MGMT promoter gene methylation and neurological scale improvement in glioma: a cohort study [version 2; peer review: 1 approved, 1 not approved]

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Abstract

Background: Glioma is one of the most common primary brain tumours and conveys a dismal prognosis despite aggressive treatment. Several biomarkers have been studied in the hope of yielding better diagnostic accuracy and improving patient management. Besides survival, functional and neurological disability are concerns that have no lesser importance. In 2017, a disease-specific assessment tool – the Neurologic Assessment in Neuro-Oncology (NANO) scale – was developed to measure neurologic function in neuro-oncology cases. We sought to determine biomarkers that might be associated with neurological scale improvement in glioma patients.

Methods: Glioma grade II-IV patients were recruited from three major hospitals in Jakarta-Tangerang. Isocitrate dehydrogenase (IDH) mutation and O6-methylguanine-DNA methyltransferase (MGMT) promoter gene methylation were tested, as well as patients’ neurological function before surgery and three months after. Improvement in neurological scale (NANO scale) was considered positive if there was a decrement of ≥1 of the scale.

Results: There were 54 patients included in the study. Mean age was 43.63 (14.723) years old, and 61.1% were male. As much as 16 (29.6%) carried a mutation in codon 132 of the IDH1 gene, and 33 (61.1%) were MGMT methylated. Median NANO scale score before and three months after surgery was 4 (0-12) and 3 (0-12), respectively. Neurological improvement was found in 44 (81.5%) of the patients. Among patients with MGMT promoter gene...
methylation, 90.9% showed neurological improvement (p=0.035; 
OR=5; 95%CI 1.122-22.272).

**Conclusions:** Gliomas with MGMT promoter gene methylation are more likely to show neurological improvement three months after surgery.

**Keywords**
MGMT promoter gene methylation; Glioma; Neurological Scale; NANO Scale
Introduction

Glioma is one of the most common primary brain tumours, in which 80% of the cases are found to be malignant (Aliferis & Trafalis, 2015; Schwartzbaum et al., 2006; Stupp et al., 2010). Grade II glioma has a median survival ranging from four to more than ten years (Bell et al., 2018; Claus & Black, 2006). However, glioblastoma – a grade IV glioma – accounts for the majority of gliomas and carries a poor prognosis with median survival of six to 14 months despite aggressive treatment with radiotherapy plus chemotherapy, and less than 12 months with radiotherapy alone (Stupp et al., 2005).

Molecular characteristics of glioma have undergone extensive research in the last decade, and some biomarkers have even been integrated into the 2016 glioma classification. Isocitrate dehydrogenase (IDH) mutation and O6-Methylguanine-DNA methyltransferase (MGMT) promoter gene methylation has been associated with better survival. Nonetheless, patients’ quality of life and neurological deficit have not been considerably measured and correlated with these prognostic biomarkers.

In 2017, the Neurological Assessment of Neuro-Oncology (NANO) scale was developed to objectively measure neurological deficit in patients with brain tumours (Nayak et al., 2017). Previous studies have shown that a good initial NANO scale score was significantly associated with improvement in neurological deficit two months after surgery (Gunawan et al., 2020) and a more powerful method to predict prognosis during initial diagnosis and disease progression (Lee et al., 2018). In this study, we aimed to elucidate biomarkers that might be associated with neurological scale improvement three months after surgery.

Methods

Patient collections and ethics statement

This is a cohort study, with a purposive sampling method. There were 72 suspected brain tumour patients, aged more than ten years old, who presented at three major hospitals in Jakarta and Tangerang over a period of one year (July 2019–July 2020). After a brain MRI, 5 patients were excluded due to tumour location (brainstem or infratentorial). Written consent was obtained from every patient or their caregiver (for patients who are unable to give consent). Karnofsky Performance Scale (KPS) as well as neurological scale were assessed before surgery. Following tumour resection, 62 patients were histologically confirmed as having glioma grade II-IV. Tumour tissue was tested for IDH mutation as well as MGMT promoter gene methylation. Patients underwent standardised therapy, and neurological function was re-assessed three months after surgery. Six patients were deceased before the second neurological scale assessment, and two patients were lost to follow-up, hence were excluded from the study. The study was approved by Medical Ethical Research Committee, Universitas Hasanuddin, No: 1232/UN4.6.4.5.31/PP36/2019.

