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Dural-based lesions: is it a meningioma?

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Abstract

Purpose Meningiomas are the most common extra-axial intracranial neoplasms with typical radiological findings. In approximately 2% of cases, histopathological reports reveal different neoplasms or non-neoplastic lesions that can closely mimic meningiomas. We describe radiological features of meningioma mimics highlighting imaging red flags to consider a differential diagnosis.

Methods A total of 348 lesions with radiological diagnosis of meningiomas which underwent to surgical treatment or biopsy between December of 2000 and September of 2014 were analyzed. We determined imaging features that are not a typical finding of meningiomas, suggesting other lesions. The following imaging characteristics were evaluated on CT and MRI: (a) bone erosion; (b) hyperintensity on T2WI; (c) hypointensity on T2WI; (d) bone destruction; (e) dural tail; (f) leptomeningeal involvement; (g) pattern of contrast enhancement; (h) dural displacement sign.

Results We have a relatively high prevalence of meningioma mimics (7.2%). Dural-based lesions with homogeneous contrast enhancement (52%) are easily misdiagnosed as meningiomas. Most lesions mimic convexity (37.5%) or parafalcine (21.9%) meningiomas. We have determined five imaging red flags that can alert radiologists to consider meningioma mimics: (1) bone erosion (22.2%); (2) dural displacement sign (36%); (3) marked T2 hypointensity (32%); (4) marked T2 hyperintensity (12%); (5) absence of dural tail (48%). The most common mimic lesion in our series was hemangiopericytomas, followed by lymphomas and schwannomas.

Conclusion The prevalence of meningioma mimics is not negligible. It is important to have awareness on main radiological findings suggestive of differential diagnosis due to a wide range of differentials which lead to different prognosis and treatment strategies.

Keywords Meningioma · Meningioma mimics · Radiological findings · Hemangiopericytoma · Lymphoma · Schwannoma

Introduction

Meningiomas are the most common extra-axial intracranial neoplasms, responsible for up to 20% of intracranial tumors in adults [1, 2]. The imaging findings of an extra-axial dural-based solid lesion with homogeneous contrast enhancement are highly suggestive of meningiomas on MRI [3]. However, in approximately 2% of cases [2], histopathological report surprises with different neoplasms or even non-neoplastic lesions [2].

The typical MRI signal intensity characteristics consist of isointensity to slight hypointensity relative to grey matter on T1-weighted sequences and isointensity to slight hyperintensity relative to grey matter on the T2 sequences. They may occasionally have the dural tail sign, areas of central necrosis, or calcifications that do not enhance and might exhibit a more infiltrating growth pattern over the dura, termed meningioma en plaque—usually along the sphenoid ridge or the convexity [4].

The main differentials are represented by metastatic lesions, hemangiopericytomas /solitary fibrous tumors, glioblastomas, or lymphoproliferative diseases, such as lymphomas [5]. Non-neoplastic lesions encompass granulomatous infectious/inflammatory diseases such as tuberculosis or sarcoidosis [6].

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Table 1 Meningioma mimics

Histologic diagnosis	Age/gender	WHO grade	Location	Radiologist impression
Basaloid carcinoma	29/M	-	Planum sphenoidale	Meningioma
Neuroendocrine carcinoma	67/F	-	Convexity	Meningioma
Chondrosarcoma	54/F	-	Petroclival	Differential – schwannoma, metastasis
Glioblastoma	70/F	IV	Convexity	Differential – high grade glioma
SFT/HPC	47/F	I	Parafalcine/parasagital	Meningioma
SFT/HPC	41/M	I	Parafalcine/parasagital	Meningioma
SFT/HPC	62/F	I	Parafalcine/parasagital	Meningioma
SFT/HPC	34/F	III	Cervical	Meningioma
SFT/HPC	39/F	III	Convexity	Meningioma
SFT/HPC	57/M	II	Convexity	Differential – hemangiopericytoma
SFT/HPC	64/F	II	Parafalcine/parasagital	Meningioma
SFT/HPC	76/F	III	Convexity	Meningioma
SFT/HPC	36/M	III	Convexity	Differential – hemangiopericytoma
SFT/HPC	31/M	III	Clinoid	Differential – hemangiopericytoma
SFT/HPC	49/F	II	Parafalcine/parasagital	Meningioma
SFT/HPC	70/F	II	Clinoid	Meningioma
Plasmoblastic lymphoma	38/F	-	Convexity	Differential – metastasis
Paraganglioma metastasis	31/M	-	Convexity	Differential – metastasis
Anaplastic oligodendroglioma	56/F	III	Parafalcine/parasagital +	Meningioma
Plasmacytoma	70/M	-	Intraosseous	Differential – metastasis or sarcoma
Undifferentiated sarcoma	15/F	III	Spheno-orbital	Meningioma
Schwannoma	18/M	I	Sphenoid wing	Meningioma
Schwannoma	63/F	I	Cerebellopontine angle	Differential – schwannoma
Diffuse large B cell lymphoma	50/F	-	Convexity	Meningioma
Diffuse large B cell lymphoma	38/M	-	Convexity	Meningioma

Although rare, the hypothesis of those differentials in pre-operative imaging might have important implications in the surgical management, which vary from minimally invasive stereotaxic biopsies to radical surgical resections [2, 3].

In attempt to search for potentially useful imaging features predictive of meningioma mimics, we describe the main imaging findings of these lesions, emphasizing similarities, as well as those features that are uncommon in meningiomas. Imaging characteristics are presented on five challenging illustrative cases.

