CLINICAL STUDY

Cortical hemosiderin is associated with seizures in patients with newly diagnosed malignant brain tumors

Ulrich Roelcke · Larissa Boxheimer ·
Ali Reza Fathi · Lucia Schwyzer · Marcos Ortega ·
Jatta Berberat · Luca Remonda

Abstract Hemorrhage is common in brain tumors. Due to characteristic magnetic field changes induced by hemosiderin it can be detected using susceptibility weighted MRI (SWI). Its relevance to clinical syndromes is unclear. Here we investigated the patterns of intra-tumoral SWI positivity (SWI_{pos}) as a surrogate for hemosiderin with regard to the prevalence of epilepsy. We report on 105 patients with newly diagnosed supra-tentorial gliomas and brain metastasis. The following parameters were recorded from pre-operative MRI: (1) SWI_{pos} defined as dot-like or fine linear signal changes; (2) allocation of SWI_{pos} to tumor compartments (contrast enhancement, central hypointensity, non-enhancing area outside contrast-enhancement); (3) allocation of SWI_{pos} to include the cortex, or SWI_{pos} in subcortical tumor parts only; (4) tumor size on T2 weighted and gadolinium-enhanced T1 images. 80 tumors (76 %) showed SWI_{pos} (4/14 diffuse astrocytoma WHO II, 5/9 anaplastic astrocytoma WHO III, 41/46 glioblastoma WHO IV, 30/36 metastasis). The presence of SWI_{pos} depended on tumor size but not on patient's age, medication with antiplatelet drugs or anticoagulation. Seizures occurred in 60 % of patients. Cortical SWI_{pos} significantly correlated with seizures in brain metastasis ($p = 0.044$), and as a trend in glioblastoma ($p = 0.062$). Cortical SWI_{pos} may confer a risk for seizures in patients with newly diagnosed brain metastasis and glioblastoma. Whether development of cortical SWI_{pos} induced by treatment or by the natural course of tumors also leads to the new onset of seizures has to be addressed in longitudinal studies in larger patient cohorts.

Keywords Brain tumor · Epilepsy · Hemorrhage · Hemosiderin · Magnetic resonance imaging

U. Roelcke (✉) · M. Ortega
Department of Neurology, Neurooncology, Cantonal Hospital,
5001 Aarau, Switzerland
e-mail: roelcke@ksa.ch

U. Roelcke
School of Medicine, University of Basel, 4031 Basel,
Switzerland

L. Boxheimer · J. Berberat · L. Remonda
Department of Neuroradiology, Cantonal Hospital, 5001 Aarau,
Switzerland

A. R. Fathi · L. Schwyzer
Department of Neurosurgery, Cantonal Hospital, 5001 Aarau,
Switzerland

Introduction

Intra-tumoral hemorrhage is frequent in brain tumors. Histopathological studies report on an incidence of 13 % in glioblastoma, and of 30 % in oligodendrogliomas [1]. Also brain metastases show hemorrhage which is present in up to 36 % of patients [1]. Acute hemorrhage can result in stroke-like symptoms [1]. On the other hand blood products such as iron and hemosiderin are epileptogenic which has been demonstrated in animal models and patients with vascular malformations [2–4]. Seizures are among the presenting symptoms in 30–50 % of patients with newly diagnosed tumors, and develop in up to 30 % during the post-operative course of disease [5]. A role for hemosiderin in the epileptogenesis of brain tumors has been postulated, however this was not yet addressed in detail [6]. As the above mentioned data on the incidence of intra-tumoral hemorrhage were derived from surgically obtained tissue specimen they do not allow to describe the exact intra-tumoral localization of hemorrhage [1]. Using

susceptibility-weighted magnetic resonance imaging (SWI) it is possible to detect signal abnormalities compatible with deposits of blood products such as hemosiderin in vivo (SWI positivity, SWI_{pos}) [7]. This technique therefore enables to characterize SWI_{pos} within tumors compartments as well as in the cortical and subcortical brain areas involved by the tumor. The aim of our study was to describe the incidence and distribution of SWI_{pos} in newly diagnosed brain tumors, and to assess whether distinct patterns of SWI_{pos} relate to the occurence of seizures.