Histopathological and molecular analysis

Histopathological and molecular analysis was carried out in Mochtar Riady Institute Tangerang and Kalgen Innolab Clinical Laboratory, Jakarta. Tissue sample dissected from the tumor was immediately put in formaldehyde 10% buffer. Six to 24 hours later, the desired tumor tissue was stored into cassettes and processed in an automatic tissue processor. After an overnight processing, tissue was put into a mold with paraffin wax. Paraffin block with the embedded tissue was cut using a microtome and placed on glass slides stained with hematoxylin and eosin to determine the diagnosis and grade of malignancy. Histopathological examination was conducted by two neuropathologists and classified according to the 2016 WHO Classification of Tumours of the Central Nervous System (Louis et al., 2016). Diffuse astrocytic and oligodendrogial tumours and other astrocytic tumours WHO grade II-IV were included in the study. Area with the most tumour accumulation along with tumour cell percentage was marked. This area was then scraped for further analysis.

DNA isolation

Genomic DNA was extracted from paraffin-embedded tumor tissues using the QIAamp®DNA MicroKit (QIAGEN, catalogue number 56304) following the manufacturers’ protocol.

IDH mutation

IDH mutation evaluation was done using high resolution melting (HRM) analysis and Sanger sequencing of polymerase chain reaction (PCR)-amplified fragments, which were generated during the HRM procedure with the PCR primers. Primers used were: IDH1-forward 5′-CGGTCTTCAAGAAGCCATT-3′ and IDH1-reverse 5′-GCAAAAATCACATTATTGCCAAC-3′ (gBlocks Gene Fragment, Integrated DNA Technologies).

HRM analysis consists of 3 steps: PCR reaction, DNA melting, and data analysis. These analyses were performed using the LightCycler® 480 High-Resolution Melting Master Kit (Roche) according to the manufacturer’s instructions. PCR amplification and HRM analysis were carried out in a LightCycler® 480 real-time PCR system (Roche Diagnostics).
Table 3 discussed and optimal scale were determined. NANO scale was
sicians, and subjective complaints not included in the scale
tion. Neurological scale was assessed by two individual phy
2 or 3, with higher score indicating worse neurologic func
sensation, visual fields, facial strength, language, level of con
patients in nine domains: gait, strength, upper extremity ataxia,
NANO scale is a simple neurological assessment evaluating
Neurological function was assessed using the NANO scale.
Neurological scale assessment and follow-up
outcomes were classified as either methylated or
DNA and unmethylated DNA (Zymo Research) served as posi
rose gel stained with SYBR Safe. Commercial methylated
DNA extracted first underwent bisulphite treatment to convert
all unmethylated cytosine to uracil, leaving 5-methylcytosine
unaltered. After being eluted in DNase-free water, methylation
analysis was commenced using real-time methylation-specific
PCR (MSP). PCR amplification was carried out in a LightCycler®
480 real-time PCR system (Roche Diagnostics). Quantifast
Multiplex PCR Kit (QIAGEN) was used for PCR and DNA was
amplified utilizing HotStarTaq Plus DNA polymerase which
was included in the kit. Primers used were: MGMT Methylated-
forward 5'-TTTCCGACGTTCTGTAAGTTTTCG-3', MGMT
Methylated reverse 5'-GCCACTCTTCCGAAAAACGAAAGC-3'
and MGMT Unmethylated-forward 5' TTGTGTGTTTGTGTT
TGTTAGGTTTGT-3', MGMT Unmethylated-reverse 5'- AA
CTCCACACTCTTCCTCCAAAAACAAAGC-3'. Cycling conditions
were 95°C for 5 min, followed by 42 cycles of amplification
(denaturation at 95°C for 30 seconds, annealing at 59°C for
30 seconds, and extension at 72°C for 30 seconds) and 72°C
for 5 min. PCR reactions (15 μl) were analyzed on a 2% agarose
gel stained with SYBR Safe. Commercial methylated
DNA and unmethylated DNA (Zymo Research) served as posi
tive controls. Outcomes were classified as either methylated or
unmethylated.

