Late onset Pneumocystis jirovecii pneumonia (PJP) in patients with ANCA associated vasculitis

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Abstract

Immunosuppression in anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) is complicated by increasing risk of infections including opportunistic infections like, Pneumocystis jirovecii pneumonia (PJP). Available evidence on risk factors and indications for prophylaxis in AAV is derived from PJP occurring early in the course of AAV. In this retrospective study, we characterized the profile of PJP in patients with AAV. PJP cases were identified retrospectively based on positive polymerase chain reaction test from electronic record followed by confirmation from medical records over 10 year period. AAV patients without PJP over the same period were used as control group. Sixteen PJP+AAV+ were identified, in fourteen of them we were able to confirm they received PJP prophylaxis during induction therapy, While in two cases data were missing. The onset of the infection was after 6 months from AAV diagnosis in 80% of cases. Escalations in immunosuppression prior to PJP were observed in six cases within 3 months prior to PJP onset. Overall mortality was 12.5%. By univariate analysis, renal involvement at AAV diagnosis was associated with PJP. These results indicate that PJP is not limited to the first six months following AAV diagnosis. Late onset infection can occur in context of augmented immunotherapy, particularly with concurrent lymphopenia. Other risk factors that can independently predict late onset PJP remains to be identified.

Key words: Penumocystis jiroveci pneumonia, Anti-neutrophil cytoplasmic antibody, Vasculitis, Prophylaxis, Co-trimoxazole

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) are chronic inflammatory conditions of unclear aetiology. They comprise three categories: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) [1]. Despite advances in both induction and maintenance treatment with less toxic regimes, infections continue to contribute heavily to morbidity and mortality[2, 3]. The majority of these infections tend to be pulmonary in origin and the severity of the infection have been linked to mortality in a number of previous studies [2–5]. Pneumocystis jirovecii pneumonia (PJP) is an opportunistic fungal infection that had been reported with the use of various induction therapies in AAV [6–8]. Interference with cell-mediated immunity, particularly with low CD4 counts predispose to the infection[9]. Optimal duration for prophylaxis against PJP remains uncertain, in our group median time from disease onset to PJP diagnosis is 8 months [10–12]. An established risk factor for PJP in GPA (and other connective tissue diseases) is the presence of lymphopenia[8, 13]. We performed a retrospective analysis on all PJP+ cases with AAV in our tertiary referral centre using unbiased case identification over a 10 years period. The primary aim was to characterize the time between AAV diagnosis and onset of PJP and examining associated risk factors.

Methods

Microbiology department database in Aberdeen Royal Infirmary was used to identify all patient with positive PJP result by polymerase chain reaction (PCR) between 31st of March 2006 until 1st of August 2016. The cases were then divided based on their underlying diagnosis to onco-haematological malignancies, HIV, renal transplants, interstitial lung disease (unrelated to systemic vasculitis), and rheumatological diseases. The latter group was then divided into rheumatoid arthritis, AAV, and other vasculitides. Data on the PJP patients were collected from case notes and electronic records. These included: age, sex, time of AAV diagnosis, AAV type, ANCA type, time of PJP, diagnosis of PJP (clinical presentation and radiologic abnormalities) and type of biological specimen used in PCR (induced sputum or bronchoalveolar lavage, BAL). Laboratory data included: renal function (at time of AAV presentation, prior to PJP diagnosis, during hospitalization, and after discharge from hospital) and lymphocyte counts (at the end of induction therapy and within 30 days before PJP infection). Lymphocyte count of <0.3 was considered as severe lymphopenia[13]. In addition, data was collected on immunosuppressive drugs used during induction and maintenance phases including corticosteroid dose at the time of PJP diagnosis. In our centre, oral Cyclophosphamide, CyC, (1.5mg/kg) with oral steroids tapering regime is the first line of treatment for organ or life-threatening AAV. Rituximab is used when Cyclophosphamide is contraindicated or not tolerated. Adjunctive plasma exchange is used in the presence of pulmonary haemorrhage, serum creatinine of >500 µmol/, or rapidly declining renal function. Mycophenolate mofetil (MMF) or Methotrexate (MTX) are used for non-organ threatening AAV. Low dose steroids with Azathioprine (AZA), Rituximab, MMF or MTX are used for maintenance remission. Major disease relapse is treated with Rituximab (or oral Cyclophosphamide if no previous exposure) and steroid dose escalation. Minor relapse is treated with either modest increase in steroid therapy or switching Azathioprine to MMF or MTX. The clinical and laboratory profile of PJP cases were compared to control group of AAV cases without PJP (n=141)

to examine potential risk factors for developing PJP. Major and minor relapse definition followed criteria previously reported in MYCYC trial[14].

