Comparison of response assessment in veterinary neuro-oncology and two volumetric neuroimaging methods to assess therapeutic brain tumour responses in veterinary patients

Giovanni Coppola, Joanna Morris, Rodrigo Gutierrez-Quintana, Shona Burnside, Roberto José-López.

School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bearsden, Glasgow, United Kingdom.

Authorship:

Coppola G.: acquisition, analysis and interpretation of data, drafting the article, revising it for intellectual content and final approval of the completed article.

Morris J.: conception and design of the study, analysis and interpretation of data, revising the article for intellectual content and final approval of the completed article.

Gutierrez-Quintana R.: acquisition, analysis and interpretation of data, revising the article for intellectual content and final approval of the completed article.

Burnside S.: acquisition of data, revising the article for intellectual content and final approval of the completed article.

José-López R.: conception and design of the study, acquisition, analysis and interpretation of data, drafting the article, revising it for intellectual content and final approval of the completed article.

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Corresponding author: Roberto José-López, School of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow, G61 1QH, UK; e-mail: Roberto.Jose-Lopez@glasgow.ac.uk

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ABSTRACT

Standardized veterinary neuroimaging response assessment methods for brain tumours are lacking. Consequently, a response assessment in veterinary neuro-oncology (RAVNO) system which uses the sum product of orthogonal lesion diameters on 1-image section with the largest tumour area, has recently been proposed.

In this retrospective study, 22 pre-treatment magnetic resonance imaging (MRI) studies from 18 dogs and four cats with suspected intracranial neoplasia were compared by a single observer to 32 post-treatment MRIs using the RAVNO system and two volumetric methods based on tumour margin or area delineation with HOROS and 3D Slicer software, respectively.

Intra-observer variability was low, with no statistically significant differences in agreement index between methods (mean AI ± SD, 0.91 ± 0.06 for RAVNO; 0.86 ± 0.08 for HOROS; and 0.91 ± 0.05 for 3D slicer), indicating good reproducibility.

Response assessments consisting of complete or partial responses, and stable or progressive disease, agreed in 23 out of 32 (72%) MRI

evaluations using the three methods. The RAVNO system failed to identify changes in mass burden detected with volumetric methods in 6 cases. 3D Slicer differed from the other two methods in 3 cases involving cysts or necrotic tissue as it allowed for more accurate exclusion of these structures.

The volumetric response assessment methods were more precise in determining changes in absolute tumour burden than RAVNO but were more time-consuming to use. Based on observed agreement between methods, low intra-observer variability, and decreased time constraint, RAVNO might be a suitable response assessment method for the clinical setting.

KEYWORDS: cat, dog, intracranial neoplasia, magnetic resonance imaging, therapeutic response metrics

INTRODUCTION

Spontaneous brain tumours in dogs and cats are responsible for severe clinical signs. Their estimated prevalence is approximately 14.5 cases per 100,000 dogs and 3.5 per 100,000 cats.[1,2]

In recent years, increased availability of advanced neuroimaging for the presumptive diagnosis of brain neoplasia in veterinary medicine has led to more frequent treatment of these tumours by different modalities including palliative corticosteroids, cytoreductive surgery, fractionated

radiotherapy, stereotactic radiosurgery, and chemotherapy, either alone or combined.[3-11]

As a result, assessment of therapeutic response of intracranial tumours using advanced neuroimaging has become an integral part of clinical management. However, no standardized neuroimaging response assessment criteria have been adopted so far in veterinary medicine. Conversely, in human medicine, magnetic resonance imaging (MRI)-based response assessments are considered acceptable surrogates of therapeutic effect and several criteria such as one- and two-dimensional diameter-based measurements and volumetric methods, have been validated.[12-15] This prompted a recent review of the advantages and challenges of published MRI-based human brain tumour therapeutic response criteria using veterinary case examples of intracranial tumours.[16] Subsequently, a response assessment in veterinary neuro-oncology (RAVNO) system was proposed and later applied in a study to objectively assess responses to irreversible electroporation ablative treatment in seven canine intracranial gliomas.[17]

The aim of the present study was to compare the RAVNO system with two volumetric MRI-based response assessment methods for brain tumours in veterinary patients, to validate the use of each of these methods and to assess the respective reliability, reproducibility, and suitability for the clinical setting.

MATERIALS AND METHODS

Case selection

This study was approved by the Research Ethics Committee of the XXX. Cases referred to the oncology and/or neurology services at the XXX between 2006-2018 were retrospectively reviewed. Inclusion criteria were a suspected intracranial neoplasia based on MRI, treatment for the lesion (any modality), and at least one post-treatment MRI evaluation including transverse T2-weighted (T2W; repetition time (RT), 3607-7785 milliseconds; echo time (ET), 84-120 milliseconds), fluid attenuated inversion recovery (FLAIR; RT, 5900-8132 milliseconds; ET, 113-160 milliseconds), and T1-weighted (T1W; RT, 464-677 milliseconds; ET, 10-15 milliseconds) images before and after intravenous administration of 0.1 mmol/kg gadopentate dimeglumine (Magnevist, Bayer Schering Pharma AG, Berlin, Germany). Additional dorsal and sagittal T2W, and transverse gradient-recalled echo sequences were routinely obtained at our institution. Magnetic resonance images were obtained under general anaesthesia with patients positioned in dorsal recumbency and using a 1.5-Tesla magnet; either Phillips Gyroscan NT 1.5 T, Phillips Healthcare, Andover, MA, US (2006-2009) or Magnetom Essenza 1.5 MRI, Siemens AG, Erlangen, Germany (2009-2018). Median slice thickness was 4mm (range, 2.5-4mm), and median interslice gap was 4.4mm (range, 2.75-4.8mm).

