From the Department of Companion Animals and Horses
University of Veterinary Medicine Vienna

Clinical Unit of Small Animal Surgery of Vetmeduni Vienna
(Head of Clinic: Ass.-Prof. Dr.med.vet. Britta Vidoni)

A literature review: pathohistological and immunohistochemical analysis of meningiomas in humans, dogs and cats- a comparison

Diploma Thesis

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submitted by
Dimitra Gkika

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Academic advisors: Dr. med. vet. Gabriele Gradner Dipl. ECVS

Clinical Unit of Small Animal Surgery of Vetmeduni Vienna

Department of Companion Animals and Horses

University of Veterinary Medicine Vienna

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1.Introduction and research questions

Meningiomas are tumours that derive from arachnoid cap cells. They are the most common intracranial tumours in humans, dogs and cats (Adamo et al. 2003). They comprise 36.6 % of all primary intracranial tumours in humans and 22.3 % and 59 % of canine and feline brain tumours (Motta et al.2012, Buerki et al.2018). According to data in humans, there is an increasing incidence with age and a female predominance (Buerki et al. 2018). In dogs there has not been proved any consistent sex predisposition but the incidence of meningioma is related with breeds like German Shepherds, Golden Retrievers, Labrador Retrievers, Collies, Scottish Terrier and Boxers (Motta et al. 2012, Kishimoto et al. 2019). Domestic shorthaired and longhaired cats seem to be overrepresented with meningioma but cases are also reported in Siamese, Persians and Maine coons without significant difference between sexes. Meningioma has been reported in dogs over seven years of age and cats over nine years of age. The most common symptoms of intracranial meningiomas are neurological dysfunctions such as headache in humans, seizures, behavioural abnormalities and vestibular dysfunction in humans, dogs and cats. (Adamo et al. 2004, Motta et al. 2012, Kishimoto et al. 2019)

The World Health Organisation (WHO) classifies meningiomas in humans according their histological features into three grades. WHO grade I meningiomas are not characterized from brain invasion and do not fulfil criteria for either atypical or anaplastic grades. The variant types of grade I are: meningothelial, fibroblastic and transitional meningiomas which are most often reported, psammomatous, angiomatous, microcystic, secretory, lymphoplasmacyte rich and metaplastic meningioma. WHO grade II meningiomas or atypical meningiomas are defined by one or more of the following criteria: 4-19 mitoses per ten high power field (HPF), brain invasion, three or more of the following histologic features: small cell change, increased cellularity, prominent nucleoli, sheet-like growth, or necrosis. Grade II includes the subtypes chordoid, clear cell and atypical meningioma. WHO grade III are anaplastic (malignant) meningiomas with 20 or more mitoses per ten high power field. Papillary, rhabdoid and anaplastic meningioma are the subtypes which belong to grade III (Backer- Grøndahl et al. 2012, Louis et al. 2016).

The WHO classification has been updated in the last years trying to improve the classification scheme with more objective and reproducible criteria. The last update in the WHO classification of CNS tumours was in 2016 (Louis et al. 2016). This classification helps researchers to make a more precise analysis of data and help to achieve a more accurate evaluation of different grades. This is important to predict the biological behaviour of meningioma and to induce effective targeted treatments (Louis et al. 2016). Most meningiomas are slowly growing and histologically benign (Shayanfar et al. 2010). However, cases with metastasis of grade I meningiomas in humans have been reported (Beutler et al.2020). Grade II and Grade III tumours are usually more aggressive and may reoccur or lead to significant complications (Commins et al. 2007). The percentage of recurrence rate in 5 years is approximately 50 % for grade II tumours and 90 % for grade III tumours (Buerki et al. 2018). The recurrence rate of grade I meningiomas varies from 2.3 % to 30 % (Shayanfar et al. 2010). High-grade meningiomas comprise 20 % of cases while grade I are more common (Mawrin and Perry, 2010).

A grading system is not developed in dogs and cats so the human classification system has been applied in domestic animals. It offers a more comprehensive list of histological subtypes and a range of morphological criteria is given in order to be assigned to the histological grade. This is a way to avoid a subjective interpretation during the evaluation of meningioma and to support a long-term prognosis based on clinical data (Motta et al. 2012). The classification of meningiomas in domestic animals based on WHO classification scheme of humans classifies them into three major groups: (1) Grad I (2) Grad II/Atypical and (3) Grad III/Malignant. The first category includes: meningothelial, fibroblastic, transitional, psammomatous, angiomatous, microcystic and secretory subtype. The second one includes the atypical subtype, the chordoid subtype and the clear cell subtype. The third group includes papillary and rhabdoid meningioma (Higgins et al. 2018). Most meningiomas are histologically benign and metastases are very uncommon in domestic animals (Motta et al. 2012). The behaviour of feline meningioma is less aggressive than the one in canine and human meningiomas (Samarani et al. 2018). In addition to this, it has been reported that the prevalence of canine atypical meningioma is much higher than in humans (Sturges et al. 2008). Cats may have concurrent benign and malignant multiple meningiomas (Motta et al. 2012, Forterre et al. 2007).

Immunohistochemistry is mainly focused on the discrimination of meningioma from its mimics (eg. hemangiopericytoma, schwannoma). Furthermore, it has been used in the diagnosis of brain tumours in order to improve diagnostic accuracy (Ramos-Vara et al. 2010, Johnson et al. 2014). The WHO 2016 classification system for meningiomas is based solely on histopathological characterisations and consequently cannot provide complete information required to understand the tumour behaviour. Therefore, additional biomarkers are used to provide additional information and more reliable prognostic details (Ma et al. 2019).

Imaging diagnostic and especially MRI plays a significant role in the diagnosis and evaluation of meningioma. The majority of meningiomas appear in CT and MRI as well-circumscribed solid masses with avid contrast uptake in a homogenous pattern in an extra-axial location (Hanft et al. 2010).

However, conventional MRI cannot usually reliably determine the pathological grade of meningioma and its growth potential. Except from that, is not a reliable tool to discern early recurrence radiographic changes from the treatment related ones (Sturges et al. 2008, Miller et al. 2019). Consequently, it is necessary the establishment of advanced MRI techniques or other imaging techniques.

The purpose of this review is to highlight and to classify the histological, immunohistochemical and imaging criteria for the meningioma grading in humans, dogs and cats using literature studies. Furthermore, the association of different markers such as cyclooxygenase (COX-2), somatostatin receptor 2 (STTR2), progesterone receptors, vascular endothelial factor (VEGF), Ki-67, osteopontin, vimentin, CD34 and p63 with meningioma grade in humans, dogs and cats will be reported. Regarding the preceding considerations the following research questions were established:

• What is like the meningioma grading in dogs, cats and humans based on current WHO-classification system?

• What alterations have been established at the WHO- classification scheme the last years? Are there pitfalls and limitations?

• Which proteins are expressed in meningioma and which proteins/markers are correlated with tumour grade and can also be used for the prognosis?

• What kind of methods based on radiology can contribute to preoperative grading?

The hypothesis reads as follows: “Meningioma grading has been established in dogs based on the WHO classification scheme for humans. Biological markers like COX-2, VEGF, progesterone receptors, Ki-67, osteopontin and STTR2 can be used to specify the classification and behaviour of meningioma. Depending on the immunohistochemical markers and staining the usefulness in the different species is discussed”.

2. Literature overview

2.1 Meningioma origin, predisposition factors and localization

2.1.1 Humans

The highest prevalence of meningioma appears in middle-aged patients with a dramatic increase in incidence after the age of 65 years. The female:male ratio is approximately 2.27:1 in young and middle-aged adults but has an insignificant role for elderly patients. The female predominance is associated with endogenous sex hormone levels and is higher during childbearing years. Furthermore, meningiomas are slightly more common among African Americans in comparison with Caucasian and Hispanic people (Beutler et al. 2020).

Some risk factors considered responsible for the development of intracranial meningioma are: ionizing radiation to the skull, history of head trauma, cigarette smoking and cell phone use. An additional factor for the predisposition to develop a meningioma has hereditary roots, due to autosomal dominant disorder, neurofibromatosis type 2 (NF2). In cases with NF2 have also been described multiple meningiomas (Forterre et al. 2007). Other syndromes which are associated with meningioma in humans are Li-Fraumeni, Gorlin, von Hippel-Lindau, Cowden disease and multiple endocrine neoplasia (MEN) type 1 (Buerki et al. 2018).

Although meningiomas can occur anywhere in the central nervous system there are predilection locations in brain such as the cerebral convexities and parasagittal localizations communicating with the falx cerebri and the venous sinuses. Atypical meningiomas were more often observed at none-skull base locations (Motta et al. 2012, Backer-Grøndahl et al. 2012).

2.1.2 Dogs and cats

Meningiomas are mostly observed in dogs over seven years old and in cats over nine years of age. However, cases in dogs younger than six months and cats older than three years of age have also been reported (Motta et al. 2011). The development of meningioma in young cats has been associated with mucopolysaccharidosis type 1 (Adamo et al. 2004). Other diseases associated with meningiomas in dogs and cats are thymic lymphoma and other unrelated neoplasia. In particular, a percentage of 13.9 % in cats and 19 % in dogs develop meningioma in addition to another intracranial tumour (Motta et al. 2012).

In contrast with humans, there has not been a significant difference between sexes in dogs and cats with meningioma reported. However, there is a breed predisposition in German Shepherds, Golden Retrievers, Labrador Retrievers, Collies, Scottish Terrier and Boxers for canine meningioma while feline meningioma is most reported in domestic shorthaired and longhaired cats, Siamese, Persians and Maine coons (Adamo et al. 2004, Motta et al. 2012).

In dogs meningiomas occur more often in the brain than in spinal cord and have also been noted in predisposed localizations in the cranial vault. In dogs most meningiomas are adjacent to the calvarium and may involve the olfactory/frontal region, the floor of the cranial cavity, the optic chiasm or the suprasellar and parasellar regions, the cerebello-pontomedullary region, the retrobulbar space and the middle ear cavity. They grow over the cerebral convexities and below the brainstem or they are attached to the falx cerebri. In cats the corresponding locations include the rostral fossa, the tela choroidea of the third ventricle, the supratentorial meninges and rarely the cerebellar meninges (Adamo et al. 2004, Montoliu et al. 2006, Motta et al. 2012). In dogs the tumours that grow in the cerebral convexity, olfactory and parasagittal regions are more common to appear in combination with diffuse edema, whereas tumours in the cerebellopontine angle and basilar regions usually show focal edema or none at all. However, there were not found any significant associations between tumour grade and location of tumour in dogs (Sturges et al. 2008).

In contrast to dogs multiple feline meningiomas are commonly reported in cats (Motta et al. 2012). In cats with multiple meningiomas one large main lesion and a secondary smaller tumour is usually noticed belonging to the same histopathological group. However, histological variations of multiple meningiomas in the same patient have been described. A theory for the development of multiple meningiomas is that they origin from the dissemination of tumour cells by subarachnoidal spread via cerebrospinal fluid or iatrogenic. Furthermore, even belonging to the same group multiple meningiomas may have different biological properties (Forterre et al. 2007).

2.2 Clinical signs

2.2.1 Humans

Meningiomas are often incidentally discovered on brain imaging. They may have no or mild symptoms to severe behavioural changes. The clinical signs depend upon their location. Despite there is not any pathognomonic presentation of meningioma some of the most common clinical symptoms are: headache due to increased intracranial pressure, focal neurological deficits or seizures caused by focal mass effect. Personality changes, confusion and level of consciousness have been also described especially in cases of frontal or parasagittal meningiomas (Buerki et al. 2018)

2.2.2 Dogs and cats

In dogs and cats the clinical signs are associated with the neuroanatomical location of meningioma. Meningiomas that grow on the cerebral cortex or diencephalon may cause epileptic seizures. In addition to this, compression or damage of diencephalon can cause altered consciousness and vestibular disorders. Other neurological deficits related with intracranial meningioma such as external and internal ophthalmoplegia have been rarely reported. To sum up, the main clinical signs associated with intracranial meningioma in dogs are seizures, while in cats are lethargy and behaviour changes (Adamo et al. 2004, Motta et al. 2012).

2.3 Histologic classification

2.3.1 Humans

World Health Organisation classifies meningioma into three grades of malignancy based on their histologic features (Hanft et al. 2010, Mawrin and Perry 2010, Backer-Grøndahl et al. 2012, Buerki et al. 2018, Zhu et al. 2019).

WHO Grade I

As grade I meningiomas are characterized tumours that do not have brain invasion and do not fulfill the criteria for grades II and III. The histologic variants of grade I (fig.1) meningiomas are:

-*Meningothelial meningioma*: it forms lobules of different size which are separated by thin collagenous septae. Its cells are polygonal or epithelioid with round to oval nuclei. In this type of meningioma nuclear changes like nuclei with clear holes or eosinophilic pseudoinclusions are common. Nuclear atypia is also possible as a result of degenerative changes.

-*Fibrous (fibroblastic) meningioma*: it consists of spindle-shaped tumor cells with elongated nuclei, forming bundles. In this subtype it is sometimes difficult for someone to find the tumor cell nests because of the collagen-rich matrix.

-*Transitional meningioma*: it has both mixed meningothelial and fibrous features with prominent formation of whorls and psammoma bodies.

-*Psammomatous meningioma*: it consists of psammoma bodies. This subtype expresses bone-related proteins like osteopontin and the extensive calcification or ossification may obscure the underlying meningothelial cell. Psammomatous meningiomas occur usually in the thoracic spinal region in elderly women.

-*Angiomatous meningioma*: in this type more than 50 % tumour volume is occupied by blood vessels. There are small or medium vascular channels and the blood vessels are hyalinized. Moreover, nuclear atypia is common.

-*Microcystic meningioma*: it consists of tumour cells with thin elongated processes which look alike arachnoidal trabecular cells and mucinous matrix. The microcystic subtype is often mixed with the angiomatous pattern. In addition to this, these tumours are highly vascularized and are accompanied by peritumoral edema.

-*Secretory meningioma*: is characterized by the presence of PAS (periodic acid-Shiff) -positive eosinophilic intracellular inclusions with gland-like spaces. These inclusions are known as pseudopsammoma bodies. Compared to the other meningioma subtypes, the presence of mast cells and histiocytic cells is prominent.

-*Lymphoplasmacyte-rich meningioma*: extensive inflammatory infiltrates are the main features and consequently the meningothelial part might not easily be seen. They have also been documented spontaneous recurrences over multiple sites as well as multifocal growth of the tumour.

-*Metaplastic meningioma*: they are characterized from mesenchymal differentiation with formation of bone, cartilage, fat and xanthomatous tissue elements.

The lymphoplasmacyte-rich and metaplastic subtype of meningiomas are rarely reported (Hanft et al. 2010, Mawrin and Perry 2010, Backer-Grøndahl et al. 2012, Buerki et al. 2018).

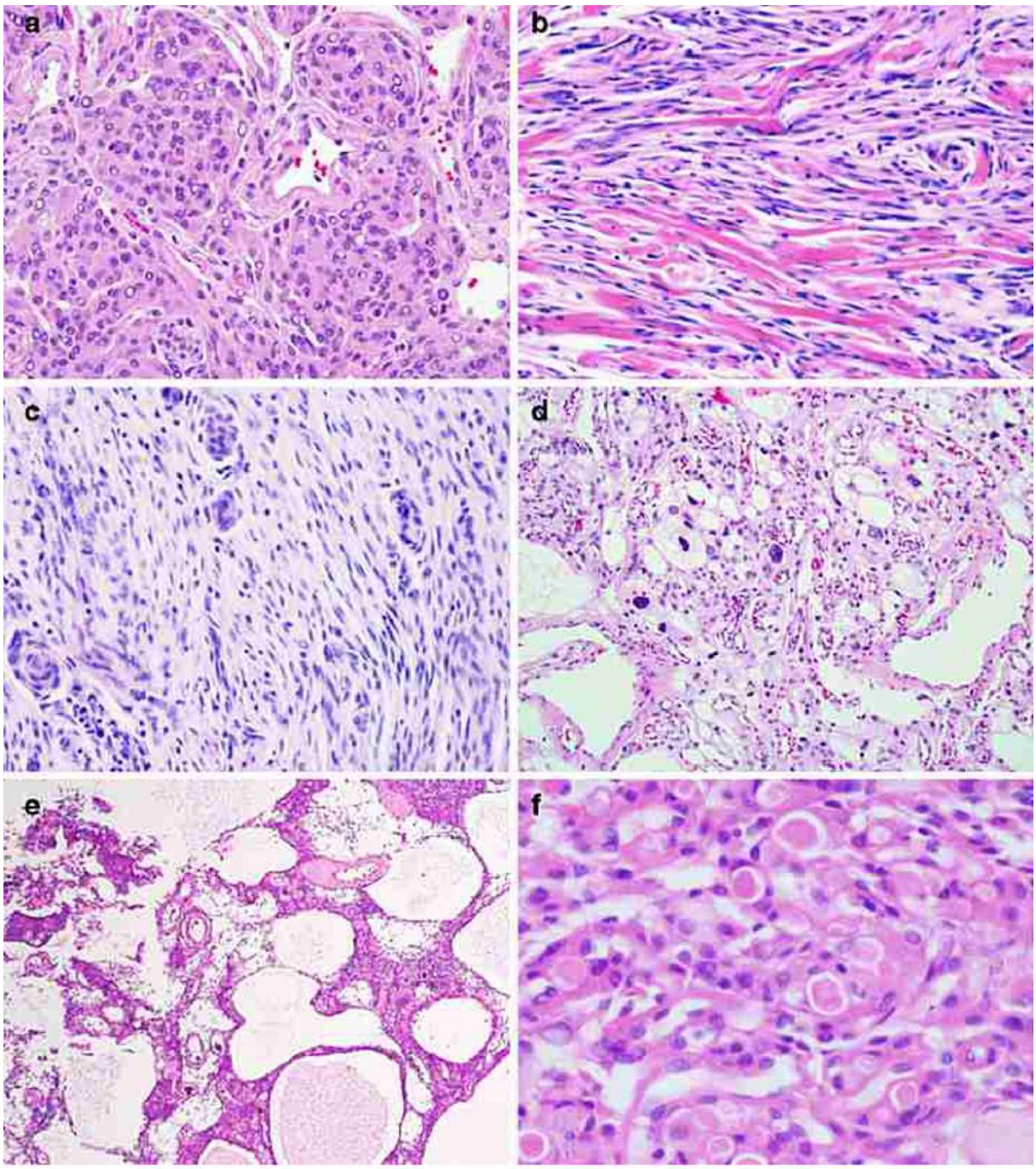


Fig. 1 Histological subtypes of WHO grade I meningiomas. a Meningothelial meningioma b Fibroblastic meningioma. c Transitional (mixed) type of meningioma d Angiomatous meningioma e Microcystic meningioma with microcysts containing pale, eosinophilic mucinous fluid. f Secretory meningioma containing numerous pseudopsammoma bodies (Mawrin and Perry, 2010)

WHO Grade II

As Grade II (atypical) meningiomas are characterized tumours with one or more of the following criteria: 1) brain invasion, 2) four to nineteen mitoses per ten hpf (high power field) and 3) three or more of the following five parameters: small cells, increased cellularity, necrosis, prominent nucleoli, sheet like growth.

The histologic variants of grade II meningiomas are (fig.2):

-*Chordoid meningioma*: it appears as chordoma with cords or trabeculae of eosinophilic epithelioid cells, mucin-rich stroma and clear vacuoles. The chordoid pattern is mixed with meningothelial or transitional tumour areas and psammoma bodies are uncommon. Chordoid meningiomas are large supratentorial tumours with high recurrence rate and a predilection for childhood.

-*Clear cell meningioma*: it consists of polygonal cells with glycogen-rich, clear cytoplasm and extensive collagen deposition. Whorl formation and psammoma bodies are rare. It is usually located in the spinal region and posterior fossa in young patients and has a high recurrence rate.

-*Atypical meningioma:* as it is has been previously referred

-*Brain invasive meningioma*: those types of meningioma should be considered as grade II even if sometimes look benign in appearance (Hanft et al. 2010, Mawrin and Perry 2010, Backer-Grøndahl et al. 2012, Buerki et al. 2018).

