Aplastic Anemia in a Patient with Anaplastic Oligodendroglioma Postradiation and Concurrent Temozolomide Therapy: Case Report and Review of the Literature

Kirollos S. Hanna, PharmD, BCPS, BCOP; Robert Mancini, PharmD, BCOP; Matthew Burtelow, MD, PhD; Benjamin Bridges, MD

BACKGROUND: Anaplastic oligodendroglioma is a malignant neoplasm of the central nervous system. Standard treatment includes complete surgical resection followed by radiation therapy and combination chemotherapy with a regimen of procarbazine, lomustine, and vincristine. Treatment with single-agent temozolomide is an acceptable alternative. Myelosuppression is typically the dose-limiting toxicity associated with temozolomide; in rare cases, aplastic anemia has also been reported.

OBJECTIVES: To review the literature of temozolomide-induced aplastic anemia and discuss the case of severe aplastic anemia in a patient with anaplastic oligodendroglioma who received temozolomide therapy.

DISCUSSION: We report a case of aplastic anemia in a 36-year-old white woman who had surgery for anaplastic oligodendroglioma followed by radiation and temozolomide therapy. Aplastic anemia is a rare, immune-mediated destruction of hematopoietic stem cells that has been reported with temozolomide therapy. Increased toxicity of temozolomide caused by limited activity of the DNA-repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) gene could contribute to prolonged marrow damage. If the MGMT promoter is methylated, cells are unable to repair DNA crosslinks and are rendered sensitive to alkylating agents. The exact mechanism of temozolomide-induced myelotoxicity is not fully understood, and no correlation has been reported between the dose and duration of temozolomide therapy.

CONCLUSION: Case series show that temozolomide can trigger severe aplastic anemia in rare cases, irrespective of the dose and duration of therapy. Clinicians should be aware of this rare and potentially devastating complication of temozolomide therapy.

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Case Report

A 36-year-old white woman with an unremarkable medical history presented in late May 2015 with progressively worsening headaches over 2 to 3 years, culminating in the abrupt development of tonic-clonic seizures. Her surgical history included a hemithyroidectomy in 2006 for a large, complex cyst that was deemed noncancerous. Her family history revealed breast cancer in an aunt at age 72 years.

The patient’s daily medications included ferrous sulfate, docusate sodium, and acidophilus, with hydrocortone and ondansetron as needed. She was prescribed levetiracetam 750 mg twice daily for seizure prophylaxis, pending further evaluation. Magnetic resonance imaging of the brain showed a frontal lobe tumor measuring 6 × 4.7 cm with infiltration of the surrounding brain, effacement of cerebral sulci, vasogenic edema, and a 7-mm midline shift. The patient underwent left-sided frontal craniotomy and gross total resection of the tumor. Pathology demonstrated an anaplastic oligodendroglioma (World Health Organization grade 3 or 4) with 1p/19q codeletion that was confirmed by fluorescence in situ hybridization.

Initial treatment with PCV or with temozolomide and concurrent radiation therapy at a dose of 60 Gy were discussed with the patient. She elected to start radiation therapy, with concurrent temozolomide monotherapy at 75 mg/m² daily, because of the better tolerability and toxicity profile of monotherapy than the standard PCV regimen; she also began pneumocystis prophylaxis with double-strength trimethoprim plus sulfamethoxazole 3 times weekly.

The patient tolerated the initial therapy well, reporting mild fatigue, nausea, headache, dizziness, and acneiform rash. After 4 weeks of radiation therapy and concurrent temozolomide monotherapy, she had a significant drop in her platelet count from 238 × 10⁹/L to 23 × 10⁹/L. Radiation therapy was continued and temozolomide was stopped as a precaution.

The patient continued to experience persistent thrombocytopenia, which required platelet transfusions. For the next 14 days, she had significant drops in all blood cell lines, including hemoglobin, 9.1 g/dL; white blood cell count, 0.7 × 10⁹/L; neutrophils, 0.22 × 10⁹/L; and platelets, 26 × 10⁹/L (Table 1). Six weeks after discontinuation of temozolomide therapy she remained significantly pancytopenic (ie, hemoglobin, 6.8 g/dL; white blood cell count, 1.1 × 10⁹/L; neutrophils, 0.58 × 10⁹/L; and platelets, 22 × 10⁹/L).

