Extension of diffuse low-grade gliomas beyond radiological borders as shown by the coregistration of histopathological and magnetic resonance imaging data

Maria Zetterling, MD, PhD,1,2 Kenney R Roodakker, MSc,3 Shala Ghaderi Berntsson, MD, PhD,3,4 Per-Henrik Edqvist, PhD,2 Francesco Latini, MD,1 Anne-Marie Landtblom, MD, PhD,3,4,11 Fredrik Pontén, MD, PhD,3 Irina Alafuzoff, MD, PhD,6,7 Elna-Marie Larsson, MD, PhD,8,9 and Anja Smits, MD, PhD3,4,10

1Department of Neuroscience, Neurosurgery, Uppsala University; 2Department of Neurosurgery, Uppsala University Hospital; 3Department of Neuroscience, Neurology, Uppsala University; 4Department of Neurology, Uppsala University Hospital; 5Department of Immunology, Genetics and Pathology, Uppsala University; 6Department of Immunology, Genetics and Pathology, Uppsala University; 7Department of Pathology and Cytology, Uppsala University Hospital; 8Department of Surgical Sciences, Radiology, Uppsala University; 9Department of Radiology, Uppsala University Hospital, Uppsala; 10Center for Medical Image Science and Visualization, Linköpings University, Linköping, Sweden; and 11Danish Epilepsy Center, Dianalund, Denmark

OBJECTIVE Magnetic resonance imaging tends to underestimate the extent of diffuse low-grade gliomas (DLGGs). With the aim of studying the presence of tumor cells outside the radiological border, the authors developed a method of correlating MRI findings with histological data in patients with suspected DLGGs in whom en bloc resections were performed.

METHODS Five patients with suspected DLGG suitable for en bloc resection were recruited from an ongoing prospective study. Sections of the entire tumor were immunostained with antibodies against mutated IDH1 protein (IDH1-R132H). Magnetic resonance images were coregistered with corresponding IDH1 images. The growth pattern of tumor cells in white and gray matter was assessed in comparison with signal changes on corresponding MRI slices.

RESULTS Neuropathological assessment revealed DLGG in 4 patients and progression to WHO Grade III glioma in 1 patient. The tumor core consisted of a high density of IDH1-R132H–positive tumor cells and was located in both gray and white matter. Tumor cells infiltrated along the peripheral fibers of the white matter tracts. In all cases, tumor cells were found outside the radiological tumor border delineated on T2-FLAIR MRI sequences.

CONCLUSIONS The authors present a new method for the coregistration of histological and radiological characteristics of en bloc–removed infiltrative brain tumors that discloses tumor invasion at the radiological tumor borders. This technique can be applied to evaluate the sensitivity of alternative imaging methods to detect scattered tumor cells at tumor borders. Accurate methods for detection of infiltrative tumor cells will improve the possibility of performing radical tumor resection. In future studies, the method could also be used for in vivo studies of tumor invasion.

http://thejns.org/doi/abs/10.3171/2015.10.JNS15583

KEY WORDS diffuse low-grade glioma; magnetic resonance imaging; tumor border; tumor cell infiltration; oncology

Diffuse low-grade glioma (DLGG) is a Grade II glioma according to the WHO classification of tumors of the central nervous system and represents a chronic infiltrative disease of the brain. It affects mainly adults with a mean age of 39 years at the time of diagnosis. Median patient survival is around 5 years.

Its incidence is 1 case (0.5–1.3 cases) per 100,000 inhabitants.

Today, there is strong evidence of a positive correlation between the extent of tumor resection and survival in patients with DLGG. Since DLGG, after varying periods of time, undergoes malignant transformation.
tumor resection should be performed early to prevent un-
controllable disease. In addition, some data support the
notion that surgery for DLGG should aim for supratotal
resection, which not only improves patient outcome, but
also inhibits the natural progression of the disease.67

Morphological MRI tends to underestimate the ex-
tent of DLGG. Previous studies using multiple biopsies
have identified tumor cells outside the radiological border
on fluid-attenuated inversion recovery (FLAIR) or T2-
weighted MRI sequences.33,53 Because of the slow growth
rate of DLGG, proliferation markers are not suitable for
histological detection of tumor cells beyond the radiologi-
ical border. Furthermore, some tumors cells invading the
peritumoral areas may escape detection because the in-
vasive properties of gliomas appear to occur at the cost of
proliferation.22

The majority of tumor cells in DLGG are characterized
by a mutation in the isocitrate dehydrogenase gene (IDH1)
at position 132 (R132H). A mutation-specific monoclo-
nal antibody recognizing tumor cells carrying the IDH1-
R132H mutated protein has been generated. Since the
complete population of tumor cells in an IDH1-positive
tumor carries the mutation, this antibody is useful in the
detection of infiltrating tumor cells.