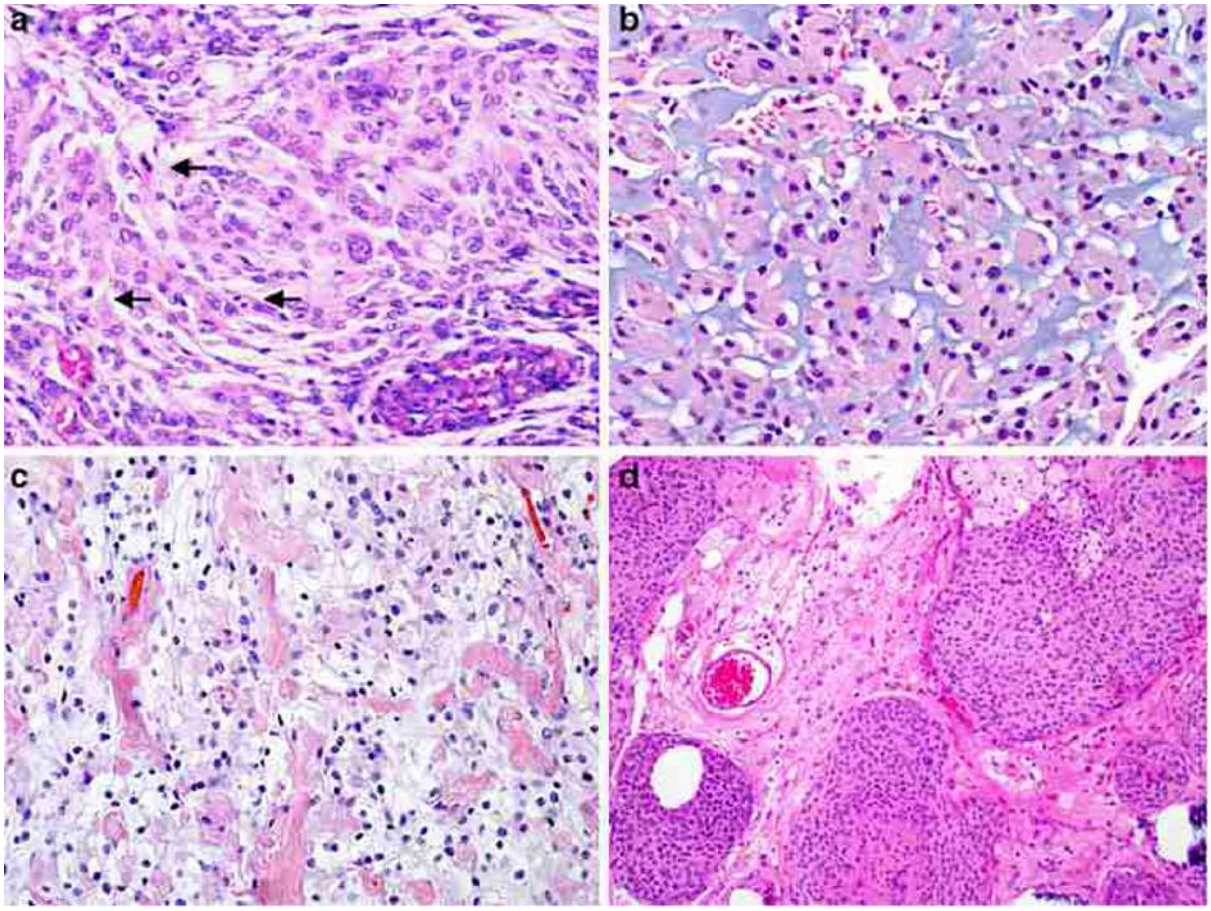


Fig. 2 a Atypical meningioma WHO grade II with sheet-like growth and increased mitotic activity (arrows). b Chordoid meningioma with abundant basophilic mucin-rich matrix and clear vacuoles in the background. c Clear cell meningioma with prominent perivascular and interstitial collagen. d Atypical meningioma showing several regions of brain invasion (arrows) (Mawrin and Perry, 2010)

WHO Grade III

As grade III (anaplastic) meningiomas are characterized tumours that mainly belong to the anaplastic variant and more rarely to papillary and rhabdoid variant (fig. 3).

-*Anaplastic meningiomas*: fulfil one of the following criteria: presence of 20 or more mitoses per ten hpf or the presence of anaplasia, defined as similar histology to the one of carcinoma, melanoma or sarcoma.

-*Papillary meningioma*: it is featured from perivascular papillary or pseudopapillary growth pattern, partial loss of cellular cohesion and ependymoma-like perivascular pseudorosettes. Classical meningothelial features may also be present. Papillary meningiomas occur usually in children and its brain invasion concerns 75 % of patients.

-*Rhabdoid meningioma*: it consists of rhabdoid cells with eccentric nuclei and prominent nucleoli. The cytoplasm is eosinophilic and includes often inclusions of whorled bundles of intermediate filaments. Malignant features like high mitotic counts, cytological atypia and necrosis are also present in most cases. This subtype of meningioma is associated with increased mortality and occasional extracranial metastasis in a median age and is very rare. (Hanft et al. 2010, Mawrin and Perry, 2010, Backer-Grøndahl et al. 2012, Buerki et al. 2018).

About 90 % of all meningiomas according to the current WHO classification are grade I tumours, 5-7 % are grade II and 1-3 % are grade III. In most cases meningiomas present a benign clinical behaviour. However, metastasis has been also reported as a rare complication. According to previous studies metastases were supposed to occur only in malignant tumours. According the study of Surov et al. (2013) was proved that metastases can also originate from histologically benign tumours. According the literature search of the present study, a recurrent transitional meningioma grade I metastasized to the liver. In the medical literature there are two more cases of transitional meningioma that metastasized in the lungs (Surov et al. 2013).

The most common sites of metastasis are the lungs, bones, spine and in liver. The location of the identified metastases indicates that the most common pathway for metastases is the hematogenous route via the jugular vein. Meningiomas may also metastasize via the paravertebral venous plexus. Seventy-five percent of cases of metastatic meningioma occur in patients with history of prior surgery or venous sinus invasion. Metastasis is also possible via the lymphatic vessels and through the cerebrospinal fluid route (Beutler et al. 2019).

Another point of view is that the prevalence of metastatic meningioma may be underreported with 31.3 % being clinically silent (Surov et al. 2013, Beutler et al. 2019).

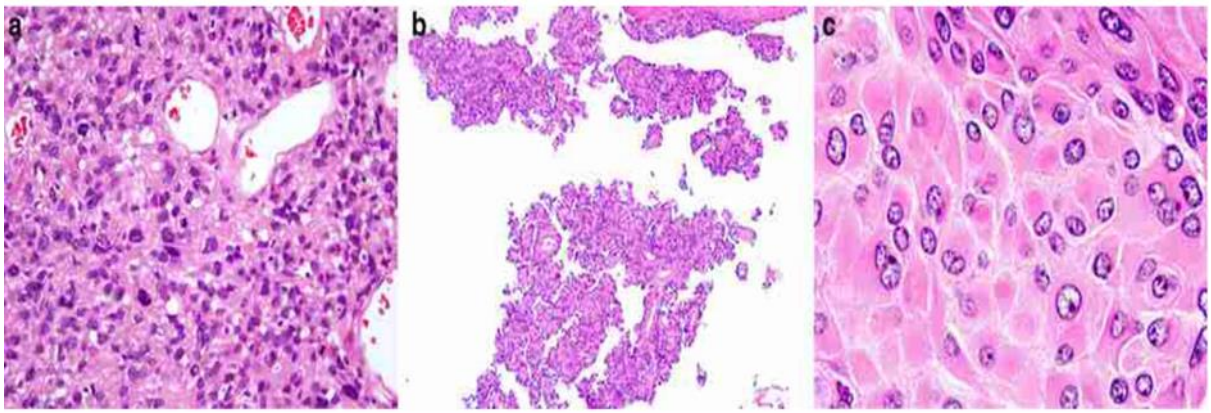


Fig. 3 WHO grade III meningiomas. a Anaplastic meningioma with sarcoma-like appearance and increased mitotic index. b Papillary meningioma. c Rhabdoid meningioma (Mawrin and Perry, 2010)

2.3.2 Dogs and cats

The current domestic animal WHO histologic classification system of meningiomas classifies them into three major groups: (1) Grade I, (2) Grad II/Atypical and (3) Grade III/Malignant. The first category includes: meningothelial, fibroblastic, transitional, psammomatous, angiomatous, microcystic and secretory subtype of meningioma. The second one includes the atypical, chordoid and clear cell subtype. The Grade III (malignant) group includes the papillary and rhabdoid meningioma subtypes. The microcystic subtype was later identified in dogs. A grading system is not applied in domestic animals. However, meningioma in domestic animals shares strong histologic and tumour behaviour similarities with human meningioma. That is the reason that the more detailed human classification scheme is used for the classification of canine and feline meningioma. In other words, the human WHO classification offers a variety of morphological criteria to assign the histological grade and allows a more precise tumour evaluation (Higgins et al. 2018, Motta et al. 2012).

-*Meningothelial meningioma*: it is formed by sheets of meningothelial cells often with vague whirling. The cells have abundant cytoplasm with large round to ovoid nuclei and prominent nucleolus.

-*Fibroblastic meningioma*: it consists of streams of spindle cells with rich collagen deposition

-*Transitional meningioma*: it combines features of both meningothelial and fibroblastic meningioma

-*Psammomatous meningioma*: there are typical mineralized concretions in the centres of meningothelial whorls. It is diagnosed when the psammoma bodies are the predominant histologic pattern.

-*Angiomatous meningioma*: they are rare variants highly vascularized

-*Microcystic meningioma*: large vacuoles in the neoplastic cells can form large cysts in the tumour. It is diagnosed when this kind of pattern is the predominant one.

-*Secretory meningioma*: this subtype has lately been recognized in dogs. It is consisted from epithelial like glands which contain distinctive eosinophilic PAS-positive globular structures mixed with conventional areas of meningioma

-*Atypical meningioma*: they may have any of the above features, more than four mitoses per ten 400x fields and three or more of the following criteria: increased cellularity, increased anisocytosis and anisocaryosis, necrosis or patternless growth patterns

-*Chordoid meningioma*: it is consisted from cells with round nuclei containing eosinophilic cytoplasm. Often are marked vacuoles which form long chords or trabeculae interrupted from interstitial matrix

-*Clear cell meningioma***:** sheets of polygonal cells are characteristic for this rare subtype

-*Rhabdoid meningioma*: it consists of meningothelial cells with abundant eosinophilic cytoplasm and small nuclei

-*Papillary meningioma*: the predominant histologic pattern is defined by the abundance of pseudorosettes. Although it is classified by WHO criteria into the slow growing benign tumours, data supports that is often an aggressive type of canine meningioma with a high recurrence rate (Higgins et al. 2018, Miller et al. 2019).

It is significant to be noticed that in canine meningioma regions of necrosis are common to be found and they should not always be associated with an atypical subtype (Miller et al. 2019). In fig. 4 are depicted the meningothelial, transitional, microcystic, psammomatous, transitional and fibrous subtypes.

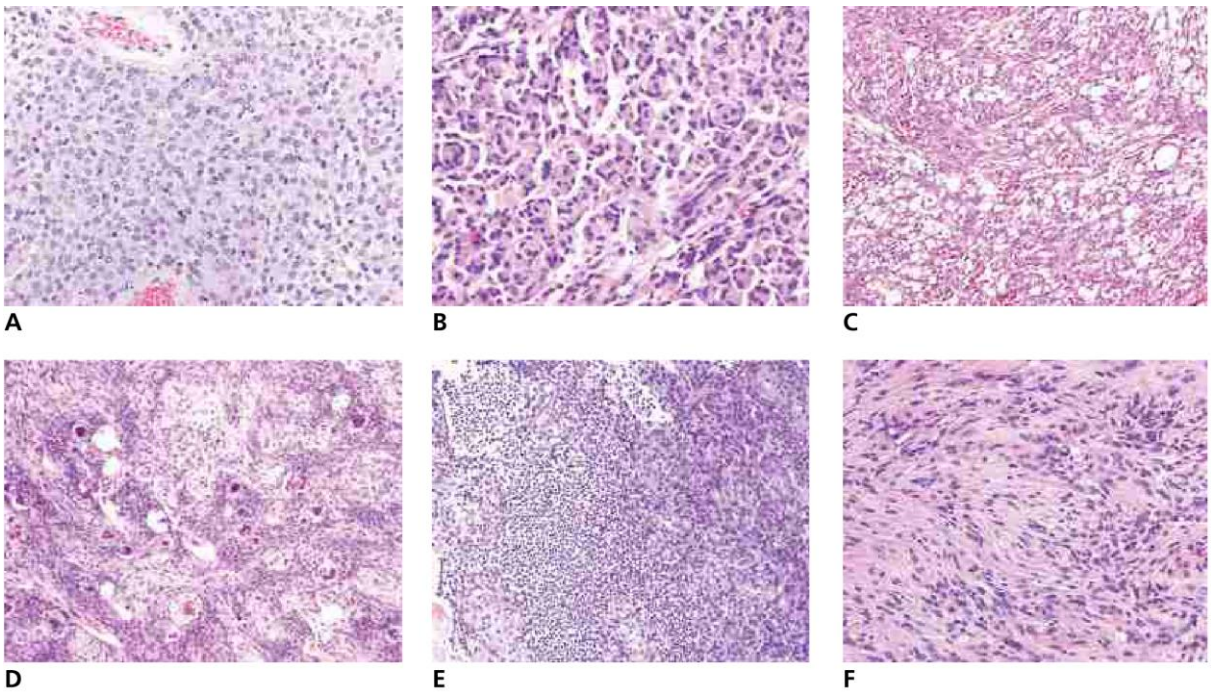


Fig. 4 Histological subtypes of grade I meningiomas, canine. (A) Meningothelial. (B) Transitional. (C) Microcystic. (D) Psammomatous. (E) Transitional with multifocal neutrophil infiltration. (F) Fibrous. H&E stain (Higgins et al. 2018)

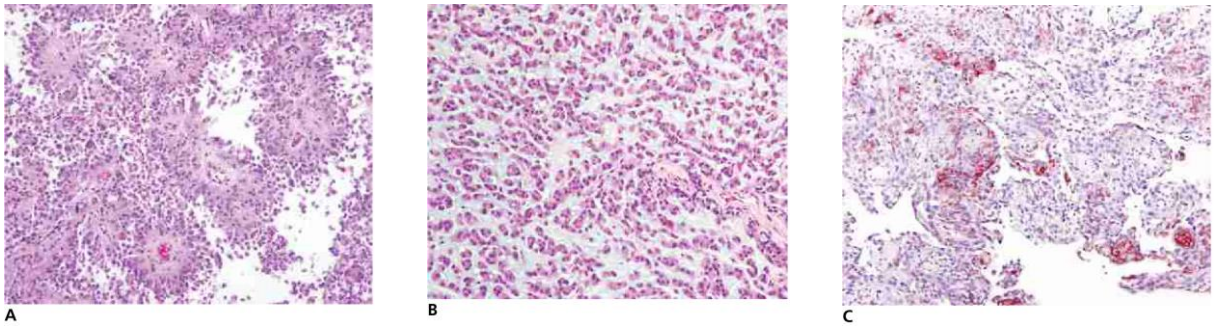


Fig.5 less common subtypes of meningioma, canine. (A) Papillary. (B) Chordoid. (C) Multifocal areas in this microcystic meningioma grade II with focal patchy pancytokeratin (AE1/AE3) immuno-reactivity (Higgins et al. 2018)

2.4 Alterations in WHO histologic classification scheme

The first published grading systems for meningiomas were in use in the late 1990s. From then the WHO classification scheme has undergone significant alterations and updates in order to impose more precise and reliable criteria for grading. The current edition of the WHO classification scheme is the 2016 WHO histologic classification system (Commins et al. 2007, Barresi et al. 2016).

The 1993 Version

One of the first classification scheme was the 1993 Version. In this scheme certain histologic features decided if a meningioma was Grade I tumour or higher grade. Grade II tumours had the following criteria: frequent mitoses, increased cellularity, small cells with high nucleus/cytoplasma ratio or/and prominent nucleoli, pattern less or sheet like growth, areas with spontaneous or focal necrosis. Grade III meningioma showed histological features of frank malignancy and the degree of the abnormalities were greater than the ones of Grade II meningiomas.

This classification system recognized only papillary meningioma as a Grade II to III tumour and considered the other histologic subtypes as benign.

The criteria for this classification were highly subjective because there was no definition for “frequent mitoses” and “increased cellularity”. That was the reason why investigators in the Mayo Clinic published two large studies in order to insert objective and reproducible criteria for grading and to make the role of brain invasion clear.

Some of their findings were adopted from the 2000 and then from the 2007 published grading schemes (Commins et al. 2007, Barresi et al. 2016).

Findings from the Mayo clinic study

They inserted as criterion for atypical meningioma four or more mitotic figures per ten high power field (HPF). The second criterion for the definition of atypical meningioma was the presence of at least three of the following four parameters: sheeting, prominent nucleoli, hypercellularity and the formation of small cells. They also noticed that some tumours invaded into the brain and brain invasion was a predictor of shorter-recurrence free survival.

In their second study they investigated the criteria which define malignant meningiomas.

These were: brain invasion, frank anaplasia and distant metastasis. There were also present excessive mitoses, twenty or more per ten HPF. Anaplastic meningiomas were defined tumours which had lost their meningothelial features and resemble a carcinoma, sarcoma or melanoma (Commins et al. 2007)

The 2000 Version

The 2000 WHO classification scheme is based on the two Mayo Clinic studies. The WHO considers necrosis as a feature for atypia but does not include the brain invasion as a criterion for atypia.

Therefore, the 2000 WHO classification scheme includes more objective grading criteria:

As Grade I meningiomas are defined the ones which do not fulfil criteria for Grade II or III. As Grade II meningiomas are defined meningiomas with at least four mitotic figures in ten high power-fields and three or more of the following parameters: increased cellularity, small cells, necrosis, prominent nucleoli and “sheeting”. As grade III meningiomas are defined meningiomas with at least 20 mitotic figures in ten high power-fields and/or malignant cytologic characteristics such that tumour resembles carcinoma, sarcoma or melanoma.

The WHO 2000 Version recognised a higher proportion of meningiomas as atypical (Commins et al. 2007; Backer-Grøndahl et al. 2012; Barresi et al. 2016).

The 2007 Version

There are no differences between 2000 and 2007 schemes except the fact that brain invasion is also included as a criterion for atypia regardless of the presence of other malignant features. Consequently, as Grade I meningiomas are defined the ones which do not fulfil criteria for Grade II or III. As Grade II meningiomas are defined meningiomas with at least four mitotic figures in ten high power-fields or/and brain infiltration or/and three or more of the following parameters: increased cellularity, small cells, necrosis, prominent nucleoli and “sheeting”. As grade III meningiomas are defined meningiomas with at least 20 mitotic figures in ten high power-fields and/or malignant cytologic characteristics such that tumour resembles carcinoma, sarcoma or melanoma as well as rhabdoid and papillary meningioma.

The criterion of brain invasion has led to further increase of the incidence of grade II meningiomas and 30 % of meningiomas are considered as atypical (Commins et al. 2007, Backer-Grondahl et al. 2012, Barresi et al. 2016).

The 2016 Version

The only update in the 2016 Version is that brain invasion is considered as a standalone criterion for the diagnosis of a Grade II meningioma. According to the current 2016 Version:

Grade I meningiomas are those with a low mitotic rate, <4 per 10 HPFs, they are characterized from absence of brain invasion and nine histologic subtypes. They are the most frequent tumours in percentage of 80-85 %.

As grade II meningiomas are defined meningiomas with mitotic rate 4-19 per ten HPF or brain invasion or three or more of the following histologic features: spontaneous or geographic necrosis, patternless sheet like growth, prominent nucleoli, high cellularity, small cells with high n:c ratio. They comprise 15-20 % of meningiomas.

As Grade III meningiomas are defined tumours with mitotic rate >20 per HPF or papillary or rhabdoid variants. They are rare in a percentage of 1-2 %. (Buerki et al. 2018)

2.5 Limitations and unresolved issues in meningioma grading

Although, the criteria for meningioma grading have been established some unresolved problems and pitfalls in meningioma grading still remain.

Mitotic count

The mitotic rate is one of the criteria that help to distinguish the different grades of meningioma. WHO suggests that all mitoses should be counted in a HPF of 0.16 mm^2. In other words, the mitotic count should be adjusted according to the microscope used for the histological evaluation and that it might be affected by cellular density in the microscopic field. One more disadvantage of mitotic counting as a grading feature is that the distinction between mitoses, apoptosis and karyorrhexis is sometimes difficult on haematoxylin and eosin stain. The use of mitosis-specific immunohistochemical stain like that against phosphohistone H3 (PHH3) protein can help to identify the mitoses and recognize aggressive meningiomas (Commins et al. 2007, Barresi et al. 2016).

Brain invasion

The brain invasion is according to 2016 WHO classification scheme a standalone criterion for Grade II meningioma diagnosis. However, brain parenchyma may be absent many times in the analysed samples. Therefore, the extensive sampling of the surgical specimen is recommended. Furthermore, it may be difficult to distinguish true brain invasion from infiltration in the intervening layer of leptomeninges (Barresi et al. 2016).

Prominent nucleoli

Although prominent nucleoli are considered as criterion for the diagnosis of grade II meningioma there is not a precise definition for them according WHO. For their identification is used a definition by Perry and Brat (2010), in which they are defined as: “nucleoli visible by using a 10x objective lens”(Barresi et al. 2016).

Preoperative embolization and radiation therapy

Preoperative embolization is used to minimize intraoperative bleeding. However, it may cause histologic changes that lead to overgrading the tumour. Kinds of example is the high frequency of macronucleoli, necrosis and increased number of mitotic figures. In order to avoid overgrading the communication between the neurosurgeon and the neuropathologist regarding the use of embolization is recommended. In addition to this, the existence of macronucleoli should be considered as present when nucleoli are visible by using a 10x objective lens and is also widespread in the tumour. Radiation therapy may also result in large foci of necrosis. Therefore, the detailed patient´s history with a possible prior radiation therapy must be known. (Commins et al. 2007, Mawrin et al. 2010, Barresi et al. 2016).

