Original Research Article

A comparative study of methods of Pneumocystis Jiroveci pneumonia in HIV patients with CD4 count less than 200 and the clinical outcome in tertiary care hospital

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International Archives of Integrated Medicine, Vol. 6, Issue 3, March, 2019.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 25-02-2019 **Accepted on:** 03-03-2019
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: G. Allwyn Vijay, S.B. Sivaraja. A comparative study of methods of Pneumocystis Jiroveci pneumonia in HIV patients with CD4 count less than 200 and the clinical outcome in tertiary care hospital. IAIM, 2019; 6(3): 148-155.

Abstract

Background: Pneumocystosis is an opportunistic fungal infection of the respiratory system leading to interstitial plasma cell pneumonia, caused by a taxonomically unique fungus *Pneumocystis jiroveci*. Major developmental stages of the organism include the small (1 to 4 μm) pleomorphic trophozoite or trophic form; the 5 to 8 μm cyst, which has a thick cell wall and contains up to eight intra cystic bodies; and the precyst, an intermediate stage. The life cycle of *P. jiroveci* probably involves asexual replication by the trophic form and sexual reproduction by the cyst, which ends in the release of the intra cystic bodies an intracellular stage has not been identified.

Aim of study: Comparing the role of clinical diagnosis, chest radiography, sputum microscopy and polymerase chain reaction for Pneumocystis jiroveci Pneumonia in HIV seropositive patients with CD4 less than 200, to know the clinical outcome of PCP patients after treatment. 151 HIV seropositive patients were recruited for study as per inclusion criteria.

Materials and methods: The study was conducted in the Department of TB and Chest Medicine, Government Stanley Medical College, Chennai from 2016-2017. Thorough clinical examination

including general and systemic examination was done meticulously with vital signs monitoring and SpO_2 was measured with pulse oximetry.

Results: Out of 151 HIV seropositive patients examined clinically, 81 individuals were diagnosed as PCP patients. But the sputum microscopy with Gomori methenamine silver staining which was taken as gold standard test, diagnosed 41 cases of PCP only. PCR was positive in 2 more patients who were missed in GMS staining. Sputum PCR was having the highest sensitivity (100%), highest specificity (97%), highest positive predictive value (93%) and also the highest negative predictive value (100%). Among 90 PCP patients diagnosed clinically, 74 of 90 (82.2%) patients recovered from the illness after treatment and 16 of 90 (17.8%) patients died due to illness.

Conclusion: As revealed in our study, induced sputum analysis is a simple procedure, without significant adverse effects, and with a good diagnostic yield for *P. jiroveci* pneumonia determination in HIV-positive patients. IFAT is very sensitive and specific, though the expensive method for the detection of this organism.

Key words

Pneumocystosis, *Pneumocystis jiroveci* , CD4 Count, HIV Patients.

Introduction

There are several unique features of Pneumocystis that make its study exciting but quite difficult. The organism cannot be cultured reliably outside the lung, complicating the investigation of its life cycle and signaling [1]. The organism's source in nature has not been identified, and so the issue of transmission has been difficult to examine. The organism is strictly hosted specific, but the antigens or mechanisms that confer specificity have not been elucidated. Finally, Pneumocystis infections are virtually always limited to the lung, but the host characteristics that allow infection are not completely understood [2]. The Delanoe¨s originated the taxonomic designation Pneumocystis carinii in 1912 when the organism was still considered to be a protist. More recent work demonstrates that Pneumocystis is a member of the fungi [3]. Each mammalian host is infected by a specific Pneumocystis that cannot infect other hosts. Therefore, efforts are being made to reclassify the various Pneumocystis organisms as separate species, and the taxonomy and nomenclature for these organisms is a focus of continuing controversy [4]. Some authorities use Pneumocystis jirovecii to refer to the Pneumocystis species that cause human disease. For Pneumocystis carinii, the organism that infects rats, the genome has been identified to be about 7.7 Mb and consists of 13 to 15 linear chromosomes that range from 300 to 700 kb. Most genes identified to date have numerous short introns [5]. An active Pneumocystis genome project exists, and it is likely that homologies between Pneumocystis and other fungi will continue to be explored to identify Pneumocystis gene function. *Pneumocystis jiroveci* is an atypical fungus that causes PCP mainly in HIV-infected individuals. PCP is still a major cause of morbidity and mortality among HIV/AIDS patients and constitutes a worldwide problem [6]. While the incidence of PCP among HIV-infected individuals has decreased in developed countries, the prevalence of AIDS-related PCP in developing countries remains high and poorly controlled. PCP is the most common opportunistic respiratory infection in patients infected with HIV [7].