Methods

Between December 2000 and September 2014, 348 patients were surgically treated at our service for dural-based lesions with pre-operative diagnosis of a probable meningioma, based on computed tomography (CT) and magnetic resonance imaging (MRI). From those patients, 25 (7.2%) did not have meningioma in the final histopathological report. All patients underwent contrast-enhanced MRI with T1, T2, FLAIR, SWI or T2 GRE, and T1 post-contrast images. CT scans were available on 18 (72%) cases.

Imaging characteristics of each case were reviewed in order to search for imaging features that could suggest a meningioma mimicker. Basic demographic data were collected for each patient: age at time of surgery, gender, need for reoperation, and the histopathological diagnosis. All patients underwent urgent or elective surgeries for tumor resection. Imaging evaluation was performed by two experienced neuroradiologists.

We included overall information about the lesions: side and type according to its location, as similarly used in meningioma (convexity, sphenoid wing, clinoid, tentorium, parafalcine/parasagital, intraventricular, spheno-orbital, petroclival, planum sphenoidale, cerebellum, and cerebellopontine angle).

The following imaging characteristics were evaluated on CT and MRI:

1) Unenhanced head CT: (a) bone erosion—area of focal bone osteolysis adjacent to the dural-based tumor; (b) hyperostosis—bone thickening and sclerosis in adjacent region to the dural-based tumor; (c) intratumoral calcifications—marked hyperdense regions, above 120 HU, inside the tumor.
2) Brain MRI: (a) dural tail sign—"tail" like dural contrast enhancement adjacent to extra-axial neoplasm in contrast-enhanced T1WI seen in at least three imaging sections; (b) tumor contrast enhancement—homogeneous or heterogeneous enhancement pattern within the tumor; (c) marked hyperintensity on T2-weighted image (T2WI)—defined as a signal close to the cerebrospinal fluid in the tumor; (d) marked hypointensity on T2WI—defined as a lower signal in the tumor compared to the white matter,

Table 2 Radiological characteristics of meningioma mimics

	MRI (n = 25)						CT (n = 18)			
	Marked hypointense on T2 n (%)	Marked hyperintense on T2 n (%)	Leptomeningeal dissemination n (%)	Dural tail n (%)	Homogeneous contrast enhancement n (%)	Dural displacement sign n (%)	Bone erosion n (%)	Hyperostosis n (%)	Calcification n (%)
General	32% (n = 8/25)	12% (n = 3/25)	0%	52% (n = 13/25)	52% (n = 13/25)	36% (n = 9/25)	22.2% (n = 4/18)	33.3% (n = 6/18)	27% (n = 5/18)
SFT /HPC[1]	58.3% (n = 7/12)	8.3% (n = 1/12)	0%	50% (n = 6/12)	33.3% (n = 4/12)	25% (n = 3/12)	12.5% (n = 1/8)	25% (n = 2/8)	37% (n = 3/8)
Lymphomas[2]	0%	0%	0%	100% (n = 3/3)	100% (n = 3/3)	66.7% (n = 2/3)	66.7% (n = 2/3)	66.7% (n = 2/3)	0% (n = 0/3)

[1] SFT/HPC: Solitary fibrous tumor / hemangiopericytoma

[2] Lymphomas: Diffuse large B cell lymphoma (n = 2) and Plasmoblastic lymphoma (n = 1)

which can be calcified or non-calcified in correlation with CT imaging (when available) or susceptibility weighted imaging; (e) leptomeningeal involvement—leptomeningeal enhancement on contrast-enhancement T1WI; (f) cystic/necrotic component—intratumoral degenerative cyst formation or necrosis with hyperintense signal on T2WI and without contrast enhancement; (g) dural displacement sign—the dural displacement sign consists of a line of T2 hypointense signal covering at least some part of the inner border of convexity dural-based lesion on T2, between tumor and cerebral cortex.

On brain MRI, two main features were evaluated on T2-weighted images, large areas of marked T2 hypointensity, which are not calcified—suggestive of large fibrous tissue component; and presence of marked T2 hyperintensity—indicating significant intratumoral water component[3]; which are not typical for most meningiomas. Extent of resection was also evaluated in patients with post-operative MRI. Additionally, data were collected from histological reports. Lesions were classified according to the World Health Organization (WHO) classification for brain tumors according to histological type and tumor grading (I, II, III, IV).

Furthermore, we have included six illustrative case reports emphasizing some radiological red flags of meningioma mimics.

Our study was previously approved by the institutional review board (protocol number 2.697.538)

Statistical analysis

A standard descriptive analysis was performed. Where applicable, 95% confidence intervals were calculated with the binomial exact method.