Only a few studies have correlated imaging techniques
with histological tumor characteristics.33,53,58,66 More accu-
rate noninvasive neuroimaging techniques are warranted
to detect the infiltration of tumor cells in the brain.9 These
issues are directly related to the unsolved neurosurgical
questions regarding radical tumor resection and the sensi-
tivity of MRI to detect the scattered tumor cells surround-
ing the tumor.

The aim of this study was to disclose the presence of
tumor cells outside the radiological border. For this pur-
pose, we developed a new method to compare MRI find-
ings with histological data in patients in whom en bloc
tumor resection was performed. We describe the applica-
tions of this method in 5 patients who underwent macro-
scopically complete resection of the tumor plus a margin
outside the MRI-defined tumor border. Consecutive T2-
weighted FLAIR images through the whole tumor were
matched with the corresponding images of IDH1-R132H-
labeled tissue sections, and the histological findings at the
radiological borders are reported.

Methods

Patients

Five patients were recruited from a longitudinal follow-
up study of adults with DLGGs at our hospital.5 The insti-
tutional review board approved the protocol for the pres-
ent analysis, and written informed consent was obtained
prior to patient participation. Among the specific inclu-
sion criteria was suspected DLGG suitable for complete
en bloc tumor resection that included a margin of normal-
appearing brain located outside the radiological border.
Tumor locations considered suitable for en bloc resection
were noneloquent areas (those outside the cortical primary
motor, somatosensory, speech, and language areas and the
insula).20 In contrast, tumors located in or in the immedi-
ate vicinity of critical white matter tracts20 that required
close monitoring of function and tumors located in central
gray nuclei were not considered suitable. Tumors that were
better approached by piecemeal removal were also not in-
cluded. The ideal tumor location for en bloc resection was
the right anterior frontal lobe, an area with a high resec-
tion probability in accordance with the “resection prob-
ability map” presented by Sarubbo et al.61 In the present
study, left-sided tumors in the anterior frontal pole were
also considered suitable for inclusion.

Preoperative MRI

Prior to surgery, patients were examined via 3-T MRI
(Philips Achieva) according to the original study proto-
col. Morphological MRI sequences included sagittal and
axial T2-weighted turbo spin echo (SE), coronal and axial
T2-FLAIR, axial T1-weighted SE before and after con-
trast injection, and sagittal T1-weighted 3D turbo field
echo after contrast injection. Perfusion and diffusion MRI
sequences were also obtained but were not used in the
present study.

A neuroradiologist manually delineated tumor borders
on T2-FLAIR sequences by using a picture archiving and
communication system (PACS; Vue PACS, Carestream
Health Inc.). The borders were drawn at the transition
between high signal intensity and normal parenchymal
signal intensity based on visual evaluation. In ambiguous
cases the signal intensity was measured in regions adja-
cent to the tumor and compared with the normal paren-
chymal signal at a distance from the tumor.

Surgery

Tumor borders with possible peritumoral margins were
delineated on preoperative T2-FLAIR MRI sequences.
The aim of tumor resection was to include the complete
tumor volume recorded on T2-FLAIR MRI plus a mar-
gin located outside the radiological border and extending
into the normal-appearing brain tissue. Magnetic reso-
nance images were loaded onto the neuronavigation sys-
tem (Curve and Kolibri, Brainlab). Intraoperatively, tumor
margins were estimated with the guidance of neuronavi-
gation and confirmed by perioperative ultrasound (Flex
Focus 700, BK Medical). Microsurgical en bloc resections
were performed. Ultrasound was used to confirm the ex-
tent of tumor resection.

