Clinical and Experimental Rheumatology 2018; 36: 490-493.

Guidelines for prophylaxis of *Pneumocystis* pneumonia cannot rely solely on CD4-cell count in autoimmune and inflammatory diseases

G. Baulier[1], N. Issa[2], F. Gabriel[3], I. Accoceberry[3], F. Camou[4], P. Duffau[1]

[1]Department of Clinical Immunology, and [2]Department of Infectious Diseases, Saint-André Hospital, CHU de Bordeaux, Bordeaux, France; [3]Department of Mycology and Parasitology, Pellegrin Hospital, CHU de Bordeaux, France; [4]Intensive Care Unit, Saint-André Hospital, CHU de Bordeaux, France.

Gildas Baulier, MD
Nahema Issa, MD
Frederic Gabriel, MD
Isabelle Accoceberry, MD
Fabrice Camou, MD
Pierre Duffau, MD, PhD

Please address correspondence and reprint requests to: Dr Gildas Baulier, Médecine Interne et Immunologie Clinique, Hôpital Saint-André, 1 rue Jean Burguet, 33000 Bordeaux, France.
E-mail: gildas.baulier@chu-bordeaux.fr

Received on July 29, 2017; accepted in revised form on December 4, 2017.

Key words: Pneumocystis pneumonia, prophylaxis, CD4-count, T-lymphocytes, autoimmune and inflammatory diseases

Competing interests: none declared.

ABSTRACT

Objective. *Guidelines for preventing Pneumocystis pneumonia (PCP) in HIV patients are based on CD4 below 200/mm³. Such cut-off value is suggested to guide prophylaxis in non-HIV conditions (NHIV) especially in autoimmune and inflammatory diseases (AD). We aimed to determine if CD4 could be used to guide PCP prophylaxis in AD.*

Methods. *CD4 and lymphocyte-count were retrospectively studied in patients diagnosed with PCP between January 2013 and February 2016.*

Results. *129 patients were included. The median CD4-count was 302/mm³ in AD, which was significantly higher than in HIV patients (19/mm³; p<0.0001). Fifty percent (n=10) of AD patients had CD4 counts greater than 300/mm³.*

Conclusion. *Prophylaxis for PCP cannot rely solely on CD4-count in NHIV patients especially in AD.*

Introduction

Pneumocystis jirovecii pneumonia (PCP) has historically been associated with human immunodeficiency virus (HIV). Nowadays, the majority of PCP infections occurs in non-HIV (NHIV) immunosuppressed patients suffering from various conditions such as haematologic and solid malignancies, solid organ transplants and autoimmune or inflammatory diseases (AD) (1). The ways in which PCP in NHIV differs from HIV include: misleading clinical presentations, delayed or difficult diagnosis due to negative microscopic examinations (ME) and worse prognosis (mortality: 30–50%) (2). Prophylaxis has proven efficient and safe for PCP in HIV while no clear recommendations have emerged regarding the target population for prophylaxis in AD (3). Existing recommendations in renal or bone marrow transplantations are not directly transposable in AD, though immunosuppressive drugs used are similar, because prophylaxis is usually recommended during a 6-month period after engraftment (4, 5). Recommendations based on duration and dosage of steroids have been proposed but the scope of patients eligible for prophylaxis would probably be to wide (1). Moreover, AD represent a heterogeneous population as underlying pathologies, immunosuppressive therapeutics and exposure to steroids differ and lead to different intrinsic risks for PCP (1). Indeed, large variations in prophylactic prescriptions for patients with AD reveal a large gap in knowledge regarding guidelines for PCP in this population (6). In HIV, it has been established that the level of immunosuppression follows a CD4 gradient: PCP prophylaxis is recommended for patients with CD4 below 200/mm³ (7). As lymphopenia is quite common in AD (8), it is tempting to use these recommendations by evaluating the CD4 count (9). However, a defined gradient of immunosuppression using the CD4 count has not been established in AD. Therefore, the aims of our study were to detail specific characteristics of AD patients developing PCP and determine if CD4 could be used to guide PCP prophylaxis in AD.

