PREVALENCE OF *PNEUMOCYSTIS JIROVECII* PNEUMONIA AMONG HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS AT MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM.

By

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Medicine (Internal Medicine) of the Muhimbili University of Health and Allied Sciences

Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for the examination of a dissertation entitled ***Prevalence of Pneumocystis jirovecii Pneumonia in Human Immunodeficiency Virus (HIV) Infected Patients at Muhimbili National Hospital, Dar es Salaam,*** in fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine) of the Muhimbili University of Health and Allied Sciences

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DEDICATION

Dedicated to my mother, **Agnes,** for her sacrifice in my education;

My beloved wife **Nelly** for her kindness, tolerance and everlasting

Support despite being similarly busy with her studies.

Their natural optimism has helped me seeing the good side of my hardworking.

ABSTRACT

Background: *Pneumocystis jirovecii* pneumonia (PCP) was said to be rare in Africa, with reported rates of 0-22% in human-immunodeficiency-virus (HIV) infected individuals with respiratory symptoms. Recent studies in sub Saharan Africa have, however, shown an increasing trend in the prevalence of PCP with rates between 11 and 43%. Similarly, empirical treatment for Pneumocystis pneumonia based on clinical and radiological features has been increasingly practiced with good clinical improvement. However, the current prevalence in our setting is not known.

Objectives: The study was done to determine the prevalence of PCP in HIV- infected patients complaining of cough.

Setting: This was a cross-sectional study conducted at the HIV clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania between May and November 2006.

Study subjects: HIV infected male and female patients with cough for at least two weeks were recruited for the study

Procedures: For every patient, sputum induction using an ultrasonic nebulizer was done and sputum examined for *P. jirovecii* using Toluidine blue stain and Polymerase chain reaction. Ziehl–Neelsen and Gram stain were also done to the induced sputa. Patients found to have PCP, pulmonary tuberculosis and bacterial pneumonia were treated accordingly.

Results: A total of 125 patients fulfilled the inclusion criteria, 3/83 (3.6%) and 13/125 (10.4%) had pneumocystis pneumonia diagnosed by PCR and Toluidine Blue chemical staining respectively. The later was unreliable as it gave discordant results with all the PCR positive sputa. Smear positive pulmonary tuberculosis was found in 9/125 (7.2%) patients. Co-infections of PCP and TB were not found. Low $CD4^+$ T lymphocyte count was associated with an increased risk for both PCP and pulmonary TB. The presence of difficult in breathing, fever, chest pain, oral candidiasis, respiratory crackles and a male sex were significantly associated with an increased risk for PTB

Conclusion: The prevalence of PCP is still low in Tanzania. Pulmonary tuberculosis remains the major respiratory problem in HIV patients.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
PCP	Pneumocystis jirovecii pneumonia
NACP	National AIDS Control Programme
PCR	Polymerase Chain Reaction
TB	Tuberculosis
HIV	Human Immunodeficiency Virus
MNH	Muhimbili National hospital
ART	Antiretroviral therapy.
HAART	Highly Active Antiretroviral Therapy
ARV	Antiretroviral
AFB	Acid Fast Bacilli
BAL	Bronchoalveolar lavage
TMP-SMX	Trimethoprim- Sulphamethoxazole
DNA	Deoxy-Nucleic Acid
RNA	Ribo-Nucleic Acid
rRNA	Ribosomal Ribo- Nucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
WHO	World Health Organization
MUHAS	Muhimbili University of Health and Allied Sciences
UNAIDS	United Nations Programme on HIV/AIDS

1 INTRODUCTION AND LITERATURE REVIEW

1.1 Definition and description

Pneumocystis carinii pneumonia (PCP) is caused by *Pneumocystis jirovecii* (formerly *P. carinii* f. sp. *hominisi);* a ubiquitous organism classified as a fungus but that share biologic characteristics with protozoa.[1] The full identification and classification of pneumocystis took many decades. Pneumocystis organisms were first identified by Carlos Chagas in the early 20th century, with the use of a guinea-pig model of trypanosome infection, and subsequently by Antonio Carinii, in infected rat lungs.[2] Both investigators believed they had identified new forms of trypanosomes.[3, 4] Several years later, however, the Delanoës recognized that Chagas and Carinii had identified a new species with a unique tropism for the lung; hence, the new species was named *Pneumocystis carinii.*[5] Pneumocystis was initially misclassified as a protozoan on the basis of the morphologic features of the small trophic form, the larger cyst form, the development of up to eight progeny within the cyst, and the rupture of the cyst to release new trophic forms. In 1988, an analysis of the small rRNA subunit from pneumocystis pneumonia established a phylogenetic linkage to the fungal kingdom, and all subsequent genomic information has corroborated pneumocystis's home within the ascomycetous fungi.[6] Biochemical studies have also suggested that the cell wall of *P.jirovecii* contain glucans and drugs that inhibit 1, 3-glucan synthesis in fungi are highly active against *P.carinii* in animal models.[2]

Pneumocystis organisms have been identified in virtually every mammalian species. In humans, serologic surveys have shown nearly universal seropositivity to pneumocystis in tested populations by two years of age.[7] Pneumocystis organisms encompass a family of organisms that have a range of genetic characteristics and that are host-specific. For example, the pneumocystis that infects humans, which was recently renamed *P. jirovecii,* cannot infect the rat, and vice versa.[8] The reason for this stringent host specificity is unclear. *Pneumocystis carinii* now refers only to the pneumocystis that infects rodents. The abbreviation PCP is still used to designate *Pneumocystis* pneumonia.