Results

The prevalence of meningioma mimics was 7.2% (IC 4.7–10.4; binomial confidence interval calculation). The mean patient age was 48.2 ± 17.1 years and 64% (n = 16/25) were women. Most lesions were frontal (n = 9/25; 36%) and right sided (n = 12/25; 48%). According to location, these lesions were classified in the following types: convexity (n = 10/25; 40%), parafalcine (n = 6/25; 24%), and skull base—petroclival, planum sphenoidale, cerebellopontine, spheno-or-

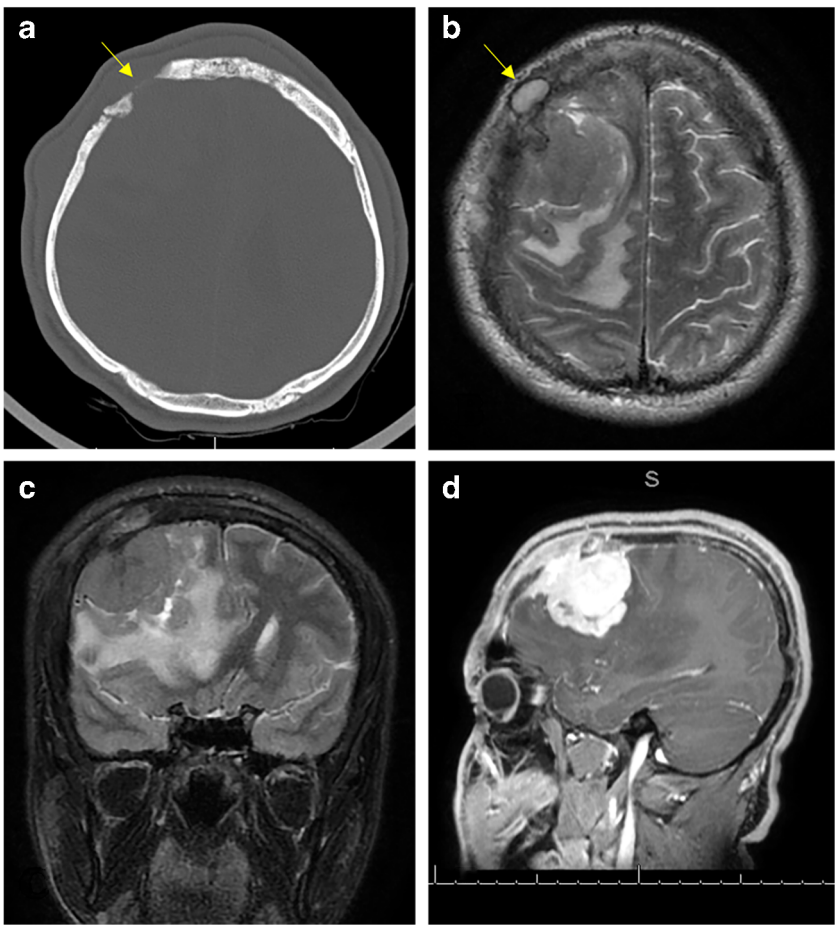

Fig. 1 A 38-year-old patient presented with headache. **a** Head CT showed a right frontal expansive lesion with osseous destruction (arrow) and an important extracranial component of the tumor. **b**, **c** On MRI, there was an apparently extra-axial isointense lesion in the right frontal lobe with significant osseous involvement. **d** The lesion showed avid contrast enhancement with lobulated contours, an evident extra-cranial growth, and dural tail sign. The patient underwent surgery with gross total resection of the lesion suggestive of meningioma. Histopathological report revealed diagnosis of diffuse large B-cell lymphoma

bital, and clinoid (n = 6/25; 24%) (Table 1). We included one intradural extramedullary tumor in the cervical spine highly suggestive of a spinal cord meningioma; however, the histological report confirmed a solitary fibrous tumor/hemangiopericytoma (HPC/SFT)—World Health Organization (WHO) grade III.

Pre-operative CT images were available for 18 patients. Four lesions presented with hyperostosis (n = 4/18; 22.2%). Two of these lesions confirmed a diagnosis of lymphoma in the histopathological report (case 1). Six (n = 6/18; 33.3%) patients presented with bone erosion and intratumoral calcifications were present on 5 cases (n = 5/18; 27%) (Table 1). Of note, two of these patients with calcifications had histological diagnosis of HPC/SFT.

On MRI, three patients (n = 3/25; 12%) presented with marked hyperintensity on T2WI and 8 patients (n = 8/25; 32%) had marked hypointensity on T2WI. Cystic/necrotic component was identified in four patients (n = 4/25; 16%). In 13 (n = 13/25; 52%) patients, lesions presented as homogeneous contrast enhancement tumors and 13 (n = 13/25; 52%) had dural tail sign. Dural displacement sign was evident in 36% of cases (n = 9/25).

On radiological reports, differential diagnosis of meningiomas was suggested for sixteen (n = 16/25; 64%) cases (Table 2).

Extent of resection was assessed in 22 patients with immediate post-operative MRI. Gross total resection was achieved in 19 cases (n = 19/22; 76%).

The main meningioma mimic was HPC/SFT representing 48% (n = 12/25) followed by lymphomas (n = 3/25; 12%, 1 plasmoblastic lymphoma; 2 diffuse large B cell lymphoma), and schwannomas (n = 2/25; 8%). The WHO histological grading was assessed in 18 central nervous system tumors; 61% (n = 11/18) of tumors presented as low grade tumors (grade I or II on the World Health Organization (WHO) classification) (Table 1).