Preparation of Tumor Samples

The resection surfaces (anterior, posterior, medial, and
lateral) were marked with tissue marking dye (Triangle
Biomedical Sciences Inc.) for spatial orientation. The en
bloc tissue sample was fixed in formalin for at least 2 days
and subsequently cut into 8–12 consecutive tissue slices
approximately 6- to 8-mm thick. The slices were photo-
ographed using a digital camera (Olympus Corp.). Tissue
slices were fixed in formalin for an additional 24 hours and
then embedded in paraffin wax. Subsequently, serial paraf-
fin-embedded 4-um-thick sections were placed on Super-
Frost Plus slides (Gerhard Menzel GmbH), rehydrated,
and stained. For diagnosis, all sections were stained with H &
E. Immunohistochemical staining was applied to the slide
containing the most representative tumor section using the
Dako Autostainer Plus (DakoCytomation) and Dako EnVision FLEX detection system (DakoCytomation). Antibodies selected for diagnostics included glial fibrillary acidic protein (DakoCytomation), microtubule-associated protein 2 (Sigma-Aldrich), oligodendrocyte transcription factor 2 (Abnova Corp.), tumor protein 53 (DakoCytomations), IDH1-R132H (Dianova), and Ki 67 (DakoCytomation). All staining was performed following the manufacturer’s recommendations. A neuropathologist (I.A.) determined all diagnoses according to the 2007 WHO classification of tumors of the central nervous system.40

In addition to the diagnostics, immunostaining with IDH1-R132H was performed on all histological sections. The IDH1-R132H–stained sections were scanned to obtain digital images using an Aperio slide scanner.

**Postoperative MRI**

Postoperative MRI was performed within 48 hours after surgery. Tumor resection was considered complete if all signal changes that had been preoperatively evaluated as tumor on T2-FLAIR MRI were removed.

**Volume Calculations**

The Vue PACS software (version 11.1.0, Carestream Health Inc.) was used to segment the lesions with the aid of a semiautomatic method (Livewire Algorithm). The software is supported by an algorithm that uses an active contour model to define and segment the lesions on each slice. The total tumor volume was calculated on preoperative axial T2-FLAIR sequences, and the volume of the postoperative cavity was calculated using postoperative axial T2-FLAIR and/or T2 sequences.

**Coregistration of Histological Data and MRI Findings**

The stepwise procedure that we used for coregistration is described in Table 1. In summary, pre- and postoperative coronal MR images were reoriented perpendicular to the longitudinal orientation of sagittal MR images of the postoperative defect in PACS so that coronal MR images were oriented in the same direction as the histological sections (Fig. 1).18 Scanned IDH1 images were overlaid on coronal radiological images using Photoshop CC (Adobe Systems Software). The best-fitting preoperative MR image was identified and matched with the IDH1 image. The IDH1 images were subsequently compared with more anterior and posterior MR images to find the best match, similar to the method used by Eriksson et al.18 The gyral branching patterns seen in IDH1 images were used in combination with color codes to identify the corresponding orientation, location, and size of the resected tumor. After matching, the IDH1 image volumes were correlated using linear registration that included translation, rotation, and scaling. Postoperative MRI was used to validate the size of the resected tumor volume. Photoshop CC was used to manually mark the radiological tumor border on the corresponding histological sections.