Materials and methods

Clinical data

We retrospectively analyzed all successive patients with newly diagnosed supra-tentorial low and high grade glioma and brain metastasis which were treated at our institution between January 2009 and July 2012. As SWI_{pos} can be found due to both the presence of blood products and calcification we excluded oligodendroglial tumors where calcification is frequently observed [7]. Patient charts were reviewed with regard to age at diagnosis of brain tumor, gender, and to the presence and semiology of seizures. Medication was recorded with particular emphasis on the administration of platelet inhibitors (aspirin, clopidogrel) or warfarin. The study was approved by the local ethics committee.

MRI data acquisition

All MR images were obtained as our routine standard diagnostic procedure in patients suspected to suffer from brain tumor using a 1.5 T Avanto or Espree scanner (Siemens Healthcare, Erlangen, Germany) using an eight channel head array coil. Among our routine MR imaging protocol of the brain which includes contrast- (Gadovist®) enhanced T1-weighted images an axial SWI sequence was obtained, which is a combination of magnitude and phase images of a 3D velocity compensated gradient echo sequence. The parameters of the T2 SWI were as follows: acquisition time 4:38 min, slice thickness 2 mm, TE/TR = 40/40 ms, 1 average and flip angle of 15°.

MRI data analysis

Pre-operative MRI scans were analyzed. SWI_{pos} was defined using the following procedure: (1) the SWI sequences were used to detect dot-like or fine linear hypointense signal changes (SWI_{pos}) [8]; (2) the minimum Intensity Projection (mIP) of the SWI sequences was reviewed to ensure that tumor signal changes are not due to

flow signal from vessels; (3) the Phase imaging sequences were used to exclude calcification as a source of intratumoral SWI_{pos} [7]. In patients with multiple brain metastases and focal epilepsy the lesion compatible with seizure semiology was chosen for assessment of SWI_{pos}. In patients without seizures the largest brain metastasis was chosen.

As there are no established criteria to classify the degree of SWI_{pos} we arbitrarily defined three categories: grade 0 = no SWI_{pos}, grade I = one to five focal signal changes (either dot-like or fine linear or both), grade II = $\geq$ six focal signal changes [8].

SWI_{pos} was also characterized by its cortical and/or subcortical localization. In patients with contrast-enhancing tumors we further assessed whether SWI_{pos} involved the enhancing tumor area, the central hypointensity within, or the non-enhancing area outside contrast-enhancement.

Tumor size was measured on axial contrast-enhanced T1 and T2 weighted images according to the RANO criteria [9]. The two maximal perpendicular diameters were calculated on the plane showing the largest tumor diameter. In patients with multiple brain metastases the lesion which corresponded to the focal seizure semiology was chosen. In patients without seizures the largest lesion was chosen.

Statistics

We tested whether SWI_{pos} was associated with age, treatment with platelet inhibitors or warfarin, or with tumor size. For comparison of categorial or continuous variables the Fisher's exact and Chi square test was applied. We used regression analysis to estimate whether the presence of seizures was associated with any of the clinical characteristics and imaging results. Statistical analyses were performed using SigmaStat 12 Software, SyStat, Inc., USA.

Results

We identified 105 patients (68 male, 37 female, age 59.5 ± 12.3 years (mean $\pm$ SD) with newly diagnosed brain tumors (14 low-grade glioma WHO II, 9 anaplastic glioma WHO III, 46 glioblastoma WHO IV, 36 brain metastasis) (Table 1). The histopathological diagnosis was established in 91 % by partial or gross total resection, 9 % of patients were biopsied only. 87 % of patients with brain metastasis suffered from multiple lesions. 25 patients (24 %) received platelet inhibitors or warfarin for cardiovascular disorders. All glioblastomas and brain metastases showed blood–brain barrier (BBB) disruption to a variable degree as evident on pre-operative gadolinium-enhanced MRI. Two low-grade gliomas and five anaplastic gliomas showed circumscribed BBB disruption.

