



Biomarker concordance between molecular stereotactic biopsy and open surgical specimens in gliomas

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ABSTRACT

Aims. To compare 1p/19q codeletion, *MGMT* promoter methylation, and *IDH* mutation status in stereotactic biopsy and open craniotomy specimens.

Clinical rationale. The latest WHO classification of gliomas requires assessment of the expression of molecular markers. Samples can be obtained for molecular assays via open craniotomy or molecular stereotactic biopsy (MSB). However, there is uncertainty as to whether MSB is representative of the entire tumour, and therefore how reliable it is for treatment planning.

Patients and methods. We examined 11 patients diagnosed with brain tumours suspicious of glioma who underwent open craniotomy after stereotactic biopsy and in whom multiple biomarkers were assessed in both sets of samples by methylation-specific multiplex ligation-dependent probe amplification. Institutional Review Board ethical approval was granted (KB 694/2018).

Results. The initial histopathological grade as determined by stereotactic biopsy was the same as in the samples obtained by open surgery. Further, the marker profile used here was valid in both high- and low-grade gliomas.

Conclusion and clinical implication. MSB is a reliable way to obtain material for precision medicine approaches.

Key words: 1p/19q codeletion; glioma; *IDH*; methylation-specific multiplex ligation-dependent probe amplification; *MGMT*; stereotactic biopsy

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Introduction

Gliomas are the most common primary malignant intracranial tumour, with an annual incidence of ~5 cases per 100,000 people [1–6]. In addition to the patient's performance status at the time of diagnosis, the main prognostic factor for glioma is the presence/absence of some common biomarkers: (i) methylation of the promoter of the gene encoding the enzyme O-6-methylguanine-DNA methyltransferase (*MGMT*); (ii) isocitrate dehydrogenase 1 (*IDH1*) mutations; (iii) isocitrate dehydrogenase 2 (*IDH2*) mutations; and (iv) chromosome 1p/19q codeletion. These molecular tests are

diagnostic, prognostic, clinical manifestations and, in some cases, predictive of responses to different therapies [7–11]. *IDH1* and *IDH2* mutations are useful in the assessment of primary and secondary gliomas and, if present, indicate at least WHO grade II glioma [12]. 1p/19q codeletion supports the diagnosis of oligodendroglioma/oligoastrocytoma [13, 14], occurring in ~75% of oligodendrogliomas and ~45% of oligoastrocytomas but seldom in fibrillary astrocytomas (~8%) [14, 15]. Molecular testing alters the diagnosis in about 3% of cases [16].

However, tumour heterogeneity may also influence the detection and value of these biomarkers [17]. 1p/19q deletions,

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MGMT methylation, and *IDH* mutations can all show heterogeneity in individual tumours, thereby limiting the routine implementation of these molecular results derived from biopsies in clinical practice [17].

Clinical rationale

Molecular stereotactic biopsy (MSB) of untreated gliomas may improve diagnostic precision and enable personalised treatment in inoperable patients. However, glioma heterogeneity and limited cell numbers in small MSB biopsy samples can give rise to diagnostic errors. Therefore, the aim of this study was to compare the molecular results derived from MSBs to those in open craniotomy specimens.

Materials and methods

Patients, consent, and ethics

The Department of Neurosurgery at the 10th Military Research Hospital (Bydgoszcz, Poland) has conducted over 2,500 stereotactic biopsies of brain tumours since 1996. MSB has been performed since 2014. Here, we retrospectively analysed data from a database of 485 patients undergoing MSB.

MSB was performed when: (1) primary tumours and recurrences were located within areas of increased risk of comorbidity without severe mass effect demanding debulking surgery because of brainstem compression and/or midline shift; (2) patients had Karnofsky performance status (KPS) <60; and (3) inflammation or lymphoma was suspected. Open craniotomy was performed after MSB when radiology and biopsy results were equivocal.

The xxx Institutional Review Board (KB 694/2018) reviewed and approved the study protocol, which was conducted according to the principles of the Declaration of Helsinki.

Biopsy and surgery procedures

CT brain scans were performed after fixation of the stereotactic frame to the head on the day of surgery. CT images were co-registered with preoperative MRI scans. Patients were consented and operated upon by a neurosurgeon who, based on the imaging results and clinical features, developed the biopsy trajectory using iPlan software (BrainLAB, Germany) (Fig. 1A). Biopsy needles were inserted, and serial samples were obtained every 1 mm using microforceps from contrast-enhancing parts of the tumour.