**TABLE 1. The coregistration procedure**

<table>
<thead>
<tr>
<th>Image</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiological</td>
<td>1. Prior to surgery, morphological MRI sequences, including sagittal &amp; axial T2-weighted turbo SE, coronal &amp; axial T2-weighted FLAIR, axial T1-weighted SE before &amp; after contrast injection, &amp; sagittal T1-weighted 3D turbo field echo after contrast injection were obtained using a 3-T scanner (Philips Achieva).</td>
</tr>
<tr>
<td></td>
<td>2. The pre- &amp; postoperative images were stored in a PACS (Carestream Health).</td>
</tr>
<tr>
<td></td>
<td>3. Using the PACS, pre- &amp; postoperative coronal &amp; sagittal FLAIR images were linked to each other &amp; aligned in a presentation.</td>
</tr>
<tr>
<td></td>
<td>4. Pre- &amp; postoperative coronal MR images were reoriented perpendicular to the longitudinal orientation of sagittal MR images of the postoperative defect using the PACS software.</td>
</tr>
<tr>
<td></td>
<td>5. Preoperative coronal FLAIR images (6-mm thickness, 3-mm spacing) of the tumor were saved as TIFF files.</td>
</tr>
<tr>
<td>Histological</td>
<td>1. After resection, the surfaces (anterior, posterior, medial, &amp; lateral) were marked with different colors for spatial orientation.</td>
</tr>
<tr>
<td></td>
<td>2. The tumor was cut into 8–12 consecutive tissue slices 6 mm thick.</td>
</tr>
<tr>
<td></td>
<td>3. Slices were formalin-fixed &amp; paraffin-embedded. Several thin (5 μm) histological sections were cut from each paraffin block.</td>
</tr>
<tr>
<td></td>
<td>4. Immunostaining with IDH1-R132H antibodies was performed on histological sections from each tumor slice, which were put on object slides for light microscopy.</td>
</tr>
<tr>
<td></td>
<td>5. Microscans were obtained using an Aperio microscope scanner &amp; saved as TIFF files (using several percentages of quality for reducing image size).</td>
</tr>
<tr>
<td>Photoshop</td>
<td>Both radiological &amp; histological images were manipulated using Photoshop CC (Adobe Systems Software).</td>
</tr>
<tr>
<td></td>
<td>1. Radiological TIFF files (480 kb) were enlarged by using the resolution tool. In this way voxels are added to the file to make scaling possible without losing quality.</td>
</tr>
<tr>
<td></td>
<td>2. Histological TIFF files (2–4 Gb) were adjusted using the Magic Wand tool to select the resection &amp; were reduced in scale while maintaining the aspect ratio.</td>
</tr>
<tr>
<td></td>
<td>3. The best fitting preoperative MR image was identified &amp; matched for each histological section separately.</td>
</tr>
<tr>
<td></td>
<td>4. The gyral branching patterns of the histological sections were used in combination with color codes to identify corresponding orientation, location, &amp; size of the resected tumor &amp; radiological image.</td>
</tr>
<tr>
<td></td>
<td>5. The histological file was overlaid on the radiological file as a separate layer to enable accurate adjustments.</td>
</tr>
<tr>
<td></td>
<td>6. The histological sections were subsequently compared with more anterior &amp; posterior MR images to find the best match.</td>
</tr>
<tr>
<td></td>
<td>7. After matching, the histological &amp; radiological tumor volumes were correlated using linear registration including translation, rotation, &amp; scaling.</td>
</tr>
<tr>
<td></td>
<td>8. Postoperative MRI was used to validate the size of the resected tumor volume.</td>
</tr>
<tr>
<td></td>
<td>9. Photoshop CC was used to manually mark the radiological tumor border on the corresponding histological sections.</td>
</tr>
</tbody>
</table>
used to manually mark the radiological tumor border on the corresponding IDH1 image.

Results

The tumor core (area with the highest density of tumor cells) was located in both gray and white matter. Infiltration of tumor cells occurred along the white matter tracts, with tumor cells occupying the peripheral fibers of the tract. In all 5 cases, tumor cells extended beyond the radiological border and IDH1-immunolabeled tumor cells were detected at a maximum distance of 1.4 cm from the radiological border. Clinical data, MRI results, and tumor characteristics of the 5 patients included in this study are summarized in Table 2. The separate cases are described in more detail below.

Case 1

Summary

This 33-year-old man was examined because of vertigo. Magnetic resonance imaging showed an incidental, nonenhancing heterogeneous tumor in the right frontal pole (Table 2 and Fig. 1A and B). A right frontal lobectomy was performed as described above, with en bloc removal of the tumor. Postoperative MRI confirmed that the tumor had been completely resected (Fig. 1C and D). Neuropathological examination showed a WHO Grade II oligodendroglioma. Figure 2 shows MR images of the preoperatively delineated tumor (Fig. 2A), the postoperative cavity (Fig. 2B), the overlay of preoperative delineated tumor and postoperative cavity illustrating the resected peritumoral margins (Fig. 2C), and the overlay of the IDH1 image with the T2-FLAIR image (Fig. 2D).

Infiltration Pattern

White Matter. The infiltrating tumor cells followed the white matter tracts. Tumor cells were concentrated in the periphery of the tracts, with the highest density in the most...
peripheral parts. The white matter tract of the peritumoral margin was occupied by a “single lane” of tumor cells (Fig. 3).

**Gray Matter.** In the tumor core, tumor cells occurred at a high density in the gray matter. Figure 4 left illustrates the core of the tumor, whereas Figs. 3 and 5 show the peripheral part of the tumor.