Major developmental stages of the organism include the trophozoite, precyst and cyst forms.[9] The small (1- 4μm) pleomorphic trophozoites or trophic form is amoeboid, thin-walled structure with a single nucleus and cellular organelles typical of a lower eukaryote; it displays close apposition to the cell surface of the flat, type I pneumocyte but cell penetration and destruction do not occur.[1] The cyst (5- to 8- μm) is a rounded, more thick-walled structure containing up to eight daughter cells or sporozoites, destined to be released as trophozoites; and the precyst, an intermediate stage.[6] The life cycle of *P.jirovecii* involves asexual replication by the trophic form and sexual reproduction by the cyst, which ends in release of intracyst bodies; an intracellular stage has not been identified.[6] Pneumocystis has a unique tropism for the lung, where it exists primarily as an alveolar pathogen without invading the host. In rare cases, pneumocystis disseminates in the setting of severe underlying immunosuppression or overwhelming infection.[1]

1.2 Epidemiology

Pneumocystis pneumonia occurs exclusively in the immunosuppressed, notably those with depression of T-lymphocyte immunity and less commonly severe hypogammaglobulinaemia.[1] It was first recognized as a cause of human disease as an epidemic pneumonia in marasmic institutionalized infants in Europe and the Middle East from 1940 to 1960.[1] As this epidemic subsided, it was found to complicate the use of immunosuppressive drug regimens in childhood leukaemia and organ transplantation. The annual attack rate for pneumocystis pneumonia was 20 per cent in each of these groups before chemoprophylaxis became a regular part of treatment schedules[1]. Initial and asymptomatic infection with *P. jirovecii* usually occurs in early childhood; two thirds of healthy children have antibody to *P.jirovecii* by age 2–4 years.[10] According to Wakefeld et al,[11] the acquisition of immunoglobulin response to *P.jirovecii* starts at 6 months of age and peaks in prevalence (80%) at about 8 years.

Though horizontal transmission between immuno-suppressed subjects is possible, the demonstration of pneumocystis-like organisms in ambient air and its fungal classification suggests that acquisition from an environmental source, as yet unidentified, is more important.[1] Disease probably occurs by new acquisition and by reactivation but the virtual absence of the organism in samplings from normal lungs, assayed by monoclonal antibody or DNA amplification, implies that clearance is highly effective in the normal human.[12] It is therefore likely that pneumonia in the immunosuppressed is the result of freshly acquired infection.[1]

PCP in the Developed World

The beginning of the AIDS epidemic in the late 1970s shifted the incidence of PCP from a rare disease to a more common pneumonia. Clusters of PCP cases in homosexual men and intravenous drug users were one of the first indications of the HIV epidemic. PCP rapidly became the leading AIDS-defining diagnosis in HIV-infected patients. In the initial stages of the epidemic, PCP rates were as high as 20 per 100 person-years for those with CD4+ cell counts <200 cells/μL.[13] PCP was responsible for two thirds of AIDS-defining illnesses, and an estimated 75% of HIV-infected patients would develop PCP during their lifetime.[14]

Before the widespread use of primary PCP prophylaxis and effective antiretroviral therapy (ART), PCP occurred in 70%–80% of patients with AIDS.[13] Incidence of PCP has declined substantially with widespread use of prophylaxis and effective ART; recent incidence rates among patients with AIDS in Western Europe and the U.S. are 2–3 cases per 100 person-years.[15] The majority of cases occur among patients who are unaware of their HIV-1 infection or are not receiving ongoing HIV care or among those with advanced immunosuppression (CD4+ T lymphocyte counts <100 cells/μL).[16] The first substantial decline in the incidence of PCP occurred after the introduction of anti-*Pneumocystis* prophylaxis in 1989.[17] The advent of highly active antiretroviral therapy (HAART) has resulted in further declines in rates of PCP and other opportunistic infections..[18] Although there is improvement, PCP still occurs in industrialized nations despite the availability of HAART and anti-*Pneumocystis* prophylaxis and remains the

most common AIDS-defining opportunistic infection in the United States. In a study done in United States on prescriptions for PCP prophylaxis in HIV-infected adults who developed PCP from 1999 through 2001, almost 44% of PCP cases occurred in patients not receiving medical care, most of whom were probably not known to be HIV-infected. [19] Forty-one percent of patients were prescribed prophylaxis but did not adhere to treatment, or PCP developed despite their taking medications appropriately. Possible explanations for this occurrence of PCP include the development of drug-resistant *Pneumocystis* or decreased efficacy of prophylaxis in those with low CD4+ cell counts.[19]