A bone marrow biopsy was performed 8 weeks after the initial onset of thrombocytopenia. Final pathology of the marrow showed extreme hypocellularity, with an estimated overall cellularity of 2% to 3%, and markedly

Table 1  Patient’s Hematologic Counts at Temozolomide Initiation and Discontinuation

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>Jul 14</th>
<th>Aug 14</th>
<th>Aug 21</th>
<th>Aug 28</th>
<th>Sept 4</th>
<th>Oct 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dL</td>
<td>10.3</td>
<td>10.9</td>
<td>11.5</td>
<td>9.1</td>
<td>9.2</td>
<td>6.7</td>
</tr>
<tr>
<td>White blood cells, × 10⁹/L</td>
<td>5.7</td>
<td>5.6</td>
<td>2.8</td>
<td>0.7</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Neutrophils, × 10⁹/L</td>
<td>3.32</td>
<td>5.10</td>
<td>2.16</td>
<td>0.22</td>
<td>0.9</td>
<td>0.58</td>
</tr>
<tr>
<td>Platelets, × 10⁹/L</td>
<td>238</td>
<td>23</td>
<td>16</td>
<td>26</td>
<td>31</td>
<td>16</td>
</tr>
</tbody>
</table>

*Initiation of temozolomide therapy.
†Discontinuation of temozolomide therapy.
Table 2: Cases of Temozolomide-Associated Aplastic Anemia Reported in the Literature

<table>
<thead>
<tr>
<th>Case reported</th>
<th>Patient characteristics</th>
<th>Temozolomide daily dose</th>
<th>Aplastic anemia onset</th>
<th>Potential contributing factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doyle et al, 2005</td>
<td>16 patients Age: ≥18 yrs</td>
<td>75-200 mg/m²</td>
<td>During radiation plus temozolomide therapy</td>
<td>Trimetoprim/sulfamethoxazole</td>
</tr>
<tr>
<td>Oh et al, 2010</td>
<td>Female Age: 63 yrs</td>
<td>N/A</td>
<td>Postresection, day 18 of radiation plus temozolomide therapy</td>
<td>Ranitidine</td>
</tr>
<tr>
<td>Villano et al, 2006</td>
<td>Male Age: 45 yrs</td>
<td>75-200 mg/m² 5 days in a 28-day cycle</td>
<td>Post-surgical resection and radiation plus temozolomide therapy</td>
<td>None</td>
</tr>
<tr>
<td>Jalali et al, 2007</td>
<td>Female Age: 30 yrs</td>
<td>75 mg/m²</td>
<td>Postresection and completion of radiation plus temozolomide therapy</td>
<td>Trimetoprim/sulfamethoxazole; phenytoin</td>
</tr>
<tr>
<td>Morris et al, 2009</td>
<td>Female Age: 16 yrs</td>
<td>90 mg/m²</td>
<td>Concurrent phase (day 24) of radiation plus temozolomide therapy</td>
<td>None</td>
</tr>
</tbody>
</table>


Discussion and Literature Review

Aplastic anemia is a rare, immune-mediated destruction of hematopoietic stem cells, with an estimated annual incidence of 5 to 12 new cases per 1 million people in the United States. Aplastic anemia is classified as inherited or acquired. The severity of aplastic anemia is categorized according to the degree of marrow cellularity and peripheral blood cytopenias.

Idiopathic, acquired aplastic anemia accounts for most cases. In some cases, environmental or infectious triggers, such as viral infection (eg, Epstein-Barr virus, cytomegalovirus, or hepatitis) medications and benzene exposure, have been associated with the onset of this disease. Marrow damage and aplasia have been reported with the use of certain medications, including antibiotics (ie, chloramphenicol), antiepileptic drugs, and alkylating agents. Levy and colleagues reported 2 cases of delayed bone marrow aplasia after administration of dacarbazine, an intravenous (IV) alkylating agent in the same drug class as temozolomide.

Temozolomide, a tetrazine derivative, is an oral and IV alkylating agent used in the treatment of several cancers, including neurologic malignancies. Myelosuppression is considered the dose-limiting side effect of temozolomide; however, myelosuppression is typically dose-dependent and reversible. Rare cases of prolonged myelosuppression after administration of temozolomide have been reported in the literature. Even fewer cases of aplastic anemia have been reported during or soon after temozolomide treatment.

The mechanism of temozolomide-induced myelotoxicity is not understood, although the postulated mechanism is thought to differ from that of other alkylating agents, such as the chloroethylyating agents nimustine, carmustine, and lomustine. O-Alkylating agents, such as nitrosoureas, damage DNA through the formation of positively charged carbonium ions that bind to electron-rich nucleophilic sites in purine and pyrimidine bases.

