A novel tool to analyze MRI recurrence patterns in glioblastoma

Wolfgang Wick, Roger Stupp, Anna-Carina Beule, Jacoline Bromberg, Antje Wick, Ulrike Ernemann, Michael Platten, Christine Marosi, Warren P. Mason, Martin van den Bent, Michael Weller, Chris Rorden, and Hans-Otto Karnath, on behalf of the European Organisation for Research and Treatment of Cancer and the National Cancer Institute of Canada Clinical Trials Group

Department of General Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany (W.W., A.-C.B., A.W., M.P., M.W.); Department of Neurooncology, University of Heidelberg, Heidelberg, Germany (W.W., M.P.); University of Lausanne Hospitals, Multidisciplinary Oncology Center, Lausanne, Switzerland (R.S.); Neuro-Oncology Unit, Daniel den Hoed Oncology Center, Rotterdam, The Netherlands (J.B.); Department of Neuroradiology, University of Tübingen, Tübingen, Germany (U.E.); Department of Internal Medicine I, Clinical Division of Oncology, Medical University Vienna, Vienna, Austria (C.M.); Princess Margaret Hospital, Toronto, ON, Canada (W.P.M.); Department of Neuro-Oncology, Erasmus MC, Rotterdam, The Netherlands (M.v.d.B.); Department of Communication Sciences and Disorders, University of South Carolina, Columbia, SC, USA (C.R.); Section Neuropsychology, Department of Cognitive Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany (H.-O.K.)

At least 10% of glioblastoma relapses occur at distant and even contralateral locations. This disseminated growth limits surgical intervention and contributes to neurological morbidity. Preclinical data pointed toward a role for temozolomide (TMZ) in reducing radiotherapy-induced glioma cell invasiveness. Our objective was to develop and validate a new analysis tool of MRI data to examine the clinical recurrence pattern of glioblastomas. MRIcro software was used to map the location and extent of initial preoperative and recurrent tumors on MRI of 63 patients in the European Organisation for Research and Treatment of Cancer (EORTC) 26981/22981/National Cancer Institute of Canada (NCIC) CE.3 study into the same stereotaxic space. This allowed us to examine changes of site and distance between the initial and the recurrent tumor on the group level. Thirty of the 63 patients were treated using radiotherapy, while the other patients completed a radiotherapy-plus-TMZ treatment. Baseline characteristics (median age, KPS) and outcome data (progression-free survival, overall survival) of the patients included in this analysis resemble those of the general study cohort. The patient groups did not differ in the promoter methylation status of *methyl guanine methyltransferase* (*MGMT*). Overall frequency of distant recurrences was 20%. Analysis of recurrence patterns revealed no difference between the groups in the size of the recurrent tumor or in the differential effect on the distance of the recurrences from the preoperative tumor location. The data show the feasibility of groupwise recurrence pattern analysis. An effect of TMZ treatment on the recurrence pattern in the EORTC 26981/22981/NCIC CE.3 study could not be demonstrated. *Neuro-Oncology 10, 1019–1024, 2008 (Posted to Neuro-Oncology [serial online], Doc. D08-00051, August 4, 2008. URL http://neuro-oncology.dukejournals.org; DOI: 10.1215/15228517-2008-058)*

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Address correspondence to Wolfgang Wick, Department of Neurooncology, University of Heidelberg, Im Neuenheimer Feld 400, D-69120 Heidelberg, Germany (wolfgang.wick@med.uni-heidelberg.de).

