ROLE OF CLINICAL METHODS, CHEST SKIAGRAM, SPUTUM MICROSCOPY AND PCR IN DIAGNOSIS OF PNEUMOCYSTIS JIROVECI PNEUMONIA IN HIV PATIENTS WITH CD4 COUNT LESS THAN 200 AND THE CLINICAL OUTCOME -A COMPARATIVE STUDY IN a TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that the dissertation on **"ROLE OF CLINICAL METHODS, CHEST SKIAGRAM, SPUTUM MICROSCOPY AND PCR IN DIAGNOSIS OF PNEUMOCYSTIS JIROVECI PNEUMONIA IN HIV PATIENTS WITH CD4 COUNT LESS THAN 200 AND THE CLINICAL OUTCOME - A COMPARATIVE STUDY IN TERTIARY CARE HOSPITAL"** is a record of research work done by **DR.S.B.SIVARAJA** in partial fulfilment for M.D.(PULMONARY MEDICINE) Examination of the Tamilnadu, Dr.M.G.R.Medical University to be held in April 2013.The period of study is from January 2012 to May 2012.

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DECLARATION

I hereby declare that the dissertation entitled **"ROLE OF CLINICAL METHODS, CHEST SKIAGRAM, SPUTUM MICROSCOPY AND PCR IN DIAGNOSIS OF PNEUMOCYSTIS JIROVECI PNEUMONIA IN HIV PATIENTS WITH CD4 COUNT LESS THAN 200 AND THE CLINICAL OUTCOME -A COMPARATIVE STUDY IN TERTIARY CARE HOSPITAL"** submitted for the Degree of Doctor of Medicine in M.D., Degree Examination, Branch XVII, PULMONARY MEDICINE is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship, fellowship or similar other titles. It had not been submitted to any other university or Institution for the award of any degree or diploma.

Place: Chennai Signature of the Scholar

Date: **(Dr.S.B.SIVARAJA)**

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ROLE OF CLINICAL METHODS, CHEST SKIAGRAM, SPUTUM MICROSCOPY AND PCR IN DIAGNOSIS OF PNEUMOCYSTIS JIROVECI PNEUMONIA IN HIV PATIENTS WITH CD4 COUNT LESS THAN 200 AND THE CLINICAL OUTCOME -A COMPARATIVE STUDY IN TERTIARY CARE HOSPITAL

Background :

Pneumocystis jiroveci Pneumonia (PCP) is one of the most predominant opportunistic infections occurring in HIV patients with CD4 count is less than 200.Even though Chest radiograph findings may be normal in 10-39% of PCP patients, it is commonly used for diagnosis in resource limited settings. The other diagnosis methods are Gomori Methanamine Silver(GMS) staining, PCR. Among them, GMS is considered as the gold standard test in diagnosing PCP.

AIM & OBJECTIVES:

1) Comparing 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.

2) To know the clinical outcome of PCP patients after treatment in our centre

STUDY DESIGN:

Prospective cohort study

INCLUSION CRITERIA:

1) All HIV seropositive inpatients with CD4 count less than 200 cells/µL

2) Age > 18 years

EXCLUSION CRITERIA:

1) HIV patients with CD 4 count > 200 cells/ µL

2) Age < 18 years

3) Patients with sputum positive pulmonary tuberculosis

METHODS:

1) Recruitment of patients as per inclusion criteria

2) Thorough clinical examination & pulse oximetry

3) Taking Chest x ray PA view

4) sputum collection after hypertonic saline nebulisation

5) Subjecting one sample of sputum for Gomori Methanamine Silver staining &another sample for Polymerase Chain Reaction

7) Follow up the clinical course of patients after treatment

8) Analysis of data using SPSS

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 is having the highest sensitivity (100%), highest specificity (97%),highest positive predictive value (93%) and also highest negative predictive value(100%).

Among 90 PCP patients diagnosed clinically ,74 of 90(82.2%) patients recovered from the illness after treatment&16 0f 90(17.8%) patients died due to illness.

Conclusion:

PCR or GMS staining should be used for diagnosing PCP whenever possible and to detect all cases of PCP in HIV patients without missing.

CONTENTS

INTRODUCTION

Pneumocystis jiroveci Pneumonia is one of the most predominant opportunistic infections occurring in immunocompromised individuals especially HIV seropositive patients.[1] Occurrence of Pneumocystis jiroveci Pneumonia is usually seen when the CD 4 lymphocyte count is less than 200 cells /microliter.

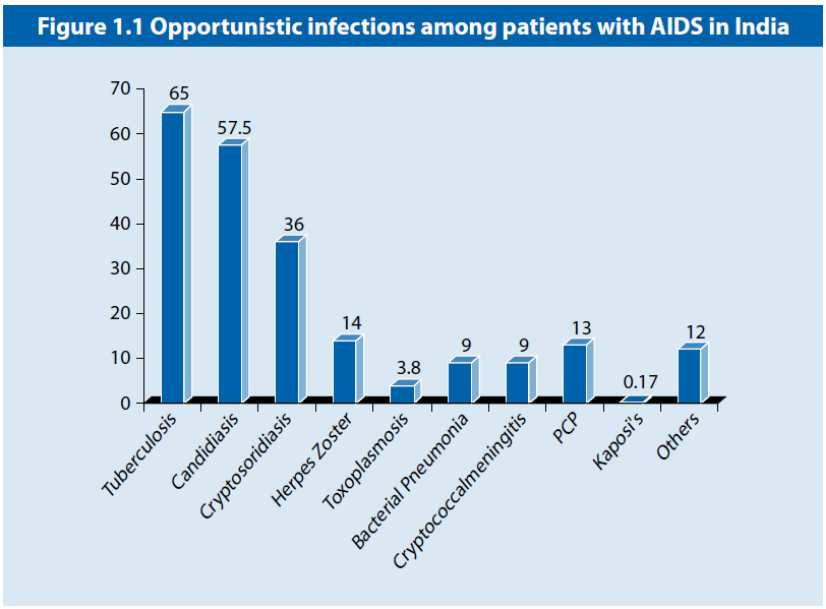
Before the era of prophylaxis, PCP was seen in 60 % of people as an AIDS defining illness and also it was noted in 80% of people living with HIV/AIDS.

The incidence of PCP is in a decreasing trend because of the prophylaxis, early diagnosis of HIV/AIDS by well-organized Government programme and HAART (Highly Active Anti-Retroviral Therapy).

An US study demonstrated that the PCP incidence had declined from 13 % in 1994 to 3 %3.[2]A Swiss study also noted the decline in PCP incidence (i.e. 30% reduction) during the period of 1995-97.[3]

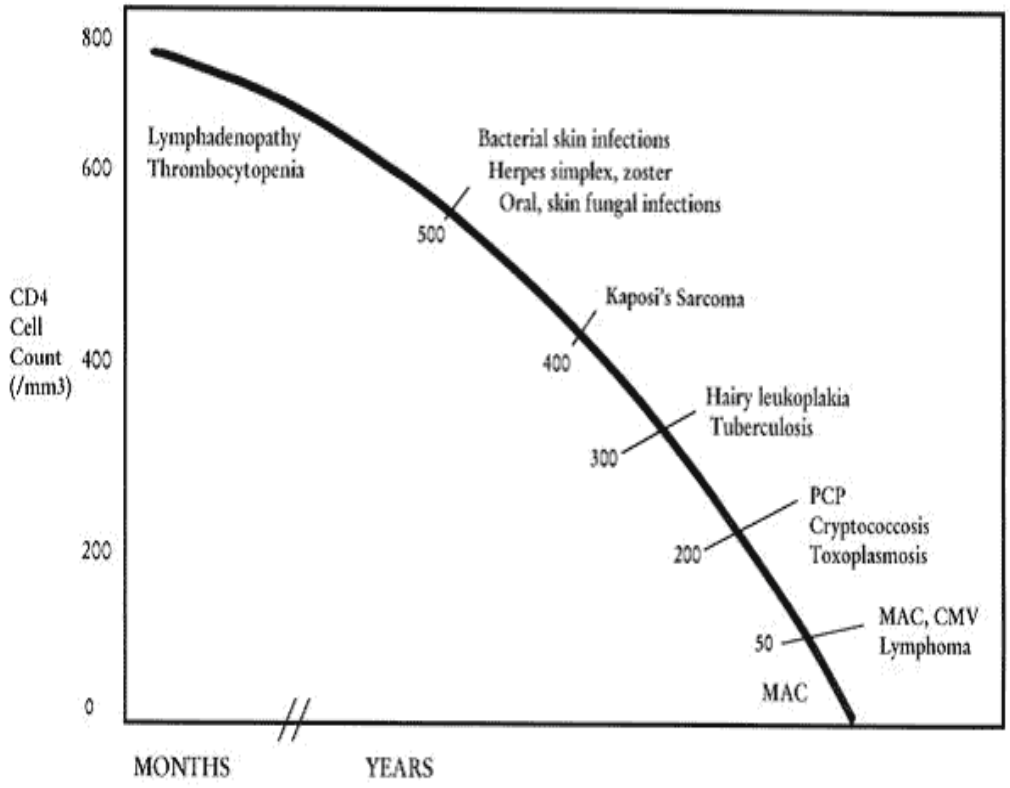
In India, PCP affects 20 to 30% of AIDS patients and the mortality due to PCP is around 20 to 40 %. Figure 1.1 shows the occurrence of various opportunistic infections in India.

Figure 1.1



PCP usually occurs when the CD 4 count becomes less than 200 cells/cubic millimeter.[fig 1.2]

Figure 1.2



The clinical features of PCP are non-specific. The onset is usually indolent. The most common symptoms are cough mostly without sputum production, breathlessness and fever.

Respiratory systemic examination may be normal at rest in mild cases. Tachypnea, tachycardia and basal crackles are the common signs elicited in clinical examination.