MGMT promoter gene methylation
DNA extracted first underwent bisulphite treatment to convert
all unmethylated cytosine to uracil, leaving 5-methylcytosine
unaltered. After being eluted in DNase-free water, methylation
analysis was commenced using real-time methylation-specific
PCR (MSP). PCR amplification was carried out in a LightCycler®
480 real-time PCR system (Roche Diagnostics). Quantifast
Multiplex PCR Kit (QIAGEN) was used for PCR and DNA was
amplified utilizing HotStarTaq Plus DNA polymerase which
was included in the kit. Primers used were: MGMT Methylated-
forward 5'-TTTCCGACGTTCTGTAAGTTTTCG-3', MGMT
Methylated reverse 5'-GCCACTCTTCCGAAAAACGAAAGC-3'
and MGMT Unmethylated-forward 5' TTGTGTGTTTGTGTT
TGTTAGGTTTGT-3', MGMT Unmethylated-reverse 5'- AA
CTCCACACTCTTCCTCCAAAAACAAAGC-3'. Cycling conditions
were 95°C for 5 min, followed by 42 cycles of amplification
(denaturation at 95°C for 30 seconds, annealing at 59°C for
30 seconds, and extension at 72°C for 30 seconds) and 72°C
for 5 min. PCR reactions (15 μl) were analyzed on a 2% agarose
gel stained with SYBR Safe. Commercial methylated
DNA and unmethylated DNA (Zymo Research) served as posi
tive controls. Outcomes were classified as either methylated or
unmethylated.

Neurological scale assessment and follow-up
Neurological function was assessed using the NANO scale.
NANO scale is a simple neurological assessment evaluating
patients in nine domains: gait, strength, upper extremity ataxia,
sensation, visual fields, facial strength, language, level of con-
sciousness, and behaviour. Each domain was scored from 0 to
2 or 3, with higher score indicating worse neurologic func-
Neurological scale was assessed by two individual phys-
cicians, and subjective complaints not included in the scale
were also noted. Any discrepancies between investigators were
discussed and optimal scale were determined. NANO scale was
first assessed before surgery and then re-assessed three months
later during their routine clinical follow up. Patients who were
deceased before the second assessment were excluded from
the study. Neurological improvement was defined as decre-
ment in NANO scale score by 1 or more, which was calculated
from difference of initial NANO and NANO three months after
surgery.

Statistical analysis
The association between baseline characteristics and biomark-
ers were analysed. Categorical data are presented as propor-
tions and interpreted using chi-square or Pearson Fisher’s
exact test. All calculations were performed using IBM SPSS
Statistics version 24. The reported p values are two-sided,
and a probability value of <0.05 was considered statistically
significant.

Results
In total, 54 patients completed the follow up and were
analysed. Half of the cases (50%) were glioma grade IV, fol-
lowed by grade II (37%) and grade III (13%). Overall mean age
was 43.63 (14.723) years old for all gliomas, and mean age for
glioblastoma was 50 (12.7) years old. Male to female ratio was
1.57. Median Karnofsky Performance Scale (KPS) score was
55 (30–80). Most of the patients underwent surgery followed by
radiation and chemotherapy (79.6%) (Table 1).

The most common presenting symptom was headache (63%).
Patients with tumours located in the frontal lobe most com-
monly presented with headache (58.8%), one-sided weakness
(35.3%), seizure (32.4%), cognitive disturbance (20.6%), and
aphasia (5.9%).

In 16 patients (29.6%) an IDH1 mutation was found and no
IDH2 mutation was found. Younger age and male gender were
significantly associated with having an IDH mutation (Table 2).

There were 33 patients (61.1%) that were MGMT methyl-
ated (Table 3). Older patients had a higher tendency to have
methylated MGMT than younger patients. Patients with IDH
mutations were more likely to harbour MGMT methylation
(p=0.049; OR=1.54; 95%CI 1.05-2.26).