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Patients with malignant glioma face a poor prognosis, with current treatments offering limited benefits. However, recent preclinical and clinical studies have improved our understanding of gliomas. Longitudinal analyses of glioma patients revealed patterns of disease progression that resemble key stages in neurogenesis, indicating that the same signaling pathways that play a critical role in regulation of forebrain neurogenesis may be involved in the control of tumor aggressiveness.[1] Clinically, neurooncologists were limited to the assessment of overall survival or immediate response to therapy[2] or to comparisons on a single-case basis.[3]

A major challenge in the management of gliomas is their propensity to infiltrate healthy brain tissue. Infiltrating neoplastic cells escape surgery and develop resistance to radiation and/or chemotherapy. Further, as a consequence of treatment, tumor cells may respond with increased migratory and invasive properties. Assessing tumor response based solely on anatomic representation of contrast enhancement on MRI by World Health Organization criteria or even when taking into account the neurological function and performance status of the patient (Macdonald criteria) will miss any of these changes in tumor behavior.[2]

Novel imaging techniques and algorithms using perfusion MRI and MR spectroscopy identify areas with enhanced perfusion and metabolism within a morphologically homogeneous tumor.[4] Diffusion-tensor imaging has helped to identify more precisely the association between tumor and anatomic fiber tracts and to correlate functions to the anatomical tumor location.[5] However, these previous studies have focused on individual patients or on a single time point. We conducted a longitudinal study of a group of patients treated within a prospective clinical trial, allowing us also to examine whether the pattern of recurrence was different after combined radiochemotherapy versus radiotherapy only.

To achieve this aim, we developed a new tool based on MR imaging to analyze the efficacy of brain tumor treatments by measuring the size and location of tumor recurrences, allowing us to understand the typical pattern of tumor development after different treatment modalities. This method uncovers common and uncommon anatomical recurrence patterns in relation to the administered treatment. This may lead to better understanding of tumor progression, and provides a radiological tool for studying and quantifying the effect of novel therapeutic interventions, in particular, regarding glioma cell migration and invasiveness.

To validate this concept, we analyzed the MRI data from a randomized trial (European Organisation for Research and Treatment of Cancer [EORTC] 26981/22981/National Cancer Institute of Canada [NCIC] CE.3) comparing standard radiotherapy with radiotherapy plus concomitant and adjuvant temozolomide (TMZ) chemotherapy in newly diagnosed glioblastoma.[6] This cohort was ideal for validating our tool since there is preclinical evidence that irradiation enhances glioma cell dissemination and that TMZ has specific antiinvasive properties in addition to its antiproliferative action.[7,8] By comparing MR data on tumor location obtained prior to any therapy and at recurrence or progression of the tumor, we addressed (1) whether there are more local recurrences in patients with combined modality treatment because TMZ might antagonize the proinvasive properties of therapeutic irradiation, and (2) whether there are more distant recurrences in patients with combined modality treatment because of the prolonged median time to progression in this arm.

Materials and Methods

Patients and Eligibility

Patients (n = 112) from Lausanne, Rotterdam, Toronto, Tübingen, Utrecht, and Vienna were available for screening from the EORTC 26981/22981/NCIC CE.3 trial.[6]

Analysis of Tumor Location

All 63 patients had a glioblastoma unilaterally affecting either the right or the left hemisphere. The brain lesions were demonstrated by contrast-enhanced T1-weighted MRI sequences. The MR scans were oriented along the bicommissural plane. Mapping of lesions was performed by two experimenters (A.-C.B. and W.W.) blinded to the clinical features of the patients. The boundary of the tumor location at baseline and at follow-up was delineated using MRIcro software[9] and mapped on the T1-template MRI from the Montreal Neurological Institute (MNI; www.bic.mni.mcgill.ca/cgi/icbm_view) that is distributed with MRIcro. The template scan provides various anatomical landmarks for precisely plotting localization of the tumor. Lesions were mapped onto the slices that correspond to MNI z-coordinates –37, –29, –21, –13, –4, 4, 13, 21, 30, 38, 47, 55, 64, and 72 mm by using the identical or the closest matching transversal slices for each individual. Tumors were mapped for each individual, with separate tumor maps generated for both the baseline and recurrence scan. By transforming each individual brain and lesion into the same stereotaxic space, the procedure allowed us to superimpose lesions of different individuals to find regions of mutual involvement and conduct subtraction analysis (Fig. 1). These techniques are well established in stroke research,[9,10] and we applied them here to tumor lesions for the first time.