Patient species, sex, breed, age at diagnosis, radiological diagnosis, histological diagnosis (where available), treatment modality,

neurological signs at presentation and at each serial assessment, and date and cause of death if deceased, were recorded from the clinical records.

Fluid accumulations associated with the tumour were categorized using T2W, FLAIR, and T1W images as either cysts or intra-tumoural accumulations of fluid (ITF) according to a previous study.[18]

When lesions exhibited irregular hypointensity on T1W and FLAIR sequences and irregular hyperintensity on T2W sequences, this was noted as suspected necrosis.[19]

Therapeutic response metrics

Three therapeutic response metrics were evaluated in this study, the RAVNO system and two different volumetric methods (Figures 1 and 2, Table 1). The RAVNO system, adapted from the response assessment in neuro-oncology criteria commonly used in humans, consisted of a two-dimensional diameter-based measurement using the sum product of the longest orthogonal diameters (d_a x d_b) of a contrast-enhancing (CE) lesion on the transverse image section with the largest tumour area, but specifically excluded incorporation of cystic or necrotic areas into measured target lesions.[12,16,20] Measurements of the tumour orthogonal diameters were obtained using an open-source software platform HOROS Software (HOROS v2.2.0, The Horos Project).

Only CE lesions with a discrete, nodular portion of ≥ 10mm in diameter were defined as target lesions and measured in two orthogonal diameters without encroachment upon any cystic or necrotic area.

Enhancing lesions with a diameter < 10mm were classified as non-measurable non-target lesions, and non-enhancing lesions, which were visible on T2W and/or FLAIR sequences, were classified as non-enhancing non-target lesions. These non-target lesions were qualitatively compared between studies in terms of size, shape, location, and number of observed abnormalities on T2W and FLAIR images, and any new lesions identified.[16,21]

Two volumetric measurement methods were adapted from human medicine for this study.[22-26] The first was performed using HOROS software (HOROS v2.2.0, The Horos Project) in which a 3D volume rendered model was generated from manual margin delineation of CE lesions on transverse T1W images post-contrast.[22,27,28] Areas of necrosis, cystic structures, and surgical scars were excluded from the contour delineation whenever possible. For non-enhancing tumours volume was defined from transverse T2W images and FLAIR images used to differentiate peritumoural oedema from tumour during segmentation.[17,29] A tumour volume in cm^3 was computed from all these sections.

In the second volumetric method, tumour segmentations were performed using 3D Slicer Software (version 4.4, Boston, MA)[30] in which a 3D volume rendered model was calculated from manually painting the pixels of all the CE areas of the tumour on transverse T1W images post-contrast.[24,31,32] As for the first volumetric method, regions of necrosis, cystic structures, and surgical scars were not included in the painted areas for volume calculation, and transverse T2W and

FLAIR images were used to calculate volume in non-enhancing tumours. Volume in cm^3 was computed from all these sections.
For all three methods, follow-up measurements were compared to the pre-treatment baseline scan to assess the response or a nadir scan, defined as the post-treatment MRI with the lowest calculated response measurement at any timepoint. Comparison to the latter is used to indicate progression from the lowest measurement.

A single observer (XX), with no previous experience on MRI reading, evaluated all the MRI studies and obtained measurements for each of the three methods after being trained by two board-certified neurologists (XX and XX). The observer was selected based on his lack of specialist diagnostic expertise, which might be more relevant for future users of the response metrics.[33] Prior to obtaining the measurements for this study, the observer underwent a workshop with the supervising board-certified neurologists where was instructed on how to identify and measure tumours using each method described above. For this, MRI studies of brain tumours not included in this series were used.

For intra-observer variability, MRI studies of all cases with target lesions (31) where quantitative measurements could be performed with all three methods, were evaluated twice. Time to obtain each measurement and time between the two sets of observation were also recorded.

Therapeutic responses were classified as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) using previously published criteria (Table 2).[12,20,34] To allow comparison of the three methods, volumetric response threshold values were extrapolated as previously suggested:[15,35] a 25% change in area was equivalent to a 40% change in volume, and a 50% decrease in area was analogous to a 65% decrease in volume. Neurological status and corticosteroid dose were also included into the evaluation of therapeutic responses (Table 2). Medication histories and serial neurological examination results were reviewed to allow for categorical scoring of neurological signs as improved, stable, or deteriorating.

According to the RAVNO criteria, non-enhancing or non-measurable non-target lesions can only be qualitatively assessed as improved, stable, or progressive. Thus, therapeutic response of non-target lesions was categorically evaluated as SD or PD (Figure 2).[12]

Statistical analysis

Data was reported as medians (patients' age, imaging slice thickness and interslice gap), means (time for response assessment), SD (agreement between methods) and ranges (ages, time for response assessment) to include all these.

Intra-observer reliability is defined as the degree of agreement or similarity between calculations made by the same observer for the same tumour.[33] For each assessment method, agreement index (AI) was

calculated to assess reliability using the following equation:

$$AI = 1- [x_a - x_b]/([x_a + x_b]/2)$$

where x_a and x_b represent the first and the second set of measurements, respectively.[31] An AI closer to 1, indicates the two measurements are less variable and therefore more reliable. The AIs for each assessment method were compared by Kruskal-Wallis test.

Time required for the measurements was recorded and median was calculated with the ranges. The statistical method used to assess the difference between the median was one-way ANOVA.