Materials and methods

The study was conducted in the department of TB and chest medicine government Stanley medical college, Chennai from 2016-2017. Thorough clinical examination including general and systemic examination was done meticulously with vital signs monitoring and SpO2 was measured with pulse oximetry.

Inclusion criteria

- All HIV seropositive inpatients with a CD4 count of less than 200 cells/µL.
- Age > 18 years.

Exclusion criteria

- HIV patients with CD 4 count > 200 cells/ µL.
- Age < 18 years.
- Patients with sputum positive pulmonary tuberculosis.

151 HIV seropositive patients were recruited for study as per inclusion criteria. Thorough clinical examination including general and systemic examination was done meticulously with vital signs monitoring and SpO2 was measured with pulse oximetry.

Clinical diagnosis

Clinical diagnosis of PCP was done based on the presence of following signs and symptoms suggestive of PCP.

Symptom

- Cough with or without sputum production for more than two weeks.
- Breathlessness.
- With or without fever.

Signs

- Tachypnea.
- With or without bibasilar crackles.
- SPO2 < 90 % in ambient air or after the exertion of 200-meter walking.

Chest X-ray PA view was taken for each and every patient. Radiological diagnosis of PCP was made based on the presence of any one of the following radiological features suggestive of PCP in chest skiagram: Pneumomediastinum, Pneumothorax,. Subcutaneous emphysema.

3 to 5 ml of sputum collection in the sterile container was done after hypertonic (3%) saline nebulization using a jet nebulizer for five minutes. This procedure was done in a separate room. One sample of sputum was subjected to Gomori Methenamine Silver staining and microscopic examination.

GMS staining method

The slides were kept in a microwave oven for 40 seconds in a solution of 10% chromic acid, washed with distilled water and then rinsed with 1% sodium metabisulphite for 30 seconds. Again the slides were washed with water and then placed in a Coplin jar containing 50 ml of Methenamine solution. Once again the slides were kept in a microwave oven for 65 seconds. After rinsing in water, the slides were treated with 1% silver chloride for 5 seconds. Again the slides were rinsed with distilled water, treated with 5% sodium thiosulfate for 1 minute and then counterstained with a light green working solution. Then they were cleared in xylene, covered with coverslips and subjected to light microscopy. Another sputum sample was subjected for Polymerase Chain Reaction (PCR) test targeting mitochondrial rRNA.

Polymerase Chain Reaction (PCR) test

Induced Sputum specimens were first exposed with a mucolytic agent 0.0065 M dithiothreitol (DTT) and then centrifuged. The pellets were resuspended in one-fifth of supernatant.200 ml of pellets were lysed in an equal volume of a lysis buffer containing 500mg of proteinase K. Then DNA extraction was done using a specific kit. The polymerase chain reaction was done by denaturation for 5 minutes at 94 degrees C followed by 35 cycles for one minute, 65 degrees C for 1 minute and 72 degrees C for one more minute in a thermocycler. Oligonucleotide primers Paz102 and Paz102 H were used. They amplify a 346 base pair region of the gene. The nested round was performed using AZ 102X as the forward primer and PAZ 102Y as reverse primer. This round amplifies a 267 base pair product. Separate rooms were used to perform DNA extraction, PCR mixture preparation, and DNA addition especially to avoid contamination. For handling reagent transfers aerosol barrier tips were utilized. All PCR products were put in 1.5% agarose gel and visualized under ultraviolet light. Sputum microscopy and PCR results were obtained in two days. All diagnosed patients were treated with oral TMP-SMX 15 mg/mg/day in three divided doses and patients on respiratory

failure were treated with corticosteroids additionally in intensive respiratory care unit along with non-invasive ventilation.

Statistical analysis

Data analysis was done using SPSS software V16. The results are expressed as percentage prevalence.

Results

Out of 151 HIV seropositive patients with low CD4 count (<200 cells/cubic mm) examined clinically with symptom analysis, respiratory system examination and pulse oximetry, 81 individuals were diagnosed as PCP patients but the sputum microscopy with Gomori methenamine silver staining which was taken as gold standard test diagnosed 41 cases of PCP only (**Table – 1**).

When Chest skiagram was as a diagnostic tool, radiological diagnosis of PCP was made in 40 patients out of 151 who had undergone chest skiagram. The calculated values for radiological diagnosis on comparing with GMS staining were as per **Table – 2**.

Table – 1: Clinical sputum microorganism sensitivity.

CLINICAL * SPUTUM_MICRO Crosstabulation

			SPUTUM_MICRO		
			NEGATIVE	POSITIVE	Total
CLINICAL	NEGATIVE	Count	61	9	70
		% within CLINICAL	87.1%	12.9%	100.0%
	POSITIVE	Count	49	32	81
		% within CLINICAL	60.5%	39.5%	100.0%
	Total	Count	110	41	151
		% within CLINICAL	72.8%	27.2%	100.0%

Table – 2: Radiological diagnosis.

