Low Peripheral Lymphocyte Count Before Focal Radiotherapy Plus Concomitant Temozolomide Predicts Severe Lymphopenia During Malignant Glioma Treatment

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Abstract

Malignant glioma patients treated with the golden standard therapy, focal radiotherapy plus concomitant daily temozolomide (radiotherapy/TMZ), often suffer severe lymphopenia. The frequency of severe lymphopenia and its predictors were analyzed by assessing adverse effects including decrease in white blood cell counts, lymphocyte counts, and neutrocyte counts according to the Common Toxicity Criteria version 3.0 (CTC) in 28 consecutive patients with pathologically verified malignant gliomas treated with radiotherapy/TMZ. Eighty-two percent of the patients suffered one or more adverse effects; lymphopenia (68%) was the most frequent adverse effect, with 32% of patients suffering CTC grade 4 lymphopenia. CTC grade 4 lymphopenia was associated with the incidence of other CTC grade 3 or 4 adverse effects and discontinuance of TMZ. Minimal lymphocyte counts during radiotherapy/TMZ and lymphocyte counts before radiotherapy/TMZ showed close linear correlation by linear regression analysis (p < 0.0001, R² = 0.569), and the most important predictor for CTC grade 4 lymphopenia was lymphocyte count before radiotherapy/TMZ less than 1200/μl by multivariate analysis (p < 0.0321, Exp = 13.2). Lymphocyte counts before radiotherapy/TMZ of less than 1200/μl predict severe lymphopenia during radiotherapy/TMZ.

Key words: lymphocytopenia, temozolomide, glioblastoma, anaplastic astrocytoma, infection

Introduction

The golden standard therapy for patients with newly diagnosed glioblastoma is maximal surgical removal and standard focal radiotherapy plus concomitant daily temozolomide (TMZ) followed by adjuvant TMZ.11,12) This therapy has also been applied as the first choice in patients with 2007 World Health Organization (WHO) classification grade III anaplastic astrocytoma. Unfortunately, patients treated with radiotherapy with concomitant TMZ (radiotherapy/TMZ) often suffer adverse effects. Common Toxicity Criteria version 3.0 (CTC) grade 3/4 lymphopenia occurred in 80% of patients undergoing radiotherapy/TMZ.10) Lymphopenia was the most frequent adverse effect after radiotherapy/TMZ, and CTC grade 2, 3, and 4 lymphopenia (lymphopenia less than 800/μl, 500/μl, and 200/μl) was observed in 22%, 38%, and 8% (total 68%) of patients, respectively.5) Severe infections including Pneumocystis jirovecii pneumonia also occur in patients receiving standard-dose TMZ therapy.9,14) On the other hand, TMZ has the potential ability to enhance immunotherapies in experimental studies,4,7) and some studies of vaccine-based immunotherapy combined with TMZ have been tried for glioblastoma.3,8) In this strategy, lymphopenia during and after radiotherapy/TMZ would be a significant factor affecting treatment results. However, predictors of severe lymphopenia have not been evaluated.

In this study, the frequency of severe lymphopenia and its predictors were investigated in patients with malignant glioma.

Materials and Methods

Twenty-eight consecutive patients, 17 males and 11...
females aged 36–75 years (mean 58.6 years), with pathologically verified malignant gliomas, 7 with 2007 WHO classification grade III gliomas and 21 with grade IV gliomas, treated with radiotherapy/TMZ for the first time between December 2007 and November 2009 were included. Only one elderly patient who stopped TMZ on day 2 because of aspiration pneumonia was excluded from the study. Histological diagnoses were made by two to four pathologists, mainly at the Department of Pathology, University Hospital of Tsukuba. Before treatment, written informed consent for radiotherapy and TMZ that explained the adverse effects and frequent blood checks was obtained from each patient.

Conformal radiotherapy with three-dimensional planning was administered for a total daily dose of 60 Gy in 2-Gy fractions on 5 days a week for 6 weeks with a linear accelerator in 20 patients, a total dose of 45 Gy in 3-Gy fractions with a linear accelerator in 3 patients, total doses of 61.2 Gy and 50 Gy with a linear accelerator in 1 patient each, and a total dose of 97 Gy with a proton beam in 3 patients. Concomitant chemotherapy consisted of TMZ at a daily dose of 75 mg/m² for 7 days a week from the first until the last day of radiotherapy, up to 49 days. Discontinuance of TMZ was decided based on a slightly modified standard protocol (neutrocyte count less than 1.5 \( \times 10^{9}/\mu l \), platelet count less than 100 \( \times 10^{9}/\mu l \), CTC grade 2 or more non-hematologic adverse effects, and prolonged lymphopenia less than 0.2 \( \times 10^{9}/\mu l \)). Prophylaxis against Pneumocystis jirovecii using trimethoprim sulfamethoxazole was mandatory during the radiotherapy/TMZ course. After a 4-week break, patients received a first cycle of adjuvant TMZ (150 mg/m²) for 5 days, assuming no hematologic toxicity. Patients then received multiple cycles of adjuvant TMZ for 5 days every 28 days. The dose was increased to 200 mg/m² beginning with the second cycle, provided no hematologic toxicity occurred.

