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ORIGINAL ARTICLE

Late-onset *Pneumocystis jirovecii* pneumonia post–fludarabine, cyclophosphamide and rituximab: implications for prophylaxis

Gabrielle M. Haeusler[1], Monica A. Slavin[1,2], John F. Seymour[2,3], Senthil Lingaratnam[4], Benjamin W. Teh[1], Constantine S. Tam[3], Karin A. Thursky[1], Leon J. Worth[1]

[1]Department of Infectious Diseases and Infection Control, Peter MacCallum Cancer Centre, East Melbourne; [2]Department of Medicine, University of Melbourne, Parkville; [3]Department of Haematology, Peter MacCallum Cancer Centre, East Melbourne; [4]Pharmacy Department, Peter MacCallum Cancer Centre, East Melbourne, Vic., Australia

Abstract

Objective: Fludarabine, cyclophosphamide and rituximab (FCR) therapy for lymphoid malignancies has historically been associated with a low reported incidence of *Pneumocystis jirovecii* pneumonia (PJP). However, prophylaxis was routinely used in early studies, and molecular diagnostic tools were not employed. The objective of this study was to review the incidence of PJP during and post-FCR in the era of highly sensitive molecular diagnostics and ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET)–computerised tomography (CT). *Methods:* All patients treated with standard FCR at the Peter MacCallum Cancer Centre (March 2009 to June 2012) were identified from a medications management database. Laboratory-confirmed PJP cases during this time were identified from an electronic database. *Results:* Overall, 66 patients were treated with a median of 5.5 FCR cycles. Eight PJP cases were identified, 6 of whom had received chemotherapy prior to FCR. In 5 cases, ^{18}F-FDG PET demonstrated bilateral ground-glass infiltrates. Median CD4$^+$ lymphocyte counts at time of PJP diagnosis and 9–12 months following FCR were 123 and 400 cells/μL, respectively. In patients receiving no prophylaxis, 9.1% developed PJP during FCR. The rate following FCR was 18.4%, with median onset at 6 months (2.4–24 months). *Conclusion:* Given the high rate of late-onset PJP, consideration should be given for extended PJP prophylaxis for up to 12 months post-FCR, particularly in pretreated patients. Further evaluation of the role of CD4$^+$ monitoring is warranted to quantify risk of disease development and to guide duration of prophylaxis.

Key words *Pneumocystis jirovecii* pneumonia; prophylaxis; cancer; fludarabine, cyclophosphamide and rituximab; lymphocyte; guideline

Correspondence Gabrielle M. Haeusler, Department of Infectious Diseases and Infection Control, Peter MacCallum Cancer Centre, Locked Bag 1, A'Beckett street, East Melbourne, Vic. 8006, Australia. Tel: +61 3 9656 5853; Fax: +61 3 9656 1185; e-mail: gabrielle.haeusler@petermac.org

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Combination chemo-immunotherapy with fludarabine, cyclophosphamide and rituximab (FCR) is highly effective against a range of indolent lymphoid malignancies (1–4). Infection is a recognised complication of FCR with reports of up to one-fifth of patients experiencing severe (Grade 3+) infections (3). Although *Pneumocystis jirovecii* pneumonia (PJP) has been associated with fludarabine-based regimens (5), our prior review of infective complications in the first twelve months of remission following FCR only identified one suspected case of PJP in 83 patients treated without prophylaxis (6). Similarly, an increased rate of PJP was not observed in the three randomised controlled trials investigating FCR chemotherapy for both previously treated (3) and untreated patients (1, 2) with lymphoid malignancies.

In immunocompromised non-HIV-infected patients, primary PJP prophylaxis is associated with a 91% reduction in the occurence of PJP and is recommended when observed rates in non-HIV-infected populations exceed 3.5% (7).

Guidelines exist for PJP prophylaxis for known risk groups including allogeneic stem cell transplant recipients (8), children with acute lymphoblastic leukaemia (9) and patients receiving ≥20 mg prednisolone equivalent for ≥1 month (10). PJP prophylaxis is also recommended during fludarabine treatment in patients with lymphoproliferative disorders (11). However, the optimal duration of PJP prophylaxis is less well defined, and few studies specifically address this issue. In particular, there are no recommendations for the duration of PJP prophylaxis following FCR chemotherapy.