RESULTS

Patients and tumour characteristics

A total of 22 patients met the inclusion criteria for this study consisting of 18 dogs (five males and 13 females) and four cats (three males and one female) (Table 3). The median age was 8 years (range, 3-14) for dogs and 10 years (range, 8-14) for cats.

Only seven patients had histological confirmation of the brain lesion, and these consisted of meningioma (transitional, 2; meningothelial, 1; and fibrous, 1), glioma (high-grade oligodendroglioma, 1; high-grade astrocytoma, 1) and lymphoma (1). The remaining lesions were classified based on MRI appearance, patient signalment, and clinical presentation as suspected glioma (7), meningioma (6), lymphoma (1), and pituitary tumour (1).[18,36-39] No patient presented with multiple brain lesions.

Eight patients were treated with fractionated radiotherapy (XRT; total dose, 4.8 Gray; total fractions, 12 to 20; fractions per week, 3 to 5); six patients received palliative therapy consisting of antiepileptic drug monotherapy (either phenobarbitone or levetiracetam) or a combination of phenobarbitone and potassium bromide or levetiracetam with or without prednisolone; four patients underwent surgical resection of the tumours as a single modality; two received surgery followed by chemotherapy (temozolomide or hydroxyurea); one patient received surgery followed by XRT, and one patient received only chemotherapy with lomustine. Treatment modalities are outlined in Table 3.

Clinical response to treatments

A total of 32 MRI scans were performed post-treatment with 15 animals having one scan, four having two and three having three scans. Initial follow-up MRI scans were obtained from the same day (i.e., immediately postoperative) to three months after the initiation of the first treatment (i.e., from the first day of palliative care, chemotherapy or radiotherapy), from 21 days to 13 months for the second post-treatment MRIs and from eight to 23 months for the third post-treatment MRIs.

In 28 medical examinations performed prior to the corresponding post-treatment MRI, clinical response was assessed as stable or improved. Only four patients were classified as having progressed clinically from the previous follow-up: three with deterioration of the existing neurological status and one with blindness as a new sign. The imaging

evaluations of these patients also showed an increase in tumour size, so their overall response was classed as PD.

Therapeutic response metrics outcome

Fifty-four MRI studies were evaluated with all three methods comparing the 32 post-treatment MRIs to 22 pre-treatment baseline MRIs (Table 4). In five cases, a nadir MRI was identified and used to assess responses in subsequent MRI studies (Table 4).

Eleven tumours contained fluid accumulations, all of which were classed as cysts. Seven of these were present on pre-treatment MRI and 6/7 were retained in the corresponding post-treatment MRI with a change in shape in 4/6. In 4/11 patients a cystic structure was detected only on post-treatment MRI.

Using the RAVNO criteria, all 54 MRI studies contained a lesion that could be evaluated; 31 considered as target lesions and 23 non-target. Six lesions (three pre-treatment and three corresponding post-treatment evaluations) were classified as non-CE non-target lesions; three of these lesions were also classified as non-measurable non-target lesions. Response assessment was variable in these three cases, SD in two and PD in the remaining one (Table 4; cases 8, 17, 18).

Seventeen (five pre-treatment and 12 post-treatment) out of 23 lesions were classified as non-measurable non-target lesions. Three of five (cases 4, 16, 21) remained non-measurable non-target post-treatment with responses assessed as SD (cases 4, 16) and PD (case 21). The

other two increased in size post treatment to become target lesions and were assessed as PD (cases 20, 22).

The remaining nine (out of 12) post-treatment non-measurable non-target lesions had a therapeutic response classified as SD (SD, n=9) from a pre-treatment target lesion (Table 4).

Volumes were calculated for all 54 lesions using both volumetric methods (Supplementary Table 1). Volumes of non-CE lesions were calculated using T2W and FLAIR sequences.

The three neuroimaging response assessment methods agreed in 23/32 post-treatment MRI evaluations (72%); RAVNO and HOROS in 26/32 (81%) evaluations; HOROS and 3D slicer in 29/32 (91%) evaluations; and RAVNO and 3D slicer in 23/32 (72%) evaluations. Table 5 depicts the proportional agreement between methods by suspected tumour type.

In five comparisons (cases 2, 8, 9, 10, 14), the RAVNO system assessed the response as stable (SD) whereas the two volumetric methods showed shrinkage (PR). These involved non-target lesions. In another case (16), RAVNO judged the response as stable (SD) whilst volumetric methods indicated progression (PD).

In three comparisons (cases 6, 13 and 18), 3D slicer differed from the other two methods due to exclusion of cystic (2) or necrotic areas (1). In one of the cases with a cystic component (case 6), 3D Slicer indicated shrinkage (PR) of the tumour whilst the other two methods did not (SD), and for the remaining case with a cyst (case 18), SD was noted with 3D Slicer compared to PD measured with the other two methods. For the case including a necrotic lesion (case 13, Figure 3),

3D Slicer indicated SD compared to PR measured with the other methods as, even though the lesion was smaller post-surgery, the percentage reduction in volume was not enough (34%) to be classed as PR.

There was a significant difference ($P<0.001$) in median time taken to evaluate responses between each method with the RAVNO system requiring less time (median, 2 minutes and 5 seconds; range, 1 minute and 18 seconds to 2 minutes and 59 seconds) than the HOROS method (median, 6 minutes 26 seconds; range, 3 minutes and 10 seconds to 12 minutes and 45 seconds) and the 3D Slicer method (median, 7 minutes and 57 seconds; range, 4 minutes and 46 seconds to 13 minutes and 21 seconds).

