International Journal of Infectious Diseases 46 (2016) 11–17

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Pneumocystis pneumonia suspected cases in 604 non-HIV and HIV patients

Anne-Lise Bienvenu [a,b,1], Karim Traore [b,1], Irina Plekhanova [b], Mourad Bouchrik [b], Cécile Bossard [a], Stéphane Picot [a,b,*]

[a] Institut de Parasitologie et Mycologie Médicale, Hospices Civils de Lyon, Lyon, France
[b] Univ Lyon, Université Claude Bernard Lyon 1, Institut de Chimie et Biochimie Moléculaire et Supramoléculaire, UMR-5246 CNRS-INSA-CPE, Malaria Research Unit, Lyon, France

ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form 16 March 2016
Accepted 18 March 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Pneumocystosis
Pneumocystis jiroveci
HIV
non-HIV
Diagnosis
Prophylaxis
Outcome
Consensus

ABSTRACT

Background: *Pneumocystis* pneumonia (PCP) is one of the most devastating fungal diseases in patients with impaired immunity. Effective antiviral therapies have reduced the burden of PCP among AIDS patients, but an increase in the prevalence of this disease among persons receiving immunosuppressive therapies has been reported.
Methods: We retrospectively reviewed HIV and non-HIV PCP patients diagnosed in our department during a nine year period. Data were collected from the local database completed during the diagnosis procedure. For each patient, demographic, clinical, radiological, biological and therapeutic data were analyzed.
Results: A total of 21,274 bronchoalveolar samples were received from patients suspected of pneumocystosis during the study period, leading to a discharge diagnosis of PCP for 604 patients (143 HIV-positive and 461 HIV-negative). The ratio of non-HIV versus HIV patients presenting PCP increased from 1.7 to 5.6 during the study period. The mortality rate at day 14 was 16%, occurring mostly in non-HIV patients (20.6% compared to 1.4%, $P < 0.0001$), while non-HIV patients were less symptomatic at diagnosis than AIDS patients.
Conclusions: This study presents one of the higher number of HIV and non-HIV patients presenting with PCP in a single center. Pneumocystosis is now a crucial health challenge for patients receiving immunosuppressive therapy, with a high mortality rate. This study highlights the need for international guidelines for prophylaxis of PCP in non-HIV patients.
© 2016 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Pneumocystis pneumocystosis (PCP) is a continuously evolving disease challenging clinicians involved in the cascade of care of patients as different as people with impaired immunity, recipients of solid organs transplants or people with hematological malignancies. The microbe responsible for that disease, *Pneumocystis jirovecii* (*P. jiroveci*), is still poorly known, while scientists decided that it belongs definitively to the kingdom of fungi based on DNA sequence homology. It is not definitively known if the disease is acquired from the environment or from colonized/infected patients when risk factors are arising, or if the disease in adults is a reactivation of dormant forms contracted during infancy, or if reactivation or de novo infection may both occur in different patients.[1] The exact role of inter-human transmission is still a matter of debate.[2,3] *P. jiroveci* is still not adapted to *in vitro* culture despite years of collaborative work to achieve this major goal for a better understanding of its biology and for improvement of its diagnosis.[4–7] The utmost importance of pneumocystosis was perceived at the time of HIV epidemic in humans, and a decade ago it was expected that HIV control will lead to pneumocystosis

Abbreviations: PCP, *Pneumocystis* pneumonia; P. jirovecii, Pneumocystis jiroveci; HIV, Human immunodeficiency Virus; Rt-PCR, Real-Time Polymerase Chain Reaction.

* Corresponding author. Pr Stéphane Picot, Institut de Parasitologie et Mycologie Médicale, Hôpital de la Croix-Rousse, 103 Grande Rue de la Croix-Rousse, 69004 Lyon, France.
E-mail address: stephane.picot@chu-lyon.fr (S. Picot).
[1] these two authors contributed equally to the study.

http://dx.doi.org/10.1016/j.ijid.2016.03.018
1201-9712/© 2016 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

control at the population level.[8] Pneumocystosis has been extensively studied in HIV positive patients and the global trend in most countries where statistics are available is a decrease of these cases for patients who have access to combination antiretroviral therapy.[9] There is clear evidence from the early 2000's that pneumocystosis is no longer restricted to HIV patients, but is mostly diagnosed in non-HIV patients, leading to new challenges for prophylaxis, diagnosis and treatment in a larger susceptible population. The number of studies with non-HIV patients is rapidly increasing.[10–13] Systematic analyses of published literature or studies conducted at country level using national health systems have confirmed this general trend.[14] Epidemiological, clinical and therapeutic backgrounds of pneumocystosis in non-HIV patients have changed and seem to be more diverse. General outcome should be considered with caution since the causes of death of these patients is often multifactorial. Higher mortality rates are reported by authors in HIV negative patient compared to HIV positive patients.[15,16]

One of the major issues for clinicians is to identify patients susceptible to P. jiroveci infection at the earlier stage of the risk period in order to promote the use of systematic prophylaxis, which has proven to be highly effective for HIV patients. A recent Cochrane study has shown that prophylaxis with trimethoprime-sulfamethoxazole should be considered for at risk non-HIV patients, with a number needed to treat to prevent PCP of 19 patients, given an event rate of 6%.[15]

In this context, it was of interest to analyze cases of non-HIV pneumocystosis during a nine year period in a single center with standardized methods of diagnosis. Thus, we conducted a retrospective study in our institution from January 2005 to December 2013 in order to describe clinical, diagnostic and treatment characteristics of *Pneumocystis* pneumonia in HIV negative and positive patients.