Necrosis

Spontaneous necrosis is considered to be a criterion for malignancy in meningiomas. Nevertheless, the distinction between spontaneous necrosis and necrosis resulting from preoperative embolization or radiotherapy may be difficult. From data, it has been reported that spontaneous necrosis appears as small foci occupying less than half of an HPF. On the other hand, induced necrosis presents as areas involving more than 1 HPF. In addition to this, spontaneous necrosis shows gradual transition from the surrounding tumour while induced necrosis presents a sharp demarcation from the surrounding tumour (Barresi et al. 2016).

Mixed Histological Characteristics

Subtypes such as papillary, chordoid and rhabdoid meningiomas often appear with a conventional pattern like meningothelial. Many times conventional patterns demonstrate features such as necrosis and increased mitosis and receive the same grade as the papillary, chordoid or rhabdoid areas while in fact are lower grade.

Such a specific case is the chordoid meningioma, which is classified as a grade II tumour due to its tendency to reoccur. Recurrence is associated with aggressive histologic features such as high mitotic count, necrosis and brain invasion. Consequently, there is the question if the chordoid variant should be downgraded as grade I and deemed as grade II only when malignant histologic features are present (Commins et al. 2007, Barresi et al. 2016).

Small Cells and Hypercellularity

Small cells and hypercellularity, like already mentioned, are criteria for meningioma grading. However, small cells are difficult to be differentiated from apoptosis or immune cells and there is not any precise definition for hypercellularity according WHO (Barresi et al. 2016).

Metastasis as a criterion of malignancy

Metastasis is a rare complication in meningiomas occurring in fewer than 1 % of cases. Previously metastasis was considered to be associated with malignant meningiomas.

According most recent studies like the one of Surov et al. 2013 proved that metastases can also originate from histologically benign and intermediary tumours. Therefore, even histologically benign tumours are classified as malignant if they metastasise (Commins et al. 2007, Surov et al. 2013).

2.6 Markers

As the meningioma grading based on exclusively histologic features leading to pitfalls and to an unreliable evaluation of the tumour grade, the diagnosis of meningioma is also supported by immunohistochemical markers. In the biological markers are included proteins, hormone receptors and growth factors. They can further specify the meningioma classification and be a helpful tool for the prediction of tumour behaviour and consequently the choice of an appropriate therapy. This literature review reports the association between biological markers, tumour grades and behaviour. The association and the usefulness of every immunohistochemical marker for human, dog and cat is described in detail in chapter “Results”.

2.6.1 Cyclooxygenase 2 (COX-2)

COX-2 is a rate limiting enzyme in the production of prostaglandins and is induced in response to mitogens and pro-inflammatory cytokines. The overexpression of COX-2 has been reported in brain tumours. This enzyme can stimulate gene transcription, tumoral growth, angiogenesis, metastasis, to inhibit apoptosis and can cause resistance to chemotherapy (Kato et al. 2014, Lee et al. 2014). Consequently, studies in human and veterinary medicine have been conducted in order to specify the relationship between meningioma and COX-2 expression and to investigate further if COX-2 inhibitors can be used as chemotherapeutic agents for patients with intracranial meningioma. In the studies found for this literature review it is discussed if COX-2 is tumour grade related in humans, dogs and cats (Rossmeisl et al. 2009).

2.6.2 Somatostatin receptor 2 (STTR2)

The neuropeptide somatostatin has an important role in the proliferation of neoplastic cells. Somatostatin receptors are considered to have antitumour activity by blocking cell division, inducing apoptosis and inhibiting secretion of growth factors or angiogenesis. Long acting somatostatin analogues like octreotide or pasireotide are recommended for the treatment of human meningiomas when surgery or radiotherapy are not indicated. There are five types of somatostatin receptors expressed in human tumours and especially the second type is identified most often in meningiomas. Most studies examine if tumour grade or subtypes are related with the second type of somatostatin (STTR2). By far there have not been found studies about STTR2 expression and feline meningioma (Foiani et al. 2019).

2.6.3 Progesterone receptors (PRs)

According to epidemiologic human data, the growth of meningiomas may be affected by the female sex hormones. In most meningiomas progesterone receptors are detected, while estrogen receptors are much less expressed. Progesterone receptors have also been identified in studies about canine and feline meningioma. These findings have a significant role for the use of antiprogesterone treatment in meningioma. Progesterone receptors are associated with meningioma grade (Adamo et al. 2003).

2.6.4 Vascular endothelial factor (VEGF)

Vascular endothelial factor (VEGF) is an angiogenic factor released by tumours and induces the growth of a capillary network (Matiasek et al. 2009). It plays an important role in angiogenesis, vascular permeability and tumour recurrence in human meningioma (Sturges et al. 2008, Matiasek et al. 2009). There have been conducted studies for further investigation between VEGF expression and canine meningioma but there have not been carried out studies for the VEGF expression in feline meningioma.

2.6.5 Ki-67

Ki-67 is a non-histone protein which is expressed in the proliferative phase of the cell cycle. It is used very often as immunohistochemical marker on formalin-fixed paraffin-embedded sections to measure cell proliferation (Babu et al. 2011). Ki-67 labelling index has been correlated with tumour grade in human medicine and have been also carried out studies in veterinary medicine in order to confirm this association in canine meningioma. However, no studies were found for feline meningioma during the literature search.

2.6.6 Osteopontin

Osteopontin (OPN) is a non-collagenous, sialic-acid-rich, glycosylated phosphoprotein, which is emitted from the osteoid matrix. Osteopontin takes part in bone remodelling, inflammation, immune responses and bone mineralization. It´s involved in tumour progression by regulation of apoptosis, proliferation, metastasis and angiogenesis. According to clinic data, OPN appears to have a high expression in tumour tissue and in the serum of some types of cancer (Arikök et al. 2014). Osteopontin may be useful as a biomarker to predict the grade and biological behaviour of meningioma. A study from veterinary medicine investigates the expression of osteopontin in different types of canine and feline tumours but not especially for meningioma (Ozmen et al. 2015)

2.6.7 Vimentin and CD34

Vimentin is a protein that is expressed in mesenchymal cells and functions as a generic marker of mesenchymal tumour. It is expressed in humans, dogs and cats in all histologic subtypes of meningiomas (Ramos-Vara et al. 2010, Johnson et al. 2014). Vimentin distinguishes meningiomas from other brain tumours such as carcinomas, as meningiomas stain positive for vimentin while carcinomas stain negative for vimentin.

CD34 is a transmembrane glycoprotein present in early myeloid cells in the bone marrow and in endothelial cells. Its function is unknown. It is found in fibroblast related mesenchymal cells and in some meningiomas and neurofibromas (Chaubal et al. 1994). CD34 is used in immunohistochemistry as a specialized marker that distinguishes meningioma from other intracranial tumours like hemangiopericytoma or schwannoma. CD34 antibody labels strongly the external limiting layer of the leptomeninges and scattered cells in the deeper layers. Vimentin and CD34 are recommended as basic immunohistochemical markers for the characterization of canine and feline meningioma (Ramos-Vara et al. 2010).

2.6.8 p63

TP53 gene is regarded as a crucial step in carcinogenesis, including the tumours of the nervous system. P53 is a suppressor gene and p63 is a structural homologue of it. DNp63, an isoform of p53 has an oncogenic role and is involved in cellular differentiation and neoplasia. P63 is used in the diagnosis of prostate and breast carcinoma, squamous-cell carcinoma, ovarian tumours and trophoblastic lesions. In human medicine p63 is evaluated as a marker that may distinguish benign from aggressive meningioma (Mittal et al. 2012). Studies reporting any association between p63 and canine or feline meningioma have not been found during literature search.

2.7 Imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) are the main diagnostic tools for the diagnosis of brain neoplasms. However, they cannot replace biopsy, which is the most reliable way for a definitive diagnosis. Imaging techniques are helpful to identify the precise anatomical location and the relationship between brain tumours and the surrounding tissues. The diagnostic accuracy of CT is about 80 % in humans and dogs but unknown for feline meningioma. On the other hand, the accuracy of diagnosis based on MRI characteristics is between 65 % and 96 % in humans. The sensitivity of MRI for intracranial meningioma varies between 66 % and 100 % in dogs and about 96 % in cats (Motta et al. 2012).

Computed tomography (CT)

Meningiomas appear in CT as solitary, well-demarcated isodense or slightly hyperdense masses in an extra-axial location. It may also be noted a displacement of the cortical grey matter in cases of large tumours or edema surrounding the tumour. Edema manifests as low-density region. Arterial narrowing may be also observed in areas, which are compressed by the tumour. About 20% of meningiomas may appear calcification in CT. According to data, strong calcification indicates a favourable prognosis. Meningiomas are demonstrated bright with homogenous enhancement in CT scan with contrast enhancement. Atypical meningioma appears heterogeneous enhancement (Beutler et al. 2020).

Magnetic Resonance Tomography (MRI)

MRI is the goldstandard for meningioma diagnosis. Tumours may appear isointense or hypointense on T1- weighted sequences (T1W1) and isointense or hyperintense on T2 -weighted sequences (T2W2). Edema is presented hypointense on T1-weighted sequences and hyperintense on T2-weighted sequences. Tumour invasion into the bone manifests a hypointense signal within the bone seen on T1-weighted sequences. The “dural tail sign” appears often in MRI, which is a thickening of the dura mater in the region adjacent to the tumour, resembling a tail extending from the lesion. It is considered that this phenomenon may be a result of hyper vascularity and/or tumoral invasion of the dura. The presence of dura tail was once pathognomonic for meningioma but now it seems to be present in up to 78 % of cases. Its presence is also associated with glioblastoma, schwannoma and other intracranial tumours (Beutler et al. 2020). Based on imaging findings some histologic subtypes of meningioma may be differentiated. For example, benign secretory meningioma is homogenously enhancing and hyperintense on T2-weighted sequences. A malignant meningioma can appear heterogeneously enhancing and hyper- to hypointense on T2 weighted sequences (Beutler et al. 2020). Grade I can be distinguished from grade II/III meningiomas with the use of six imaging features according to a study. These features are: intratumoral cystic change, hyperostosis of the adjacent skull, bony destruction, extracranial tumour extension through the skull base, arterial encasement and peritumoral brain edema. Especially, intratumoral cystic change and extracranial tumour extension through the skull base foramina were the two features most commonly reported in the Grade II/III (Hanft et al. 2010). Furthermore, it is necessary to correlate the radiologic findings with the clinical history, physical examination and biopsy because the radiologic features of meningioma may be similar to those of other intracranial lesions (Beutler et al., 2020).

Dogs and cats present in CT and MRI a similar picture to that of humans. Features like intratumoural fluid, large cystic regions, intratumoural mineralization, calvarial hyperostosis or dural tail is some of its characteristics. In canine meningiomas peritumoral edema is present in >90 % of cases. As the specific subtypes of meningioma cannot be distinguished using conventional MRI techniques and there has not been found any significant association between tumour grade and MRI features, new advanced imaging techniques must be used. (Sturges et al. 2008, Miller et al. 2019).

2.7.1 Advanced imaging techniques for meningioma grading

It has already been reported that CT and conventional MRI techniques cannot provide a reliable prediction for the tumour grade.

However, a new study from Spille et al. (2020) concluded that using routine preoperative MRI can predict the risk of postoperative recurrence and high-grade histology in patients with intracranial meningiomas. Previous studies have correlated findings in MRI like edema, tumour volume, and disruption of arachnoid layer or lobulated growth with tumour recurrence after surgery and higher grade meningiomas histology. However, the results were not considered reliable because of the small samples of the studies. Therefore, Spille et al. (2020) investigated cases from 565 patients. Their findings were that preoperative MRI features such as peritumoral edema volume, heterogenous contrast enhancement and irregular tumour shape were associated with histology of high grade meningiomas. Furthermore, disruption of the arachnoidal layer, heterogenous contrast enhancement and irregular, mushroom-like tumour shape were correlated with increasing recurrence. Disruption of arachnoidal layer has been proved to be the strongest risk factor of recurrence. Increased risk of recurrence is also correlated with a rising tumour volume or a postoperative residual tumour volume. It has also been noted that variables associated with recurrence are not necessarily congruent with variables for high-grade histology (Spille et al.,2020). In fig. 5 are referred the radiological variables associated with high-grade histology and recurrence.

Radiological variable	High-grade histology	Recurrence
Tumor location: Convexity/falcine vs other (ref.)	(✓)	×
Tumor volume (in ccm)	×	✓
Edema volume (in ccm)	✓	×
Intensity on T2-weighted MRI	×	×
Isointense vs hyperintense (ref.)	×	×
Hypointense vs hyperintense (ref.)	×	×
Arachnoid layer: interrupted vs intact (ref.)	×	✓
Contrast enhancement: heterogeneous vs homogeneous (ref.)	✓	✓
Tumor shape: irregular vs regular (ref.)	✓	✓
Tumor calcifications: present vs absent (ref.)	×	×
Capsular contrast enhancement: present vs absent (ref.)	×	×

Several risk factors were associated with both endpoints. However, tumor volume and, most remarkably, disruption of the arachnoid layer are strongly correlated with recurrence but not with histology; borderline significant correlations in brackets

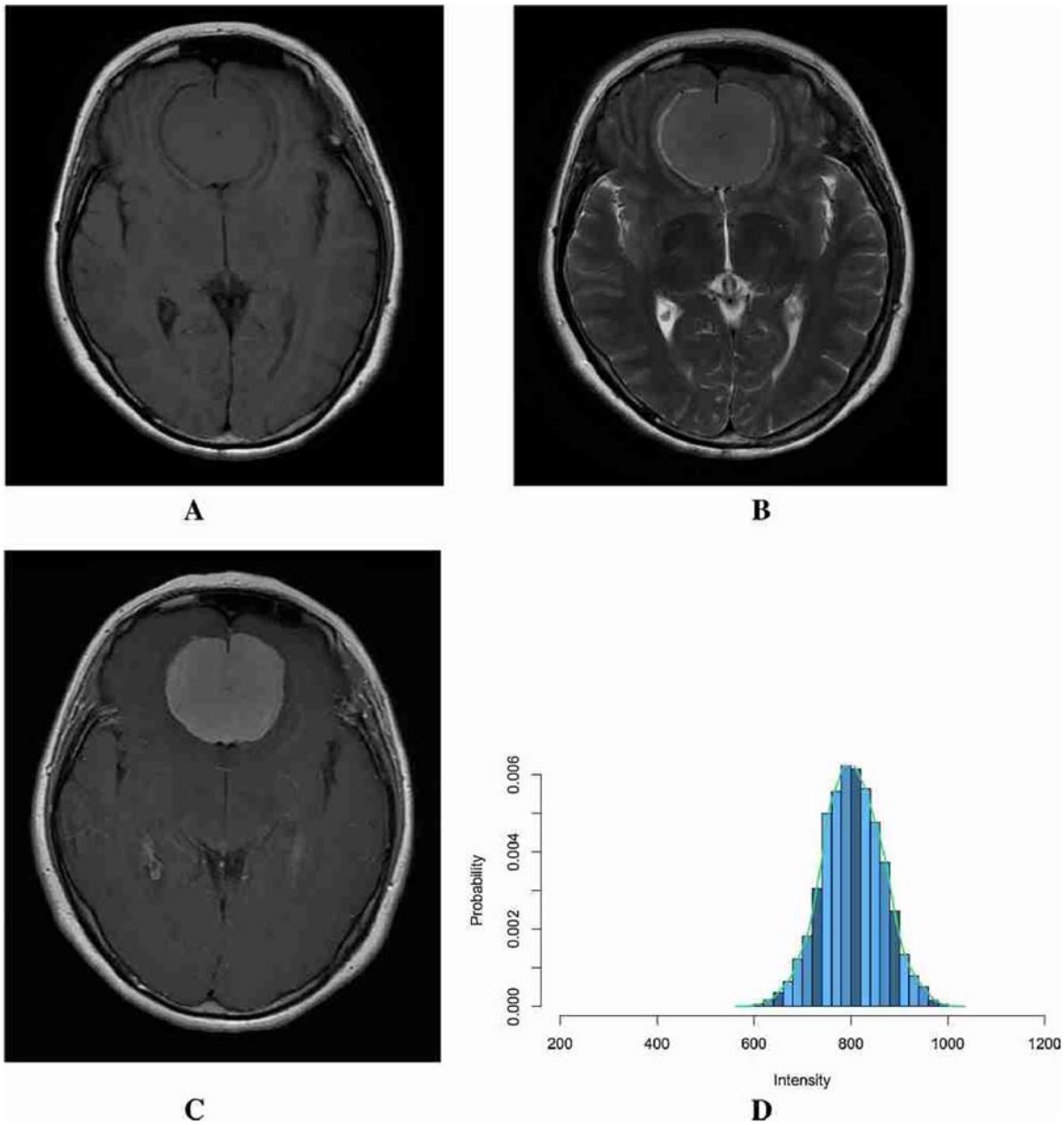
×, no correlation; ✓, significant correlation; ref., reference

Fig. 6 Comparison of variables for high grade histology and recurrence in humans (Spiller et al.,2020)

Moreover, techniques and models which may be a useful tool in the preoperative prediction of meningioma grade have been developed.

Conventional MRI histogram analysis based on 3D tumour measurement

According Yao et al. 2018, T2W1 signal intensity offers a predictive measurement of tumour consistency whereas T1W1 seems to have not any predictive value. The use of naked eye and local ROI (region of interest) selection in tumours limit the objectivity for measurements (Li et al. 2019). Region of interest (ROI) is a tumor lesion area used for quantitative analysis (Zhu et al. 2019). Later, Yusuhn Kang et al. (2011) noticed that ROIs covering the entire volume of the tumour would provide more objective information about tumour characteristics and heterogeneity. Histogram can provide this kind of information using the mean value or the standard deviation. Areas in the tumour with different signal sequence characteristics can be depicted on the histogram, be compared side by side and offer a more clear diagnosis about tumour grade. Then Li et al. (2019) carried out a study trying to explore the application of histogram analysis of conventional MRI based on 3D tumour measurement. They observed significant differences between high and low grade meningiomas regarding features such as histogram volume count, uniformity of T1W1, T2W1 and contrasted T1W1 sequences, range of T1W1 and T2W1, kurtosis, standard deviation, variance, max intensity of T2W1, skewness, mean deviation, minimum intensity, mean value and the 5th, 10th, 25th, 50th, 75th, 90th percentile of contrasted T1W1. The most reliable parameters for the discrimination of high- from low grade meningiomas were volume count and uniformity. Histogram volume count and uniformity changed with the increase of tumour grade on T1W1, T2W2 and contrasted T1W1 images. In detail, volume count increased with increased tumour grade, whereas uniformity tended to decrease with increasing tumour grade. Higher-grade meningiomas presented higher heterogeneity due to larger range and lower uniformity compared to low grade meningiomas. Histograms of higher grade meningiomas are represented in fig. 7. Skewness and kurtosis present the distribution of histogram curves, which are significant indicators for the tumour heterogeneity. Lower grade meningiomas show higher kurtosis and skewness. Histograms of lower grade meningiomas are presented in fig. 6. The optimal MRI histogram model was achieved with a sensitivity of 71.4 % and a specificity of 78.6 % in the test set. Consequently, histogram analysis of conventional MRI based on 3D tumour measurement can be used in the assessment of meningioma grading (Li et al. 2019).