The logic of the analysis is straightforward. First, tumor lesions for a patient group at baseline and lesions for this group at recurrence are defined on the template image described above. Next, the lesions at baseline are added together, creating an overlap image showing the regions of mutual involvement. The same is carried out for the lesions at recurrence. Finally, the lesion overlap image at recurrence is subtracted from the lesion overlap image at baseline. This method creates an image that shows regions that are commonly damaged in the

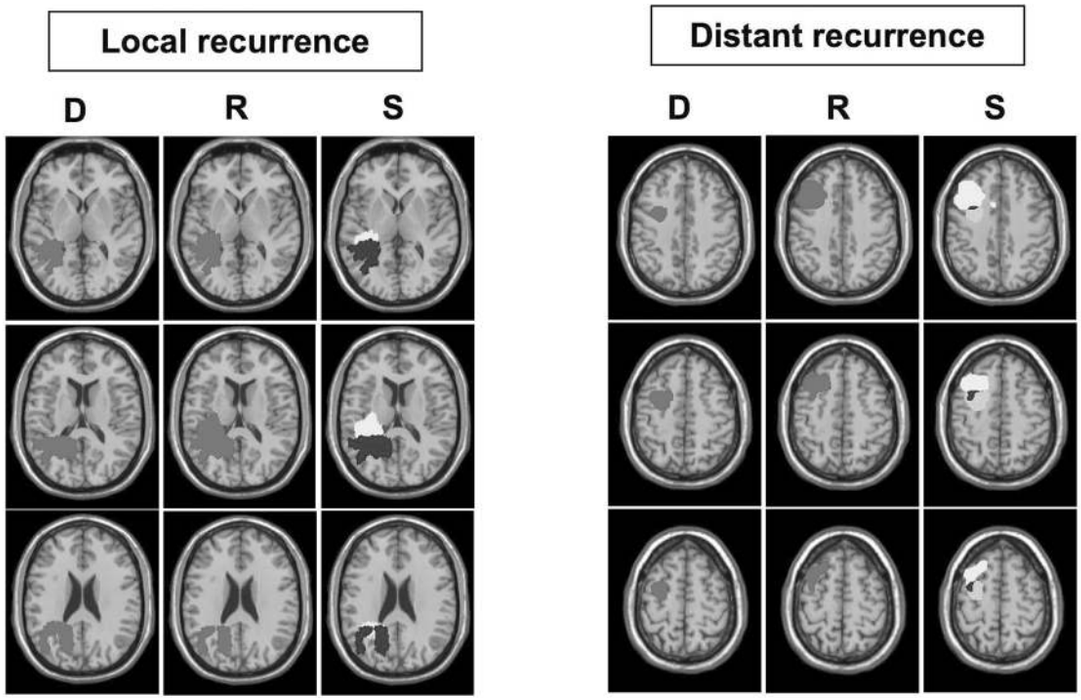

Fig. 1. Overlay tumor location plots of a patient with local versus distant recurrence: initial tumor locations in three transverse sections at diagnosis (D), at recurrence after treatment (R), and superimposed (S) for a prototypic local recurrence (left) as well as a prototypic distant recurrence (right).

patient group at recurrence but are typically spared in this group at baseline (coded as positive values), regions specifically damaged at baseline (coded as negative values), and regions that are damaged/spared in equal proportions between the two stages (values near zero). For example, a brain region that is affected by the tumor in all patients at recurrence (100%) and three-quarters of patients at baseline (75%) would have a value of +25% (100% – 75%) in the subtraction plot. The results can be graphically plotted on the same template image; we used progressively brighter shades from red to white to highlight positive values and progressively brighter shades of blue to illustrate negative values. Regions with a value of zero (with either equal numbers of lesions at baseline and recurrence, or no observed lesions at baseline or recurrence) remain uncolored.