2. Methods

2.1. Study population and sampling

This study was conducted at the Lyon teaching hospital (approximately 5000 bed-facility) where bone marrow and solid organ transplantation activities are common, as well as surgery, intensive care and infectious diseases including HIV patients. During the period from January 1st, 2005 to December 31st, 2013, 21274 samples (swab, broncho-aspiration, broncho-alveolar lavage, lung biopsy) from patients suspected of pneumocystosis were collected. After confirmation of the biological diagnosis, all information from patients including age, sex, risk factors, symptoms, chest computed tomography scan, biological diagnosis (microscopic examination and real-time PCR), treatment and outcome at day 14 post-diagnosis, were prospectively collected from patient medical records and laboratory database.

2.2. Diagnosis procedure

The same diagnosis procedure has been followed during the study period. Suspicion of pneumocystosis was based on epidemiological, clinical and radiological findings. Symptoms considered to be common during PCP were: progressive dyspnea, nonproductive cough, and low-grade fever. Radiological signs considered to be associated with PCP were bilateral perihilar interstitial infiltrates. Samples collected from these patients were systematically submitted to microscopic examination (ME) after conventional staining methods (Diff-Quick and Grocott-methamine silver stain) by two experienced microscopists. Discrepancies were resolved by consensus.

Samples showing no trophic forms or cysts by microscopic examination were tested using real-time PCR (RT-PCR) according to the method developed locally.[18,19] Briefly, DNA was extracted using a QIAamp DNA Mini Kit according to the manufacturer's recommendations. Positive and negative controls were tested simultaneously, and two different external quality controls were performed twice a year. Positive threshold of the method was 35 cycles.

The diagnosis of pneumocystosis was considered as confirmed if ME and/or real-time PCR were positive, or if anti-pneumocystis treatment has been prescribed to patients based on clinical conviction. Patients considered as being affected by pneumocystosis were specifically treated according to international guidelines.[8,20]

Systematic PCR confirmation of the positive microscopic diagnosis was not required since quality controls performed regularly showed no false-positive results from microscopy.

2.3. Statistical analysis

Categorical variables were analyzed with Chi-square test or with Fisher's exact test, as appropriate. Continuous variables were analysed by Student's t test. Data were analyzed by SPSS for Windows version 11 (Chicago, USA). Difference was considered significant if p-value was below 0.05 (risk at 5% and confidence interval at 95%).

3. Ethical considerations

This research involved anonymized records and datasets where it is not possible to identify an individual from the information provided. De-identification and removing of protected health information from clinical narratives were performed according to the European Textbook on Ethics in research (http://ec.europa.eu/research/swafs/pdf/pub_archive/textbook-on-ethics-report_en.pdf). Data used in this study were collected for the routine diagnosis and clinical management of patients in the Lyon teaching hospital, and no additional intervention was made on patients for research purposes. Therefore, ethical clearance was not needed for that study.

4. Results

4.1. Patient characteristics

From January 2005 to December 2013, 21274 samples were received from patients suspected of pneumocystosis. A total of 604 patients with a diagnosis of pneumocystosis recorded on clinical narratives at discharge [143 HIV-positive (23.7%) and 461 HIV-negative (76.3%)] were included in this study (Figure 1). The overall mortality rate was 16% (97/604), mostly in non-HIV patients (95/97).

Fifty-four patients (8.9%) did not present symptoms usually associated with pneumocystosis (fever, cough or dyspnea), while biological tests were positives for *Pneumocystis jroveci* (2 by microsopic examination, and 52 by RT-PCR). Most of them were HIV negative, 76% received anti-pneumocystis treatment, and 22.2% died. These patients should have been classified as colonized by *Pneumocystis*.

Among the 550 patients with symptoms commonly associated with pneumocystosis, 62 (11.3%) showed no Xray/TDM signs suggestive of pneumocystosis. Finally, 488 patients (24.8% HIV-positive and 75.2% HIV-negative) presented conventional clinical symptoms and radiological signs compatible with pneumocystosis (Figure 2).

The median age of the patients was 59 years (ranging from 3 months to 88 years of old) and the sex ratio male/female was 2.02 (404/200) (Table 1). Only 6.6% (40/604, including 13 HIV positive