A
B
C
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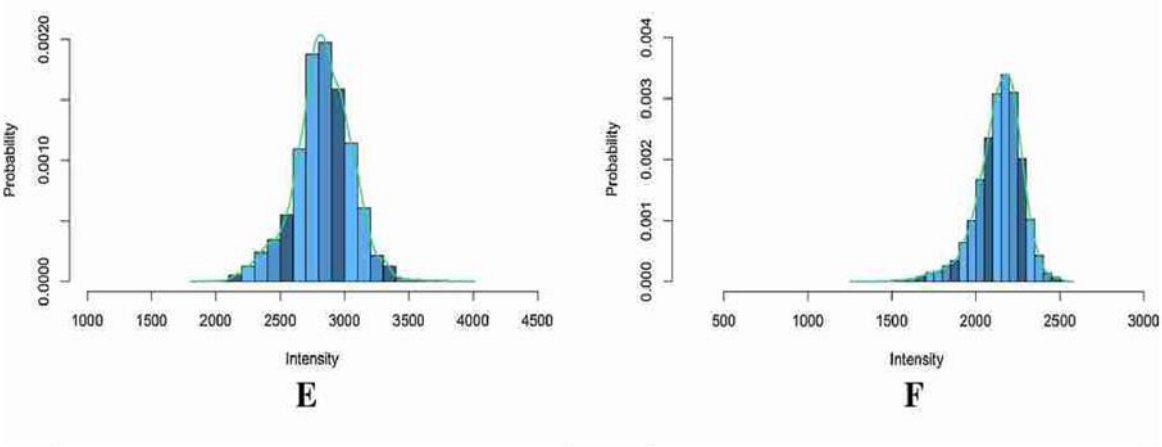
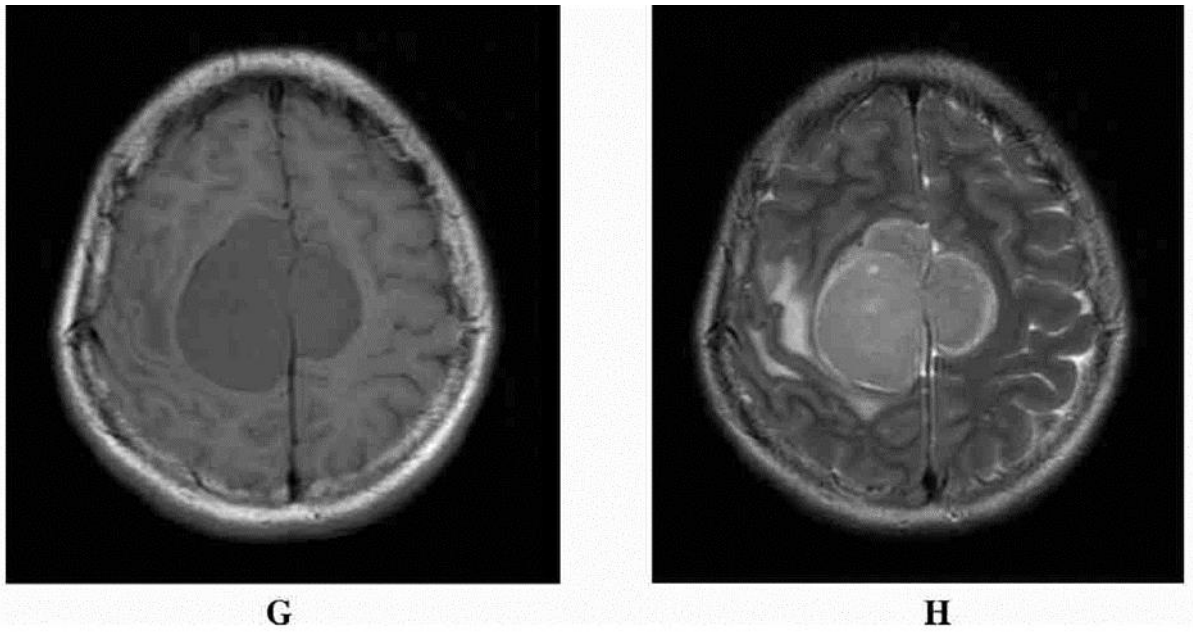


Fig.7 Image A–F are T1WI, T2WI, contrasted T1WI, and their respective histograms of LGM. Tumour is isointense on both T1WI(A) and T2WI(B), there is significantly homogeneous enhancement on contrasted T1WI(C). Histogram fitting curves of LGM are high and sharp (Li et al.,2019)



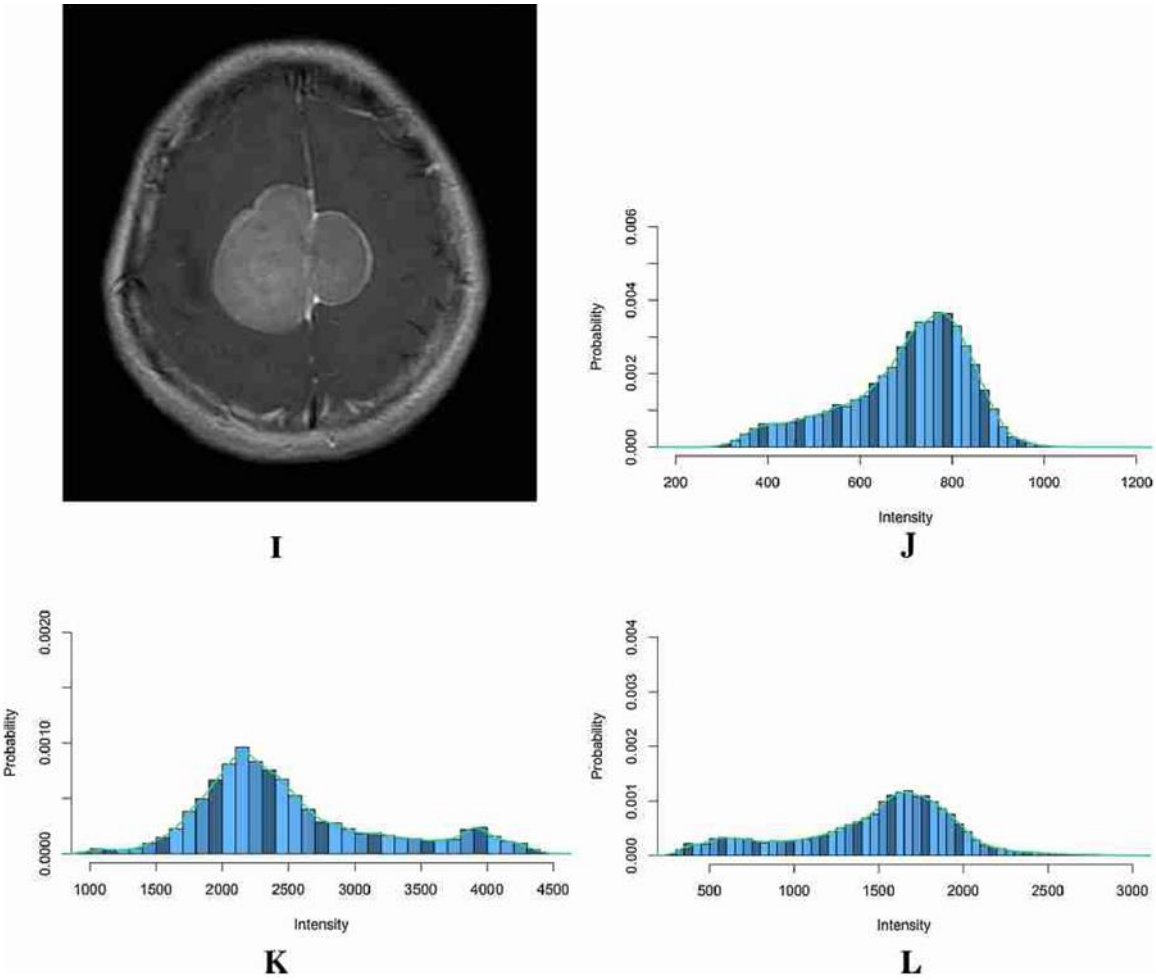


Fig. 8 Image G–L are T1WI, T2WI, contrasted T1WI and their respective histograms of HGM. Tumour is iso- or hypointense on T1WI(G), and iso- or hyperintense on T2WI(H). Tumour shows homogeneous enhancement on contrasted T1WI(I). The histogram fitting curves of HGM are wide and flat compared to LGM, and the morphology of histogram is unstable (i.e. a partial or a double peak). The MRI manifestations of HGM and LGM are similar, but the histogram are different. Therefore, histogram conduces to meningioma classification (Li et al.,2019).

Clinical application of 3D-CISS MRI sequences in dogs

Although, MRI is the preferred imaging technique for the diagnosis of myelopathic changes and spinal cord abnormalities it cannot detect early lesions such as arachnoid adhesions and diverticula. The study of Tauro et al. (2018) investigates the clinical application of 3D-CISS MRI sequences for diagnosis and surgical planning of spinal arachnoid diverticula and adhesions in dogs. The use of Half-Fourier Acquisition Single-shot Turbo Spinecho (HASTE) sequences has been reported in dogs for the identification of arachnoid diverticula. However, severe artefacts and poor resolution of the image has been described and for this reason HASTE has to be evaluated in combination with the standard images of the spinal cord. Newer pulse sequences such as three-dimensional (3D) constructive interference in ready state (CISS) have been proposed as more reliable than HASTE to improve the quality of routine MRI imaging methods. The 3D-CISS sequence is flow-compensated, high contrast and high-spatial resolution and provides a detailed visualization and discrimination of the various neural structures surrounded by cerebrospinal fluid (CFS). This advantage is due to the high-signal-to-noise ratio, high contrast-to-noise ratio and intrinsic sensitivity to motion with minimal signal because of the CFS pulsations.

In human medicine 3D-CISS is used in the investigation of disorders like cystic spinal tumours and their adhesions, for the assessment of cerebellar-pontine angle lesions, inner ear structures, CSF rhinorrhoea, hydrocephalus and neurovascular compression in patients with trigeminal neuralgia and gives the neurosurgeons the possibility to identify accurately obstructions to CSF flow and to plan the surgical intervention (Tauro et al., 2018). Consequently, if further investigation carried out to confirm it, it may be a useful tool for the meningioma diagnosis.

Deep learning radiomics model

Radiomics is a field of medical study with the purpose to extract a large number of quantitative features from medical images using data characterization algorithms. This offers the possibility to uncover tumour characteristics, which are difficult to identify by human vision only. Radiomic features include volume, shape, surface, density, and intensity, texture, location, and relations with the surrounding tissues. Radiopaedia. https://Radiomics | Radiology Reference Article | Radiopaedia.org (Access 10/09/2020) and they have been recognized as effective tools in differentiating tumour grades (Zhu et al. 2019). Conventional radiomic features are based on hand-crafted radiomic (HCR) features and can offer information about tumour shape, intensity and texture information based on imaging. However, intrinsic characteristics may be difficult to define by low-order HCR features. Therefore, high-order features like deep learning radiomics (DLR) features have to be explored in order to improve the prognosis performance of traditional radiomics model. Deep learning methods are composed from “layers”, which are multi -types of self-learning units. It has been proved in the study of Zhu et al. (2019) that DLR models depict the tumour heterogeneity and differentiate meningioma grades better than HCR models. Meningiomas appearing heterogeneity with necrosis and/or hemorrhage mostly belong to higher grade meningiomas. In their study the patients´ tumours were based on post-contrast axial T1 weighted images from which 2048 deep learning features were extracted by the convolutional neural network (fig. 8). An algorithm was used to select features with importance values over 0.001 and a deep radiomic signature was built by an analysis classifier. DLR model had a 0.769 sensitivity and a 0.898 specificity. Calibration curves of DLR model showed good correlation between the prediction probability and outcome of high-grade meningioma. So, a DLR model with good performance for prediction of meningioma grades with a quantization capability better than this of hand-crafted features was developed using routine MRI data (Zhu et al. 2019).

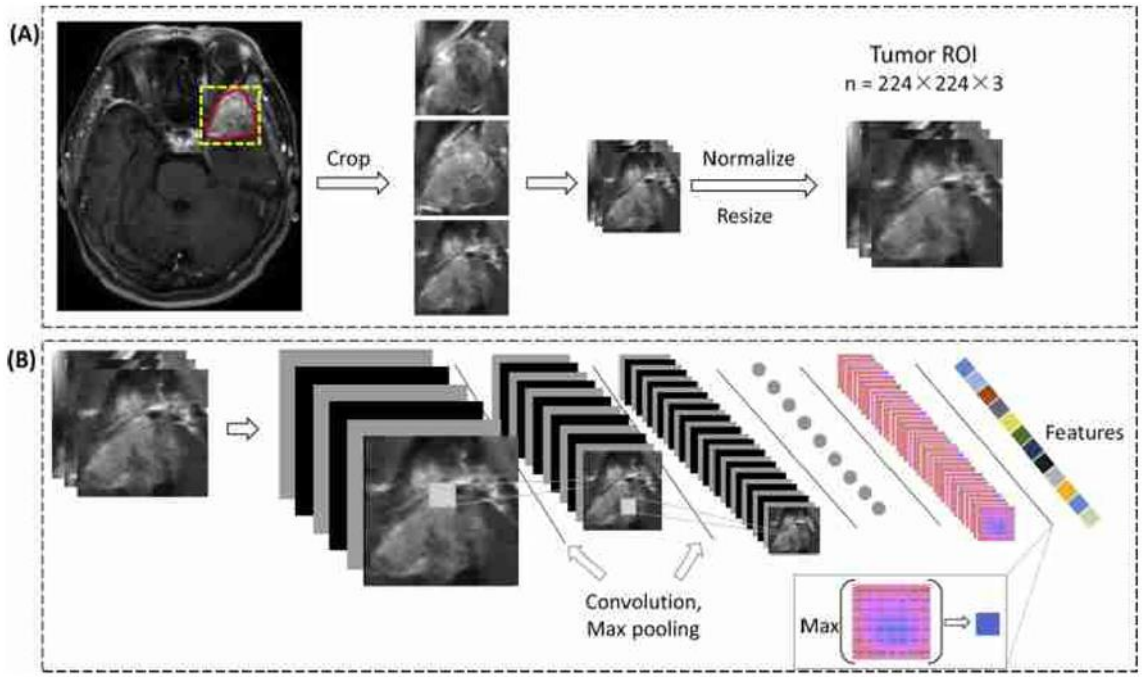


Fig.9 (A) the extraction process of region of interest (ROI) in the tumor (B) deep learning feature extraction process (Zhu et al. 2019)

Texture analysis of magnetic resonance images

In dogs the MRI features do not seem to have any significant correlation with histologic subtype or grade. Texture analysis describes mathematically the relationship between pixel intensity within a given region of interest (ROI) of an image. This technique overcomes the restricted ability of the vision of human eyes to distinguish the various shades of grey and increase the amount of information taken from diagnostic images. In the study of Banzato et al. (2017) texture analysis was used to predict histologic grade of meningioma in dogs. They grouped the tumours histologically into three grades and divided the MRI sequences into a training set (with higher number of atypical anaplastic tumours) and a test set (with higher number of benign tumours). Specialised software was used for the performing of texture analysis on T2 weighted and pre- and postcontrast T1 weighted images. Then a set of 30 texture features providing the highest discriminating ability was selected by the texture analysis software in order to be calculated the most discriminant factor (MDF). The conclusions of the study were that texture analysis of precontrast T1W images offered the best diagnostic accuracy and a sensitivity and specificity of 97.4 % and 95.0 % for discriminating benign from atypical and anaplastic meningiomas. The use of postcontrast T1W and T2W images resulted to lower quality diagnostic performance because the providing information about the structure of the tissue are poor. Texture analysis of MRI scans are useful for the prediction of meningioma grade in dogs but texture analysis cannot be applied immediately as a diagnostic test. Future studies are required in order to evolve the existing model (Banzato et al. 2017).

Thallium-SPECT and FDG-PET

Fluorine-18 fluorodeoxyglucose (FDG) positron emission tomography (PET) and thallium-201 (T1) single photon emission computed tomography (SPECT) are non-invasive imaging methods, which can differentiate preoperative the grades of meningioma with the following procedure. The maximum FDG uptake values of the tumour are measured, are standardized to the whole body (SUVmax) and normalize as grey matter ratio (SUVR-max). The mean and maximum (TURmean and TURmax) T1 uptake ratios of the tumours are measured and normalized as ratios to those of the contralateral normal brain. The differentiation between high and low grade meningiomas comes from the curve analysis of the four indexes and the areas under the curve (AUC) are compared. Then correlation coefficients are calculated between the indexes and Ki 67. The values of the four indexes are significantly higher in high-grade meningioma. The grading capability of FDG-PET and TI-SPECT is comparable to each other. However, FDG-PET examination requires a cyclotron or a local FDG distribution network as well as an expensive PET system itself and not many hospitals have access to an FDG-PET examinaton. On the other hand, TI-SPECT examination is widely available and compared to PET scanners, much more SPECT scanners are distributed all over the world, providing an important tool for meningioma grading (Okuchi et al.

2015).

2.8 Therapy options

2.8.1 Human

Surgical treatment

An acceptable option for asymptomatic tumours is the constant surveillance with routine imaging. The safer treatment option for tumours that grow or cause symptoms is surgical resection if it is possible. The surgical approach to meningioma depends on its neuroanatomic location and may be limited or impossible when venous sinuses, arteries and cranial nerves are involved or there is an extensive involvement of the skull. The measurement of the extent of resection has been the Simpson grade and is defined by the neurosurgeon's assessment and postoperative imaging (Buerki et al. 2018).

Simpson Grade (Buerki et al. 2018)	**Description**
Grade I	Gross total resection of tumour, dural attachment and involved bone
Grade II	Gross total resection of tumour, coagulation of dural attachment
Grade III	Gross total resection of tumour without resection of coagulation of dural and extradural components
Grade IV	Partial resection of tumour
Grade V	Biopsy only

Five-year recurrence rates after gross total resection in grade I meningiomas are 7-23 %, in grade II are 50-55 % and in grade III are 72-78 %. With subtotal resection of the tumour the possibility for recurrence increases (Buerki et al. 2018). For WHO grade tumours that are not completely resected or for tumour grades II and III an adjuvant therapy like radiation is necessary to prevent or delay recurrence (Hanft et al. 2010, Buerki et al. 2018).

Radiation therapy

Radiation therapy has been the treatment of choice for growing tumours that are not surgically approachable and is also used as adjuvant therapy after tumour resection in order to prevent or delay recurrence or to prevent a progression to higher grade meningioma. Both fractionated external beam RT (EBRT) and single-fraction stereotactic radiation (SRS) are used. Single fraction SRS is utilized in cases of meningiomas <30 mm diameter and for tumours which are not attached on sensitive structures like optic chiasm. Multifraction SRS is applied for larger tumours.

Using EBRT, WHO grade I tumours are irradiated at a dose of about 50 Gy and grades II/III at a dose of about 60 Gy daily over 5-6 weeks. Radiation dose is a predictor for outcome, as 42 % of patients who underwent radiation therapy at a dose of 50 Gy or more after surgery had a 5-year cause-specific survival while there were not found any patients surviving 5 years with a dose less than 50 Gy. This shows how significant is the utility of radiation therapy in the post operative treatment of meningioma (Hanft et al. 2010; Buerki et al. 2018). Side effects of radiation therapy may be alopecia, fatigue and acute toxicities. Radiation treatment does not offer a relief of meningioma related neurological symptoms like surgery does (Buerki et al. 2018).

It was proved that patients with WHO Grade III meningioma who underwent near-total resection with EBRT had a survival time of 107 months, whereas patients who underwent gross total resection and EBRT had a survival time of 50 months, suggesting that more aggressive efforts at gross- total resection may be harmful (Forterre et al. 2007, Hanft et al. 2010).

According the Current National Comprehensive Cancer Network (NCCN) for CNS tumours, radiation therapy is recommended for small (<30 mm) asymptomatic meningiomas, if there is a “potential symptom” in cases of grade II and subtotal resected or grade III regardless of resection volume and in grade I subtotal resected tumours. Radiation therapy is applied to large (>30 mm) grade III tumours and is also considered for WHO grade II or incompletely resected WHO grade I. For symptomatic meningiomas radiation therapy is recommended after surgery for any grade III tumours and should be considered for grade II tumours or >30 mm grade I incompletely resected tumors. For patients who cannot be operated or with inaccessible tumours RT alone is also recommended (Buerki et al. 2018). The European association of neuro-oncology guidelines for evaluation and treatment of meningioma are described in fig. 9.

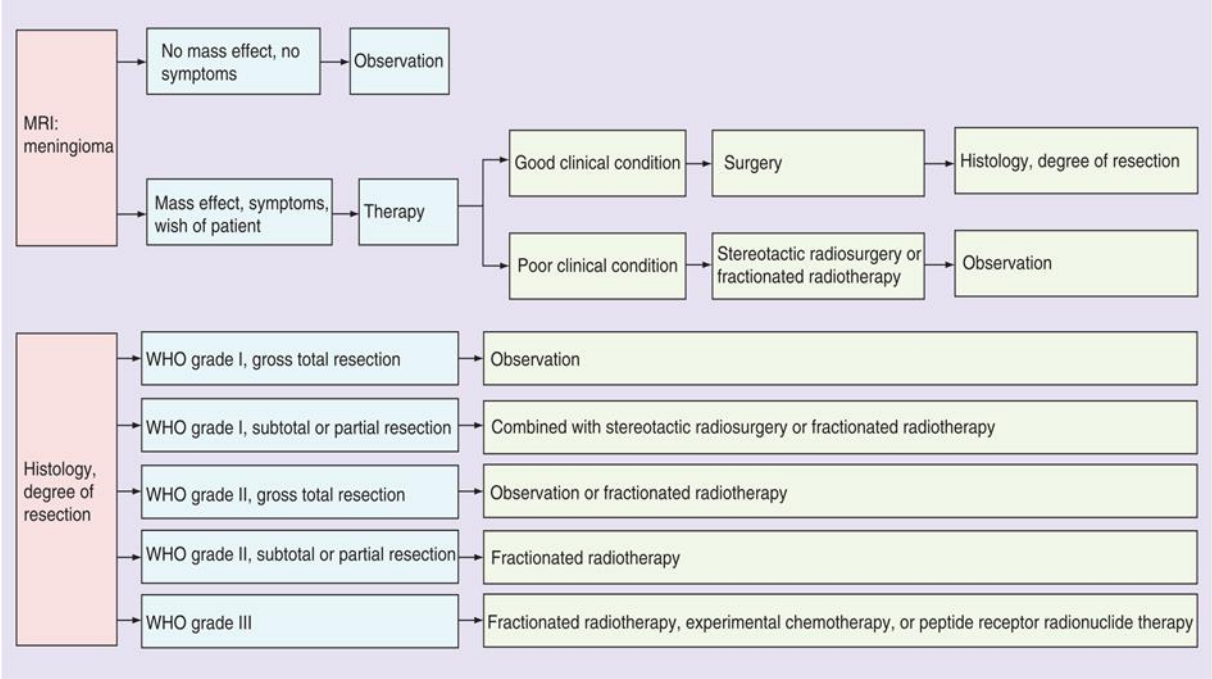


Fig.10 European association of neuro-oncology guidelines for evaluation and treatment of meningioma (Buerki et al. 2018)

Systemic treatments

Systemic treatments are not widely used as an option choice because they are not so effective according to the current clinical data (Hanft et al. 2010, Buerki et al. 2018). Chemotherapy is recommended only in cases of recurrent meningiomas when radiation therapy and further surgical approaches are not possible. The three classes of meningioma systemic therapy recommended by National Comprehensive Cancer Network are Interferon-α (α-IFN), somatostatin receptor agonists and VEGF inhibitors (Buerki et al. 2018). Cytotoxic chemotherapeutic agents like hydroxyurea are not recommended because their effect do not last for a long time in progressive grade I or grade II/III meningioma. It has shown a 6-month progression- free survival (PFS-6) of 10 % and 3 % in two retrospective series (Buerki et al. 2018). According the study of Hanft et al. (2010), hydroxyurea prevented the growth of recurrent malignant meningiomas for 24 months in patients after gross total resection (Hanft et al. 2010). Temozolomide showed little activity in recurrent grade I meningioma with PFS-6 of 0 % in a small series of 16 patients and no efficacy in malignant meningiomas (Hanft et al. 2010, Buerki et al. 2018).