Further, by using MRIcro,[10] tumor volume and the location of the center of mass of the tumor for each individual were computed. The center of mass is the mean position for all tumor-affected voxels in each of the three spatial dimensions, resulting in a single Cartesian coordinate (x,y,z-position). In the case of a single spherical tumor, the center of mass thus will be located right in its center; with, for example, a U-shaped configuration, the center of mass may lie outside the tumor itself. Likewise, this measure is influenced by satellites of the main tumor mass and thus sensitive to the development of satellites between baseline and recurrent images. Because all lesions were coded in the same stereotaxic space, as described above, this procedure allowed us to calculate the anatomical distance between the initial and the recurrent tumor on the group level.

For an additional case-by-case analysis, a distant recurrence was defined as a recurrence with at least 50% of the tumor mass located outside the borders of the contrast-enhancing tumor on T1-weighted images plus 2 cm margin.

Ethics

All participating centers obtained approval for the conduct of the study and further studies by their institutional review board according to local and national regulations. All patients provided written informed consent.

Statistics

A two-tailed t-test was conducted to determine whether the two treatments influenced the size of recurrent tumors and/or the distance of the center of mass between the baseline and follow-up scan. A sample size of 30 per group would be sufficient to detect a 20% difference for the movement of the center of mass with a power of 80% that would be regarded clinically relevant.

Results

Because of different imaging modalities at diagnosis and recurrence or because MRI scans were missing, 72 patients with localized focal contrast-enhancing disease

Table 1. Baseline characteristics of analyzed patients

Variable	Radiotherapy ($n = 33$)	Radiotherapy plus TMZ ($n = 30$)
Median age	56 years	56 years
KPS (*n*)	70/80 (13)	70/80 (12)
	90/100 (20)	90/100 (18)
Debulking surgery (*n*)	19	18
Methyl guanine methyltransferase promoter:[14] methylated/unmethylated	10/12	10/13
Median progression-free survival (95% confidence interval)	5.2 (4.2–5.5) months	7.1 (5.8–8.2) months
Median overall survival (95% confidence interval)	12.0 (11.2–13.2) months	14.4 (13.4–16.8) months

were included in our analyses, all of whom exhibited primarily unilocular contrast-enhancing tumors. Nine patients had to be excluded because marked mass effect and midline shift did not allow the use of the MNI template for the present anatomical analysis. Thus, 63 patients with glioblastoma treated in the radiotherapy group ($n = 33$) or in the radiotherapy-plus-TMZ group with concomitant and adjuvant TMZ ($n = 30$) were analyzed. Clinical characteristics are summarized in Table 1. Importantly, patients in the two groups did not differ with respect to extent of resection or *methyl guanine methyltransferase* (*MGMT*) promoter methylation status. The median time between imaging used for the baseline MRI scanning and first histological diagnosis was 0.2 months in both groups. The median time between baseline MRI and the MRI demonstrating recurrence was 5.4 months in the radiotherapy group and 7.3 months in the radiotherapy-plus-TMZ group, comparable to that for the whole study cohort.

Groupwise Analysis of Recurrence Patterns

Groupwise analysis demonstrates wide spread of tumor location at baseline and at recurrence in the two treatment groups. Tumors affected the temporal, parietal, and frontal cortices in the left and right hemisphere without obvious preferred locations of tumor origin or growth (Figs. 2a and 3a). To investigate whether there were preferred directions of tumor growth or of tumor reduction after treatment in the radiotherapy versus the radiotherapy-plus-TMZ group, the tumor locations at baseline were subtracted from the superimposed tumor locations after treatment in each treatment group (for details concerning the subtraction method, see Rorden and Karnath[11]).For both treatment groups, we found no marked anatomical shift of tumor locations after treatment (Figs. 2b and 3b). The overlap frequencies after subtraction did not exceed 40% of overlap at any location, indicating that the anatomical differences between baseline and follow-up measurement were small and not directionally specific.

