Brain tumours, brain metastases, and neuroinflammation: Insights from neuroimaging studies.

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September 24, 2021

Abstract

Brain tumors and brain metastases induce changes in brain tissue remodelling that lead to immunosuppression and trigger an inflammatory response within the tumor microenvironment. These immune and inflammatory changes can influence invasion and metastasis. Other neuroinflammatory and necrotic lesions may occur in patients with brain cancer or brain metastases as sequelae from treatment with radiotherapy. Glioblastoma (GBM) is the most aggressive primary malignant brain cancer in adults. Imaging methods such as positron-emission tomography (PET) and different magnetic resonance imaging (MRI) techniques are highly valuable for the diagnosis and therapeutic evaluation of GBM and other malignant brain tumors. However, differentiating between tumor tissue and inflamed brain tissue with imaging protocols remains a challenge. Here, we review recent advances in imaging methods that have helped to improve the specificity of primary tumor diagnosis versus evaluation of inflamed and necrotic brain lesions. We also comment on advances in differentiating metastasis from neuroinflammation processes. Recent advances include the radiosynthesis of 18F-FIMP, an L-type amino acid transporter 1 (LAT1)-specific PET probe that allows better differentiation between tumor tissue and inflammation compared to previous probes; and the combination different advanced imaging protocols with the inclusion of radiomics and machine learning algorithms.

Clinical & Experimental Immunology

Invited Review for Special Issue on "Imaging Immunological Processes in Neuroinflammatory Diseases"

REVIEW ARTICLE

Neuroinflammation and immunoregulation in glioblastoma and brain metastases: recent developments in imaging approaches

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Summary

Brain tumors and brain metastases induce changes in brain tissue remodelling that lead to immunosuppression and trigger an inflammatory response within the tumor microenvironment. These immune and inflammatory changes can influence invasion and metastasis. Other neuroinflammatory and necrotic lesions may occur in patients with brain cancer or brain metastases as sequelae from treatment with radiotherapy. Glioblastoma (GBM) is the most aggressive primary malignant brain cancer in adults. Imaging methods such as positron-emission tomography (PET) and different magnetic resonance imaging (MRI) techniques are highly valuable for the diagnosis and therapeutic evaluation of GBM and other malignant brain tumors. However, differentiating between tumor tissue and inflamed brain tissue with imaging protocols remains a challenge. Here, we review recent advances in imaging methods that have helped to improve the specificity of primary tumor diagnosis versus evaluation of inflamed and necrotic brain lesions. We also comment on advances in differentiating metastasis from neuroinflammation processes. Recent advances include the radiosynthesis of ^{18}F-FIMP, an L-type amino acid transporter 1 (LAT1)-specific PET probe that allows better differentiation between tumor tissue and inflammation compared to previous probes; and the combination different advanced imaging protocols with the inclusion of radiomics and machine learning algorithms.

Keywords Glioblastoma; Brain metastases; Magnetic Resonance Imaging; Positron-Emission Tomography; Imaging; Brain tumor

Introduction

Neuroimaging techniques are crucial tools for diagnosing, staging, and monitoring the treatment effects in patients with brain cancers [1]. Structural magnetic resonance imaging (MRI) helps to identify, classify, and grade brain tumors, as well as to guide surgery [2]. Complementary information is obtained with positron emission tomography (PET) imaging, where insights on tissue metabolism can be evaluated, which is particularly valuable for the measurement of fast cell proliferation in tumors and investigation of early-stage tumors [3-6].