Table 1 Clinical data and imaging results

Histology	n	Age[a] years	PI Warf %	Tumor size (T1 + CE[b])[a] mm²	Tumor size (T2[b])[a] mm²	SWI$_{pos}$ tumors n (%)	SWI$_{pos}$ located in BBBD[d] %
Low-grade glioma	14	53 ± 12	14	3.1 ± 1.6	20.5 ± 14.1	4 (29)	50
Anaplastic astrocytoma	9	58 ± 15	44	4.8 ± 8.4	21.2 ± 11.0	5 (56)	100
Glioblastoma	46	59 ± 12	15	13.6 ± 8.6	29.9 ± 15.7	42 (91)	90
Brain metastasis[c]	36	63 ± 11	33	7.5 ± 8.0	19.5 ± 12.9	29 (83)	97

[a] Data are presented as mean ± SD. PI, Warf: percentage of patients receiving platelet inhibitors or warfarin

[b] T1 + CE = contrast enhanced T1-weighted MRI; T2 = T2-weighted MRI; SWI$_{pos}$: MRI susceptibility changes compatible with the presence of hemosiderin (see "Materials and methods" section)

[c] Primary tumor: small cell lung cancer (n = 4), non-small cell lung cancer (n = 13), breast cancer (n = 5), malignant melanoma (n = 4), prostate (n = 2), urothel (n = 2), colon (n = 1), esophagus (n = 1), uterus (n = 1), unknown (n = 3)

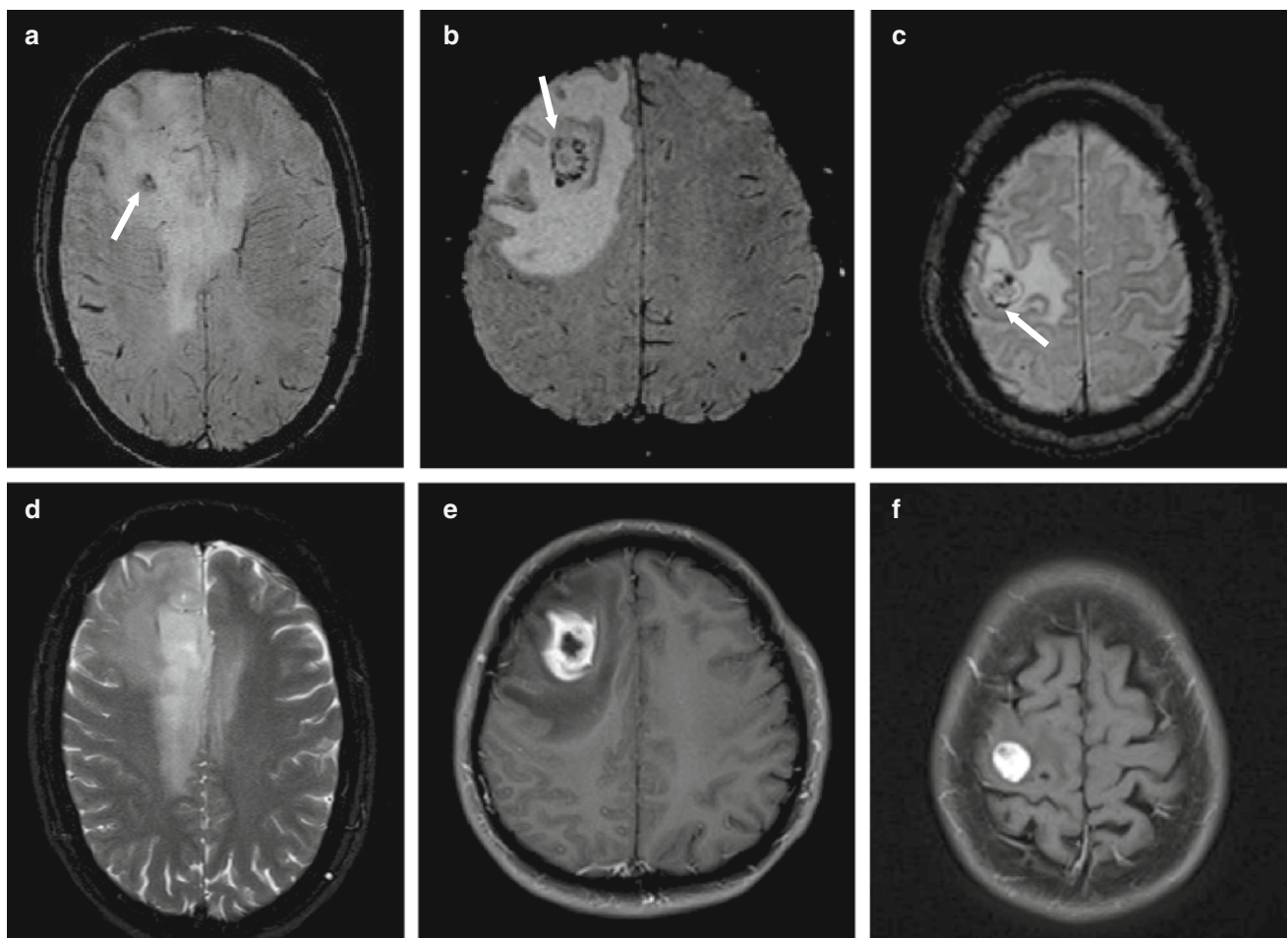[d] BBBD: tumor area with blood–brain barrier disruption and/or central hypointensity