On T2-FLAIR sequences, we noted a round area in the central part of the tumor with relatively low signal intensity surrounded by a ring of high signal intensity. These radiological areas corresponded to histological areas with a low density of tumor cells and vacuoles (low FLAIR signal, central part) and a high density of tumor cells (high FLAIR signal, peripheral ring; Fig. 4). The vacuoles within the central part were considered to represent tumor edema, a phenomenon in DLGGs that has been described by Gerin et al.\(^2^1\)

Tumor cells did not cross the pia mater in the sulcus, resulting in a notable difference in tumor cell density in the gray matter of two adjacent gyri.

**Radiological Versus Histological Tumor Border**

The maximum radiological peritumoral margin (that is, tissue outside the radiological border on T2-FLAIR sequences) measured 2 cm. Tumor cells were found outside the radiological border (Figs. 3 and 5) at a maximum distance of 1.4 cm from the radiological border.

An area with high tumor cell density and low signal on T2-FLAIR MRI was found outside the radiological border in the white matter (Fig. 5 left), which had not been detected on preoperative FLAIR sequences (Fig. 5 right).

**Case 2**

**Summary**

The MRI studies in this 39-year-old man, who had presented with headache, showed a nonenhancing, left frontal, slightly heterogeneous tumor. Left frontal en bloc resection was performed, and postoperative MRI confirmed complete tumor resection. Neuropathological examination showed a WHO Grade II oligodendroglioma (Table 2).

**Infiltration Pattern**

**White Matter.** Similar to Case 1, tumor cells followed the white matter tracts and were slightly more concentrated in the peripheral parts of the tracts (Fig. 6A).

**Gray Matter.** In the tumor core, tumor cells were found at high density in the gray matter (Fig. 6B). Farther away from the core, fewer tumor cells were present in the gray matter (Fig. 6A).

The density of tumor cells was lower in the gray matter of the adjacent gyrus, which was most pronounced at the tumor borders. Thus, tumor cells followed the route depicted by the white matter tracts at the bottom of the sulcus (Fig. 6C), resulting in a higher density of tumor cells in the bottom of the sulcus (Fig. 6D), compared with the more rostral part of the gyrus (Fig. 6E).

**Radiological Versus Histological Tumor Border**

The tumor volume was large (Table 2), and some tissue corresponding to the medial part of the resection was lost. The other tumor portions were well preserved, enabling reliable coregistration with the radiological data.

The maximum radiological margin on T2-FLAIR sequences measured 2 cm. In the lateral and posterior portion of the tumor, in the white matter as well as the gray, tumor cells were identified outside the radiological border (Fig. 7). In fact, tumor cells were detected 1 cm from the radiological border. However, the opposite situation (radio-
logical tumor border outside the histological tumor border) was also encountered. Thus, it was difficult to appreciate the correct tumor border on T2-FLAIR sequences in the gray matter because tumor cells were seen outside the radiological border and the radiological border extended into tumor-free tissue.

**Case 3**

**Summary**

This 60-year-old man had partial seizures followed by a transitory postictal left-sided hemiparesis. Magnetic resonance imaging showed a right-sided, homogeneous non-enhancing tumor in the premotor area (Fig. 8A and B). En bloc tumor resection was performed, and postoperative MRI confirmed complete resection (Fig. 8C). After surgery, the patient suffered from an inability to initiate voluntary movements, most likely due to resection of the supplementary motor area, but he made a full recovery within 1 month.

During tissue preparation, some technical difficulties resulted in some tissue loss and distortion of the en bloc sample at the lateral tumor side. Neuropathological examination revealed WHO Grade II astrocytoma (Table 2).

**Infiltration Pattern: White and Gray Matter**

The tumor core was located in gray matter as well as white and showed a high density of tumor cells (Fig. 9A). At the periphery of the tumor, we saw a pattern similar to the one described before, with tumor cells primarily infiltrating the white matter tracts, at a somewhat higher cell density at the border of the gray matter, and partly infiltrating the white matter tracts (Fig. 9E).

Figure 9B–D illustrates the difference in tumor cell density in the gray matter of 2 adjacent gyri: There is a high cell density in the proximal gray matter, in connection with the pia overlaying the sulcus (Fig. 9B). In the adjacent gyrus, there are only a few scattered tumor cells, declining in number farther from the bottom of the sulcus (Fig. 9C and D).

