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Original Article

# Correlation between contrast enhancement on intraoperative magnetic resonance imaging and histopathology in glioblastoma

Pieter L. Kubben, Pieter Wesseling[1,2], Martin Lammens[1,3], Olaf E.M.G. Schijns, Mariël P. ter Laak-Poort, Jacobus J. van Overbeeke, Henk van Santbrink

Departments of Neurosurgery, [3]Pathology, Maastricht University Medical Center, Maastricht, [1]Department of Pathology, Radboud University Medical Centre, Nijmegen, [2]Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands

E-mail: *Pieter L. Kubben - pieter@kubben.nl; Pieter Wesseling - P.Wesseling@pathol.umcn.nl; Martin Lammens - martin.lammens@uza.be; Olaf E.M.G. Schijns - o.schijns@mumc.nl, Mariël P. ter Laak-Poort - m.ter.laak@mumc.nl; Jacobus J. van Overbeeke - jj.van.overbeeke@mumc.nl; Henk van Santbrink - h.van.santbrink@mumc.nl
*Corresponding author

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## Abstract

**Object:** Glioblastoma is a highly malignant brain tumor, for which standard treatment consists of surgery, radiotherapy, and chemotherapy. Increasing extent of tumor resection (EOTR) is associated with prolonged survival. Intraoperative magnetic resonance imaging (iMRI) is used to increase EOTR, based on contrast enhanced MR images. The correlation between intraoperative contrast enhancement and tumor has not been studied systematically.

**Methods:** For this prospective cohort study, we recruited 10 patients with a supratentorial brain tumor suspect for a glioblastoma. After initial resection, a 0.15 Tesla iMRI scan was made and neuronavigation-guided biopsies were taken from the border of the resection cavity. Scores for gadolinium-based contrast enhancement on iMRI and for tissue characteristics in histological slides of the biopsies were used to calculate correlations (expressed in Kendall's tau).

**Results:** A total of 39 biopsy samples was available for further analysis. Contrast enhancement was significantly correlated with World Health Organization (WHO) grade (tau 0.50), vascular changes (tau 0.53), necrosis (tau 0.49), and increased cellularity (tau 0.26). Specificity of enhancement patterns scored as "thick linear" and "tumor-like" for detection of (high grade) tumor was 1, but decreased to circa 0.75 if "thin linear" enhancement was included. Sensitivity for both enhancement patterns varied around 0.39-0.48 and 0.61-0.70, respectively.

**Conclusions:** Presence of intraoperative contrast enhancement is a good predictor for presence of tumor, but absence of contrast enhancement is a bad predictor for absence of tumor. The use of gadolinium-based contrast enhancement on iMRI to maximize glioblastoma resection should be evaluated against other methods to increase resection, like new contrast agents, other imaging modalities, and "functional neurooncology" – an approach to achieve surgical resection guided by functional rather than oncological-anatomical boundaries.

**Key Words:** Glioblastoma, image guided surgery, intraoperative magnetic resonance imaging, neurooncology, neuropathology

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## INTRODUCTION

Glioblastoma is a highly malignant brain tumor that often shows extensive infiltrative growth in the surrounding brain parenchyma. Standard treatment consists of surgery, radiotherapy, and chemotherapy, leading to a median survival of 14.6 months.[29,28] Although the role of surgery is still under debate, mounting evidence suggests that increased extent of tumor resection (EOTR) is associated with prolonged survival.[23] Intraoperative magnetic resonance imaging (iMRI) is a technique that can help to increase EOTR, comparable to the use of 5-aminolaevulinic acid (5-ALA).[26]

The added value of iMRI in increasing EOTR for glioblastoma is based on visualizing remaining contrast enhancement on T1-weighted scans at the border of the resection cavity. This contrast enhancement is supposed to indicate residual tumor, which can be resected in the same procedure. In a few studies the additionally resected tissue was sent separately for histological analysis, leading to varying reports on tumor presence.[25,20,16] However, due to the infiltrative nature of a glioblastoma, tumor cells are often present outside the contrast enhancing area.[5,1] If contrast enhancement on T1-weighted iMRI is to be used as a marker for high grade glioma, then contrast enhancing tissue should exhibit more high grade tumor characteristics than non (contrast) enhancing tissue. Serial stereotactic biopsies have been performed on preoperative computed tomography (CT) and MRI, demonstrating tumor cells outside the contrast enhancing area.[9] To our knowledge, such studies have not been performed systematically on iMRI, in which contrast agent is administered after tumor resection (i.e., after possible iatrogenic damage to the blood–brain barrier). Therefore, histologic results correlated to preoperative imaging might not correlate to iMRI.

This is the first study that systematically compares contrast enhancement on iMRI with histopathological characteristics in glioblastoma. The study objective is to determine to what extent contrast enhancement on T1-weighted iMRI can be used as a marker for presence of (high grade) glioma, and therefore as a valid indicator to assess EOTR.

## MATERIALS AND METHODS

The study protocol is registered at ClinicalTrials.gov under number NCT00780819 and has been approved by the institutional ethics research board.

### Patient selection

For this prospective cohort study, we recruited 10 patients with a supratentorial brain tumor suspect for a glioblastoma. We determined the number of patients to be included based on consensus in the study committee. Inclusion criteria were: Indication for tumor resection, minimum age of 18 years, World Health Organization (WHO) Performance Scale 2 or better, American Society of Anaesthesiologists (ASA) class 3 or better, understanding of the Dutch language, and informed consent. Exclusion criteria were: Recurrent tumor, multiple tumor locations, prior radiotherapy on the skull, and prior chemotherapy.