Chest skiagram may be normal in 10 to 39% cases. The common radiological manifestations seen in chest skiagram or computed tomogram are

1. Bilateral diffuse perihilar infiltrates
2. Bilateral airspace consolidations
3. Pneumotoceles
4. Pneumomediastinum
5. Pneumothorax
6. Subcutaneous emphysema

Clinical manifestations and radiological features are used as diagnostic methods in resource limited settings. However, the diagnostic method of choice is demonstration of organism in clinical specimen. If sputum is not available, induction of sputum production with hypertonic saline or bronchoalveolar lavage is done.

Since Pneumocystis jiroveci is a non-cultivable fungus, sputum microscopy using various staining methods including Gomori's

Methenamine Silver(GMS), Giemsa, toluidine blue and certain fluorescent brightners like calcoflour white and is used worldwide. Among them Gomori's Methenamine Silver staining is considered as gold standard.

PCR can be more efficient than immunofluorescence technique.[4]It was first evaluated by Wakefield et al. to increase the sensitivity and specificity to the PCP diagnosis.[5]

In resource limited developing countries, because of lack of availability and cost factor, instead of microbiological and PCR testing, clinical manifestations and radiological features are used for diagnosing PCP. And also microscopic examination and PCR testing are labour intensive requiring trained personnel and infrastructure.

Our NACO (National AIDS Control Organization) guidelines are also following the presumptive diagnosis of PCP and insist on earlier treatment.

So there is a necessity to evaluate the role of clinical diagnosis, radiological diagnosis, sputum microscopy using Gomori's Methenamine silver staining and PCR technique in diagnosis of PCP in HIV

seropositive individuals especially with CD4 T lymphocytes count < 200 cells/cubic mm.

This study has been done in Government Hospital of Thoracic Medicine (GHTM), Tambaram Sanatorium, Chennai. The hospital has 33 wards, of which 11 wards are especially for HIV patients, and 22 wards have patients with TB and other respiratory diseases in addition to the Outpatient Departments (OPDs).

GHTM is not only a premier institution attached to Stanley Medical College but also is uniquely placed as a Centre of Excellence (COE) in HIV/AIDS training because of the lot of complex clinical cases presenting every day at GHTM. This institution is having NABL accredited microbiology laboratory.

This is a reputed tertiary care center for HIV / AIDS patients in Tamilnadu. GHTM is well equipped to deal with every type of HIV-related illness and to provide high-level care and support for all chest diseases.

So there is necessity to evaluate the role of clinical diagnosis, radiological diagnosis, sputum microscopy using Gomori's Methenamine silver staining and PCR technique in diagnosis of PCP in HIV seropositive individuals especially with CD4 T lymphocytes count < 200 cells/cubic mm.

REVIEW OF LITERATURE

Pneumocystis jiroveci:

Pnuemocystis organism was first identified and reported by Chagas in 1909**(6).** It was mistaken for Trypanasoma cruzi's morphological form, but in further studies it was well established that the organism was not a trypanazoma and it was a new species, named Pneumocystis carinii [7]**.**

Because of molecular biological techniques, Phylogenetic determinations based on gene sequences allowed the fungal identity of the genus and gave the species distinction. Among them, Pneumocystis carinii infects rat but Pneumocystis jiroveci is the species infecting humans. [6]

In 1976 Frenkel was the first researcher who proposed the name Pneumocystis jiroveci.

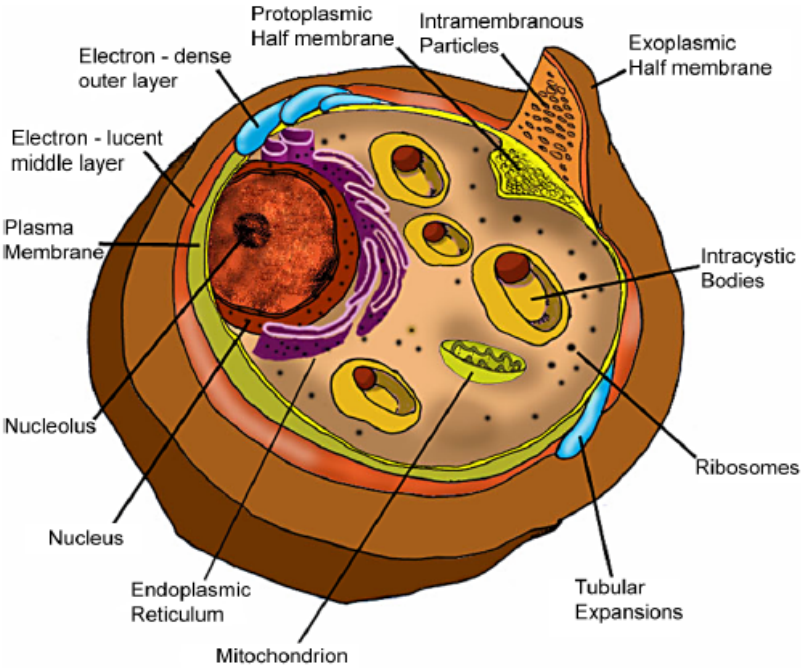
Many clinicians and researchers raised issues about the nomenclature change from Pneumocystis carinii to Pneumocystis jiroveci. Inspite of these controversies, scientific and biological evidences made general acceptance of the current nomenclature. [7]

Three Structural forms of Pneumocystis jiroveci are

1. Cyst form
2. Sporozoite
3. Trophozoite

Cyst form is spherical or ovoid in shape and diameter is 4-6 micro meter and contains up to 8 sporozoites. The structure of the cystic form of PCP is shown in fig 2.1

Figure 2.1



Trophozoite is thin walled represent as excysted sporozoite. This pathogen is specific to humans; it will not infect other animals. Other species parasitize in animals have not shown to infect human.[8]

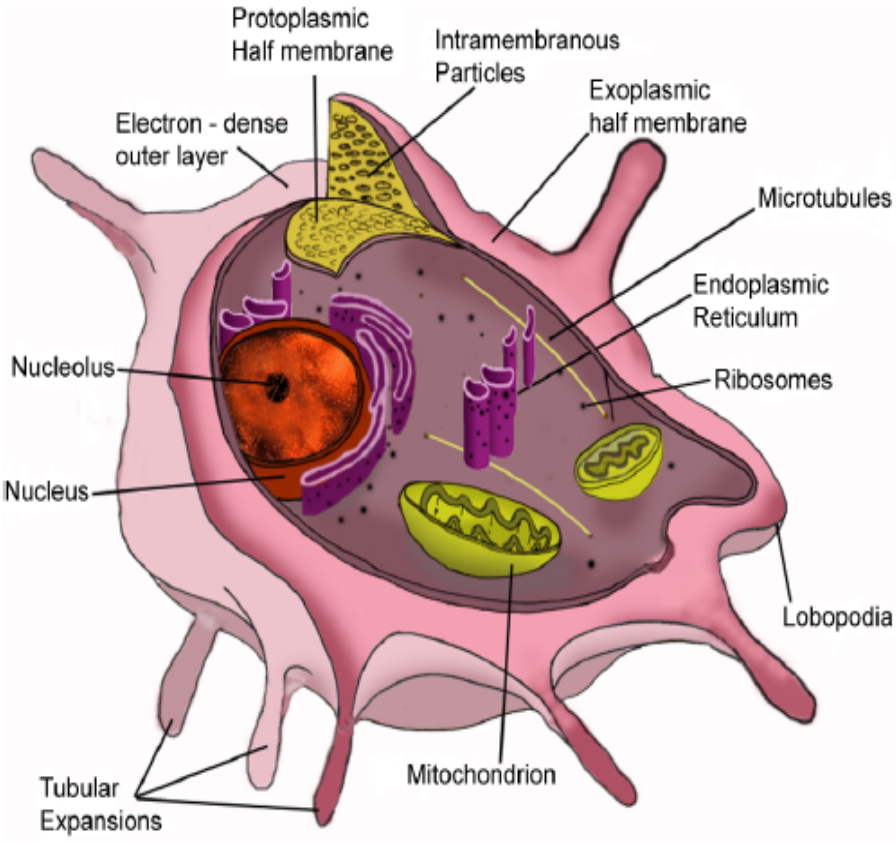
Structure of Pneumocystis jiroveci:

Cell wall consists of 3 layers, outer layer made up of glycoprotein. It helps in the attachment of pneumocystis to host cell [type 1 pneumocyte]. Surface glycoprotein has different molecular weight these are 110 to 120, 45 to 50, 35 to 45, 20 to 27 KD.

Most prominent feature of human derived pneumocystis is staining intensely in band between 35 to45 KD and it is the most common band found in lung and bronchoalveolar fluid of patient infected with pneumocystis.

The structure of Pneumocystis jiroveci trophozoite is shown in figure 2.2.

Figure 2.2



The cell wall contains cholesterol not ergosterol. Although classified as fungus, P.jirovecii does not contain ergosterol, instead it synthesizes two distinct sterols called 24 alkyl sterol [(24-methylcholest-7-en-3β-ol and 24-ethylcholest-7-en-3β-ol] which it can scavenge from lung macrophages.[9]Thus pathogen specific sterol is important for survival and proliferation.

The inefficacy of azoles against p.jirovecii is explained because of 24 alkyl sterol synthesis by the organisms. Mammals cannot synthesize 24 alkyl sterol so their biosynthesis in p.jirovecii is attractive target for the development of drugs against it.

Cell wall is carbohydrate rich with glucose, mannose and β-1, 3-glucon. β-1, 3-glucon synthase which mediate polymerization, so inhibitors of β-1, 3-glucon synthase are effective in clearing cyst of pneumocystis from lung of infected patient.

Signal transduction found in pneumocystis are cdc2 cyclin derived kinase, cdc13 B-type kinase, cdc25 mitotic phosphatase and pneumocystis mitogen activated protein kinase.