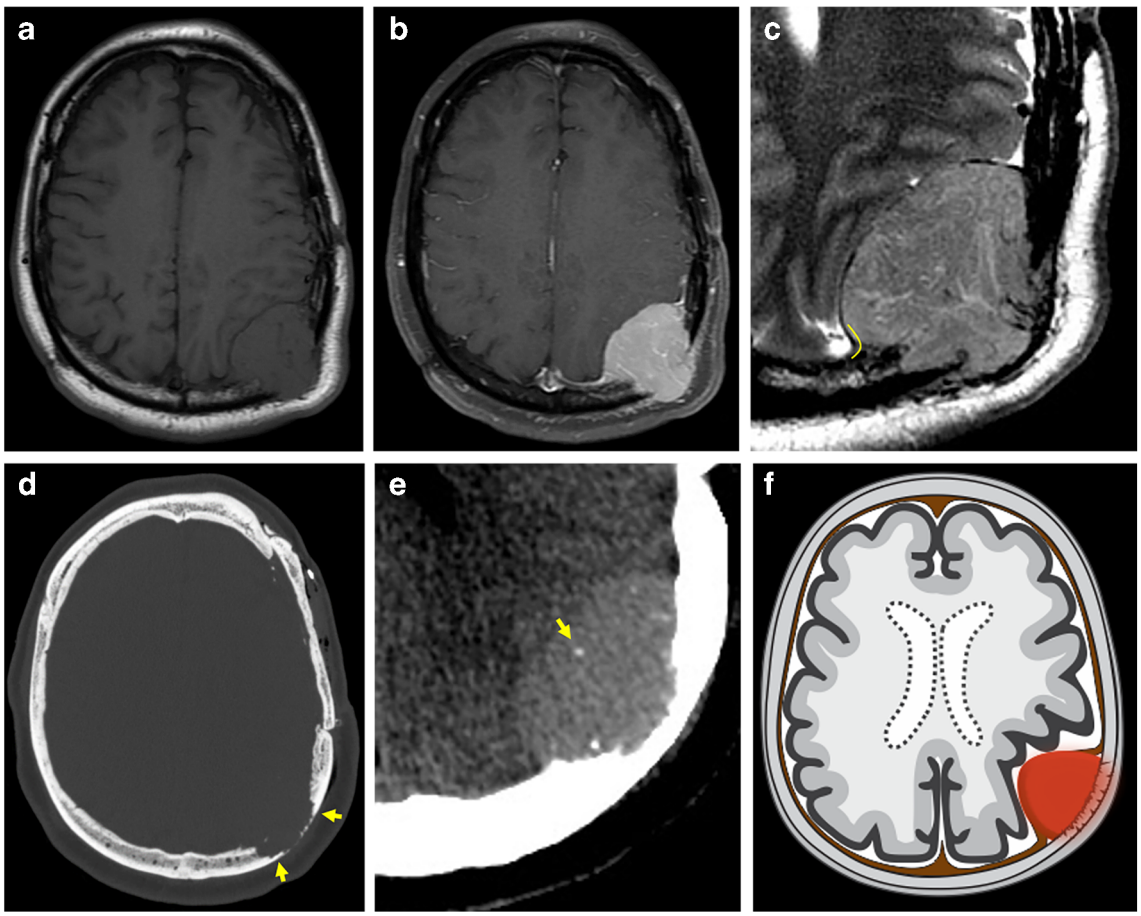

Fig. 2 A 31-year-old patient with a previous surgery for resection of a brain tumor at an external service. Admitted for neurosurgery follow-up. **a**, **b** At first brain MRI, there was a left parieto-occipital extra-axial tumor with homogeneous contrast enhancement and bone erosion and part of the tumor outside the cranial vault. **b** On T2WI, the dural displacement sign is evident with the dural layer dislocated along the interface of the tumor with cortex (line). On head CT, there was evident osseous destruction (**d**; arrows) by the tumor growth in addition to calcifications within the tumor (**e**; arrows). On figure **f**, the illustrative image shows an extra-axial tumor with an epidural growth pattern or displacement of the dural layer which may be responsible for the hypointense line on T2WI. Histopathological report revealed HPC/SFT (WHO grade II)

Case 1: Lymphoma with bone erosion (Fig. 1)
Case 2: HPC/SFT with the dural displacement sign, calcifications and bone erosion (Fig. 2)
Case 3: HPC/SFT with marked hyperintensity and hypointensity on T2WI (Fig. 3)
Case 4: HPC/SFT with calcification and the dural displacement sign (Fig. 4)
Case 5: Plasmacytoma with dural displacement (Fig. 5)
Case 6: Dural metastasis with dural tail and dural displacement sign (Fig. 6)

Discussion

Meningiomas are dural-based masses with contrast enhancement and extra-axial origin in different places such as convexity, falx cerebri, or the tentorium; they might as well be located in deep sites along the skull base such as the clivus, olfactory groove, clinoid process, and planum sphenoidale [7].

Osseous involvement with hyperostosis on CT is a suggestive feature of meningioma, as well as isointensity to slight hyperintensity on T2WI and avid contrast enhancement on MRI, with rounded and well circumscribed or "en plaque" lesions [3]. Additionally, intratumoral calcifications are seen in 15–20% of these tumors on CT [8]. Usually, radiological diagnosis is straightforward for experienced radiologists. Cystic and necrotic areas, ring enhancement, hemorrhage, and metaplastic changes are unusual, although can be present in typical, atypical, and anaplastic meningiomas, and can be misleading [9]. Ghosal et al. [2] described 2% of meningioma mimics in their series, with preoperative diagnosis also based on imaging findings [2].

In our study, from 348 patients with preoperative diagnosis of meningioma, 25 (7.2%) were meningioma mimics after histopathological report. HPC/SFT are the most common followed by metastatic lesions (case 6) and schwannomas.