### Study endpoints

Primary endpoint of this study was the correlation between contrast enhancement at the border of the resection cavity on T1-weighted iMRI and presence of high grade tumor according to the WHO classification.[15] Secondary endpoints of this study were: Correlation between contrast enhancement at the border of the resection cavity on T1-weighted MRI and other histopathologial tissue characteristics, postoperative clinical condition, and survival.

### Surgical procedure

All study participants were operated by a neurosurgeon (OS, MtLP, or HvS) sufficiently experienced with the 0.15 Tesla iMRI system used in our hospital (PoleStar N20 with Stealth Station extension; Medtronic Navigation, Louisville, CO). After patient installation in the headclamp, a contrast enhanced preoperative (high-field strength) MRI was loaded for surgical planning and initial neuronavigation. Before incision, a nonenhanced iMRI scan was made as a baseline scan that intraoperatively acquired scans could be compared with.

During tumor resection, resected tissue was sent for standard histopathological analysis. As soon as the neurosurgeon considered the intended tumor resection to be complete, T1-weighted iMRI scans were acquired using the so-called "T1 7 min 4 mm"-protocol in axial orientation: First a nonenhanced scan, then a contrast enhanced scan using gadopentetate dimeglumine (Magnevist; Bayer-Schering Pharma AG, Berlin, Germany). Contrast dose was 0.4 ml/kg (0.2 mmol/kg) – a so-called "double-dose" – provided no renal failure was present. The contrast enhanced scan was made immediately after intravenous contrast administration.

After scanning neuronavigation was continued on the contrast enhanced iMRI scan, which was imported in the Stealth Station neuronavigation system. In all directions where gross total resection was intended, neuronavigation-guided biopsies were taken at the border of the resection cavity. A screen capture from the neuronavigation system was saved for each biopsy to relate contrast enhancement with histopathology. Each biopsy was sent separately for histopathological analysis, labeled with a number corresponding to the screen capture. After taking the biopsies, surgery was continued to resect any contrast enhancement in a

direction where gross total resection was intended. Scanning was repeated if this goal was considered to be achieved, and additional biopsies were taken if safely possible. Contrast administration was only repeated if the previous iMRI scan was performed more than 2 hours back, in a dose of 0.2 mmol/kg (0.1 mmol/kg) – a "single-dose" – provided no renal failure was present.

## Perioperative procedure

Preoperative and postoperative MRI scans were made with the Intera 1.5 Tesla MRI system (Release 11.1; Philips, Best, The Netherlands). Preoperative neuronavigation scans were contrast-enhanced T1-weighted volume scans (isovoxel 1 mm, gap thickness 0 mm). Postoperative multiple sequences were acquired in a standardized fashion, including contrast enhanced T1-weighted sequences. Gadopentetate dimeglumine was used as a contrast agent in a dose of 0.2 ml/kg (0.1 mmol/kg) provided no renal failure was present.

All preoperative and postoperative scans were performed within 72 hours before and after surgery, respectively.

WHO Performance Scale was measured the day before surgery, and one week after surgery.

## Determination of contrast enhancement

The screen captures from the biopsy locations were independently reviewed by a neurosurgeon and a senior resident in neurosurgery (HvS and PK). Both have ample experience in interpreting PoleStar images. Contrast enhancement was scored according to a four-tier classification [Table 1] described by Ekinci *et al*.: None, thin linear, thick linear, and (suspected) residual tumor.[4] Screen captures that were scored differently by the reviewers were reviewed together to obtain consensus.

## Determination of histopathological characteristics

The biopsy tissue samples were independently reviewed by two experienced neuropathologists (PW and ML), blinded for corresponding contrast enhancement. Histopathological characteristics were scored for 10 parameters (most of these in a semiquantitative fashion): Amount of tissue, quality of tissue, preexistent tissue, increased cellularity, tumor presence, mitoses, vascular changes, necrosis, inflammation, and WHO grade in the sample. To each individual biopsy specimen in which tumor was present a WHO grade was assigned according to the WHO 2007 classification of tumors of the central nervous system[15]: Grade II = no mitotic activity, no necrosis and no florid microvascular proliferation found; grade III = mitotic activity present, but absence of necrosis and florid microvascular proliferation; grade IV: Presence of necrosis and/or florid microvascular proliferation. The values for each parameter are displayed in Table 2. The so-called "Tier 1 items" to be reported according to the "Biospecimen reporting for improved study quality" (BRISQ) recommendations are displayed in Table 3.[17] Tissue samples that were scored different by the neuropathologists were reviewed together to obtain consensus.

## Statistics

Interobserver agreement for contrast enhancement, WHO classification and histopathological parameters were

**Table 1: Ekinci classification for scoring contrast enhancement**

| Description | Definition |
|---|---|
| None | No visible contrast enhancement |
| Thin linear | Resembles normal dural enhancement (<5 mm) |
| Thick linear | Thicker than typical dural enhancement (5-10 mm) |
| Suspected residual tumor | >10 mm in any imaging plane |

**Table 2: Classification for histopathological characteristics**

| Parameter | Values |
|---|---|
| Preexistent tissue | White matter, gray matter, combination, indeterminate |
| WHO grade | No WHO grade: Nonneoplastic (normal or reactive changes); WHO grade II: Low grade diffuse astrocytoma; WHO grade III: Anaplastic astrocytoma; WHO grade IV: Glioblastoma |
| Vascular changes | No apparent, vasodilatation, hypertrophic endothelium, florid microvascular proliferation |
| Necrosis | Absent, indeterminate, focal, local, extensive |
| Mitoses | No, sparse, moderate, frequent |
| Increased cellularity | No apparent, limited, moderate, marked |