PCP in the Developing World

In contrast to the dramatic improvements in the industrialized world, persons living in developing nations continue to be devastated by HIV. Large portions of the populations of Southeast Asia and sub-Saharan Africa are infected with HIV. In 2006, UNAIDS estimated that 34.1 million to 47.1 million people worldwide were living with HIV. Sub-Saharan Africa continues to bear the brunt of the global epidemic. Despite being a home of about 10% of the world's population, Sub- Saharan Africa had about two thirds (63%) of the world's HIV infected adults and children by the end of 2006.In 2006 it is also estimated that 2.8 million people were newly infected with HIV while 2.1 millions (72%) died of the disease in Sub-Saharan Africa alone. In Southeast Asia HIV is similarly increasing. For instance, in India it is estimated that 5.7 million people are HIV-infected among the 1.1 billion Indian population. This is probably the highest country's number of HIV infected people. South Africa is a home of about 5.5 million HIV infected

people.[20]

Among patients with AIDS, *Pneumocystis jirovecii* pneumonia (PCP) has been noted to occur less frequently in patients in developing regions than in those in industrialized countries.[21] Autopsy reports also showed that disseminated infection with *Mycobacterium avium* complex and PCP occurred less frequently among HIV-infected patients in Africa than among such patients in developed countries.[22] Other studies have showed that tuberculosis was the most common diagnosis in selected HIV-infected patients who had abnormal chest radiography findings that were consistent with PCP.[23] Recent reports, however, have noted a significantly higher prevalence of PCP among HIV-infected patients with respiratory symptoms who live in Africa and other regions of the developing world than was noted in the past. Studies from Thailand show a prevalence of 27% to 40% among HIV-infected patients treated at a university hospital clinic.[24] Central and South America also have a large number of PCP cases. One Brazilian study found a prevalence of 55% among HIV-infected persons with respiratory complaints. [25] Other studies in this region report PCP prevalence rates of 24% to 29%, depending on the population studied.[26, 27]

PCP in Adults in Africa

Pneumocystis pneumonia is certainly prevalent in Africa; it had been diagnosed in infants with kwashiorkor prior to AIDS epidemic and a recent serologic study in Gambia documented a high prevalence of PCP antibodies among children.[11] In contrast to the

situation in many other developing regions, PCP has been thought to be rare in the African adult. Most early studies during or before 1992 reported prevalence rates of 0% to 11%[28-32] in HIV-infected patients, although one early study found a rate of 33%.[23] In a prospective study conducted in 1990 in Rwanda PCP was found to account for 4% of pneumonia cases.[31] *Mycobacterium tuberculosis* and other bacteria were more frequent causes of pneumonia than was PCP.[33] Similarly, in another prospective study in 1991 in Tanzania among 83 HIV seropositive people with difficult breathing, cough, fever of at least 2 weeks duration, or overt pneumonia; 3.6% of the sputum samples were confirmed positive for *P. jirovecii*; 38.5% of preparations and 13.2% of cultures tested positive for Mycobacterium tuberculosis.[34] In Zambia, two prospective studies done in 1990 and 1988; and another study from Congo which was performed before 1990 showed that PCP accounted for 9% to 15% of cases of pneumonia.[30,35,36] An additional study performed in Zimbabwe during 1986 to1987 reported a relatively higher percentage of PCP (22%) than did other studies conducted during this period.[37] In another study done in 1996 in Tanzania to determine pulmonary complications in HIV-1-infected patients, Pneumocystis jirovecii pneumonia was diagnosed only in one patient (0.8%) out of the 127 HIV infected patients studied. Tuberculosis was the most common cause of pulmonary disease as it was found in 75% of these patients.[38]

HIV-infected African adults have high rates of bacterial pneumonia and tuberculosis, diseases that may result in death at higher CD4+ cell counts and prevent many HIV-infected patients from reaching a stage at which they would be susceptible to PCP. Environmental factors, such as seasonal variations and tropical climate, might contribute

to a low rate of PCP in Africa. However, high rates of anti-*Pneumocystis* antibodies among African children suggest that exposure to the organism is common.[11] Regional strains may be less virulent, or the population may be more resistant, as HIV-infected African Americans have been shown to have lower PCP rates compared to white Americans.[39] Similarly in a study from South Africa of 67 HIV-infected patients whose sputum smears were negative for TB and who had a fiberoptic bronchoscopic evaluation; PCP was diagnosed in 27% among those of African origin and in 59% among those of European origin.[40] More recent clinical studies that used sensitive methods to diagnose PCP have shown that *P. jirovecii* accounts for a greater percentage of cases of pneumonia among African adult HIV-infected patients than had been noted in earlier reports. The above-sited South African retrospective study that used BAL and monoclonal antibody methods for patients with pneumonia between 1988 and 1993 showed that PCP accounted for 43% of episodes.[40] In another prospective study that used similar diagnostic methods Malin et al. studied a group of 64 hospitalized HIV-infected patients in Zimbabwe in 1995.[23] These patients had pneumonia unresponsive to penicillin, and sputum samples were smear-negative for acid-fast bacilli (AFB). All patients underwent bronchoscopy with bronchoalveolar lavage (BAL). Twenty-one (33%) of these patients had PCP and 24 (39%) had tuberculosis (TB); six of these had both infections. Five patients had Kaposi's sarcoma (KS) associated with PCP, TB, or another infection. KS was the only finding in one patient, and no pathogens were identified in 21 patients. Another study in Uganda examined 83 patients hospitalized with respiratory symptoms.[41] All patients had sputum cultures that were negative for AFB and underwent