Statistical analysis

Descriptive statistics included the number (n), percentage (%) or median with interquartile range (IQR). Categorical variables were compared using Chi square test. Continuous variable were compared using Mann-Whitney U test. A p value of <0.05 was considered significant. Binary logistic regression was used to test potential predictors. Two tailed p value of less than 0.05 was considered to be significant. All statistical analysis was done using SPSS version 24.

Results

This study included 16 PJP+AAV+ patients out of total of 142 PJP cases over 10 years period. This group comprised 11% of the total PJP population. Other clinical backgrounds with PJP included; oncohaematology 48%, HIV 18%, renal transplants 9%, rheumatoid arthritis 7%, interstitial lung disease 4%, and glomerulonephritis 2%. All PJP+AAV+ cases had clinical presentation and radiologic evidence of PJP. Table 1 shows summary of characteristics of these patients. The median age at PJP diagnosis was 66.5 years. The majority of AAV patients had GPA (75%). Almost all (15 out of 16) PJP+AAV+ cases received oral CyC for induction therapy. Data on PJP prophylaxis were available in 14 cases, all of whom received Co-trimoxazole (CTX) prophylaxis during induction treatment. AZA was the predominant agent used during maintenance phase. The median time from AAV diagnosis to PJP was 8 months (25^{th} -75^{th} percentile 7-127.5months). The median Prednisolone dose at the time when PJP diagnosed was 10mg. Four patients were receiving more than 20 mg of Prednisolone at the time of PJP diagnosis. Increments in immunotherapy were observed in 6 cases within 3 months from diagnosis of PJP infection. Further details about disease relapses and augmentation on immunotherapy are available in Table 2.

Lymphopenia was present in all cases with a median lymphocyte count of 0.35 cells/L.CTX was used to treat PJP in 14 patients. Two patients were intolerant to CTX and were treated with combination of Clindamycin & Primaquine. Two patients required invasive ventilation and both did not survive accounting for 12.5% mortality.

Comparison between PJP+ and control (n=141) groups revealed no differences in age or sex at the time of vasculitis diagnosis. Comparison of maintenance treatment between both groups was not possible due to lack of good quality data for control group, however, available data for 129 control cases (out of 141 cases) revealed that Azathioprine was used as maintenance treatment in 88 cases (68%), MMF in 24 cases (19%), Methotrexate in 6 cases (4.7%) while Rituximab was used in 8 cases (6%).

Higher frequency of renal involvement, lymphopenia, and use of oral CyC were observed in the PJP+AAV+ compared to the PJP-AAV+ controls (Table 3). Univariate binary logistic regression of renal involvement at the time of diagnosis of AAV, lymphopenia at the end of induction and oral CyC use showed that absence of renal involvement was associated with less risk of PJP (OR 0.18, 95% CI 0.04-0.84, p=0.03). None of these covariates were predictors of PJP in multivariate analysis.

Discussion

In this study, the majority of PJP infections occurred beyond six months from AAV diagnosis, often preceded by augmenting immunotherapy. Lymphopenia was observed in all patients that developed PJP prior to the onset of infection. Although CTX prophylaxis was almost uniformly used during induction treatment, re-introducing CTX was not evident during escalation of immunosuppression. Traditional risk factors such as renal involvement at the time of AAV diagnosis, use of oral CyC, and lymphopenia at the end of induction therapy were not predictors of acquiring PJP in this study.

Previous studies on PJP in AAV were primarily retrospective observational studies focusing on GPA [10, 11, 13, 15, 16]. The onset of PJP in most of these studies was closely related to the induction phase of therapy. The European League Against Rheumatism (EULAR) guidelines reflected this observation and encouraged the use of CTX during treatment with CyC[17]. In our study the majority of cases happened beyond the period of induction therapy with median time between AAV diagnosis and PJP of 8 months. This observation suggests that extended prophylaxis might be of value, particularly in the presence of persistent lymphopenia. In our series, more than a third of PJP infections occurred in context of escalation of immunotherapy. Only one case had received CTX prophylaxis along with treatment escalation, this also can be interpreted, as CTX prophylaxis is effective.