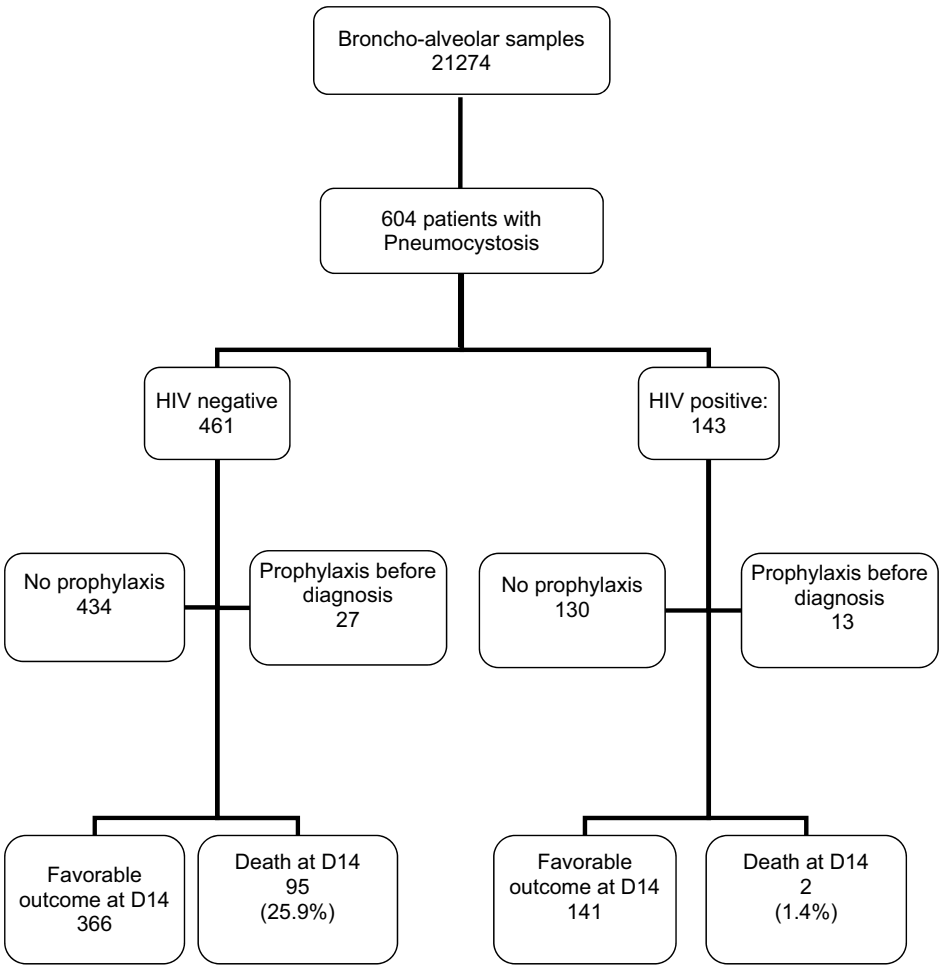

Figure 1. Enrollment, classification and outcome of patients suspected of Pneumocystosis.

and 27 HIV negative) of the patients received a prophylaxis prior to the diagnosis of pneumocystosis.

In HIV negative patients, hematological malignancies constituted the predominant risk factor for pneumocystosis (39%), followed by cancer of different organs (26.9%). More than eighty two per cent (82.4%) of patients were using immunosuppressive therapy. Risk factors for pneumocystosis of HIV negative patients are shown in Table 2.

From 2005 to 2013, the total annual number of pneumocystosis cases has increased by 39.5%. During the same period, the rate of pneumocystosis has increased significantly from 63% to 85% in non-HIV patients ($p = 0.004$) and decreased from 37% to 15% in HIV-positive patients (Figure 3).

4.2. Radiological findings

Radiological findings were suggestive of pneumocystosis for 86.4% (522/604) of patients. There was no difference between HIV-positive (124/143) and HIV-negative (398/461) patients ($p > 0.1$). In the group of patients under prophylaxis, 77.5% (31/40) had a suggestive radiology compared to 87% (491/564) of patients without prophylaxis ($p = 0.08$).

4.3. Clinical manifestations

The most frequent symptom was dyspnea 398/604 (65.9%), followed by fever 395/604 (65.4%) and cough 337/604 (55.4%). Thirty two percent of the patients (n = 195) presented with these 3 symptoms, 31% (n = 188) with 2 symptoms (70 with fever and dyspnea, 64 with dyspnea and cough, 54 with fever and cough) and 27.6% (n = 167) with 1 symptom (76 with fever, 68 with dyspnea and 23 with cough).

Non-HIV patients presented less frequently with cough ($p = 0.0003$) and dyspnea ($p < 0.00001$) compared to HIV patients. Fever was not a discriminant symptom between the two groups of patient ($p = 0.08$).

Only 54 patients (8.9%) were asymptomatic and should have been considered as colonization, but among them 61% (33/54) showed radiological abnormalities and 75.9% (41/54) received anti-pneumocystis treatment. Asymptomatic presentation of pneumocystosis was significantly ($p = 0.009$) more frequent in non-HIV patients (10.6%) compared to HIV-positive patients (3.4%),

4.4. Biological diagnosis

Biological diagnosis of pneumocystosis was performed on samples from 604 patients, including 521 broncho-alveolar lavages, 56 swabs, 26 broncho-aspirations and 1 lung biopsy. ME was positive for 181/604 patients (29.9%) and the 423 samples from patients with a negative ME were all PCR positive. Since both methods were used for 80 patients, Real-time PCR tests were positive for a total of 503 patients. The positivity rates of ME were 32.6% for broncho-alveolar lavages (170/521); 10.7% for swab (6/56) and 19.2% for broncho-aspiration (5/26). It was significantly more frequent to observe a positive ME from HIV-positive patients