Methods

Data collection

This single centre French study was conducted at the Bordeaux University Hospital. As a retrospective survey, no ethics board approval was needed, in accordance of the policy of our institution. Data collection was performed with the permission of the National Commission of Information Technology and Freedom (CNIL, Commission Nationale de l'Informatique et des Libertés) (declaration number: 2032553v0). Retrospective data collection covered the period from January 2013 to February 2016. All qPCR *Pneumocystis jirovecii* analyses performed during this period were included. All cases with at least a single significant positive qPCR sample (>1000 cp/mL) were reviewed (sputum, bronchial aspiration fluid (BA), bronchoalveolar lavage fluid (BAL)). All cases were analysed by three independent specialists from the departments of infectious diseases, intensive care, and clinical immunology.

Diagnosis of PCP infection was confirmed using the following criteria: clinical signs of pneumonia (cough, fever, dyspnea), radiological findings compatible with PCP (ground glass opacities and/or acute interstitial lung disease), and introduction of specific

Table I. PCP patient characteristics.

	HIV n=37	AD n=27	NHIV n=65	AD *vs*. HIV *p*-value	AD *vs*. NHIV *p*-value
Median age (years)	45 (8.16)	71 (12.65)	62 (13.06)	**<0.0001**	**0.018**
Sex ratio (M/W)	4.3	1.1	2.1	**0.0127**	0.16
Biological parameters (mean value, SD):					
Haemoglobin (g/dL)	11.29 (1.88)	11.62 (1.73)	10.36 (1.88)	0.4754	**0.0036**
Polynuclear cells (/mm^3)	6441 (5883)	7660 (3340)	5601 (4082)	0.3381	**0.0231**
Creatinine (µmol/L)	61.4 (17.26)	119.4 (108.4)	147 (153.9)	**0.0021**	0.3992
Gamma globulin (g/L)	18.4 (6.98)	7.41 (4.27)	7.18 (4.50)	**<0.0001**	0.8392
Albumin (g/L)	28.6 (6.09)	30.8 (5.70)	31.9 (6.66)	0.1839	0.4997
CRP (mg/L)	77 (92.51)	102.3 (67.57)	118.3 (88.93)	0.2450	0.4140
Underlying lung disease, n (%)	11 (2.7%)	12 (44%)	13 (20%)	**<0.0001**	**0.0164**
Corticosteroid exposure, n (%)	-	26 (97%)	37 (57%)	-	**<0.0001**
Median CS dose (mg)	-	22.5 (23.16)	7.5 (32.74)	-	0.8700
CS ≥ 20mg, n (%)	-	17 (62.9%)	13 (35.1%)	-	**0.0420**
CS ≥ 40mg, n (%)	-	10 (37%)	1 (31.4%)	-	0.6000
CS alone, n (%)	-	6 (22.2%)	4 (6.2%)	-	0.0691
Median exposure to CS (months)	-	6 (206)	17 (217)	-	0.6900
Immunosuppressive drug exposure, n (%)					
Chemotherapy	-	-	20 (30.7%)	-	**0.0005**
Rituximab	-	7 (25.9%)	10 (15.4%)	-	0.2355
Cyclophosphamide	-	3 (11.1%)	3 (4.6%)	-	0.2505
Anti-metabolite (AZA, MTX)	-	13 (40.7%)	3 (4.6%)	-	**<0.0001**
Anti-Calcineurin	-	1 (3.7%)	25 (38.5%)	-	**0.0007**
Other biologics	-	1 (3.7%)	-	-	0.1187
Lymphocyte count (/mm^3)					
Median (SD)	595 (582)	680 (856)	480 (784)	0.3601	0.1630
Range	100-2640	120-4300	50-4120	-	-
Ly > 600/mm^3, n (%)	18 (48.6%)	17 (63%)	25 (41%)	0.2559	0.0569
Ly > 1200/mm^3, n (%)	7 (18.9%)	5 (18.5%)	5 (8.2%)	0.9677	0.1594
CD4 count (/mm^3)					
Number of CD4-count available	n=36	n=20	n=39		
Median (SD)	19 (63)	302 (280)	236 (235)	**<0.0001**	0.2200
Range	0-268	12-1202	7-1138	-	-
CD4 > 200/mm^3, n (%)	2 (5.5%)	14 (70%)	21 (54%)	**<0.0001**	0.2318
CD4 > 300/mm^3, n (%)	-	10 (50%)	14 (36%)	-	0.2966
CD4 > 450/mm^3, n (%)	-	5 (25%)	8 (20.5%)	-	0.6938
CD4/CD8, mean (SD)	0.15 (0.28)	3.6 (7.78)	1.7 (1.62)	**0.0087**	0.1700
PCP prophylaxis, n (%)	-	1 (3.7%)	-	0.2380	0.2900
PCR value (copies/mL)					
Median	513000	44050	20725	**0.0432**	0.7857
SD	2.7x10^7	2.5x10^6	2.8x10^6		
Range	1510-1x10^8	1080-1x10^7	1120-2x10^7		
Positive microscopic examination, n (%) (pneumocystosis-specific)	28 (75.7%)	8 (29.6%)	18 (27.1%)	**0.0002**	0.8509
Clinical progression, n (%)					
Intensive care unit	13 (35.1%)	15 (55.5%)	26 (40%)	0.1039	0.1717
Orotracheal intubation	8 (21.6%)	9 (33.3%)	12 (18.5%)	0.2948	0.1217
3-months mortality after PCP	7 (18.9%)	12 (44.4%)	17 (26.1%)	**0.0273**	0.0855