The use of corticosteroids is suspected to be a risk factor for PJP [8]. The cut off value for corticosteroid dose is not entirely clear and can be between 15-40 mg Prednisone equivalent per day[18–20]. In our study, 75% of the cases were on less than 20 mg Prednisolone per day prior to occurrence of PJP. Lymphopenia has been consistently reported as a useful predictor of PJP. The cut off value for lymphopenia $<600/mm^3$ at 3 months of treatment[13] or a lymphocyte count off <500 cells/µl at two weeks after starting corticosteroid therapy (>30 mg/day) [19]. Other traditional PJP risk factors in AAV such as renal involvement and CyC usage were not predictors of PJP in our study. It is tempting to speculate that the apparent differences that we observed between PJP+AAV+ and controls were reflecting more aggressive initial AAV rather than representing risk factors for late onset PJP.

There are a number of limitations to our study. First, this is a single centre, retrospective study with inherent potential for selection & recall bias and it is relatively small sample size group. Second, BVAS scores are not available for the patients included in this study to directly compare the contribution of disease severity to PJP infections. Thirdly, we were unable to assess the impact of immunosuppression agents used as maintenance treatment in both groups and also to assess the impact of combination and consecutive use of different agents.

A limited number of risk factors were considered in this study and we cannot rule out the presence of other potential confounding factors e.g. ethnicity, serum albumin, or severe malnutrition.

In conclusion, PJP can occur beyond the early induction period in AAV, especially in context of escalating immunosuppression. Extended PJP prophylaxis might be of value when augmented immunosuppression is considered, especially in the presence of lymphopenia. Previously recognized risk factors that were proved to be useful predictors for PJP occurring early in the course of AAV, were not predictor of late onset PJP. Registry data or multi-centre observational studies are needed to stratify infection risks and prophylaxis strategies in AAV.

Compliance with ethical standards

This study was approved by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Disclosures

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The other authors have disclosed no conflicts of interest.

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Table 1: Characteristics of vasculitis patients who developed PJP infection

Age, median (IQR)	66.5 (52.5-73.7)
Sex (Female/Male)	8/8
Diagnosis GPA/MPA/EGPA	 12/2/2
Escalation of immunosuppression (see table 3 for more details)	6/16
Daily prednisolone dose (mg) at time of PJP diagnosis, median (IQR)	10 (8.1-18.7)
Disease duration at event, months, median (IQR)	8 (7-127.5)
Test to confirm diagnosis & Method used to obtain sputum sample DNA PCR Induced sputum BAL	 16 11 5
Lymphocytes count*x10^9 median (IQR)	0.35 (0.2-0.7)
Neutrophils count*x10^9 median (IQR)	6.8 (5.7-9)
Serum Creatinine µmol/L, median (IQR)	111 (84-180)
Hospital stay, days (IQR)	11 (6-19.2)

GPA=granulomatosis with polyangiitis, MPA=microscopic polyangiitis, EGPA=eosinophilic granulomatosis with polyangiitis, BAL: Bronchoalveolar Lavage.

*Lymphocytes or neutrophils count within 30 days from the onset of PJP. Categorical variables values are number (%) and continuous variables are median (IQR).