Our objective was to review the incidence of PJP during and post-FCR in the era of highly sensitive molecular diagnostics and ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET)–computerised tomography (CT). Timing of onset, clinical features and outcomes of PJP were also determined.

Materials and methods

All patients treated with a standard FCR regimen (12) at the Peter MacCallum Cancer Centre between March 2009 and June 2012 were identified from a medications management database. Laboratory-confirmed PJP cases during this time were also identified from an electronic database. PJP prophylaxis was administered according to physician preference. During the study period, institutional guidelines recommended PJP prophylaxis with oral trimethoprim–sulfamethoxazole for patients with lymphoma who had evidence of impaired cell-mediated immunity due to disease or cumulative exposure to systemic anticancer therapy (e.g. alemtuzumab therapy) and for all patients receiving corticosteroids at a daily dose equivalent to or greater thana minimum average dose of 20 mg of prednisolone for more than 1 month (10, 13).

Pneumocystis jirovecii pneumonia was defined in the presence of a radiologically confirmed pulmonary infiltrate, identification of *P. jirovecii* on respiratory tract sampling [bronchoalveolar lavage (BAL) or induced sputum] by polymerase chain reaction (PCR) and absence of other pathogenic causes of diffuse pulmonary infiltrates. Investigations performed on BAL were according to a standardised protocol and included bacterial and fungal culture, viral PCR and non-culture techniques for fungal infection (Table 2).

Semiquantitative PCR targeting the *P. jirovecii* major surface glycoprotein gene was performed (maximum 40 cycles for detection) according to previously published methods (14). Radiological investigations included X-ray, CT and/or FDG PET/CT imaging. Median duration of follow-up from FCR completion was 17 (range 0–39) months.

To differentiate early- and late-onset infections, rates of PJP were calculated for patients who did not receive PJP prophylaxis and (i) who developed PJP during FCR chemotherapy and (ii) who developed PJP after the completion of FCR chemotherapy. The incidence of PJP following FCR was calculated per 10 000 patient-days.

Results

Sixty-six patients were treated with a median of 5.5 FCR (range 3–6) cycles. Underlying conditions included chronic lymphocytic leukaemia (CLL) (n = 43), mantle cell lymphoma (n = 7), follicular lymphoma (n = 6), Waldenström macroglobulinaemia (n = 5) and other indolent lymphomas (n = 5). Twenty-nine patients had received prior chemotherapy regimens including chlorambucil (n = 5); autologous stem cell transplant (n = 3); FC/FCR (n = 7); cyclophosphamide, vincristine, doxorubicin and dexamethasone (n = 2); rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) (n = 9); and unknown (n = 3). Fifty-five patients (83%) received PJP prophylaxis with trimethoprim–sulfamethoxazole (n = 54) or dapsone (n = 1) during and for up to 1 month following FCR. Prophylaxis was continued after completion of FCR (i.e. beyond 1 month) in 27 patients (41%) for a median of 85 (range 50–653) days.

Eight PJP cases were identified. No cases were clustered epidemiologically. Clinical findings and outcomes are summarised in Tables 1 and 2. In all cases, a new pulmonary infiltrate was identified on imaging. In addition, an elevated C-reactive protein (CRP) (range 35–217 mg/L, normal <2 mg/L) was documented in all patients, consistent with an infective process. Following treatment, the CRP normalised in the six patients who had this test repeated.

Pneumocystis jirovecii pneumonia was diagnosed during FCR treatment in one patient (between cycles 3 and 4) and at a median of six (range 2.5–24) months after the completion of FCR treatment in the remaining seven patients. Six patients had received prior chemotherapy regimens, and the patient who developed PJP 24 months following FCR had recently received investigational drug ABT-199 (patient 6) (15). No episodes occurred in patients receiving concurrent PJP prophylaxis. In patients who did not receive PJP prophylaxis, one of 11 [9.1%, 95% binomial confidence interval (CI) 0.2–41.2] and seven of 38 (18.4%, 95% CI 7.7–34.3) developed PJP during and post-FCR, respectively. The incidence of PJP following FCR chemotherapy was 3.3 per 10 000 patient-days.

Cycle thresholds for *P. jirovecii* detection by PCR spanned 21–32 (median 28). In six patients, additional molecular or culture-positive BAL results were considered to represent colonisation (*Aspergillus* spp. and cytomegalovirus) or inconsistent with the clinical presentation (Herpes simplex virus, *Staphylococcus aureus*, *Moraxella catarrhalis*) (Table 2). In the setting of fever, elevated inflammatory markers and new onset pulmonary infiltrate, an alternative chronic or non-infective process was considered unlikely.