Interferone- α inhibits the growth of meningioma cells and has shown effectivity in patients with recurrent grade I and in higher grade meningioma, by demonstrating PFS-6 of 54 % and 17 % in small studies (Hanft et al. 2010, Buerki et al. 2018).

VEGF inhibitors show more promising results as meningiomas are highly vascularized tumours. Sunitinib, a small molecule included in this group demonstrated a PFS-6 of 42 % in the second phase of clinical trial of patients with grade II/III refractory meningioma. Another molecular inhibitor of VEGF, valatinib showed a PFS-6 of 37.5 % in a study of 22 patients with recurrent high-grade meningioma. The anti-VEGF monoclonal antibody, bevacizumab showed a PFS-6 of 87 %, 77 % and 46 % for recurrent meningioma grade I,II and III respectively in the second phase of a clinical trial. However, the trial is ongoing (Buerki et al. 2018).

Hormone receptors, like the receptors for somatostatin (SSTR2) have been identified in meningiomas but therapy with inhibitors and analogues of hormone receptors did not demonstrate a sufficient efficacy in clinical trials. In detail, in the phase II trial of the somatostatin receptor pasireotide long-acting release in recurrent meningioma without previous surgery or radiation therapy it was shown PFS-6 of 17 % in grade II and III meningiomas and 50 % in grade I meningiomas. During the clinical study of intramuscular octreotide there was not prove of any clinical response for meningiomas grade II and III but it was reported a PFS-6 of 44 %. It has been concluded that somatostatin may have a therapeutic role for grade I recurrent meningiomas but the results of the treatment of recurrent malignant meningiomas are not promising (Hanft et al. 2010, Buerki et al. 2018).

Other clinical trials for the effectiveness of new agents like trabectedin (ET-743) an antineoplastic agent inhibiting transcription and cell cycle progression was investigated in recurrent grade II and III meningiomas but the results have not been published by far. The same thing occurs investigating immunotherapy agents in meningioma such as the inhibitors nivolumab, pembrolizumab or avelumab. There are not yet available results for these studies (Buerki et al. 2018).

2.8.2 Dogs and Cats

Medical treatment

Like in human medicine, the treatment of meningioma combines tumour resection, chemotherapy and radiation therapy. However, surgical intervention and radiation therapy are often not possible options for meningioma treatment due to tumour location, patient age, financial and ethical reasons. So, the medical treatment is used to provide a better quality of life and a prolonged survival time. In dogs and cats the most common medical treatment plan includes the combination of corticosteroids, antiepileptic drugs and/or different chemotherapeutic agents (Motta et al. 2012, Miller et al. 2019).

Corticosteroids reduce the permeability of tumour capillaries and vasogenic oedema and consequently the intracranial pressure (Adamo et al. 2004, Motta et al. 2012, Miller et al. 2019). However, it has been proved that even if edema is not present the corticosteroid therapy alone may reduce the tumour volume (Miller et al. 2019). The palliative corticosteroid therapy offers in dogs a median survival time 2.5 months (range: one day to 13 months) and 3.9 months (range: 2.8 to 6 months) (Adamo et al. 2004).

Antiepileptic drugs should reduce the frequency and the intensity of the seizures caused by meningioma. Popular clinical anticonvulsant monotherapy choices are phenobarbital, levetiracetam and zonisamid but ideal anticonvulsant protocols for the treatment of meningioma associated seizures are currently unknown (Motta et al. 2012; Miller et al. 2019). However, the administration of medical treatment is a palliative therapy and does not consist of a radical solution of the problem (Adamo et al. 2004, Motta et al. 2012).

Chemotherapy

Chemotherapy in dogs has been proved that has a limited efficacy in treating brain tumours like meningiomas (Adamo et al. 2004, Miller et al. 2019). The most commonly used chemotherapeutic drugs are the alkylating agents lomustine (CCNU), carmustine (BCNU) and tenzolomide (TMZ) or the antimetabolite hydroxyurea, which all penetrate the blood-brain barrier (Adamo et al. 2004, Miller et al. 2019). It was proved in a previous study, that dogs treated with lomustine had a median survival time of three months whereas dogs that received palliative therapy had a median survival time of two months. This fact confirms that lomustine treatment does not present survival benefits compared to palliative treatment. In another study dogs treated with lomustine showed a significant median survival time of 4.5 months compared to dogs treated with palliative therapy only that had a median survival time of a month. In general, the effects of lomustine are not so efficient when it is used as a monotherapy (Miller et al. 2019).

Oral hydroxyurea (50 mg/kg three times a week) used as adjuvant therapy after surgical resection of intracranial canine meningioma seems to have promising results (Adamo et al. 2004). Hydroxyurea was administered in cats with multiple meningiomas in the study of Forterre et al. (2007) in order to prevent tumour regrowth. This attempt was based on in vitro studies that showed that the multiplication of feline meningioma cells was slowed or stopped after application of hydroxyurea and this in vitro effect was confirmed (Forterre et al. 2007). Side effects associated with CCNU, TMZ and hydroxyurea occur commonly but they are seldom life threatening (Motta et al. 2012, Miller et al. 2019). Characteristics and adverse effects of chemotherapeutic agents are described in fig. 10.

Characteristics of chemotherapeutic agents used to treat meningiomas.[a]

Chemotherapeutic agent	Characteristics	Adverse effects
Hydroxyurea	It is an antimetabolite that specifically affects the S stage of the cell cycle. It is a drug that inhibits the growth of tumours with low mitotic indices, such as meningiomas (Hoshino et al., 1986)	Toxicity in dogs common but usually not life threatening (Tamura et al., 2007) No adverse affects noted in cats (Forterre et al., 2006, 2007)
Lomustine (1-[2-chloroethyl]-3-cyclohexyl-1-nitrosourea)	Alkylating antineoplastic agent belonging to the nitrosourea group High lipid solubility of lomustine and its metabolites results in wide distribution to tissues and penetration across the blood–brain barrier (Carter and Newman, 1968)	Toxicity in dogs common but is usually not life threatening (Jung et al., 2006a,b) No studies in cats
Nitrosylcobalamin	Vitamin B12 analogue which includes nitric oxide as an axial ligand that uses receptor-mediated cobalamin uptake to target nitrosylcobalamin causing apoptosis of cancer cells (Bauer et al., 2002)	No adverse affects noted in dogs (Bauer et al., 2010) No studies in cats

Fig.11 Characteristics and adverse effects of chemotherapeutic agents (Motta et al. 2012)

Non-steroidal anti-inflammatory drugs (NSAIDs), especially COX-2 inhibitors may play a role in meningioma therapy. In addition to analgesia, NSAIDs may have antitumor, antiedema and immunomodulatory effects in human and dogs (Rossmeisl et al. 2009, Samarini et al. 2018, Miller et al. 2019). VEGF antiogenic agents like bevacizumab or the receptor tyrosine kinase inhibitors mastinib or toceranib phosphate may be useful also in canine meningioma therapy (Miller et al. 2019).

Surgical treatment

The surgical resection of meningiomas offers the quick reduction or elimination of the tumor and consequently beneficial effects like the reduction of intracranial pressure and histopathological diagnosis of the tumor (Miller et al. 2019).

Most surgical studies of canine meningiomas have included in their majority superficially located rostrotentorial tumours. The surgical treatment of basilar, foramen magnum and tentorial meningiomas is more complicated and has not been reported often. When conventional surgery techniques are used the median survival time of dogs is between seven and ten months. However, the use of techniques or technologies that allow removal of infiltrative tumor or intraoperative visualization like cortical resection, use of ultrasonic aspirator or endoscopic assisted resection in canine meningiomas refer a median survival time of 16-70 months, significantly longer compared to that of conventional surgery techniques (Adamo et al. 2004, Miller et al. 2019). For cats with meningioma, the surgical excision is the treatment of choice because tumours are well encapsulated and can be easily removed from brain. The median survival time after surgical resection in cats is 26 months (Adamo et al. 2004, Forterre et al. 2007, Motta et al. 2012). The surgical treatment of multiple meningiomas in cats has also good results and the number of tumors does not seem to affect the prognosis. However, would be necessary more case series to confirm this theory (Forterre et al. 2007).

For the resection of meningiomas in dogs and cats different techniques have been reported. For the complete removal of a rostro-temporal basal meningioma in a cat, a suprazygomatic temporobasal approach has been performed. Feline medial tentorial meningiomas may be successfully removed using a unilateral temporal supracerebellar transtentorial approach. The “trap door” technique has been used for the total resection of feline rostrotentorial meningiomas with dural and bone attachment. In dogs a modified bilateral transfrontal sinus craniotomy seems to be the most appropriate access to the canine olfactory bulbs and frontal lobes (Miller et al. 2019)

Post-surgical complications in dogs have been reported only sporadically and these are intraventricular tension pneumocephalus, cervical subarachnoid pneumorrhachis, aspiration pneumonia, intracranial hemorrhage, transient or permanent neurological disability, electrolyte and osmotic disturbances and thermoregulatory dysfunction. In cats have been reported central blindness, anaemia and acute renal failure (Motta et al. 2012, Miller et al. 2019).

Radiation therapy

Radiation therapy can be applicated alone, in combination with medical treatment or as adjuvant therapy following surgical excision of intracranial meningiomas (Adamo et al. 2004). Radiation therapy alone indicates a median survival time of about 16 months in dogs. Dogs treated with surgery and adjuvant radiation therapy had longer median survival times than dogs treated with surgery alone. Combinations of surgical treatment and radiation therapy have been reported median survival times of 16-30 months in dogs and that makes it the best therapy option (Adamo et al. 2004, Motta et al. 2012, Miller et al. 2019). In cats radiation therapy is recommended only when surgical resection is incomplete or the tumour recurs. Side effects of the radiation therapy like epilation and otitis or conjunctivitis and corneal ulcers when eyes included have also been reported in dogs but they subside within 3-5 weeks after treatment. Reported protocols have included five weekly fractions or 12-16 fractions of radiation given daily or three times a week (Adamo et al., 2004).

Alternative therapy options such as immunotherapy and hormonal therapy requires further investigation because the effects are not clear yet (Adamo et al. 2004).

3.Materials and Methods

3.1 Literature research

In order to answer the research questions a literature review was implemented using RCVS Knowledge as guideline for Evidence based medicine (RCVS Knowledge. https:// EBVM Toolkit - RCVS Knowledge (Access 30.03. 2020)).

Through the 'PICO method' the following keywords were defined:

Patient/Population	Dogs, cats, canine, feline, puppies, humans, (veterinary) patients with intracranial meningioma/brain tumor, intracranial meningioma, brain tumor
Intervention	Grading, histological/histopathological classification
Comparison/Control	WHO-grading system, Immunohistochemistry, Osteopontin, Ki-67, progesterone/estrogen receptors, somatostatin receptors, COX-2, p63
Outcome	Classification, classification system

The database CAB was used, because it has the highest coverage (90.2 %) of veterinary journals, whereas Medline only has 36.5 % (RCVS Knowledge. https:// EBVM Toolkit - RCVS Knowledge (Access 30.03. 2020)).

Starting on 25th February until March on 30th 2020, a search using the combinations of the keywords above resulted in total 1383 articles. 633 articles were found in Pubmed, 461 articles were found in Scopus and 289 articles were found in CAB. After 240 duplicates were removed, 1143 articles were screened. Then 841 articles were excluded because the content was not focused on grading/classification of meningioma or was irrelevant to the topic. 302 articles were screened for eligibility. 256 articles were excluded because their topic was focused on genetics (n=88) and spinal or other forms of meningiomas (n=61), surgical treatment of meningioma (n=44), CNS tumours without/ negligible or irrelevant mention of meningioma (n=37), described radiological findings only (n=16), were not written in English or German (n=10). The procedure is also described in the flow chart below (fig.1).

The criteria for the articles included in the literature review were the following: species included were humans, dogs and cats with the main disease of an intracranial meningioma. The topic was focused on intracranial meningioma grading based on histopathological, pathological, immunohistochemical or radiological criteria. There were also articles with intracranial tumours as topic included but with a clear and detailed reference to meningioma grading such as articles which describe the function of immunohistochemical markers to the grading procedure. The articles are case series, case reports and reviews. The writing language of the papers is English or German (fig.3).

A significant resource used for this study was also the book: Meuten-Tumours in domestic animals (2018), from the library of Veterinary University of Vienna.

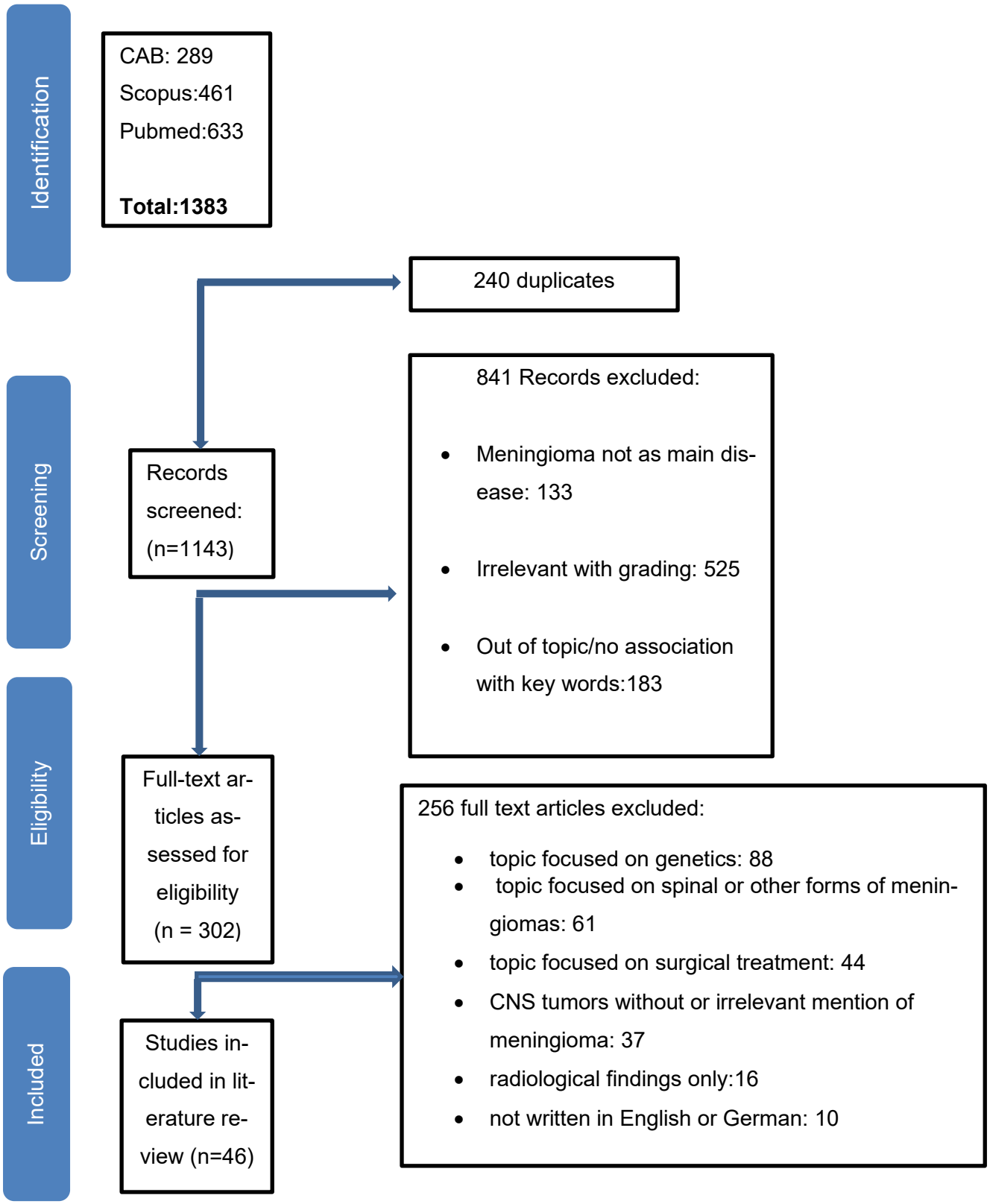


Fig.1. Study flow chart. Exclusion process and identification of articles included for this literature review

In this literature review, there are 46 articles included: 30 case series, 2 case reports and 14 reviews (fig.2)

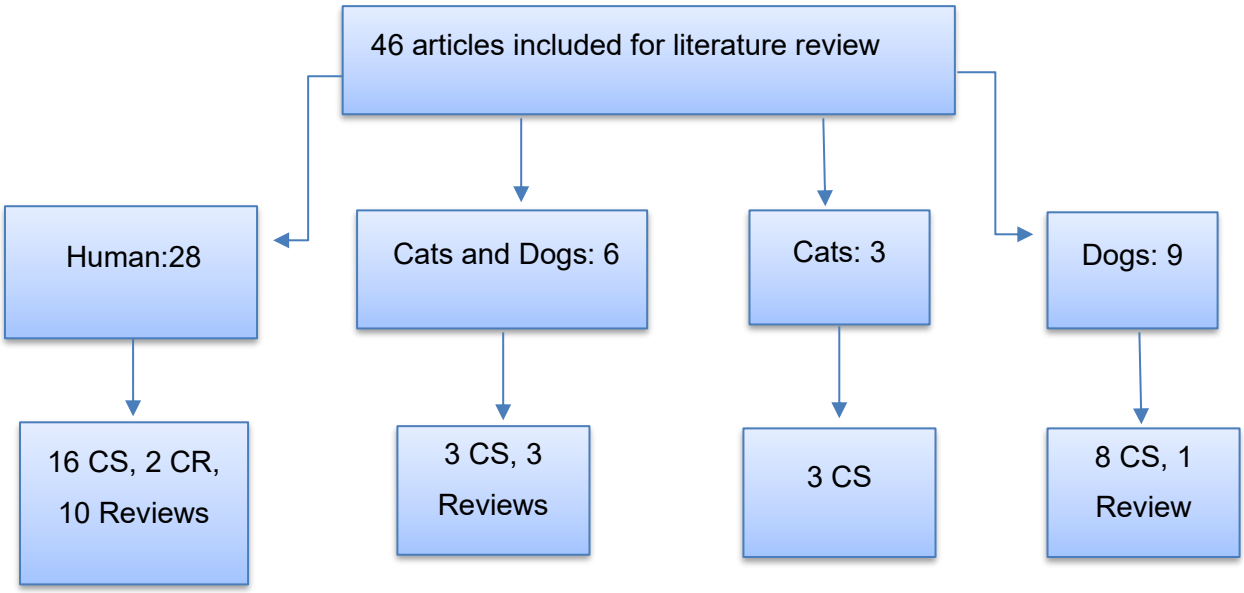


Fig.2: Flow chart representing the number of case series (CS), case reports (CR) and reviews

Inclusion criteria:

- case series, case reports, reviews for humans, dogs, cats
- intracranial meningioma as main disease
- Focus on meningioma grading based on histopathological, pathological, immunohistochemical or radiological classification
- Written in English or German

Fig. 3: Inclusion criteria for studies included in this literature review

In order to evaluate quality and reliability of the evidence a categorisation of the studies was arranged according to the level of evidence (Burns et al. 2011). The articles were placed into IV category as the majority of the articles are case series. Among the 46 articles that were reviewed there were 30 case series, 2 case reports and 14 reviews. The case series described 2060 humans, 613 dogs and 57 cats. The case reports included only two humans. In the part "Results" in Tables 1, 2, 3 are found in detail the kind of articles from the literature search.

Level of evidence for prognostic studies (Burns et al. 2011)

Level of evidence	**Type of evidence**
I	High quality prospective cohort study with adequate power or systematic review of these studies
II	Lesser quality prospective cohort, retrospective cohort study, untreated controls from an RCT, or systematic review of these studies
III	Case control study or systematic review of these studies
IV	Case series
V	Expert opinion; case report or clinical example; or evidence based on physiology, bench research or "first principles"

In order to determine the usefulness of the different immunohistochemical markers, different tables for each marker were outlaid and the results were written down. For the identification of the most common subtype of meningioma in dogs, cats and humans the different species were separated and different tables were outlaid. The same procedure was followed for the description of meningioma grades and subtypes in humans and domestic animals. Tables were also outlaid for the record of alterations in WHO classification and for meningioma grading criteria and frequency.

