple slice dynamic series in 42 of 86 (48.8%) dogs (Fig. 1). The pituitary flush was visible in both single and multiple slice dynamic series in 33 of 86 (38.4%) dogs (Fig. 1). The position of the pituitary flush was similar during single and multiple slice dynamic series in 19 dogs, whereas in the other 14 dogs multiple slice dynamic series gave additional information on the position and extension of the flush compared to single slice dynamic series. In these dogs the multiple slice dynamic series revealed rostrocaudal displacement of the flush or a change in its position when moving from rostral to caudal through the gland (Fig. 2).

In 30 of 40 (75%) dogs with non-enlarged pituitary glands, a pituitary flush was detected. In 24 of the 30 dogs the pituitary flush was evident in both single and multiple slice dynamic series (Figs. 2, 3), whereas in 2 dogs the flush was evident only in single slice dynamic series and in 4 dogs the flush was visible only in multiple slice dynamic series (Fig. 1). In 7 of 40 (17.5%) dogs with non-enlarged pituitary glands, multiple slice dynamic series provided more information than single slice dynamic series on the enhancement pattern of the pituitary gland, marked by hypo- or hyperdense areas in the pituitary gland different from the flush.

In 15 of 46 (32.9%) dogs with enlarged pituitary glands, a pituitary flush was detected. In nine dogs the pituitary flush was evident in both single and multiple slice dynamic scanning, whereas in one dog the flush was visible only in single slice dynamic scanning and in five dogs the flush was detectable only in multiple slice dynamic scanning. Among the dogs with enlarged pituitary gland and no flush detected, eight dogs showed an irregular hyperdensity of the pituitary gland in multiple slice dynamic scanning during and after the arterial phase, usually at the moment when the pituitary flush should have been detected (Fig. 4). In 24 of 46 (52.2%) dogs with enlarged pituitary glands, multiple slice dynamic series provided more detailed information than single slice dynamic series on the enhancement pattern of the pituitary gland, marked by hypo- or hyperdense areas in the pituitary gland different from the flush.

Overall, the pituitary flush was not detected significantly more frequently in MSDS than in SSDS (χ^2 -test, $P = 0.53$). However, when