Despite the current efficiency of these imaging techniques in brain tumor detection, some limitations remain to be overcome. For example, differentiating among relapsed brain tumors, solitary brain metastases, and inflammatory and necrotic lesions resulting from chemo- and radiotherapy remains a challenge. For example, the most commonly used PET probe for tumor imaging is 2-Deoxy-2-^{18}F-fluoro-D-glucose (FDG), which is actively transported into cells via glucose transporters (GLUTs), accumulating not only in tumor tissue, which contains inflammatory processes itself, but also in other inflamed areas, where glucose metabolism is crucial for neuroinflammatory and neuroimmune responses. This can lead to false positive results for tumor diagnosis [7-9]. Also, both tumors and metastatic sites can display MRI patterns featuring peritumoral hyperintensities with similar intratumoral texture, thus representing a potential confounder for neuroradiologists [10]. In this review, we will discuss selected recent advances in imaging approaches aimed at improving the evaluation of brain tumors and brain metastases in relation to immunomodulation and neuroinflammatory processes. We will focus specifically on one type of brain tumor, glioblastoma (GBM), given that it represents the most aggressive primary malignant brain cancer in adults.

Glioblastoma (GBM)

The current World Health Organization (WHO) classification divides GBM tumors into three groups depending on the status of the isocitrate dehydrogenase (*IDH*) gene: *IDH* wild-type (IDHwt) GBM, mutated *IDH* , or not specified GBM (NOS, unevaluated status). IDHwt GBMs represent around 90% of cases and typically appear in older patients, whereas around 10% of patients present tumors with an*IDH* mutation, which correspond to secondary GBMs which originate from lower grade gliomas, occur in younger patients, and have a better prognosis. Furthermore, GBMs are defined by other genetic biomarkers, including O6-methylguanine DNA methyltransferase (*MGMT*) promoter methylation, chromosome 1p/19q deletion, mutations of telomerase reverse transcriptase (*TERT*) promoter, tumor protein P53 (*TP53*), and phosphatase and tensin homolog (*PTEN*), and amplification of epidermal growth factor receptor (*EGFR*) and platelet-derived growth factor receptor A (*PDGFRA*). The prognosis for patients with GBM is dismal; current treatment, based on combining surgical resection, radiotherapy and adjuvant chemotherapy still

results in a median overall survival of less than 2 years [11-15].

Immunoregulation and inflammation in glioblastoma: brain tumors as neuroinflammatory diseases

Inflammation is an integral component of cancer pathology, contributing to carcinogenesis and tumor progression. One common feature of GBM is tissue necrosis accompanied by microenvironment inflammation. Immunosuppressive inflammation with associated necrosis is typical of GBMs that display higher resistance to therapies and worst prognosis [16-20]. GBM cells express and secrete immune suppressive chemokines and cytokines including interleukin-6, interleukin-10, transforming growth factor (TGF)-β, galectin-1, and prostaglandin-E, which act on infiltrating immune cells to hijack them by inducing a pro-tumor cellular phenotype. Thus, a population of non-neoplastic cells consisting mostly of tumor-associated macrophages, is established in the tumor site. These immunosuppressive changes enabled by inflammatory mediators stimulate GBM cell proliferation, migration, angiogenesis, and resistance to treatment. For example, signaling mediated by CXCR3 and CCR2 receptors recruit tumor-promoting immune cells such as T cells and myeloid-derived suppressor cells [16, 21-28]. In GBM cancer stem cells (CSCs), hypoxic tissue triggers expression of genes of the inflammatory reparative response [25, 29]. Cytokines such as interleukin (IL)-1β and TGF-β cooperate to induce inflammatory gene expression and a proinflammatory phenotype in GBM CSCs, which in turn facilitates cellular proliferation and migration [30].

Neuroinflammation as a consequence of GBM treatment

Inflammation and necrosis of healthy brain tissue is one of the main unwanted effects of radiotherapy used to treat GBM. Studies have found incidences of radiation-induced necrosis ranging from 2.5% to about 30% of GBM patient cohorts [31-33]. Necrosis can include underlying inflammation together with occlusive vasculopathy and perivascular parenchymal changes. Alterations in the integrity of the blood-brain barrier in inflamed sites leads to immune cell infiltration, fluid transudation into the interstitial space and brain edema. Infiltrated immune cells as well as reactive glial cells increase expression of mediators, amplifying the response [31, 34-36].