PCP: *Pneumocystis* pneumonia; ME: microscopic examination; CS: corticosteroid; AZA: azathioprine; MTX: methotrexate; AD: auto-immune and inflammatory diseases; NHIV: non-HIV; SD: standard deviation.

treatment against *P. jirovecii*. In the absence of these criteria, cases were considered as colonisation and excluded.

Sample treatment

The specimen treatment protocol is described in the supplementary method

Statistical analysis

Independent *t*-tests and Chi-squared-tests were used to compare the characteristics between groups. A *p*-value less than 0.05 (two-tailed) was considered statistically significant. Tests were performed using GraphPad Prism software (GraphPad, San Diego, CA, USA).

Results

Population characteristics

3,554 qPCR were performed during the study period (Suppl. fig. 1). We identified a total of 129 patients who presented with PCP (54 certain PCP with positive ME, and 75 possible PCP with negative ME). Among them, 37 (28.7%) patients were HIV, 27 (20.9%) had AD, 65 (50.4%) had other NHIV conditions (haematologic malignancies (n=31), solid organ transplants (n=24), solid malignancies (n=7), and pulmonary fibrosis (n=3)). Detailed patient characteristics are shown in Table I.

AD patients were older than HIV or

Table II. Characteristics of autoimmune and inflammatory disease patients (AD).