The number of patients with MGMT methylation who under-
went surgery plus chemoradiation, surgery plus radiation only,
and surgery only were 26 patients (78.8%), one patient (3%)
and six patients (18.2%), respectively.

Median NANO scale score before surgery was 4 (0–12) and
three months after surgery was 3 (0–12). Improvement in
neurological function, measured using the NANO scale, was
found in 44 (81.5%) of the patients. Age, gender, initial KPS,
tumour location, grade of glioma and IDH mutation were
not associated with improvement in neurological function. Among
patients with MGMT promoter gene methylation, 90.9% showed
improvement in neurological function. A multivariate analysis
using logistic regression confirmed MGMT methylation as an
independent factor towards NANO scale improvement (p=0.035;
OR=5; 95%CI 1.122-22.272) (Table 4).
Further analysis shows that coexistence of IDH mutation and MGMT methylation were mostly found in grade II patients (53.8%) and 100% of patients with coexistence of both biomarkers showed improvement in neurological scale ($p=0.032$) (Table 5).

We did a subgroup analysis of patients with glioblastoma. Baseline characteristics of patients with glioblastoma were depicted in Table 6. IDH mutation and MGMT methylation did not show any relation towards neurological scale improvement (Table 7).

**Discussion**

Glioma remains to be a challenging tumour, with diverse clinical presentation, phenotype and molecular parameters (Louis et al., 2016). It is not unusual for clinicians to encounter cases of higher-grade glioma with longer survival than...
### Table 3. Characteristics of patients and MGMT methylation.

<table>
<thead>
<tr>
<th>Variables</th>
<th>MGMT methylation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methylated</td>
<td>Unmethylated</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥45 years old</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>&lt;45 years old</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>Initial KPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–50</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>60–100</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>IDH Mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH mutant</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Wild type</td>
<td>20</td>
<td>18</td>
</tr>
</tbody>
</table>

KPS: Karnofsky Performance Scale; IDH: isocitrate dehydrogenase; MGMT: O6-Methylguanine-DNA methyltransferase.

### Table 4. Characteristics of patients and neurological improvement.

<table>
<thead>
<tr>
<th>Variables</th>
<th>NANO scale improvement</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥45 years old</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>&lt;45 years old</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Initial KPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–50</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>60–100</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Tumour Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>28</td>
<td>6</td>
</tr>
<tr>
<td>Other than frontal lobe</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Grade of glioma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>17</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>NANO scale improvement</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>IDH mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH mutant</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Wild type</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>MGMT methylation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylated</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>Unmethylated</td>
<td>14</td>
<td>7</td>
</tr>
</tbody>
</table>

KPS: Karnofsky Performance Scale; IDH: isocitrate dehydrogenase; MGMT: O6-Methylguanine-DNA methyltransferase; NANO: Neurologic Assessment in Neuro-Oncology.

lower-grade glioma during conventional treatment. Despite survival, clinical and functional status is another concern in treatment initiation and disease progression. KPS has been generally used to evaluate brain tumours' performance status. However, like tumour grade, there have also been cases in which those with a low KPS score survived longer than those with a high KPS score (Lee et al., 2018).
Table 5. Combination of IDH mutation and MGMT methylation towards neurological improvement.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>NANO scale improvement</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>(-)</td>
</tr>
<tr>
<td>IDH mutant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGMT methylated</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>MGMT unmethylated</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Wild type IDH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGMT methylated</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>MGMT unmethylated</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

IDH: isocitrate dehydrogenase; MGMT: O6-Methylguanine-DNA methyltransferase; NANO: Neurologic Assessment in Neuro-Oncology.