The second set of observations used to calculate intra-observer variability was performed over 12 months after the first evaluation. There was no significant difference ($P = 0.09$) in intra-observer agreement between the three methods; (mean AI ± SD, 0.91 ± 0.06 for RAVNO; 0.86 ± 0.08 for HOROS; and 0.91 ± 0.05 for 3D slicer).

DISCUSSION

Numerous studies in human and veterinary medicine have used MRI to characterise brain tumours and assess their response to treatment.[20, 36,40,41] In human neuro-oncology, image-based therapeutic response assessment is well established and criteria to assess the response in

high-grade gliomas have been validated. In veterinary neuro-oncology, conversely, various clinical and research studies have evaluated brain tumour responses on MRI in a similar fashion to human medicine, but no method has been described in sufficient detail to allow replication or standardized response assessment.[27,40,42] In this study, we validate and compare the use of RAVNO and two volumetric MRI-based response assessment methods in a series of brain tumours in dogs and cats.

The volumetric methods were more precise than RAVNO in determining changes in tumour burden, and in overcoming difficulties with cystic structures and necrotic tissue, especially 3D Slicer which was most precise in excluding these. The latter allowed for exclusion of central necrosis or central cystic components, whereas HOROS volumetric method only permitted exclusion of superficial cysts or necrosis during margin delineation of the lesion. Overall, the disadvantage of the volumetric methods was that they were more time-consuming and technically challenging than RAVNO.

In human neuro-oncology, volumetric methods may be better for detecting changes in slowly evolving tumours,[14] and have a stronger association with overall survival and lower inter-observer variability.[15] However, in canine intracranial gliomas no association between MRI pre-operative tumour volume and post-surgical survival time or predictive value of outcome following surgery and adjunctive therapy was found.[43] Thus, further validation in future clinical trials is needed

in order to recommend replacement of two-dimensional methods with volumetric methods.

Results of this study indicate the RAVNO system is a reliable method which is less time consuming as it requires less technical expertise: selection of target lesions and performing two-dimensional measurements require limited training, with electronic callipers for measurements available in numerous open-source digital imaging software. Thus, the RAVNO system may be a more suitable method for use in the clinical setting.

Although retrospective studies comparing therapeutic response assessment methods in human gliomas did not find statistically significant differences between diameter-based or volumetric methods [22-26,44], the RAVNO method failed to identify tumour size variation and assess response correctly in this study when lesions changed in volume but maintained stable diameters on the transverse image section with the largest tumour area or when they contained cysts.

There were several limitations to this study. A relatively small number of cases were included; however, multiple response assessments were made for eight cases. Since the response criteria for all three methods used CE images for quantitative measurements, these could have been influenced by other CE secondary lesions such as inflammation, necrosis, seizure-induced changes and infarction.[12,20,45-51] Similarly, assessment of non-contrast enhancing lesions using T2W and FLAIR sequences may have led to further inclusion of these and other secondary changes, such as oedema, resulting in additional bias in

tumour response evaluations. To account for variations in these secondary changes in association with treatment, RAVNO's qualitative assessment of non-enhancing non-target lesions excludes complete and partial responses as categorical responses.

Other limitations of this study are intrinsic to its retrospective nature, including the lack of standardized record-keeping to optimize clinical data compilation, and the absence of standardized MRI acquisition protocols or interval for post-treatment imaging. The optimal interval for post-treatment imaging in veterinary patients is currently unknown. However, in human medicine, recommendation is that immediate post-operative MRI studies are obtained within 72 hours of surgery, as in the cases included herein, to reduce inclusion of post-operative reactive enhancement and allow for optimal serial evaluations of tumour responses. [12,16,20,49,51] In veterinary medicine, an interval between baseline imaging and entry into clinical trials of 4-6 weeks for patients with slow growing tumours, such as meningiomas, and follow-up imaging every 8-12 weeks in dogs with glioma, have been suggested.[16]

The lack of a standardized image acquisition protocol in this study could represent a potential source of bias. In human medicine a consensus with recommended sequences and parameters has been published to improve standardization of image acquisition in clinical trials.[52] In veterinary medicine, no cut-off values have been clearly described for the quantification of tissue CE. This could be influenced by dose of contrast agent administered, administration rate, and time to

acquisition of the post-contrast images as well as magnetic field strength, T1W sequence acquisition parameters, and even patient positioning. [53] Although these were not standardised in this study, all MRIs were performed with 1.5T magnets and in a single institution, where the contrast dose, timings, sequences run, and patient positioning are relatively consistent. Slice thickness and interslice gap variability; however, might have inferred some bias to our evaluation as it may have increased error identifying the largest tumour diameter for RAVNO measurements and decreased accuracy of tumour volume calculations in some of the cases included herein.

In this study a single observer made all the measurements and there was no assessment of inter- as opposed to intra-observer variability. The intra-observer AI was similar for all methods; however, this contrasts with another study where significant differences between response assessment methods were reported.[33] The low number of cases in our study could have affected the statistical results and further studies with a larger population are advised to clarify this.

Although inter-observer variability was not assessed here, other studies have shown considerable variability in defining a region of interest manually even among expert reviewers.[26,47,54] To improve the variability associated with different observers, use of computer automation for volumetric measurements has shown interesting potential.[25]

In human medicine, separate response assessment systems are emerging for different tumour types such as high-grade glioma and

meningioma.[12,14,15,22] The therapeutic brain tumour response evaluation methods studied here could present limitations associated with certain tumour phenotypes. However, the lack of histological confirmation in most of the brain tumours included herein precluded any conclusion in this regard.