After adhering to alveolar epithelium it activates signalling pathway which is responsible for mating and proliferation of organism. Currently some of the molecules are under intense study which includes dihydrofolate reductase, thymdylate synthase, inosine monophosphate dehydrogenase and lanosetrol 14α-demethylase.

	PCP cases	Patients	Rates (%)
Lymphomas	34	9,907	0.34
Acute lymphocytic leukemia	5	2,929	0.17
Other leukemia	16	5,023	0.32
Solid tumors	30	26,085	0.11
Cerebral tumors	21	3,098	0.68
Bone marrow transplantation	22	1,348	1.63

LIFE CYCLE:

There are various articles on the life cycle of *P.jirovec*ii with different views and developmental stages. Wanderley de Souza et al have demonstrated a life cycle by incorporating the details and data were obtained using electron microscopy. This has been widely accepted (Figure 2.3).

There is no clear data about the infective form of *P.jirovec*ii. But the well accepted route of transmission is airborne.

Life cycle is characterised by two different developmental stages [17]

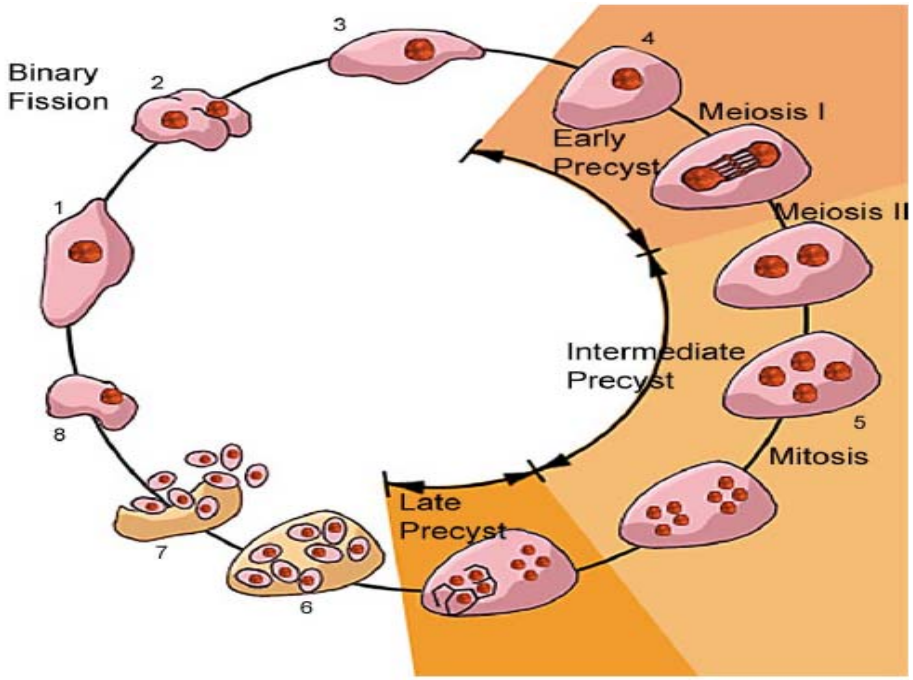
1. Mature cyst
2. Trophozoite

The origin of Trophozoite form is directly from the mature cyst which contains eight spherical intracystic bodies, which in turn developed into 8 trophozoites.

Two other intermediate cystic forms have been demonstrated including developing or empty cysts. Some of the trophozoites mature and develops into cyst. Thus the cycle is repeated.

Sexual and asexual intermediate forms have been also described in-between.

Figure 2.3



Most of the scientific papers postulating the life cycle are based on animal studies or by passing the organism in tissue culture. But the organism especially *P.jirovecii* the human derived species has not shown growth consistently in vitro

MOLECULAR BIOLOGY:

Both unique and some common antigens are expressed by *P.jirovecii* in different hosts. By genetic differentiation so many surface glycoprotein (MSG) types has been demonstrated in humans. And also GP 45-55 may have significant role in human infection

Each genomic copy of an MSG has an upstream highly conserved expression site. And also it includes a specific downstream segment encoding the differentiating antigenic property of each clone.

EPIDEMOLOGY:

PCP occurs in four groups of immune compromised host:

1. Congenital, caused by defective antibody synthesis
2. Induced, by drugs causing immunosuppression including corticosteroids
3. Acquired, affecting HIV population as an opportunistic infection

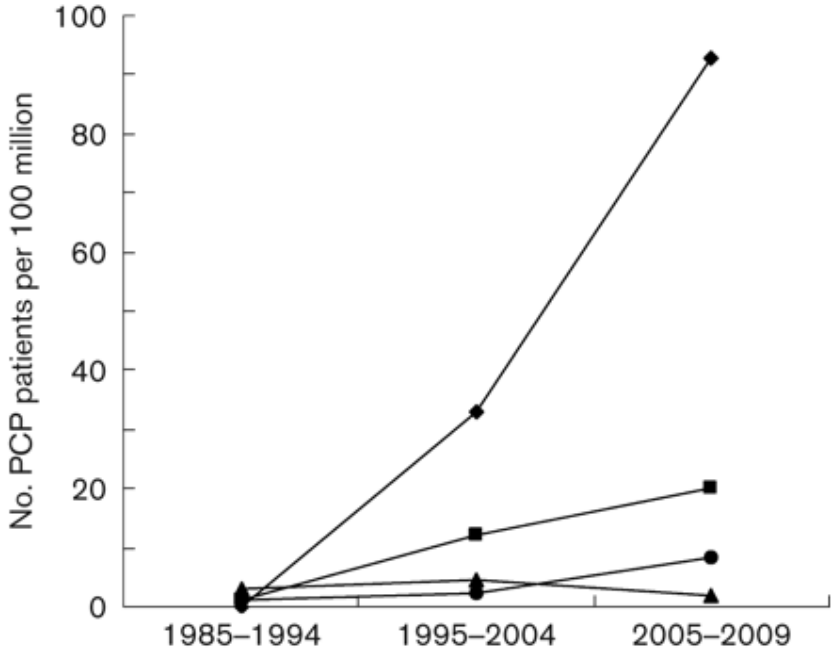
4. Nutritionally deficient, usually seen in neonates as epidemic

CONDITIONS WITH RISK OF PCP:

1. HIV/AIDS
2. Organ transplantation
3. Hematologic manifestations
4. Prolonged steroid therapy
5. Radiotherapy
6. Congenital immunodeficiencies
7. Protein energy malnutrition
8. Prematurity

A retrospective study in 116 HIV seronegative individuals with PCP shows 30.2% associated with hematologic malignancies, 25% with organ transplant, 12.9% with solid malignant tumours and 9.5% with other disorders.

A retrospective study in China showed the increase in incidence of PCP.



Epidemiologic research in paediatric population suggests that PCP can occur in children as a result of simultaneous viral infection.

Beard et al described different strains in infants on comparing to HIV patients with PCP.

With the help of genetic probes several molecular studies conducted in animals and humans have suggested that both reinfection and reactivation of latent *P.jirovecii* infection play a role in incidence of disease in treated PCP patients.

Virulence and pathogenesis of p.jirovecii

Attachment of trophozoite form of p.jirovecii to lung epithelial cells (type 1 pneumocyte) plays critical role in development of disease in human .Attachment is mediated through variety of molecules including fibronectin, vitronectin, laminin, and mannose receptor.

Binding is facilitated by p.jirovecii extracellular matrix. Majority of alveolar surface is covered by type 1 pneumocytes and it is responsible for gas exchange.[18]

In the susceptible host attachment of p.jirovecii causes secretion of proteolytic enzymes such as chymase or reactive oxygen species which leads to proliferation of the organism and impaired lung cell replication

P.jirovecii induces alteration in bio physiology of lung surfactant and may have effect on alveolar macrophages and lung monocyte.

Organism by coating itself by host derived glycoprotein such as surfactant protein – A and soluble form of the macrophage mannose receptor leads to delay in recognition and destruction by host immune system.

P.jirovecii replicates extracellularly causes host cell damage, resulting in cell lysis and rupture which leads to impairment in oxygen diffusion. Lung basement membrane damage generates a characteristic foamy exudates and interstitial leukemic infiltration.

Granulomatous inflammation is seen in up to 5% of lung biopsy from human immunodeficiency virus (HIV)-infected patients

P.jirovecii fails to cause disease in normal host because it is an opportunistic pathogen .It causes disease only when host immune system is compromised.

Dr Icenhour has shown that p.jirovecii has melanin in their cell wall it causes alteration in host immune response and melanised pneumocystis retains viability better than native pneumocystis when incubated with phagocytes

CLINICAL FEATURES:

The symptoms and signs of PCP may be subtle. High index of suspicion is required for diagnosis especially in HIV negative individuals. Only 50% of patients report the classic triad of dry cough, exertional dyspnoea and fever.

In a clinical review of 93 PCP patients, only 53% had the classical triad of cough, breathlessness and fever.

Usually the progression of symptoms is slow. It takes over weeks to months to progress. But in HIV seropositive patients there is rapid onset and progression of symptoms.

The patients who had the prior history of PCP episode can mostly identify a recurrent episode but the patients who are having a new episode of PCP usually refer it as non-resolving severe cold.

Systemic examination of respiratory part may be normal or fine dry crackles can be heard on auscultation. Spontaneous pneumothorax may be seen in 2-6% of cases.

Serum LDH elevation can occur but its non-specific. It can also be used as evidence of severity. It also has a prognostic role as demonstrated by Forrest et al.[11]

Chouaid et al found hypoxia in almost all patients with PCP in a study.[12]

SpO2 measurement may show a decrease <90% in rest or during exercise. Arterial blood gas analysis usually shows reduced PaO_2 (hypoxemia) or an increased alveolar to arterial difference ([A-a] DO_2).