**Table 3:Biospecimen reporting for improved study quality Tier 1 items**

| Data element | Value |
|---|---|
| Biospecimen type | Perifocal brain tumor parenchyma |
| Anatomic site | Cerebrum |
| Disease status of patients | WHO performance scale≤2 |
| Clinical characteristics of patients | Neurological deficit dependent on tumor location |
| Vital state of patients | Alive |
| Clinical diagnosis of patients | Supratentorial intra-axial brain tumor, suspect for high grade glioma |
| Pathology diagnosis | Glioblastoma |
| Collection mechanism | Tumor forceps |
| Type of stabilization | Saline 0.9% solution |
| Type of long-term preservation | Formalin-fixed, paraffin embedded |
| Constitution of preservative | 10% neutral-buffered formalin |
| Storage temperature | Room temperature |
| Storage duration | 6-18 months |
| Relocation temperature | Room temperature (after embedding in paraffin) |
| Composition assessment and selection | Scored as "adequate" regarding "amount of tissue" and "quality of tissue" |

expressed as kappa-squared values, calculated with an in-house made application. Correlation between contrast enhancement and histopathological parameters was expressed as Kendall's tau with a one-tailed significance, calculated in PASW Statistics version 18.0.3 for Mac (IBM Corporation, Armonk, NY). Further analysis consisted of creating crosstables to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) for contrast enhancement in relation to WHO grade. Two definitions were used for presence of contrast enhancement: "Thick linear + tumor-like" versus "thin linear + thick linear + tumor-like". In addition, two definitions were used for presence of tumor: "Grade III + grade IV" versus "grade II + grade III + grade IV". Calculations were performed with Microsoft Excel for Mac version 2011 (Microsoft, Redmond, WA), as well as the graphical representation of the interobserver agreement.

## RESULTS

### Patient characteristics

Table 4 shows relevant characteristics of the 10 participants included in this study, including information on tumor location, and preoperative and postoperative WHO Performance Status (WPS). Patients were recruited between October 2008 and July 2009, and follow-up lasted until all patients died. Age varied between 46 and 71 years, with a mean of 59.7 ± 9.0 years. Six patients were male and four patients were female. The main tumor mass was located in the left versus right cerebral hemisphere in six and four patients, respectively, and the tumor was most frequently located in the frontal lobe ($n$ = 4), followed by the temporal lobe ($n$ = 3), parietal lobe ($n$ = 2), and occipital lobe ($n$ = 1).

All patients but one were administered a double-dose of contrast agent, the remaining patient received a single-dose because of preexisting renal dysfunction, which was, however, not a contraindication for gadolinium-based contrast agent. Standard histopathological examination of the resected tumor revealed glioblastoma as the clinical diagnosis for all patients. The total number of study biopsy samples of all patients was 42. The number of biopsy samples per patient varied between 2 and 8, with a mean of 4.2 ± 1.9 samples. In two patients, biopsy samples were taken in two different phases during surgery. In those cases the delay between the first dose of contrast administration and the second iMR scan was 115 and 90 minutes, respectively, and according to our protocol no new dose of contrast agent was administered before the second iMR scan. Preoperative WPS varied between 0 and 2, with a mean of 0.7 ± 0.7. Postoperative WPS varied between 0 and 2, with a mean of 0.9 ± 0.6. Two patients (BZS02 and BZS03) suffered from transient neurological deficit postoperatively, one due to a supplementory motor area (SMA) syndrome and another due to postoperative hemorrhage. Both patients recovered within a few days from WPS 3 - 4 to WPS 1. All patients received the standard treatment consisting of radiotherapy and chemotherapy postoperatively.[29,28] Postoperative survival varied between 40 and 721 days, with a mean of 350 ± 215 days (median 372 days). Of note, the patient with the shortest survival (BZS05) opted for euthanasia. Postoperative survival is displayed as a Kaplan–Meier curve in Figure 1.

### Interobserver agreement

A total of 42 samples were available for further analysis, 39 of these were scored as "adequate" both on "amount of tissue" and "quality of tissue". Table 5 shows the results for both observers for contrast enhancement and tissue characteristics for these 39 samples. Interobserver agreement is calculated for each parameter and expressed as kappa-squared with 95% confidence intervals in Figure 2. For all biopsies, tumor parameters that were scored differently by both observers were

**Table 4: Study demographics**

| Code | Sex, age (y) | Tumor location | WPS pre/post | Contrast dose* | Second scan delay | NoS | NoATS |
|---|---|---|---|---|---|---|---|
| BZS01 | M, 51 | L frontal | 0/0 | Double | N/A | 5 | 5 |
| BZS02 | M, 60 | R frontal | 0/1 | Double | 115 min | 5+1 | 4+1 |
| BZS03 | M, 62 | L temporal | 0/1 | Single | N/A | 2 | 2 |
| BZS04 | F, 69 | L frontal | 1/1 | Double | 90 min | 5+3 | 5+3 |
| BZS05 | M, 46 | L temporal | 1/1 | Double | N/A | 3 | 2 |
| BZS06 | F, 61 | L parietal | 1/1 | Double | N/A | 5 | 4 |
| BZS07 | F, 47 | R temporal | 2/2 | Double | N/A | 3 | 3 |
| BZS08 | M, 71 | R occipital | 1/1 | Double | N/A | 4 | 3 |
| BZS09 | F, 69 | L frontal | 1/1 | Double | N/A | 4 | 3 |
| BZS10 | M, 61 | R parietal | 0/0 | Double | N/A | 2 | 1 |