bronchoscopy with BAL for diagnosis. Thirty-two patients (38.6%) were found to have PCP. In a study conducted in Kenya between June 1999 and August 2000, a total of 51 patients with the presumptive diagnosis of PCP were involved. Bronchoalveolar lavage fluid (BALF) was obtained by fiberoptic bronchoscopy and examined for cysts of P. jirovecii using Toluidine blue stain and immunofluorescent antibody test (IFAT). Pneumocystis jirovecii stain was positive in 37.2%.[42]

Not all recent studies have found high rates of PCP. In Ethiopia, Aderaye et al [43] reported that of 119 outpatients with respiratory symptoms and negative AFB cultures, only 10.9% had PCP. Similarly, in another recent autopsy study in Botswana; PCP was found positive in 11% of HIV positive inpatients that died of respiratory symptoms.[29]

1.3 Pathogenesis

Impaired cellular immunity is the major host factor that predisposes to pneumocystosis, although defects in B cell function also play a role. It has long been thought that PCP develops by reactivation of latent infection, yet recent molecular evidence suggest that at least some episode of the disease represent new bouts of infection. Sequencing gene fragments derived by PCR showed that genetically distinct isolates were associated with each episode of PCP.[11, 12] Roux et al [43] also showed that patients with negative Pneumocystis PCR in bronchoalveolar lavage (BAL) fluid had had a first positive PCR in BAL about 3 weeks earlier. This also illustrates that *P.jirovecii* can be cleared. All these data support and concern the hypothesis that PCP that develops in immunocompromised patients may be a new infection resulting from exposure to an

exogenous source of P. jirovecii and not from reactivation of latent infection. Therefore, patients who had two episodes of PCP separated by at least 4 months have different *P.jirovecii* isolates in the first and second disease episodes. These cumulative data support the hypothesis that recurrence of Pneumocystis infection occurs in immunocompromised hosts and may result from de novo acquisition of the organism from an exogenous source.

1.3.1 Alveolar epithelial cells and proteins response

Within the lung, P. jirovecii attaches firmly to the alveolar type I pneumocyte through interdigitation of their membranes with those of the host.[45] The binding of pneumocystis to the epithelium is facilitated by interactions of proteins in the host, such as fibronectin and vitronectin that bind to the surface of pneumocystis and mediate the attachment to integrin receptors present on the alveolar epithelium.[1] In infected tissues, type I alveolar cells with adherent pneumocystis appear vacuolated and eroded.[46] However, studies of cultured lung epithelial cells have shown that the adherence of pneumocystis alone does not disrupt the structure or barrier function of alveolar epithelial cells, though proliferative repair of the epithelium is reduced.[47] Pulmonary surfactant phospholipids, which contribute to the low surface tension in the alveoli, are reduced during pneumocystis pneumonia, and abnormalities in the composition and function of the surfactant are the result of the host's inflammatory response to pneumocystis, rather than direct effects of the organisms on the surfactant. It is therefore unlikely that the adherence of pneumocystis to alveolar epithelium is by itself responsible for the diffuse

alveolar damage in severe pneumonia. Rather, the inflammatory responses in the host are primarily responsible for the compromise of the alveolarcapillary surface. Effective inflammatory responses in the host are required to control pneumocystis pneumonia. Exuberant inflammation, however, also promotes pulmonary injury during infection. Severe pneumocystis pneumonia is characterized by neutrophilic lung inflammation that may result in diffuse alveolar damage, impaired gas exchange, and respiratory failure. Indeed, respiratory impairment and death are more closely correlated with the degree of lung inflammation than with the organism burden in pneumonia.[48]

1.3.2 Lymphocyte response.

Immune responses directed against pneumocystis involve complex interactions between CD4+ T lymphocytes, alveolar macrophages, neutrophils, and the soluble mediators that facilitate the clearance of the infection. In particular, the activity of CD4+ T cells is pivotal in the host's defenses against pneumocystis, in both animals and humans, and the risk of infection increases with a CD4+ count of less than 200 per cubic millimeter.[13] CD4+ cells are important in recruitment and activation of other immune effector cells, including monocytes and macrophages, to attack the organism. Mice with severe combined immunodeficiency (SCID) have progressive pneumocystis infection, despite the presence of otherwise functional macrophages and neutrophils.[49] When the immune system is reconstituted with the use of CD4+ spleen cells, however, the mice regain the ability to clear infection effectively.[50]

