Incidence of *Pneumocystis jirovecii* pneumonia after temozolomide for CNS malignancies without prophylaxis

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Summary points

- There is no consensus on use of *Pneumocystis jirovecii* pneumonia (PJP) prophylaxis for patients receiving temozolomide, nor duration of temozolomide, for glioblastoma multiforme (GBM) or high-grade glioma patients.
- Evidence for PJP during chemoradiotherapy (CRT) is very limited. However, adverse events were associated with the use of PJP prophylaxis during CRT, for example, Green *et al.* reported a 15.2% of adverse reactions and 3.1% of severe adverse reactions (mainly leukopenia) in non-HIV adults.
- This group of brain tumor patients were treated with temozolomide without prophylaxis since 1999, and temozolomide was given until progression.
- A single case of PJP occurred among 240 consecutive patients with GBM and other brain tumors treated at a single institution (0.4%). The proportion was one in 127 (0.8%) among patients receiving concurrent CRT.
- The patient that developed PJP also had herpes zoster and aspergillus suggestive of underlying immunodeficiency.
- The rate of PJP is low even if the patients had multiple risk factors in addition to the use of temozolomide.
- For patients receiving temozolomide until tumor progression, 10% had over 30 courses and no significant toxicity was observed among all patients.
- A recent meta-analysis by Green *et al.* suggests PJP prophylaxis should be used only when risk of PJP greater than 3.5%.

SUMMARY Aims: Prophylaxis against *Pneumocystis jirovecii* pneumonia (PJP) is currently recommended for patients receiving chemoradiation with temozolomide for newly diagnosed glioblastoma multiforme. At our institution, PJP prophylaxis during temozolomide treatment has not been routinely given because of the paucity of supporting data. We investigated the rate of PJP infections in our patients. **Patients & methods:** We conducted a retrospective

KEYWORDS

- brain radiation
- glioblastoma
- *Pneumocystis jirovecii*
- temozolomide

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chart review of 240 brain tumor patients treated between 1999 and 2012 with temozolomide and no PJP prophylaxis, 127 of which received concurrent chemoradiation. **Results:** One in 240 patients (0.4%; 95% CI: 0.01–2.00; median total dose: 7375 mg/m²; interquartile range: 1300) were diagnosed with PJP. **Conclusion:** There was a <1% rate of PJP for brain tumor patients treated with temozolomide until progression without PJP prophylaxis.

Temozolomide (TMZ) is a drug used against CNS malignancies because of its superior ability to cross the blood–brain barrier relative to other chemotherapeutic agents [1]. In an early study involving TMZ, two out of 15 patients treated with TMZ developed *Pneumocystis jirovecii* pneumonia (PJP) [2]. Consequently, upon the approval of TMZ, the US FDA required the use of PJP prophylaxis in conjunction with TMZ and concomitant radiotherapy for newly diagnosed glioblastoma multiforme (GBM) patients. As a result, PJP prophylaxis became common practice for all high-grade gliomas, despite the relative paucity of supporting data. Indeed, in the landmark study that ultimately resulted in the approval of TMZ for use against GBM, co-trimoxazole (trimethoprim-sulfamethaxazole [TMP-SMX]; Bactrim®, Roche, Basel, Switzerland) was routinely used in the TMZ treatment arm, with radiation, and then given for 6 months per FDA guidelines [3].

Co-trimoxazole is effective in the prevention of PJP [4]. However, adverse reactions to TMP-SMX used in prophylactic doses in non-HIV adults is 15.2%, with severe adverse reactions (mainly leukopenia) occurring in 3.1% of adults [5]. The drug is therefore not without risk. At our institution, the division of neuro-oncology, in consultation with pharmacy, decided that the evidence for prophylactic co-trimoxazole due to lymphopenia [6,7] for brain tumor patients treated with TMZ was insufficient to justify its routine use. This study examined the outcomes of 240 consecutive brain tumor patients treated with TMZ without concurrent medication with prophylactic activity against PJP. In addition, contrary to FDA guidelines for GBM to stop TMZ after six courses after chemoradiotherapy (CRT), TMZ is given until progression at our institution since no good second-line therapy exists for malignant high-grade brain tumors. Clinical observations in our group [8] and others [9,10] support such use. Therefore, this study also evaluated the incidence of PJP without prophylaxis under longer treatment of TMZ beyond six courses.

Patients & methods

The study was registered with and approved by Oregon Health and Science University institutional review board (eIRB# e8365).