Table 6. Baseline Characteristics of Glioblastoma patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N = 27 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>≥45 years old</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td>&lt;45 years old</td>
<td>8 (29.6)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (55.6)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (44.4)</td>
</tr>
<tr>
<td>Tumour location</td>
<td></td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>18 (66.7)</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Initial KPS</td>
<td></td>
</tr>
<tr>
<td>0–50</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td>60–100</td>
<td>8 (29.6)</td>
</tr>
</tbody>
</table>

The NANO scale is a relatively new scale that serve as an objective and quantifiable metric of neurologic function in brain tumour patients (Nayak et al., 2017). A previous study found that performance status estimated by the NANO scale was significantly associated with overall survival, and was a more powerful method to predict the prognosis of GBM than the KPS during both initial diagnosis and disease progression (Lee et al., 2018). It was also found that initial NANO scale possesses a stronger correlation neurological scale improvement than initial KPS towards functional scale improvement (Gunawan et al., 2020). In this study, we used NANO scale to objectively measure neurological scale improvement. A decrement in NANO scale score by 1 or more was used as a cut-off for neurological improvement considering the results and definition used in previous studies. One study showed a mean progression of NANO scale as much as 4.1 points during 18.9 months follow up (Lee et al., 2018) and another study defined neurologic response of NANO scale as ≥2 level of improvement (Nayak et al., 2017). Taking into account that this is a short follow-up study, a decrement of ≥2 will be unlikely, especially if the mean progression of NANO scale is 4.1 points in 18.9 months, which means a progression of 1 point in 4.7 months. Thus, decrement of ≥1 score of NANO scale was considered as seemingly relevant.

From 54 patients included in the study, most of the cases were glioma grade IV, glioblastoma, IDH-wildtype (44.4%), followed by grade II and III. This distribution is similar to previous studies analysing different grades of gliomas (Malueka et al., 2020; Ostrom et al., 2018; Theresia et al., 2020). Mean age in this study was 43.63 years old, and patients with glioblastoma have a mean age of 50 (12.7) years old, which is comparable to previous studies conducted in Asian (Lee et al., 2018; Malueka et al., 2020; Theresia et al., 2020), African (Senhaji et al., 2017), and Amsterdam (Molenaar et al., 2014) populations and lower than mean age of studies in the United Kingdom (Philips et al., 2018; Sehmer et al., 2014), Greece (Aliferis & Trafalis, 2015) and United States (Ostrom et al., 2018; Schwartzbaum et al., 2006). The male to female ratio was 1.57, which is comparable to previous studies (Malueka et al., 2020; Ostrom et al., 2018; Sehmer et al., 2014). The proportion of low and high initial functional status was equivalent. Tumours were mostly located in the frontal lobe, followed by temporal, parietal, and occipital. The most common presenting symptom was headache, and 58.8% of tumours located in the frontal lobe presented with this symptom. Surgery, radiation, and chemotherapy were underwent by 79.6% of patients.
It was found that 29.6% of patients were positive for IDH1 mutation. Being of a younger age and male were factors associated with having an IDH mutant. This is similar to previous studies that found younger age to be associated with IDH mutation in glioblastoma (Molenaar et al., 2014; Songtao et al., 2012). However, a study in Indonesia showed no differences in age and gender towards IDH mutation (Malueka et al., 2020). Dissimilarity may be caused by differences in characteristics of patients included in their study (which also included grade I glioma). Grade I glioma is frequently found in children, with different characteristics, therapeutic interventions, and prognosis. Therefore, in this study, we did not include grade I glioma and excluded patients less than 10 years old.

MGMT methylation was found in 61.1% of patients. Patients with IDH mutation were more likely to have an MGMT methylation as well (p=0.049; OR=1.54; 95%CI 1.05-2.26). These findings are in accordance with previous studies (Molenaar et al., 2014; Songtao et al., 2012).

Improvement in NANO scale within three months after surgery was found in 81.5% of the patients. From the variables studied, only MGMT promoter gene methylation revealed a significant link to NANO scale improvement three months after surgery (p=0.033; OR=5; 95%CI 1.122-22.272).

Although IDH mutation alone was not significantly related to scale improvement, the combination of IDH mutation and MGMT methylation was associated with improvement in neurological function three months later. This might be due to the significant association of MGMT methylation towards NANO scale improvement, which influenced the relation between its combination with IDH mutation towards scale improvement.

MGMT promoter gene methylation has been commonly accepted to confer survival advantage regardless of therapy (Reifenberger et al., 2014). It carries both prognostic and predictive value. In this study, it was found that gliomas with MGMT promoter methylation are five times likely to show neurological improvement three months after surgery. However, a subgroup analysis of patients with glioblastoma, did not show significant association between MGMT methylation status and neurological scale improvement. This might be apparently due to the small sample size of glioblastoma patients in this study.