4. Results

4.1 Literature search

Among the 46 articles that were reviewed there were sixteen case series, two case reports and 10 reviews for humans, three case series and three reviews including dogs and cats, three case series including only cats and eight case series and one review including only dogs. The tables 1, 2 and 3 below present in detail the articles included in our study.

In the case series were included 2060 humans, 613 dogs and 57 cats (Tab.1)

Tab. 1: Literature Search revealing Case Series for Humans, Dogs and Cats

Case series	Number of humans	Number of dogs	Number of cats
Spille et al. 2020	565	0	0
Foiani et al. 2019	0	46	0
Kishimoto et al. 2019	0	186	0
Li et al. 2019	90	0	0
Ma et al. 2019	34	0	0
Zhu et al. 2019	181	0	0
Alamir et al. 2018	15	0	0
Samarini et al. 2018	0	0	15
Tauro et al. 2018	0	19	0
Banzato et al. 2017	0	58	0
McBride et al. 2017	0	0	17
Telugu et al. 2016	224	0	0
Okuchi et al. 2015	67	0	0
Ozmen et al. 2015	0	32	8
Silva et al. 2015	60	0	0
Arikök et al. 2014	84	0	0
Kato et al. 2014	76	0	0
Lee et al. 2014	88	0	0
Mittal et al. 2012	85	0	0
Babu et al. 2011	300	0	0
Combs et al. 2011	62	0	0

Tab. 1: Literature Search revealing Case Series for Humans, Dogs and Cats			
Case series	**Number of humans**	**Number of dogs**	**Number of cats**
Ramos-Vara et al. 2010	0	28	8
Matiasek et al. 2009	0	70	0
Rossmeisl et al. 2009	0	24	0
Shayanfar et al. 2010	78	0	0
Durand et al. 2008	51	0	0
Sturges et al. 2008	0	112	0
Forterre et al. 2007	0	0	4
Montoliu et al. 2006	0	30	0
Adamo et al. 2003	0	8	5
Total	**2060**	**613**	**57**

The publication bibliography of this study includes nine human reviews, one dog review and three reviews including dogs and cats (Tab.2)

Tab. 2: Published Reviews for Humans, Dogs and Cats			
Reviews	**Human**	**Dogs**	**Cats**
Miller et al. 2019		+	
Buerki et al. 2018	+		
Da Silva et al. 2017	+		
Schulz-Schaeffer, 2017	+		
Barresi et al. 2016	+		
Louis et al. 2016	+		
Johnson et al. 2014		+	+
Backer-Grøndahl et al. 2012	+		
Motta et al. 2012		+	+
Tab. 2: Published Reviews for Humans, Dogs and Cats			

Reviews	**Human**	**Dogs**	**Cats**
Hanft et al. 2010	+		
Mawrin et al. 2010	+		
Commins et al. 2007	+		
Adamo et al. 2004		+	+
Chaubal et al. 1994	+		
Total	**10**	**4**	**3**

In the case reports were included only two humans (Tab.3)

Tab. 3: Case Reports published for Humans, Dogs and Cats			
Case reports	**Humans**	**Dogs**	**Cats**
Beutler et al. 2020	1	0	0
Surov et al. 2013	1	0	0
Total	**2**	**0**	**0**

4.2 WHO classification – human

4.2.1 Alterations in WHO grading scheme

The histological classification of human meningiomas is based on a scheme published from the World Health Organisation (WHO). This scheme is not stable but is updated when new criteria are inserted. The alterations from the 1993 edition to the latest 2016 edition are cited in the table 4. In the late 1990s were in use different published grading systems for meningiomas, which suffered from weaknesses and subjectivity of criteria (Commins et al. 2007). One of those grading systems was the 1993 WHO classification, the criteria of which was not that precise. For example, for atypical meningioma grading, “several features” like spontaneous necrosis, increased cellularity, high nuclear/cytosolic ratio, nuclear atypia or frequent mitosis were required by the WHO 1993 classification system (Combs et al. 2011). That version recognised only one histological type of meningioma that was considered a Grade II to III meningioma: the papillary subtype. The other variants were recognised as benign grade I meningioma (Commins et al. 2007). Then the WHO 2000 classification was an improvement over the previous classification with more objective criteria and it led to recognition of a higher proportion of meningiomas as atypical (Backer-Grøndahl et al. 2012). Atypical meningiomas are meningiomas with at least four mitotic figures in ten high power-fields and anaplastic meningiomas have 20 or more mitotic figures in ten high power fields (Combs et al. 2011). Brain invasion was then controversially debated and finally was removed from 2000 classification due to the observation that did not allow prediction of a malignant clinical course (Combs et al. 2011). The 2007 classification introduced brain invasion as criterion for classification of grade II meningiomas. This modification has led to further increase in the incidence of grade II meningiomas (Backer-Grøndahl et al. 2012). The latest WHO classification system for brain tumours is the 2016 version, in which brain invasion can be used alone for the diagnosis of Grade II meningioma (Schulz-Schaeffer. 2017, Louis et al. 2016).

Tab. 4: Alterations in WHO Classification from 1993 to 2016 Version	
WHO Classification edition	**Classification criteria**
1993	Highly subjective classification criteria-mitotic figures were defined as “frequent” for WHO grade II and as “high” for WHO grade III
2000	Objective classification criteria- at least four mitotic figures in ten high power-fields for WHO grade II (atypical) meningiomas and 20 mitotic figures in ten high power fields for WHO grade III (anaplastic) meningiomas
2007	Brain invasion is included as a criterion for atypia (no other differences with 2000 version)
2016	Brain invasion alone is an adequate criterion to diagnose a meningioma as atypical (Grade II)

4.2.2 Meningioma grading criteria according WHO 2016 guidelines and frequency

In the table 5 are cited the criteria for the meningioma grading according the latest update of WHO classification, the WHO 2016 classification. As Grade I are described meningiomas with mitotic rate < 4 per ten high power fields, no brain invasion and nine histologic subtypes (meningothelial, fibrous, transitional, psammomatous, angiomatous, microcystic, secretory, lymphoplasmatic rich and metaplastic). Meningiomas are classified as grade II “atypical” tumors if they have either ≥4 mitoses per ten high power fields, or brain invasion. If none of these features are present, then at least three of the following criteria must be evident for the diagnosis of a grade II meningioma: spontaneous or geographic necrosis, patternless sheets of tumor cells, prominent nucleoli, high cellularity and small cells with scant cytoplasm relative to nuclear size (small cells changes). Grade III anaplastic meningiomas show most or all of the features of grade II atypical meningiomas, but the only required finding is 20 or more mitoses per ten high power fields. Rhabdoid and papillary meningiomas are grade III (Buerki et al. 2018).

Tab. 5: Meningioma Grading Criteria and Frequency		
WHO grade	**Description**	**Frequency**
Grade I	Low mitotic rate, <4 per ten HPFs Absence of brain invasion nine histologic subtypes	80-85 %
Grade II atypical	Mitotic rate 4-19 per ten HPF OR Brain invasion OR ≥3 of five specific histologies: -Spontaneous or geographic necrosis -Patternless sheet-like growth -Prominent nucleoli -High cellularity -Small cells with high n:c ratio	15-20 %
Grade III anaplastic (malignant)	Mitotic rate >20 per ten HPF OR Specific histologies: papillary or rhabdoid	1-2 %

4.2.3 Meningioma grades and subtypes

The following classification on table 6 is according WHO grading scheme 2007 and 2016. The only difference between 2007 and 2016 scheme is that in 2016 brain invasion is alone sufficient as a criterion for the diagnosis of atypical meningioma, WHO Grade II.

Tab.: 6 WHO Classification Human		
WHO -grade	**Histological subtype**	**Articles**
		Barresi et al. 2016 Louis et al. 2016 Backer-Grøndahl et al. 2012 Mittal et al. 2012 Mawrin and Perry, 2010 Hanft et al. 2010 *the subtype brain invasive meningioma exists only in classification of Barresi et al. 2016, Mawrin and Perry.,2010
I	Meningothelial	
	Fibrous (fibroblastic)	
	Transitional (mixed)	
	Psammomatous	
	Angiomatous	
	Microcystic	
	Secretory	
	Lymphoplasmacyte-rich	
	Metaplastic	
II	Chordoid	
	Clear cell	
	Atypical	
	(Brain invasive)	
III	Papillary	
	Rhabdoid	
	Anaplastic	

4.2.4 Most common meningioma subtype in humans

According different studies, cases of meningioma were classified according WHO 2007 grading criteria and statistically the most common subtype in humans was identified.

The total meningioma samples from the referred studies were 907. The most common subtype was the transitional subtype (252/907; 27.8 %) followed by the the atypical subtype (176/907; 19.4 %), while meningothelial and fibroblastic represent 18 % (163/907) and 13 % (118/907) of meningiomas respectively. Consequently, transitional meningioma is the most common subtype appearing in humans (Tab.7).

Tab. 7: Most common Meningioma Subtype in Humans

Article	Meningioma Subtype	Absolute Number of Subtypes	Percentage of Subtypes	Number of intracranial Meningiomas included in the study
Telugu et al. 2016	Meningothelial	53	23.66 %	224
	Transitional	40	17.85 %	
	Psammomatous	32	14.28 %	
	Fibroblastic	28	12.5 %	
	Angiomatous	11	4.91 %	
	Metaplastic	6	2.7 %	
	Other subtypes (secretary,atypical,clear cell,anaplastic, papillary)	54	24.1 %	
Silva et al. 2015	Meningothelial	25	41.5 %	60
	Atypical	11	18.3 %	
	Transitional	9	15 %	
	Fibroblastic	2	3.3 %	

	other subtypes (psammomatous, angiomatous, microcystic, secretory, rhabdoid, anaplastic)	13	21.7 %	
Kato et al. 2014	Atypical	27	35.5 %	76
	Meningothelial	19	25 %	
	Fibroblastic	15	19.7 %	
	Transitional	2	2.6 %	
	other subtypes (angiomatous, microcystic, psammomatous, secretory, anaplastic, unknown)	13	17.1 %	
Backer-Grøndahl et al. 2012	Transitional	78	40 %	196
	Atypical	57	29.1 %	
	Meningothelial	34	17 %	
	Fibroblastic	14	7 %	
	Other subtypes (psammomatous, angiomatous, microcystic, secretory, clear cell, anaplastic)	13	6.6 %	
Babu et al. 2011	Transitional	106	35.3 %	300
	Atypical	70	23.3 %	
	Fibroblastic	47	15.7 %	
	Meningothelial	27	9 %	
	other subtypes (psammomatous, angiomatous,microcystic,secretoy,clear cell,chordoid)	50	16.7 %	

Durand et al. 2008	Transitional	17	33.3 %	51
	Fibroblastic	12	23.5 %	
	Atypical	11	21.6 %	
	Meningothelial	5	9.8 %	
	Microcystic	6	11.8 %	
	Subtype	**Total number**	**Total percentage**	**Total number of intracranial meningiomas included in the study**
	Transitional	252	27.8 %	907
	Atypical	176	19.4 %	
	Meningothelial	163	18 %	
	Fibroblastic	118	13 %	

4.3 Classification in domestic animals

4.3.1 WHO- Classification in dogs and cats

At this moment there is not a grading system, which is applied to domestic animal meningiomas (Motta et al. 2012). The classification of meningioma in dogs and cats is based on the WHO grading scheme for humans. It classifies them into three major groups: (1) Grade I tumours including seven subtypes, Grade II including three subtypes and Grade III including two subtypes (table 8). Subtypes similar to those recognised in human meningioma (microcystic, chordoid, lipomatous and secretory were recently identified in dogs (Motta et al. 2012, Higgins et al. 2018).

Tab. 8: WHO-Classification Dogs and Cats

Meningioma grade	**Histological subtype**	**Articles**
Grade I	Meningothelial	Higgins et al. 2018 Johnson et al. 2014 Motta et al. 2012 Ramos-Vara et al. 2010 Montoliu et al. 2006 Adamo et al. 2004
	Fibrous (fibroblastic)	
	Transitional (mixed)	
	Psammomatous	
	Angiomatous	
	Microcystic	
	Secretory	
Grade II/Atypical	Atypical	
	Chordoid	
	Clear cell	
Grade III/Malignant	Papillary	
	Rhabdoid	

4.3.2 Most common meningioma subtype in dogs

The most common subtypes of meningioma in dogs and cats are identified based on the WHO classification scheme (Tab.9,10).

In summary all 135 intracranial meningiomas participated in those studies more common are meningothelial subtype (38/135; 28,1 %) and transitional (31/135; 23 %). Fibroblastic and anaplastic subtypes follow consisting each of them (15/135; 11.1 %) of the total number. The number of samples of the study of Miller et al. (2019) were not included in the total number of meningiomas because there are not given any absolute numbers. However, their conclusion is that transitional and meningothelial subtype of meningiomas are the most common in dogs as well, representing 20-30 % or more (Tab.9).

Tab. 9: Most common Subtype in Dogs

Article	**Meningioma Subtype**	**Absolute Number of Subtypes**	**Percentage of Subtypes**	**Number of intracranial Meningiomas included in the study**
Foiani et al. 2019	meningothelial	10	21.7 %	46
	Fibroblastic	9	19.6 %	
	Anaplastic	8	17.4 %	
	Papillary	7	15.2 %	
	Transitional	5	10.9 %	
	Atypical	5	10.9 %	
	other subtypes (angiomatous, psammomatous)	2	4.3 %	
Kishimoto et al. 2019	meningothelial	23	57.5 %	40
	Transitional	6	15 %	

	granular cell	4	10 %	
	Fibroblastic	2	5 %	
	other subtypes (psammomatous, papillary)	5	12.5 %	
Ramos-Vara et al. 2010	Transitional	13	54.2 %	24
	Anaplastic	5	20.8 %	
	Fibroblastic	2	8.3 %	
	meningothelial	1	4.2 %	
	other subtypes (myxoid, microcystic)	3	12.5 %	
Montoliu et al. 2006	Transitional	7	28 %	25
	meningothelial	4	16 %	
	psammomatous	3	12 %	
	anaplastic	2	8 %	
	fibroblastic	2	8 %	
	Other subtypes (angiomatous, microcystic, papillary, optic nerve)	7	28 %	
	Meningothelial	38	28.1 %	135
	Transitional	31	23 %	
	Fibroblastic	15	11.1 %	
	Anaplastic	15	11.1 %	

4.3.3 Most common meningioma subtype in cats

From the 29 intracranial meningiomas participating in those studies the most common subtype in cats are transitional meningiomas (16/29; 55.2 %) and psammomatous (5/29; 17.2 %).

Tab. 10: Most common Subtype in Cats

Article	Meningioma Subtype	Absolute Number of Subtypes	Percentage of Subtypes	Number of Meningiomas included in the study
McBride et al. 2017	Transitional	12	70.6 %	17
	Fibroblastic	4	23.5 %	
	Psammomatous	1	5.9 %	
	Meningothelial	0	0 %	
Ramos-Vara et al. 2010	Psammomatous	4	50 %	8
	Transitional	2	25 %	
	Angiomatous	1	12.5 %	
	Fibroblastic	1	12.5 %	
	Meningothelial	0	0 %	
Forterre et al. 2007	Meningothelial	2	50 %	4
	Transitional	2	50 %	
	Transitional	16	55.2 %	29
	Meningothelial	2	6.9 %	
	Psammomatous	5	17.2 %	

4.4 Immunohistochemical markers participating in meningioma classification

4.4.1 Cyclooxygenase 2 (COX-2)

COX-2 is used as immunohistochemical marker in meningioma diagnosis and it is also used to discriminate high from low grade meningiomas. Since the tumorigenic activity of COX-2 has been proved for some types of tumours, including brain tumours several studies have been trying to evaluate the COX-2 expression in human meningioma and how it is correlated with tumour grade (Kato et al. 2014). In human studies it is referred that COX-2 is correlated with WHO grade but there is no significant correlation with patient prognosis after the immunohistochemical analysis of 76 cases of low and high grade human meningiomas. COX-2 is higher expressed in high grade meningiomas (Kato et al., 2014). Lee et al. published in 2014 after the evaluation of 80 human intracranial meningiomas that COX-2 expression is significantly correlated with necrosis and brain or adjacent soft tissue invasion. For canine and feline meningioma there are not so many informations documented about COX-2 expression. The study of Rossmeisl et al. (2009) supports that there is not any significant association between COX-2 immunoreactivity and tumour grade in canine meningioma after the examination of 24 canine meningiomas. In case of feline meningioma there are no differences in COX-2 immunoreactivity patterns between low and high grade meningioma according to Samarini et al. (2018) after the evaluation of COX-2 expression in 15 feline meningiomas. The findings of the previously referred studies are presented in tab. 11.

Tab. 11: COX-2 Expression in Meningioma				
Article	**Cases of intracranial meningioma**	**Human**	**Dog**	**Cat**
Samarini et al. 2018	15			COX-2 expression is not correlated with tumour grade
Kato et al. 2014	76	COX-2 is correlated with WHO grade but there is no significant correlation with patient prognosis		
Lee et al. 2014	80	COX-2 expression is significantly correlated with necrosis and brain or adjacent soft tissue invasion		
Rossmeisl et al. 2009	24		No significant association between COX-2 immunoreactivity and tumour grade	

COX-2 expression is correlated with WHO tumour grade, necrosis and brain invasion in humans but is not significantly correlated with tumour grade in dogs and cats.

4.4.2 Somatostatin receptor 2 (SSTR-2)

On tab.12 is described the association of Somatostatin receptor 2 expression with meningioma. Durand et al. (2008) have examined 51 cases measuring the levels of mRNA coding for SSTR2 and 26 cases measuring the levels of immunohistochemistry in humans. They concluded that SSTR2 was not grade related and could not be used as predictive marker for tumour malignancy but was histotype related, as significantly higher levels of STTR2 were found in the meningothelial subtype. A different tumorigenesis process in this meningioma subtype is possible. Silva et al. (2015) published a case series of 60 human meningioma cases. SSTR-2 expression was detected in 100 % of cases but without correlation with the tumour grade. In addition, there was not found any difference between the expression of SSTR2 and histological subtypes. Due to lacking data for SSTR2 expression in veterinary medicine, Foiani et al. (2019) published a study that investigates the STTR2 expression in canine meningioma at protein and mRNA level in 46 cases. According to the results, SSTR2 protein expression was not correlated with the tumour histological grade or subtype. STTR2 expression was detected in 81 % of cases by immunohistochemistry. Although no significant correlation between STTR2 expression and meningioma subtype could be proved, meningothelial meningiomas showed the most extensive STTR2 immunolabelling area and the highest gene expression level compared with the other histological subtypes. It was also concluded that STTR2 is not an appropriate marker to predict tumour progression in dogs, because no significant differences were detected between tumour grades (Foiani et al.,2019). Unfortunately, no data for SSTR2 expression in feline meningioma was found during literature search.

Tab. 12: Somatostatin Receptor 2 (SSTR2) Expression in Meningioma				
Article	**Cases of intracranial meningioma**	**Human**	**Dog**	**Cat**
Foiani et al.,2019	46		No significant correlation between tumour histological subtype or grade	
Silva et al.,2015	60	No correlation between SSTR2 expression and tumour grade No difference between STTR2 expression and histological subtype		
Durand et al.,2008	77	SSTR2 is not grade-related but is histotype related (higher levels in the meningothelial subtype		

SSTR2 expression in humans is not grade related and it is ambiguous if it is subtype related or not. In dogs there is no evidence for correlation between tumour histological subtype or grade. No studies found for correlation between STTR2 expression and meningiomas in cats.

4.4.3 Progesterone receptors (PRs)

Meningiomas are considered as potentially hormone-sensitive tumours so the expression of progesterone receptors was necessary to be investigated. Shayanfar et al. (2010) after the examination of 78 cases of human meningiomas, supported that WHO grades II and III expressed fewer progesterone receptors than grade I meningiomas. In other words, there was a significant correlation between progesterone receptors and lower grade of meningiomas. Higher progesterone staining was also noted in female than in male patients and in meningothelial tumours more than other subtypes. PRs can also be used as predictive marker, as negative PR meningiomas are very likely to be atypical or malignant and can be potentially considered to be recurrent (Shayanfar et al. 2010). Although the expression of PR receptors is well established in humans, there have been few studies conducted in veterinary medicine. Adamo et al. (2003) examined the progesterone receptors expression in 8 canine and 5 feline intracranial meningiomas. The results from this study were that PR expression in meningiomas of dogs was inversely correlated with aggressive behaviour of this type of tumour. In cats, it was also proved a higher PR expression in benign meningiomas and lower portion in malignant meningiomas. Another interesting finding of this study was the high expression of PRs and absence of estrogen receptors in meninges of clinically normal dogs and cats. According statistical analysis nuclear pleomorphism, necrosis and histologic subtype were significantly correlated with the proportion of PRs in tumour tissue. In other words when the proportion of cells with PRs immunoreactivity was high (>72%) severe necrosis and high nuclear pleomorphism were absent. In cats no correlation between these variables was proved from the study results. It was also marked that meningothelial and transitional meningiomas in dogs and transitional meningiomas in cats presented higher PRs expression than that of granular cells (Adamo et al. 2003). The findings of the previously referred studies are presented in table 13.