In conclusion, our results suggest that although the volumetric methods may be more precise, the RAVNO system may be the most suitable for use in the clinical setting requiring less time and less training. Prospective studies including larger case numbers and standardised imaging protocols are necessary to confirm the most appropriate method to assess therapeutic brain tumour responses in veterinary patients.

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TABLES

Table 1. Summary of the RAVNO and volumetric response assessment metrics methodology.

Therapeutic response metric	Software	2D or 3D measurement method	MRI sequence used for measurements	Method	Cystic and necrotic tissue
RAVNO	HOROS (v2.2.0, The Horos Project)	2D	• Target lesion†: Transverse T1W post-contrast • Non-target lesion†: Transverse T2W/FLAIR	1. Evaluation of clinical data‡ Target lesions: 2. Search for the transverse section with the largest contrast-enhancing tumour area on T1W post-contrast images 3. Draw the largest two orthogonal lesion diameters on the selected T1W post-contrast transverse image 4. Calculate the product of the measured diameters (and the sum of products if more than one lesion present) Non-target lesions: 2. Qualitatively evaluate the lesion burden on T2W and/or FLAIR transverse images	Avoided on orthogonal diameter drawing
HOROS volumetric measurement method	HOROS (v2.2.0, The Horos Project)	3D	• Transverse T1W post-contrast or transverse T2W/FLAIR in non-enhancing tumours	1. Evaluation of clinical data‡ 2. Detect all contrast-enhancing tumour areas on T1W post-contrast transverse images or define the tumour area using T2W and FLAIR transverse images in non-enhancing tumours 3. Delineate the margins of these areas 4. Use a repulsor instrument to close the margins of the lesion 5. Compute the volume of the delineated areas	Excluded from tumour contour delineation
3D Slicer volumetric measurement method	3D Slicer Software (v 4.4, Boston, MA)	3D	• Transverse T1W post-contrast or transverse T2W/FLAIR in non-enhancing tumours	1. Evaluation of clinical data‡ 2. Detect all contrast-enhancing tumour areas on T1W post-contrast transverse images or define the tumour area using T2W and FLAIR transverse images in non-enhancing tumours 3. Paint all the pixels of these areas with a paint effect tool	Excluded from tumour area painting

				4. Generate the volume of the painted areas through model maker option	

Abbreviations: FLAIR, fluid attenuated inversion recovery; MRI, magnetic resonance imaging; RAVNO, Response assessment in veterinary neuro-oncology; T1W, T1-weighted; T2W, T2-weighted; 2D, two-dimensional; 3D, three-dimensional.

† See Figure 2 for definition of target and non-target lesion.

‡ Medication history and serial neurological examination results were reviewed to allow for categorical scoring of neurological status as improved, stable, or deteriorating.

Table 2. Comparison of response criteria for target lesions according to RAVNO and volumetric methods.

		RAVNO[12,20]	Volumetric response criteria[16,34,47]
Complete response	Clinical	• Stable or improved clinical status • Patient not receiving steroids	• Stable or improved clinical status • Patient not receiving steroids
	Imaging	• Elimination of all enhancing tumour • Stable or decreased T2W/FLAIR lesion burden • No new lesions	• Elimination of all enhancing tumour • Elimination of all T2W/FLAIR lesion burden
Partial response	Clinical	• Stable or improved clinical status • Stable or decreased steroid dose	• Stable or improved clinical status • Stable or decreased steroid dose
	Imaging	• ≥ 50% decrease in enhancing tumour PDs • Stable or decreased T2W/FLAIR lesion burden • No new lesions	• ≥ 65% decrease in enhancing tumour or T2W/FLAIR lesion burden
Stable disease	Clinical	• Stable or improved clinical status • Stable or decreased steroid dose	• Stable or improved clinical status • Stable or decreased steroid dose
	Imaging	• <50% decrease or <25% increase in enhancing tumour PDs • Stable or decreased T2W/FLAIR lesion burden • No new lesions	• < 65% decrease or < 40% increase in enhancing tumour or T2W/FLAIR lesion burden
Progressive disease	Clinical	• Clinical deterioration and new neurological signs[†]	• Clinical deterioration and new neurological signs
	Imaging	• ≥25% increase in enhancing tumour PDs • Increased in T2W/FLAIR lesion burden • New lesion(s) present	• ≥40% increase in enhancing tumour or T2W/FLAIR lesion burden

Abbreviations: FLAIR, fluid attenuated inversion recovery images; PDs, product of diameters; RAVNO, response assessment in veterinary neuro-oncology; T2W, T2-weighted images.

[†] Note that in the absence of corroborating imaging or clinical evidence, an increased corticosteroid requirement did not constitute grounds for assignment of progressive disease.[33-35]

Table 3. Signalment, diagnosis and treatment details of the twenty-four cases included in the study.

Case	Age (years)	Species	Breed	Sex	Diagnosis	Treatments
1	11	Dog	Cross breed	FN	Meningioma	RT
2	7	Dog	Labrador	FN	Glioma	RT
3	8	Dog	Boxer	MN	Glioma	RT
4	12	Dog	Cross breed	FN	Meningioma	RT
5	8	Dog	Labrador	F	Pituitary tumour	RT
6	8	Dog	Boxer	FN	Glioma	RT
7	3	Dog	Border collie	FN	Meningioma	RT
8	11	Dog	Jack Russell Terrier	M	Glioma	RT
9	14	Cat	DSH	MN	Transitional meningioma†	SX
10	9	Cat	DSH	MN	Lymphoma†	SX
11	7	Dog	German Shepherd	F	Transitional meningioma†	SX
12	7	Dog	Cross breed	FN	Meningioma	SX
13	11	Cat	DSH	FN	Fibrous meningioma†	SX/RT
14	8	Dog	Boxer	FN	High-grade oligodendroglioma†	SX/CXT‡
15	10	Dog	Boxer	MN	Meningothelial meningioma†	SX/CXT§
16	5	Dog	Boxer	FN	Meningioma	CXT¶
17	9	Dog	Labrador	FN	Glioma	P
18	4	Dog	Boxer	M	Glioma	P
19	11	Dog	Border collie	FN	Meningioma	P
20	3	Dog	Whippet	FN	Glioma	P
21	8	Cat	Bengal	MN	Lymphoma	P
22	8	Dog	Boxer	MN	High-grade astrocytoma†	P

