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CLINICAL MYCOLOGY LAB ISSUES (S CORDOBA, SECTION EDITOR)

Epidemiology of *Pneumocystis jirovecii* Pneumonia in Venezuela

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Abstract

Purpose of the Review The aim of this work is to contribute to the knowledge of the epidemiology of pneumocystosis or *Pneumocystis jirovecii* pneumonia (PCP) in Venezuela, by an updated review of the information currently available.

Recent Findings The PCP epidemiology in Venezuela is not formally known. There is no national surveillance program for mycosis, and systemic mycoses are not mandatory notification diseases. Efforts made to record the disease in our country have not been sufficient; therefore, there is a sub-registry. All the information carried out prevents comparisons with other reports due to the variations in study designs, heterogeneity of the studied patients, and diagnostic methods.

Summary This review summarizes the current knowledge about PCP epidemiology in Venezuela based on available national publications. The contributions of these studies are very valuable, since they reflect the need for systematic PCP study, not only in AIDS patients, but in cancer and COPD patients.

Keywords *Pneumocystis jirovecii* · *Pneumocystis jirovecii* pneumonia (PCP) · Venezuela · Direct immunofluorescence assay (DIF) · Nested PCR (nPCR) · Epidemiology

Introduction

Pneumocystis jirovecii (formerly known as *Pneumocystis carinii*) is an opportunistic fungus of universal distribution and pulmonary tropism, which causes a severe respiratory infection in humans, called pneumocystosis or *Pneumocystis jirovecii* pneumonia (PCP) considered one of the most common diseases affecting immunocompromised patients. It is the most prevalent opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS), also affecting patients with hematological malignancy, solid organ transplant, congenital T and B lymphocyte immunodeficiency, malnutrition, and chronic obstructive pulmonary disease (COPD), among others [1••, 2, 3, 4••, 5•, 6].

In Venezuela, PCP is scarce and not formally known. This is due to several factors. (1) Traditionally, PCP diagnosis has been clinical and histopathological, causing a significant impact on both morbidity and mortality of this disease. (2) The absence of mycological diagnosis in hospital centers. In Venezuela, hospital centers that have mycological diagnosis, apart from those in the capital (Caracas), are confined to state-level health agencies (both public and private) or to the Working Groups in Mycology, distributed in eight states of the country, which do not completely cover the entire national territory and which are mostly no longer operational [5•, 7••, 8••, 9]. (3) There are no complementary microbiological tests that neither confirm the etiological diagnosis nor qualified personnel in hospital centers, especially at the state level. (4) PCP is not included in the differential diagnosis of acute respiratory diseases, especially in immunosuppressed patients without infection by human immunodeficiency virus (HIV) or in patients with long treatments with antibiotics, cytotoxic

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drugs, and corticosteroids, without adequate posttreatment evolution; if you do not think about PCP, it is not diagnosed. (5) There is no national surveillance program for systemic mycoses, and for this reason, there are no official data; systemic mycoses are not mandatory notification diseases. Although there is a national program for bronchopulmonary diseases surveillance, it does not consider respiratory diseases caused by fungi. (6) Published reports about PCP in Venezuela are scarce; they are mostly casuistic reports of some internal medicine, infectology or pathological anatomy hospital services [10–15•, 16–23], research results [24•, 25–27•, 28•, 29], or product of passive surveillance in reference centers [5•, 30•].

PCP studies in Venezuela are compiled in Table 1. Most of them have been published in national peer-reviewed journals and in Spanish language. We also included summaries of papers presented at conferences and meetings nationwide, which are mostly not available online. Following, we will briefly describe the studies and provide some relevant historical data.

Epidemiology of *P. jirovecii* Pneumonia by in Venezuela

The first published study was conducted by Duran et al., [10] who analyzed nationwide incidence of 60 first cases of AIDS, finding that PCP was the first opportunistic infection (30%), followed by Kaposi's sarcoma (14%); combination of both pathologies was found in 9%. Garcia Tamayo et al. [11] evaluated 120 autopsies of AIDS patients and described in detail lung parenchyma alterations caused by opportunistic microorganisms, finding a 30% of PCP frequency. The authors conclude, when comparing their results with those described by other researchers, that the opportunistic microorganism's frequency found seems to be related to geographical and socioeconomic factors. In addition, this study grouped and described the autopsies results evaluated in two previous works carried out by the author, which are not available online.