Fig. 1 Distribution of hemosiderin in newly diagnosed brain tumors. Fibrillary astrocytoma (*left*), glioblastoma (*middle*), brain metastasis (*right*). *Top* **a–c** intra-tumoral SWI$_{pos}$. *Bottom* T2 weighted (**d**) and gadolinium enhanced T1 weighted (**e, f**) MRI. *Arrow* subcortical (**a, b**) and cortical (**c**) SWI$_{pos}$

Frequency and distribution of SWI$_{pos}$

SWI$_{pos}$ was observed in 80 patients (76 %) and was significantly higher in glioblastoma and brain metastasis compared to the other subgroups (Chi square 9.455, $p = 0.024$) (Table 1). The distribution of SWI$_{pos}$ within the tumors and with regard to subcortical location and cortical involvement is illustrated in Fig. 1. In the majority

of patients with malignant tumors SWI_{pos} was located to the area of BBB disruption or the central hypointensity and was of grade II (Tables 1, 2). SWI_{pos} significantly correlated with tumor size on T2 weighted and gadolinium enhanced T1 MRI ($p < 0.001$), but neither with patient's age ($p = 0.371$) nor with treatment with platelet inhibitors or warfarin ($p = 0.290$).

Seizures, tumor size and SWI_{pos}

For the whole group of 105 patients the presence of seizures correlated with tumor size on MRI T2 and gadolinium enhanced T1 weighted MR ($p = 0.009, 0.002$), but not with age ($p = 0.127$) and medication with platelet inhibitors or warfarin ($p = 0.423$). SWI_{pos} revealed a significant correlation with seizures as onset symptoms ($p = 0.005$, likelihood ratio 0.62), but with moderate sensitivity (0.5) and specificity (0.2) (Fisher's exact test). A stronger association with seizures was observed when SWI_{pos} involved the cerebral cortex ($p = 0.003$, likelihood ratio 2.2). Here we found a sensitivity of 0.67 and a specificity of 0.7. Involvement of the cortex by tumor alone was of marginal significance ($p = 0.051$).

SWI_{pos} with regard to seizures in the histological subgroups is detailed in Table 2 and is summarized in Fig. 2. Seizure frequency and cortical involvement by tumor was highest in low-grade glioma, but cortical SWI_{pos} was lowest in this subgroup (Fig. 2). Vice versa 95 and 77 % of patients with glioblastoma and brain metastasis showed cortical involvement by tumor, but only 65 and 73 % showed cortical SWI_{pos}, and 43 and 72 % of these patients experienced seizures. Testing all clinical and imaging variables we observed a significant correlation between cortical SWI_{pos} and the presence of seizures for brain metastasis ($p = 0.044$), and a trend for glioblastoma ($p = 0.062$). Tumor SWI_{pos} per se did not correlate with the presence of seizures. Logistic regression analysis was not possible for
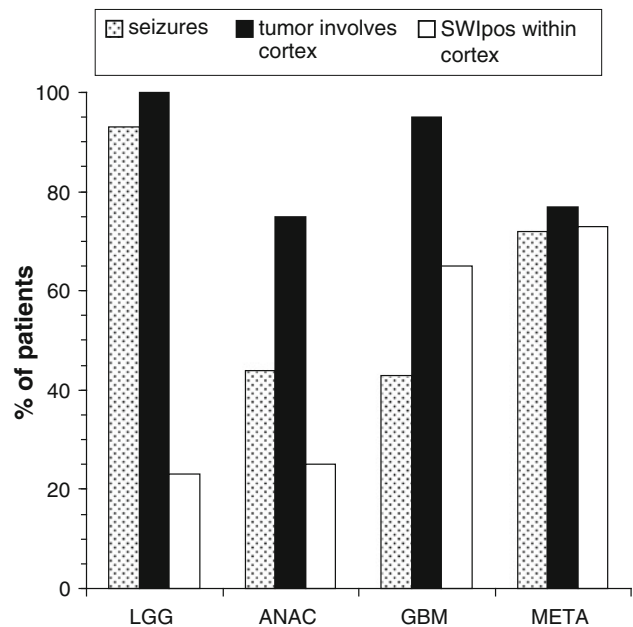