**Radiological Versus Histological Tumor Border**

The radiological tumor-free zone included in the en bloc resection measured 2.3 cm. Tumor cells were found at a maximal distance of 1.2 cm from the radiological border.

**Case 4**

This 50-year-old woman had a tumor in the left frontal pole with heterogeneous signal characteristics and cystic areas on MRI (Table 2). The tumor had been diagnosed 10 years earlier, and she was referred to the neurosurgical department because of a slow progression in tumor size. The tumor was completely removed en bloc. Postoperatively she suffered from dysphasia that spontaneously resolved within a few days. Neuropathological examination showed WHO Grade II oligodendroglioma with densely packed IDH1-labeled tumor cells mainly in the gray matter. A relatively high concentration of tumor cells was also found at the border between gray and white matter in the peripheral part of the tumor (not shown). Tumor cells were identified...
at a distance of 1.1 cm from the radiological border, which corresponded to the maximum tissue margin (not shown).

**Case 5**

This 53-year-old man had a left frontal pole tumor with minimal contrast enhancement that was compatible with a DLGG on initial MRI (Table 2). However, MRI 1 month later showed signs of tumor progression with some hemorrhagic transformation and the appearance of atypical gyral contrast enhancement in the medial part of the tumor. An en bloc resection was performed, and the patient was neurologically intact after surgery. The tumor volume was large, and there was some loss of white matter tissue on the medial/inferior side of the tumor after en bloc resection. Neuropathological examination verified focal progression to WHO Grade III oligodendroglioma. The tumor core was situated in both gray and white matter, and as in the previous cases, tumor cell concentration was high at the border between gray and white matter. Tumor cells were identified at a distance of 1.3 cm from the radiological border, corresponding to the maximal resected peritumoral margin (not shown).

**Discussion**

In this study we developed and applied a new method for the coregistration of MRI and histological data in DLGG. Our findings show that this method is a useful aid in evaluating the growth pattern of these tumors in relation to radiological findings because it illustrates the characteristic tumor infiltration of the white matter tracts and the extension of tumor cells outside the radiological border. Application of this new technique in larger series of patients will enable a more reliable assessment of tumor in-
There are no previous reports on the coregistration of histological data with MRI of en bloc–removed DLGGs as described here. However, the correlation of tumor characteristics on MRI with histological findings of DLGGs has been the focus of previous studies.30,33,41 In these studies tumor cells have been demonstrated outside the radiological tumor borders, that is, in normal-appearing brain on FLAIR or T2-weighted images. In a postmortem study of 4 patients with high-grade glioma, IDH1-positive tumor cells were found to infiltrate diffusively in the brain at a great distance from the primary tumor location, supporting the notion that a high-grade tumor is a surgically untreatable systemic brain disease. The goal when treating DLGG is to postpone or, ideally, to prevent the ominous progression from low-grade tumor to high-grade tumor.4,11,14,46,60,63,62

Because the extent of resection positively influences the time to anaplastic transformation4,14,46,60,65 and tumor cells infiltrate beyond the radiological border,33,53 it is likely that a supratotal resection—that is, resection of the tumor together with a margin outside the radiological border—will improve the prognosis of patients with DLGG.51 This was actually shown by Yordanova et al.67 and Duf-fau. Anaplastic transformation was avoided in patients with DLGG who had undergone supratotal resection, a significantly better result compared with that in their control group in which only complete resection was performed.67,1

The optimal treatment of DLGG is probably resection to the functional borders during awake surgery, a strategy well described by Duffau and colleagues.10,15,16 Based on their extensive experience with awake surgery and direct electrical stimulation, probability maps on the resectability of different cortical and subcortical regions have been created and most likely represent what is possible to achieve in terms of complete resections of DLGGs.29,61

It is well accepted that DLGGs prefer to infiltrate along white matter tracts.22,23,44,62 Indeed, infiltrating tumor cells were detected in the white matter tracts in all cases in the present study. Interestingly, in 2 of the cases the white matter tracts were only partly infiltrated. In 4 cases tu-

Fig. 7. Case 2. Tumor cells are present outside the radiological border, which is indicated by a black line. The area marked with an asterisk is outside the radiological border. Fewer tumor cells occur farther away from the border. Tumor cells outside the radiological border occur more frequently closer to the border (lower inset). Farther from the border no tumor cells are found (upper inset). Figure is available in color online only.