F: Female, L: Left, M: Male, min: Minutes, N/A: Not applicable, NoATS: Number of Adequate Tumor Samples, NoS: Number of Samples, post: Postoperative, pre: Preoperative, R: Right, WPS: WHO Performance Status, y: Years, *A single dose of contrast is 0.1 mmol/kg, a double dose is 0.2 mmol/kg

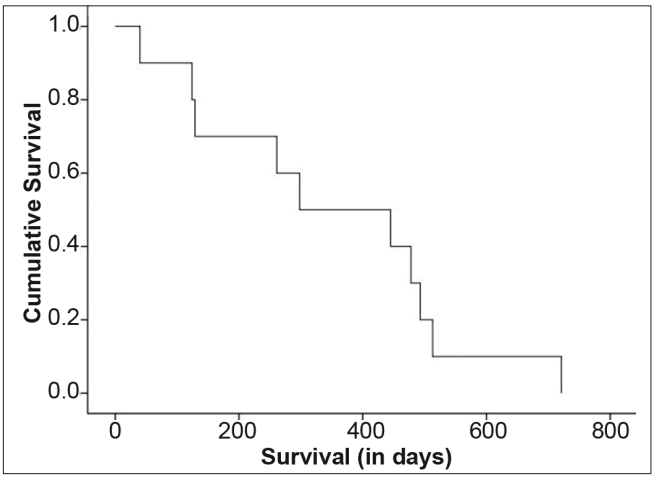



**Figure 1: Kaplan–Meier curve displaying postoperative survival**

**Table 5a: Crosstable for "contrast enhancement"**

| | None | Thin linear | Thick linear | Residual tumor |
|---|---|---|---|---|
| None | 20 | 1 | 0 | 0 |
| Thin linear | 2 | 5 | 2 | 4 |
| Thick linear | 0 | 1 | 2 | 3 |
| Residual tumor | 0 | 0 | 0 | 2 |

**Table 5b: Crosstable for "WHO grade"**

| | Normal/NT | RC | Grade II | Grade III | Grade IV |
|---|---|---|---|---|---|
| Normal/NT | 6 | 1 | 0 | 0 | 0 |
| RC | 1 | 0 | 0 | 0 | 0 |
| Grade II | 0 | 1 | 4 | 0 | 0 |
| Grade III | 0 | 0 | 3 | 0 | 4 |
| Grade IV | 0 | 0 | 2 | 2 | 12 |

Abbreviations: NT: No Tumor, RC: Reactive changes

**Table 5c: Crosstable for "preexistent tissue"**

| | Gray+white | White | Gray | Indeterminate |
|---|---|---|---|---|
| Gray+white | 9 | 2 | 1 | 1 |
| White | 3 | 4 | 0 | 2 |
| Gray | 5 | 1 | 0 | 1 |
| Indeterminate | 0 | 1 | 0 | 12 |

**Table 5d: Crosstable for "increased cellularity"**

| | Extreme | Marked | Moderate | Limited | No apparent |
|---|---|---|---|---|---|
| Extreme | 0 | 2 | 0 | 1 | 0 |
| Marked | 0 | 6 | 9 | 1 | 0 |
| Moderate | 0 | 2 | 4 | 3 | 0 |
| Limited | 0 | 0 | 0 | 1 | 0 |
| No apparent | 0 | 0 | 0 | 4 | 9 |

**Table 5e: Crosstable for "vascular changes"**

| | No apparent | Vasodilatation | +HT | +Florid MVP |
|---|---|---|---|---|
| No apparent | 10 | 1 | 0 | 0 |
| Vasodilatation | 7 | 2 | 1 | 0 |
| +HT | 2 | 1 | 3 | 4 |
| +Florid MVP | 0 | 0 | 1 | 7 |

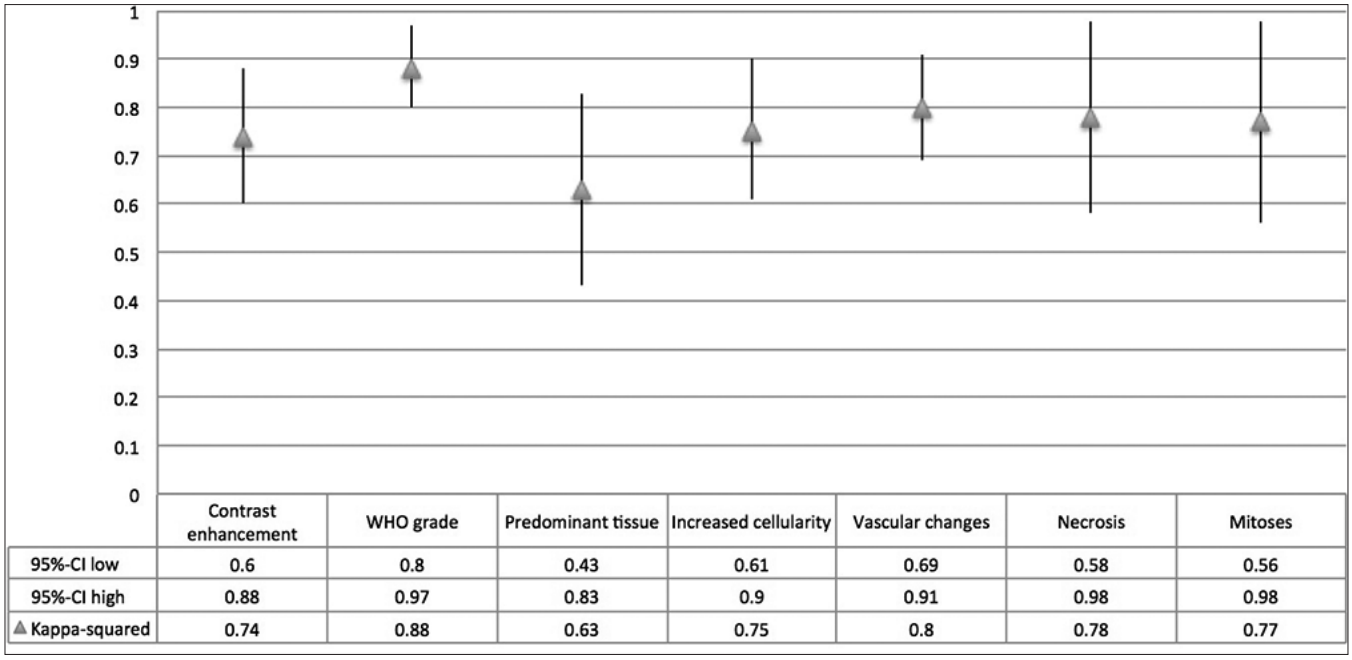
Abbreviations: HT: Hypertrophic endothelium, MVP: Microvascular proliferation