For MSB, the first biopsy sample was stained with methylene blue for intraoperative histopathological examination. The neuropathologist classified samples according to the World Health Organisation (WHO) criteria [18]. Every second sample was taken for histopathology assessment and immunohistochemistry as necessary. The same sample was examined by methylation-specific (MS-) multiplex ligation-dependent probe amplification (MLPA) for molecular alterations (see below). The sample intraoperatively assessed as the largest (on average 50 cells) was selected for molecular testing.

During craniotomy, samples for molecular and histopathological assessment were chosen arbitrarily by the neurosurgeon based on macroscopic evaluation (e.g. highly vascularised areas), and radiological changes visualised by MRI used for neuronavigation +/- application of 5-ALA fluorescence marker (Fig. 2). Tumour samples for postoperative molecular examination were much larger than those taken for intraoperative biopsy. After surgery, MRI was used to assess the resection status using a 3T (Philips) device, T1 and T2 sequences, and after intravenous administration of gadobutrol contrast medium (1 mmol/ml) at a dose of 0.1 ml/kg body weight [19] (Fig. 1B).

Molecular analysis

MS-MLPA allows simultaneous analysis of multiple molecular markers using small amounts (100 ng) of DNA isolated

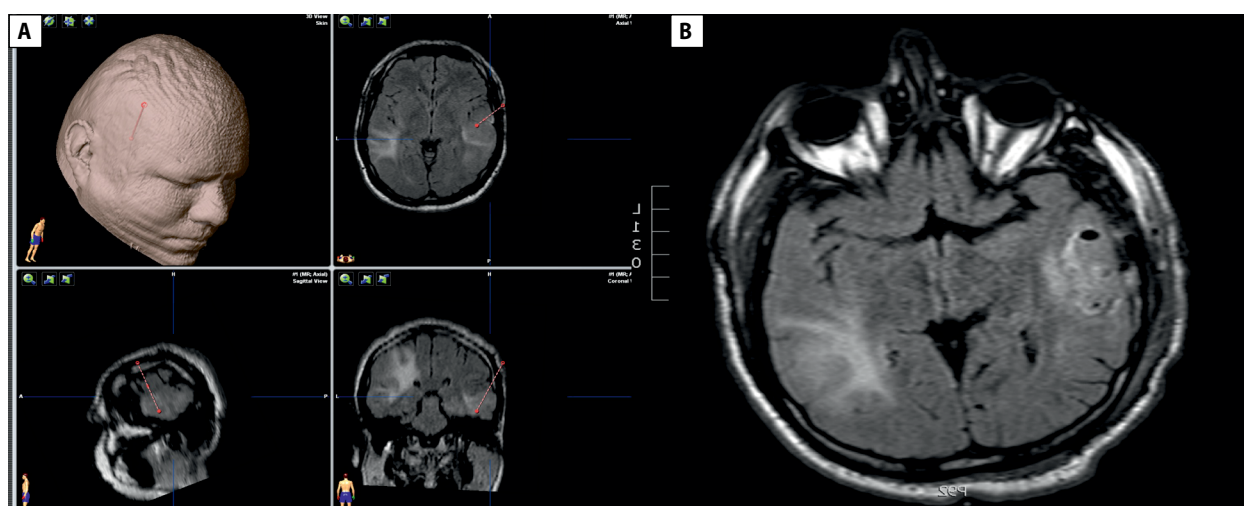


Figure 1. A. biopsy planning based on T2-Flair MRI (patient 2). B. Post surgery T2-Flair MRI (patient 2)