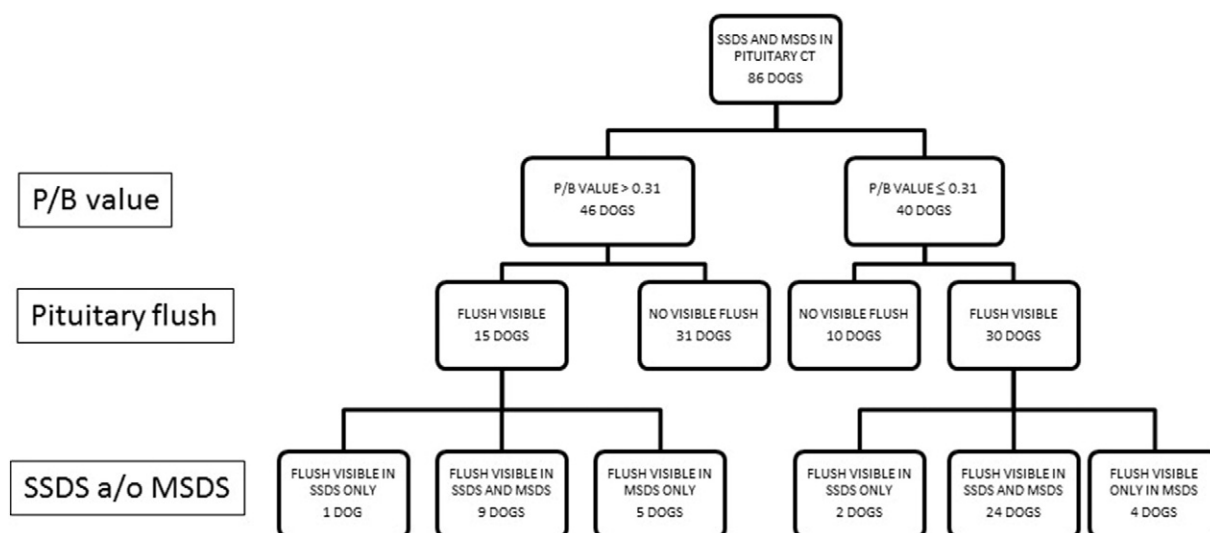


Fig. 1. Organogram showing breakdown of 86 dogs with pituitary-dependent hypercortisolism that underwent computed tomography (CT) with a single slice CT scanner using single slice dynamic scanning (SSDS) and multiple slice dynamic scanning (MSDS) protocols. Dogs were subdivided according to the pituitary height/brain area (P/B) value, the presence or absence of the pituitary neurohypophyseal flush, and visualization of the flush on SSDS and/or MSDS series.

differentiated according to pituitary enlargement, the flush was seen significantly more frequently in non-enlarged pituitaries than in enlarged pituitaries ($P < 0.0001$), but in both groups separately there

was no statistical significant difference between SSDS and MSDS for the detection of the pituitary flush (enlarged pituitaries, $P = 0.47$; non-enlarged pituitaries, $P = 0.81$).