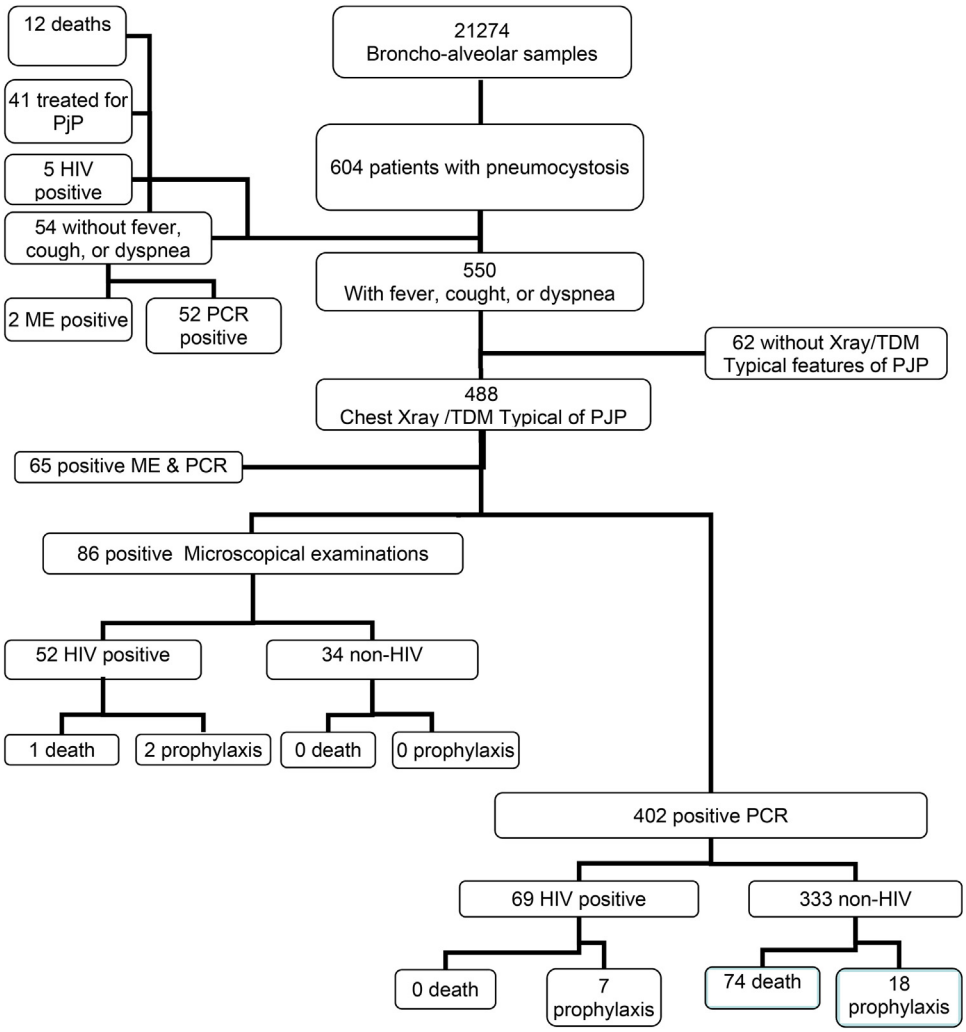

Figure 2. Detailed description of patient's characteristics, including clinical, radiological and biological data. 604 patients with a final diagnosis of Pneumocystis pneumonia were included, retrospective analysis was performed to classified patients according to the presence or lack of clinical or radiological signs. Patients were finally separated in groups according to the method used for the biological diagnosis, and the HIV status.

(94/143; 65.7%) compared to non-HIV patients (87/461; 18.9%), ($p < 0.001$). No difference was observed in the positivity rate of ME of broncho-pulmonary samples between patients presenting radiological abnormalities suggestive of the diagnosis of pneumocystosis (158/522; 30.3%) compared to those with normal chest X-ray (23/82; 28.0%), ($p > 0.1$).

The ME positivity rates for patients presenting 3, 2 or 1 symptoms (fever, dyspnea, cough) (29.7% (58/195) for 3 symptoms, 33% (62/188) for 2 symptoms, 31.1% (52/167) for 1 symptom), were not different from asymptomatic patients (16.6%, 9/54) ($p > 0.05$).

Prophylaxis did not decrease the positivity rate of ME: 13/40 (32.5%) patients under prophylaxis had a positive ME compared to 168/564 (29.7%) patients without prophylaxis ($p > 0.1$).

Table 1
Patient characteristics, on inclusion, classified by HIV status

	Total	HIV-negative	HIV-positive	P
n	604	461	143	
Median age (years)	59 [<1 - 88]	59 [<1 - 88]	59 [<1 - 88]	> 0.5
Sex ratio (M/F)	2.02	1.66	4.29	< 0.005
Prophylaxis before diagnosis	40 (6.6%)	27 (5.8%)	13 (9%)	> 0.5
Radiological findings	522 (86.4%)	398 (86.3%)	124 (86.7%)	> 0.5
Dyspnea	398 (65.9%)	297 (64.4%)	101 (70.6%)	0.00001
Fever	395 (65.4%)	293 (63.5%)	102 (71.3%)	0.08
Cough	337 (55.4%)	238 (51.6%)	99 (69.2%)	0.0003
Other symptoms	54 (8.9%)	49 (10.6%)	5 (3.4%)	0.009

4.5. Treatment

Around 97% (560/604) of patients received anti-*Pneumocystis* treatment. Sulfamethoxazole + Trimethoprime was the most frequent treatment (93.6%, 524/560), followed by atovaquone (5.1%, 29/560) and pentamidin (1.3%, 7/560). Anti-*Pneumocystis* treatment was initiated more frequently ($p < 0.02$) in HIV-positive patients (97.2%, 139/143) compared to non-HIV patients (91.3%, 421/461).

As expected, positive ME led more frequently to anti-*Pneumocystis* treatment than negative ME (97.2% (176/181) versus 90.7% (384/423)), ($p = 0.005$).

Initiation of treatment was lower when patients presented no symptoms (79.6% (43/54), $p = 0.0004$), compared to patients presenting 1 symptom (91.0% (152/167), $p = 0.0003$), 2 symptoms (94.1%; 177/188) or 3 symptoms (96.4%; 188/195). Among the 21 patients without symptoms and without imagery findings, 62% (13/21) were treated for pneumocystosis.