Abbreviations: CXT, chemo; DSH, domestic short hair; F, female; FN, female neutered; M, male; MN, male neutered; n.c., non-completed; P, palliative; RT, radiotherapy treatment; SX, surgery.
† Histopathologically confirmed diagnosis.
‡ Temozolomide-single agent protocol: 60 mg/m^2 PO q 24hr for 5 days every 4 weeks.
§ Hydroxyurea-single agent protocol: 50 mg/kg PO q48hr.
¶ Lomustine-single agent protocol: 50 mg/m^2 PO q3weeks.

Table 4. Classification of the pre-treatment lesions according to RAVNO criteria and response evaluation post-treatment using RAVNO criteria, and HOROS and 3D Slicer volumetric criteria.

Case	Pre-treatment RAVNO Target lesion	Response Criteria	Follow-up 1 RESPONSE	RAVNO Target lesion	Follow-up 2 RESPONSE	RAVNO Target lesion	Follow-up 3 RESPONSE	RAVNO Target lesion
1	CE TL	RAVNO	SD	CE TL	SD	CE TL	SD	CE TL
		Horos volumetry	SD		SD		SD	
		3D Slicer volumetry	SD		SD		SD	
2	CE TL	RAVNO	SD†	NM nTL	NA	NA	NA	NA
		Horos volumetry	PR					
		3D Slicer volumetry	PR					
3	CE TL	RAVNO	SD	NM nTL	SD†	T2 evaluation	NA	NA
		Horos volumetry	SD		PR			
		3D Slicer volumetry	SD		PR			
4	NM nTL	RAVNO	SD	NM nTL	NA	NA	NA	NA
		Horos volumetry	SD					
		3D Slicer volumetry	SD					
5	CE TL	RAVNO	SD	CE TL	NA	NA	NA	NA
		Horos volumetry	SD					
		3D Slicer volumetry	SD					
6	CE TL	RAVNO	SD	NM nTL	SD	NM nTL	PD ‡	CE TL
		Horos volumetry	SD		SD		PD ‡	
		3D Slicer volumetry	SD		PR†		PD ‡	
7	CE TL	RAVNO	SD	CE TL	NA	NA	NA	NA
		Horos volumetry	SD					
		3D Slicer volumetry	SD					
8	NE nTL	RAVNO	SD†	NM NE nTL	NA	NA	NA	NA
		Horos volumetry	PR					
		3D Slicer volumetry	PR					
9	CE TL	RAVNO	SD†	NM nTL	PD ‡	CE TL	NA	NA
		Horos volumetry	PR		PD ‡			
		3D Slicer volumetry	PR		PD ‡			
10	CE TL	RAVNO	SD†	NM nTL	NA	NA	NA	NA
		Horos volumetry	PR					
		3D Slicer volumetry	PR					
11	CE TL	RAVNO	SD	NM nTL	SD	NM nTL	NA	NA
		Horos volumetry	SD		SD			
		3D Slicer volumetry	SD		SD			
12	CE TL	RAVNO	PR	CE TL	NA	NA	NA	NA

		Horos volumetry	PR					
		3D Slicer volumetry	PR					
13	CE TL	RAVNO	SD	CE TL	PR	CE TL	PD ‡	CE TL
		Horos volumetry	SD		PR		PD ‡	
		3D Slicer volumetry	SD		SD†		PD ‡	
14	CE TL	RAVNO	SD†	NM nTL	PD ‡	CE TL	NA	NA
		Horos volumetry	PR		PD ‡			
		3D Slicer volumetry	PR		PD ‡			
15	CE TL	RAVNO	SD	CE TL	SD ‡	CE TL	NA	NA
		Horos volumetry	SD		SD ‡			
		3D Slicer volumetry	SD		SD ‡			
16	NM nTL	RAVNO	SD†	NM nTL	NA	NA	NA	NA
		Horos volumetry	PD					
		3D Slicer volumetry	PD					
17	NM NE nTL	RAVNO	SD	NM NE nTL	NA	NA	NA	NA
		Horos volumetry	SD					
		3D Slicer volumetry	SD					
18	NE nTL	RAVNO	PD	NE nTL	NA	NA	NA	NA
		Horos volumetry	PD					
		3D Slicer volumetry	SD†					
19	CE TL	RAVNO	SD	CE TL	NA	NA	NA	NA
		Horos volumetry	SD					
		3D Slicer volumetry	SD					
20	NM nTL	RAVNO	PD	CE TL	NA	NA	NA	NA
		Horos volumetry	PD					
		3D Slicer volumetry	PD					
21	NM nTL	RAVNO	PD	NM nTL	NA	NA	NA	NA
		Horos volumetry	PD					
		3D Slicer volumetry	PD					
22	NM nTL	RAVNO	PD	CE TL	NA	NA	NA	NA
		Horos volumetry	PD					
		3D Slicer volumetry	PD					

Abbreviations: CE TL, contrast enhancing target lesion; NE nTL, non-enhancing non target lesion; NM nTL, non-measurable non target lesion; NM NE nTL, non-measurable non-enhancing non target lesion; PR, partial response; RAVNO, Response assessment in veterinary neuro-oncology; SD, stable disease; PD, progressive disease.