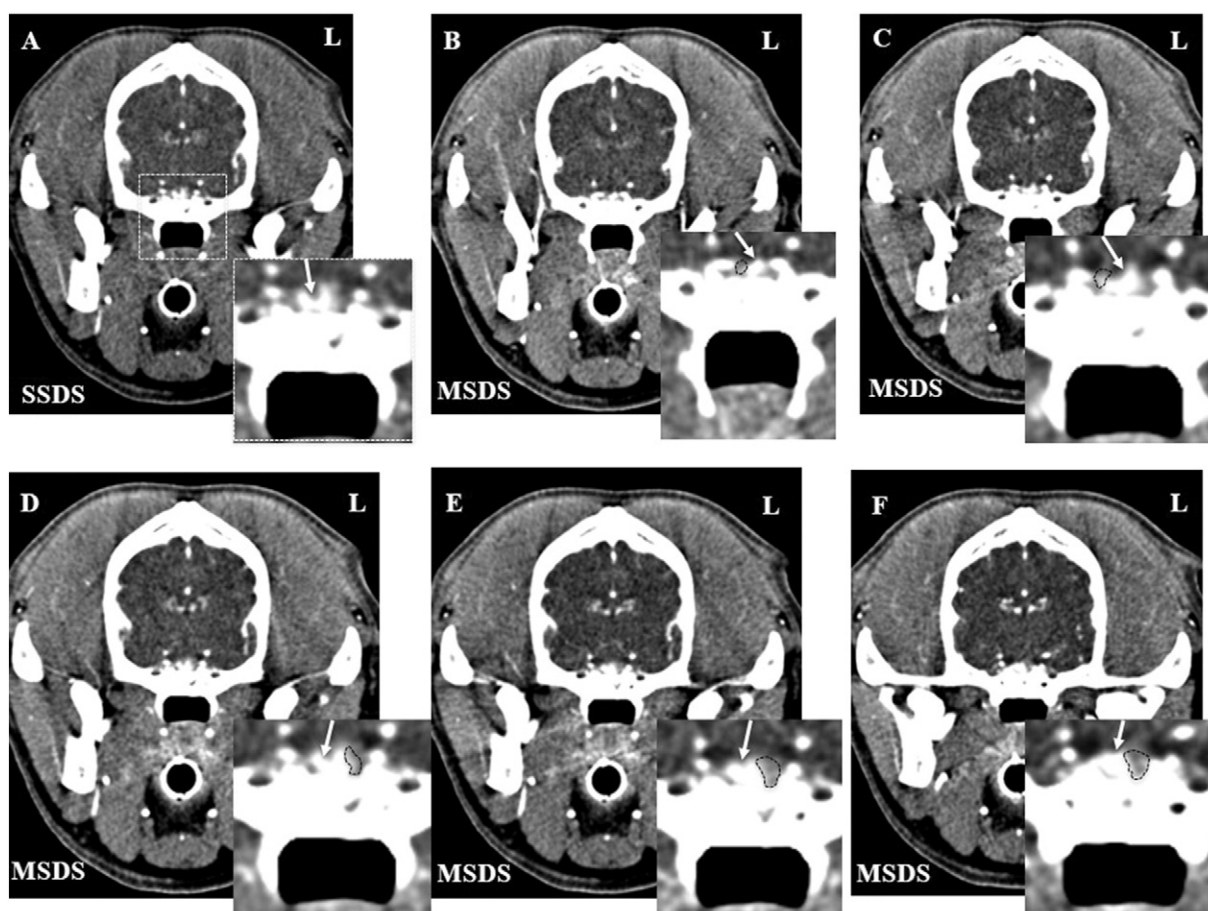


Fig. 2. Transverse CT images during single slice dynamic scanning (SSDS) (A) and the third series of multiple slice dynamic scanning (MSDS) (B–F), at arterial enhancement of the pituitary fossa in a 9-year-old female Labrador retriever with pituitary-dependent hypercortisolism. The pituitary gland is not enlarged with a pituitary height/brain area (P/B) value of 0.23. The pituitary flush (arrow) appears normal on the single slice dynamic series (A) but representative images of the multiple slice dynamic series (B–F) reveal that the flush starts on the left side rostrally (B,C), is in the midline halfway the pituitary fossa (D) resembling the single slice dynamic scanning (A), and is displaced to the right side caudally (E,F). Inserts are magnified images with schematic outlining (dashed line) of the pituitary adenoma. Window width = 250, window level = 80.