IDH mutation is one of the molecular tests well known for its prognostic and predictive implications in high-grade as well as some low-grade gliomas. Patients with IDH mutation have been shown to convey a longer overall survival and progression-free survival compared to those in the wild-type group (Malueka et al., 2020; Molenaar et al., 2014; Reifenberger et al., 2014; Songtao et al., 2012). However, it is still unclear if IDH mutational status is a prognostic and predictive measure of response to treatment. Some studies have concluded that IDH mutation is an independent factor towards response to treatment (Hartmann et al., 2011; Houiller et al., 2010), while others linked the relation to other markers such as 1p19q deletion (Iwamoto et al., 2008; Jenkins et al., 2006; Mariani et al., 2006) and G-CIMP phenotype (Reifenberger et al., 2014). Our study found no association between IDH mutation and neurological improvement. This might be due to the difference in study outcome, which is improvement in neurological function rather than survival or progression-free survival. Secondly, since we used the difference between initial NANO scale score and score three months later as a measure for neurological improvement, it is possible that patients with low initial scale scores had a persistent score three months later, which is then assessed as not having improvement after three months. Third, the NANO scale as a measure for neurological improvement does not integrate headaches into the scale, which is the most common presenting symptom in this study.

There are several limitations of this study. First, we did not analyse other predictive biomarkers such as 1p19q deletion and G-CIMP phenotype. Second, the follow up period of three months after surgery might not reveal changes in neurological improvement, since some patients might still be in chemotherapy and radiation therapy. Hopefully, future studies could integrate other biomarkers as well and conduct a longer period of follow up.

**Conclusions**

MGMT promoter gene methylation as well as coexistence of IDH mutation and MGMT methylation shows a significant link towards improvement in NANO scale score three months after surgery. Glioma patients with MGMT gene promoter methylation are five times more likely to show neurological improvement three months after surgery.

**Data availability**

**Underlying data**

Zenodo: MGMT promoter gene methylation and Neurological Scale Improvement in Glioma. [https://doi.org/10.5281/zenodo.4482094](https://doi.org/10.5281/zenodo.4482094) (Gunawan et al., 2021)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Open Peer Review

Current Peer Review Status: ✅ ❌

Version 2

Reviewer Report 12 May 2021
https://doi.org/10.5256/f1000research.55656.r82485

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Young Zoon Kim
Department of Neurosurgery, Samsung Changwon Hospital, Changwon, South Korea

I think that the revised manuscript has reached the level for indexing. Authors did their best to improve the quality of the manuscript for further understanding by readers.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 13 May 2021
Pricilla Yani Gunawan, Faculty of Medicine, Universitas Pelita Harapan, Lippo Village, Indonesia

Dear Mr Young Zoon Kim,
I sincerely appreciate your time and kind support.

Gratefully yours,
Pricilla

Competing Interests: No competing interests were disclosed

Reviewer Report 13 April 2021
https://doi.org/10.5256/f1000research.55656.r82484
Ery Kus Dwianingsih
Department of Anatomical Pathology, Dr Sardjito Hospital, Yogyakarta, Indonesia

Thank you for providing the revised manuscript. However as I mentioned before, the length of follow up in the patients (only 3 months post-surgery) is not long enough to determine the patients’ outcome. As this issue cannot be addressed by this revision I could not recommend for this paper to be indexed at this time. Thank you.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Pathology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 1

Reviewer Report 16 March 2021

https://doi.org/10.5256/f1000research.54356.r80085

Ery Kus Dwianingsih
Department of Anatomical Pathology, Dr Sardjito Hospital, Yogyakarta, Indonesia

The manuscript described MGMT methylation status and IDH1/2 mutation in glioma patients in Indonesian population which showed neurological improvement three months post-surgery.

- The manuscript is well described, however, there are some rooms for improvement. As we know that the IDH1/2 mutation and MGMT methylation status in glioma patients in asian population have been reported previously in some other publications and its associations with prognosis have also been widely published. The authors may add other highlights in the manuscript to make it more appealing, such as radiological features or other clinical parameters of the subjects or even additional molecular analysis.