No Change in the Size or Recurrence Pattern of the Recurrent Tumors

Tumor volumes of the recurrent tumors measured in voxels on a per-patient basis in both treatment arms were 1.32-fold (±0.36) larger than the original tumors in the radiotherapy and 1.29-fold (±0.37) larger in the combined modality arm. Hence, the sizes of the recurrent tumors did not differ according to treatment ($p = 0.81$). The center-of-mass measure allowed us to examine whether treatment influences the recurrence at distant sites. For each individual, the distance between baseline and follow-up centroids was computed, providing a measure of whether the tumor exhibited little movement (e.g., when changes primarily reflect growth or remission of the initial tumor) or a large shift in location (e.g., when new satellite tumors have developed). A *t*-test was

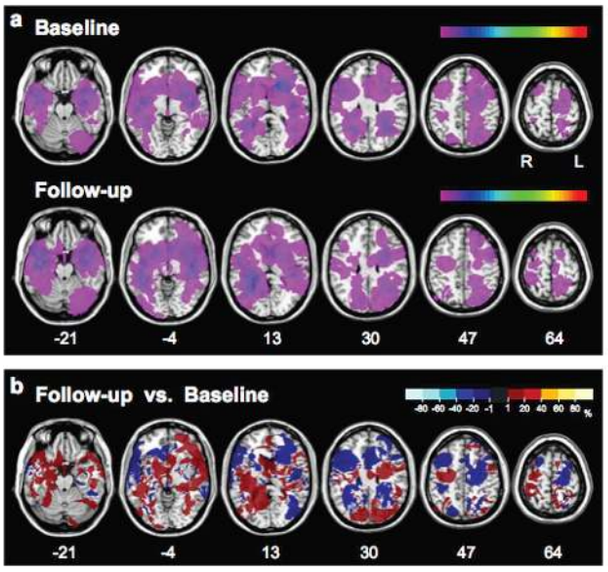

Fig. 2. (a) Overlay tumor location plots of the patients treated with radiotherapy ($n = 33$) at baseline and after treatment. The number of overlapping lesions is shown by different colors coding increasing frequencies from violet ($n = 1$) to red ($n = 33$). (b) Overlay subtraction plot for the group of patients treated with radiotherapy: superimposed tumor locations after treatment (follow-up) minus tumor locations at baseline (baseline). The percentage of overlapping lesions after subtraction is illustrated by five different colors coding increasing frequencies from dark red (difference = 1%–20%) to white-yellow (difference = 81%–100%), in 20% increments. The different colors from dark blue (difference = –1% to –20%) to light blue (difference = –81% to –100%) indicate regions affected by the tumor more frequently before treatment compared to tumor progression. Montreal Neurological Institute *z*-coordinates of the transverse sections are given.

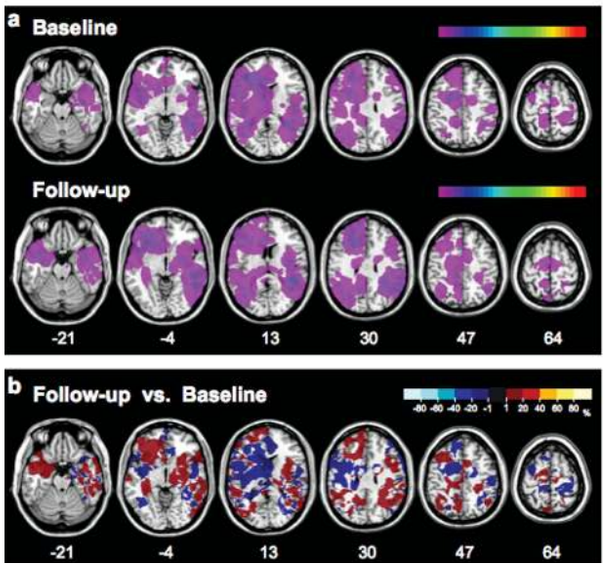

Fig. 3. (a) Overlay tumor location plots of the patients treated in the radiotherapy-plus-temozolomide (TMZ) group ($n = 30$) at baseline and at recurrence. The number of overlapping lesions is shown by different colors coding increasing frequencies from violet ($n = 1$) to red ($n = 30$). (b) Overlay subtraction plot for the group of patients treated with radiotherapy plus TMZ: superimposed tumor locations after treatment (follow-up) minus tumor locations at baseline (baseline). The percentage of overlapping lesions after subtraction is illustrated as in Fig. 2. Montreal Neurological Institute *z*-coordinates of the transverse sections are given.