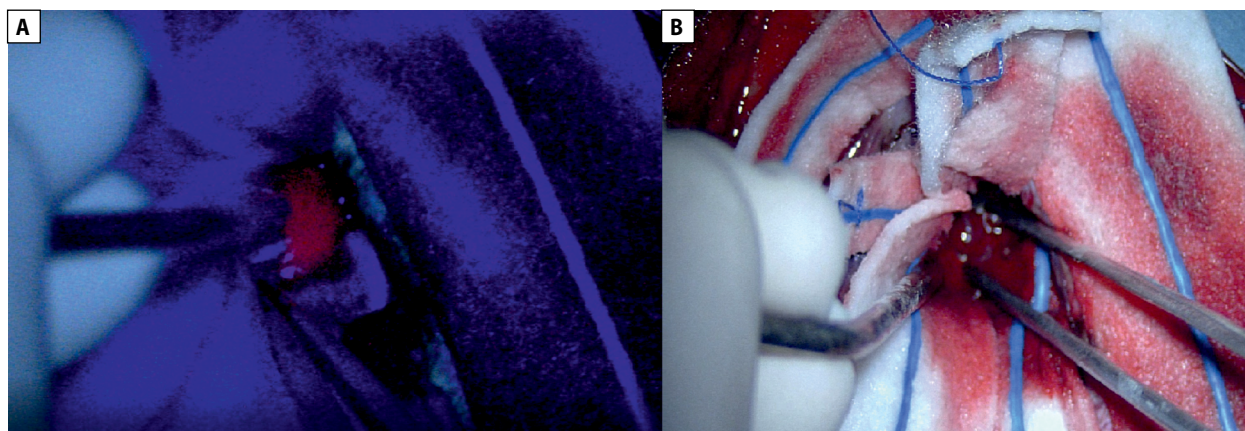


Figure 2. A. Tumour in 5-ALA induced fluorescence (patient 9). B. Tumour in white light (patient 9)

from paraffin-embedded tissue. The MS-MLPA method is described in detail elsewhere [20]. Briefly, DNA was extracted from the fixed samples by the magnetic method using a Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Promega, Madison, WI, USA). The MLPA method was validated prior to clinical use on 50 archival tissue samples, with methylation verified using pyrosequencing and methylation PCR. Co-deletion was not verified using other methods because there is known to be a high correlation between MLPA and FISH test results [21–23].

MLPA is a comparative method, i.e. the result of a given test sample is compared to a control. According to the manufacturer's recommendations (MRC, Holland), for each experiment a minimum of four reference tests was used when the number of test samples did not exceed 20; when there were > 20 tests performed, one reference sample was added for every seven tests in accordance with the manufacturer's recommendations. In addition, MLPA has a number of internal controls to assess each reaction and the purity and amount of DNA. The results were analysed using the dedicated cofalyser.net software (MRC, Holland). Repeatability and reproducibility were established as 100%.

Statistical analysis

The results were described using descriptive statistics. Cohen's kappa coefficient and intraclass correlation coefficient (ICC) was used to assess the agreement between MSB and open biopsy molecular results (Statistica v 13.0).

Results

Of 531 patients with molecular results, only 11 (2.1%) patients had molecular tests from both the MSB and subsequent surgery; five women (45.5%) and six men (Table 1). The median age was 35 years (IQR 21–60 years). The initial KPS ranged from 60 to 100. After treatment, two patients experienced a deterioration in their quality of life.

Only one case had been biopsied and diagnosed as a glioma prior to surgery to determine the nature of the MRI changes. In this case, the MSB was performed to determine whether the visible lesion represented recurrence or necrosis after radiotherapy. In the MSBs, four (36.4%) patients had WHO II astrocytoma diffusum, one patient (9.1%) had oligodendroglioma, and the other six were classified as high-grade malignant gliomas. Of these, four (36.4%) were diagnosed with anaplastic astrocytoma, and two (18.2%) with glioblastoma multiforme. There were no other treatments between procedures, and the median interval between procedures was 57.5 days (16–132 days) (Tab. 1).

In all cases, the molecular tests were conclusive (Tab. 2). No perioperative complications were noted, and the neurological status did not change after MSB. After the procedure, each patient had a follow-up CT examination that did not reveal any bleeding. After surgery, the patients had a control MRI with contrast, which also showed no clinically significant incidents of bleeding.

A total absence of contrast-enhancing lesion or a contrast-enhancing lesion with < 2% of the baseline change was considered to be a complete tumour resection. Contrast-enhancing lesions with a volume > 2% of the initial change were considered partial resections. In the post-surgery MRIs, total resection was demonstrated in seven (63.6%) cases and partial resection in four cases (36.4%) (Tab. 2). Two patients experienced a deterioration in neurological status accompanied by a deterioration in quality of life after craniotomy (18.2%).