Table 2: Details of Cases with confirmed PJP

	Age at time of PJP infection (Years)	Sex	GPA/MPA/ EGPA	ANCA type	Organ involvement at presentation	Induction Immunosuppression	PJP prophylaxis at induction	Maintenance Immunosuppression Prior to PJP infection	Any relapses Within 3 months?	Type of Escalation or change of immunosuppression	Duration between: I) Vasculitis diagnosis & PJP infection II) Immunosuppression escalation & PJP infection
Case 1	74	F	GPA	Anti-PR3	Kidneys, Peripheral Nervous System	Oral CYC Prednisolone	Yes	AZA	Yes - Minor	AZA to MMF	I) 210 days II) 35 days
Case 2	54	M	EGPA	ANCA-Negative	Lungs	Oral CYC Prednisolone	Yes	AZA, MTX	No		I) 210 days
Case 3	64	F	GPA	Anti-PR3	Lungs	Oral CYC Prednisolone	Yes	AZA	No		I) 270 days
Case 4	52	M	GPA	Anti-PR3	ENT, Scleritis, Arthritis	Oral CYC Prednisolone	Unable to confirm	MTX, AZA MMF	No		I) 5640 days
Case 5	77	F	GPA	Anti-PR3	Kidneys	Oral CYC Prednisolone	Yes	AZA	No		I) 240 days
Case 6	47	F	GPA	Anti-PR3	Kidneys	IV CYC Prednisolone	Unable to confirm	AZA, MMF	No		I) 9120 days
Case 7	75	F	MPA	Anti-MPO	Kidneys	Oral CYC Prednisolone	Yes	AZA	No		I) 105 days
Case 8	73	M	GPA	Anti-PR3	Lungs, Kidneys	Oral CYC Prednisolone	Yes	AZA	No		I) 180 days
Case 9	49	M	GPA	Anti-PR3	Kidneys	Oral CYC Prednisolone	Yes	AZA,	Yes - Minor	AZA to MMF	I) 4080 days II) 85 days
Case 10	67	F	GPA	Anti-PR3	Lungs	Oral CYC Prednisolone	Yes	AZA, MMF	No		I) 3060 days
Case 11	66	M	GPA	Anti-PR3	Joints, Kidneys	Oral CYC Prednisolone	Yes	AZA, MMF	No		I) 330 days
Case 12	54	F	GPA	Anti-PR3	ENT, Kidneys	Oral CYC Prednisolone	Yes	AZA	Yes - Minor	AZA to MTX then adding Rituximab[]	I) 240 days II) 68 days
Case 13	71	M	GPA, Anti-GBM	Anti-PR3, Anti GBM	Lungs, Kidneys	Oral CYC Prednisolone	Yes	AZA	No		I) 135 days
Case 14	70	M	MPA	Anti-MPO	Kidneys	Oral CYC Prednisolone	Yes	AZA, MTX	Yes - Minor	MTX to MMF	I) 4650 days II) 32 days
Case 15	37	F	EGPA	ANCA-Negative	GI	Oral CYC Prednisolone	Yes	AZA	Yes - Minor	AZA to MMF	I) 210 days II) 36 days
Case 16	76	M	MPA	Anti-MPO	Kidneys	Oral CYC Prednisolone	Yes	AZA	Yes- Major	AZA to Rituximab	I) 210 days II) 34 days

GPA: Granulomatosis with Polyangiitis, EGPA: Eosinophilic Granulomatosis with Polyangiitis, MPA: Microscopic Polyangiitis, CYC: Cyclophosphamide, AZA: Azathioprine, MMF: Mycophenolate Mofetil, MTX: Methotrexate.

[]Use of Rituximab in this case for minor relapse though is inconsistent with our local protocol but given severity of disease at presentation and multiple symptoms reported the treating clinician opted to treat as major Relapse

Table 3: Participant characteristics-cases and controls

	PJP (n=16)	Control (n=141)	Significance
Age, median (IQR)	66.5 (52.5-73.7)	62.6 (51.7-71.1)	0.96
Sex (Female/Male)	8/8	57/84	0.46
Organ involvement at presentation & later during follow up period (%)			
Renal	14 (87)	79 (56)	0.01
Lung	10 (62)	63 (45)	0.18
ENT	8 (50)	51 (36)	0.29
PR3/MPO/negative	11/3/2	63/63/15	0.07
Creatinine at presentation µmol/L, median (IQR)	150 (79-533)	100 (74-225)	0.26
Lymphocytes $x10^9$, median (IQR)	0.4 (0.1-0.8)	0.6 (0.4-1.1)	0.04
Neutropenia during induction therapy (%)	4 (27)	19(14)	0.4
Induction therapy (%)			
Oral Cyclophosphamide	15(94)	93(66)	0.02
Pulse Cyclophosphamide	1 (6)	5 (3.5)	0.5
Rituximab	0	10 (7)	0.6
Mycophenolate mofetil	0	19 (13.5)	0.2
Methotrexate	0	6 (4.3)	1.0
Azathioprine	0	1 (0.7)	1.0
Steroid only	0	7 (5)	1.0

PJP prophylaxis at induction (%)	14 (87.5)	108 (76.5)	0.7
Relapse, number of patients with at least one relapse episode *	10 (62.5)	55 (39)	0.1
Major relapse	3 (30)	27 (49)	0.7**
Minor relapse	7 (70)	28 (51)	0.7
Time from diagnosis to relapse in months (IQR)	12 (6-50)	11 (5-18)	0.4
Relapse treatment			
Rituximab	4 (40)	23 (42)	0.8
Mycophenolate mofetil	4 (40)	9 (16.4)	0.2
Methotrexate	3 (30)	9 (16.4)	0.7
Azathioprine	1 (10)	3 (5.5)	1.0
Cyclophosphamide	0	4 (7)	1.0
Steroid increment only	1 (10)	6 (11)	1
Mortality	2 (13)	13 (14)	0.6

Categorical variables values are number (%) and continuous variables are median (IQR).

*Episodes of relapse are counted from diagnosis of Vasculitis to onset of PJP infection

**Chi square used to test differences in the frequencies of major and minor relapses between PJP and control groups.