Adverse effects including decreased white blood cell (WBC) count, lymphocyte count, and neutrocyte count in the peripheral blood, abdominal symptoms such as nausea and appetite loss, skin problems, depression, and liver dysfunction were checked according to CTC in all 28 patients treated with radiotherapy/TMZ. Changes in the counts of WBC, lymphocytes, and neutrocytes, from the start of radiotherapy/TMZ to the first cycle of adjuvant TMZ were examined. To determine the most important associating factors for patients suffering severe lymphopenia, the association between CTC grade 4 lymphopenia and several factors including age, sex, Karnofsky performance status (KPS) before surgical removal, WHO grading of gliomas, preoperative maximal size of tumors, removal ratio, dose of radiotherapy, WBC count before radiotherapy/TMZ (pre WBC), lymphocyte count before radiotherapy/TMZ (pre lymph), neutrocyte count before radiotherapy/TMZ (pre neutro), steroid use (10 mg or more dosage of prednisolone during 7 days or more), discontinuance of TMZ, and other severe adverse effects (CTC grade 3–4 complications including severe infections) were analyzed.

Statistical analysis was performed using the unpaired Student’s t-test to test for significance in differences between group mean values for continuous variables. The Mann-Whitney U (MWU) test or Fisher’s direct method was used for differences in intermittent or categorical variables. To determine the most important factors for CTC grade 4 lymphopenia, possible factors (p values nearly equal to 0.1 or less, by univariate analysis) were evaluated by logistic regression analysis. Statistical significance in all analyses was recognized at p < 0.05.

Results

Twenty-three of the 28 patients suffered one or more adverse effects. Nineteen patients suffered lymphopenia (with or without other adverse effects), and only 5 of these had only lymphopenia. CTC grade 2, 3, and 4 lymphopenia were observed in 5, 5, and 9 patients, respectively. The average of pre lymphocyte count and average minimal lymphocyte count during radiotherapy/TMZ (minimal lymph) were 1618.2 ± 807.6 and 721.3 ± 909.0/\( \mu l \). On the other hand, 18 of the patients suffered one or more other adverse effects with or without lymphopenia. CTC grade 2, 3, and 4 decreases in WBC were observed in 6, 2, and 1 of the CTC grade 2, 3, and 4 lymphopenia cases, abdominal symptoms including nausea in 5, 4, and 0, neutropenia states in 6, 0, and 1, infections including a case of Pneumocystis jirovecii pneumonia in 0, 3, and 3, skin problems including stomatitis in 2, 3, and 0, liver dysfunctions in 2, 2, and 0, depression states in 3, 0, and 0, and hand tremor in 1, 0, and 0. Platelet count decreased mildly in many cases, but not to CTC grade 2 level nor to clinically meaningful levels.

Changes in counts of WBCs, lymphocytes, and neutrocytes from the start of radiotherapy/TMZ to the first cycle of adjuvant TMZ are shown in Fig. 1. These 3 types of cells decreased during radiotherapy/TMZ and stabilized until the first cycle of adjuvant TMZ. The mean value of lymphocyte counts 5 weeks after the start of radiotherapy/TMZ (790/\( \mu l \)) reached less than 800/\( \mu l \), which corresponded to CTC grade 2. Subtypes of the lymphocytes could be examined before and after radiotherapy/TMZ in

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Fig. 1 Changes in counts of white blood cells (WBC), lymphocytes, and neutrophils from the start of concomitant radiotherapy and temozolomide (TMZ) to the first cycle of adjuvant TMZ.