Fig. 8. Case 3. Preoperative coronal T2-FLAIR (A) and sagittal T1-weighted (B) MR images showing a right-sided nonenhancing tumor in the premotor area. Postoperative coronal T1-weighted MR image (C) showing complete tumor resection.
tumor cells were concentrated in the peripheral fibers of the tracts. It is likely that this limited infiltration of the white matter reflects an early stage of tumor invasion. To spread to the adjacent gyrus, tumor cells followed the white matter tracts. They reached the molecular layer of cortex but did not infiltrate the pia mater. The short and intracortical U-fibers are the most probable way of diffusion for tumor cells between 2 adjacent gyri.42,49,54

Differences in tumor genotype and phenotype are associated with different invasion patterns in DLGG.25 In addition, the site of origin is also thought to affect the growth properties of the tumor.24,44,68

The migration properties of oligodendrocyte precursors during early development are influenced by their specific origin in the brain.26 and different proteins can guide the migration of cells.8,25,48 In a transgenic mouse model, oligodendroglialomas showed a preference for the white matter regions, while low-grade astrocytomas were more often associated with the lateral ventricles.8 Interestingly, metalloproteinases, expressed by the tumor cells, may enhance the spread of glioma cells in the white matter.19,47 In a study using tumor cell lines, low-grade astrocytomas were sensitive to the inhibitory proteins present in myelin, whereas oligodendrogliomas were able to overcome the inhibitory effects.1 Similar factors may be involved in tumor cell migration and tumor spread.23 By obtaining large resections including tumor margins, as described here, we can study the expression of proteins that may be involved in tumor growth and spread in the brain.

Our method allowed a detailed and reliable comparison between the histological properties of specific intra- and peritumoral regions and the corresponding signal changes on MRI. Tumor cells were identified outside the radiological border in all cases. In Case 1, we found a relatively large area of high-density tumor cells outside the radiological border that had appeared normal on preoperative T2-weighted FLAIR images and was therefore interpreted as normal tissue (Fig. 5).

The tumor is expected to be visible on FLAIR MRI if the tumor cell density is above a certain value, that is, the “visibility threshold.” In addition to the cell density, the MRI signal is influenced by several other factors, including the water content of the tissue.43 Data regarding the threshold for detecting tumor cells on FLAIR MRI or diffusion tensor imaging (DTI) are scarce.19 Tumor cell concentrations and edema fraction were correlated in biopsy samples derived from inside and outside T2-defined abnormalities in a recent study.21 The tumor cell density is inversely proportional to the MRI apparent diffusion coefficient value.17,26,36 In a DTI study of gliomas, the isotropic and the anisotropic diffusion components could differentiate between normal and tumor-infiltrated brain.58

Our results showing tumor cells outside the radiological border on FLAIR MRI support the notion that FLAIR sequences are not sensitive enough to accurately detect infiltration of tumor cells. Our method provides a new tool for further studies on how the signal changes on different MRI sequences are affected by the concentration of tumor cells, the water content of the tumor, and other factors present in the tissue. Moreover, the sensitivity of diffusion MRI with regard to detecting infiltrating tumor cells can be further evaluated.

Positron emission tomography with amino acid tracers is another potential tool for identifying the infiltrative “penumbra” of tumor cells. Because of the low background activity of 11C-l-methionine and 18F-fluorooctyl-l-tyrosine, these tracers are considered better than FDG in delineating gliomas.22,54 A threshold for the detection of tumor cells in the infiltration area has been discussed,64 but a common cutoff value has been difficult to define because of tumor heterogeneity.27,35,37 Coregistration of PET and histological data in the way described here enables an evaluation of the potential of these PET tracers to identify scattered tumor cells within the infiltrative tumor zone.