Tab. 13: Progesterone Receptor (PR) Expression in Meningioma			
Article	**Cases of intracranial meningioma**	**Human**	**Dogs and Cats**
Shayanfar et al. 2010	78	Progesterone receptors positivity is significantly correlated with lower grade of meningiomas PR positive meningiomas are correlated with low recurrence	
Adamo et al. 2003	8 dogs 5 cats		High proportion of PR receptors in dogs and cats In dogs low PRs expression has a significant correlation with high nuclear pleomorphism, severe necrosis and with histological subtype but this correlation was not found in cats. Inverse association between PRs and cell proliferation PRs expression more common in benign meningiomas

Progesterone receptors positivity in humans is related with lower grade meningiomas. In dogs the proportion of PRs in tumour tissue has a significant correlation with high nuclear pleomorphism, severe necrosis and with histological subtype. In cats there was no correlation found between these variables.

4.4.4 Vascular endothelial factor (VEGF)

A marker that is also associated with meningioma is the vascular endothelial factor. Increased vascular permeability and angiogenesis are essential for tumorigenesis. Vascular endothelial growth factor also known as vascular permeability factor, is a regulator of vascular permeability and angiogenesis (Lee et al., 2014). The study of Lee et al. (2014) used 88 cases of human meningioma to prove an association between tumour grade in meningiomas and VEGF expression. The conclusion was that VEGF protein in humans was correlated with increased tumour grade. The overexpression of VEGF was associated with poor prognosis but a significant association between VEGF expression and tumour recurrence was not observed. VEGF has been correlated with the formation of peritumoral brain oedema. In veterinary medicine the majority of intracranial meningiomas in dogs stain positively for VEGF with diffuse distribution of staining and with moderate or mild intensity (Matiasek et al. 2009). In the study of Matiasek et al (2009) after the immunostaining of 70 intracranial canine meningiomas a VEGF expression was detected in 96 % of cases. However, there was not proved any association between VEGF as a marker of angiogenesis and tumour proliferation. In particular, in meningiomas with high tumour proliferation rate only 25-50 % of cells stained positive for VEGF with mild intensity. As VEGF expression is associated with survival, angiogenesis may be an important predictor of canine meningioma activity (Matiasek et al. 2009). In the literature no studies that are relevant with VEGF expression in feline meningioma were found. The findings of each study are described in table 14.

Tab. 14: Vascular Endothelial Growth Factor Expression in Meningioma				
Article	**Cases of intracranial meningioma**	**Human**	**Dog**	**Cat**
Lee et al. 2014	80	VEGF protein expression is correlated with increased tumour grade and poor prognosis		
Matiasek et al. 2009	70		No association between VEGF expression and tumour proliferation	

VEGF protein expression in humans is correlated with increased tumour grade and poor prognosis. In dogs no association between VEGF expression and tumour proliferation could be proved. No studies for VEGF expression in feline meningiomas could be found.

4.4.5 Ki-67

One of the most widely used immunohistochemical marker is Ki-67, a none-histone protein that is expressed in the proliferative phase of the cell cycle and is available to measure cell proliferation (Babu et al. 2011). There are many studies in human medicine that investigate the expression of Ki-67 and its correlation with tumour grade and recurrence (tab.15). Shayanfar et al. (2010) and Babu et al. (2011) assumed that there was an increasing Ki-67 expression with increase in meningioma grades after the examination of 78 meningioma cases. According to them, a high Ki-67 labelling index indicated a higher grade of meningioma and possible recurrence in 300 human cases. In 2016, Telugu et al. conducted a study in which 100 human meningiomas were immunostained and classified. The results confirmed that there was an increasing mean Ki-67 labelling index with increasing meningioma grades and indicated the biological behaviour of meningioma. Ki-67 was according that study the most important criterion for distinguishing anaplastic meningioma from benign meningioma as brain invasive meningiomas had a higher Ki-67 labelling index than non-invasive meningiomas. Ki-67 can be used as prognostic factor for tumour recurrence according Ma et al. (2019) after the immunostaining of 34 frozen meningiomas. The expression level of Ki-67 was positively correlated with tumour volume, recurrence and mortality in intracranial meningiomas. For this reason, studies in veterinary medicine have been conducted in order to control if the previous findings are valid. Matiasek et al. (2009) studied VEGF and Ki-67 expression in meningioma of 70 dogs. Positive staining for Ki-67 was detected in 91 % of the canine meningiomas. Ki-67 could be used to measure cell proliferation in intracranial meningiomas in dogs, but did not predict outcome like in humans. However, the study of Matiasek et al. (2009) was limited due to the small sample size and maybe that was a reason that there were no information about recurrence of the patients. More studies have to been conducted for more accurate results. During literature search no studies for Ki 67 expression in feline meningioma were found.

Tab. 15: Ki-67 Expression in Meningioma				
Article	**Cases of intracranial meningioma**	**Human**	**Dog**	**Cat**
Ma et al. 2019	34	Ki-67 expression increased in high grade WHO meningiomas and was higher in recurrent meningiomas		
Telugu et al. 2016	100	Ki 67 correlated with increasing histological grade and biological behaviour of meningioma		
Babu et al. 2011	300	Ki 67 correlates with histological grade- indicates higher grade of meningioma and possible recurrence		
Tab. 15: Ki-67 Expression in Meningioma				

Article	Cases of intra-cranial menin-gioma	Human	Dog	Cat
Shayanfar et al. 2010	78	Ki 67 increases with increasing meningioma grades		
Matiasek et al. 2009	70		No association between Ki 67 and meningi-oma recurrence in patients was proved	

Ki 67 expression in humans increases with increased WHO grade and correlates with tumour recurrence. In dogs no association with recurrence has been proven. There are no published data for cats.

In the study of Matiasek et al. (2009) the association between Ki-67 staining variables and survival from the date of onset of clinical signs as well as survival from the date of diagnosis was investigated in 15 dogs. The median survival time from the date of beginning of clinical signs was 749 days while the median survival time from the date of diagnosis was 514 days. However, there was not found any association between Ki-67 and these two variables as the p value was .44 and .74 respectively (tab.16).

Tab.16: Association of Ki-67 and Survival in Dogs				
Article and number of dogs	**Median Survival Time from the date of onset of clinical signs**	**Confidence Interval**	**Median Survival Time from the date of diagnosis**	**Confidence Interval**
Matiasek et. al. 2009 n= 15 dogs	749 days	95 % [CI]: (470-1,486)	514	95 % [CI]: (393-1,409)
	Association between Ki-67 staining variables and survival from the date of onset	**P**	**Association between Ki-67 staining variables and survival from the date of diagnosis**	**P**
	no significant	.44	no significant	.74

There is no significant association between between Ki-67 staining variables and survival from the date of onset or diagnosis.

4.4.6 Osteopontin

Another marker, which is associated with WHO grade and recurrence in meningiomas is osteopontin. Osteopontin is a non-collagenous, protein which regulates osteoblast function during bone formation (Arikök et al. 2014). Arikök et al. published in 2014 a study, in which the osteopontin correlation with WHO grade and recurrence has been investigated. Eighty-four cases of human meningiomas were immunostained and classified. The results were that there was a significant correlation between OPN expression and meningioma grade and recurrence. OPN expression was significantly lower for benign meningiomas and higher for atypical and anaplastic meningiomas and it seemed to be a valuable biomarker for prediction of recurrence of benign meningiomas. In veterinary medicine, there are few studies for the immunohistochemical expression of osteopontin in canine and feline tumours, but not for meningioma. Ozmen et al. (2015) evaluated in 32 dogs and 8 cats 40 different kinds of canine and feline tumours (15 malign mammary tumours, 5 benign mammary tumours, 9 benign soft tissue tumours, 5 malign soft tissue tumours, 2 malign skin tumours, 3 malign bone tumours and 1 hematopoietic tissue tumour). They noticed that OPN immunoreaction was strong in most cells in malignant tumours, while weak and a few immunopositive cells were seen in benign ones. That was the first study that showed the localization of OPN expression in canine and feline tumours by immunohistochemical method. The findings of Arikök et al. 2013 are described in table 17.

Tab. 17: Osteopontin Expression in Meningioma			
Article	**Cases of intracranial meningioma**	**Human**	**Dogs and cats**
Arikök et al. 2013	84	Significant correlation between OPN expression and meningioma grade. OPN expression lower for benign meningiomas Correlation between OPN expression and recurrence	

OPN expression in humans increases in higher-grade meningiomas and is correlated with tumour recurrence in patients. There are no studies for the association between the expression of osteopontin in meningioma in dogs and cats

4.4.7 Vimentin

A protein that is highly associated with meningioma and can be used to distinguish meningioma from other intracranial tumours is vimentin. Meningiomas stain positive for vimentin, while sarcomas for example will stain positive for cytokeratin and negative for vimentin (Commins et al. 2007). Alamir et al. (2018) after the immunostaining of 15 human intracranial meningiomas reported that there were large areas in meningiomas with positive cells for vimentin. They also concluded that vimentin expression was higher in grade II/III than grade I meningiomas. As for veterinary medicine, Montoliu et al. (2006) conducted an immunohistochemical study of 30 cases of canine meningioma. The 30 meningiomas showed strong immunolabelling for vimentin. The two fibrous meningiomas were weakly positive for vimentin. In meningothelial, transitional and papillary meningiomas vimentin expression was higher in concentric whorls of cells than in bundles of spindle-shaped cells. In papillary and angiomatous meningiomas, cells in the perivascular areas showed particularly intense vimentin immunolabelling. According to Ramos-Vara et al. (2010), vimentin was expressed in all types of meningiomas. All tumour samples in the study (28 canine and 8 feline meningiomas) were positive for vimentin. The findings of each study are represented in table 18.

Tab. 18: Vimentin Expression in Meningioma				
Article	**Cases of intracranial meningioma**	**Human**	**Dog**	**Cat**
Alamir et al. 2018	15	Vimentin positive in large areas of meningiomas higher number of cells immunostained with vimentin in grade II and III than grade I meningiomas		
Ramos-Vara et al. 2010	28 dogs 8 cats		Vimentin expressed in all cases	

Article	**Cases of intracranial meningioma**	**Human**	**Dog**	**Cat**
Montoliu et al. 2006	25		Vimentin expression strong in all meningiomas (except two fibrous) In meningothelial, transitional and papillary meningiomas vimentin expression is higher in concentric whorls of cells than in bundles of spindle-shaped cells In papillary and angiomatous meningiomas, cells in the perivascular areas showed particularly intense vimentin immunolabelling	

Vimentin is highly expressed in human, canine and feline meningiomas. In humans is also correlated with higher tumour grade. In dogs there have been noticed differences in different subtypes of meningioma. In feline meningiomas vimentin is expressed in all cases.

4.4.8 CD34

More specialised markers like CD34 are used in immunohistochemistry to distinguish meningioma from other intracranial tumours like hemangiopericytoma or schwannoma. CD34 antibody labels strongly the external limiting layer of the leptomeninges and scattered cells in the deeper layers. (Ramos-Vara et al., 2010). Ramos-Vara et al. (2010) published a study in order to interpret the immunohistochemical detection of CD34 and other novel markers in 28 canine and 8 feline meningiomas. They mark that CD34 is strongly expressed in all types of canine and feline meningioma in a percentage of 94 %. Based on this result they recommend CD34 as a basic immunohistochemical marker, which may be used to distinguish meningiomas from peripheral nerve sheath tumours. On the other hand, the expression of CD34 in human meningiomas varies from 0 % to 40 % and is typically observed in the fibroblastic type only (tab.19). In the study of Chaubal et al. (1994) is marked that CD34 is expressed in 16 of 103 meningiomas and especially fibroblastic meningiomas show strong immunoreactivity for CD34. On the contrary, all cases of meningeal hemangiopericytoma show increased expression of CD34. Traumatic neuromas, show increased expression of CD34 as well, while schwannomas are stained negative for CD34 (Chaubal et al. 1994).

Tab.19: CD34 Expression			
Article	**Cases of intracranial meningioma**	**Human**	**Dogs and Cats**
Ramos-Vara et al. 2010	28 dogs 8 cats	CD34 expression is observed in the fibroplastic type of meningioma	CD34 expression in all types
Chaubal et al. 1994	103	16 from 103 meningiomas show intense staining for CD34 in more than 75% of tumour cells (fibroblastic, transitional and meningothelial subtype). Fibroblastic meningiomas show strong immunoreactivity for CD34	

CD34 expression in humans is observed in the fibroplastic type of meningioma while is expressed in all types in dogs and cats.

4.4.9 p63

Mittal et al. (2012) supported that the prediction of tumour behaviour based exclusively on morphological features remains difficult. That is why several immunohistochemical markers should be evaluated. They came up with a study that examined the expression of p63 protein in 85 cases of human meningiomas. The results were that although there was a considerable number of grade I meningiomas that expressed p63, the expression was higher in higher grade of meningiomas. P63 cannot be considered as sole marker in discriminating benign from malignant meningioma (Mittal et al.,

2012). Unfortunately, there were not found any studies about p63 expression in canine and feline meningioma (tab.20).

Tab. 20: P63 Expression in Meningioma

Article	**Cases of intracranial meningioma**	**Human**	**Dog**	**Cat**
Mittal et al. 2012	85	P63 expression is associated more with higher grade of meningiomas		

In humans p63 expression is associated more with higher grade of meningiomas but it cannot be used to discriminate benign from malignant meningiomas. Results relevant with p63 expression in meningiomas in dogs and cats have not been found.

In table 21 is summarized which markers are evaluated as useful for meningioma grading in the different species according our study.

Tab.21 Markers evaluated as useful for Grading of Meningiomas			
Marker	**Humans**	**Dogs**	**Cats**
COX-2	+	-	-
SSTR-2	-	-	no studies
PRs	+	can be used together with other markers	can be used together with other markers
VEGF	+	-	no studies
Ki-67	+	+	no studies
Osteopontin	+	no studies	no studies
Vimentin	can be used together with other markers	-	-
CD34	-	-	-
p63	Can be used together with other markers	no studies	no studies

(+):useful for grading, (-): useless for grading

For humans COX-2, Ki-67 and progesterone receptors are useful markers for meningioma grading. Osteopontin and VEGF can be also utilized for meningioma grading in humans, while vimentin and p63 can be used together with other markers and not as sole markers. For SSTR2 is not proved any association with meningioma grade. For canine meningioma are useful for grading Ki-67 and progesterone receptors. A combination of both markers would be ideal because PRs expression is also high in the meninges of clinically normal dogs and cats. Between COX-2, SSTR2, VEGF, vimentin expression and tumour grade is not found any significant association. For feline meningiomas there are not many studies by far. Progesterone receptors can be used for grading of feline meningioma as PRs expression is higher in benign meningiomas but it might not be as reliable as in dogs because there is not found any correlation between low progesterone receptors expression and high nuclear pleomorphism and severe necrosis. CD34 cannot be used as a marker for meningioma grading in humans, dogs and cats but it can be used for meningioma diagnosis especially for fibroblastic subtype in humans.

5. Discussion

5.1 Classification in domestic animals is based on WHO classification scheme for humans

Our hypothesis that meningioma grading has been established in dogs based on the WHO classification scheme for humans is confirmed.

According the literature search results, the classification of meningioma in dogs and cats follows the WHO grading scheme for humans, as there is not any available grading system applied to domestic animals. WHO classifies meningioma into three major groups:

1. Grade I meningiomas are divided into the following subtypes: meningothelial meningioma, fibrous (fibroblastic) meningioma, transitional (mixed) meningioma, psammomatous meningioma, angiomatous meningioma, microcystic meningioma, secretory meningioma.

2. Grad II/ Atypical meningiomas are divided into atypical meningioma, chordoid meningioma and clear cell meningioma.

3. Grad III/ Malignant meningiomas include papillary and rhabdoid meningioma.

Human and canine meningiomas have common characteristics and there have been attempts to improve the domestic animal´s classification scheme based on significant pathological, immunological, molecular and MRI similarities.

Like in humans, it is reported that a mitotic index of four or more mitoses per ten HPF and brain invasion are sufficient criteria for the identification of grade II meningiomas. Furthermore, patternless sheets alone could be assumed as a criterion to attribute a grade II to canine meningiomas. In cats meningiomas of grade III are not detected and this fact confirms that the behaviour of feline meningioma is less aggressive. Consequently, no grading system can be applied to feline meningioma.

The prevalence of canine atypical meningioma is much higher than in humans whereas benign meningiomas in dogs occur less frequently (Motta et al. 2012). Moreover, meningiomas arising from the optic nerve should be included due to their distinct morphological features as a separate type in the meningioma classification of domestic animals (Montoliu et al. 2006)

Articles included in the present study show that the most common meningioma subtypes in dogs are the meningothelial subtype and transitional subtype without significant difference, whereas in cats the most common subtype is the transitional one followed by meningothelial subtype. According to the results of our study, the most common subtype in humans is the meningothelial subtype followed by the atypical subtype. However, meningothelial and transitional subtypes are the most frequent subtypes of grade I.

Metastases are neither common in domestic animals nor in humans. Occasionally metastases have been reported from intracranial meningioma into the lungs and/or heart in dogs while in cats metastases in kidneys and uterus have been reported (Motta et al. 2012).

The application of human WHO grading system in domestic animals offers a range of morphological criteria and histological subtypes in order to assign the histological grade. This helps to reduce any subjective interpretation in evaluating the malignancy potential of meningioma and supports a long-term prognosis based on clinical data.

However, an improved classification scheme and grading relevant to the biological behaviour of meningiomas could be a benefit for the veterinary oncology (Motta et al. 2012).

5.2 Markers as a tool for meningioma grading

According to our hypothesis biological markers like COX-2, VEGF, progesterone receptors, Ki 67, Osteopontin and STTR2 can be used for the classification and behaviour of meningioma. Depending on the immunohistochemical markers and stainings the usefulness in the different species is discussed.

Cyclooxygenase 2 (COX-2)

According to the literature the COX-2 expression in human meningiomas is associated with high grade meningiomas. There is a significant correlation with necrosis and invasion into the surrounding tissues (Kato et al. 2014, Samarini et al. 2018). It has not been proved if there is significant correlation between COX-2 expression level and patient prognosis (Kato et al. 2014). However, when COX-2 is expressed in breast tumours it is a negative prognostic indicator for women (Samarini et al. 2018). There is a clinical benefit from the usage of NSAIDs for patients with malignant meningiomas due to inhibition of the positive feedback loop of COX-2 expression among the tumour and inflammatory cells in the stroma. It is also reported that it carries a favourable prognosis for patients with colon cancer after the administration of NSAIDs including aspirin (Kato et al. 2014). COX-2 inhibitors decrease meningioma growth with evidence of decreased microvascular density, increased apoptosis and decreased COX-2 expression (Lee et al. 2014). These results indicate NSAIDs administration in patients with higher grade meningiomas in combination with chemotherapy or radiotherapy (Kato et al. 2014).
In canine meningioma it is assumed that COX-2 participates in the development of choroid plexus and maintenance of the blood-cerebrospinal fluid barrier. There is no proof between COX-2 immunoreactivity and tumour grade in dogs. However, COX-2 is expressed in the majority of canine meningiomas. It is common in canine intracranial meningiomas and in many constitutive tissues of the normal canine brain. In other words, dogs can be a good animal model for studies of the functions of COX-2 within CNS and effects of COX-2 inhibition in the treatment of intracranial meningioma. COX-2 selective NSAIDs are not expensive and generally safe and easy to administrate (Rossmeisl et al. 2009).

In feline meningioma the COX-2 immunoreactivity is not associated with tumour grade as low and high grade meningiomas do not present significant differences in COX-2 expression. COX-2 inhibitors can also be used as adjuvant treatment of feline meningioma because they seem to have antineoplastic effects on several tumour cell lines in vitro (Samarini et al. 2018)

COX-2 can be used as marker for prediction of tumour grade in human meningioma but not for canine and feline meningioma.

Somatostatin Receptor 2 (SSTR2)

According our results, SSTR-2 expression is associated neither with tumour grade nor with tumour subtype in humans and SSTR-2 expression is not considered as a predictor for malignancy (Silva et al. 2015). On the other hand, Durand et al. (2008) support that SSTR-2 is not grade related but is related with the histotype as it is expressed much more in meningothelial subtype. This fact indicates that a different tumour process of that subtype is possible. The high SSTR2 expression noticed in meningothelial meningiomas might be useful in clinical set ups. The SSTR2 receptors can be detected and visualized by labelled somatostatin analogues such as octreotide. Moreover, a targeted chemotherapy for recurrent meningiomas or non-surgical tumours would be indicated for meningothelial meningiomas, which present high levels of STTR2 expression (Durand et al. 2008). Long-acting SST analogues such as octreotide, bind with high affinity to SSTR2. Somatostatin receptors present antitumour activity by blocking cell division, including apoptosis, inhibiting growth factor and angiogenesis. Somatostatin analogues are recommended from the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology for human (Foiani et al. 2019).

In canine meningioma there is not any correlation proved between STTR2 expression and tumour grade or subtype. However, SSTR2 is detected in 81 % of canine meningiomas and therefore SSTR2 targeted therapy may be efficacious for dogs with meningioma in inappropriate areas for resection or post-radiation recurrence or when surgical and radiation therapies are refused by the owners or animals of advanced age or poor condition (Foiani et al. 2019).

The plan of a systemic therapy with somatostatin analogues demands previous assessment of SSTR2 expression by the individual tumour. This is possible in humans with SSTR2 scintigraphy in vivo, an early diagnostic tool recommended also in veterinary medicine (Foiani et al. 2019).