Table 1 Associations between several factors and lymphopenia during concomitant radiotherapy and temozolomide (radiotherapy/TMZ)

<table>
<thead>
<tr>
<th></th>
<th>CTC grade 4 lymphopenia</th>
<th>CTC grade 0–3 lymphopenia</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>9</td>
<td>19</td>
<td>—</td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>59.1</td>
<td>58.4</td>
<td>0.8740*</td>
</tr>
<tr>
<td>Sex (men/women, cases)</td>
<td>4/5</td>
<td>13/6</td>
<td>0.4087**</td>
</tr>
<tr>
<td>Median preop KPS (%)</td>
<td>70</td>
<td>70</td>
<td>0.7450***</td>
</tr>
<tr>
<td>WHO grade 4 (cases (%))</td>
<td>7 (78%)</td>
<td>14 (74%)</td>
<td>0.9999**</td>
</tr>
<tr>
<td>Mean tumor size (mm)</td>
<td>54.1</td>
<td>59.9</td>
<td>0.5065***</td>
</tr>
<tr>
<td>Mean removal ratio (%)</td>
<td>74.9</td>
<td>47.8</td>
<td>0.1034***</td>
</tr>
<tr>
<td>Mean radiation dose (Gy)</td>
<td>60.1</td>
<td>62.9</td>
<td>0.5165***</td>
</tr>
<tr>
<td>Mean pre WBC (×10^3/μl)</td>
<td>5.07</td>
<td>7.17</td>
<td>0.0549***</td>
</tr>
<tr>
<td>Mean pre lymph (×10^3/μl)</td>
<td>1.09</td>
<td>1.87</td>
<td>0.0085***</td>
</tr>
<tr>
<td>Mean pre neutro (×10^3/μl)</td>
<td>3.48</td>
<td>4.65</td>
<td>0.1985***</td>
</tr>
<tr>
<td>With/without steroid (cases)</td>
<td>3/6</td>
<td>6/13</td>
<td>0.9999**</td>
</tr>
<tr>
<td>Other severe adverse effects (A and/or B, cases (%))</td>
<td>8 (89%)</td>
<td>2 (11%)</td>
<td>0.0001***</td>
</tr>
<tr>
<td>A: severe infections (cases (%))</td>
<td>4 (44%)</td>
<td>2 (11%)</td>
<td>0.0638***</td>
</tr>
<tr>
<td>B: others excluding A (cases (%))</td>
<td>6 (67%)</td>
<td>2 (11%)</td>
<td>0.0048***</td>
</tr>
<tr>
<td>Discontinuance of TMZ (cases (%))</td>
<td>8 (89%)</td>
<td>3 (16%)</td>
<td>0.0004***</td>
</tr>
</tbody>
</table>

*Student's t-test, **Fisher's direct method, ***Mann-Whitney U test. CTC: Common Toxicity Criteria version 3.0, other severe adverse effects: CTC grade 3 or 4 adverse effects including severe infections and excluding severe lymphopenia, others excluding A: CTC grade 3 or 4 adverse effects excluding severe lymphopenia and severe infections, pre lymph count before radiotherapy/TMZ, pre neutro: neutrophil count before radiotherapy/TMZ, preop KPS: Karnofsky performance status score before surgical removal, pre WBC: white blood cell count before radiotherapy/TMZ, severe infections: CTC grade 3 or 4 infections, WHO: World Health Organization, with/without steroid: long-term use or disuse of steroid before or during radiotherapy/TMZ.

only 5 cases. Mean ratio of CD4^+ T cells was decreased (39% before radiotherapy/TMZ and 32% just before the first cycle of adjuvant TMZ) and mean ratio of CD8^+ T cells was increased (14% before radiotherapy/TMZ and 24% just before the first cycle of adjuvant TMZ). Other subtypes including natural killer cells and B cells were unchanged.

The statistical findings of the association between CTC grade 4 lymphopenia and several factors are shown in Table 1. The pre lymph level (1.09 × 10^3/μl) in patients with CTC grade 4 lymphopenia was significantly lower than that (1.87 × 10^3/μl) in patients with CTC grade 0–3 lymphopenia (p = 0.0085). Pre WBC (5.07 × 10^3/μl) and the tumor removal ratio (74.9%) in patients with CTC grade 4 lymphopenia tended to be different from that (7.17 × 10^3/μl and 47.8%) in patients with CTC grade 0–3 lymphopenia, although without statistical differences. Steroid use was not associated with CTC grade 4 lymphopenia (Table 1), but was associated with KPS (51% with steroid versus 73% without steroid, p = 0.0027, MWU test), removal ratio (35%
Table 2  Results of univariate and multivariate logistic regression analysis

<table>
<thead>
<tr>
<th>Candidate predictors causing CTC grade 4 lymphopenia</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p Value</td>
<td>Exp</td>
</tr>
<tr>
<td>High removal ratio (≥60%)</td>
<td>0.2891</td>
<td>—</td>
</tr>
<tr>
<td>Lower pre WBC (&lt;6.0 × 10³/μl)</td>
<td>0.0890</td>
<td>4.33 (0.799–23.3)</td>
</tr>
<tr>
<td>Lower pre lymph (&lt;1.2 × 10³/μl)</td>
<td>0.0124</td>
<td>10.6 (1.67–66.7)</td>
</tr>
</tbody>
</table>

CTC: Common Toxicity Criteria version 3.0, Pre lymph: lymphocyte counts before concomitant radiotherapy and temozolomide (radiotherapy/TMZ), Pre WBC: white blood cell counts before radiotherapy/TMZ.