At the Hospital Universitario de Caracas, a study described the clinical and epidemiological characteristics of 500 patients with AIDS, finding 3.6% of PCP frequency [12]. Another study evaluated 45 lung tissue specimens coming from autopsies of AIDS patients by optical microscopy, using hematoxylin-eosin and Gomori-Grocott stains. *P. jirovecii* was detected in 11 specimens; thereafter, the specimens were evaluated by electron transmission microscopy, in order to observe at an ultrastructural level their different morphological states in the lung, correlate these findings with the information published internationally so far, and contribute with its possible taxonomic classification. The results coincided with those reported by other researchers, placing *P. jirovecii* (*P. carinii* at that time) within the Fungi kingdom, relating it to the *Ascomycetes* [13].

In a retrospective study, 131 autopsies of AIDS patients and their respective medical records were evaluated, to correlate both findings and discrepancies between them. The results revealed that PCP had the highest clinical suspicion, with a 9% of frequency at autopsies, while *Cytomegalovirus* infection was the least clinically suspected but the most frequent at autopsies [14].

The introduction of DIF for *P. jirovecii* detection in Venezuela was in 1999, when the first comparative study between DIF and Gomori-Grocott stain was carried out at the Instituto Nacional de Higiene Rafael Rangel (Rafael Rangel National Institute of Hygiene, RRNIH). DIF was more sensitive than the Gomori-Grocott stain for *P. jirovecii* detection in 30 spontaneous sputum (SS) specimens of AIDS patients coming from three hospital centers located in the capital, obtaining a 40% PCP frequency. It is important to highlight that in this study, DIF was standardized using the previously named specimens [24•].

Hernandez [25] conducted a prevalence study of neoplasms and opportunistic infections in AIDS patients between January 1985 and December 1997 at the Hospital Vargas in Caracas. The study included 260 patients and was divided into two periods: 1985–1993 (200 patients) and 1994–1997 (60 patients), finding that PCP was the second most frequent opportunistic infection in both periods (13% and 8.3%, respectively), preceded by candidiasis. The limitation of this study was the absence of microbiological confirmation of opportunistic infections, which influenced the obtained results.

To determine the types and prevalence of oral lesions and opportunistic infections associated with AIDS, an investigation was conducted in a group of 208 patients, of which 108 (66%) presented oral lesions. The most common was oral candidiasis (48.7%). Among opportunistic diseases, PCP was in second place (11.5%) [26].

A cross-sectional and descriptive study was designed to determine PCP frequency in 69 patients with lower respiratory tract infection from the Hospital General del Oeste. *P. jirovecii* detection was performed by DIF in induced sputum (IS) and bronchoalveolar lavage (BAL) specimens, obtaining a 14.5% PCP frequency. Among the patients with PCP, four had AIDS and six other comorbidities, such as COPD, diabetes mellitus, and chronic lung diseases. The great contribution of this work was to calculate the diagnostic capacity (sensitivity, specificity, predictive values, and likelihood ratios) of the signs, symptoms, radiological findings, enzyme lactic dehydrogenase (LDH) values, and arterial gases, among others, based on DIF's results. Pleuritic pain, hypoalbuminemia, elevated LDH levels, and radiological findings compatible with PCP obtained moderate diagnostic capacity values for the disease. The authors concluded that PCP should be suspected in patients without AIDS and risk factors for the disease, recommending DIF as the choice technique for diagnosis [15•].