GRADING PCP BY ABG:

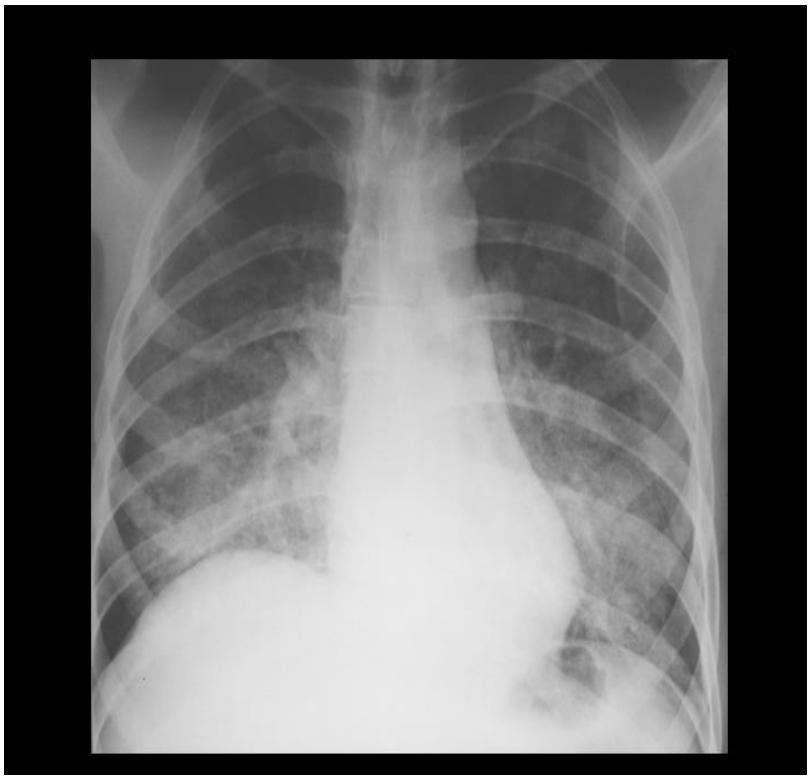
SEVERITY GRADING	[A-a]DO_2	PaO_2(mmHg)
MILD	<35	>70
MODERATE	35-45	>70
SEVERE	>45	>50

RADIOLOGICAL FEATURES:

The chest skiagram plays a vital role in the diagnosis of PCP. There is no pathognomonic radiological feature or pattern for PCP diagnosis.[19] Chest X-ray may be normal in 10-39% individuals.

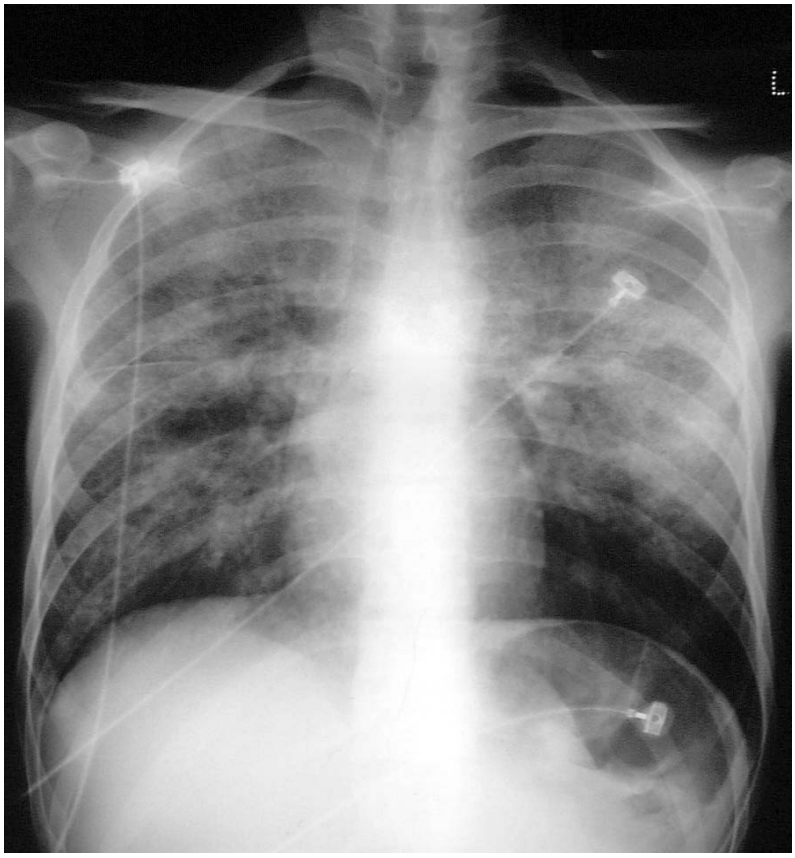
The common radiological pattern seen in chest skiagram is bilateral, perihilar, diffuse infiltrate. It may progress to consolidation pattern involving upper and lower zones (Figure 2.4). Air bronchogram may be appreciated. Pneumatoceles and cyst may be seen.

Figure 2.4



Depending upon severity and duration of illness, pneumothorax, pneumomediastinum, and subcutaneous emphysema can occur (Figure 2.5).

Figure 2.5

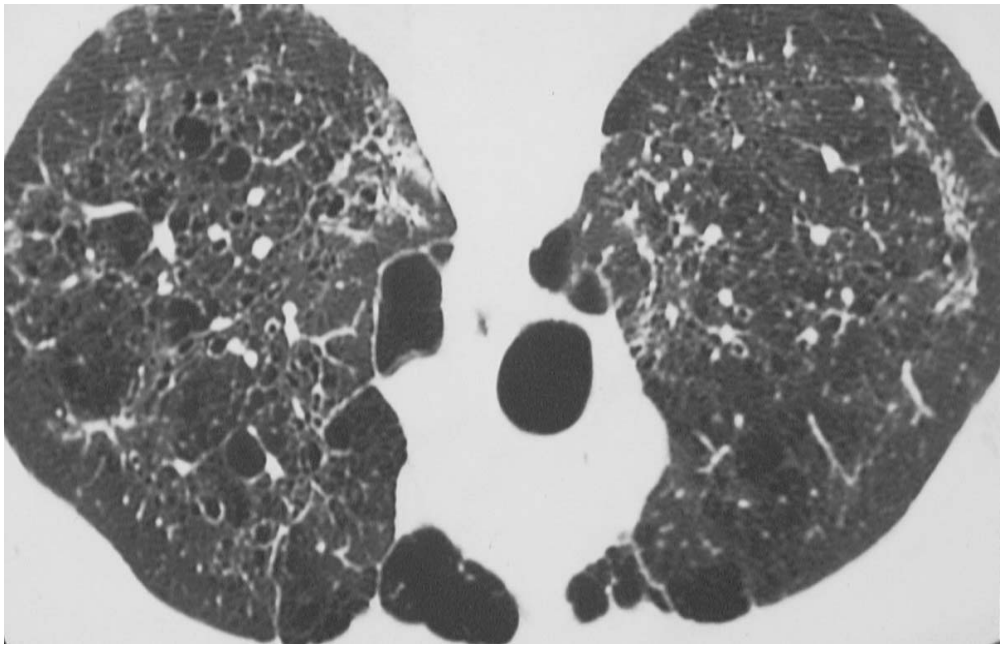


Unusual patterns like nodules, unilateral infiltrates or lobar consolidation may mimic atypical pneumonia

Upper lobe predominance is noted with the presence of cystic changes in patients those who underwent prophylaxis or therapy with inhaled pentamidine.

In HRCT images the ground glass attenuation is the hallmark of PCP. More than 90% of patients show GGO which denotes an exudative alveolitis. GGO refers to parenchymal opacification not obscuring underlying pulmonary architecture (Figure 2.6).

Figure 2.6



Mosaic attenuation is seen in around 56% patients. Interlobar septal thickening, small cysts, mediastinal emphysema, pneumothorax, subcutaneous emphysema can be seen in HRCT chest images.

A review of HRCT chest in 39 patients by Kuhlman et al showed GGO (26%), ILD (18%) and bilateral patchy parenchymal opacity (56%) in PCP patients.

Along with clinical features the presence of GGO on HRCT chest images in HIV patients with CD4 count less than 200 cells/cumm is virtually diagnostic of PCP with around 94% accuracy.

Staining methods:

Gomori Methenamine blue [GMS], Giemsa, toludine O blue, papanicolaou, fungi fluor & immunofluorescent antigen[IFA].

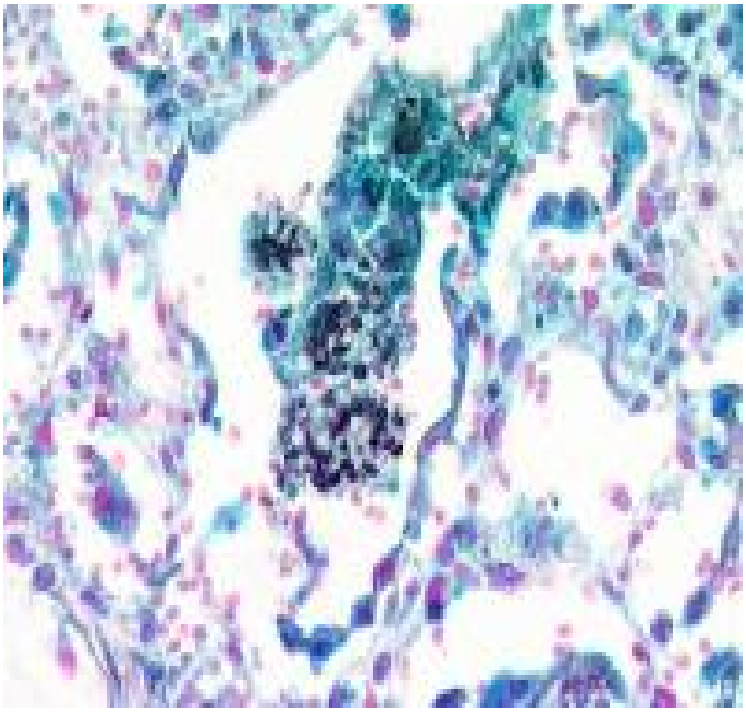
However GMS &IFA are gold standards for detection of p.jirovecii. In fungi fluor stain cyst shows parenthesis or comma like internal dots surrounded by cyst walls, one must know these two structures before diagnosing them as p.jirovecii.