Two patients presented with pyrexia of unknown origin (PUO) and bilateral FDG-avid pulmonary infiltrates were

Table 1 PJP cases: underlying and predisposing conditions

Patient	Age/ sex	Diagnosis (disease status)	Comorbidities	Pre-FCR treatment	Completed FCR cycles	Corticosteroid within 1 month of PJP diagnosis	PJP prophylaxis during and post-FCR
1	58/M	CLL (remission)	Nil	Chlorambucil	6/6	No	During: Yes Post: No
2	71/M	Mantle cell lymphoma (remission)	Myelodysplasia	Hyper-CVAD	3/4	No	During: No Post: No
3	64/M	Follicular lymphoma (remission)	Mild pulmonary fibrosis Lung cancer RUL lobectomy	Chlorambucil, Autologous SCT	6/6	Prednisolone 25 mg/d (3 wk)	During: Yes Post: No
4	80/M	Mantle cell lymphoma (active)	Prostate cancer Hypothyroidism	R-CHOP, R-CEOP	6/6	No	During: No Post: No
5	66/F	CLL (remission)	Nil	No	6/6	No	During: Yes Post: No
6	62/F	CLL (active)	Hypogammaglobulinaemia	FCR	3/3	No	During: Yes Post: No
7	70/F	Mantle cell lymphoma (remission)	Ovarian cancer Splenectomy	No	5/5	No	During: Yes Post: No
8	66/M	Follicular lymphoma (remission)	COPD	R-CHOP	4/4	No	During: Yes Post: No

M indicates male; CVAD, cyclophosphamide, vincristine, doxorubicin, dexamethasone; RUL, right upper lobe; SCT, stem cell transplant; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; R-CEOP, rituximab, cyclophosphamide, doxorubicin, etoposide, prednisolone; d, day; w, weeks; F, female; COPD, chronic obstructive pulmonary disease.

identified on FDG PET/CT. The median $CD4^+$ lymphocyte count at time of PJP diagnosis was 123 cells/μL (six patients tested, range 60–440 cells/μL), compared with a median count of 400 cells/μL when tested nine to 12 months following FCR (three patients tested, range 300–590 cells/μL). Seven patients had complete response to PJP treatment, and one patient died of respiratory failure.

Discussion

Our findings suggest that the risks of PJP during and following FCR may be higher than previously appreciated. In the three randomised trials investigating FCR for lymphoid malignancies, PJP prophylaxis was mandated during FCR (3), during and 6 months following FCR (1) or in the presence of lymphopenia beyond 7 days (2). Therefore, an increased risk of PJP during treatment was not reported. An extended risk of PJP following FCR has similarly not been reported with PJP being identified infrequently in the first 12 months of remission in both previously treated and untreated patients (6, 16). Of note, these studies were conducted before the routine use of PCR for diagnosis of PJP.

The availability of newer diagnostic tools with improved sensitivity for the detection of PJP, in part, explains the higher rate observed in our study (17). Both conventional and quantitative PCR are sensitive and specific for identifying *Pneumocystis* in respiratory secretions (18), but are unable to distinguish infection from colonisation. *Pneumocystis* colonisation is described in patients with haematological malignancies, although rates are unknown (18). Quantitative PCR has been proposed as one method for differentiating infection from colonisation with one study showing copy numbers were significantly higher in definite and probable PJP patients than in colonised patients (19). Although clinically relevant cut-off values have not been validated (17, 19), the low median cycle threshold for *P. jirovecii* DNA detection (and hence high organism burden) in our cohort is suggestive of infection rather than colonisation. The presence of respiratory symptoms, compatible radiological changes, elevated inflammatory markers, and response to treatment further favours infection over colonisation.

FDG PET is also emerging as a tool for identification of otherwise occult infection in neutropenic patients with prolonged or unexplained fever (20–22). However, the contribution of FDG PET to the diagnosis of PJP has only previously been described in three case reports (23–25). In our cohort, bilateral FDG uptake in lungs was seen in all patients with PJP who underwent FDG PET, including two patients with PUO and normal chest X-ray and CT findings, suggesting utility of FDG PET for early PJP detection.