All histopathological findings after craniotomies were the same as the MSB results. The molecular results were also the same in all cases (absolute agreement of 1.00 ICC). *MGMT* promoter methylation was present in two patients, and *IDH* mutations were present in seven cases (Cohen's kappa coefficient 1, $p < 0.05$).

Discussion

Here we report that molecular genetics results derived from MSB are reliable and representative of both low- and

Table 1. Clinicopathological features of the study population

Variable		Value
Age		Median 35 years (IQR 21–60 years)
Gender	Female	5 (45.5%)
	Male	6 (54.5%)
KPS before resection of a tumour	100	7 (63.6%)
	90	2 (18.2%)
	80	1 (9.1%)
	70	0 (0%)
	60	1 (9.1%)
KPS after resection of a tumour	100	6 (54%)
	90	2 (18.2%)
	80	0 (0%)
	70	2 (18.2%)
	60	1 (9.1%)
Performance status deterioration after surgery		2 (18.2%)
Histopathological diagnosis	Astrocytoma WHO II	4 (36.4%)
	Oligodendroglioma WHO II	1 (9.1%)
	Astrocytoma anaplasticum WHO III	4 (36.4%)
	GBM WHO IV	2 (18.2%)
Interval between biopsy and resection		Median 57 days (range 16–132 days)

Table 2. Biopsy and surgery histopathology and molecular results of the entire group

Number	Histopathological diagnosis (biopsy)	Histopathological diagnosis (craniotomy)	MGMT	IDH1 (R132)	Deletion (1p/19q)	Tumour localisation	Type of resection
Patient 1	Astrocytoma anaplasticum (WHO III)	Astrocytoma anaplasticum (WHO III)	-	Mutated	Negative	Left frontal lobe	Total
Patient 2	Astrocytoma diffusum (WHO II)	Astrocytoma diffusum (WHO II)	-	Mutated	Negative	Right temporal lobe	Total
Patient 3	Astrocytoma AAASDADAS-DADASDSADASDADDA-astrocytoma anaplasticum (WHO III)	AAASDADASDASDSDSA-DASDAstrocytoma anaplasticum (WHO III)	-	No mutation	Negative	Left parietal lobe	Total
Patient 4	Astrocytoma diffusum (WHO II)	Astrocytoma diffusum (WHO II)	-	Mutated	Negative	Right insular lobe	Partial
Patient 5	Astrocytoma diffusum (WHO II)	Astrocytoma diffusum (WHO II)	-	No mutation	Negative	Left cerebellar lobe	Partial
Patient 6	Oligodendroglioma (WHO II)	Oligodendroglioma (WHO II)	-	No mutation	Positive	Right frontal lobe	Partial
Patient 7	Astrocytoma anaplasticum (WHO III)	Astrocytoma anaplasticum (WHO III)	-	No mutation	Negative	Left temporal lobe	Partial
Patient 8	Astrocytoma diffusum (WHO II)	Astrocytoma diffusum (WHO II)	-	Mutated	Negative	Cerebellar vermis	Total
Patient 9	Glioblastoma multiforme (WHO IV)	Glioblastoma multiforme (WHO IV)	Methylated	Mutated	Negative	Left frontal lobe	Total
Patient 10	Astrocytoma anaplasticum (WHO III)	Astrocytoma anaplasticum (WHO III)	Methylated	Mutated	Negative	Left occipital lobe	Total
Patient 11	Glioblastoma multiforme (WHO IV)	Glioblastoma multiforme (WHO IV)		No mutation	Negative	Right occipital lobe	Total

high-grade gliomas. MLPA testing on stereotactic biopsy material and tissue derived after craniotomy appears to be equivalent for genetic analysis.

Molecular diagnostic testing should be considered whenever changes in tumour molecular biology might be expected, i.e. after primary therapy and where the results might justify the use of adjuvant systemic therapy. At the time of asymptomatic recurrence, the value of an open craniotomy should be balanced against the risk of complications and prolonging the time before starting systemic or radiation therapy [24–28]. Additionally, safe resection or macroscopic tumour removal is not feasible when gliomas are localised in eloquent areas. The role of subtotal surgery, especially in recurrent high-grade gliomas or previously unirradiated low-grade gliomas, remains unclear. It has been shown that subtotal tumour removal has no advantage over chemoradiation in the treatment of glioblastomas. Nevertheless, partial resections have generally been considered to be the only reliable method to obtain precise molecular results, and from this point of view our results are of great practical significance.