Table 2
Risk factors for HIV negative patients (n = 461)

Hematologic malignancies	**181 (39.3%)**
Lymphoma	84
Leukemia	66
Myeloma	15
Hodgkin	13
Myelodysplasia	3
Cancer	**124 (26.9%)**
Autoimmune disorders	**52 (11.3%)**
Rheumatoid polyarthritis	20
Dermatomyositis	5
Lupus	5
Anemia	4
Rectocolitis	4
Wegener	3
Others	11
Transplantation	**34 (7.4%)**
Kidney	19
Heart or heart and lung	10
Liver	5
Immunosuppressive therapies	**380 (82.4%)**
Corticosteroids	153
Chemotherapy	120
Corticosteroids + Chemotherapy	84
Other therapies	23
Other risk factors	**48 (10.4%)**
Pulmonary fibrosis	13
Cirrhosis	5
DICS	3
Miscalleous	13
No risk factor identified	14

- Other autoimmune disorders: Berger, Crohn, Horton and Gougerot's diseases, Arteritis, Sarcoidosis, Cryoglobulinemia.
- Other therapies: methotrexate, tacrolimus, mycophenolate and others.

4.6. Outcome

The overall cases mortality rate at day 14 was 16% (97/604), mostly in non-HIV patients, comparable to a previous study.[21] There was no significant difference in death rate ($p > 0.1$) between patients receiving a prophylaxis (3/40; 7.5%) or not (94/564; 16%). Non-HIV patients had a higher mortality rate (20.6%; 95/461) compared to HIV positive patients (1.4%, 2/143), ($p < 0.00001$).

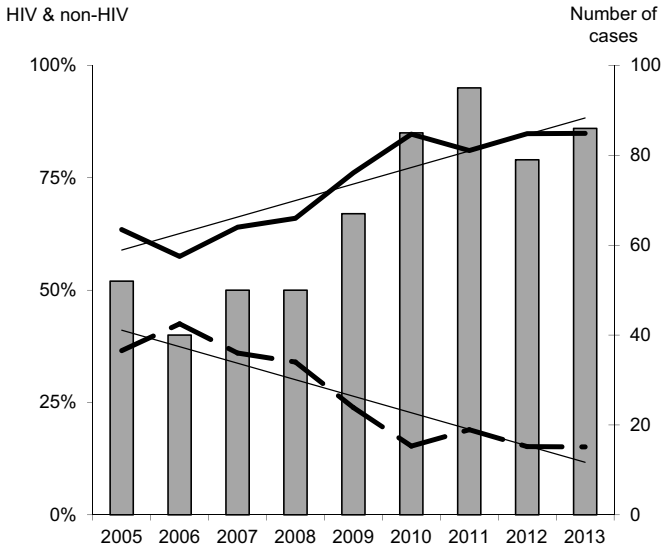

Figure 3. Annual number of Pneumocystis pneumonia (bars). Percentage of HIV-positive (dashed line) and non-HIV patients (black line) per year.

Clinical symptoms and radiological signs were not associated with a higher death rate ($p > 0.1$ and $p = 0.09$ respectively). For asymptomatic patients without radiological abnormalities (n = 21), 3 patients died, two of them with an anti-*Pneumocystis* treatment. Among the deceased patients, 87.6% (85/97) have a negative direct examination.

5. Discussion

In our institution, we showed that pneumocystosis rate is increasing among non-HIV patients. In less than ten years, the ratio of non-HIV versus HIV patients presenting pneumocystosis dramatically increased from 1.7 to 5.6. Most of the non-HIV patients (357/461; 77.4%) were receiving corticosteroids and/or chemotherapy, and the most frequent underlying diseases were hematological malignancies and cancer (305/461; 66.1%).

Surprisingly, in our study, anti-*Pneumocystis* treatment was less frequent in non-HIV patients (91.3%) compared to HIV patients (97.2%) ($P < 0.02$). However, the mortality rates at day 14 were higher in non-HIV patients compared to HIV patients, as previously reported.[15,22–24] Many confounding factors need to be taken into account to avoid a misinterpretation of mortality rates in patients with severe comorbidities. At the time of diagnosis, Pneumocystosis was less symptomatic in non-HIV patients compared to HIV positive patients. The absence of symptoms was described as an independent predictor of mortality.[25] Moreover, longer symptom duration was described in HIV patients compared to non-HIV patients whose symptoms could occur less than 3 days.[26] Consequently, these results suggested that it is better not to rule out the diagnosis of pneumocystosis in case of limited clinical expression in non-HIV patients and to initiate the treatment as early as possible for non-HIV patients suspected of Pneumocystosis,[27] while specific drugs may have significant adverse effects.

We demonstrated, as previously described, that in non-HIV patients, real-time PCR was more frequently positive than microscopic examination. This could be explained by fewer organisms present in non-HIV patients[28,29] and by the higher sensitivity of real time PCR compared to direct examination.[30,31] Conventional PCR is not recommended as a molecular diagnostic method for *Pneumocystis* amplification due to its lack of sensitivity[32] and specificity[33] compared to real-time PCR. Quantitative real-time PCR was claimed to be more specific than real-time PCR avoiding the detection of colonized patients.[34,35] However, in light of the recently published studies, a standardized quantitative method and a discriminant cut-off between colonized and infected patients are still lacking.[36–38]

Negative Real-time PCR is highly contributive to rule out pneumocystosis diagnosis in non-HIV patients due to its high negative predictive value, close to 100% and thus sufficient to reasonably discontinue an anti-*Pneumocystis* treatment.[39]

It is known that *Pneumocystis* colonization does exist and is defined, in contrast to *Pneumocystis* infection, by the detection of *Pneumocystis*, mostly by PCR-based techniques, in a patient without signs or symptoms of acute pneumonia.[1] According to authors, colonization is more common in non-HIV patients and could be a warning value for *Pneumocystis* prophylaxis in immunosuppressed patients, especially if an increase in immunosuppression is planned.[40,41] In our study, 21 patients had no symptoms typical of pneumocystosis and no compatible imagery findings, but only 8 of them did not receive specific treatment.