conducted to determine if the two treatments influence movement of the center of mass between the baseline and follow-up scan. A two-tailed *t*-test revealed no significant difference: $t(62) = 0.869$, $p = 0.387$, with a mean movement of 11.67 mm (±0.42) for the radiotherapy arm and 12.03 mm (±0.39) for the radiochemotherapy arm.

An additional comparison on a case-by-case basis suggested the same conclusion. The frequency of distant recurrences was 23% in the radiotherapy group and 18% in the radiochemotherapy group (chi squared = 1.18, $p = 0.056$).

Discussion

This study was conducted to implement a novel tool for the analysis of brain tumor studies by analyzing whether the anatomical recurrence pattern of glioblastoma in patients treated with combinational radiochemotherapy with TMZ and adjuvant TMZ in the EORTC 26981/22981/NCIC CE.3 study[6] is altered compared with that of patients treated with radiotherapy alone. Although the analysis was technically feasible, the heterogeneity of imaging modalities (CT and MRI) used in the original study limited the number of eligible patients for this analysis since it depends on equal imaging modalities for comparison and requires MRI scans during the entire follow-up.

From 5% to 10% of recurrent or progressive glioblastomas have been reported to be out of the primary tumor field defined by contrast enhancement.[12,13] Our data using a pairwise comparison for the individual patient with a center-of-mass approach revealed an approximate incidence of out-of-field recurrences of 20%. This higher number could be due to methodological differences because the determination of distant recurrences in older studies often did not rely on MRI. Preclinical studies had suggested antiinvasive properties of TMZ.[8] In addition, glioma growth patterns, more invasive or more angiogenic, may correlate with molecular glioblastoma signatures, for example, activation of the protein kinase-3/Akt or Notch pathways.[1] However, our clinical data do not support the preclinical hypothesis that the improved outcome with combined TMZ chemoradiotherapy is due to reduced tumor cell mobility. The pattern of recurrence, although delayed in TMZ-treated patients, is comparable, and independent of the analyzed clinical and molecular factors (*MGMT* promoter methylation according to Hegi et al.).[14] Similarly, one may have expected an increased frequency of distant (out of radiation field) recurrences due to the presumed radiosensitizing effect and longer survival. Again, this has not been observed when looking at the site of first recurrence or progression. Our findings indicate that yet to be identified molecular and cellular determinants independent of current treatment are the main drivers for tumor progression and pattern of recurrence.

New treatment approaches aiming at molecular targets of both tumor and stroma or vasculature (e.g., bevacizumab, cilengitide, enzastaurin, or epidermal growth factor receptor inhibitors) may also alter the invasiveness of tumor cells, including the risk of increasing cell motility, during antiangiogenic treatment. Thus, systematic evaluation of recurrence pattern as in our study may be needed to identify changes in tumor behavior, and adequate prospective imaging surveillance should be implemented in future studies. This could also mean performing the present analysis not only on postcontrast MRI but also with sequences likely to visualize infiltrative tumor, for example, T2-weighted or fluid-attenuated inversion recovery sequences, as well as metabolic imaging, for example, amino acid positron emission tomography.

In summary, the present study used tumor boundaries depicted on MRI sequences that were transposed onto the MNI template and analyzed using MRIcro. It allows detection of changes of site and distance between the initial and the recurrent tumor on the group level. We did not observe a difference in recurrence pattern between the patients treated with radiochemotherapy and those treated with radiotherapy alone. This recurrence pattern analysis may aid the development of combinational therapies that counteract the putative proinvasive adverse effects of therapeutic irradiation or antiangiogenic strategies.

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