RADIOLOGICAL * SPUTUM_MICRO Crosstabulation

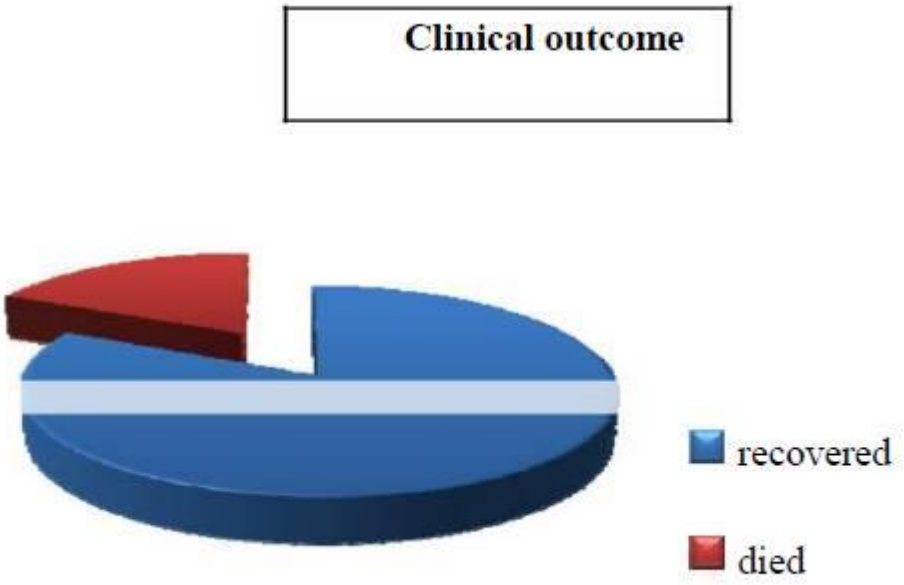
			SPUTUM_MICRO		
			NEGATIVE	POSITIVE	Total
RADIOLOGICAL	NEGATIVE	Count	95	16	111
		% within RADIOLOGICAL	85.6%	14.4%	100.0%
	POSITIVE	Count	15	25	40
		% within RADIOLOGICAL	37.5%	62.5%	100.0%
	Total	Count	110	41	151
		% within RADIOLOGICAL	72.8%	27.2%	100.0%

Table – 3: Polymerase chain reaction (PCR).

PCR * SPUTUM_MICRO Crosstabulation

			SPUTUM_MICRO		
			NEGATIVE	POSITIVE	Total
PCR	NEGATIVE	Count	107	0	107
		% within PCR	100.0%	.0%	100.0%
	POSITIVE	Count	3	41	44
		% within PCR	6.8%	93.2%	100.0%
	Total	Count	110	41	151
		% within PCR	72.8%	27.2%	100.0%

Graph - 1: Outcome of the study.



A confidence interval for sensitivity, specificity, positive predictive value, and a negative predictive value was calculated. Chi-square test showed the results of Radiological diagnosis of PCP were significant.

Out of 151 patients who had undergone induced sputum for PCR, Diagnosis of PCP was made in 44 patients (**Table – 3**).

Among the three methods including clinical evaluation, radiological examination, and induced sputum PCR, sputum PCR was having the highest sensitivity (100%), highest specificity (97%), highest positive predictive value (93%) and also highest negative predictive value (100%). Chi-square test showed the results of the PCR test were significant. PCR was positive in 2 patients who were missed in GMS staining. Those two showed a good clinical response to TMP-SMX therapy. Among them, in one patient clinical diagnosis was made and in another one was missed in a clinical and radiological evaluation.

Of the total 151 HIV seropositive patients included in our study, 81 were clinically diagnosed as suspected PCP. Of these 41 patients were confirmed microbiologically by sputum examination using Gomeri Methenamine Silver staining. 9 patients who were clinically negative for PCP (CD4 < 200) were positive microbiologically. These 90 patients were treated with TMP-SMX (20 mg/kg/day of TMP in three

divided doses). Patients who had severe PCP were treated with adjuvant corticosteroid along with non-invasive ventilation using a CPAP (Continuous Positive Airway Pressure) machine.

The total number of PCP patients diagnosed clinically was 90.74 of 90(82.2%) patients recovered from the illness after treatment (**Graph – 1**). 16 of 90(17.8%) patients died due to illness during the treatment. The average length of stay in hospital as inpatient was 21 days. The average length of stay in ICU (Intensive Respiratory Care Unit) was 7.5 days (n = 30). Among patients admitted in IRC, 15 of 90(16.7%) required Non Invasive ventilation (NIV).