In Gram stain Cyst appears as vacuolated unstained round structure &trophozoites as pinkish darts

In Giemsa stain Trophozoites as dotted particles that are small and pleomorphic showing pale blue cytoplasm and nucleus as purplish in colour. Cysts appear as rounded and vacuolated.

In Gomori Methenamine stain Cyst appears dark staining (greyish brown or black), collapsed with cup shaped appearance. Sometimes black dots appear in cysts (Figure 2.7).

Figure 2.7



Papanicolaou stain:

It is non-fluorescence stain so cyst structure appear clusters of haloes.

Immunofluorescence stain:

Round to oval shaped cyst embedded in extracellular matrix trophozoite and sporozoite appear as fluorescent apple green colour

Stain	sensitivity	specificity
calcofluor	73.8%	99.6%
Gomori methenamine silver	79.4%	99.2%
Diff –quik	49.2%	99.6%
Merifluor	90.8%	81.9%

Only Calcofluor and Gomori's Methenamine stain had positive and negative predictive values of $\geq 90\%$.

Calcofluor white is a fluorescent stain, in which the active ingredient is cellufluor. The cellufluor non-specifically binds to beta-linked polysaccharides like chitin and cellulose. This method is used for the direct visualization of fungi in clinical samples.

Immunofluorescent staining methods utilizing antibodies directed against Pneumocystis jiroveci is also being used in many centres to directly visualise the organism in clinical samples.

Procop et al., demonstrated in their study that the Calcofluor white stains and GMS stains had the best parameters to use routinely in PCP diagnosis. Even though these staining methods were less sensitive than the immunofluorescent assay, they had high specificity and more acceptable predictive values.[13]

LUNG BIOPSY:

VATS affords the same yield as open lung biopsy done via thoracotomy.

Especially in HIV seronegative patients, the invasive techniques are often needed to diagnose PCP.

The demonstration of organism is essential in transplant recipients. In the adult patients, the disease predominantly occurs as an alveolar process. The airspaces are usually filled with foamy eosinophilic exudative material and appear to be honeycombed.

The alveolar exudate consists of organisms itself, lot of surface glycoprotein, proteinaceous exudate and debris of macrophages and other inflammatory cells. Simultaneously, the alveolar interstitium is infiltrated by polymorphonuclear leukocytes and lymphocytes.

PULSE OXIMETRY:

Pulse oximetry used in exercise test may be useful in excluding PCP in doubtful cases.

In a study, exercise testing in 85 HIV seronegative patients with suspected PCP found that 3% desaturation with exercise was 100% sensitive and 70% specific[12].

This can be used as a good screening tool in outpatient department.

Arterial blood gas analysis can be done and utilized as a tool for severity grading.

PCR:

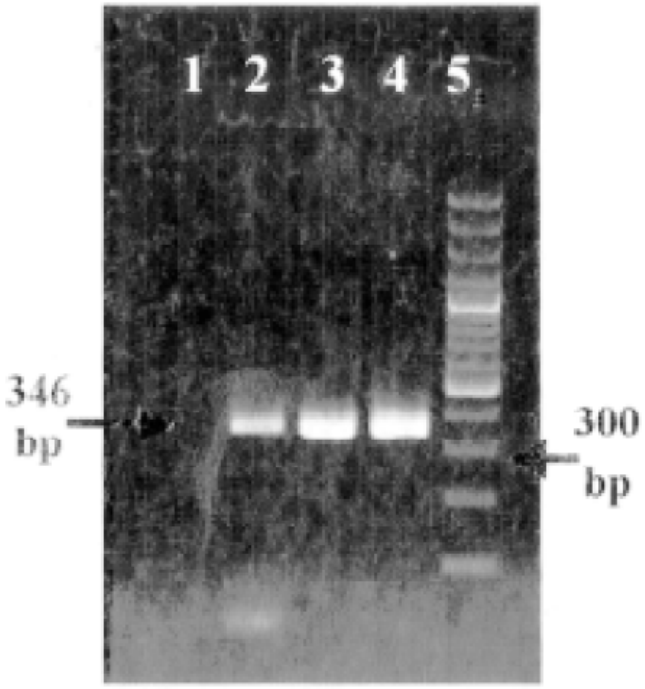
In the 1990s, PCR was introduced and it facilitated the diagnosis of P. jiroveci by showing increased sensitivity, specificity and predictive values, and compared to staining techniques and monoclonal antibodies and by reducing the necessity for trained personnel to differentiate P. jiroveci from common artefacts and other non-specific staining.

Even though the results of PCR on routine sputum examination, induced sputum with hypertonic saline are differing from BAL specimens, it is likely to be more efficient in diagnosis of PCP when comparing to the immunofluorescent test.

The use of PCR for diagnosis of PCP has been increasingly reported on in the past ten years.

PCR is valuable tool for describing natural history of disease and the PCP epidemiology (Figure 2.8).

Figure 2.8



The prophylactic therapy given for PCP may decrease the yield of microscopic diagnostic method

Various clinical studies showed that PCR is more sensitive than conventional diagnostic methods[14, 15].

Meja Rabodonirina et al have documented the sensitivity and specificity for the nested PCR method as 100% and 77% respectively. The same study showed the positive predictive value and negative predictive value were 54% and 100% respectively[16].

DNA extraction and amplication are the two essential steps of PCR.

After DNA extraction by denaturation in a thermocycler, DNA amplication using primers, T_{99} polymerase were done usually in two steps. DNA extraction and amplication are usually done in three separate rooms to avoid contamination with the specimen.

At last, PCR end products are subjected to agarose gel electrophoresis. Then they are stained with ethidine bromide and finally visualised under ultra violet light.

Barbara Alexander et al has reported the sensitivity (93%), specificity (91%), positive predictive value (59%) and negative predictive value (99%) for PCR diagnosis of PCP using pneumocystis MLSU (mitochondrial ribosomal large sub-unit) as the target specimen.

In recent years, large number of research papers is demonstrating *P.jirovecii* genes amplication by PCR.[20]

The gene encoding mitochondrial rRNA, chromosomal rRNA and thymidylate has been implicated in various PCR studies. They have improved the sensitivity of detecting *Pneumocystis jiroveci.*[21]

Cartwright et al have showed in a study, in 133 BAL specimens the sensitivity of PCR was 100% (21 of 21 samples) and specificity of 99% (132 of 133 samples). In that same study, the specificity of PCR in induced sputum was 98%.

In some studies comparing microscopy and PCR for PCP diagnosis, no real advantage of nested PCR, with regard to sensitivity and specificity compared to those of microscopic diagnosis, could be reported when bronchoalveolar samples were examined.[45, 46, and 47]

But for HIV patients the majority of studies reveal higher sensitivity for both single and nested PCR than for microscopic staining when induced sputum is obtained.

The first step in the diagnosis of PCP is the realization that the patient is at risk for the opportunistic infection.

The very first procedure should be a routine sputum smear examination for Gram staining, Acid fast staining and fungal staining and cultures.

Then, the choice of diagnostic test depends on the patient status that is the ability to cooperate with sputum induction, the distribution of respiratory disease, and the urgency of diagnosis.

Induced sputum should be obtained whenever possible to diagnose PCP. [23]

If the patient is on respiratory failure or the sputum examination is negative, BAL should be done as earlier as possible[24]

EXTRAPULMONARY PNEUMOCYSTOSIS:

Two reviews from New York revealed that incidence of extra pulmonary pneumocystis jiroveci infection is 0.5 % from 3%, but it is usually underdiagnosed if patient dies without post mortem.

The common sites involved are reticuloendothelial system (lymph nodes, spleen, liver and bone marrow) Gastro intestinal tract and genito-urinary tract.[25]

It may develop concomitantly with PCP or independently.

Differential diagnosis of P.jiroveci pneumonia

Tuberculosis

Viral Pneumonia

Mycobacterium interacellulare (MAC) Infection

Acute Respiratory Distress Syndrome

Mycoplasma Infections

Cytomegalovirus

Lymphocytic Interstitial Pneumonia

Pulmonary Embolism

Legionellosis

TREATMENT:

Therapy of HIV infection has undergone remarkable changes in the past 10 years with advances in HAART and prophylactic therapy and associated significant improvements in survival. With these advances, the incidence of PCP has remarkably fell down, but it is still the most common opportunistic infections among HIV seropositive patients.

Treatment should be initiated as soon as the presumptive diagnosis of PCP is aimed because the infection is rapidly progressive and the mortality rate is quite high.

The drug of choice of PCP is trimethoprim-sulfamethoxazole. It is the agent of choice for extra pulmonary infection in all individuals.[26]

TMP-SMX has the tremendous penetration in tissues and expeditious clinical response.

The usual dose is 15 to 20 mg/kg/day of trimethoprim. Oral administration is having comparable bioavailability with that of intravenous therapy.

TMP-SMX sequentially blocks two enzymes in folate metabolism essential for DNA synthesis: 1.Dihydrofolate reductase (DHFR) 2.Dihydropteroate synthetase (DHPS)[27.]

If there is severe hypoxia or gastro-intestinal disturbances, intravenous TMP-SMX therapy administration should begin.

Serum SMX level above 200microgram/ml is associated with adverse effects particularly bone marrow suppression.

It is not necessary to discontinue therapy because of mild side effects including rash, neutropenia, and liver enzymes elevation if the patients is tolerating well.

Nephrotoxicity is the major adverse event occurring frequently in the renal transplant recipients. This can be both dose related event and also idiosyncratic. The dose of TMP-SMX reduction should be done. 3-5 mg/kg/day are sufficient for patients with GFR around 10-50 ml/min.