Finally, large tumor resections provide more reliable representative samples for histopathological tumor diag-

---

**FIG. 9.** Case 3. IDH1 images from the core and the periphery of the tumor. A: In the tumor core, the tumor cells occur at a high density in both gray and white matter. B: There is a high tumor cell density in the proximal gray matter in connection to the pia overlaying the sulcus. In the adjacent gyrus, tumor cells are scarce, with a declining number of tumor cells farther from the bottom of the sulcus (that is, from left to right in the figure, magnified in insets C and D, respectively). C: More tumor cells are found close to the bottom of the sulcus. D: No tumor cells are found farther from the bottom of the sulcus. E: In the tumor periphery, tumor cells primarily follow the white matter tracts, at a slightly higher density at the border of the gray matter, and partly infiltrate the white matter tracts.
nosis. When tissue loss is minimized and the whole tumor can be analyzed, the probability of a correct diagnosis with respect to tumor grade and focal signs of anaplastic transformation is augmented. The importance of intact tumor samples was illustrated in a recent study in which a subset of WHO Grade II gliomas showed microfoci of high cellularity and with molecular and genetic aberrations, indicating early anaplastic transformation. The presence of these microfoci was associated with a shorter survival than in patients with homogeneous DLGGs.55

Limitations of the Study

The main shortcoming of this study is the limited number of cases included. Therefore, undue extrapolation of our findings should be avoided, and our results should be confirmed by larger series. Another limitation of our method is that tumor localization permitting an en bloc resection technique is mandatory; however, this technique cannot be used for all tumor locations. Tumors in brain areas that are better approached by piecemeal removal are not appropriate, and tumors in or in close connection to eloquent areas with extended subcortical involvement that require close monitoring of motor or speech function are not suitable for en bloc removal. As tumor characteristics can be expected to vary for tumors with different localizations,13,24,25,38,44 this bias by tumor location is important to bear in mind.

For optimal assessment of infiltrated brain outside the radiological border, a larger margin of peritumoral tissue is desirable but must be weighed against the risk of introducing neurological deficits in the event that eloquent areas are breached. We also encountered a few technical obstacles. To achieve an optimal fit between the coronal MRI and the histological slices, a correct angle (perpendicular to the horizontal plane when cutting the tissue) must be achieved. The technical problems encountered in Case 3 were probably attributable to a mismatch between the cutting angle and the angle of the coronal MRI slices. There is the risk of tissue loss during tumor resection and/or preparation of the slices, which was the case at the medial resection border in the patients in Cases 2 and 5. The tissue can also be distorted during surgery or preparation, and great care is warranted to avoid damage to vulnerable brain tissue. Finally, detection of tumor cells with the IDH1-R132H antibody is only possible in IDH1-mutated tumors. It has been shown that 68%–94% of all Grade II oligodendroglomas and 59%–72% of all Grade II astrocytomas have IDH1 mutations.28,39 For the remaining IDH1-negative tumors, another method for detecting tumor cells will be necessary. To restrict the tumor population to only IDH1-positive tumors also introduces a risk of “selection bias.”

Conclusions

In summary, we present a method for the coregistration of histological and radiological tumor characteristics. This method provides a valuable tool to study the invasive growth of DLGGs and to detect infiltrating tumor cells at the radiological border. Our results are encouraging and will contribute to a deeper understanding of the biological behavior of DLGGs and the intrinsic limitations of MRI.

Application of the method on a larger scale offers the possibility to evaluate the sensitivity of alternative imaging methods in detecting scattered tumor cells in the infiltration zone. The method also allows in vivo studies of factors with a potential role in regulating tumor growth and tumor spread in the brain.

Acknowledgments

This work was supported by research funds from the Erik, Karin och Gösta Selanders Stiftelse (A.S.), Hanna Eklund Stiftelsen (A.S.), and the Uppsala County Council (M.Z., A.S.).

References

15. Duffau H, Mandonnet E: The “onco-functional balance” in


Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions
Conception and design: Zetterling, Smits. Acquisition of data: Zetterling, Berntsson, Latini, Larsson, Smits. Analysis and interpretation of data: Zetterling, Roodakker, Latini, Alafuzoff, Larsson, Smits. Drafting the article: Zetterling, Roodakker, Berntsson, Latini, Alafuzoff, Larsson, Smits. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Zetterling. Administrative/technical/material support: Roodakker

Correspondence
Maria Zetterling, Department of Neuroscience, Neurosurgery, Uppsala University, Uppsala 75185, Sweden. email: maria.zetterling@akademiska.se.


64. Smits A, Baumert BG: The clinical value of PET with amino acid tracers for gliomas WHO Grade II. Int J Mol Imaging 2011:372509, 2011