Fig. 2 Frequency of seizures, cortical involvement by tumor and cortical SWI_{pos} LGG: low-grade glioma, ANAC: anaplastic astrocytoma, GBM: glioblastoma, META: brain metastasis

low-grade glioma and anaplastic astrocytoma due to the small number of patients in these subgroups. The Chi square test showed no correlation between the grade of SWI_{pos} and the presence of seizures in all subgroups (Table 2).

Discussion

Our data indicate that intra-tumoral hemorrhage as studied with susceptibility weighted MRI (SWI_{pos}) occurs more frequent than estimated from histopathological analysis [1]. This difference is most likely explained by sampling errors in patients who underwent partial resection or biopsy only [1]. In line with earlier reports the incidence of SWI_{pos}

Table 2 SWI_{pos} and seizures

Histology	Presence of seizures n (%)		SWI_{pos} in tumor subgroups n (%)	Grade of SWI_{pos} (0/1/2)[a] n	Tumor involves cortex n (%)	Tumors with cortical SWI_{pos} n (%)
Low-grade glioma	Yes	13 (93)	4 (31)	9/2/2	13 (100)	3 (23)
	No	1 (7)	0 (0)	1/0/0	0 (0)	0 (0)
Anaplastic astrocytoma	Yes	4 (44)	1 (25)	3/1/0	3 (75)	1 (25)
	No	5 (56)	4 (80)	1/1/3	5 (100)	2 (40)
Glioblastoma	Yes	20 (43)	16 (85)	4/0/16	19 (95)	13 (65)
	No	26 (57)	25 (96)	1/1/24	24 (92)	11 (42)
Brain metastasis	Yes	26 (72)	21 (81)	5/5/16	20 (77)	19 (73)
	No	10 (28)	9 (90)	1/0/9	9 (90)	4 (40)

[a] The grade of SWI_{pos} did not correlate with seizures (low-grade glioma $p = 0.422$, anaplastic astrocytoma $p = 0.283$, glioblastoma $p = 0.159$, brain metastasis $p = 0.209$; Chi square test)

exceeded 80 % in patients with glioblastoma and brain metastasis, and was low in patients with low-grade glioma [8, 10]. Therefore the presence of SWI_{pos} on MRI may serve as a non-invasive marker of tumor malignancy. As the majority of patients with glioblastoma and brain metastasis show SWI_{pos} within the area of BBB disruption or the central tumor core it is likely that SWI_{pos} represents a non-specific marker of vascular leakage common to malignant brain tumors. The abnormal vascular structure in malignant gliomas is composed by immature and retiform capillaries as well as by thin walled sinusoids which may predispose to hemorrhage [11]. The origin of SWI_{pos} in non-enhancing low grade glioma is unclear. It may be hypothesized that circumscribed SWI_{pos} lesions (Fig. 1a) represent malignant tumor foci, and that the detection of new or enlarging SWI_{pos} during the long-term course of low-grade tumors indicates subsequent transformation into malignant glioma. Biopsy of SWI_{pos} tumor regions aiming at the detection of malignant glioma may unravel vascular changes within these lesions.