Fig. 2  Left: Correlation between minimal lymphocyte counts during concomitant radiotherapy and temozolomide (radiotherapy/TMZ) and white blood cell counts before radiotherapy/TMZ (pre WBC). The correlation was shown using linear regression analysis (p = 0.0001, R² = 0.433, y = −214.943 + 0.125x). Right: Correlation between minimal lymphocyte counts during radiotherapy/TMZ and lymphocyte counts before radiotherapy/TMZ (pre lymph). The correlation was shown using linear regression analysis (p < 0.0001, R² = 0.569, y = −175.981 + 0.476x).

versus 66%, p = 0.0279, MWU test), and pre WBC (8.1 × 10³/μl versus 5.8 × 10³/μl, p = 0.0113, MWU test). KPS was also not associated with CTC grade 4 lymphopenia (Table 1), but was associated with tumor size, steroid use, and severe infections (p < 0.05, MWU test or linear regression analysis).

CTC grade 4 lymphopenia was positively associated with the frequencies of other severe adverse effects including severe infections (CTC grade 3 or 4, p = 0.0001, Fisher’s method) and discontinuance of TMZ (p = 0.0004, Fisher’s method). Severe infection was very weakly related with both CTC grade 4 lymphopenia and steroid use (p > 0.1, Fisher’s method). Multivariate logistic analysis showed that CTC grade 4 lymphopenia was the most important factor associated with both severe adverse effects (p = 0.0056, Exp = 95.3 in lymphopenia and p > 0.05 in other factors) and severe infection (p = 0.0489, Exp = 16.1 in lymphopenia and p > 0.05 in other factors).

To determine the most important predictive factors for CTC grade 4 lymphopenia, the associations between CTC grade 4 lymphopenia and 3 factors (60% removal or more, less than 6.0 × 10³/μl of pre

Fig. 3  Mean values of minimal lymphocyte counts during concomitant radiotherapy and temozolomide (radiotherapy/TMZ) in 19 patients with high lymphocyte counts (high pre lymph, 1200/μl or more) and 9 patients with low lymphocyte counts (low pre lymph, less than 1200/μl) before radiotherapy/TMZ. The two values (782 and 203/μl) are different statistically (p = 0.0017, Mann-Whitney U test).

WBC, a pre lymph less than 1.2 × 10³/μl were evaluated by univariate and multivariate logistic analysis, as shown in Table 2. The pre lymph value was
the most important factor for CTC grade 4 lymphopenia (p < 0.05, Exp > 10 both in univariate and multivariate analysis). The correlations between minimal lymph and pre WBC or pre lymph was evaluated as shown in Figs. 2 and 3. Minimal lymph and pre lymph had a close linear correlation (R² = 0.569). Sixty-seven percent of cases with pre lymph less than 1200/µl developed CTC grade 4 lymphopenia and 16% of cases with high pre lymph (1200/µl or more) developed CTC grade 4 lymphopenia.

**Discussion**

In this study, 82% of patients suffered one or more adverse effects, and lymphopenia (68%) was the most frequent adverse effect. Surprisingly, the mean lymphocyte count 5 weeks after the start of radiotherapy/TMZ reached the level of CTC grade 2, and 32% of patients suffered CTC grade 4 lymphopenia. CTC grade 4 lymphopenia was associated with the occurrence of other severe (CTC grade 3 or 4) adverse effects including severe infections and discontinuance of TMZ, and was the most important factor associated with severe adverse effects and severe infection. Severe neutropenia occurred only in one patient, and there was no association between neutropenia (CTC grades 2–4) and severe adverse effects. These findings suggest that severe lymphopenia might be a crucial adverse effect inducing other complications in the treatment of radiotherapy/TMZ. Severe neutropenia and/or thrombopenia occurred in more than 20% of patients receiving nitrosourea or platinum-based chemotherapies.\(^1\,2\,6\) The neutropenia and thrombopenia are key adverse effects in these chemotherapies, but no detailed data about lymphopenia has been obtained. Thus, TMZ is a unique agent especially affecting lymphocytes rather than neutrophils. Although adverse effects during radiotherapy/TMZ have been reported in several previous studies, lymphopenia had not been evaluated. The incidence of lymphopenia in our study (68%) is compatible with previous data (68%),\(^5\) although the frequency of CTC grade 4 lymphopenia in the present study was higher than in previous studies. We speculate that the high frequency of low pre lymph in our patients induced the high rate of CTC grade 4 lymphopenia.