6. Conclusion

In regard to these results, we observed that pneumocystosis outcome is more unfavorable in non-HIV patients compared to HIV

patients, probably because symptoms are less frequent and treatment is less common, maybe subsequent to the scarcity of positive microscopic examination results. Real-time PCR is a useful tool for the diagnosis of Pneumocystis pneumonia in non-HIV patients. Hence, a positive Pneumocystis real-time PCR should be taken into account even if the patient does not have symptoms, but has a risk factor (cancer or hematological disorder) and/or receives an immunosuppressive therapy (corticosteroids and/or chemotherapy). For the patients who do not present these risk factors, the biological diagnosis using ME and real-time PCR may constitute the key elements to rule out the diagnosis of pneumocystosis. This study also raises another major question related to the lack of consensus on pneumocystosis prophylaxis in non-HIV patients more prone to suffer severe pneumocystosis. Except for patients undergoing allogeneic bone marrow transplantation, solid organ transplantation, acute lymphoblastic leukemia and collagen vascular diseases, little data are available for the other at-risk patients.[17]

Acknowledgments

Guarantor statement: Anne-Lise Bienvenu had full access to all the data in the study and takes responsibility for the integrity and the accuracy of the data analysis.

Collaborators: Aubert E (Service d'anesthésie et de réanimation, Centre Léon Bérard, Lyon), Avrillon V (Department of Medical Oncology, Centre Léon Bérard, Lyon), Broussolle C (Department of Internal Medicine, Hospices Civils de Lyon, Lyon), Chidiac C (Service de Maladies Infectieuses et Tropicales, Hospices Civils de Lyon, Lyon), Devouassoux G (Respiratory Medicine, Hospices Civils de Lyon, Lyon), Durieu I (Department of Internal and Vascular Medicine, Hospices Civils de Lyon, Lyon), Fayette J (Department of Medical Oncology, Centre Léon Bérard, Lyon), Fellahi JL (Service d'Anesthésie-Réanimation, Hospices Civils de Lyon, Lyon), Guerin C (Service de Réanimation Médicale, Hospices Civils de Lyon, Lyon), Lehot JJ (Service de Réanimation Neurologique, Hospices Civils de Lyon, Lyon), Michallet M (Hématologie Clinique, Hospices Civils de Lyon, Lyon), Mornex JF (Service de pneumologie, Hospices Civils de Lyon, Lyon), Persat F (Institut de Parasitologie et Mycologie Médicale, Hospices Civils de Lyon, Lyon), Piriou V (Service d'Anesthésie Réanimation, Hospices Civils de Lyon, Lyon), Rebattu P (Department of Medical Oncology, Centre Léon Bérard, Lyon), Salles G (Service d'hématologie, Hospices Civils de Lyon, Lyon), Souquet PJ (Acute Respiratory Medicine and Thoracic Oncology Department, Hospices Civils de Lyon, Lyon)

Conflict of interest: none

Other contributions: We thank biologists and technicians for their help in data acquisition.