The overall clinical impact of hemorrhage in brain tumor patients has not been addressed in detail. Hemorrhage with stroke-like symptoms is rare and occurs in 2 to 3 % of patients [1, 12]. These clinical events are clearly less frequent than the histopathological detection of hemosiderin or SWI_{pos} on MRI (our data) [1, 11]. Small intra-tumoral hemorrhage which does not result in stroke-like symptoms may be present for a hitherto unknown period of time. The evolution of the characteristic signal changes due to hemorrhage has been studied in T1 and T2 weighted MRI in various conditions including brain tumors [13, 14]. Hemorrhage induced hypointensity on T2 weighted sequences can be already observed 1 day after bleeding. As SWI are derived from T2 weighted sequences a similar behaviour of intra-tumoral SWI_{pos} can be assumed. Intra-tumoral hemorrhage at the time of tumor detection may therefore be present for several days or even longer. Whether single or multiple small intra-tumoral hemorrhages contribute to persisting or increasing neurological dysfunction as e.g. in patients with amyloid angiopathy cannot be answered at the present time [15]. However cortical SWI_{pos} in our study was significantly associated with the presence of seizures in brain metastasis, and as a trend in glioblastoma. This would be in line with studies in animals and patients with vascular malformations. Intra-cortical injection of hemoglobin or ferric chloride in rats leads to persisting spike activity and seizures which occur 2 to 8 days after injection [2, 3]. In patients with resected cavernoma areas with hemosiderin deposits show neuronal loss, glial proliferation and upregulation of NMDA receptor subunits to a magnitude considered to be able to promote seizures [16]. Accordingly cortical SWI_{pos} may indicate a role for hemosiderin in the epileptogenicity of

glioblastoma and brain metastasis. It has been proposed that disruption of the BBB per se induces seizures by extravasation of albumine and activation of transforming growth factor beta (TGFβ) [17]. Whether this assumption is relevant to patients with brain tumors has not been investigated. In our series all patients with glioblastoma or brain metastasis showed BBB disruption, but only 43 and 72 % respectively experienced seizures. Our findings therefore do not support the view that BBB disruption or cortical involvement alone explain the epileptogenicity of these brain tumors.

Cortical tumor location represented a strong factor associated with pre-operative seizures in a series of 508 patients with low-grade glioma [18]. In line with this finding we found in our small subgroup of low-grade glioma patients a high percentage of cortical involvement, but only few tumors with SWI_{pos}. Therefore biochemical alterations other than hemosiderin deposits may account for the occurrence of seizures in these tumors. In general changes in pH, amino acid composition, NMDA receptor status have been discussed to be involved in epileptogenesis [6]. However, all these metabolic changes cannot be assessed in a non-invasive manner in human patients. Therefore they cannot serve as in vivo markers to determine the risk for seizures. From a methodological point of view it can be assumed that SWI_{pos} in fact reflects hemosiderin if mIP and Phase images exclude tumor calcification and flow signal. However, prospective tissue validation of SWI_{pos} in brain tumors is required. Preliminary data of our group obtained from a retrospective series indicate a high specificity to identify hemosiderin using susceptibility weighted MRI [19].

The limitation of our study is the small number of patients, particularly of low-grade gliomas and anaplastic astrocytomas, which however reflects their incidence in the general population and therefore in our clinical routine. Cortical SWI_{pos} as an independent predictor of subsequent seizures should lead to re-evaluate the use of prophylactic anticonvulsants in patients showing this feature. From our results we propose to longitudinally investigate the presence and evolution of SWI_{pos} with particular focus on glioblastoma and brain metastasis in larger patient cohorts. Several issues may contribute to hemosiderin deposits during the course of brain tumors. SWI_{pos} is often detected after brain tumor surgery. Irradiation can result in vascular leakage and microbleeds [20, 21]. In addition hemorrhage may also result from anti-angiogenic therapy, uncontrolled tumor growth and from the appearance of new brain metastasis. In a recent study on patients with newly diagnosed brain metastasis from malignant melanoma 94 % of lesions involved the cerebral cortex, and 54 % were hemorrhagic [22]. In those patients who initially did not present with seizures the risk to develop seizures within three

months after the time of the diagnosis was 17 % if no prophylactic anticonvulsants were administered. In contrast no patient treated with prophylactic anticonvulsants suffered from seizures during that period. It appears worthwhile to prospectively assess whether cortical SWI_{pos} represents a risk factor for the occurrence of seizures, and whether cortical SWI_{pos} determines the risk for seizure relapse once anticonvulsants are discontinued after surgery.

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