Pentamidine isethionate is the alternate drug for PCP treatment. The usual dose is 4mg/kg/day as slow intravenous infusion in 5% dextrose.

Pentamidine attains the therapeutic concentrations in the lung parenchyma slowly (in a week) because the extrpulmonary tissue binding is high.it also has long half-life (6.4 hrs.). Because of above said reasons the accumulation and toxicity is common with the drug.

The adverse events including pancreatitis, diabetes, transient hypoglycaemia, hypotension, renal insufficiency are due to idiosyncrasy. It is better to avoid this drug in pancreatic transplant patients.

A meta-analysis study suggested that therapy with clindamycin-primaquine combination is associated with a more favourable outcome when compared with pentamidine therapy.

Breakthrough infection has been seen in a few patients receiving aerosolized and intravenous pentamidine therapy as prophylaxis (especially in the upper lobes). These breakthrough infections are in patients receiving primary prophylaxis after lung transplantation or in HIV seropositive patients who have not yet received antibiotic, in Cytomegalovirus -infected persons, or in secondary prophylaxis after inadequate clearance of infections.

When TMP-SMX, dapsone-TMP and clindamycin-primaquine were compared in mild to moderate PCP patients, there were no significant differences in terms of either treatment failure or therapy-limiting toxicity; 54 % of the patients did not finish a complete course of treatment, predominantly because of drug toxicity.[27]

Fred R Sattler et al have compared co-trimaxazole therapy with pentamidine therapy for treating PCP in HIV seropositive individuals and concluded that survival rate was more and quick improvement in oxygenation was seen with co-trimaxazole therapy.[28]

HIV seropositive patients who are clinically diagnosed as PCP are similar to those who had microscopic diagnosis and that empirical therapy for PCP in this group results in similar outcomes.[29]

Dapsone along with trimethoprim is another option for treatment of PCP.

ATOVAQUONE (750 mg PO TDS) has also been approved by FDA treatment of PCP

Trimetrexate, clindamycin- primaquine combination have also been approved for treatment of PCP.

Helweg-Larsen J et al. in a tri-centre cohort study have compared pentamidine therapy with clindamycin/primaquine combination therapy for PCP treatment and showed that clindamycin/primaquine was superior to pentamidine therapy and that combination could be used for patients not tolerating or failing with co-trimoxozole therapy.[29]

Interferon, colony stimulating factors like (G-CSF), (GM-CSF) have been studied in animals and showed some response in terms of reduction in the amount of pneumocystis.[30, 31]

The recommended duration of treatment is 3weeks in HIV seropositive patients and 2 weeks in HIV seronegative individuals.

Treatment of *P. carinii**	
Agent(s)(route)†	**Dose**
Trimethoprim and sulfamethoxazole (TMP-SMX) (IV/PO)	15 mg/kg/day TMP (to 20) 75 mg/kg/day SMX (to 100)
Pentamidine isethionate (IV)	4 mg/kg/day 300 mg/day max.
Dapsone (PO) with TMP (PO/IV)	100 mg/day 15–20 mg/kg/day (900 mg)
Clindamycin (IV/PO) diarrhea and primaquine	450–600 mg q6h 15–30 mg base qd
Trimetrexate (IV) with folinic acid (Not available)	30–45 mg/m/day 80–100 mg/m day
Pyrimethamine (PO) with sulfadiazine (PO)	Load 50 mg bid × 2d, then 25–50 mg qd Load 75 mg/kg, then 100 mg/kg/day
Piritrexim (IV) with folinic acid	Max. 4 gm in two doses; up to 8 g
Atovaquone (PO) suspension	750 mg (PO) tid to 1500 bid

Bennett et al., compared PCP patients treated empirically with PCP patients treated after microscopic confirmation in 56 hospitals and revealed that empirical treatment had a significant greater death rate.[32]

The therapeutic response is usually excellent in patients receiving a diagnosis before respiratory insufficiency. The ability to reduce immune suppression also improves the rapidity of infection clearance.

Deciding whether a patient is responding to therapy may be difficult. It is vital to remember that oxygenation typically reaches a nadir approximately 72 hours after treatment is initiated. Because it generally takes several days for clinical improvement, therapy usually should not be changed for at least four to five days.

If clinical improvement is not seen by days 4 to 5 of TMP-SMX therapy or 5 to 7 of pentamidine therapy should suggest the presence of some another process: Fibrosis, ARDS, abscess, bronchial obstruction, drug hypersensitivity and bronchogenic carcinoma.

Bronchoalveolar lavage and biopsy for microbiologic staining and pathology, or computed tomography of chest may be revealing in these individuals.

Prognosis of P.jirovecii pneumonia:

Worst prognosis is noted in patient without HIV infection and mortality rate is 30%-50%. In patients with HIV infection mortality

depends on severity of disease at presentation and mortality rate is 10%-20%.

In various studies, worst prognosis is showed in patients presented with concurrent pulmonary disease, in patients with pneumothorax and who require mechanical ventilation

The higher mortality is mainly because of delay in diagnosis and delay in initiation of appropriate treatment regimen.

Complications

The common complications of PCP are

- Respiratory failure

  (May occur despite with appropriate treatment)
- Acute respiratory distress syndrome
- Pneumothorax

  (May be due to spontaneous or secondary to barotraumas during positive –pressure ventilation)
- Pneumomediastinum
- Worsening of condition after starting therapy
- Pulmonary cyst formation
- Haematogenous spread

- Extra pulmonary infection
- Dissemination can occur in both HIV non HIV infected patients
- Most common sites of involvement are lymph nodes, bone marrow, liver, spleen, gut and eye lesion (choroiditis)

PROPHYLAXIS:

NACO guidelines in India and CDC guidelines of USA recommend primary prophylaxis for PCP in HIV seropositive patients with CD4 count <200/micro litre, Secondary prophylaxis is recommended after treatment of PCP. For both primary and secondary prophylaxis, the drug of choice is TMP-SMX.

The other agents used for prophylaxis are dapsone, dapsone + pyrimethamine combination, pentamidine, atovaquone etc.

Drug(s), Dose, Route
First Choice
TMP-SMX, 1 DS tablet or 1 SS tablet qd PO
Other Agents
Dapsone, 50 mg bid or 100 mg qd PO
Dapsone, 50 mg qd PO; plus pyrimethamine, 50 mg weekly PO; plus leucovorin, 25 mg weekly PO
Dapsone, 200 mg weekly PO; plus pyrimethamine, 75 mg weekly PO; plus leucovorin, 25 mg weekly PO
Pentamidine, 300 mg monthly via nebulizer
Atovaquone, 1500 mg qd PO
TMP-SMX, 1 DS tablet three times weekly PO

A large cohort study done by Phair et al in asymptomatic HIV seropositive homosexuals not under prophylaxis for PCP showed 8.4% of patients with CD 4 count less than 200 cells/microliter developed PCP within 6 months and 18.4%developed PCP within 12 months.

CORTICOSETROIDS IN PCP TREATMENT:

Corticosteroids administration in the first 72 hours of PCP management can prevent respiratory failure and death in AIDS patients. Patients with hypoxia (PaO_2<70 mmhg) and [A-a] DO_2 > 35 mmhg should get corticosteroid adjuvant therapy.[33,34]

Some studies demonstrated corticosteroids usage in mechanically ventilated patients with PCP has shown reduction in the mortality from 84 % to 39%.

PREDNISOLONE	DAYS	DOSE
	1-5	40mg BD
	6-10	40mg OD
	11-21	20mg OD

Oral prednisolone if tolerable or intra venous methyl prednisolone can be given.

Steroid therapy should be initiated within 72 hours of initiating antimicrobial treatment in an HIVseropositive patient with PCP if the partial pressure of arterial oxygen (PaO2) is less 70 mmHg or the alveolar-arterial oxygen difference (AaDO2) is more than 35.

60 to 79 % mortality was reported for severe PCP in HIV seropositive patients who required ICU care in the pre – HAART (Highly Active Anti-Retroviral Treatment) era. But it has been reduced after HAART.

Sabha Radhi et al. have demonstrated overall mortality of 11.6% for hospitalised PCP patients and 29% mortality especially for patients admitted in ICU.[35]

Helweg-Larsen J et al. have demonstrated in a study done in three different centres including Copenhagen, Milan and London that the rate of concomitant LRI with co-trimaxazole was 10%, clindamycin/primaquine combination was 5% and with pentamidine was quite high (15%). [36]

A study in San Francisco showed 55 % mortality for patients with PCP admitted in ICU compared to 63% shown in the same hospital previously in pre HAART era.

In the recent HAART era, predictors of clinical outcome especially death have not changed. Development of pneumothorax and pneumomediastinum, hypoalbuminemia, need for mechanical ventilation are the predictors of poor outcome in many studies.

Mallal SA et al demonstrated in pre HAART era ,PCP found in newly diagnosed HIV patients had more severe form of PCP with low PaO2 and higher requirement of mechanical ventilation comparing to PCP in known HIV patients.[37]

The clinical features and outcomes of PCP differ in HIV seropositive and HIV seronegative patients. But, there are only limited data on the efficacy of adjunctive corticosteroid therapy in HIV seronegative patients with PCP.

No randomized trials have been conducted. In contrast to the data for HIV seropositive patients, the data for HIV seronegative PCP patients showed that adjuvant corticosteroid therapy might not improve outcomes.

J Randall Curtis et al., conducted a study about the outcome of PCP with respiratory failure requiring assisted ventilation .They found the hospital survival rate was approximately 40 % which was twofold improvement from the years 1992 to 1995.[38]

There is no dedicated prognostic system with significant accuracy to determine which patients will be benefitted by mechanical ventilation.

AIM & OBJECTIVES

1) Comparing role of clinical diagnosis, chest radiography, and sputum microscopy and polymerase chain reaction for Pneumocystis jiroveci Pneumonia in induced sputum in HIV seropositive patients with CD4 less than 200.