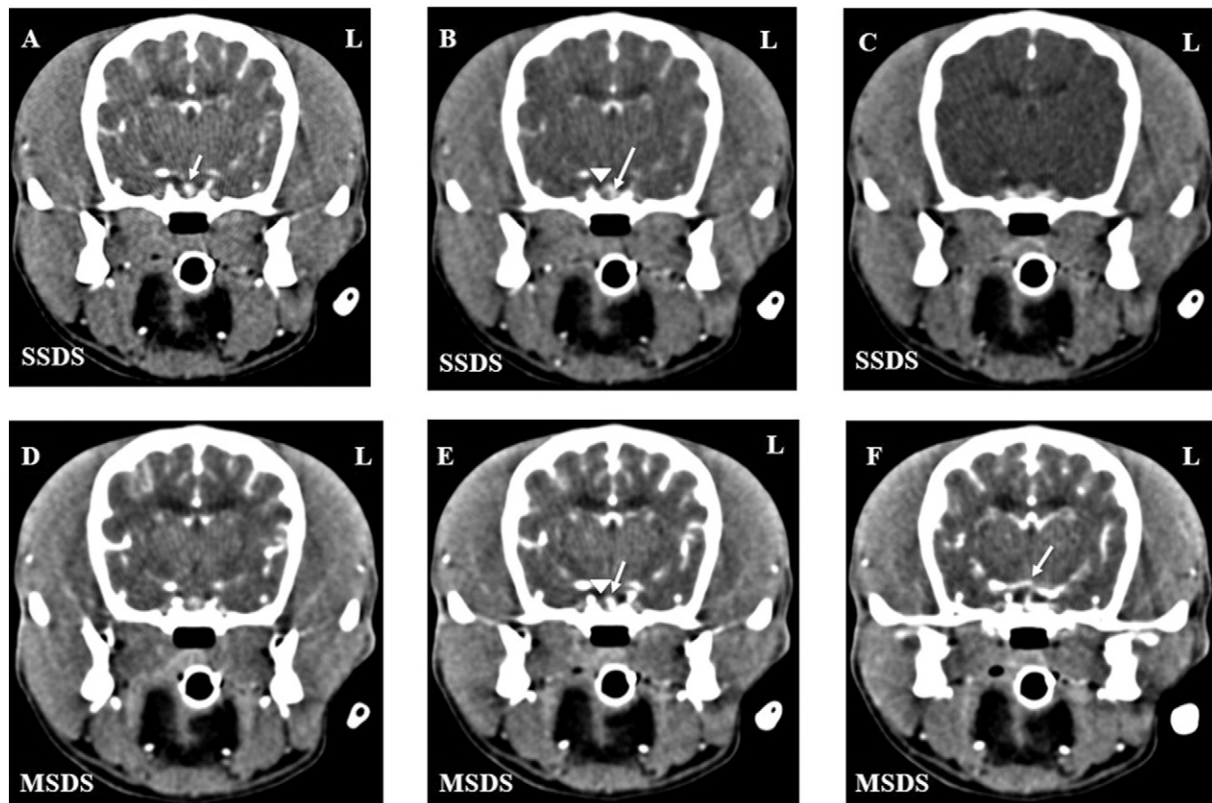


Fig. 3. Transverse CT images during single slice dynamic scanning (SSDS) (A,B,C) and the first series of multiple slice dynamic scanning (MSDS) (D,E,F), at arterial enhancement of the pituitary fossa in an 8-year-old male castrated Maltese with pituitary-dependent hypercortisolism. The pituitary gland is not enlarged with a pituitary height/brain area (P/B) value of 0.29. In single slice dynamic series the pituitary flush is clearly visible during early enhancement (A,B) and displaced to the left (arrow) indicating an adenoma on the right side (arrowhead). In multiple slice dynamic series the flush is only visible at slice position depicted in E which is comparable to the position at single slice dynamic series (A–C); however if single slice dynamic series would have been performed at slice position depicted in D (more rostral) or F (more caudal), the flush and (the adenoma) would have been missed. The slice position at F shows the arterial enhancement of the connection between basilar artery and arterial cerebral circle of Willis (arrow). Window width = 250, window level = 80.

3.3. Relation between surgical, imaging and histopathological findings

In the cohort of 30 dogs that underwent hypophysectomy the pituitary gland was not enlarged in four dogs (P/B value 0.23, 0.25, 0.27, 0.29) and enlarged in 26 dogs (median P/B value 0.55; ranging from 0.32 to 0.99). The pituitary flush was visible only in 10 dogs, i.e. in three of four dogs with non-enlarged pituitary glands and in seven of 26 dogs with enlarged pituitary glands.

In two of these 10 (20%) dogs with a visible pituitary flush, there was agreement between the position of the adenoma, detected indirectly by the position of the pituitary flush on single and/or multiple slice dynamic series, and the position of the adenoma detected during surgery. In the other eight dogs there was incomplete or no agreement between the surgical and imaging findings. In 20 of 30 (66.7%) dogs in which no pituitary flush was detected, the adenoma was identified by the surgeon in the central part of the pituitary gland.

During hypophysectomy the surgeon collected pituitary tissue samples and identified them as adenomatous adenohypophyseal tissue (tumor sample, 28 dogs), mixed adenomatous adenohypophyseal and unaffected adenohypophyseal tissue (mixed sample, five dogs), and neurohypophyseal tissue (neurohypophysis sample, six dogs). There was complete correspondence between the surgeon's view and the histological diagnosis of these samples in 16 of 30 (53.3%) dogs. The 28 tumor samples collected by the surgeon indeed contained adenoma on histology. However, in two of these 28 tumor samples, histology also revealed adenoma infiltrating in neurohypophyseal tissue. In four of the 28 tumor samples, histology showed neurohypophyseal tissue, normal adenohypophyseal tissue, and adenomatous adenohypophyseal tissue. In 12 of the 28 tumor samples, histology showed that normal