Consequences of inflammation for imaging diagnosis: differentiating between tumors and inflammatory lesions

Conventional neuroradiological techniques do not always allow a differentiation between radiation necrosis and GBM recurrence, given that both recurrent tumors and necrosis (pseudoprogressions) may appear as similar lesions in MRI protocols such as T2- and gadolinium-enhanced T1-weighted imaging, with an increase in contrast enhancement and the image of a mass [31, 37-42]. PET imaging using ^{18}F-Fluorodeoxyglucose (FDG-PET), in turn, can be useful in differentiating radiation-induced lesions from recurrent GBM [31, 43]. However, with this method false-positive and false-negative PET scan results can lead to low sensitivity and pose a severe limitation [44].

Imaging advances for the evaluation of brain tumors and neuroinflammation

The measurement of metabolic and cell physiology parameters with improved MRI techniques provides more detailed information on tissue biochemical composition and blood perfusion, facilitating the distinction between tumors and necrosis. Proton magnetic resonance (MR) spectroscopic imaging is used to evaluate cell metabolism through the detection of proton metabolites and their distribution. Chemicals detected include compounds containing choline, creatine, lactate, lipid, and N-acetylaspartate. Choline, a component of cell membrane phospholipids, is particularly useful for differentiating tumors from non-tumoral tissues because its content is increased in highly proliferative cell populations. Overall, higher choline levels are associated with disease progression or recurrence, whereas low levels of choline are found in necrotic lesions. The analysis of increased ratios of choline content in relation to other chemicals can result in an accuracy of up to 97% in separating tumors from non-tumoral necrotic tissues [31, 42].

Conventional MRI, with the T1/T2 mismatch criterion, had a specificity of 75% and a sensitivity of only 44% in distinguishing between tumors and inflamed lesions. PET scan combined the best sensitivity and speci-

ficity, respectively of 92% and 69%. PET remained superior compared to NMR spectroscopy for choline/N-acetylaspartate and choline/creatin ratios across different thresholds [45]. A retrospective study of 57 GBM patients examined with T2*-weighted dynamic susceptibility-weighted contrast material–enhanced (DSC) MRI found mean, maximum, and minimum relative peak height and relative cerebral blood volume were significantly higher in GBM cases compared to radiation necrosis cases. In contrast, mean, maximum, and minimum relative percentage of signal intensity recovery values were significantly lower in recurrent GBM compared to radiation necrosis [46]. Proton MR spectroscopy showed a temporary elevation of choline in 4 of 9 cases of necrosis, creating a confounding factor that could result in false positive findings for tumor recurrence [47]. Another study using proton MR spectroscopy in 11 patients who received high-dose radiotherapy revealed that cases of radiation necrosis had either increased lactate/creatine and phosphocreatine (Cr) ratio and decreased choline-containing compounds/phosphocreatine ratio compared to recurrent GBM, or reductions in all major metabolites [48]. A meta-analysis of 397 patients in 13 studies examined roles of several metabolites. MR spectroscopy and MR perfusion using Cho/NAA and Cho/Cr ratios and rCBV may increase the accuracy of differentiating necrosis from recurrent tumor in patients with primary brain tumors or brain metastases [49].

A meta-analysis of 6 studies with 118 patients and 134 scans indicated^{11}C-choline PET as an accurate diagnostic method for the differentiation of tumor relapse from radiation induced necrosis in gliomas [50]. A study with 55 patients, followed up for at least 11 months, with suspected brain tumor recurrence or necrosis after radiotherapy, examined MRI, (^{18})F-FDG, and^{11}C-choline PET/CT, concluding for the superiority of^{11}C-choline PET/CT [51]. In a F98 orthotopic rat model of GBM, PET using (^{18})F-FDG and (^{18})F-FET PET were effective in discriminating GBM from radiation necrosis, with (^{18})F-FDG delayed PET being particularly useful [52]. A study with 50 patients showed that (^{11})C-methionine-PET was superior to both (^{11})C-choline and (^{18})F-FDG -PET for distinguishing GBM recurrence from radiation necrosis [53]. The LAT1 tumor-specific PET tracer 2-[^{18}F]FELP PET is able to differentiate glioblastoma from radiation necrosis, and 2-[^{18}F]FELP uptake is less likely to be contaminated by the presence of inflammation than the [^{18}F]FDG signal [35].