2) To know the clinical outcome of PCP patients after treatment in our centre.

MATERIALS AND METHODS

STUDY DESIGN:

Prospective cohort study

INCLUSION CRITERIA:

1) All HIV seropositive inpatients with CD4 count less than 200 cells/μL

2) Age > 18 years

EXCLUSION CRITERIA:

1) HIV patients with CD 4 count > 200 cells/ μL

2) Age < 18 years

3) Patients with sputum positive pulmonary tuberculosis

4) Unconsented patients

5) Comatose patients

SITE OF INVESTIGATION:

Govt. Hospital of Thoracic Medicine, Tambaram Sanatorium, Chennai

STUDY PERIOD:

January 2012 to May 2012

METHODS:

1) 151 HIV seropositive patients were recruited for study as per inclusion criteria

2) Thorough clinical examination including general and systemic examination was done meticulously with vital signs monitoring & SpO2 was measured with pulse oximetry

Clinical diagnosis:

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

Symptoms

A. Cough with or without sputum production for more than two weeks

B. Breathlessness

C. With or without fever

Signs

A. Tachypnea

B. With or without bibasilar crackles

C. SPO2 < 90 % in ambient air or after exertion of 200 meter walking

3) Chest x ray PA view was taken for each and every patient.

Radiological diagnosis:

Radiological diagnosis of PCP was made based on the presence of any one of the following radiological features suggestive of PCP in chest skiagram.

- ❑ Bilateral perihilar infiltrates
- ❑ Bilateral airspace consolidations
- ❑ Pneumatoceles
- ❑ Pneumomediastinum
- ❑ Pneumothorax
- ❑ Subcutaneous emphysema

4) 3 to 5 ml of sputum collection in sterile container was done after hypertonic (3%) saline nebulisation using jet nebulizer for five minutes. This procedure was done in a separate room.

5) One sample of sputum was subjected for Gomori Methenamine Silver staining and microscopic examination

6) **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 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 cover slips and subjected to light microscopy.

7) Another sputum sample was subjected for Polymerase Chain Reaction (PCR) test targeting mitochondrial rRNA

8) **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 equal volume of a lysis buffer containing 500mg of proteinase K.

Then DNA extraction was done using a specific kit.

Polymerase chain reaction was done by denaturation for 5 minutes at 94 degree C followed by 35 cycles for one minute, 65 degree C for 1 minute and 72 degree 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 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 visualised under ultra violet light.

9) Sputum microscopy and PCR results were obtained in two days.

10) 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.

11) All patients under inpatient treatment were followed up clinically upto the completion of treatment for PCP and outcome were analysed

12) Analysis of data was done using SPSS

ETHICAL JUSTIFICATION

The various investigations and procedures that will be used in this study will be as per protocol. The identity of each patient will be kept confidential. This study will not violate medical ethics in anyway and it will help to know the role of diagnostic methods in pneumocystis jiroveci pneumonia patients.

OBSERVATION AND RESULTS

In the study on ROLE OF CLINICAL METHODS, CHEST SKIAGRAM, SPUTUM MICROSCOPY AND PCR IN DIAGNOSIS OF PNEUMOCYSTIS JIROVECI PNEUMONIA IN HIV PATIENTS WITH CD4 COUNT LESS THAN 200 AND THE CLINICAL OUTCOME between January 2012 to May 2012

The following observations were made.

Out of 151 HIV seropositive patients with low CD4 count (<200cells/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.

2 x 2 tabulation was used for calculation and following results were made for clinical diagnosis.

Sensitivity 0.78 95% Confidence Interval (0.63 to 0.88)

Specificity 0.55 95% Confidence Interval (0.46 to 0.64)

Positive PV 0.40 95% Confidence Interval (0.30 to 0.50)

Negative PV 0.87 95% Confidence Interval (0.77 to 0.93)

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
CLINICAL * SPUTUM_MICRO	151	100.0%	0	.0%	151	100.0%

Chi square test was done.

Confidence interval for sensitivity, specificity, positive predictive value and negative predictive value were calculated.

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%

Chi square test showed the results of clinical diagnosis were significant.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	13.482[a]	1	.000		
Continuity Correction[b]	12.168	1	.000		
Likelihood Ratio	14.192	1	.000		
Fisher's Exact Test				.000	.000
N of Valid Cases	151				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 19.01.

b. Computed only for a 2x2 table

RADIOLOGICAL DIAGNOSIS

When Chest skiagram is 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:

Sensitivity 0.60 95% Confidence Interval (0.59 to 0.82)

Specificity 0.86 95% Confidence Interval (0.79 to 0.92)

Positive PV 0.62 95% Confidence Interval (0.60 to 0.83)

Negative PV 0.86 95% Confidence Interval (0.78 to 0.93)

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
RADIOLOGICAL * SPUTUM_MICRO	151	100.0%	0	.0%	151	100.0%

Confidence interval for sensitivity, specificity, positive predictive value and negative predictive value were calculated.

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%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	34.373[a]	1	.000		
Continuity Correction[b]	31.985	1	.000		
Likelihood Ratio	32.119	1	.000		
Fisher's Exact Test				.000	.000
N of Valid Cases	151				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 10.86.

b. Computed only for a 2x2 table

Chi square test showed the results of Radiological diagnosis of PCP were significant.

Polymerase chain reaction (PCR):

Out of 151 patients who had undergone induced sputum for PCR, Diagnosis of PCP was made in 44 patients. The following results are derived from 2 x 2 tabulation.

Sensitivity 1.0 95% Confidence Interval (0.91 to 1.0)

Specificity 0.97 95% Confidence Interval (0.92 to 0.99)

Positive PV 0.93 95% Confidence Interval (0.82 to 0.98)

Negative PV 1.0 95% Confidence Interval (0.97 to 1.0)

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
PCR * SPUTUM_MICRO	151	100.0%	0	.0%	151	100.0%

Among the three methods including clinical evaluation, radiological examination and induced sputum PCR , sputum PCR is having the highest sensitivity (100%), highest specificity (97%),highest positive predictive value (93%) and also highest negative predictive value(100%).

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%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	136.867[a]	1	.000		
Continuity Correction[b]	132.197	1	.000		
Likelihood Ratio	154.696	1	.000		
Fisher's Exact Test				.000	.000
N of Valid Cases	151				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.95.

b. Computed only for a 2x2 table

Chi square test showed the results of PCR test were significant.

PCR was positive in 2 patients who were missed in GMS staining.

Those two showed good clinical response to TMP-SMX therapy. Among them, in one patient clinical diagnosis was made and in another one was missed in clinical and radiological evaluation.

OUTCOME:

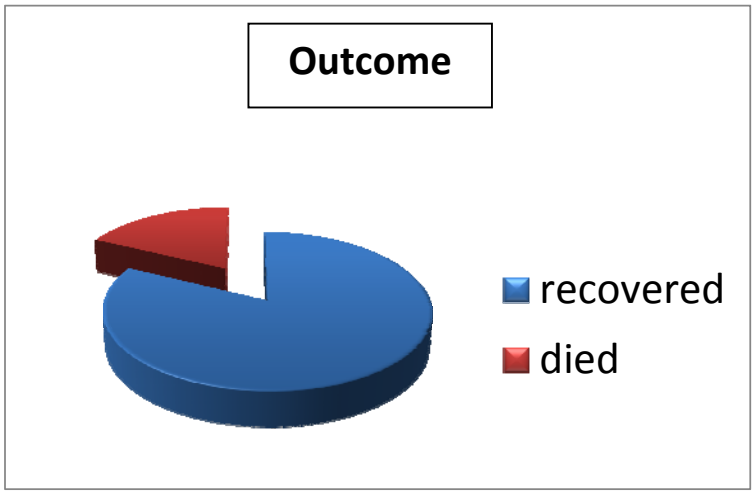
Of the total 151 HIV seropositive patients included in our study, 81 were clinically diagnosed as suspected PCP.

Of these 41 patients were confirmed microbiologically by sputum examination using Gomeri Methanamine Silver staining.

9 patients who were clinically negative for PCP (CD4 < 200) were positive microbiologically.

These 90 patients were treated with TMP-SMX (20mg/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.



Total number of PCP patients diagnosed clinically was 90.

74 of 90(82.2%) patients recovered from the illness after treatment.

16 0f 90(17.8%) patients died due to illness during the treatment.

Average length of stay in hospital as inpatient – 21 days

Average length of stay in IRCU (Intensive Respiratory Care Unit) – 7.5 days (n = 30)

Among patients admitted in IRCU, 15 0f 90(16.7%) required Non Invasive ventilation (NIV)

DISCUSSION

I.de Toro-Peinado et al have compared microscopic diagnosis and a real time PCR for the diagnosis of PCP with induced sputum and BAL. They analysed (39) patient with strong clinical suspicion of PCR. The concordance was 100% for BAL, but all the discrepant cases happened with induced sputum.

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+. 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).[39]

Liebovitz et al., showed that staining methods and PCR having similar detection data in Broncho alveolar lavage specimen but in bronchial washing specimen, PCR was superior.[40]

Lu et al., have compared 6 different PCR techniques, using BAL samples and showed nested PCR technique was the most sensitive assay for PCP diagnosis.[41]

A different single band 'touch down' PCR technique was demonstrated by Helweg lavsen et al., as having sensitivity and specificity for diagnosing PCP in AIDS patients.

Rabodonirina et al., 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.[42]

But in our study we clinically and radiologically diagnosed PCP before doing PCR and staining. We used GMS staining as gold standard.

Azouley E et al 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%, negative predictive value of 98.7%, positive predictive value of 51.5%. They concluded PCR performed similar to conventional methods for PCP diagnosis with high negative predictive value.[43]

Like all the above mentioned studies, our data are also showing high negative predictive value (100%) with negative predictive value (100%) with confidence interval between 0.97 and 1.0.