• Patient population & chart review

A cohort of patients was created from a retrospective chart review of patients that received TMZ chemotherapy between 1999 and 2012 (see **Supplementary Table 1** for definitions; see online at www.futuremedicine.com/doi/full/10.2217/cns.14.24). Inclusion criteria to the study were high-grade glioma patients who received TMZ chemotherapy for brain tumor treatment at our institution between 1999 and 2012. Exclusion criteria were patients who received medication with prophylactic activity against PJP (Bactrim, pentamidine, dapsone and primaquine) and absence of sufficient records for analysis. Included patients were evaluated at 3 months to 13 years of follow-up after initiation of TMZ (specifically, charts were reviewed from time of initiation of TMZ until the time of chart review in April 2012). All charts were reviewed by AJ Neuwelt or TM Nguyen. A total of 10% of randomly selected charts were dual reviewed and abstracted to assure consistency in the data abstraction. Inter-reviewer differences were discussed among the co-authors.

• Data collection & variables

Data for patient characteristics, risk factors for immunosuppression (diabetes, smoking, extended steroid use, radiation therapy [6,7], receiving other chemotherapy (**Supplementary Table 2**), severe malnutrition and HIV status), infections associated with T-cell dysfunction (herpes simplex virus, varicella-zoster virus and fungal infections) and data for blood counts (lymphocytes and white blood count) were abstracted for each patient (**Supplementary Table 1**). Doses of TMZ [11] administered to each patient were recorded. Blood counts were routinely obtained on days 21 and 28 after initiation of TMZ, and as complications arose.

• Data analysis

All analyses are descriptive. Continuous variables were summarized using mean and standard

deviation, or median and interquartile range (IQR), and discrete variables were summarized using proportions.

Results

A total of 259 neuro-oncology patients received TMZ chemotherapy between 1999 and 2012 at Oregon Health and Science University. Of these patients, 252 had records available for analysis. An additional 12 patients received medication with activity against PJP during their treatment course (most commonly for treating urinary tract infection or were started on prophylactic co-trimoxazole by a referring physician), and were excluded. Thus, 240 patients were included in the final analysis (Figure 1).

• Patient demographics

The median age of the patients was 52 years (range: 6–88 years) and 62% of patients were male (Table 1). The most common diagnosis was GBM ($n = 95$; 40%). Most patients received other treatments in addition to TMZ, including surgery, radiation and/or additional chemotherapeutic drugs. A total of 127 patients had concurrent chemoradiation with TMZ. For the vast majority of patients, concurrent TMZ is identified as the daily 75 mg/m² of TMZ with radiation therapy for 6 weeks. After concurrent TMZ, patients were put on maintenance TMZ on an approximately 28-day cycle. Per the package insert [11], standard TMZ dose was 150–200 mg/m² once a day $\times$ 5 days, monthly for 6 months after concurrent radiation. Monthly TMZ was continued beyond the recommended 6 months, but doses were adjusted for toxicities, and the median final cumulative dose was 7375 mg/m², which is slightly less than the maximum recommended dose of 8900 mg/m². However, the median number of courses of TMZ patients received was seven (range: 1–54) and 25 patients (10%) were treated with at least 30 courses of TMZ.

In addition to TMZ, the patients had risk factors for PJP infection. Particularly significant was 213/240 (89%; 95% CI: 84–92) of the patients received extended steroids as defined in Supplementary Table 1. The median nadir lymphocyte count during treatment was $0.7 \times 10^9/l$ (IQR: 0.6).

• Infections associated with T-cell dysfunction

In order to assess the immune status of the studied patient population, information was

gathered regarding infections contracted by the patients that may be associated with impaired T-cell function (Table 2). Two patients were diagnosed with herpes simplex virus infections and six patients were diagnosed with herpes zoster. None of these infections were disseminated.

• Patient infected with PJP

A single patient out of the 240 patients who received TMZ (0.4%; 95% CI: 0.01–2.00) was diagnosed with PJP pneumonia (Table 3). The proportion was one in 127 (0.8%; 95% CI: 0.02–4.00) among patients receiving concurrent chemoradiation with TMZ. This patient was a 41-year-old female with GBM. She underwent surgical resection and then was treated with TMZ at 75 mg/m² over 6 weeks and concomitant radiation (a standard dosing regimen). During this time, the patient also received multiple doses of steroids for symptomatic relief of headaches. She completed 6 weeks of TMZ with radiation and 3 months later developed PJP pneumonia. She did not receive adjuvant TMZ. In the interim between completion of TMZ and diagnosis of PJP, she received two courses of 10 mg/kg bevacizumab. The PJP was diagnosed by bronchoscopy and Grocott's methenamine silver stain. In addition, she concomitantly developed herpes zoster and pulmonary aspergillosis. At the time of PJP infection, the lymphocyte count was $0.1 \times 10^9/l$. These infections resulted in a hospitalization. The patient was treated with TMP-SMX and fully recovered from these infectious complications. After diagnosis of PJP, the patient was maintained on prophylactic TMP-SMX.