adenohypophyseal tissue and adenomatous adenohypophyseal tissue were mixed. In all the five samples, where the surgeon indicated that normal adenohypophyseal tissue was collected together with adenomatous adenohypophyseal tissue (the mixed sample), histology confirmed the presence of both tissue types. Neurohypophyseal tissue was confirmed by histology in all the six samples classified by the surgeon as neurohypophysis. In one of these six neurohypophyseal samples, histology also showed normal adenohypophyseal tissue and in another case, where the surgeon thought the neurohypophysis also contained a microadenoma, a cystic lesion was found on histology. The pars intermedia was not recognized by the surgeon as a separate entity but in 4 of 30 dogs histology showed that the adenoma originated from the pars intermedia. In three of these four dogs no flush was detected on single and multiple slice dynamic scanning, while in two of these four dogs multiple slice dynamic scanning showed hyperdense areas during the arterial phase.

In all 30 surgical samples a pituitary adenoma was diagnosed on histology. The adenomatous adenohypophyseal tissue that was found showed strong (20 dogs), weak (three dogs), or negative (seven dogs) immunoreactivity for ACTH and α -MSH.

4. Discussion

The first part of the present study showed that, using a single slice CT scanner in dogs with pituitary-dependent hypercortisolism, multiple slice dynamic scanning does not more frequently detect the pituitary flush than single slice dynamic scanning, which does not confirm our initial hypothesis.