Characteristics of meningioma mimics

Meningioma mimics presented as dural-based contrast enhancing masses in typical locations of meningiomas—convexity and parafalcine [10]. Most lesions presented with radiological findings of extra-axial lesions—cerebral spinal fluid (CSF) cleft sign in T2WI. Additionally, we had a high

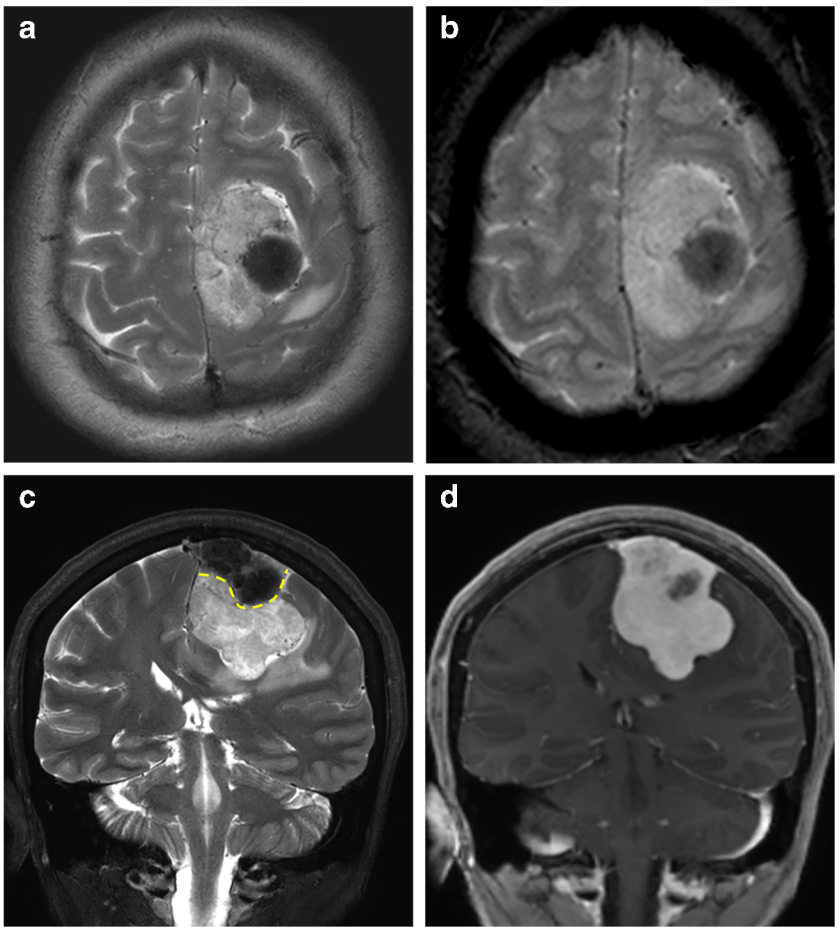

Fig. 3 A 62-year-old female patient presented with progressive right hemiparesis. On investigation, brain MRI showed a left frontoparietal parafalcine mass with relatively homogeneous contrast enhancement, irregular shape, and dural-tail sign (**d**). **a**, **b**, **c** On T2WI, there were different areas of hyperintensity and marked hypointensity in the tumor with a "Ying-yang" appearance (stippled line in **c**) [23, 26]. **b** On T2-gradient echo sequence, there is no magnetic susceptibility effect in the areas of marked hypointensity, suggesting solid fibrous tissue, rather than calcification or hemorrhage. After surgical resection, histopathological report revealed HPC/SFT (WHO grade III) [22].

prevalence of homogeneous contrast enhancement lesions (52%) with a high prevalence of the dural tail sign (52%), which made these lesions even more similar to meningiomas.

There are radiological findings highly suggestive of typical meningioma. On CT, osseous involvement with hyperostosis is one of those [11], but can occur in lymphoma and chronic inflammatory processes like IgG4-related disease [12, 13]. We had only two cases with marked hyperostosis both related to lymphoma. Intratumoral calcification is another typical finding of meningiomas.

Red flags of meningioma mimics

Star et al. [3] suggested five imaging red flags on differential diagnosis of meningiomas: (1) marked T2 hypointensity; (2) marked T2 hyperintensity; (3) osseous destruction; (4) leptomeningeal extension; (5) absence of dural tail.

We suggest another differential imaging feature, the dural displacement sign. This may represent epidural growth or displacement of the periosteal from the meningeal dural layer, seen as a T2-hypointense line covering at least some part of the inner border of the tumor, between tumor and cerebral cortex. Convexity meningioma typically grows adhered to dura mater, so the dural layer is not usually depicted over tumor inner surface on T2 imaging. To our knowledge, this is the first study to report this dural displacement sign as a red flag, suggesting a meningioma mimicker. We speculate that some extra-axial lesions that mimic meningiomas may have more peripheral dural, epidural, or bone origin and therefore displace the meningeal dural layer or both meningeal and periosteal dural layers from the bone over the convexity. Additionally, we believe that this sign is not so reliable where dural layers are naturally splitted, like the normal infoldings at falx cerebri and venous sinus.