The simultaneous development of molecular diagnostics and the possibility of adopting effective conservative treatment in patients with low-grade gliomas with *IDH1* mutations encourages the use of MSB [29, 30]. Our data suggests that it is reasonable to guide targeted therapies based on MSB results rather than open surgery. The most beneficial genetic alteration in gliomas is coexistent *IDH1* mutation and *MGMT* promoter methylation [31]. Here, both *IDH* mutation and *MGMT* promoter methylation status established by MSB and surgery were conclusive, irrespective of tumour grade.

In a recent report, macroscopic gross total resection was achieved in approximately 80% of glioma cases [32]. In patients initially qualifying for stereotactic biopsy, complete surgical resection was more difficult, being achieved in 63.6% of cases, and as a consequence of preselection of patients for MSB in our department.

In this study, MS-MLPA was used due to its high reproducibility, accuracy, and robustness [20–23, 33]. MS-MLPA has a number of advantages over conventional MS-polymerase chain reaction (PCR) [20]. Regardless of the method used, it should be emphasised that *MGMT* promoter methylation may change over time [34, 35], which must be taken into account when there is a long gap between examinations.

Glioma heterogeneity has been evaluated in several studies [36–41]. Heterogeneous *MGMT* promoter methylation status has mainly been noted in high-grade tumours [42, 43], raising concerns as to whether single MSB results are representative. Some studies have reported differences in the molecular and histopathological diagnosis in different samples from the same tumour, missing anaplastic foci in stereotactic samples [39, 44]. Furthermore, the phenomenon of pseudo-heterogeneity should be considered when there are large amounts of intratumoural necrosis, inflammation, or normal tissue in glioma samples. A recent study

[38] detected no *MGMT* promoter heterogeneity between different samples taken from the same tumours in 24 out of 25 patients; the one false negative that was detected was due to DNA dispersing into necrotic tissues. This is consistent with our results and the results of a serial MSB study into WHO II gliomas, where *MGMT* methylation and *IDH* mutations were homogeneous inside and outside hotspots of all 14 tumours tested [44].

A recent comparison of biopsy and surgery molecular results [45] reported similar results to ours, the main differences being the histopathological diagnosis and molecular diagnostic test (MS-PCR) chosen [16]. Gessler et al. [45] did not examine low-grade gliomas, the group instead being limited to three anaplastic astrocytomas and 19 glioblastomas. In both studies, samples were examined after a short interval, mainly due to the non-specific radiological appearances. Conclusive molecular biomarkers were obtained from small biopsy samples (< 1 mm³) by selecting many probes and by using a highly controlled sampling technique, with intraoperative neuropathological examination to exclude any contamination. Eigenbrod et al. [16] collected at least two samples from different tumour sites to determine *MGMT* promoter methylation, while we showed that the results are the same in samples obtained by biopsy and open craniotomy. We also showed that MSB is effective in both low- and high-grade gliomas.

Many papers have reported the safety and diagnostic value of materials obtained using stereotactic biopsy [46–50]. The number of perioperative complications occurring during surgical resections of brain tumours remains significant [51]. Even in our neurosurgery department, a facility with a high level of experience, the performance status decreased in two out of 11 patients.

Our study is limited by the small number of cases analysed; however, this also meant that of 531 biopsies, only 11 had clinical indications for subsequent open surgery. This uncommon scenario is also reflected in the number of patients recruited in other series [45]. It should be noted that the proportion of normal, non-tumour cells and neoplastic cells may falsely influence the *MGMT* methylation result in MS-MLPA. Moreover, there was an almost two month gap between MSB and open craniotomy in some cases, although histopathological and molecular results did not change despite this interval.

Clinical implications/future directions

Given our small group, cautious conclusions should be drawn. However, we have shown that the material obtained for MSB from both low- and high-grade gliomas can be used for the precise assessment of molecular status, and that MSB provides material for the valid and reproducible examination for multiple biomarkers without compromising the quality of histological classification and grading. MSB can be used for definitive molecular diagnosis and represents the final stage

of neurosurgical activities in some cases, especially tumours in which there is little chance of total removal without consequential neurological deficits. Further large-scale concordance studies are required to further validate molecular diagnoses on MSB specimens.

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