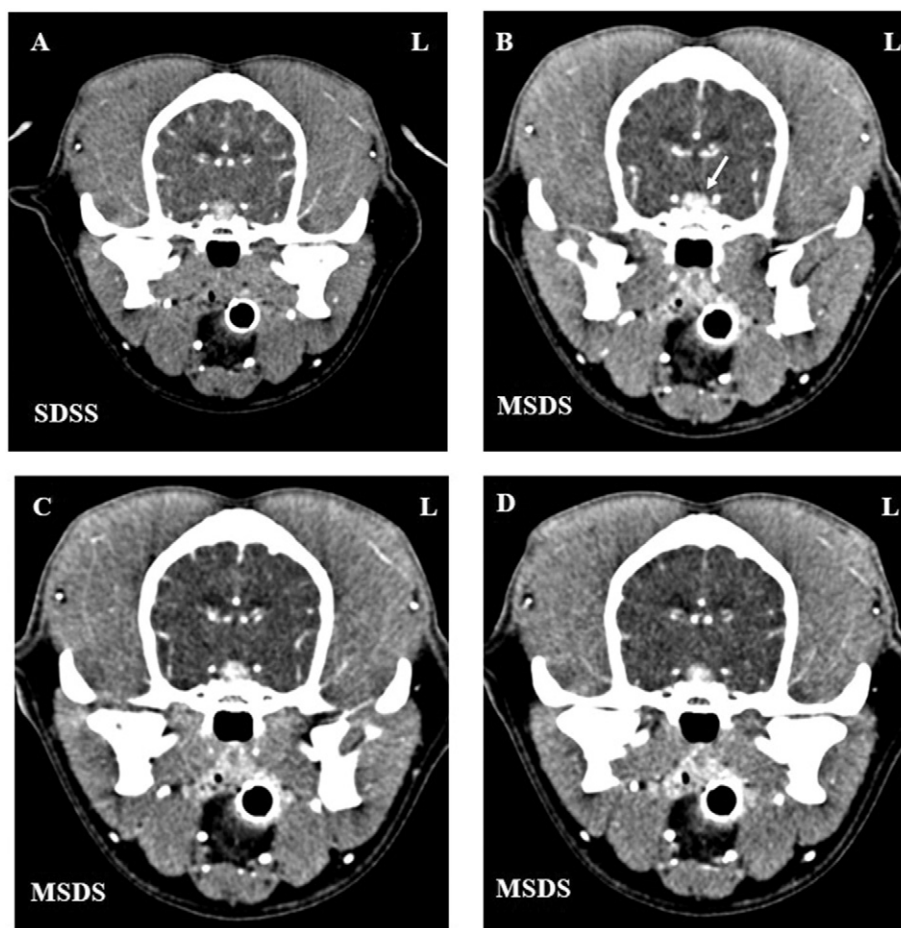


Fig. 4. Transverse CT images during single slice dynamic scanning (SSDS) (A) and the second series of multiple slice dynamic scanning (MSDS) (B–D), at arterial enhancement of the pituitary fossa in a 5-year-old female castrated beagle dog with pituitary-dependent hypercortisolism. The pituitary gland is enlarged with a pituitary height/brain area (P/B) value of 0.46 with irregular hyperdense enhancement pattern (arrow). The slice in single slice dynamic scanning (A) is at the same position as the slice (D) during multiple slice dynamic scanning. Window width = 250, window level = 80.

However, more pituitary flushes were visible with SSDS of non-enlarged pituitary glands than with SSDS of enlarged pituitary glands which was previously reported (van der Vlugt-Meijer et al., 2003). Most likely this is caused by the limited compression that a microadenoma exerts on the neurohypophysis and its arterial supply. The neurohypophysis thus remains visible, but it is usually displaced. In contrast, when the pituitary gland is enlarged the tumor may disturb the enhancement of the neurohypophysis and the pituitary flush (van der Vlugt-Meijer et al., 2003). Pituitary flushes (9/86 dogs) were detected in the MSDS series that were not seen in the SSDS series (Fig. 1). Our hypothesis that multiple slice dynamic scanning could identify more frequently the pituitary flush was not supported by the statistical analysis for the whole group. However, dynamic scanning using SSDS and MSDS protocols to detect the flush was especially useful in non-enlarged pituitaries and for individual cases MSDS could identify more precisely the likely position of the adenoma. In fact the rostral or caudal displacement of the flush was only evident in MSDS in several cases. In multiple slice dynamic scanning the complete pituitary gland was repeatedly scanned every few seconds during contrast administration whereas in single slice dynamic scanning, the wrong slice may be chosen missing the pituitary flush completely.

In three dogs the flush was visible in the SSDS series but not in the MSDS series. This is probably caused by discrepancy between the time of scanning and the arrival of the contrast medium in the neurohypophysis. The pituitary flush is usually only visible for some seconds and more time is usually needed with a single slice helical CT scanner to examine the complete gland in the MSDS protocol than in SSDS protocol.

The time of contrast injection is fixed in relation to the time of scanning, but the contrast distribution can vary due to the circulatory state of the dog and due to the effect of anesthetic agents. As a consequence the enhancement of the neurohypophysis can reach its peak between series during multiple slice dynamic scanning and may already have disappeared in the next series. Another possible explanation may be the difference in slice thickness between single slice dynamic scanning (2 mm) and multiple slice dynamic scanning (1 mm) which could lead to accumulation of contrast medium in the SSDS series resembling the flush.

Irregular hyperdense areas were seen in the arterial contrast phase (eight dogs), especially in the MSDS series. The possible reasons are an anomalous flush due to the compression of the tumor, accumulation of contrast medium because it is administered the first time for single slice dynamic scanning and the second time for multiple slice dynamic scanning, or a direct arterial supply to the adenoma. Two of the eight dogs underwent hypophysectomy and there were no apparent differences at histology compared to other dogs. In humans a rare arterial vascular supply has been reported in ACTH-secreting adenomas (Racadot et al., 1986), but, to our knowledge, no data are available in the veterinary literature. It was proposed that a direct arterial supply to the tumor reduces the control of the hypothalamus on the pituitary gland and on the adenoma, but in the eight dogs with hyperdense areas in the multiple slice dynamic scanning series, no different behavior was seen with respect to the dexamethasone suppression.