Table 1 Studies on pneumocystosis in Venezuela

Study period	Hospital center	Sample size/study design	Population/PCP diagnosis	PCP frequency	Reference
ND	ND	60/retrospective	AIDS/clinical	30% PCP + KS 9%	[10]
1987–1992	Instituto Anatomopatológico Dr. José Antonio O′ Daly - HUC (Caracas)	120/retrospective	AIDS/autopsies	30%	[11]
1993–1995	Hospital Universitario de Caracas (Caracas)	500/retrospective	AIDS/clinical	3.6%	[12]
January 1995–July 1996	Instituto Anatomopatológico Dr. José Antonio O′ Daly - HUC (Caracas)	45/retrospective	AIDS/autopsies	24.4%	[13]
1988–1997	Hospital Universitario de Caracas (Caracas)	131/retrospective	AIDS/autopsies	9%	[14]
July–September 1999	Instituto Nacional de Higiene Rafael Rangel (Caracas)	30/prospective	AIDS/clinical + DIF	40%	[24•]
1985–1997	Hospital Vargas (Caracas)	260/retrospective	AIDS/clinical	12%	[25]
November 1998–July 2000	Servicio de Atención a Pacientes con Enfermedades Infectocontagiosas “Dra. Elsa La Corte”. Universidad Central de Venezuela (Caracas)	108/retrospective	AIDS/clinical	11.5%	[36]
2004–2005	Hospital General del Oeste Dr. José Gregorio Hernández (Caracas)	69/prospective	AIDS and other comorbidities/clinical + DIF	General: 14,5% AIDS: 40% Other comorbidities: 60%	[15•]
2001–2006	Instituto Nacional de Higiene Rafael Rangel (Caracas)	129/retrospective	AIDS-cancer-Non-AIDS non-cancer/DIF	General: 23.3% AIDS: 36.6% Cancer: 35.3% Non-AIDS non-cancer: 10.9%	[5•]
2000–2001	Complejo Hospitalario Dr. Ruíz y Páez (Bolívar State)	40/retrospective	HIV + non-HIV/clinical + DIF	General: 35% HIV: 15% CRI: 5% RT: 2.5% CN: 12.5%	[16]
January–July 2006	Hospital Universitario Dr. Ángel Larralde (Carabobo State)	44/retrospective	AIDS/clinical	29.5%	[17]
2005–2006	Hospital Vargas (Caracas)	83/retrospective	AIDS/clinical	10%	[18]
January–June 2008	Hospital Universitario Dr. Ángel Larralde (Carabobo State)	36/retrospective	AIDS/clinical	34% (TB y PCP)	[19]
2007	Instituto Nacional de Higiene Rafael Rangel (Caracas)	62/retrospective and comparative	AIDS-Cancer-Non-AIDS non-cancer/DIF + nPCR	22.6% (DIF + nPCR positives)	[27•]
September 2007–July 2008	Instituto Nacional de Higiene Rafael Rangel (Caracas)	31/retrospective	Cancer/DIF	25.8%	[28•]
January–July 2010	Hospital Universitario Dr. Ángel Larralde (Carabobo State)	58/retrospective	AIDS/clinical	8.6%	[20]
2010	Hospital Vargas (Caracas)	42/retrospective	AIDS/clinical	7%	[21]
February–September 2009	Hospital Universitario Dr. Ángel Larralde (Carabobo State)	35/retrospective	AIDS/clinical + pathological anatomy	57%	[22]
2011–2012	Complejo Hospitalario Universitario “Dr. Luis Razetti” (Anzoátegui State)	52/prospective	HIV and other comorbidities/clinical-Giemsa-DIF	General: 15.4% HIV: 3 DM2: 1 ND: 2	[23]

Table 1 (continued)

Study period	Hospital center	Sample size/study design	Population/PCP diagnosis	PCP frequency	Reference
2017	Hospital Universitario de Los Andes (Mérida State)	36/prospective	AIDS/clinical + Histopathological	Epilepsy: 1 AHT: 1 64%	[29]
2007–2013	Instituto Nacional de Higiene Rafael Rangel (Caracas)	161/retrospective	AIDS/DIF + nPCR	39% (DIF + nPCR positives)	[30•]

PCP Pneumocystis jirovecii pneumonia, *DIF* Direct Immunofluorescence, *CRI* chronic renal insufficiency, *RT* renal transplant, *CN* chronic pneumopathy, *DM2*diabetes mellitus type 2, *AHT* arterial hypertension, *KS* Kaposi's sarcoma, *ND* no data available

The first report of PCP incidence through passive surveillance corresponds to the national reference center, the RRNIH. In a 6-years period, 129 referred respiratory specimens from several hospital centers of the capital were processed by DIF. All patients presented acute pulmonary symptoms and were divided into three groups according to clinical diagnosis: AIDS, cancer, and non-AIDS non-cancer. Thirty cases of PCP (23.3%) were diagnosed, and the frequency varied according to the patients group: AIDS = 36.6%; cancer = 38%; and non-AIDS non-cancer = 10.4%. This study showed that there are differences in PCP presentation related to the patients' underlying disease and that the study of this disease should be extended in patients with cancer and COPD [5•].

The first report of PCP frequency outside of metropolitan area of Caracas was made in Bolívar State, through the analysis by DIF of 40 sputum specimens coming from patients with signs and symptoms of pneumonia. *P. jirovecii* was detected in 35% of the specimens, and the frequency varied according to the patients' group studied [16]. At the Hospital Universitario Dr. Angel Larralde, also at the state level, a retrospective study was conducted to know the morbidity and mortality in 44 hospitalized AIDS patients. The most frequent opportunistic infections were disseminated histoplasmosis (40.9%), cerebral toxoplasmosis (34.1%), PCP (29.5%), and disseminated *Cytomegalovirus* infection (27.3%). Forty of them were in the AIDS C3 stage, and eight (18.2%) died from acute respiratory failure [17].