**Table 5f: Crosstable for "necrosis"**

| | Extensive | Local | Focal | Indeterminate | Absent |
|---|---|---|---|---|---|
| Extensive | 4 | 0 | 0 | 0 | 1 |
| Local | 2 | 1 | 0 | 0 | 0 |
| Focal | 0 | 1 | 0 | 0 | 1 |
| Indeterminate | 0 | 0 | 0 | 2 | 3 |
| Absent | 0 | 0 | 3 | 1 | 23 |

**Table 5g: Crosstable for "mitoses"**

| | Frequent | Moderate | Sparse | No |
|---|---|---|---|---|
| Frequent | 1 | 2 | 3 | 0 |
| Moderate | 1 | 3 | 0 | 0 |
| Sparse | 0 | 0 | 4 | 4 |
| No | 0 | 0 | 4 | 20 |




| | Contrast enhancement | WHO grade | Predominant tissue | Increased cellularity | Vascular changes | Necrosis | Mitoses |
|---|---|---|---|---|---|---|---|
| 95%-CI low | 0.6 | 0.8 | 0.43 | 0.61 | 0.69 | 0.58 | 0.56 |
| 95%-CI high | 0.88 | 0.97 | 0.83 | 0.9 | 0.91 | 0.98 | 0.98 |
| ▲ Kappa-squared | 0.74 | 0.88 | 0.63 | 0.75 | 0.8 | 0.78 | 0.77 |

**Figure 2: Interobserver agreement for each tumor parameter expressed in kappa-squared (CI = confidence interval)**

scored again, now by both observers simultaneously. This consensus-based final value was chosen for further analysis.

### Correlation between contrast enhancement and tumor

Of the 39 adequate biopsy samples, 3 had an uncertain diagnosis regarding WHO grade. Correlations between contrast enhancement and tumor were calculated using the remaining 36 samples and displayed in Table 6. Four tumor parameters demonstrated a significant correlation with contrast enhancement: WHO grade, vascular changes, necrosis and increased cellularity. In particular the first three demonstrated highly significant but only moderately strong correlation, with Kendall's tau values around 0.50. The parameter "tumor presence" was found to have no additional value over the information obtained by combining grade II, III and IV lesions in the parameter "WHO grade". Furthermore, substantial interobserver variation for the parameter "inflammation" prevented meaningful analysis of this parameter. Therefore, the parameters "tumor presence" and "inflammation" are not further incorporated in the tables and results.

### Subgroup analysis

To gain more insight in the type of correlation, crosstables are created to relate the values for contrast enhancement with the respective values for each tumor parameter. The results are presented in Table 7, and used to calculate sensitivity, specificity, PPV, NPV, LR+ and LR-. Table 8 shows these values for the correlation between "contrast enhancement" and "WHO grade" using two definitions for each parameter (see also "Materials and Methods" section). If "contrast enhancement" is defined as "thick linear" plus "tumor-like" then the PPV is 1 and the LR+ goes to infinity, regardless of tumor definition (only high grade components versus low grade components as well). Sensitivity is 0.48 if only high grade components are included, and 0.39 if low grade components are included as well. If "contrast enhancement" is defined including "thin linear" enhancement, then specificity falls to circa 0.75 and sensitivity rises to 0.70 (for only high grade components) or 0.61 (including low grade components). Figure 3 contains a web diagram illustrating the sensitivity and specificity of contrast enhancement (using two definitions) for all significantly correlated tumor parameters.

Sensitivity, NPV and LR-all vary around 0.50. This means that half of the histologically confirmed "tumor samples" show contrast enhancement, and half do not. Moreover, half of the contrast enhancing samples are classified as "tumor", and half are not.