Differentiation of brain tumors from brain infections and abscesses

Brain abscesses caused by aerobic bacteria can mimic GBM tumors in MRI image appearance and single voxel proton MR spectroscopy, leading to misdiagnosis. Based on the hypothesis that metabolite levels of choline would be decreased in the ring-enhancing portion of abscesses compared to GBM, one study using MR spectroscopic imaging found that metabolite ratios and maximum choline/choline-n, choline/creatine, and choline/ N-acetylaspartate ratios of the contrast-enhancing rim could be useful differentiating aerobic abscesses from GBM. In MRI procedures, the apparent diffusion coefficient (APC) may be useful in differentiating GBM from brain fungal abscess. Combining diffusion tensor imaging (DTI) and dynamic susceptibility contrast perfusion-weighted imaging (DSC-PWI) provides better results in differentiating lesions from brain infections than each technique used alone [54-56].

Brain metastases, inflammation, and imaging

It is worth noting that CSCs constitute the main cell population mediating metastasis [57]. Brain metastases are more common than tumors originating primarily in the brain and confer grave prognosis to patients with various types of primary cancers originating in other sites, such as lung, breast, colorectal, and melanoma, given that available treatments show very limited efficacy. The tumor microenvironment is crucial to determine the establishment of brain metastases, and within this context, neuroinflammation plays a central role [58].

Brain metastases can produce a tissue lesion that induces a response comprising persistent astrocyte and microglia activation with cytokine and chemokine release, increased blood vessel permeability and recruitment of immune cells, resulting in neuroinflammation [58-61]. Also, non-neoplastic inflamed sites in the brain may facilitate the adhesion of circulating tumor cells from peripheral tumors to the activated endothelium in brain vessels, which is one of the possible mechanisms promoting metastasis initiation [62]. Inflammation

at distant sites promotes adhesion of CTCs to the activated endothelium and then initiates the formation of metastases. Many different types of mediators and immune cells are involved in brain metastasis, depending among other factors on the type of primary tumor of origin, metastatic site in the brain, and differential astrocyte and microglial activation, resulting in high biological heterogeneity [58, 63-67].

In terms of imaging, differentiating brain tumors from brain metastases pose a challenge by itself, as both present imaging patterns with similar peritumoral hyperintensities and intratumoral texture on MRI. Improvements have been obtained with the use of relative cerebral blood flow and volume analyses, diffusion tensor imaging, neurite orientation dispersion, density imaging to examine intracellular volume fraction, isotropic volume fraction, and extracellular volume fraction, and metabolite analysis with MR spectroscopy. Also, intratumoral creatine suggests GBM, whereas its absence indicates metastasis when single-voxel proton MR spectroscopy is used for imaging [68, 69]. A study examining the use of the mean apparent diffusion coefficient and absolute standard deviation derived from apparent diffusion coefficient measurements based on cellularity levels could not find marked differences between GBM tumors from brain metastasis [70]. However, another study found that the apparent diffusion coefficient could differentiate GBM from metastasis, and that homogeneity and the inverse difference moment of GBM were significantly higher than those of metastases in the regions of interest placements examined [71, 72].