Macrophage-derived tumor necrosis factor *a* (TNF*a*) and interleukin-1 are believed to be

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necessary for initiating pulmonary responses to pneumocystis infection that are mediated by CD4+ cells. The cells proliferate in response to pneumocystis antigens and generate cytokine mediators, including lymphotactin and interferon gamma.[50] Lymphotactin, a chemokine, acts as a potent chemoattractant for further lymphocyte recruitment in pneumocystis pneumonia.[51] Interferon gamma strongly activates the macrophage production of TNF*a*, superoxides, and reactive nitrogen species, each of which is implicated in the host defense against pneumocystis.[52]

Aerosolized interferon gamma reduces the intensity of infection in rats infected with pneumocystis, regardless of the degree of CD4+ depletion.[52] Although T lymphocytes are essential for the clearance of pneumocystis, data suggest that T-cell responses may also result in substantial pulmonary impairment during pneumonia. For instance, in SCID mice infected with pneumocystis, normal oxygenation and lung function occur despite active infection until the late stages of the disease.[49] When the immune systems in these animals are reconstituted with the use of intact spleen cells, an intense T-cell–mediated inflammatory response ensues, resulting in substantially impaired gas exchange. Pneumocystis pneumonia also results in the marked accumulation of CD8+ T lymphocytes in the lung.[53]

1.3.3 Macrophages in host defense against pneumocystis

Alveolar macrophages are the principal phagocytes mediating the uptake and degradation of organisms in the lung. When there are no opsonins in the epithelial-lining

fluid, the uptake of pneumocystis is mediated mainly through the macrophage mannose receptors, pattern-recognition molecules that interact with the surface mannoprotein, glycoprotein A.[54]

After they have been taken up by macrophages, pneumocystis organisms are incorporated into phagolysosomes and degraded.[55] The function of macrophages is impaired in patients with AIDS, malignant disease, or both, resulting in the reduced clearance of pneumocystis.[56] In macrophage-depleted animals the resolution of pneumocystis infection is impaired. Macrophages produce a large variety of proinflammatory cytokines, chemokines, and eicosanoid metabolites in response to phagocytosis of pneumocystis.[55] Although these mediators participate in eradicating pneumocystis, they also promote pulmonary injury.

1.3.4 Cytokine and chemokine networks

TNF-*a* has important effects during pneumocystis pneumonia infection.[57] The clearance of pneumocystis is delayed when TNF-*a* is neutralized by antibodies or inhibitors in animals with pneumocystis pneumonia.[57] TNF-a promotes the recruitment of neutrophils, lymphocytes, and monocytes. Although their recruitment is important for clearance of the organisms, these cells injure the lung by releasing oxidants, cationic proteins, and proteases. TNF-*a* also induces the production of other cytokines and chemokines, including interleukin-8 and interferon gamma, which stimulate the recruitment and activation of inflammatory cells during pneumocystis pneumonia. The cell wall of pneumocystis contains abundant beta-glucans, and studies have confirmed

that the production of TNF-*a* by alveolar macrophages is mediated by recognition of the beta-glucan components of pneumocystis.[58] Macrophages display several potential receptors for glucans, including CD11b/CD18 integrin (CR3), dectin-1, and toll-like receptor 2. The activation of macrophages by pneumocystis is augmented by proteins in the host such as vitronectin and fibronectin that bind the glucan components on the organism.[58] The cysteine–X amino acid–cysteine chemokines, such as interleukin-8, macrophage-inflammatory protein-2, and the interferon-inducible protein of 10 kD, which are potent chemoattractants for neutrophils, are important during pneumocystis infection. Interleukin-8 is correlated with both neutrophil infiltration of the lung and impaired gas exchange during severe pneumocystis pneumonia. Furthermore, the level of interleukin-8 in bronchoalveolar- lavage fluid may serve as a predictor of severe respiratory compromise and death from the disease.[59] Isolated pneumocystis beta-glucan stimulates alveolar macrophages and alveolar epithelial cells to produce marked quantities of macrophage inflammatory protein-2.[58] The neutrophils recruited into the lungs release reactive oxidant species, proteases, and cationic proteins, which directly injure capillary endothelial cells and alveolar epithelial cells. [58]

1.4 Clinical features

Pneumocystis pneumonia is often the AIDS-defining illness in patients infected with HIV, occurring most frequently when the T-helper cell count (CD4+) is less than 200 cells per cubic millimeter.[13] The clinical features of *P. jirovecii* infection are consistent but not diagnostic. Clinical syndrome can be mimicked by other infections including