Aplastic anemia is a rare, immune-mediated destruction of hematopoietic stem cells, with an estimated annual incidence of 5 to 12 new cases per 1 million people in the United States.

The effectiveness of O-Alkylating agents is limited by the activity of gene encoding for the DNA-repair enzyme O-Methylguanine-DNA methyltransferase (MGMT) gene. Crosslinking of double-stranded DNA by alkylating agents is inhibited by this DNA repair enzyme. If the MGMT promoter is methylated, cells are unable to repair DNA crosslinks and are rendered sensitive to this class of alkylating agents. The MGMT promoter methylation is common in tumors with 1p/19q codeletions, because of the presence of isocitrate dehydrogenase-1 mutations, which are associated with CpG island methylator phenotypes.

Hypermethylation of the MGMT promoter in gliomas is considered a positive predictor of responsiveness to alkylating agents. The difference in toxicity seen...
with temozolomide could be a result of its byproduct, 5-(3-methyltriazeno)imidazole-4-carboxamide, which causes DNA methylation of several bases (eg, O\textsubscript{6}-guanine, N\textsubscript{3}-adenine, or N\textsubscript{2}-guanine), leading to severe marrow damage in certain patients.\textsuperscript{16}

Jansen and colleagues evaluated the protection of hematopoietic cells from different O\textsubscript{6}-alkylating agents by MGMT gene transfer.\textsuperscript{16} Several studies have assessed the feasibility of this approach, and the benefit of MGMT overexpression in hematopoietic cells.\textsuperscript{19-22} They reported in vitro and animal models supporting a positive impact of MGMT on marrow protection with O\textsubscript{6}-alkylating agents; however, temozolomide had substantially fewer benefits than those seen with chloroethylnitrosoureas, suggesting the involvement of different myelotoxicity mechanisms.\textsuperscript{16} MGMT gene transfer is not part of standard practice, and is beyond the scope of this report.

Other investigators have evaluated genetic and clinical factors that may contribute to myelosuppression associated with temozolomide and radiation therapy. Lombardi and colleagues enrolled 87 patients (32 women, 55 men) diagnosed with glioblastoma who received temozolomide and radiation therapy in their clinical trial.\textsuperscript{23} Four patients (all women) had grade 3 or 4 myelosuppression, with pretreatment platelet counts ≤300 x 10\textsuperscript{9}/L (P = .05) and methylated MGMT.

Lombardi and colleagues identified 3 polymorphisms associated with myelotoxicity in the methionine adenosyltransferase 1 (MAT1A) and the cytochrome (CY) P450 oxidoreductase (POR) genes—rs17102596 and rs7087728 in MAT1A, and rs17685 in POR. S-adenosylmethionine is produced by MAT1A enzymes; low concentrations of methionine from MAT1A polymorphisms are associated with increased sensitivity to alkylating agents through deactivation of MGMT.\textsuperscript{24} POR enzyme mutations may also contribute to myelosuppression by inhibiting the CYP450 system from proper drug metabolism.

By contrast, Sylvester and colleagues present 2 patients with different genetic profiles of the MGMT gene mutation who had prolonged myelosuppression.\textsuperscript{25} In their report, 1 patient had the GG or GA mutations (rs2308321 and rs2308327, respectively), which increases the risk for myelosuppression, but is inconsistent with Lombardi and colleagues’ findings. They reported no significant difference in these mutations; their review included 9 control patients, 3 of whom were positive for GG or GA mutations.\textsuperscript{25}

**Patient Case Revisited**

Our patient had severe aplasia associated with the use of temozolomide. Despite discontinuation of temozolomide, cytopenias persisted 12 weeks posttherapy. Before the onset of pancytopenia, levetiracetam therapy was discontinued, because the patient no longer needed seizure prophylaxis. Environmental and hereditary factors were unlikely contributory factors to severe aplasia. Cumulative exposure to temozolomide might have been the trigger, but this does not seem to be a trend from other reports pertaining to the dose (range, 75-200 mg/m\textsuperscript{2}) and duration (range, day 18 of therapy to after completion of therapy) of therapy (Table 2).\textsuperscript{12,14,15,26,27}

The use of antiepileptic drugs (ie, felbamate, carbamazepine, valproic acid, and phenytoin) has been associated with rare cases of aplastic anemia; however, although a deep search of the medical literature and medication safety databases for levetiracetam revealed rare cases of eosinophilia, agranulocytosis, and lymphocytosis, no cases of levetiracetam-associated aplastic anemia were described.\textsuperscript{28-30} Trimethoprim plus sulfamethoxazole was discontinued 6 weeks after temozolomide discontinuation, but our patient continued to experience prolonged aplasia. Myelosuppression has been reported with trimethoprim plus sulfamethoxazole, but was reversible upon treatment discontinuation.\textsuperscript{28-30}