Bone erosion is an uncommon finding in meningioma. We have a relatively high prevalence of this radiological finding; diagnosis of skull vault metastasis [3], plasmacytomas [3], lymphoma [3], and HPC/SFT should be considered as differentials. Due to hypercellularity of lymphoma, water diffusion is often highly restricted on DWI [14], although this can also be seen in some atypical meningiomas [15]. Lack of

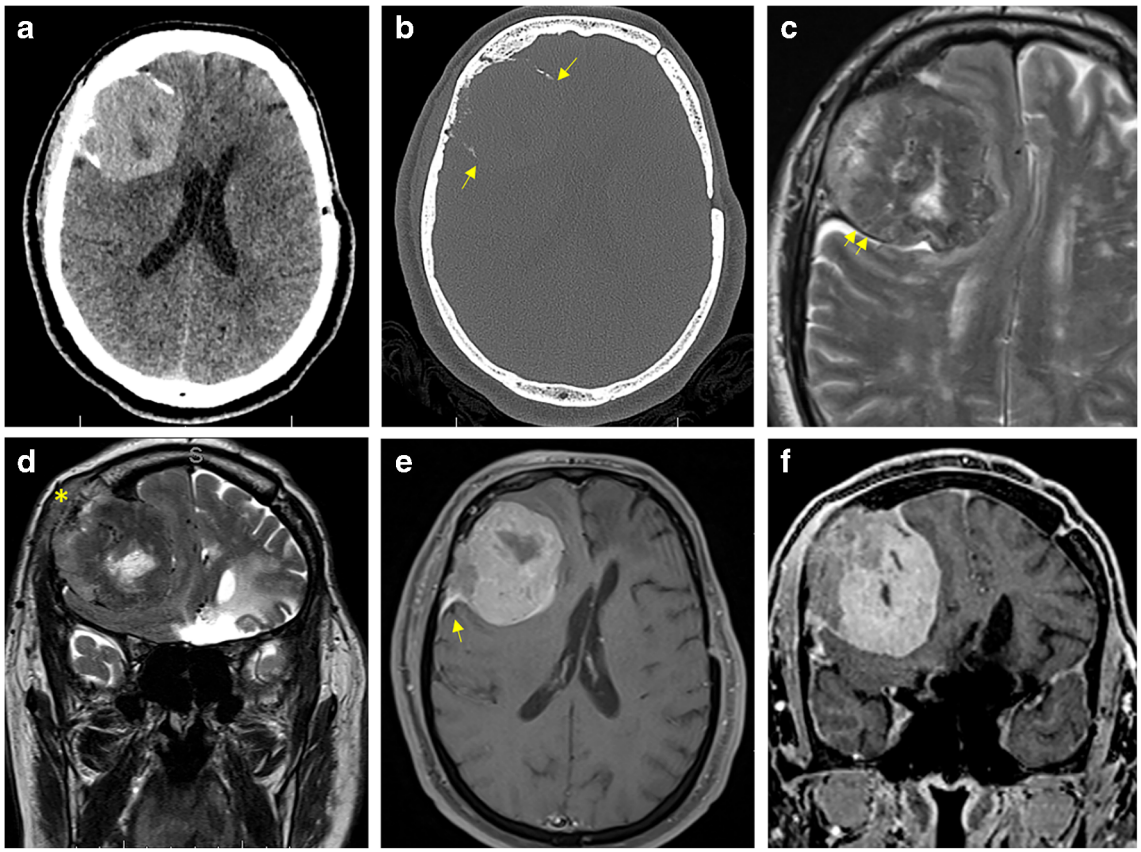

Fig. 4 A 57-year-old male patient with a previous left pterional craniotomy for aneurysm clipping admitted at our emergency service with seizures. **a**, **b** On brain CT, there was a right frontal lesion on the convexity with bone invasion and areas of calcifications on tumor boundaries (arrows). **c** On Axial T2WI, it was a dural-based extra-axial tumor with CSF cleft sign and the dural displacement sign (arrows). In addition, there are intratumoral flow-voids and areas of marked hypointensity (areas that are darker than the white matter) without calcification in the tumor. **d** On coronal T2WI, it is evident the dural displacement sign and bone invasion (*) caused by the tumor. **e**, **f** T1 post-contrast images show the dural tail sign (arrow) and areas of heterogeneous contrast enhancement which suggests the diagnosis of HPC/SFT. After surgical gross total resection, the histological report confirmed diagnosis of HPC/SFT (WHO grade II)

neoangiogenesis is another histological feature of lymphomas [16]. Therefore, on dynamic susceptibility contrast perfusion, the maximal rCBV values of lymphoma are far lower than meningioma (1.68 × 7.16) [17]. Large areas of marked T2 hypointensity, which are not calcified, suggest large areas of extensive fibrous tissue component such as in HPC/SFT cases (case 3) [3]. Marked hyperintensity on T2WI might represent cystic degeneration in microcystic meningiomas and are also frequent in chordoid and angiomatous meningiomas; however, diffuse hyperintensity on T2WI at skull base lesions might represent cartilaginous-matrix in chondrosarcomas or fluid gelatinous material in chordomas [18].

Dural tail sign (case 4)

The dural tail is a hallmark for meningiomas since it was first described in 1989 [19, 20]. It is prevalent in almost 60% of meningiomas; however, several dural masses also present this sign. In our series, lesions with an avid contrast enhancement (HPC/SFT, lymphomas, or high grade tumors) in parafalcine or convexity locations were more likely to present typical dural thickening. The absence of the dural tail sign might be considered as a red flag for radiological differentials of meningiomas due to its high prevalence (case 4).