**Table 6: Correlation coefficients for "contrast enhancement" related to other tumor parameters**

| Tumor parameter | Kendall's tau | Significance* | NoS |
|---|---|---|---|
| WHO grade | 0.50 | <0.01 | 36 |
| Vascular changes | 0.53 | <0.01 | 38 |
| Necrosis | 0.49 | <0.01 | 39 |
| Mitoses | 0.09 | 0.27 | 39 |
| Increased cellularity | 0.26 | 0.03 | 39 |

NoS: Number of Samples (available for statistical analysis per tumor parameter), *The 1-tailed significance is expressed in a *P* value

**Table 7a: Crosstable with "WHO grade"**

| | Normal/NT | Grade II | Grade III | Grade IV |
|---|---|---|---|---|
| No enhancement | 6 | 4 | 3 | 4 |
| Thin linear | 2 | 1 | 2 | 3 |
| Thick linear | 0 | 0 | 1 | 5 |
| Suspected tumor | 0 | 0 | 0 | 5 |

Abbreviations: NT: No Tumor

**Table 7b: Crosstable with "increased cellularity"**

| | No apparent | Limited | Moderate | Marked |
|---|---|---|---|---|
| No enhancement | 5 | 4 | 6 | 4 |
| Thin linear | 2 | 1 | 3 | 2 |
| Thick linear | 1 | 0 | 3 | 3 |
| Suspected tumor | 0 | 0 | 3 | 2 |

**Table 7c: Crosstable with "vascular changes"**

| | No apparent | Vasodilatation | +HT | +Florid MVP |
|---|---|---|---|---|
| No enhancement | 11 | 2 | 4 | 2 |
| Thin linear | 2 | 3 | 2 | 1 |
| Thick linear | 0 | 2 | 1 | 3 |
| Suspected tumor | 0 | 0 | 0 | 5 |

Abbreviations: HT: Hypertrophic endothelium, MVP: Microvascular proliferation

**Table 7d: Crosstable with "necrosis"**

| | Absent | Indeterminate | Focal | Local | Extensive |
|---|---|---|---|---|---|
| No enhancement | 17 | 0 | 2 | 0 | 0 |
| Thin linear | 4 | 1 | 1 | 0 | 2 |
| Thick linear | 1 | 1 | 0 | 2 | 3 |
| Suspected tumor | 2 | 0 | 2 | 0 | 1 |

## DISCUSSION

The goal of a "gross total resection", or "complete resection of enhancing tumor" (CRET)[30] of a glioblastoma is to resect the contrast enhancing part as visualized on T1-weighted MRI. However, tumor cells are known to be present outside this contrast enhancing area,[5,1] and recent studies comparing contrast enhancement on T1-weighted MRI with diffusion weighted MRI and Positron Emission Tomography have demonstrated a considerable nonoverlap between tumor delineation using these different techniques.[7,21] Our study compares contrast enhancement at the border of the resection cavity with histopathological tumor

**Table 8: Subgroup analysis correlating "contrast enhancement" and "WHO grade" using two definitions per parameter**

| Contrast definition | Thick linear+ tumor-like | Thick linear+ tumor-like | Thin linear+thick linear+ tumor-like | Thin linear+thick linear+ tumor-like |
|---|---|---|---|---|
| Tumor definition* | III+IV | II+III+IV | III+IV | II+III+IV |
| Sensitivity | 0.48 | 0.39 | 0.70 | 0.61 |
| Specificity | 1.00 | 1.00 | 0.77 | 0.75 |
| PPV | 1.00 | 1.00 | 0.84 | 0.89 |
| NPV | 0.52 | 0.32 | 0.59 | 0.35 |
| LR+ | ∞ | ∞ | 3.01 | 2.43 |
| LR- | 0.52 | 0.61 | 0.40 | 0.52 |

LR- : Negative Likelihood Ratio, LR+ : Positive Likelihood Ratio, NPV: Negative Predictive Value, PPV: Positive Predictive Value, thick linear: Thick Linear enhancement, thin linear: Thin Linear enhancement, tumor-like: Suspected residual tumor enhancement, *Tumor definition is expressed in "WHO grade" as explained in the methods section