Fludarabine, cyclophosphamide and rituximab induces a profound and sustained T-lymphocytopenia. In patients with CLL undergoing FCR, the median times to reach $CD4^+$ lymphocyte counts >200 cells/μL and 400 cells/μL post-treatment were six and 24 months, respectively (26). This correlates with the risk of late infection being 10% in the first year of remission after FCR and 4% in the second year (4). In patients infected with human immunodeficiency virus (HIV), there is a strong association between $CD4^+$ counts <140 cells/μL and PJP risk (27). Monitoring of $CD4^+$ counts

Table 2 PJP cases: clinical presentation, diagnostic features and outcomes

Patient	Presentation	PJP diagnosis, months post-FCR	Lung Imaging	CD4 Monitoring cells/μL		Respiratory specimen	Treatment	Outcome
				At PJP dx	Post-FCR[1]			
1	PUO	2.5	CXR: N HRCT: N FDG PET/CT: diffuse low-grade FDG uptake	140	ND	*Positive*[2]: PJP PCR (Ct 28) *Negative*[2]: Routine diagnostic panel[3]	TMP-SMX po 15 mg/kg/d (3 wk) Azithromycin po 500 mg/d (3 d) Prednisolone po 50 mg/d (then weaning dose)	Full recovery
2	Fever, dyspnoea, hypoxia	Between cycles 3 and 4	CXR: N CT: diffuse ground-glass infiltrate FDG PET/CT: patchy bilateral low-grade FDG uptake	ND	ND	*Positive*[2] PJP PCR (Ct 32) Aspergillus PCR (serum neg.) CMV PCR (serum neg.) *Negative*[2] Routine diagnostic panel[3]	TMP-SMX iv 20 mg/kg/d (2 wk) Voriconazole po 6 mg/kg/d Pip-Taz iv 4.5 mg tds Methylprednisolone iv 1 g/d (5 d)	ICU admission Invasive ventilation Died from respiratory failure
3	Fever, dyspnoea	7.5	CXR: bilateral ground-glass infiltrates CT: patchy bilateral ground-glass infiltrate FDG PET/CT: Patchy bilateral FDG uptake	60	ND	*Positive*[2] PJP PCR (Ct 28) HSV-I PCR *Negative*[2] Routine diagnostic panel[3]	TMP-SMX po 15 mg/kg/d (3 wk) Valaciclovir po 1 g tds (1 wk) Prednisolone po 50 mg/d (then weaning dose)	Full recovery
4	Fever, hypoxia	6	CXR: bilateral ground-glass infiltrates HRCT: bilateral mild upper lobe infiltrates FDG PET/CT: bilateral moderate FDG uptake in upper zones	126	ND	*Positive*[2] PJP PCR (Ct 21) *Negative*[2] Routine diagnostic panel[3]	TMP-SMX po 20 mg/kg/d (3 wk) Azithromycin iv 500 mg/d (3 d) Meropenem 1 g tds (5 d) Prednisolone po (20 mg d then weaning dose)	ICU admission, non-invasive ventilation Full recovery
5	Fever, cough and hypoxia	7	CXR: diffuse ground infiltrates HRCT: diffuse ground-glass infiltrates FDG PET/CT: ND	120	300	*Positive*[2] PJP PCR (Ct 29) CMV PCR (serum neg.) *Negative*[2] Routine diagnostic panel[3]	Atovaquone po 750 mg bd (3 wk) Azithromycin po 500 mg/d (5 d) Prednisolone po 50 mg/d (then weaning dose)	Full recovery
6	PUO	24	CXR: N CT: N FDG PET/CT: bilateral FDG uptake	440	ND	*Positive*[2] PJP PCR (Ct 38) *Staphylococcus aureus* *Negative*[2] Routine diagnostic panel[3]	TMP-SMX po 15 mg/kg/d (3 wk)	Full recovery
7	Fever, cough and dyspnoea	5	CXR: mild ground-glass infiltrate HRCT: diffuse ground-glass infiltrate FDG PET/CT: ND	120	590	*Positive*[2] PJP PCR (Ct 26) CMV PCR (serum neg.) HSV-1 PCR *Negative*[2] Routine diagnostic panel[3]	TMP-SMX po 20 mg/kg/d (3 wk) Pip-Taz iv 4.5 g tds (3 d) Azithromycin po 500 mg bd (5 d) Valaciclovir po 500 mg tds (1 wk) Prednisolone po 50 mg/d (then weaning dose)	Full recovery