SSTR2 cannot be used as predictive tool for meningioma grading and biological behaviour in humans and dogs. It is possible that can be used to identify tumour subtype in humans but more studies have to be conducted for a definitive result. Unfortunately, there are not studies in the literature for the expression of SSTR2 in feline meningioma and we recommend further studies to investigate this relation.

Progesterone receptors (PRs)

In humans, WHO grade II and III meningiomas have significantly fewer progesterone receptors than grade I meningioma. In other words, progesterone receptor positivity is significantly correlated with a lower grade of meningiomas. In regard to the biological behaviour of meningioma, PRs positive meningiomas are correlated with low recurrence, while meningiomas which are PRs negative are biologically more aggressive and especially malignant meningiomas may not present PRs at all. Clinically, cell proliferation indices like Ki-67 and hormone receptor status may be used as tool in grading of meningioma and prediction of recurrence. Meningiomas presenting higher proliferation index and negative PR are possibly grade II or III and may considered to be recurrent (Shayanfar et al. 2010). The identification of progesterone receptors in the meningeal neoplastic cells evokes that meningioma growth is an expression of hormone stimulation. This is confirmed from the fact that pregnant women show predisposition for the meningioma occurrence or recurrence and the female to male ratio is about 2:1 (Motta et al. 2012).

In canine meningiomas a high proportion of PR receptors is expressed. Low PRs expression is significantly correlated with high nuclear pleomorphism, severe necrosis and with histological subtype (meningothelial and transitional meningiomas present greater PR expression than granular cell meningiomas). No correlation between PRs and age, sex or tumour location was found. Furthermore, the inverse association noticed between PRs and cell proliferation indicates a relation between the proportion of PRs and tumour aggressive behaviour like in humans (Adamo et al. 2003).

In feline meningioma PR receptors are expressed in high levels as well. However, there is no association between the proportion of PR positive cells in tumour with high nuclear pleomorphism, severe necrosis and histological subtype like in dogs. Correlation between sex and progesterone expression is not found, too (Adamo et al. 2003).

In human with meningiomas hormonal treatment has been used with some success. Nevertheless, in veterinary practice this approach has not been investigated sufficiently. In some animal studies, the treatment with antiprogesterone agents has inhibited meningioma growth, but the efficacy of this kind of treatment in cases of dogs and cats with unresectable or recurrent meningiomas warrants investigation. (Adamo et al. 2003, Commins et al. 2007).

Consequently, PR expression in humans and dogs may be a reliable factor to distinguish malignant from benign meningiomas and predict recurrence rate.

VEGF

According to our results, VEGF protein expression is correlated with tumour grade in humans. VEGF has an important role in the neovascularization of human meningiomas. The frequently presented intratumoral haemorrhages, may be seen as haemosiderin depositions and are associated with more aggressive and recurrent meningiomas. However, neither the haemosiderin deposits nor high vascularization is not correlated to atypical features (Backer-Grøndahl et al. 2012). VEGF is also a critical factor in the formation of peritumoral brain edema. In the clinical setting VEGF can be used as a prognostic marker for meningiomas as overexpression of VEGF is correlated with poor prognosis of meningiomas in humans (Lee et al. 2014). If VEGF is associated with recurrence is ambiguous. In the study of Lee et al. (2014), no significant association between VEGF expression status and tumour recurrence is observed. On the other hand, according Motta et al. (2012), VEGF overexpression is now considered a marker for recurrence for meningiomas after surgery and potential malignancy. Other studies are needed in order to come to a definitive conclusion.

VEGF has a significant role in the regulation of angiogenesis in tumours and consequently is expressed in intracranial canine meningiomas in a great percentage. VEGF in dogs is not associated with high proliferation like in humans (Matiasek et al. 2009). Moreover, it is not correlated to peritumoural edema but it is related to tumour malignancy and poorer prognosis (Miller et al. 2019). VEGF overexpression is a prognostic factor for survival in dogs, as it is bound with a shorter survival rate even in benign meningiomas (Sturges et al. 2008, Matiasek et al. 2009, Motta et al. 2012).

For feline meningiomas no studies have been found and consequently arise the necessity for more investigation.

So, in practice VEGF is useful as a prognostic factor for human meningioma, as a factor for grading and maybe for recurrence. In dogs, VEGF cannot be used for grading but it can be used for the prognosis of survival rate.

It is also suggested that VEGF is a potential target for antiangiogenic therapy in all WHO grades of meningiomas (Motta et al. 2012).

Ki-67

Ki-67 is increased in higher grade meningiomas in humans and is associated positively with tumour volume and aggressive biological behaviour (Telugu et al. 2016, Ma et al. 2019). Ki-67 is associated with the proliferative activity of meningioma and is considered the most important criterion for distinguishing anaplastic meningiomas from the benign ones. In addition to this, brain invasive meningioma presents higher Ki-67 labelling index than non-invasive meningioma (Telugu et al.2016). Ki-67 in humans is a good marker of proliferation potential and grading as the Ki-67 labelling index increases with grade of meningiomas (Babu et al. 2007). As for recurrence, in some studies the mean Ki-67 labelling index in recurrent tumours is higher than the respective of the non- recurrent ones (Babu et al. 2007, Matiasek et al. 2009). However, it is not completely reliable for predicting recurrence, because different techniques and interpretation can result in different cut-offs from one laboratory to another (Arikök et al. 2014).

Ki-67 in canine meningiomas can be used to document cell proliferation in intracranial meningiomas in dogs but does not predict outcome for biological behaviour and recurrence. However, this statement may not be so reliable because it was concluded from a study with a small sample size (Matiasek et al. 2009). Moreover, no correlation between Ki 67 expression and survival in dogs has been found (Matiasek et al. 2009, Motta et al. 2012).

Ki- 67 can be used for the prediction of meningioma grade and possibly for recurrence in humans after studies establishing cut off values for Ki-67. For canine meningioma Ki-67 may be helpful to identify tumour proliferation but cannot give information about biological behaviour and prognosis of meningioma according the current studies and new studies have to be conducted.

Studies to investigate Ki-67 expression in feline meningioma have to be carried out.

Osteopontin

There is a significant correlation between OPN expression and meningioma grade in humans as OPN expression is significant lower for grade I meningiomas than for grade II and III meningiomas. Moreover, a good association between OPN expression and recurrence is observed. On the contrary, with Ki-67, OPN can be more reliable as marker for recurrence as the same cut-off values were obtained from different studies for recurrent benign meningiomas.

Osteopontin is involved in tumour progression, by regulation of apoptosis, proliferation, adhesion, migration, invasion, metastasis and angiogenesis. In prostate tissue for example, OPN levels are elevated in carcinoma compared with normal and benign prostatic hyperplasia tissue. OPN is associated with recurrence of prostate cancer. In patients with high grade glioblastoma OPN expression is elevated in comparison to patients with low grade glioblastoma (Arikök et al. 2014). OPN overexpression in humans is also related with breast cancer progression and metastasis (Ozmen et al. 2015). This fact indicates that osteopontin may be a marker for grading in several types of tumours and consequently for malignancy.

For feline and canine meningioma there are not any studies, which investigate OPN expression in meningioma. There are not many studies investigating OPN expression in other types of tumours as well. There is only the study of Ozmen et al. (2015) which supports that OPN expression by immunohistochemical methods is increased in malignant tumours in comparison with benign tumours especially in malignant mammary tumours. Moreover, OPN can be distributed heterogeneously especially more in areas composed with highly proliferated cells. OPN is probably more than just a marker of malignancy, because this protein may play a functional role in malign-gene expression and cancer cell behaviour in cats and dogs. Anti-OPN treatment may be used for cancer in humans and animals. There is strong association between OPN expression and lower mean survival time for patients.

Consequently, OPN can be used in practice for meningioma grading, recurrence and prognosis in human meningioma and other types of tumours. In canine and feline tumours OPN can be used as marker for grading and prognosis but there are not exclusive studies for meningioma. It may be that OPN expression in meningioma is analog to the human one but studies must be carried out to confirm it.

P63

P63 expression in humans is associated more with higher grade of meningiomas. It has an important role in tumourgenesis in various tumours such as squamous cell carcinomas of the oral cavity head and neck, adenoid cystic carcinomas of the salivary gland, Merkel cell carcinoma and cervical dysplasia. High expression of p63 is associated with aggressive phenotype, poor prognosis and lower survival rates. P63 is considered as a biomarker in separating benign from atypical and malignant meningiomas. There is a difference in p- value, which is significant when meningiomas grade I are compared with higher grade meningiomas and when meningiomas grade I are compared separately with atypical and anaplastic tumours. Then is the p value non-significant. Therefore, p63 expression cannot be considered as a sole immunohistochemical marker in discriminating benign from malignant meningiomas despite the fact that is associated more with higher grade of meningiomas. The expression in feline and canine meningiomas warrants investigation as there are not studies found in the literature (Mittal et al. 2012).

Vimentin and CD34

Vimentin is a commonly expressed immunohistochemical marker in meningioma. It is a marker of mesenchymal cells. Consequently, other intracranial tumours of mesenchymal origin cannot be distinguished using this marker. Vimentin is expressed in large areas of human meningiomas and is grade associated as higher number of cells are immunostained with vimentin in grade II and III meningiomas (Alamir et al. 2018). In canine meningiomas vimentin is expressed in all tumours regardless of subtype with a percentage of immunostained cells >70%, with exception of fibrous subtypes which are weaker immunostained. Different subtypes of meningiomas reveal differences in the way of immunostaining. In meningothelial, transitional and papillary subtypes, vimentin expression is higher in concentric whorls of cells than in bundles of spindle-shaped cells. In papillary and angiomatous subtype, cells in the perivascular areas show intense expression of vimentin (Montoliu et al., 2006). In feline meningiomas vimentin is expressed in all cases (Ramos-Vara et al., 2010). In other words, vimentin expression in canine and feline meningiomas can be used infallibly to confirm the existance of mesenchymal tumours.

In human meningiomas CD34 is not always expressed in meningiomas (its expression varies from 0-40 %) and is strongly expressed in fibroblastic subtype of meningioma. On the other side, CD34 is expressed in all histologic subtypes of canine and feline meningioma. CD34 can be used as immunohistochemical marker for meningioma diagnosis and distinguishment from peripheral nerve sheath tumours as CD34 cannot be expressed in nerval sheath tumours. In humans may be also helpful to identify the fibroblastic subtype (Chaubal et al. 1994; Ramos-Vara et al.,2010).

Vimentin and CD34 are recommended in praxis for meningioma diagnosis and discrimination from other intracranial tumours (but not for other tumours of mesenchymal origin) in humans, dogs and cats but are not appropriate as predictors for meningioma grading and biological behaviour and their use need further investigation.

5.3 Conventional and advanced imaging techniques for meningioma grading

Conventional imaging techniques like CT and especially MRI are the goldstandards for meningioma diagnosis. In general, malignant meningiomas may appear heterogeneously enhancing and hyper- to hypointense on T2 weighted sequences while benign secretory meningiomas for example are homogenously enhancing and hyperintense on T2-weighted sequences (Beutler et al., 2020). Imaging features like intratumoural cystic change, hyperostosis of the adjacent skull, bony destruction, extracranial tumour extension through the skull base, arterial encasement and peritumoral brain edema can distinguish grade I from grade II and III meningioma. Especially, intratumoral cystic change and extracranial tumour extension through the skull base foramina are most commonly reported in grade II and III meningioma (Hanft et al. 2010).

In dogs and cats the appearance of meningioma in CT and MRI is similar to that of humans. Intratumoural fluid, large cystic regions, intratumoural mineralization, calvarial hyperostosis or dural tail is some of its characteristics. In canine meningiomas peritumoural edema is present in >90% of cases. As the specific subtypes of meningioma cannot be distinguished using conventional MRI techniques, the necessity for more advanced imaging techniques has arised (Sturges et al., 2008, Miller et al., 2019).

Using routine preoperative MRI in humans, high grade intracranial meningiomas can be discriminated and the risk of postoperative recurrence can be predicted. Imaging findings such as irregular tumour shape, heterogeneous contrast enhancement and a non-skull base tumour location are associated with high grade histology. Peritumoural edema volume is considered as limited risk factor for histology because its sensitivity and specificity are low. In addition to this, characteristics like disruption of arachnoid layer, irregular tumour shape and tumour volume (especially postoperative residual tumour volume) are related with increased tumour recurrence. In cases with arachnoid layer disruption, an almost tenfold risk for recurrence is confirmed and that makes it remarkable factor for the biological behaviour of meningioma.

Consequently, routine MRI techniques can be used to distinguish lower from high grade meningioma and can predict the biological behaviour in humans in clinical praxis especially when disruption of arachnoid layer occurs (Spille et al. 2020). A respective study about dogs and cats should be conducted in order to be confirmed if the imaging features for prediction of tumour grade and recurrence are the same.

<u>Conventional MRI histogram analysis based on 3D tumour measurement</u>

Conventional MRI histogram analysis based on 3D tumour measurement can be used in the assessment of meningioma grading in clinical practice. Histogram features such as histogram volume count and uniformity are reliable parameters for the discrimination of benign from malignant meningiomas. Higher-grade meningiomas appear an increased volume count and lower uniformity in histogram, whereas lower grade meningiomas show decreased volume count and increased uniformity. In clinical praxis, this technique is important as routine MRI is available in most care health facilities and hospitals. The logistic regression model acquired from contrasted T1W1 histogram parameters is a very promising assessment for meningioma grading (Li et al., 2019).

Clinical application of 3D-CISS MRI sequences in dogs

The 3D-CISS sequence is used for the diagnosis and surgical planning of spinal arachnoid diverticula and adhesions in dogs. In human medicine it is used to identify accurately obstructions of CSF flow and to plan the surgical intervention (Tauro et al.,2018). If further investigation is carried out it might be proved a tool for meningioma diagnosis. However, it is not a technique that can be used for meningioma grading by far.

Deep learning radiomics model

Another tool in the imaging diagnostic is the development of a deep learning radionics model for the prediction of meningioma grades, using routine MRI data. This technique is able to differentiate meningioma grades based on meningioma heterogeneity, as heterogenous meningiomas with necrosis and/or hemorrhage belong most likely to high grade. Deep learning networks present advantages in quantifying the prognostic features of meningioma grading that cannot be manually defined. Using routine post contrast MRI as base of the deep learning model is suggested for the diagnosis of intracranial meningioma and providing prognostic information for the patients (Zhu et al., 2019).

Texture analysis of magnetic resonance images

Texture analysis (TA) is a technique used to increase the amount of information that can be obtained from diagnostic images. For example, the best classification results for canine meningioma are obtained with precontrast T1-weighted images where meningioma appears heterogenous. In T2-weighted images canine meningiomas appear homogenously providing poor information about tissue structure. Texture analysis quantifies tissue heterogeneity through evaluation of local signal intensity variations. In human medicine the combination of TA and dynamic contrast-enhanced MRI provides good results in the differentiation of low from high grade gliomas but is not tried out for human meningiomas. Consequently, TA can theoretically be used as technique for the discrimination of low grade from higher grade meningiomas but cannot be immediately applied as a diagnostic test. Data from previously existing database of histologically confirmed meningiomas and corresponding MRI scans are demanded to build this predictive model. Therefore, more studies involving larger databases for the development of an advanced software, which provides accurate diagnosis for meningioma are necessary (Banzato et al., 2017).

Thalium-SPECT and FDG-PET

Positron emission tomography (PET) can play a role in the monitoring of meningioma recurrence and grading (Buerki et al., 2018). Grading using Fluorine-18 fluorodeoxyglucose (FDG-PET) can be conducted because higher FDG uptake is correlated with higher grade of meningiomas and biological aggressiveness. Thalium-201 single photon emission computed tomography (TI-SPECT) imaging is also used for meningioma grading and has higher grading capability compared to FDG-PET at the early phase. Thalium-201 (T1) is a potassium analogue presenting affinity to the sodium- and potassium-activated adenosine triphosphatase (Na+-K+ ATPase) pump and is distributed in many organs but shows little uptake in the normal brain. Na+-K+ATPase activity contributes to higher T1 uptake in high grade meningioma than the low grade one. Both TI-SPECT and FDG-PET are appropriate for meningioma grading. However, FDG-PET requires a cyclotron or a local FDG distribution network, as well as a relatively expensive PET system itself. Therefore, its use is limited as not many hospitals have access to it. On the other hand, much more SPECT scanners are distributed all over the world with an equivalent role in meningioma grading (Okuchi et al. 2015).

6. Summary

The aim of this literature overview was to assess what we know by far from the literature about meningioma grading in humans and domestic animals, to compare the role of different immunohistochemical markers in meningioma grading and biological behaviour prediction in the different species and to refer which imaging techniques are appropriate for meningioma grading.

The grading of meningioma and the prediction of its biological behaviour is very important for an accurate diagnosis and the planning of an appropriate and efficacious therapy. The WHO classification scheme was updated over the years trying to provide more objective and reliable criteria for grading. The incorporation of molecular markers is a helpful tool for intracranial tumours but more studies for meningioma should be carried out. WHO classification scheme can be applied to domestic animals offering a more detailed system for these tumours, but attempts for improvements are accepted.

The role of immunohistochemical markers in meningioma grading plays a significant role as grading based only in histologic features may have pitfalls and limitations. However, we conclude that the use of biological markers for the prediction of meningioma grade and recurrence rate may not be the same in humans, dogs and cats. For example, malignant (grade III) meningiomas are associated with high COX-2, VEGF, Ki-67, OPN and low PRs expression in humans. On the other hand, there was not found any correlation between COX-2 and VEGF expression and tumour grade in dogs. Furthermore, feline meningioma warrants further investigation because the evidence from literature is insufficient for a conclusion.

MRI and techniques based on it are reliable for the distinguishment of benign from malignant meningiomas and especially MRI is recommended as predictor for recurrence especially in cases with disruption of arachnoid layer. Except from that, MRI is used widely in clinical praxis compared with other imaging techniques.

The restriction of this study is the low number of cats participating in the study in comparison with humans and dogs and the little number of articles found for feline meningioma in the literature. Furthermore, our study includes more case reports than literature overviews. Meta-analysis, randomized controlled trials as well as cohort studies and case control studies, with a higher level in evidence, are not used in this literature review.

7. Zusammenfassung

Das Ziel dieser Literaturübersicht war zu bewerten, was wir bis jetzt aus der Literatur über die Einstufung von Meningiomen bei Menschen und Haustieren wissen, die Rolle verschiedener immunhistochemischer Marker bei der Einstufung von Meningiomen aufzuzeigen und die Vorhersage des biologischen Verhaltens bei den verschiedenen Arten zu vergleichen und zu präsentieren welche Bildgebung Techniken für die Klassifizierung von Meningeomen geeignet sind.

Die Einstufung des Meningioms und die Prognose seines biologischen Verhaltens ist sehr wichtig für die genaue Diagnose und die Planung einer geeigneten und wirksamen Therapie. Das Klassifizierungsschema der WHO wurde im Laufe der Jahre aktualisiert, um objektivere und zuverlässigere Kriterien für die Einstufung bereitzustellen. Die Eingliederung molekularer Marker ist ein hilfreiches Instrument für intrakranielle Tumore, es sollten jedoch weitere Studien zum Meningiom durchgeführt werden. Das WHO-Klassifizierungsschema kann auf Haustiere angewendet werden, weil es ein detaillierteres System für diese Tumore bietet. Verbesserungsversuche werden jedoch akzeptiert.

Die Nutzung immunhistochemischer Marker bei der Klassifizierung von Meningeomen spielt eine bedeutende Rolle, da die Einstufung, die nur auf histologischen Merkmalen basiert, Fallstricke und Einschränkungen aufweisen kann. Wir schließen jedoch, dass die Verwendung von biologischen Markern zur Vorhersage des Meningiomgrades und der Rezidivrate bei Menschen, Hunden und Katzen möglicherweise nicht gleich ist. Zum Beispiel sind maligne (Grad III) Meningeome mit einer hohen COX-2-, VEGF-, Ki-67-, OPN- und niedrigen PR-Expression beim Menschen verbunden. Andererseits wurde bei Hunden keine Korrelation zwischen der COX-2- und VEGF-Expression und dem Tumorgrad gefunden. Darüber hinaus erfordert das Katzenmeningiom eine weitere Untersuchung, da die Literaturangaben für eine Schlussfolgerung nicht ausreichen.

MRT und darauf basierende Techniken sind zuverlässig für die Unterscheidung von gutartigen von bösartigen Meningeomen, und insbesondere die MRT wird als Prädiktor für ein Wiederauftreten empfohlen, insbesondere bei Fällen mit Unterbrechung der Arachnoidalschicht. Überdies wird die MRT in der klinischen Praxis im Vergleich zu anderen bildgebenden Verfahren häufig eingesetzt.

Die Einschränkung dieser Studie ist die geringe Anzahl von Katzen, die an der Studie im Vergleich zu Menschen und Hunden teilnehmen, und die geringe Anzahl von Artikeln, die in der Literatur für Katzenmeningeome gefunden wurden. Darüber hinaus enthält unsere Studie mehr Fallberichte als Literaturübersichten. Metaanalysen, randomisierte kontrollierte Studien sowie Kohortenstudien und Fallkontrollstudien mit einem höheren Evidenzniveau werden in dieser Literaturübersicht nicht verwendet.

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