References

1. Morris A, Norris KA. Colonization by Pneumocystis jirovecii and Its Role in Disease. *Clin Microbiol Rev* 2012;**25**(2):297–317. http://dx.doi.org/10.1128/CMR.00013-12
2. Miller RF, Ambrose HE, Novelli V, Wakefield AE. Probable Mother-to-Infant Transmission of Pneumocystis carinii f. sp. hominis Infection. *J Clin Microbiol* 2002;**40**(4):1555–7. http://dx.doi.org/10.1128/JCM.40.4.1555-1557.2002
3. Vargas SL, Hughes WT, Santolaya ME, Ulloa AV, Ponce CA, Cabrera CE, et al. Search for Primary Infection by Pneumocystis carinii in a Cohort of Normal, Healthy Infants. *Clin Infect Dis* 2001;**32**(6):855–61. http://dx.doi.org/10.1086/319340
4. Le Marchand-Auffret B, Polianski J, Roux P. Attempts at in vitro cultivation of Pneumocystis carinii from human bronchoalveolar lavage fluids without feeder cells. *J Protozool* 1991;**38**(6):116S–7S.
5. Polack B, Ceré N, Le Rhun D, Guillot J, Chermette R. Cultivation of rabbit Pneumocystis carinii on cells derived from rabbit (Oryctolagus cuniculus). *J Eukaryot Microbiol* 1997;**44**(6):22S.
6. Kaiser K, Rabodonirina M, Mayencon M, Picot S. Culture of Pneumocystis carinii sp.f. hominis. *J Eukaryot Microbiol* 1999;**46**(5):122S.
7. Kaiser K, Rabodonirina M, Mayencon M, Picot S. Cdc2 gene of Pneumocystis carinii hominis and its expression during culture. *J Eukaryot Microbiol* 1999;**46**(5):130S.
8. Masur H, Kaplan JE, Holmes KK. U.S. Public Health Service, Infectious Diseases Society of America. Guidelines for preventing opportunistic infections among HIV-infected persons–2002. Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. *Ann Intern Med* 2002;**137**(5 Pt 2):435–78.
9. Maini R, Henderson KL, Sheridan EA, Lamagni T, Nichols G, Delpech V, et al. Increasing Pneumocystis pneumonia, England, UK, 2000–2010. *Emerg Infect Dis* 2013;**19**(3):386–92. http://dx.doi.org/10.3201/eid1903.121151
10. Roux A, Gonzalez F, Roux M, Mehrad M, Menotti J, Zahar JR, et al. Update on pulmonary Pneumocystis jirovecii infection in non-HIV patients. *Médecine Mal Infect* 2014;**44**(5):185–98. http://dx.doi.org/10.1016/j.medmal.2014.01.007
11. Ainoda Y, Hirai Y, Fujita T, Isoda N, Totsuka K. Analysis of clinical features of non-HIV Pneumocystis jirovecii pneumonia. *J Infect Chemother Off J Jpn Soc Chemother* 2012;**18**(5):722–8. http://dx.doi.org/10.1007/s10156-012-0408-5
12. Kofteridis DP, Valachis A, Velegraki M, Antoniou M, Christofaki M, Vrentzos GE, et al. Predisposing factors, clinical characteristics and outcome of Pneumonocystis jirovecii pneumonia in HIV-negative patients. *J Infect Chemother Off J Jpn Soc Chemother* 2014;**20**(7):412–6. http://dx.doi.org/10.1016/j.jiac.2014.03.003
13. Fillatre Pierre, Decaux Olivier, Jouneau Stéphane, Revest Matthieu, Gacouin Arnaud, Robert-Gangneux Florence, et al. Incidence of Pneumocystis jiroveci pneumonia among groups at risk in HIV-negative patients. *Am J Med* 2014;**127**(12):1242.e11–e. http://dx.doi.org/10.1016/j.amjmed.2014.07.010
14. Morris Alison, Lundgren Jens D, Masur Henry, Walzer Peter D, Hanson Debra L, Frederick Toni, et al. Current epidemiology of Pneumocystis pneumonia. *Emerg Infect Dis* 2004;**10**(10):1713–20. http://dx.doi.org/10.3201/eid1010.030985
15. Asai Nobuhiro, Motojima Shinji, Ohkuni Yoshihiro, Matsunuma Ryo, Nakashima Kei, Iwasaki Takuya, et al. Early diagnosis and treatment are crucial for the survival of Pneumocystis pneumonia patients without human immunodeficiency virus infection. *J Infect Chemother Off J Jpn Soc Chemother* 2012;**18**(6):898–905. http://dx.doi.org/10.1007/s10156-012-0441-4
16. Festic Emir, Gajic Ognjen, Limper Andrew H, Aksamit Timothy R. Acute respiratory failure due to pneumocystis pneumonia in patients without human immunodeficiency virus infection: outcome and associated features. *Chest* 2005;**128**(2):573–9. http://dx.doi.org/10.1378/chest.128.2.573
17. Stern Anat, Green Hefziba, Paul Mical, Vidal Liat, Leibovici Leonard. Prophylaxis for Pneumocystis pneumonia (PCP) in non-HIV immunocompromised patients. *Cochrane Database Syst Rev* 2014;**10**:CD005590. http://dx.doi.org/10.1002/14651858.CD005590.pub3
18. Kaiser Karine, Rabodonirina Meja, Picot Stéphane. Real time quantitative PCR and RT–PCR for analysis of Pneumocystis carinii hominis. *J Microbiol Methods* 2001;**45**(2):113–8. http://dx.doi.org/10.1016/S0167-7012(01)00241-X
19. Rabodonirina M, Cotte L, Boibieux A, Kaiser K, Mayencon M, Raffenot D, et al. Detection of Pneumocystis carinii DNA in blood specimens from human immunodeficiency virus-infected patients by nested PCR. *J Clin Microbiol* 1999;**37**(1):127–31.
20. Limper Andrew H, Knox Kenneth S, Sarosi George A, Ampel Neil M, Bennett John E, Catanzaro Antonino, 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**(1):96–128. http://dx.doi.org/10.1164/rccm.2008-740ST
21. Hardak Emilia, Neuberger Ami, Yigla Mordechai, Berger Gidon, Finkelstein Renato, Sprecher Hannah, et al. Outcome of Pneumocystis jirovecii pneumonia diagnosed by polymerase chain reaction in patients without human immunodeficiency virus infection. *Respirol Carlton Vic* 2012;**17**(4):681–6. http://dx.doi.org/10.1111/j.1440-1843.2012.02158.x
22. Su Ying-Shih, Lu Jang-JihJ, Perng Cherng-Lih, Chang Feng-Yee. Pneumocystis jirovecii pneumonia in patients with and without human immunodeficiency virus infection. *J Microbiol Immunol Infect Wei Mian Yu Gan Ran Za Zhi* 2008;**41**(6):478–82.
23. Roux Antoine, Canet Emmanuel, Valade Sandrine, Gangneux-Robert Florence, Hamane Samia, Lafabrie Ariane, et al. Pneumocystis jirovecii Pneumonia in Patients with or without AIDS. *France Emerg Infect Dis* 2014;**20**(9):1490–7. http://dx.doi.org/10.3201/eid2009.131668
24. Monnet Xavier, Vidal-Petiot Emmanuelle, Osman David, Hamzaoui Olfa, Durrbach Antoine, Goujard Cécile, et al. Critical care management and outcome of severe Pneumocystis pneumonia in patients with and without HIV infection. *Crit Care Lond Engl* 2008;**12**(1):R28. http://dx.doi.org/10.1186/cc6806
25. Guo Fei, Chen Yong, Yang Shuang-Li, Xia Huan, Li Xing-Wang, Tong Zhao-Hui. Pneumocystis Pneumonia in HIV-Infected and Immunocompromised Non-HIV Infected Patients: A Retrospective Study of Two Centers in China. *PLoS ONE* 2014;**9**(7.). http://dx.doi.org/10.1371/journal.pone.0101943
26. Bollée Guillaume, Sarfati Claudine, Thiéry Guillaume, Bergeron Anne, de Miranda Sandra, Menotti Jean, et al. Clinical picture of Pneumocystis jiroveci pneumonia in cancer patients. *CHEST J* 2007;**132**(4):1305–10.
27. Sokulska Magdalena, Kicia Marta, Wesołowska Maria, Hendrich Andrzej B. Pneumocystis jirovecii–from a commensal to pathogen: clinical and diagnostic review. *Parasitol Res* 2015;**114**(10):3577–85. http://dx.doi.org/10.1007/s00436-015-4678-6
28. Alanio A, Desoubeaux G, Sarfati C, Hamane S, Bergeron A, Azoulay E, et al. Real-time PCR assay-based strategy for differentiation between active Pneumocystis jirovecii pneumonia and colonization in immunocompromised patients. *Clin Microbiol Infect* 2011;**17**(10):1531–7. http://dx.doi.org/10.1111/j.1469-0691.2010.03400.x