(continued)

Table 2 (continued)

Patient	Presentation	PJP diagnosis, months post-FCR	Lung Imaging	CD4 Monitoring cells/μL		Respiratory specimen	Treatment	Outcome
				At PJP dx	Post-FCR[1]			
8	Fever, cough and dyspnoea	4	CXR: bilateral lower lobe patchy infiltrate HRCT: not done FDG PET/CT: ND	ND	400	*Positive*[4] PJP PCR (Ct 24) *Moraxella catarrhalis*	TMP-SMX po 15 mg/kg/d (3 wk)	Full recovery

PUO indicates pyrexia of unknown origin; N, normal; PCR, polymerase chain reaction; Ct, cycle threshold; TMP-SMX, trimethoprim–sulfamethoxazole; po, per oral; ND, not done; neg., negative; CMV, cytomegalovirus; iv, intravenous; ICU, intensive care unit; tds, three times daily; HSV-1, herpes simplex virus-1; Pip-Taz, piperacillin–tazobactam; bd, twice daily. Abbreviations d and w are explained in Table 1.

[1]$CD4^+$ count measured 9–12 months post-FCR.

[2]BAL specimen.

[3]Routine diagnostic panel includes bacterial, fungal and *Mycobacterium* microscopy and culture; typical and atypical *Mycobacterium* PCR; viral respiratory PCR (including influenza A and B, parainfluenza 1, picornavirus, adenovirus, rhinovirus, respiratory syncytial virus, human metapneumovirus and coronaviruses); herpes virus PCR (including CMV, HSV-1 and HSV-2, varicella zoster virus); aspergillus PCR and galactomannan. Results were negative unless otherwise stated as positive.

[4]Induced sputum specimen.

has also been used to identify immunocompromised HIV-negative patients who may be at risk of PJP (28). In our cohort, where $CD4^+$ counts were measured at the time of PJP diagnosis, five of six patients (83%) had values <140 cells/μL. Of interest also is the recovery of $CD4^+$ counts within 12 months following FCR in the three patients. Our observations suggest that further evaluation of the role of immune markers in risk stratification is warranted.

Confirming a direct causal relationship between possible risk factors and the development of PJP is difficult. This is particularly relevant in heavily pretreated patients where consecutive chemotherapy regimens may contribute additive immune suppressive effects. This may explain the predominance of PJP in pretreated patients as compared to treatment-naive patients in our series. However, while PJP has been reported in association with chemotherapy regimens such as R-CHOP (29), FCR was the most recently administered chemotherapy in the majority of our patients and therefore considered to exert the greatest risk.

A limitation of this study is the retrospective interpretation of diagnostic results. Drug-related pulmonary toxicity is an important differential diagnosis for pulmonary infiltrates. Fludarabine-related pulmonary toxicity was reported at a single US centre in up to 8.6% of treated patients (30), with onset usually within 1 week following FCR. However, this putative phenomenon has not been reproduced by other centres or larger prospective studies (1–5). Even if accepted as a possible cause, such fludarabine-related pneumonitis would not explain the later presentations observed in our series. An alternative infective diagnosis was also considered unlikely given the extensive investigation for co-pathogens on BAL specimens. The absence of detectable cytomegalovirus in blood (by PCR) or typical radiological changes in invasive fungal infection or bacterial pneumonia suggest the additional organisms were unlikely to be of clinical significance. Finally, PJP prophylaxis was instituted according to physician discretion, and this bias may have impacted the calculated rates.

Results of this study indicate that the risks of developing PJP during and following FCR treatment for lymphoid malignancies have been previously underappreciated. Newer and more sensitive diagnostic modalities are now available to identify PJP as a potential cause of respiratory symptoms or PUO. Further study is required before molecular techniques can be reliably used to identify patients with *Pneumocystis* infection as distinct from colonisation. Similarly, further evaluation of the role of $CD4^+$ monitoring is warranted to quantify risk of disease development and to guide duration of prophylaxis. Until thresholds are validated for this population, we suggest the use of primary PJP prophylaxis during FCR treatment cycles and for 1 year following FCR completion. This is particularly relevant to pretreated patients.

Disclosure of conflict of interest

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