Pierre Flori 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.[44]

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 University of California expert panel showed the reduction in mortality, oxygenation and respiratory failure in moderate –to-severe PCP.

In a clinical study, Sabha Radhi et al. has shown overall mortality of 11.6% for hospitalised PCP patients and 29% mortality especially for patients admitted in ICU. Several studies have demonstrated the same results.

On comparing to this data, our study have showed the 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.

Mallal SA et al demonstrated in pre HAART era, PCP found in newly diagnosed HIV patients had more severe form of PCP with low PaO2 and higher requirement of mechanical ventilation on comparing to PCP in known HIV patients.

The reason postulated in Mallal et al. study may be the explanation for the high mortality in our study.[37]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.

LIMITATIONS

The gold standard respiratory specimen for diagnosing PCP is Broncho Alveolar Lavage (BAL). But induced sputum was the respiratory specimen used in our study.

Other staining methods, immunofluorescence methods were not compared in our study.

The treatment outcome was measured only clinically. Sputum microscopy or PCR was not utilised for follow up after completion of treatment. Our study was done in a short period (five months).

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%), specificity86%), PPV (62%) and NPV (86%) when comparing to GMS staining.

For PCR in 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, mortality rate was 17.8 % and 82.2 % of patients showed clinical improvement.

These findings need further studies with large population to confirm.

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ROLE OF CLINICAL METHODS, CHEST SKIAGRAM, SPUTUM MICROSCOPY AND PCR IN DIAGNOSIS OF PNEUMOCYSTIS JIROVECI PNEUMONIA IN HIV PATIENTS WITH CD4 COUNT LESS THAN 200 AND THE CLINICAL OUTCOME -A COMPARATIVE STUDY IN a TERTIARY CARE HOSPITAL Dissertation submitted In Partial Fulfilment of the Requirements for the Degree of DOCTOR OF MEDICINE PULMONARY MEDICINE Branch - XVII 2011-2013 DEPARTMENT OF PULMONARY MEDICINE Government Stanley Medical College & Hospital Chennai-600 001 THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY CHENNAI-600 032 APRIL 2013 CERTIFICATE This is to certify that the dissertation on "ROLE OF CLINICAL METHODS, CHEST SKIAGRAM, SPUTUM MICROSCOPY AND PCR IN DIAGNOSIS OF PNEUMOCYSTIS...

ROLE OF CLINICAL METHODS, CHEST SKIAGRAM, SPUTUM MICROSCOPY AND PCR IN DIAGNOSIS OF PNEUMOCYSTIS JIROVECI PNEUMONIA IN HIV PATIENTS WITH CD4 COUNT LESS THAN 200 AND THE CLINICAL OUTCOME -A COMPARATIVE STUDY IN A TERTIARY CARE HOSPITAL

Dissertation submitted In Partial Fulfilment of the Requirements for the Degree of

DOCTOR OF MEDICINE

PULMONARY MEDICINE

Branch - XVII

2011-2013

DEPARTMENT OF PULMONARY MEDICINE
Government Stanley Medical College & Hospital
Chennai-600 001

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI-600 032

APRIL 2013

PROFORMA

Name :

Age :

Gender :

Occupation :

Address :

Phone No :

Height/Weight :

Smoking : Yes / No

Brand/quantity/Duration :

If stopped when? :

Alcohol use : Yes / No

Brand/quantity/Duration :

If stopped when? :

Tobacco use : Chew/Snuff

If yes no. of times/day :

COMORBID CONDITIONS

Pregnancy : Yes/No

Peptic ulcer : Yes/No

Bronchial Asthma : Yes/No

Hypertension : Yes/No

Diabetes mellitus : Yes/No

SYMPTOMS

Cough:

Fever:

Sputum:

Chest pain:

Haemoptysis:

Wheeze:

Contact history of Tuberculosis: Yes/No

Previous history of Tuberculosis treatment:

If yes, Number of times of treatment:

Duration of treatment:

Regular /irregular:

GENERAL EXAMINATION :

BMI

Anaemia

Lymphadenopathy

Jaundice

Spine

Pedal oedema

VITALS

PR: BP:

RR: Temp:

PULSE OXIMETRY:

EXAMINATION OF RESPIRATORY SYSTEM

Inspection :

Palpation :

Percussion :

Auscultation :

PER ABDOMEN :

CARDIOVASCULAR SYSTEM :

CENTRAL NERVOUS SYSTEM :

INVESTIGATIONS

Haemoglobin :

WBC Count :

Chest X-Ray :

MANTOUX :

SPUTUM for AFB :

ELISA FOR HIV :

CD4 COUNT :

SPUTUM MICROSCOPY FOR PNEUMOCYSTIS JIROVECCI:

(GMS STANINIG)

PCR :

FINAL DIAGNOSIS :

CONSENT FORM

I Mr / Mrs / Miss / _____ have understood the procedure read by the Doctors. I in my whole conscious awareness give consent for the procedure. I understand that the procedure is done in good faith for the best therapeutic results possible. I fully understand the consequences of the procedure. I can resign from the study at any point of time.

Signature

Name :

Date and Time :

Signature of Researcher :

சுயஒப்புதல்படிவம்
ஆய்வுசெய்யப்படும்தலைப்பு

எச்.ஐ.வி.
நோயாளிகளுக்கானநியுமோசிச்டிஸ்ஜெரோவெசிநிமோனி யாகண்டறிதல்பரிசோதனைகள்மற்றும்நோயாளிகளின்உடல் நிலைபற்றியஆய்வு

ஆராய்ச்சிநிலையம்: அரசுநெஞ்சகநோய் மருத்துவமனை ,
தாம்பரம்சானடோரியம், சென்னை.

பங்குபெறுபவரின்பெயர் :

பங்குபெறுபவரின்எண் :

பங்குபெறுபவர் ( ) இதனைக்குறிக்கவும் :

மேலேகுறிப்பிடப்பட்டுள்ள ஆய்வின்விவரங்கள்எனக்குவிளக்கப்பட்டத. என்னுடையசந்தேகங்களைக்கேட்கவும், அதற்க்கானதகுந்தவிளக்கங்களைப்பெறவும்வாய்ப்பளிக்கப்பட்டது. ☐
நான்இவ்வாய்வில்தன்னிச்சையாகத்தான்பங்கேற்கிறேன். எந்தகாரணத்தினாலோஎந்தகட்டத்திலும்எந்தசட்டச்சிக்கலுக்கும்உட்படாமல்நான்இவ்வாய்வில்இருந்துவிலகிக் கொள்ளலாம்என்றும்அறிந்துகொண்டேன்.

இந்தஆய்வுசம்பந்தமாகவோ, இதைச்சார்ந்தமேலும்ஆய்வுமேற்கொள்ளும்போதும் இந்தஆய்வில்பங்குபெறும்மருத்துவர்என்னுடையமருத்துவஅறிக்கையைபார்ப்பதற்கு☐ண்அனுமதிதேவையில்லைஎனஅறிந்துகொள்கிறேன்.
நான் ஆய்வில் இருந்துவிலகிக்கொண்டாலும்இதுபொருந்தும்எனஅறிகிறேன்.

இந்தஆய்வுமூலம்கிடைக்கும்தகவல்களையும்பரிசோதனைமுடிவுகளையும்மற்றும்சிகிச்சைதொடர்பானதகவல்களையும்மருத்துவ☐மேற்க்கொள்ளும்ஆய்வில்பயன்படுத்திக்கொள்ளவும்அதைப்பிரசுரிக்கவும்எண்முழுமனதுடன்சம்மதிக்கிறேன்.

இந்தஆய்வில்பங்குகொள்ளஒப்புக்கொள்கிறேன்.
எனக்குக்கொடுக்கப்பட்டஅறிவுரைப்படிநடந்துகொள்வதுடன்இந்தஆய்வைமேற்கொள்ளும்மருத்துவஅணிக்குஉன்னமையுடன்இருப்பேன்☐றுஉறுதிஅளிக்கின்றேன்.
என்உடல்நலம்பாதிக்கப்பட்டாலோஅல்லதுஎதிர்பாராதவழக்கத்திற்கு

மாறானநோய்க்குறிதென்பட்டாலோஉடனேஅதைமருத்துவஅணிக்குத் தெரிவிப்பேன்எனஉறுதிஅளிக்கிறேன் .

பங்குபெறுபவரின்கையொப்பம் ----------

இடம் ---------- தேதி ----------

கட்டைவிரல்ரேகை

பங்குபெறுபவரின் பெயர்மற்றும்விலாசம் ----------

----------ஆய்வாளரின்கையொப்பம் ----------

---- இடம் ---------- தேதி ----------ஆய்வாளரின்பெயர் ----------

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நோயாளிக்கானதகவல்படிவம்

மதிப்பிற்குரியஐயா/அம்மையீர்,

உங்கள்விருப்பத்தின்பேரில் எச் ஐ வி நோயாளிகளுக்கானநியுமோசிச்டிஸ்ஜெரோவெசிநிமோனியாகண்டறிதல்பரிசோதனைகள்மற்றும்நோயாளிகளின்உடல்நிலைபற்றியஆய்வில்பங்கேற்கும்படிஅன்புடன்கேட்டுக்கொள்கிறோம்.

இந்தஆய்வில்ஆரய்ச்சிநோக்கத்துக்காகதாங்கள்பரிசோதனைக்குஉட்படுத்தப்படுவீர்கள்.தகுந்தசிகிச்சைதங்களுக்குதொடங்கப்படும்.

தங்களுக்குஇந்தஆய்வில்பங்கேற்கவிருப்பம்இருந்தால்தாங்கள்அருள்கூர்ந்துஒப்புதல்படிவத்தைப்படித்துப்பார்த்துக்கையொப்பம்இடும்படிக்கேட்டுக்கொள்கிறேன்.

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