29. Thomas Charles F, Limper Andrew H. Pneumocystis Pneumonia. *N Engl J Med* 2004;**350**(24):2487–98. http://dx.doi.org/10.1056/NEJMra032588
30. Wilson John W, Limper Andew H, Grys Thomas E, Karre Theresa, Wengenack Nancy L, Binnicker Matthew J. Pneumocystis jirovecii testing by real-time polymerase chain reaction and direct examination among immunocompetent and immunosuppressed patient groups and correlation to disease specificity. *Diagn Microbiol Infect Dis* 2011;**69**(2):145–52. http://dx.doi.org/10.1016/j.diagmicrobio.2010.10.021
31. Fillaux Judith, Berry Antoine. Real-time PCR assay for the diagnosis of Pneumocystis jirovecii pneumonia. *Methods Mol Biol Clifton NJ* 2013;**943**:159–70. http://dx.doi.org/10.1007/978-1-60327-353-4_11
32. Flori Pierre, Bellete Bahrie, Durand Fabrice, Raberin Hélène, Cazorla Céline, Hafid Jamal, et al. Comparison between real-time PCR, conventional PCR and different staining techniques for diagnosing Pneumocystis jiroveci pneumonia from bronchoalveolar lavage specimens. *J Med Microbiol* 2004;**53**(Pt 7):603–7. http://dx.doi.org/10.1099/jmm.0.45528-0
33. Fujisawa Tomoyuki, Suda Takafumi, Matsuda Hiroyuki, Inui Naoki, Nakamura Yutaro, Sato Jun, et al. Real-time PCR is more specific than conventional PCR for induced sputum diagnosis of Pneumocystis pneumonia in immunocompromised patients without HIV infection. *Respirol Carlton Vic* 2009;**14**(2):203–9. http://dx.doi.org/10.1111/j.1440-1843.2008.01457.x
34. Fan Li-Chao, Lu Hai-Wen, Cheng Ke-Bin, Li Hui-Ping, Xu Jin-Fu. Evaluation of PCR in Bronchoalveolar Lavage Fluid for Diagnosis of Pneumocystis jirovecii Pneumonia: A Bivariate Meta-Analysis and Systematic Review. *PLoS ONE* 2013;**8**(9). http://dx.doi.org/10.1371/journal.pone.0073099
35. Lu Yuan, Ling Guoya, Qiang Chenyi, Ming Qinshou, Wu Cong, Wang Ke, et al. PCR Diagnosis of Pneumocystis Pneumonia: a Bivariate Meta-Analysis. *J Clin Microbiol* 2011;**49**(12):4361–3. http://dx.doi.org/10.1128/JCM.06066-11
36. Robert-Gangneux Florence, Belaz Sorya, Revest Matthieu, Tattevin Pierre, Jouneau Stéphane, Decaux Olivier, et al. Diagnosis of Pneumocystis jirovecii Pneumonia in Immunocompromised Patients by Real-Time PCR: a 4-Year Prospective Study. *J Clin Microbiol* 2014;**52**(9):3370–6. http://dx.doi.org/10.1128/JCM.01480-14
37. Botterel Françoise, Cabaret Odile, Foulet Françoise, Cordonnier Catherine, Costa Jean-Marc, Bretagne Stéphane. Clinical Significance of Quantifying Pneumocystis jirovecii DNA by Using Real-Time PCR in Bronchoalveolar Lavage Fluid from Immunocompromised Patients. *J Clin Microbiol* 2012;**50**(2):227–31. http://dx.doi.org/10.1128/JCM.06036-11
38. Louis M, Guitard J, Jodar M, Ancelle T, Magne D, Lascols O, et al. Impact of HIV Infection Status on Interpretation of Quantitative PCR for Detection of Pneumocystis jirovecii. *J Clin Microbiol* 2015;**53**(12):3870–5. http://dx.doi.org/10.1128/JCM.02072-15
39. Azoulay Elie, Bergeron Anne, Chevret Sylvie, Bele Nicolas, Schlemmer Benoît, Menotti Jean. Polymerase chain reaction for diagnosing pneumocystis pneumonia in non-HIV immunocompromised patients with pulmonary infiltrates. *Chest* 2009;**135**(3):655–61. http://dx.doi.org/10.1378/chest.08-1309
40. Fily F, Lachkar S, Thiberville L, Favennec L, Caron F. Pneumocystis jirovecii colonization and infection among non HIV-infected patients. *Médecine Mal Infect* 2011;**41**(10):526–31. http://dx.doi.org/10.1016/j.medmal.2011.07.006
41. Tasaka Sadatomo, Tokuda Hitoshi. Pneumocystis jirovecii pneumonia in non-HIV-infected patients in the era of novel immunosuppressive therapies. *J Infect Chemother Off J Jpn Soc Chemother* 2012;**18**(6):793–806. http://dx.doi.org/10.1007/s10156-012-0453-0