cytomegalovirus, tuberculosis, and other fungi; and by non-infectious pulmonary complications in the immuno-suppressed, including drug-induced disease or pulmonary oedema or pulmonary Kaposis sarcoma.[1] The onset of disease ranges between the acute and insidious (over 2-4 weeks); the latter is a more regular feature in AIDS. Common symptoms of pneumocystis pneumonia include the subtle onset of progressive dyspnea, nonproductive cough, and low-grade fever. Acute dyspnea with pleuritic chest pain may indicate the development of a pneumothorax. Physical examination typically reveals tachypnea, tachycardia, and normal findings on lung auscultation. Patients with AIDS and pneumocystis pneumonia have a significantly increased number of pneumocystis organisms in their lungs, with fewer neutrophils, than do patients with pneumocystis pneumonia in the absence of AIDS.[48] This greater organism burden results in a higher diagnostic yield of induced sputum and bronchoalveolarlavage fluid to confirm pneumocystis pneumonia in patients with AIDS as compared with other conditions associated with immunosuppression. The smaller number of inflammatory cells in patients with AIDS-related pneumocystis pneumonia also correlates with better oxygenation and survival as compared with pneumocystis pneumonia in patients without AIDS.[48] Among most patients with AIDS and pneumocystis pneumonia, the mortality rate is 10 to 20 percent during the initial infection, but the rate increases substantially with the need for mechanical ventilation.[60] Patients who have pneumocystis pneumonia without AIDS typically present with an abrupt onset of respiratory insufficiency that may correlate with a tapered or increased dosage of immunosuppressant medications.[61] Affected patients have more neutrophils and fewer pneumocystis organisms in their

lungs during an episode of pneumocystis pneumonia than do patients with AIDS who have pneumocystis pneumonia.[48] The mortality rate among patients with pneumocystis pneumonia in the absence of AIDS is 30 to 60 percent, depending on the population at risk, with a greater risk of death among patients with cancer than among patients undergoing transplantation or those with connective-tissue disease.[62] In patients with AIDS, rare cases of disseminated pneumocystis infection are well documented. Tissues involved in disseminated disease include lymph nodes, spleen, liver, bone marrow, the choroid, and retina. Clinical features associated with such spread include general malaise, fever, painless lymphadenopathy and splenomegaly, anaemia and visual disturbance. [1]

1.5 Diagnosis of PCP

The diagnosis of pneumocystis pneumonia requires microscopical examination in order to identify pneumocystis from a clinically relevant source such as specimens of induced sputum, bronchoalveolar fluid, or lung tissue; because pneumocystis cannot be cultured.[63]

Sputum induction with hypertonic saline has a diagnostic yield of 50 to 90% and should be the initial procedure used to diagnose pneumocystis pneumonia, particularly in patients with AIDS.[64] If the initial specimen of induced sputum is negative for pneumocystis, then bronchoscopy with bronchoalveolar lavage should be performed. Bronchoalveolar lavage (BAL) alone has sensitivity of more than 95%[65]. When combined with BAL, transbronchial biopsy (TBB) has a sensitivity of almost 100%. However, it is best avoided for fear of pneumothorax and pulmonary hemorrhage.[65]

Trophic forms can be detected with modified Papanicolaou, Wright–Giemsa, or Gram–Weigert stains. Cysts can be stained with Gomori methenamine silver, cresyl echt violet, toluidine blue O, or calcofluor white. [66] Monoclonal antibodies for detecting pneumocystis have a higher sensitivity and specificity in induced-sputum samples than conventional tinctorial stains have, but the difference is much less in bronchoalveolar-lavage fluid. [67] An advantage of monoclonal antibodies is their ability to stain both trophic forms and cysts, which is important because the trophic forms are generally more abundant during pneumocystis pneumonia. [67] PCR has been shown to have greater sensitivity and specificity for the diagnosis of pneumocystis pneumonia from specimens of induced sputum and bronchoalveolar-lavage fluid than conventional staining when PCR primers for the gene for pneumocystis mitochondrial large-subunit ribosomal RNA (rRNA) are used.[68] In patients with positive PCR results in bronchoalveolar-lavage fluid or sputum but with negative smears, clinical management of the disease remains a challenge. However, it is recommended to treat these patients if immunosuppression is ongoing.[69] Although an elevated serum lactate dehydrogenase level has been noted in patients with pneumocystis pneumonia, it is likely to be a reflection of the underlying lung inflammation and injury rather than a specific marker for the disease.[70]

The plain chest radiograph and arterial blood gas estimates are the essential investigations for preliminary assessment. While chest x- ray may reveal a variety of patterns, the most common pattern is either a normal film, if the disease is suspected

early or a faint interstitial infiltrate.[6] Typical radiographic features of pneumocystis pneumonia are bilateral perihilar interstitial infiltrates that become increasingly homogeneous and diffuse as the disease progresses.[71] Less common findings include solitary or multiple nodules, upper-lobe infiltrates in patients receiving aerosolized pentamidine, pneumatoceles, and pneumothorax. Pleural effusions and thoracic lymphadenopathy are rare. Normal films may be found in 5 –10% of patients with PCP.[1] When chest radiographic findings are normal, a high-resolution computed tomography, which is more sensitive than chest radiography, may reveal extensive groundglass attenuation or cystic lesions. [71] Blood gas analysis show varying degrees of hypoxaemia. The use of percutaneous oximetry is an effective and simple alternative to arterial blood gas measurement in the assessment of initial hypoxaemia and response to treatment. Diagnosis of PCP must be differentiated from pulmonary *Mycobacterium tuberculosis,* atypical mycobacteriosis, bacterial pneumonia caused by *streptococcus pneumoniae or Hemophilus pneumonie,* mycosis (pulmonary cryptococcosis), Cytomegalovirus infection and Kaposis sarcoma.[72]