† Differing response assessment result from remaining methods.

‡ Response assessed comparing with the nadir.

Table 5. Proportional agreement between methods by suspected tumour type.

Tumour type (suspected or confirmed)	Number of cases	Number of comparisons	RAVNO and Horos method agreement	RAVNO and 3D Slicer agreement	Horos and 3D Slicer agreement	Overall agreement
Meningioma	10	17	88%	82%	94%	82%
Glioma	9	12	75%	58%	83%	58%
Other tumours[†]	3	3	66%	66%	100%	66%

Abbreviation: RAVNO, Response assessment in veterinary neuro-oncology.
[†] Other tumours include pituitary tumour (1) and lymphoma (2).

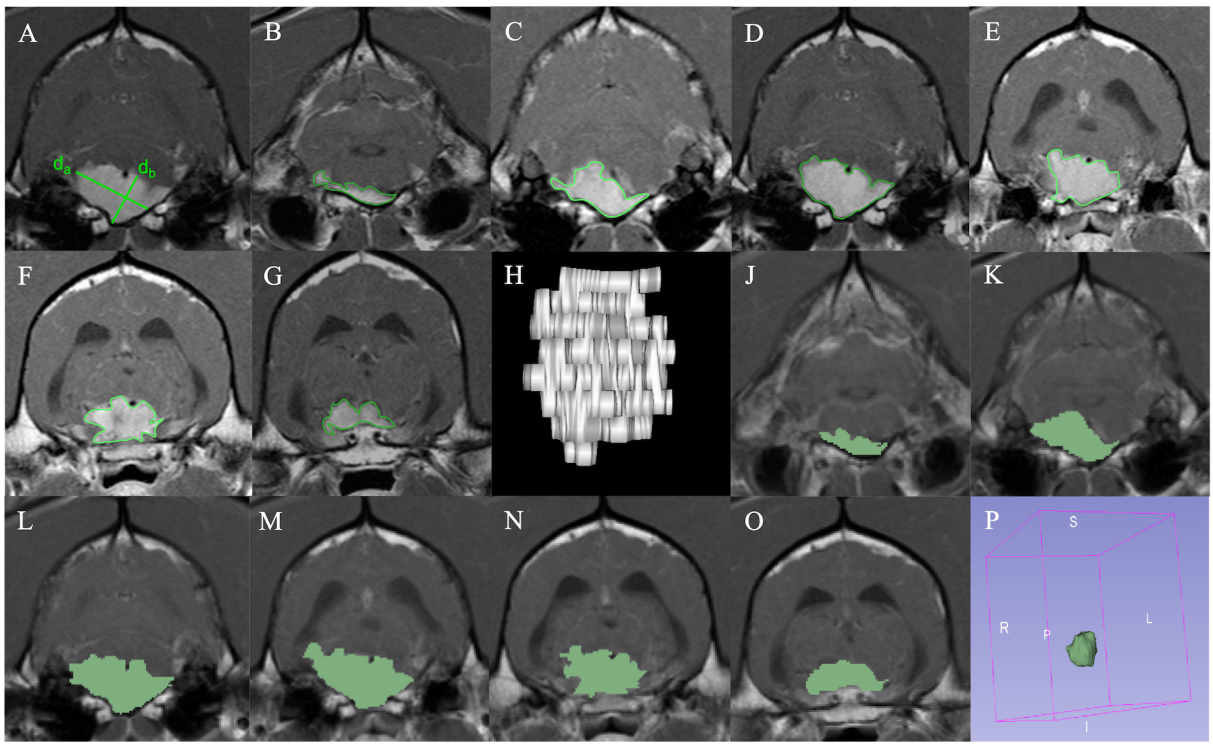
FIGURE LEGENDS

Figure 1. Post-contrast T1-weighted transverse MR images (case 1) illustrating brain lesion measurement using (A) the RAVNO system based on the sum product of the longest orthogonal diameters (d_a x d_b) on the transverse section showing the largest lesion area, (B-H) the HOROS volumetric method based on margin delineation of sequential transverse images and tumour segmentation and (J-P) the 3D slicer volumetric method based on pixel painting of sequential transverse images and tumour segmentation.

Figure 2. Algorithm for defining lesions with MRI using RAVNO criteria and corresponding response assessments. *Note that therapeutic response of non-target lesions can only be categorised as SD or PD. Abbreviations: CR, complete response; FLAIR, fluid attenuated inversion recovery images; PD, progressive disease; PR, partial response; SD, stable disease; T1W+GAD, T1-weighted images post-gadopentate dimeglumine administration; T2W, T2-weighted images.

Figure 3. Pre-treatment (A-C), post-operative (D-F) and second follow-up (G-I) post-contrast T1-weighted transverse MR images of a meningioma in the left frontal lobe of a cat treated with surgery followed by radiotherapy (case 13). The lesions were measured using RAVNO (A,D,G) and volumetric methods using margin delineation with HOROS (B,E,H) and area pixel painting with 3D Slicer (C,F,I). Central areas of mixed intensities suggestive of necrosis could be excluded with the 3D Slicer volumetric method. Post-operative MRI revealed incomplete cytoreduction of the meningioma and further reduction in tumour burden was noted on second follow-up MRI after radiotherapy completion. Both RAVNO and HOROS volumetric measurements indicated PR whereas volume reduction with 3D Slicer

(34%) indicated SD. Note that the second MRI in this case became the nadir for response assessment in its subsequent follow-up MRI study.