Solitary fibrous tumor/hemangiopericytoma (cases 2, 3, and 4)

HPC/STF is the main meningioma mimic [3]. In 66.6% (*n* = 8/12) of cases of HPC/SFT, meningioma was the only hypothesis suggested in the radiological reports. It is relatively rare in the CNS with an estimated incidence of 0.4% among all CNS tumors [21]. Recently, it has been considered that solitary fibrous tumor and hemangiopericytoma are parts of the same spectrum of a mesenchymal neoplasm and were grouped in new combined entity, namely HPC/SFT, ranging from grade I to III [22]. Radiologically, it consists a dural-based lesion with homogeneous contrast enhancement and different findings on MRI—with both hypo- and hyperintensity on T2WI, placed in typical locations of meningiomas and dural tail sign in sporadic cases [6, 23]. The main challenge is to differentiate atypical meningiomas with lobulated irregular outlines and cystic changes from

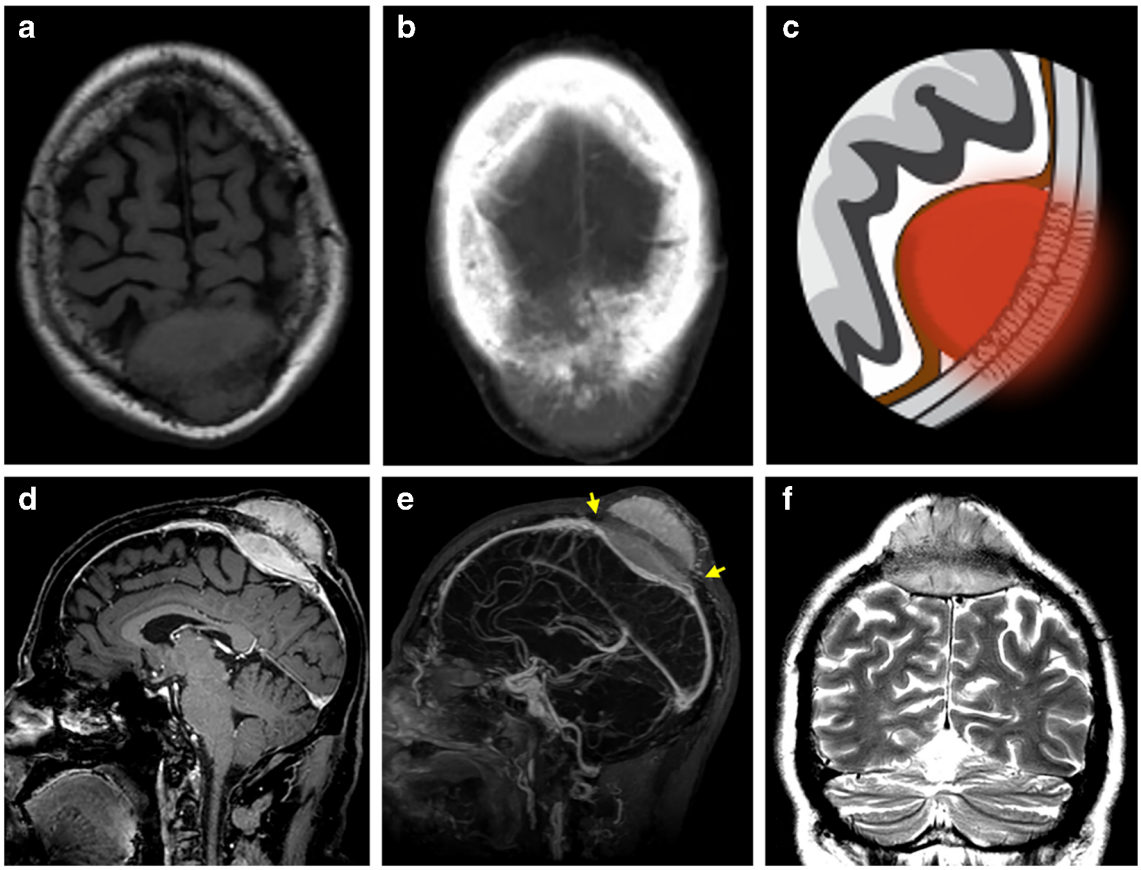

Fig. 5 A 70-year-old male patient presented with a bulging mass in the head. **a**, **b** On head CT, there is evident tumor through a bone erosion area with intra- and extracranial masses. The MRI showed extra-axial tumor with homogeneous contrast enhancement (**d**) and displacement of the superior sagittal sinus, dislocated downward on MR angiography (**e**). **f** On coronal T2WI, a hypointense line on the inferior limit of the tumor represents the dural displacement sign represented on the illustrative image (**c**)

HPC [21]. However, some imaging features may help thinking of this tumor like more osseous destruction (cases 1, 2, 5) than hyperostosis and the collagenous component in the tumors that might appear as larges areas of non-calcified marked hypointensity in T2WI (case 3) [24]. Another differential is the intratumoral calcification which is rarely seen in HPC. Despite this, in our sample, 3 HPC/STF (two in the same patient) cases presented with areas of calcification confirmed on pre-operative CT (cases 2, 4). Intratumoral irregular and tortuous vessels are described [25]—as opposed to the typical radially oriented "spoke wheel" appearance of meningioma.

Impact on treatment strategy

The main implication of an accurate preoperative image-based diagnosis is regarding to treatment planning. For such potentially benign lesions, gross total resection is mandatory for curative treatment. However, in suspicion of secondary lesions or even lymphoproliferative diseases (case 1), a biopsy with systemic staging might be the best decision for appropriate oncologic treatment.