1.6 Treatment of PCP

1.6.1 Trimethoprim and sulphamethoxazole (TMP-SMX)

The treatment of first choice is the combination of trimethoprim and sulphamethoxazole given orally (20mg trimethoprim, 100mg sulphamethoxazole/kg body weight daily in three to four divided doses) or intravenously (trimethoprim 15mg, sulphamethoxazole 75mg/kg daily, again as three to four divided doses). As opposed to HIV negative

patients where treatment for 14 days is adequate, treatment is generally given for 21 days in AIDS patients. It is also prudent to wait at least 7 days before concluding that therapy has failed.[1]

1.6.2 Steroids

Clinical studies have demonstrated that adjunctive steroid therapy initiated within 72 hours in patients with PCP who have moderate to severe hypoxemia significantly reduced the risk of transfer to the intensive care unit, intubation and death.[71] Skin rashes also appear to occur less frequently in TMP-SMX recipients when steroid is given.[74] The steroid may be given orally or intravenously. One regimen that proved successful was the use of prednisone (or prednisolone) 40 mg twice daily for 5 days; 40 mg daily for 5 days and 20 mg daily in the remaining days of therapy. It must be cautioned that the use of steroids in AIDS could have detrimental effects in the presence of Kaposi's sarcoma, tuberculosis and some fungal infections.

1.6.3 Intravenous pentamidine

Compared to TMP-SMX, intravenous pentamidine yields inferior results in term of survival rates.[75] Furthermore, it is fraught with complications e.g. hypoglycemia, hyperglycemia, hypotension, nephrotoxicity, and neutropenia. Substitution of pentamidine in case of TMP-SMX failure is often ineffective; neither does it increase the efficacy of TMP-SMX when given in combination. Nevertheless, in severe PCP, it is the second line agent to use in the event of TMP-SMX intolerance.[75]

1.6.4 Alternative agents

All alternative agents are inferior to TMP-SMX in terms of therapeutic success but may be more tolerable. Their role is therefore limited to mild-to-moderate episodes of PCP, with the possible exception of trimetrexate. In combination with folinic acid, intravenous trimetrexate may be used for severe PCP that has failed TMP-SMX and pentamidine.

For mild to moderate PCP

Trimethoprim-dapsone - in one small study,[76] TMP-dapsone was as effective as TMP-SMX and had fewer adverse events. Dapsone alone is not effective. TMP-dapsone is especially valuable in the event of sulfa allergy

Clindamycin-primaquine - neither alone is effective against PCP. In a study [77] of mild to moderate PCP, this combination however yielded comparable rates of response and intolerance as TMP-SMX.

Atovaquone - In a multicenter trial, [78] use of atovaquone in mild-to-moderate PCP, as compared against TMP-SMX, was associated with lower rates of clinical efficacy and survival but better tolerated. Therapeutic success correlated with plasma concentrations. The new liquid formulation of atovaquone allows it to be given twice a day only.

For severe PCP

Trimetrexate - trimetrexate (in combination with folinic acid) is inferior to TMP-SMX. Its role is limited to patients who have failed or are intolerant to both TMP-SMX and pentamidine. In all patients, supportive treatment for hypoxaemia is required.

Supplementation of inspired oxygen via a nasal cannula (flow 3-4 l/min) is simple and often effective. If not, an oxygen-mask capable of delivering 60% inspired oxygen may be required. In unrelieved hypoxaemia, temporary artificial ventilation may be considered, although it is not currently recommended for patients with AIDS because of the greater than 90% mortality for such patients with pneumocystis pneumonia requiring ventilation and because of the currently unrescueable immune deficiency of that disease.[1]

1.7 Prevention of Recurrence

In fact, effective PCP prophylaxis alone, in the absence of HAART, delays the onset of AIDS by 6-12 months.[76] Patients who have a history of PCP should be administered secondary prophylaxis (chronic maintenance therapy) with TMP-SMX unless immune reconstitution occurs as a result of ART. Despite its efficacy between 20 and 50% of patients with AIDS discontinue TMP-SMX prophylaxis or therapy. Toxicity most commonly in the form of cutaneous hypersensitivity is due to the accumulation of metabolites as a result of a deficient glutathione system in HIV-infected patients.[80] Most adverse effects can be managed with supportive care. However, discontinuation of TMP-SMX is necessary when there is severe rash with mucosal involvement, drug-induced hypotension, or anaphylaxis. For patients who are intolerant of TMP-SMX, alternatives are dapsone; dapsone combined with pyrimethamine, atovaquone, or aerosolized pentamidine. [80]

Secondary prophylaxis should be discontinued for adult and adolescent patients after