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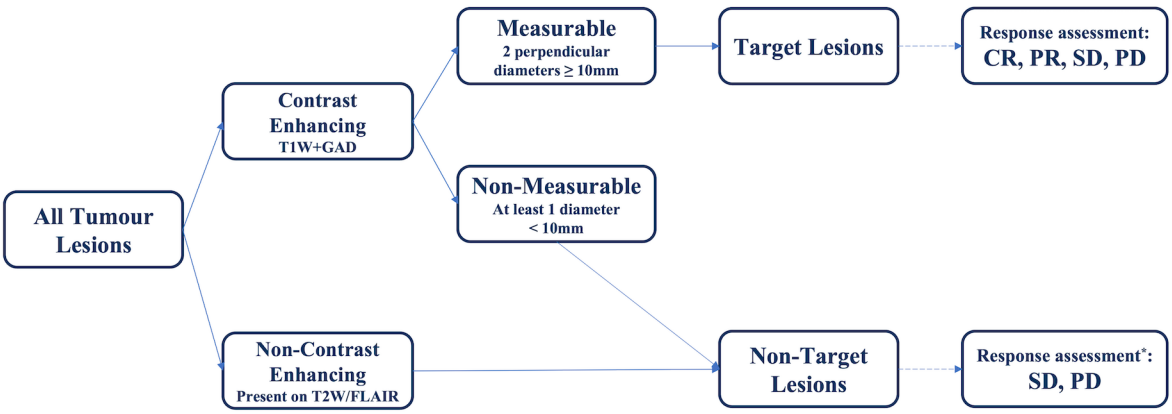

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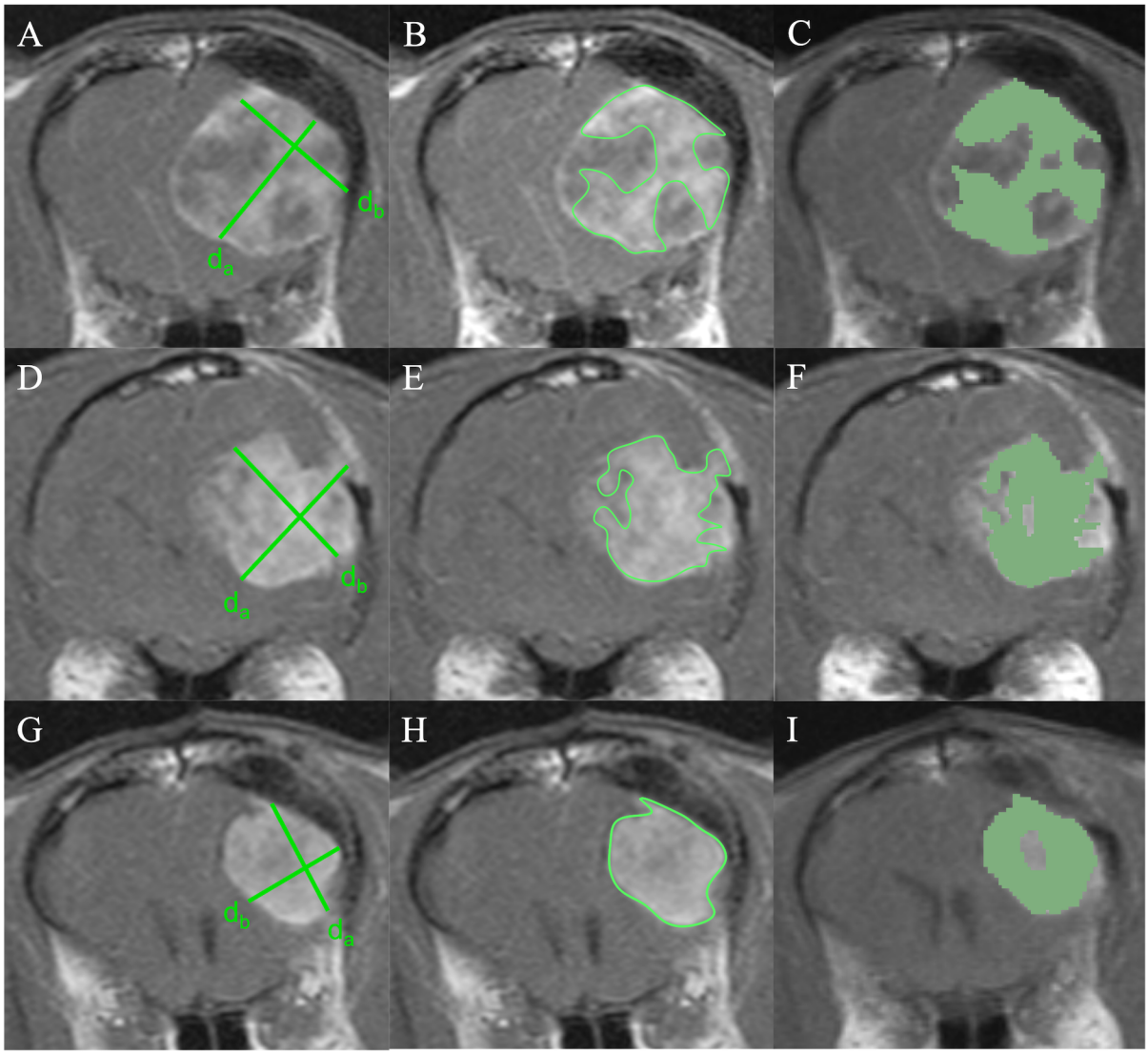

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