We used the Naranjo probability scale for causality

<table>
<thead>
<tr>
<th>Table 3</th>
<th>The Naranjo Adverse Drug Reaction Probability Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>1. Are there previous conclusive reports on this reaction?</td>
<td>+1</td>
</tr>
<tr>
<td>2. Did the adverse event occur after the suspected drug was administered?</td>
<td>+2</td>
</tr>
<tr>
<td>3. Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?</td>
<td>+1</td>
</tr>
<tr>
<td>4. Did the adverse reaction reappear when the drug was readministered?</td>
<td>+2</td>
</tr>
<tr>
<td>5. Are there alternative causes (other than the drug) that could have on their own caused the reaction?</td>
<td>-1</td>
</tr>
<tr>
<td>6. Did the reaction reappear when a placebo was given?</td>
<td>-1</td>
</tr>
<tr>
<td>7. Was the drug detected in the blood (or other fluids) in concentrations known to be toxic?</td>
<td>+1</td>
</tr>
<tr>
<td>8. Was the reaction more severe when the dose was increased or less severe when the dose was decreased?</td>
<td>-1</td>
</tr>
<tr>
<td>9. Did the patient have a similar reaction to the same or similar drugs in any previous exposure?</td>
<td>+1</td>
</tr>
<tr>
<td>10. Was the adverse event confirmed by any objective evidence?</td>
<td>+1</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
</tr>
</tbody>
</table>

The adverse drug reaction is assigned to a probability category from the total score as follows: definite, if overall score is ≥8; probable, score of 5-7; possible, score of 1-4; doubtful, score is 0. Source: Naranjo CA, Busto U, Sellers EM, et al. Clin Pharmacol Ther. 1981;30:239-245.
therapies.11-14 Currently, our patient is transfusion-dependent, and the question of how to treat the aplasia remains. During review of our patient case at our tumor board, varied opinions were given on whether immune-suppression therapy with cyclosporine and antithymocyte globulin were options. No literature is available to support this approach for temozolomide-induced aplasia in adults, because this is not a T-cell–mediated complication. Reported cases of temozolomide-induced aplastic anemia have led to disease progression, transfusion dependence, and palliative services.11-14

Clinicians should be aware of this rare and potentially devastating complication of temozolomide therapy. Morris and colleagues reported a case of severe aplastic anemia in an adolescent girl with a high-grade glioma after radiation therapy and concurrent temozolomide therapy.27 Twenty-three months after diagnosis, treatment with immunosuppressive therapy for severe aplastic anemia (ie, antithymoglobulin, cyclosporine, and corticosteroids) was initiated but proved inadequate. The patient eventually underwent an 8/8 HLA-matched unrelated donor transplant that led to a significant improvement in her quality of life and reduced the number of transfusions.

For our patient, the decision was made to seek expert opinion from a large academic institution. In the meantime, our patient continued to receive weekly transfusions as needed, and started filgrastim and epoetin alfa therapy; one study suggests a response to these drugs.14 Our patient’s severe pancytopenia continues at this time. Hematologic counts 3 weeks after the initiation of therapy continued to show no significant improvement (Table 4).

One could argue that having the patient’s MGMT methylation status could help to determine optimal therapy; however, this is not currently the standard of care, and no recommendations are available on how to proceed with treatment when the MGMT status is known. MGMT status was not obtained in our patient before treatment initiation, and would not have benefited the patient at the time.

Conclusion
Temozolomide, an oral and IV alkylating agent, is used for the treatment of patients with central nervous system tumors. It is generally well-tolerated as a single agent or with concurrent radiation compared with the PCV regimen.

Multiple, small case series show that temozolomide very rarely triggers severe aplastic anemia, regardless of the dose and duration of therapy. Genetic and clinical factors may exacerbate such a condition, by increasing the sensitivity to temozolomide, but large cohorts are needed to provide a definitive answer. Clinicians should be aware of this rare and potentially devastating complication of temozolomide therapy.

Author Disclosure Statement
Dr Mancini is on the Speaker’s Bureau for Takeda Pharmaceuticals and Pfizer Pharmaceuticals, and is a Consultant for Taiho Pharmaceuticals; Dr Hanna, Dr Bartelow, and Dr Bridges have no conflicts of interest to report.

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12. Doyle TJ, Mikkelsen T, Croteau D, et al. Fatal hematologic toxicity with...