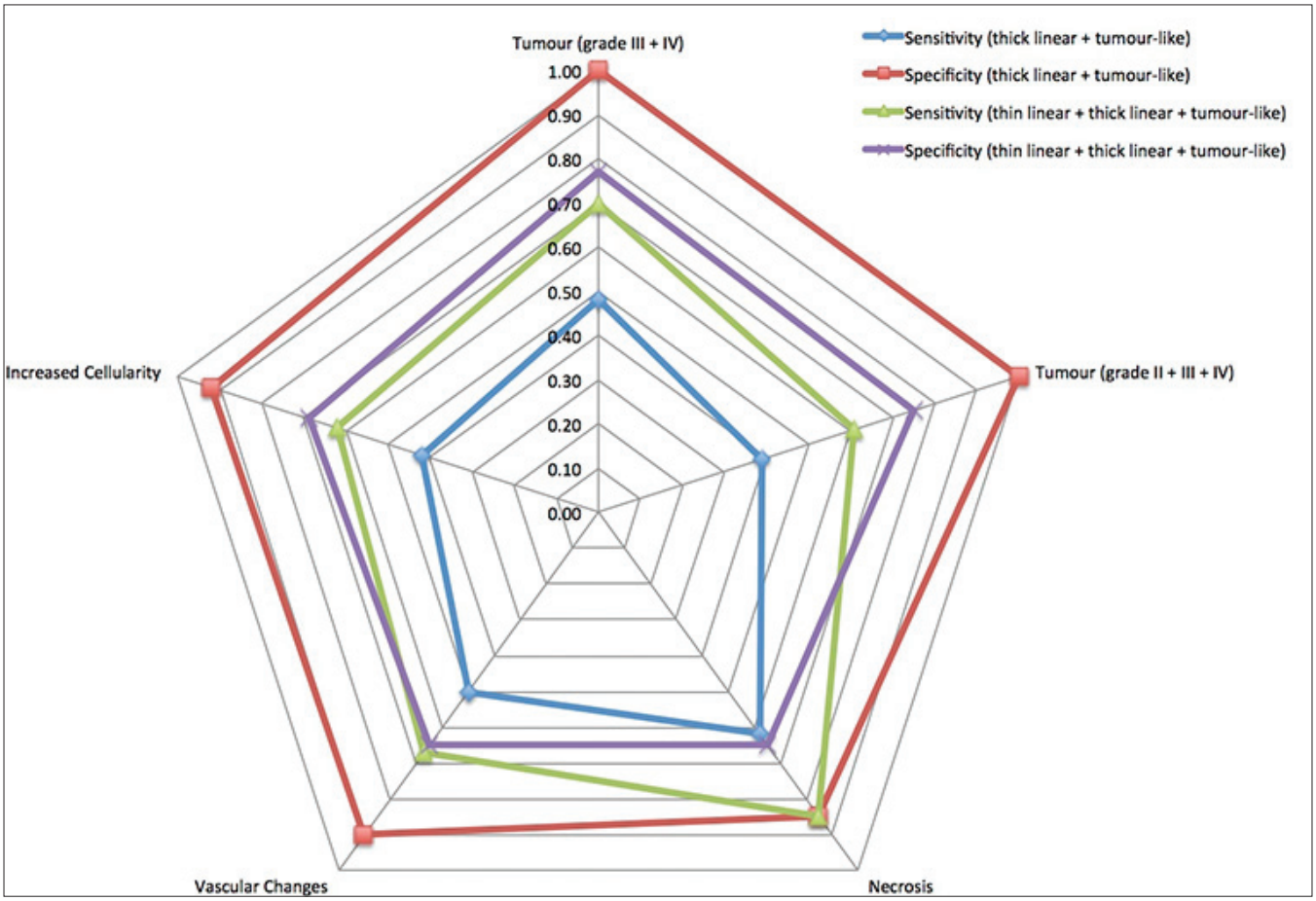



**Figure 3: Web diagram demonstrating sensitivity and specificity of contrast enhancement (using two definitions) for all significantly correlated tumor parameters**

characteristics to determine to what extent contrast enhancement correlates with tumor.

The classification we used for contrast enhancement is derived from Ekinci *et al*.[4] They describe that thin linear "dural like" enhancement leads to tumor regrowth in only 1 out of 16 cases, whereas thick linear enhancement leads to tumor regrowth in all cases, in particular when nodular (tumor-like) components are present. The study by Ekinci studied postoperative 1.5 Tesla MRI using 0.1-0.2 mmol/kg gadolinium-DTPA. Intraoperative contrast enhancement may differ due to iatrogenic manipulation and direct damage to the blood-brain barrier. Therefore we used two definitions for "contrast enhancement" in our study, both with and without thin linear enhancement.

Four histological parameters are significantly correlated with contrast enhancement at the border of the resection cavity: WHO grade, vascular changes, necrosis, and increased cellularity. Of note, these histological features are interrelated. For instance, diffusely increased cellularity

in a brain biopsy sample is an important indicator for the presence of a diffuse glioma, and the presence of necrosis and/or florid microvascular proliferation are the histological hallmarks of high malignancy grade in a diffuse glioma. Furthermore, the correlation itself is relatively weak, with Kendall's tau values of 0.49-0.53 for WHO grade, vascular changes and necrosis, and a value of 0.26 for increased cellularity. As the Kendall's tau is a nonparametric test that describes correlation but provides no detailed information on the kind of correlation, we calculated sensitivity and specificity for the relation between contrast enhancement and tumor presence, using two definitions for each. Our results are consistent with Ekinci *et al*. for thick linear + tumor-like enhancement: Specificity is 1, regardless whether tumor definition includes what we defined as "WHO grade II". Importantly, all patients in our study had a histologically proven glioblastoma. With the histological designation WHO grade II to biopsy samples of these patients we refer to samples in which the tumor lacked histological features of high grade malignancy in that particular (small) specimen. Of note, glioblastomas often show areas (e.g., in the diffuse infiltrative, peripheral parts) in which such histological features of high grade malignancy are lacking, but this does not necessarily mean that the glioblastoma originated from a less malignant precursor lesion. Likewise, PPV and LR+ are maximal for thick linear + tumor-like enhancement regardless of tumor definition. Sensitivity is rather low: 0.48 when including only high grade tumor components, and 0.39 when including grade II components as well. Comparable conclusions can be drawn for NPV and LR-. If we expand our definition of contrast enhancement to include thin linear enhancing tissue, specificity falls to circa 0.75 regardless of tumor definition, PPV varies around 0.84-0.89 and LR+ varies around 2.4-3.0. Sensitivity rises to 0.70 for only high grade components and 0.61 if grade II components are included. NPV and LR- also improve slightly.

Translating these numbers into practical conclusions, one can say that presence of evident contrast enhancement (thick linear + tumor-like) always refers to presence of tumor, regardless of whether histologically less malignant components are included. This is an interesting finding because "iatrogenic damage" to the blood–brain barrier is thought to cause false-positive intraoperative contrast enhancement. Our results demonstrate that this is not the case for thick linear enhancement, but it might be an explanation for the lower specificity when thin linear enhancement is included. Note that we refrained from contrast administration before incision to prevent residual contrast enhancement after tumor resection, which possibly can cause contrast enhancement in nontumorous tissue.[6]

Absence of tumor is always correlated with absence of thick linear enhancement and tumor-like enhancement. Unfortunately, our study shows that absence of contrast enhancement is not useful for predicting absence of tumor. In our study 41-68% of the biopsy samples showed tumor despite absence of contrast enhancement, depending on definition of enhancement (thin linear + thick linear + tumor-like versus thick linear + tumor-like).