Assessing the heterogeneity of both the tumor masses and peritumoral edema with MRI texture analysis revealed that the heterogeneity of the GBMs peritumoral edema was significantly higher than the edema surrounding MET, differentiating them with a sensitivity of 80% and specificity of 90% [73]. Combining arterial spin labeling perfusion (ASL)- and diffusion tensor imaging (DTI)-derived metrics showed to be promising in differentiating GBM and solitary brain metastases [74]. The use of 2D texture features extracted from images obtained with MRI may be a useful alternative for discriminating between GBM and brain metastases [75]. Computational-aided quantitative analysis of MRI images may improve the accuracy in differentiating GBM from metastases, and texture features are more significant than fractal-based features for that purpose [76]. Increasingly, machine learning algorithms have been applied to imaging data to improve the differentiation between GBM and brain metastases [77-81]. Novel diagnostic support systems based on radiomic features extracted from post-contrast 3DT1 MR images may help improving the distinction between solitary brain metastases and GBM with high diagnosis performance and generalizability [77]. Machine learning and deep learning-based models applied to conventional MR images may support preoperative discrimination between GBM and solitary brain metastasis conventional MR images [78-80], and deep learning network models that allow automated, on-site analysis of resected tumor specimens based on confocal laser endoscopic techniques image datasets have been developed [81]. Other parameters such as the cerebral blood volume gradient in the peritumoral brain zone may enable the differentiation of GBMs from metastases [82]. Another approach is to evaluate peritumoral areas with color map of phase difference enhanced imaging (Color PADRE) [83].

As with tumors originating in the brain, metastases are treated with radiotherapy and stereotactic radiosurgery, making incidence of radiation necrosis an important issue, and the distinction between metastasis and inflamed and necrotic tissue by MRI can be challenging. One study examined the hypothesis that methionine levels could be increased in metastatic tissue, whereas the inflammation marker translocator protein (PBR-TSPO), which can be quantified with specific PET tracers, would be increased in necrosis. Thus, the use of the [^{11}C]methionine and [^{11}C]PBR28 tracers in PET was evaluated in 5 patients previously treated brain metastases showing regrowth. The use of [^{11}C]methionine could accurately identify pathologically confirmed tumor regrowth in all 7 lesions examined, whereas [^{11}C]PBR28 could only identify 3 of 7 lesions, indicating that the former, but not the later tracer can be used as a reliable marker [84].

Concluding remarks

As discussed above, the differentiation between GBM recurrence and radiation necrosis remains a crucial challenge (Fig. 1), given that the need for additional surgery in a previously operated brain region increases surgical risk in addition to raising treatment costs. An important current tool is multiparametric MRI using a combination of perfusion and diffusion parameters. Although previous studies [85-91] had found that apparent diffusion coefficient, volume transfer constant, and relative cerebral blood volume are very useful,

and the latter is the strongest parameter for differentiating radiation necrosis from GBM tumors [92]. Nael et al. [93] achieved a diagnostic accuracy of 92.8% by combining the use of volume transfer constant and relative cerebral blood volume.

In daily neuro-oncological practice, multiparametric MRI is the most used tool to achieve an accurate differential diagnosis between GBM, inflamed sites, and other brain diseases. Despite its advantages, the use of PET Scan remains limited in many regions of the world due to economical and logistic shortcomes. Overall, the integrated use of different MR and PET approaches, including multiparametric MRI, in addition to perfusion and diffusion parameters [94] may enable increasingly accurate diagnosis based on brain imaging.

Acknowledgements

The writing of this article was supported by the National Council for Scientific and Technological Development (CNPq, MCTI, Brazil) grant 305647/2019-9 (R.R.); and The Center for Advanced Neurology and Neurosurgery (CEANNE)-Brazil (S.A.D., G.R.I.).

Disclosure

The authors declare no conflicts of interest.

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Legend for Figure 1

Fig. 1. Illustrations of **A.** A pseudoprogression resulting from radiation-induced inflamed necrosis. MR images taken post-operatively show axial T2, FLAIR, T1 contrast-enhanced, and perfusion, with enhancement in the right temporal lobe, areas of T2/FLAIR hyperintensity in the surrounding parenchima, indicating a lesion induced by radiation therapy. There is no increase in relative cerebral blood volume in the enhancing portion of the lesion, which further suggests pseudoprogression. **B.** A tumor relapse shown by images taken from the same patient three months later. MR images taken post-operatively show axial T2, FLAIR, T1 contrast-enhanced, and perfusion, with heterogeneous enhancement in the right temporal lobe and great extension of the T2/FLAIR hyperintensity area, indicating a combination of tumor spread and vasogenic edema involving the right peritrigonal and insular regions. There is increased relative cerebral blood volume in the enhancing portion of the lesion, representing tumoral progression.