In another retrospective study, Franco Ricart [18] determined the frequency of opportunistic infections in AIDS patients hospitalized at the Hospital Vargas in Caracas. The most frequent infections were TB (24.64%), toxoplasmosis (19.87%), candidiasis (15.94%), and PCP (10.14%), and they concluded that respiratory infections were the most frequent. Naranjo et al. [19] determined that the most frequent opportunistic infections in 36 AIDS patients hospitalized at the Hospital Central Dr. Miguel Pérez Carreño in Caracas were TB and PCP (34%). Eighty percent of the patients were in AIDS C3 stage, and the associated mortality was 5.6%.

The introduction of nPCR for *P. jirovecii* detection also corresponds to the RRNIH [27•]. A comparative study was conducted between DIF (chosen as the gold standard test) and nPCR in 62 respiratory specimens, mostly SS, from patients with PCP clinical suspicion referred by several hospital centers located in Caracas. Sensitivity value = 100%, specificity value = 79.2%, positive predictive value = 58.3%, negative predictive value = 100%, and an agreement of 84% between nPCR and DIF were obtained. One of the most important contributions of this work was that both sensitivity and specificity values obtained for nPCR were excellent, taking into account that noninvasive respiratory specimens such as SS were used, which supported the standardization process of this type of samples in the RRNIH [5•, 16]. In addition, it was concluded that nPCR successfully predicts the absence of

PCP, but given a positive result, the patient's clinical condition should be taken into account, since the test is not able to discriminate between colonization and infection. These results are similar to those obtained by other researchers and members of IBEROPNEUMOCYSTIS [4••, 31••, 32–36].

A study by Moreno et al. [28•] focused on knowing the PCP frequency in 31 cancer patients, using DIF with IS, BAL, and tracheal aspirates specimens. The general frequency of PCP was 25.8%, with variations according to the patients' group: 27.8% in patients with solid tumors, 22.2% in patients with lymphomas, and 25% in patients with leukemia. They got excellent results with serial specimens of IS, recommending its use.

Another state report determined the frequency of opportunistic infections in 58 AIDS hospitalized patients at the Hospital Universitario Dr. Angel Larralde. Eighty-two percent (82%) of patients were admitted with respiratory infections and CD4 cell count < 200 cell/mm^3. PCP ranked third in frequency with 8.6%, preceded by cryptococcosis and toxoplasmosis [20].

A retrospective study conducted in the Medicine III service of the Hospital Vargas in Caracas reported that HIV infection and AIDS accounted for 16% of the hospitalization causes. The most frequent reasons for consultation were respiratory infections, where TB was the most frequent (16.7%), followed by PCP (7%) and histoplasmosis (7%) [21].

In the Pulmonology and Thoracic Surgery Department of the Hospital Universitario de Los Andes, a retrospective study was carried out to know the most frequent lung pathogens in AIDS patients. Thirty-five patients were evaluated and 57% presented PCP, which was diagnosed by BAL histopathological evaluation plus radiological evidence of respiratory infection on high-resolution chest tomography. The majority of patients did not receive highly active antiretroviral therapy (HAART) or prophylactic treatment for PCP [22]. Another study in Anzoategui State determined PCP frequency in hospitalized immunocompromised patients with respiratory symptoms, using clinical, epidemiological, radiological, and laboratory criteria. Fifty-two SS specimens were evaluated by Giemsa staining and DIF, obtaining eight positive results (15.4%) [23].

In the study conducted by Fuenmayor et al., [29] different infectious etiologies and histological findings were described in a group of 36 patients with pneumopathies (23% with HIV infection and 13% with AIDS). These patients underwent fibrobronchoscopy to obtain BAL specimens, bronchial brushing, and transbronchial biopsy, which were subsequently stained with hematoxylin-eosin, Gomori-Grocott, Pap smear, and Ziehl-Neelsen. With the Gomori-Grocott staining, cysts (ascus) and trophozoites (ascospores) of *P. jirovecii* were observed in 64% of the samples ($n = 23$). In 25% of the samples ($n = 9$), *P. jirovecii* was the only etiologic agent observed, while in 39% ($n = 14$), *P. jirovecii* was associated to other agents such as *Histoplasma capsulatum*, *Candida* spp., and bacteria. Fifty-three (53%) of these patients did not receive HAART, so it was concluded that both histological findings and infectious diseases were closely related to AIDS and the lack of HAART.