## Strengths and limitations

To the best of our knowledge this is the first report with a prospective systematic comparison of intraoperative contrast enhancement and histopathological tumor characteristics, with comparison of biopsies from contrast enhancing and nonenhancing tissue as a particular added value. This is in contrast with previously published work.[16,20,25] Another strength is that both neuropathologists, evaluating the histological parameters in the biopsies, were blinded for the pattern of contrast enhancement.

As far as we know, no validated scoring systems exist for contrast enhancement or for assessment of the histopathological characteristics of glial tumors as assessed in the present study. The scale we used for grading contrast enhancement has been published in an evaluation of tumor regrowth on postoperative MRI, and the scale we used for grading histopathological characteristics has been developed by two experienced neuropathologists (PW, ML). To increase reliability we assessed interobserver agreement for all measurements, and found this to be satisfactory except for the parameter "inflammatory changes". Consensus-based outcomes were used for further analysis, thereby decreasing subjectivity and variation in measurements.

The sample number of 10 patients may be relatively low, but the number of biopsies that was adequate for further analysis ($n$ = 39) was satisfactory. The number of biopsy samples per patient varied because (as also described in the inclusion criteria) the neurosurgeon only took biopsies in those directions where it was considered to be safely possible.

Magnetic field strength is related to spatial resolution of the MR images and capacity of obtaining other imaging modalities (e.g. diffusion weighted imaging, MR spectroscopy). A limitation of this study is that our results cannot automatically be transferred to high-field strength iMRI. However, we do not expect that using a high-field strength iMRI would result in a substantially different outcome as this would only increase spatial resolution. Of course, the use of additional imaging modalities could be of added value.

We used gadopentetate dimeglumine for this study, and gadolinium-based contrast agents are commonly used for (intraoperative) MRI. An interesting alternative for neurosurgeons might be the use of so-called "ultrasmall particles of iron oxide" (USPIO)-based agents, which have been tested on iMRI as well.[18,19,8] These might be less susceptible for iatrogenic damage of the blood–brain barrier, and could offer better correlations between thin

linear contrast enhancement and tumor. Of note, in case of thick linear and tumor-like enhancement we found no indications for imaging artefacts (in particular false-positive contrast enhancement) related to damage of the blood–brain barrier. Specificity for high grade tumor in this pattern of contrast enhancement equals 1.

Our study is limited to assessment of remaining tumor using iMRI. A recent study investigated the use of 5-ALA as a marker for representative stereotactic biopsy samples in several types of tumor, and found better values compared with our study for specificity (1.00) and sensitivity (0.69) in case of strong 5-ALA fluorescence.[32] Another study used 5-ALA to differentiate between necrosis, (fluorescent) "tumor cells", and (nonfluorescent) "margin cells". They found that margin cells do not possess a 'stem-cell molecular signature' but retain tumor-initiating ability *in vivo*.[22] This finding is important, as it contradicts the belief that especially these margin cells are highly tumorigenic. To what extent 5-ALA fluorescence correlates with contrast enhancement on iMRI (and therefore – indirectly – with tumorigenicity of cells at the resection cavity, is currently being investigated (Senft *et al*., personal communication September 2012). The consequences of these findings on surgical strategy regarding EOTR remain to be seen.

### Implications for the future

Contrast enhancement on low-field strength iMRI at the border of the glioblastoma resection cavity has a high specificity but low sensitivity for high grade tumor. Absence of contrast enhancement is unreliable to assess absence of tumor, and from that perspective the rationale for CRET becomes debatable. Especially in glioma surgery complication avoidance is of critical importance. Increasing sensitivity of tumor detection to increase EOTR may be undesirable if a corresponding lower specificity is associated with a higher incidence of (and/or more severe) postoperative neurological deficit. Furthermore, the definition of "tumor" is being discussed to include more than the contrast enhancing part,[31,30] and this may change the philosophy about maximizing tumor resection. There is class 1 evidence that iMRI offers increased EOTR compared with a population of high grade glioma patients that were operated with or without conventional neuronavigation.[26] In a posthoc exploratory analysis Senft *et al*. found no difference between both treatment arms in the control group, which is consistent with the Willems *et al*. study.[33,26] Based on other literature there is, at best, class 2 evidence that iMRI-guided surgery is more effective than conventional neuronavigation-guided surgery in increasing EOTR, enhancing quality of life, or prolonging survival after glioblastoma resection.[10]

Recently, the concept of 'functional neurooncology' was introduced by Duffau *et al*. in low grade gliomas as a method to achieve optimal surgical resection guided by functional rather than by oncological-anatomical boundaries.[2,3] This approach does not suffer from imaging-related limitations. If increased EOTR is associated with prolonged survival, the "functional neurooncology" approach might also be an alternative in high grade glioma surgery to determine resection borders compared with an imaging-based approach.

## CONCLUSIONS

Our present study on glioblastomas shows that evident contrast enhancement (thick linear + tumor-like) as detected on iMRI always reflects presence of high grade tumor and may thus be used as a parameter to increase EOTR. Furthermore, absence of tumor is always correlated with absence of such contrast enhancement. Unfortunately absence of contrast enhancement and presence of thin linear enhancement on iMRI is not useful for predicting absence of tumor. Obviously, diffuse gliomas including glioblastomas are neoplasms that cannot be cured surgically. An (arbitrary) minimally required resection threshold to improve survival, like the widely cited 98% as described by Lacroix *et al*.[13] is debatable, and a valid method to measure this threshold still has to be established.[30,11,24,12,27,14] The use of 5-ALA or a "functional neurooncology" approach may be interesting alternatives for high grade glioma surgery using gadolinium-based contrast agents to increase EOTR safely.

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