Patient	Sex	Age (years)	AD	Duration (months)	Lung disease	Active AD	CS dosage (mg/day)	CS duration (months)	IS exposure	Ly count/ mm^3	CD4 count/ mm^3	Prophylaxis	BAL	PCP	ICU	OTI	3-month mortality
1	W	76	RA	69	-	Yes	NA	NA	MTX	400	359	-	-	possible	-	-	-
2	M	56	RA	342	-	-	9	288	MTX	630	12	-	-	possible	Yes	-	-
3	M	64	RA	332	-	-	40	2	CYC/RTX	160	62	-	-	possible	Yes	Yes	Yes
4	W	71	RA	274	-	-	7	198	MTX/RTX	910	208	-	Yes	possible	-	-	-
5	M	73	RA	-	Yes	Yes	5	-	MTX	1360	282	-	-	possible	-	-	-
6	W	47	RA	111	-	Yes	40	2	-	4300	95	-	-	possible	-	-	Yes
7	M	57	ANCA vasculitis	7	Yes	-	80	6	CYC	1170	186	-	Yes	certain	-	-	Yes
8	W	53	ANCA vasculitis	18	Yes	-	45	18	MTX	670	390	-	Yes	possible	-	Yes	-
9	M	73	ANCA vasculitis	191	Yes	-	10	191	AZA	1110	582	-	Yes	possible	-	-	-
10	W	78	Giant cell arteritis	28	-	-	20	4	MTX	560	ND	-	-	possible	Yes	-	-
11	W	86	Giant cell arteritis	3	-	Yes	40	3	-	640	240	-	-	possible	-	-	Yes
12	M	89	Giant cell arteritis	75	Yes	-	2	75	MTX/TOCI	1020	655	-	-	possible	-	-	-
13	M	71	Type II cryoglobulinaemia	5	Yes	-	60	3	-	118	26	Pentamidin	Yes	certain	Yes	Yes	Yes
14	M	51	Type II cryoglobulinaemia	6	-	-	30	6	RTX	430	ND	-	-	possible	-	-	-
15	W	65	Type II cryoglobulinaemia	3	-	-	30	3	RTX	310	322	-	-	possible	-	-	-
16	W	57	Dermatomyositis	1	Yes	Yes	80	1	AZA	856	532	-	Yes	certain	Yes	Yes	Yes
17	W	72	Dermatomyositis	58	Yes	Yes	5	20	-	1430	599	-	Yes	possible	Yes	-	-
18	M	64	Autoimmune hepatitis	3	-	-	40	3	AZA	550	ND	-	Yes	certain	Yes	Yes	Yes
19	M	66	Autoimmune hepatitis	8	-	Yes	20	8	AZA	130	ND	-	Yes	certain	Yes	Yes	Yes
20	M	69	Glomerular nephropathy	3	-	Yes	20	3	ACN	500	ND	-	-	certain	Yes	Yes	-
21	M	32	Neurosarcoidosis	4	Yes	-	60	3	MTX	680	62	-	Yes	certain	Yes	-	Yes
22	W	72	Spondyloarthritis	225	Yes	-	5	221	MTX	840	257	-	Yes	certain	Yes	Yes	Yes
23	W	71	Autoimmune haemolytic anaemia	15	-	-	60	2	RTX	750	369	-	-	possible	Yes	Yes	Yes
24	M	85	Acquired haemophilia	3	-	-	25	3	RTX	710	342	-	-	possible	-	-	-
25	W	71	Myasthenia gravis	50	-	-	20	47	MMF	1510	ND	-	-	possible	Yes	-	-
26	W	79	Interstitial pneumonia	55	Yes	-	15	6	-	2620	ND	-	Yes	possible	Yes	-	-
27	M	61	Systemic sclerosis	64	Yes	Yes	10	60	CYC/RTX	489	392	-	Yes	possible	Yes	-	Yes

M: man; W: woman; RA: rheumatoid arthritis; IS: immunosuppressive treatment; Ly: lymphocyte; CS: corticosteroid;MTX: methotrexate; RTX: rituximab; CYC: cyclophosphamide; AZA: azathioprine; TOCI: tocilizumab; ACN: anticalcineurin; MMF: mycophenolate mofetil; ICU: intensive care unit; OTI: orotracheal intubation; BAL: bronchoalveolar lavage.

NHIV patients (median ages: 71, 45 and 62 years respectively; AD *vs*. HIV $p<0.0001$; AD *vs*. NHIV $p=0.0127$). Underlying lung diseases were more frequent in AD than HIV or NHIV patients (44%; 2.7% and 20%; AD *vs*. HIV $p<0.0001$; AD *vs*. NHIV $p=0.0164$). AD patients had heavier exposure to corticosteroids than NHIV patients (97% *vs*. 57%; $p<0.0001$). Detailed characteristics of each AD patient are described in Table II.

The most common underlying diseases were rheumatoid arthritis (n=6; 22%), giant cell arteritis (n=3; 11%), anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (n=3; 11%), and cryoglobulinaemia vasculitis (n=3; 11%). Diagnosis of the underlying disease was made an average of 6 years before PCP infection (range: 19 days–28 years). Activity of AD was not controlled at the time of PCP in 33% of the cases (n=9). 97% of the patients (n=26) were taking corticosteroids at the time of PCP with a median dosage of 22.5 mg/day (as prednisone equivalent) and for a median duration of 6 months. Corticosteroids were prescribed for more than one month in 96% of the cases (n=25), and 62.9% (n=17) had CS dosage superior to 20mg/day. Corticosteroids were the only immunosuppressant treatment in 5 cases (18.5%), and were often associated with other immunosuppressant drugs including methotrexate in 33.3% (n=9), rituximab in 26% (n=7), azathioprine in 14.8% (n=4), cyclophosphamide in 11% (n=3), mycophenolate in 3.7% (n=1), cyclosporine 3.7% (n=1) and tocilizumab in 3.7% (n=1). None of the AD patients were exposed to anti-TNF treatment. From the 129 cases studied here, only one patient (n=1; 0.77%) had prophylaxis before PCP.