Additional information, besides the pituitary flush, was recorded in the multiple slice dynamic scanning series. The hypo- or hyperdense

areas may suggest the presence of a necrotic portion of the tumor, a cystic lesion, or a mineralized portion. None of these lesions were proved to be linked to the prognosis in the literature, but they can give the surgeon a more accurate indication of the type of tissue that will be encountered during surgery.

No pituitary flush was detected during single and multiple slice dynamic scanning in the 20 of 30 dogs that underwent hypophysectomy. In enlarged pituitary glands it is more likely that the adenoma had sufficient time to disrupt the neurohypophyseal integrity explaining the absence of the pituitary flush on dynamic CT. On expansion the tumor may affect the complete pituitary gland, with the center of the tumor becoming necrotic over time. Indeed, the surgeon identified the tumor frequently in the central part of the gland.

In the last decade, more dogs with enlarged than non-enlarged pituitary glands were operated in the institution where the present study was performed despite the fact that surgery for microadenomas is reported to give better curative effect in the long term with less post-operative complications (Hanson et al., 2007). This is probably linked to sufficient control of hypercortisolism in dogs with microadenomas by medical therapy with trilostane (Cho et al., 2013) and the difficulty to visualize directly the microadenoma on CT. Since microadenomas tend not to grow fast and there is no compression on the brain tissue, this may seem a fair strategy. But, to our knowledge, no information is available on the growth rate of pituitary adenomas in the dog and there are no controlled clinical trials available comparing results of surgery and trilostane therapy (Teshima et al., 2009).

The second part of the present study was undertaken to investigate whether selective adenectomy (Ciric et al., 2012) is an option in dogs with pituitary-dependent hypercortisolism. Selective adenectomy reduces the need for lifelong hormone supplementation which is a direct consequence of complete hypophysectomy (Hanson et al., 2007). Adenectomy spares the normal adenohypophyseal tissue and the neurohypophysis and it is the gold-standard therapy of Cushing's disease in humans. To reach this aim, accurate pre-operative tumor localization is necessary by pituitary imaging and the (micro)adenoma should be distinguished separately from normal pituitary tissue during surgery. In only two of 10 dogs with a visible pituitary flush there was agreement between the position of the adenoma on CT and during surgery and this explained the low correlation in our study.

In this study an experienced surgeon attempted to collect the normal adenohypophyseal tissue, the adenomatous adenohypophyseal (adenoma) tissue, and the neurohypophyseal tissue in separate vials. When the surgeon identified adenoma tissue macroscopically, histological examination frequently also detected normal and/or neurohypophyseal tissues besides the adenoma tissue. When the surgeon identified a mixed sample macroscopically, it indeed was normal and adenoma tissue on histology. When the surgeon identified the neurohypophysis macroscopically, it was confirmed histologically and just in one case also normal adenohypophyseal tissue was included in the sample. From these findings it can be concluded that in the cases where the surgeon recognized the neurohypophysis (six of 30 dogs), leaving the neural lobe in place would have been an option to prevent the post-operative lack of vasopressin. No tumor was found in the neurohypophyseal samples, while in our previous study (van der Vlugt-Meijer et al., 2003), leaving the neurohypophysis in situ was considered an important limitation to selective adenohypophysectomy. This may reflect a continued learning effect and increased experience on the part of the surgeon. It remains to be seen whether leaving the neurohypophysis during pituitary surgery in dogs would indeed reduce the frequency of diabetes insipidus since selective adenohypophysectomy may necessitate manipulation of the neurohypophysis during removal of the adenomatous adenohypophysis. This in itself may lead to hypo- or hypersecretion of vasopressin with hyponatremia (diabetes insipidus) or hyponatremia (syndrome of inappropriate ADH secretion), respectively.