TMZ has the potential ability to enhance acquired immunotherapies in experimental studies.\(^4\,7\) On the other hand, TMZ is associated with CD4+ T-cell dysfunction,\(^8\,13\) which might be associated with decline of the immune system. To avoid the immune-compromised state during radiotherapy/TMZ, the identifying the predictive factors for severe lymphopenia as pre lymph less than 1200/µl and pre WBC less than 6000/µl, which were associated with CTC grade 4 lymphopenia, and the former was the most important predictor. Therefore, physicians should pay special attention to patients with a pre lymph less than 1200/µl. However, CTC grade 4 lymphopenia occurred even in patients with a high pre lymph.

Among the limitations of the present study, we could not prove whether severe lymphopenia affected the clinical outcome of patients with malignant glioma. This can be resolved by following up these and future patients. Furthermore, procedures for preventing severe lymphopenia (and the related adverse effects) for patients with a low pre lymph were not explored in this study. Although dose reduction of TMZ might be one solution, the influence on the outcome has not been examined. On the other hand, prophylaxis against Pneumocystis jirovecii during radiotherapy/TMZ might be unnecessary for some patients with high pre lymph.

In conclusion, a pre lymph (lymphocyte count before radiotherapy/TMZ) less than 1200/µl is a predictor of severe lymphopenia during radiotherapy/TMZ in patients with malignant glioma.

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**References**


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Commentary

This article written by Ishikawa et al. is a retrospective analysis of lymphocyte counts in patients receiving radiotherapy and concomitant temozolomide after complete resection of a malignant glioma. It is well known that lymphocyte counts decrease significantly after such a combined therapy, and regular blood tests are mandatory in this treatment setting. The presented series of 28 patients confirms this experience with lymphopenia in 68% of all cases. Another finding was that low lymphocyte counts before radio- and chemotherapy constituted a statistically significant risk of severe lymphopenia as an adverse event. However, an association of this adverse event with worse outcome could not be confirmed. The authors concluded that lymphocyte counts less than 1200/μl before radiotherapy and temozolomide treatment are a predictor of severe lymphopenia in patients with malignant glioma. They also advised to turn attention to patients with such low lymphocyte counts before further treatment, and did not suggest changing the treatment strategy to only radiotherapy. Instead, concomitant treatment was proposed as the golden standard. As patients with initially high lymphocyte counts were at lower risk to suffer from Pneumocystis jirovecii infection, prophylaxis was discussed critically.

The proposed concomitant radiotherapy and temozolomide treatment is nowadays, indeed, very common, and neurosurgeons are sensitized to pseudoprogression and severe lymphopenia in early post-treatment controls. In our opinion it would be interesting to investigate whether low or high lymphocyte counts also play a role in pseudoprogression. In summary, this article is an important contribution to the treatment of malignant glioma, and the authors should be congratulated for their valuable results.
Temozolomide has become an important adjunct in the management of malignant gliomas. For most patients, the recommended dose and dose schedule are well tolerated. Although adverse effects can occur, as with any chemotherapeutic agent, they usually consist of headache and fatigue, and ordinarily they are not dose limiting or dangerous. The most important side effect of this drug is progressive lymphopenia, which can be dose limiting or not tolerated. This complication can at times lead to serious complications or even death. The complications related to the drug may also be compounded by the effect of concomitant radiation therapy. Until now we have not been able to predict reliably which patients may be at risk for this problem.

In a carefully done, well designed study, Ishikawa and colleagues have investigated the phenomenon of post treatment lymphopenia. They have demonstrated that the preoperative lymphocyte count can predict the development of post treatment severe lymphopenia. This is an important study, and it will be useful in guiding more effective multimodality therapy in patients with malignant gliomas.

Clearly, patients with pre treatment lymphopenia will need to be monitored very carefully. They may require modification in dosage and scheduling of temozolomide therapy. Some will not be able to tolerate this drug at all, and alternative regimens may need to be considered. The findings of this study will be of benefit to many of our glioma patients.

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