Certain PCP (positive ME) were less frequent in AD (n=8; 29.6%) than in HIV (n=28; 75.7%) ($p=0.0002$), without any significant difference between AD and NHIV (n=18; 27.1%) ($p=0.8509$).

Lymphocyte and CD4 cell counts

Total lymphocyte counts were available for each of the 129 patients (Table I). The median value was 680/mm^3 (SD: 856/mm^3; range: 120–4300/mm^3) in AD. The total lymphocyte counts were not significantly different when compared with HIV (median: 595/mm^3; SD: 582/mm^3; range: 100–2640/mm^3; $p=0.3601$) or NHIV patients (median: 480/mm^3; SD: 784/mm^3; range: 50–4120/mm^3; $p=0.1630$).

The CD4 lymphocyte counts were available for 20 AD patients (74%) at the time of PCP infection. The median count in AD was 302/mm^3 (SD: 280/mm^3; range: 12–1202/mm^3) and was

significantly higher when compared to HIV patients (median: 19/mm^3; SD: 63/mm^3; range: 0–268/mm^3; p<0.0001). While 50% (n=10) of AD patients had >300 CD4/mm^3, and 25% had normal CD4 counts (>450/mm^3), no difference was observed in CD4 counts between AD and NHIV patients (median: 236/mm^3; SD: 235/mm^3; p=0.22).

Discussion

In NHIV, some PCP prophylaxis strategies have been inspired by HIV and based on CD4 or total lymphocytes counts. Value limits such as 200 CD4/mm^3 and total lymphocyte count less than 600/mm^3 have been suggested (10). Our study demonstrates that in AD and other NHIV, total lymphocyte and CD4 counts are not reliable tools to guide prophylaxis. A majority (60%) of AD with PCP had more than 600 lymphocytes/mm^3 and 300 CD4/mm^3. CD4 counts were normal (>450/mm^3) in 25% of the cases in AD. A systematic review looking at CD4 counts and PCP in HIV negative patients was recently published (11). The authors found that almost 75% of PCP patients had CD4<200/mm^3. Of note, very few CD4 counts were available and diagnostic criteria for PCP differ in multiple ways as qPCR was not used in the majority of the studies analysed (11). As most of the PCP in AD showed negative ME, PCR is a crucial tool in this population. Lack of specificity due to detection of colonised patients with PCR, can be overcome by combined diagnosis strategies using clinical and radiological criteria as done in our study (3).

The difficulties in delineating the target population for prophylaxis are emphasised by the extreme heterogeneity among NHIV. In most of the studies, AD are pooled with other NHIV such as renal transplants or leukaemia (3). To our knowledge, only one study has compared AD patients to other NHIV patients but CD4 counts were not assessed in this study and sample size was small (n=11) (12). Moreover underlying conditions in AD demonstrated extreme diversity in our study. Of 27 AD cases, we identified no less than 14 different AD conditions, adding complexity to the analysis. We found that 62.9% of our AD patients had more than 20 mg/day of steroids which is less than previously described by Mecoli *et al*. (86%) (13). This is certainly due to immunosuppressive drugs used as steroid sparing-agent widely prescribed in our cohort. Rituximab and methotrexate were the most frequent immunosuppressive treatments prescribed and they both target humoral and cellular immunity. Giant cell arteritis (GCA) is considered as a low risk pathology for PCP (1) though steroid are often prescribed at dosage >20 mg/day. We found three patients developing PCP during GCA treatment. All of them had more than 560 total lymphocytes/mm^3 contrary to patients reported in the study of Berger *et al*. where patients had always total lymphocytes count less than 400/mm^3 (14). This could be due to cumulative immunosuppression as 2 out of 3 patients of our cohort had steroids associated with other immunosuppressive drugs.