The second incidence report of PCP through passive surveillance corresponds to the RRNIH. Over a period of 6 years, 161 respiratory specimens were received from hospitalized patients with HIV infection, lower respiratory tract infection, and PCP clinical suspicion. *P. jirovecii* was detected by IFD in 76 of 161 specimens (47.2%); nPCR was performed in parallel in 36 of 76 specimens, obtaining positive results in all of them; nPCR was negative in 8 of 76 specimens; and in the remaining 32 of 76 specimens, this technique could not be performed, because the necessary reagents were not available. DIF was negative in 85 of 161 specimens; nPCR was negative in 65 of 85, while in 20 of 85 nPCR was positive (23.5%). In the end, the detection of *P. jirovecii* was positive in 96 patients (39%) [30•].

Trying to put into perspective the PCP frequency reported in these studies, taking into account the hospital center, sample size, study design, studied population, and how PCP diagnosis was made, it was possible to confirm several aspects: (1) Most of the studies were conducted in AIDS patients from hospital centers located in Caracas, with few contributions from state studies. (2) Variations in PCP frequency are particularly due to differences in the study design, sample size, methods used to make PCP diagnosis, and the heterogeneity of the studied patients. (3) The authors of this work are pretty sure that there are more studies nationwide, which, despite of the search, could not be found. The fact that the studies have been published in national journals and in Spanish language, it reflects the need to demonstrate the presence of this disease in our country, but it also demonstrates the lack of knowledge of these data at regional and world level, due to the barrier caused by language and the lack of indexing the journals in databases and global repositories, which allow them for a greater massification. (4) The obvious PCP sub-registration, in addition to prevent its proper study, prevents evaluation and implementation of therapeutic and precautionary measures, so necessary for any infectious disease.

The contributions of these studies are very valuable, since they reflect the need for systematic PCP study, not only in AIDS patients, but in cancer and COPD patients [37•]. In addition, these studies were the pioneers who started preliminary investigations of PCP in immunocompetent individuals in contact with patients suffering from this disease, to know if they were colonized by *P. jirovecii* [38•, 39•]. Our working group has demonstrated the need to study PCP from clinical, histopathological, and microbiological point of view, by DIF and molecular techniques, which confirm *P. jirovecii* supporting initial clinical diagnosis [5•, 15•, 24•, 27•, 28•, 30•, 37•, 38•, 39•]. All the information presented above corroborates the analysis of PCP epidemiological situation in Venezuela carried out in the first paragraph of this section and prevents comparisons with other reports due to the variations already described.

PCP frequency reported in Latin America in AIDS patients is very variable, with ranges between 5.9 and 55%; the variation is due to the same characteristics observed in Venezuelan studies: study design, heterogeneity of the studied patients, and diagnostic methods. Compared to reports of PCP incidence decrease in developed countries, after introducing HAART, PCP frequency in AIDS patients of Latin America seems to remain stable, but with a tendency to increase over time, according to two reports of the IBEROPNEUMOCYSTIS group. PCP epidemiological situation in Venezuela is very similar to that of Latin American countries, and it is very unfortunate. Systemic mycoses are not of mandatory notification, so there are no official data; this happens not only in Venezuela but in other countries of the continent [7••, 8•].

When reviewing PCP frequency reported in AIDS patients from Europe, Asia, and Africa, it depends, as in Latin America, on the same variables mentioned above. We observed that it is high, and additional common findings in these studies were the absence of the patient's immune status data, evaluation of signs and symptoms, radiological images, and treatment records, which prevent comparisons [40–46].

Conclusions

The underestimation of PCP in Venezuela is evident. Reports of PCP from different regions of our country are not enough to visualize a real picture. In this way, our country needs to establish an epidemiology surveillance program for mycoses in Venezuela including PCP, in order to get incidence and prevalence epidemiological data that will be used for the formulation of public policies for its control and prevention. Moreover, PCP could be a disease of mandatory notification since marks AIDS presence, which would further favor getting reliable data.

We do not know how long time will take to make this happen. Meanwhile, we will continue working to learn more about this paradigmatic disease.

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Compliance with Ethical Standards

Conflict of Interest María Mercedes Panizo, Giuseppe Ferrara, Nataly García, Xiomara Moreno, Trina Navas, and Enrique Calderón declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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