Figure 1. Flow chart of patient selection.
PJP: *Pneumocystis jirovecii* pneumonia.

Table 1. Patient demographics (n = 240).

Characteristic	n (%; unless otherwise defined)
Age, median years (range)	52 (6–88)
Male	149 (62)
Diagnosis[†]	
Glioblastoma multiforme	95 (40)
Anaplastic astrocytoma	58 (24)
Anaplastic oligodendroglioma	89 (37)
Treatment other than temozolomide	
Surgery	225 (94)
Radiation	177 (74)
Bevacizumab	69 (29)
Carboplatin	85 (35)
Etoposide phosphate	38 (16)
Cyclophosphamide	39 (16)
Irinotecan	14 (6)
Melphalan	36 (15)
Other [‡]	60 (25)
Temozolomide treatment	
Number courses; median (range)	7 (1–54)
Total dose (mg/m ²); median (IQR)	7375 (1300)
Patients receiving >30 courses	25 (10)
Comorbid conditions	
Smoker	14 (6)
Diabetes	63 (26)
HIV	0
Bone marrow transplant	0
Dialysis	0
Solid organ transplant	0
High steroid exposure	213 (89)
History of lymphoma	2 (0.8) [§]
Other hematologic malignancy/dysplasia	3 (1.2) [¶]
Severe malnutrition	1 (0.4)
Primary immunodeficiency	1 (0.4)
Nadir median lymphocytes (IQR) [#]	0.7 × 10 ⁹ /l (0.6)
Nadir median WBC (IQR) [#]	4 × 10 ⁹ /l (2.9)

[†]The percentages do not add up to 100 because some patients had more than one pathology diagnosed on biopsy.

[‡]5 procarbazine, lomustine, and vincristine, 10 procarbazine, 1 sunitinib, 19 etoposide, 5 lomustine, 6 methotrexate, 2 rituximab, 3 carmustine, 7 lomustine and 1 ibritumomab tiuxetan.

[§]1 Non-Hodgkin's lymphoma and 1 large B-cell lymphoma.

[¶]1 acute myeloid leukemia, 1 chronic lymphocytic leukemia and 1 myelodysplasia.

[#]WBCs were available for 96% (230/240) of patients, and total lymphocyte counts for 81% (195/240) patients.

IQR: Interquartile range; WBC: White blood count.

Discussion

The important finding of this study was <1% of 240 consecutive patients treated with TMZ until progression for malignant brain tumor developed PJP despite receiving no PJP prophylaxis. In addition, <1% of 127 patients treated with concurrent chemoradiation and TMZ for newly diagnosis malignant glioma developed PJP.

The FDA approval for concurrent chemoradiation with TMZ [11] stipulated PJP

prophylaxis was required because two of the 15 study patients developed PJP [2]. Additional case reports have described sporadic PJP in unprophylaxed patients receiving TMZ for malignant brain tumor [12–16]. As PJP is a known complication of TMZ chemoradiation, some cases may not be reported in the literature. Nevertheless, it was on the basis of the limited evidence of these prior case reports that our institution decided not to use PCP prophylaxis for the patient cohort described in this study.

Table 2. Incidence of infections usually associated with T-cell deficiency.

Infection	Number of patients
PJP pneumonia (%)	1 (0.4)
Herpes simplex virus (%)	2 (0.8)
Herpes zoster (%)	6 (2.5)
Invasive fungal disease (%)	1 [†] (0.4)
[†] Invasive pulmonary aspergillosis. PJP: <i>Pneumocystis jirovecii</i> pneumonia.	

While daily or three times a week TMP-SMX may appear simple for preventing a life-threatening infection, there are published concerns about side effects [5,17]. One study reports adverse reactions to TMP-SMX used in prophylactic doses in non-HIV adults as 15.2%, with severe adverse reactions (mainly leukopenia) occurring in 3.1% of adults [5]. There is also the need to minimize antibiotic use in an era of increasing multidrug-resistant organisms. A recent meta-analysis concludes that benefits of PJP prophylaxis outweigh the risks in populations only when there is a >3.5% risk of infection [5]. Our retrospective review showed <1% incidence of PJP in 270 unphylaxed patients.