The immunoreactivity for ACTH and α -MSH was confirmed for 23 pituitary adenomas, but the absence of immunoreactivity for ACTH

and α -MSH in seven cases seems to be in contrast with the clinical diagnosis of pituitary-dependent hypercortisolism. However, apart from technical problems (e.g. duration of fixation, faults in the staining process), the corticotrophs of the adenoma may be so productive in secretion of excess hormones that this results in insufficient storage of ACTH and α -MSH to produce visible staining above the immunohistochemical detection limit.

A limitation of the study was the fact that the majority of the dogs that underwent hypophysectomy had enlarged pituitary glands. It would have been more appropriate to include an equal number of dogs with enlarged and non-enlarged pituitary glands to effectively correlate the surgical and CT findings about the position of the adenoma. However, since this was a descriptive and retrospective study this factor was not well controlled and this may have introduced a bias in this study.

The single slice helical CT scanner used in the present study is nowadays not the most advanced CT modality available in veterinary medicine and is a limitation of our study. More advanced technology like multiple slice CT scanners and high field MRI scanners may well give more positive results for the detection of pituitary adenomas in dogs. In human medicine the preferred diagnostic imaging technique to diagnose a pituitary adenoma is high-field MRI (De Rotte et al., 2016) whereas CT is performed in cases where MRI failed in the identification of the tumor (Kinoshita et al., 2015). MRI is also used to guide the surgeon in the preoperative planning of the endoscopic transsphenoidal surgery and can also be used intraoperatively but this requires expensive adaptations to the surgical room which are not feasible in veterinary medicine at this moment. Recently 3D reconstruction by CT imaging permitted to create an anatomical precise model of the region of the sphenoid sinus and of the sella turcica. This technology helps the surgeon in the accurate preoperative planning in each case, because detailed anatomical landmarks and vital structures are represented (Inoue et al., 2015). Other groups prefer to use CT to detect residual tumor intraoperatively (Tosaka et al., 2015).

Mamelak and his group described a surgical protocol in dogs which included preoperative MRI and intraoperative CT to precisely localize the *fossa hypophysialis*. CT was performed intraoperatively after two holes were drilled in the sphenoidal bone, to check the precise location of the pituitary gland in relation to the drill holes and bony landmarks (Mamelak et al., 2014).

High-field MRI can be useful for the detection of the adenoma and its position, whereas multiple slice CT scanners can be more precise to guide the surgeon during the approach. The techniques can be complementary and both should ideally be performed in every case, also in veterinary medicine. However detailed information about high-field MRI for pituitary adenoma and in particular for microadenoma in dogs is still scarce (Son et al., 2015). It is expected that especially with the current trend in veterinary medicine to faster helical CT scanners with multiple slice modalities and high field MRI scanners, dynamic pituitary imaging will provide even more detailed information on the three dimensional characteristics of the pituitary flush or the adenoma itself in case of MRI and this may give the surgeon more stronghold to go for selective removal of pituitary adenoma tissue.

5. Conclusions

Considering the scarce overlap between the findings on diagnostic imaging and during surgery with respect to the localization of the adenoma, and the difficulty to undisputedly distinguish the different portions of the pituitary tissue (normal versus adenoma), complete hypophysectomy is still the most safe and efficient way to achieve remission of Cushing's disease. Only in cases where the neurohypophysis is visualized on imaging pre-operatively and is subsequently identified during surgery, it may be attempted to perform a selective adenohypophysectomy. Multiple slice dynamic scanning series give a panoramic overview in place and time of the enhancement of the

pituitary gland and of the pituitary flush and thereby indirectly of the position of the adenoma which may help the surgeon in pre-operative planning. Accordingly, dynamic pituitary imaging should remain an essential part of the pituitary imaging protocol, especially for non-enlarged pituitaries. Future studies are recommended for dynamic pituitary imaging using faster helical CT scanners with multiple slice modalities and high field MRI scanners.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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