Humoral and cellular immunity against *Pneumocystis jirovecii* are often impaired in AD by the underlying disease or iatrogenic immunosuppression, but first and foremost by corticosteroids (15). Since AD patients have multiple alterations of their immune system, it cannot be summarised with a single CD4-count. Taken together, the CD4 count should not be the only guiding factor for prophylaxis in AD patients and other well known risk factors for PCP, including type of immunosuppressive drugs, CS dosage, age, underlying lung disease, type of AD should be assessed to evaluate individual risk for PCP. More studies specifically targeting AD patients are needed.

Acknowledgements

We thank JetPub Scientific Communications for English language editing and helpful comments.

References

1. FILLATRE P, DECAUX O, JOUNEAU S *et al*.: Incidence of Pneumocystis jiroveci pneumonia among groups at risk in HIV-negative patients. *Am J Med* 2014;127: 1242. e11-17.
2. ROUX A, CANET E, VALADE S *et al*.: Pneumocystis jirovecii pneumonia in patients with or without AIDS, France. *Emerg Infect Dis* 2014; 20: 1490-7.
3. STERN A, GREEN H, PAUL M, VIDAL L, LEIBOVICI L: Prophylaxis for Pneumocystis pneumonia (PCP) in non-HIV immunocompromised patients. *Cochrane Database Syst Rev* 2014 Oct 1; (10): CD005590.
4. CHAPMAN JR: The KDIGO clinical practice guidelines for the care of kidney transplant recipients. *Transplantation* 2010; 89: 644-5.
5. MAERTENS J, CESARO S, MASCHMEYER G *et al*.: ECIL guidelines for preventing Pneumocystis jirovecii pneumonia in patients with haematological malignancies and stem cell transplant recipients. *J Antimicrob Chemother* 2016; 71: 2397-404.
6. CETTOMAI D, GELBER AC, CHRISTOPHERSTINE L: A survey of rheumatologists' practice for prescribing pneumocystis prophylaxis. *J Rheumatol* 2010; 37: 792-9.
7. NELSON M, DOCKRELL D, EDWARDS S *et al*.: British HIV Association and British Infection Association guidelines for the treatment of opportunistic infection in HIV-seropositive individuals 2011. *HIV Med* 2011; 12 (Suppl. 2): 1-140.
8. SOWDEN E, CARMICHAEL AJ: Autoimmune inflammatory disorders, systemic corticosteroids and pneumocystis pneumonia: a strategy for prevention. *BMC Infect Dis* 2004; 4: 42.
9. LIMPER AH, KNOX KS, SAROSI GA *et al*.: An official American Thoracic Society statement: Treatment of fungal infections in adult pulmonary and critical care patients. *Am J Respir Crit Care Med* 2011; 183: 96-128.
10. TADROS S, TEICHTAHL AJ, CICIRIELLO S, WICKS IP: Pneumocystis jirovecii pneumonia in systemic autoimmune rheumatic disease: A case-control study. *Semin Arthritis Rheum* 20167; 46: 804-9.
11. MESSIAEN PE, CUYX S, DEJAGERE T, VAN DER HILST JC: The role of CD4 cell count as discriminatory measure to guide chemoprophylaxis against Pneumocystis jirovecii pneumonia in human immunodeficiency virus-negative immunocompromised patients: A systematic review. *Transpl Infect Dis Off J Transplant Soc* 2017 [Epub Feb 15].
12. TEICHTAHL AJ, MORRISROE K, CICIRIELLO S, JENNENS I, TADROS S, WICKS I: Pneumocystis jirovecci pneumonia in connective tissue diseases: Comparison with other immunocompromised patients. *Semin Arthritis Rheum* 2015; 45: 86-90.
13. MECOLI CA, SAYLOR D, GELBER AC, CHRISTOPHER-STINE L: Pneumocystis jiroveci pneumonia in rheumatic disease: a 20-year single-centre experience. *Clin Exp Rheumatol* 2017; 35: 671-3.
14. BERGER CT, GREIFF V, JOHN S *et al*.: Risk factors for pneumocystis pneumonia in giant cell arteritis: a single-centre cohort study. *Clin Exp Rheumatol* 2015; 33 (Suppl. 89): S122-25.
15. BASCHANT U, LANE NE, TUCKERMANN J: The multiple facets of glucocorticoid action in rheumatoid arthritis. *Nat Rev Rheumatol* 2012; 8: 645-55.