- How was the pathological examination performed? What are the criteria for sample to be included in this study? These are not clearly explained in the manuscript.
In this study the patients were followed for only 3 months post-surgery, which is not long enough to determine the patients' outcome. Longer follow up is needed to give better insight of the MGMT profile related to its prognostic evaluation as seen in previous study conducted by lee et al, in 2018, which was also cited in the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Pathology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Mar 2021
Pricilla Yani Gunawan, Faculty of Medicine, Universitas Pelita Harapan, Lippo Village,, Indonesia

Dear Mrs. Ery Kus Dwianingsih.
First of all, I would like to express my gratitude for having you as one of my expert reviewer. It is a great honour to have your suggestions regarding my manuscript. Below are my responses to your suggestions.

1. IDH mutation and MGMT methylation are unquestionably related with better prognosis in glioma. However, the significance of these biomarkers towards neurological scale improvement has not been extensively studied, especially with the NANO scale. The aim of the study is to pin point the relation between the biomarkers and the short term improvement in neurological scale.
2. The details of how the pathological examination was performed, as well as the criteria for sample to be included in this study was added in the Methods section, under the
'Histopathological and molecular analysis' heading.
3. We agree that the length of follow-up was of short term, hence further studies with longer period of follow-up may give a better insight. For that reason, we have stated this matter under the Discussion section, at the last paragraph.

Thank you for your kind and valuable suggestions.

Best Regards,
Pricilla et al

Competing Interests: I have no competing interests

Reviewer Report 04 March 2021
https://doi.org/10.5256/f1000research.54356.r80084

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Young Zoon Kim
Department of Neurosurgery, Samsung Changwon Hospital, Changwon, South Korea

First of all, I'd like to give a great congratulation to them for nice and successful study. They identified Gliomas with MGMT promoter gene methylation are more likely to show neurological improvement three months after surgery. It is well known for MGMT promoter methylation status to play an important role in predicting prognosis of glioma patients. They showed that MGMT promoter methylation status also have important role in improving the neurological outcome which is estimated by NANO scale. I think that the topic and idea is much novel enough to attract great interest to the readers. Their study was well designed and methods were also reasonable and scientific as well.

1. Authors determined the improvement of neurological status if there was a decrement of ≥1 of the NANO scale. The cut-off value should be validated by use of other neurologically estimating scale such as KPS or WHO performance scale.

2. Readers may wonder if MGMT methylation status can be independent factor for influencing on NANO scale in glioma patients or not. More statistical analysis such as multivariate analysis using Cox regression model is mandatory to prove their conclusions. Because the patients with methylated MGMT promoter had also IDH-mutation which is favourable prognostic factor in glioma.

3. Although the sample size of glioblastoma patients is small, subgroup analysis focused on glioblastoma patients may be informative to readers.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
I cannot comment. A qualified statistician is required.

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.


I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Author Response 23 Mar 2021**

**Pricilla Yani Gunawan**, Faculty of Medicine, Universitas Pelita Harapan, Lippo Village,, Indonesia

Dear Mr Young Zoon Kim.
I would like to express my gratitude for having you as one of my expert reviewer. It is such an honour to have your detailed and wonderful comments on my manuscript. Here are my responses to your suggestions.
1. The **cut-off value** of neurological improvement as decrement in NANO scale by 1 or more was made through consideration of the results and definition used in previous studies. I have added the explanation of this subject in the **Discussion** section, at the end of the second paragraph.
2. **Multivariate analysis** has been done using a logistic regression analysis to confirm that MGMT methylation status is an independent factor influencing neurological scale improvement. This statement has been added in the **Results** section, before Table 4.
3. A **subgroup analysis** focused on glioblastoma patients was added in the **Results** section, along with Table 6 and Table 7 to further describe the analysis. It was also added in the **Discussion** section, at the end of the eight paragraph.

Thank You for your kind and valuable suggestions.

Best Regards,

Pricilla et al

**Competing Interests:** I have no competing interests.