In our series, only 20% of mimics were WHO grade I tumors. Within the high grade lesions, there were glioblastoma, anaplastic hemangiopericytoma, and undifferentiated sarcoma. Our series addresses meningioma mimic lesions; however, these were not straightforward homogeneous contrast enhancement, dural-based lesion with regular contours. There were certainly atypical lesions that were indicative of meningioma but in 36% of cases, there were not discarded differentials in radiological reports which might explain the relatively high frequency of tumors with aggressive biological behavior.

Our study has limitations. Its retrospective design brings some limitations especially in the management and investigation work-up of cases. We had a high prevalence of meningioma mimics (7.2%) within patients who underwent surgical treatment of tumors with presumed diagnosis of meningiomas, which might be explained by the academic nature of our institution—a reference center in the public health system. In addition, our limited sample size that did not permit accuracy tests to compare imaging findings of these "mimics" lesions with the other meningiomas from our series. Nevertheless, our study is a representative series of radiological differential diagnosis of meningiomas which radiologists and neurosurgeons should be aware.

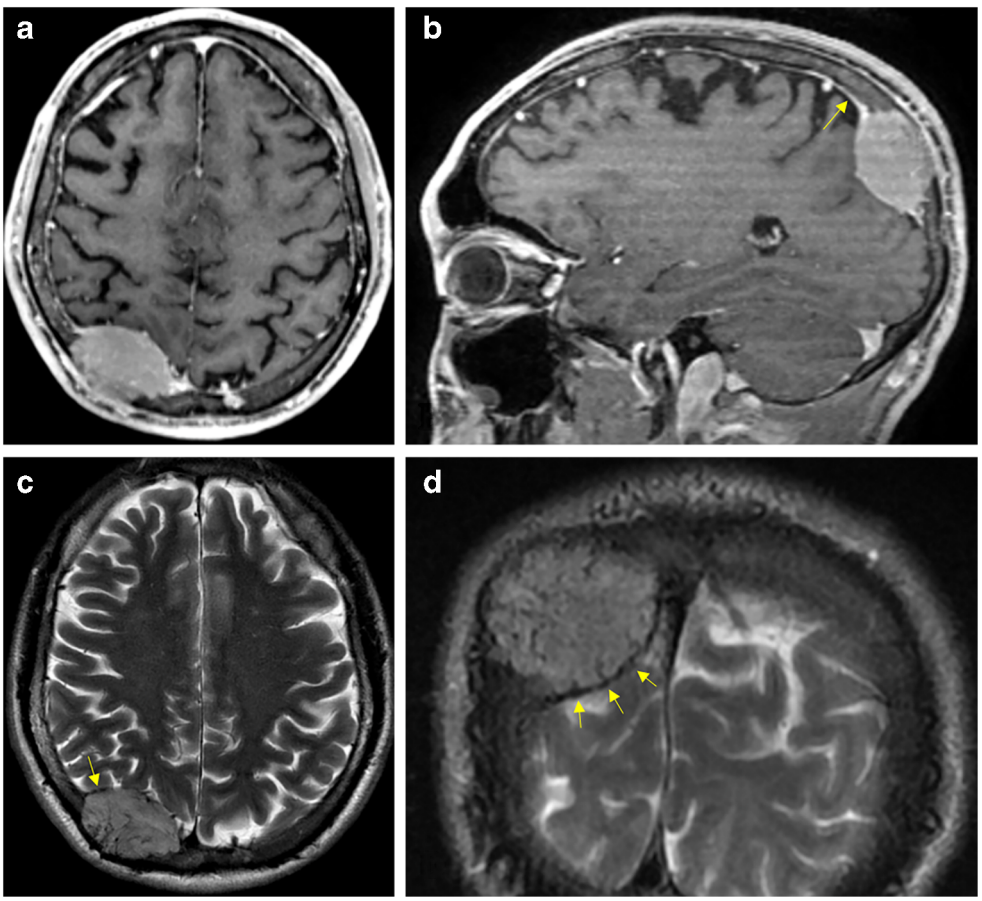

Fig. 6 A 31-year-old male patient that has been in follow-up for a retroperitoneal paraganglioma operated 1 year before. **a**, **b** On T1WI post-gadolinium, there was a dural-based mass with bone invasion in the right parietal region with homogeneous contrast enhancement and dural tail sign (**b**, arrow). **c**, **d** T2WI shows an extra-axial lesion with intermediate signal, bone invasion, and an evident hypointense line in the deep border of the tumor—the dural displacement sign (arrows)

Conclusion

Meningiomas are relatively common neoplasms for neurosurgeons that surprises in histological reports in 7% of cases. Hemangiopericytomas, schwannomas, and lymphomas were the main differentials. Complete imaging work-up should be performed preoperatively for treatment planning and eventually oncologic staging for patients with atypical lesions; therefore, multidisciplinary rounds are necessary for decision-making in treatment of "meningioma-like" neoplasms.

Author Contribution List V.N.Y., G.A.B., L. F. S. G., L.T.L, I.S.N. and W.S.P. contributed to design and implementation of the research;

V.N.Y., G.C.L. and D.J.F.S. contributed to retrospective review of data, statistical analysis and reported results;

G.A.B., L. F. S. G. and L.T.L reviewed neuroimages and confirmed radiological signs reported on results;

V.N.Y. and L. F. S. G. wrote the paper;

W.S.P. and M.J.T. supervised the project;

All the authors critically reviewed the manuscript before submission

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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