Discussion

Kaneshiro ES, et al. have compared microscopic diagnosis and a real-time PCR for the diagnosis of PCP with induced sputum and BAL. They analyzed 39 patients with strong clinical suspicion of PCR. The concordance was 100% for BAL, but all the discrepant cases happened with induced sputum [8, 9]. Juan Torres, et al. have done a blind comparison of PCR in HIV patients. In that study, PCR was done without the knowledge of the diagnosis. Depending upon the intensity of the banding pattern, PCR results were graded from 'negative' to 3+. A positive result at grade 1 or higher for all 18 individuals (100% sensitivity), at grade 2 or higher for all 18 individuals (86.2% specificity). Fishman J A., et al. showed that staining methods and PCR having similar detection data in Bronchoalveolar lavage specimen but in bronchial washing specimen, PCR was superior [10]. Forrest DM, et al. [11], have compared 6 different PCR techniques, using BAL samples and showed nested PCR technique was the most sensitive assay for PCP diagnosis. A different single band 'touch down' PCR technique was demonstrated by Helweg Larsen, et al. as having sensitivity and specificity for diagnosing PCP in AIDS patients [11]. Rabodonirina, et al. [16] showed very good sensitivity (100%) but less specificity (77%) for rapid nested PCR technique in BAL samples of HIV patients. But in this study PCP diagnosis was done retrospectively. But in our study, we clinically and radiologically diagnosed PCP before doing PCR and staining. We used GMS staining as the gold standard [12]. G.W. Procop, et al. [13] have done a prospective observational study in non-HIV patients with pulmonary infiltrates. They compared PCR with the Giemsa staining and indirect immunofluorescence antibody. Results of their study showed the sensitivity of 87.2%, the negative predictive value of 98.7%, the positive predictive value of 51.5%. They concluded PCR performed similarly to conventional methods for PCP diagnosis with high negative predictive value. Like all the above-mentioned studies, our data are also showing high negative predictive value (100%) with negative predictive value (100%) with a confidence interval between 0.97 and 1.0 [13]. Elvin K, et al. have done a comparison study of RT-PCR, conventional PCR and different staining methods in BAL samples for PCP diagnosis sensitivity was 60% and specificity was 100% for staining, 100% sensitivity and 87% specificity for conventional PCR and 100% sensitivity and 84.9% specificity for RT-PCR respectively [14]. Moreover, they concluded RT-PCR is an expeditious method, which took less than three hours with high sensitivity and having the utility of determining a cut-off for differentiating carrier state and disease. Our study showed higher sensitivity than most of the studies for GMS staining in induced sputum samples to diagnose PCP. A consensus statement by the University of California expert panel showed the reduction in mortality, oxygenation and respiratory failure in moderate-to-severe PCP [15]. In a clinical study, M. Rabodonirina, et al. has shown overall mortality of 11.6% for hospitalized PCP patients and 29% mortality especially for patients admitted in ICU. Several studies have demonstrated the same results [16]. On comparing to this data, our study has shown slightly higher mortality (17.8%). The patients who presented with PCP and diagnosed as HIV seropositive are (48) more than known HIV seropositive patients came with PCP on comparing to those studies [17]. Bartlett MS, et al. demonstrated in the pre HAART era, PCP

found in newly diagnosed HIV patients had a more severe form of PCP with low PaO2 and higher requirement of mechanical ventilation on comparing to PCP in known HIV patients [18]. The reason postulated in Fishman JA, et al. [19] study may be the explanation for the high mortality in our study. The known HIV seropositive patients with CD 4 lymphocyte count less than 200 cells/cubic mm were on cotrimoxazole prophylaxis along with HAART according to NACO guidelines [19, 20].

Conclusion

The sensitivity (78%), specificity (55%) and positive predictive value (40%), negative predictive value (87%) for clinical diagnosis of PCP in HIV seropositive patients are less on comparing with sputum GMS staining. The chest x-ray diagnosis of PCP in HIV seropositive patients is also having low sensitivity (60%), specificity (86%), PPV (62%) and NPV (86%) when comparing to GMS staining. For PCR in the diagnosis of PCP in HIV seropositive patients, sensitivity (100%), specificity (97%), PPV (93), NPV (100%) are all showing highest values than radiological and clinical diagnosis. PCR or Gomori Methenamine silver staining should be used for diagnosing PCP whenever possible and to detect all cases of PCP in HIV patients without missing. Among 90 patients who had treatment for PCP, the mortality rate was 17.8% and 82.2% of patients showed clinical improvement.

Acknowledgments

The authors would like to thank the Professors, Associate Professor, and Postgraduate students, Department of Pulmonary Medicine, Govt. Stanley Medical College, Chennai for helping with data collection and laboratory analyses.

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