Pneumocystis colonizes healthy individuals. Risk factors for developing PJP are conditions in which there is lymphocyte dysfunction [18] or depletion (HIV, radiation, treatment with lymphocyte inhibiting/depleting agents [19] and patients with hematological or solid malignancies) [17]. It was striking that the cohort of patients presented in this study had multiple PJP risk factors in addition to TMZ (steroids, cranial radiation therapy, other chemotherapy and lymphopenia). The single patient who did develop symptomatic PJP in the study was exposed to multiple courses of steroids in addition to other chemotherapy agents. However, this was not unique to this patient and it is unclear why this

individual developed PJP and other infections that suggested profound lymphocyte dysfunction (multidermatome recalcitrant herpes zoster and invasive pulmonary aspergillosis), while the rest of the cohort did not. These results therefore suggested that future studies of the ‘co-risk factors’ for PJP during TMZ therapy are needed. However, with the potential for a <1% incidence of disease, a very large prospective study cohort will be needed for definitive results.

At our institution, we have extended the use of TMZ until definitive evidence of tumor progression [8], which is significantly longer than the FDA recommended (6 months after CRT) [11]. Other groups have also adopted this practice. In the Stupp study in which patients received adjuvant TMZ for up to 6 months, overall survival (OS) was 14.6 months [3]. In contrast in the Brandes *et al.* study [9], patients with GBM received adjuvant TMZ until complete response (a minimum of 12 months) or tumor progression had an OS of 20.7 months. Another retrospective study showed improved OS in GBM patients treated with adjuvant TMZ for more than 6 months compared with less than 6 months (due to institutional policy and not tumor progression), 24.6 versus 16.5 months ($p = 0.031$), respectively [10]. Recent large randomized studies (RTOG 0525 [20] and RTOG 0825 [21]) were not designed to assess the survival benefit of

Table 3. Patient infected with *Pneumocystis jirovecii* pneumonia.

Characteristic	Variable
n (%)	1 (0.4)
Age (years)	41
Sex	Female
Diagnosis	GBM
Treatment	Surgery, chemoradiation with 75 mg/m ² temozolomide, bevacizumab ×2 (10 mg/kg)
Nadir lymphocytes	0.1 × 10 ⁹ /l
Other risk factors for immunosuppression	Malnutrition (albumin 1.6), multiple courses of steroids
Infections acquired during treatment	Herpes zoster, fungal pneumonia, PJP
Method of PJP diagnosis	GMS stain from bronchoscopy
GBM: Glioblastoma multiforme; GMS: Grocott's methenamine stain; PJP: <i>Pneumocystis jirovecii</i> pneumonia.	

extended TMZ treatment beyond 6 months, but are consistent with the results that extended use of TMZ may be associated with survival benefit. Our work provides additional data showing that extended use of TMZ without the use of PJP prophylaxis will not lead to extra cases of PJP, but will aid in the care of these patients.

This study was limited by its retrospective design, which could have led to inaccurate identification of PJP cases. However, it is likely that this bias was minimal because there was close follow-up of the patients until death from their brain malignancy. Another limitation of the study was most patients did not take a continuous routine dose of steroids. Doses were adjusted by patients based on need for symptom control. Thus, accurate records of the exact amount of steroids used by the patient population were unavailable. The fact the study analysis was blinded to steroid doses made graduated steroid-related risk assessment for the development PJP impossible. Future studies must include prospective accurate documentation of steroid doses.

Also of note, 12 patients were excluded from the analysis because they received agents with anti-*Pneumocystis* activity for non-*Pneumocystis*-related issues, such as TMP-SMX for urinary tract infection. There was no evidence these patients were at higher risk of PJP infection, but their exclusion may have biased the results (data not shown). Finally, this was a single-center study, and the results cannot be directly extrapolated to other centers. It is possible some subtle differences in the treatment regime rendered these patients less susceptible to symptomatic PJP compared with other centers. *Pneumocystis* is an environmental organism and it is unknown whether our geographical location resulted in less exposure, although PJP is seen in other vulnerable populations at our institution (data not shown).

Conclusion

In summary, this study showed the incidence of PJP in 240 patients treated with TMZ for malignant brain tumor with no PJP prophylaxis

was <1%. The study did not answer the question whether PJP prophylaxis is indicated during TMZ therapy for malignant brain tumor. Instead, it suggested further investigation is warranted to identify the patients most at risk of developing PCP during TMZ therapy, perhaps by examining the other co-risk factors.

Future perspective

The mandate for PJP prophylaxis on the package insert may be a two-edged sword and needs to be evaluated in other institutions and other geographical locations. The use of PJP prophylaxis needs further study especially when the absolute lymphocyte count goes below 100 cell/mm³; however, since prophylaxis can cause leukopenia, it may suppress inflammation needed for pseudoprogession to develop, which is associated with increased survival as shown by Nasseri *et al.* [8]. When severe leukopenia develops, then the use of dapsone rather than bactrim should be considered with low CD4 counts.

In addition, inflammation, which may be key to increased survival with CRT (i.e., development of pseudoprogession) may be suppressed by the rare leukopenia sometimes seen with prophylaxis and affect patient care.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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