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successful use of HAART, when the CD4 count has risen above 200/ul for at least 3 months and re-introduced if the CD4+ T lymphocyte count decreases to <200 cells/μL or if PCP recurs at a CD4+ T lymphocyte count of >200 cells/μL.[81]

1.8 Co-Infection of PCP with other Respiratory Pathogens

The percentage of individuals with PCP who are co infected with other pathogens is higher in developing countries than in industrialized countries; rates of co infection with any pathogen ranging from 20% to 70% have been reported.[34, 35] *Mycobacterium tuberculosis,* which is the most common co pathogen, is present in association with 13%-66% of cases of PCP.[28,34, 35, 40] One study from Mexico showed that tuberculosis was found at the time of post-mortem examination of 24% of patients with AIDS, compared with 6% of US patients with AIDS and 5% of Italian patients with AIDS.[27]. Malin et al found a co- infection of tuberculosis and PCP in 23.8% of all patients diagnosed to have PCP in his study.[23] One report noted the frequency of co-infection with non-tuberculous pathogens for example, cytomegalovirus (68%), *Streptococcus pneumoniae* (50%), *Staphylococcus aureus* (25%), and *Cryptococcus neoformans* (25%).[37] Autopsy series of 18 children from South Africa showed a high frequency of co-infection with cytomegalovirus (44%), which possibly reflected the low mean CD4 cell count (22 cells/ μL) of the patients and the routine use of corticosteroids for the treatment of PCP.[82]

2 PROBLEM STATEMENT

Pneumocystis pneumonia (PCP) has historically been one of the leading causes of disease among persons with AIDS in the North. [13,15,16,18,19] The introduction of highly active antiretroviral therapy in industrialized nations has brought about dramatic declines in the incidence of AIDS-associated complications, including PCP. [18] In the adult population, the incidence of PCP has significantly decreased, but it remains among the most common AIDS-defining infections.[16] In contrast to the situation in developed regions, PCP has been thought to be rare in African adults with HIV/AIDS. Most early studies reported prevalence rates of 0% to 11% in HIV-infected patients.[28-32] The incidence of PCP in Africa may be growing as the AIDS epidemic progresses as more recent studies have been showing an increasing trend though the actual prevalence remain debatable. Malin and colleagues have reported finding PCP among 33% of 64 patients in Zimbabwe observed with diffuse pneumonia unresponsive to penicillin.[17] Recently a trend has also emerged whereby an increasing number of HIV infected patients are empirically treated for *Pneumocystis jirovecii* pneumonia (PCP) based on clinical and radiological features with improvement. This trend is common in many African countries where HIV/AIDS is prevalent and diagnostic tests are unavailable due to limited resources. In such circumstances, a treatment algorithm independent of extensive diagnostic testing and targeted against locally prevalent pathogens is called for. Untreated PCP is lethal. The high incidence of PCP raises concerns that in certain parts of Africa treatment algorithms that do not consider PCP may need to be re-evaluated and if the incidence of PCP is rising, even in selected areas, then prophylaxis in such areas

with co-trimoxazole may be a cost-effective management approach which may also decrease the incidence of bacterial infections. Alternatively, early empirical therapy with co-trimoxazole at high doses may be an effective approach for treating both PCP and bacterial infections before the initiation of empirical anti-TB therapy.

3 RATIONALE

In our setting the number of HIV infected adults presenting with respiratory complaints and who respond to anti-pneumocystic medications is increasing. Though debatable, prevalence of PCP in neighbouring African countries has also shown an increasing trend. Early reports reported lower prevalence in adults despite high seropositivity in community and relatively higher prevalence in HIV infected African children. As the known prevalence in Tanzania is relatively low, [34] this study therefore intends to determine the actual prevalence of *Pneumocystis jirovecii* pneumonia (PCP) among HIV infected adults presenting with cough at our HIV care and treatment clinic.

4 HYPOTHESIS

The prevalence of pneumocystis pneumonia among HIV infected patients presenting with cough in Dar es Salaam is low.

5 OBJECTIVES

5.1 Broad Objective

To determine the prevalence of *pneumocystis jirovecii* pneumonia among HIV- infected patients presenting with cough at Muhimbili National Hospital, Dar es Salaam.

5.2 Specific Objectives

1. To determine the prevalence of PCP among HIV- Infected patients complaining of cough at Muhimbili National Hospital's HIV clinic.
2. To determine the prevalence of PCP co-infection with pulmonary tuberculosis among HIV patients complaining of cough at Muhimbili National Hospital's HIV clinic.
3. To determine the prevalence of PCP co-infection with bacterial pathogens among HIV patients complaining of cough at Muhimbili National Hospital's HIV clinic.
4. To determine the association of CD4+ cells with PCP among HIV patients complaining of cough at Muhimbili National Hospital's HIV clinic.

6 MATERIALS AND METHODS

6.1 Study design

It was a descriptive cross sectional study which aimed at determining the prevalence of PCP among HIV- infected patients who presented with cough at the HIV clinic at MNH.

6.2 Study setting

The study was done at MNH, the largest consultant and University teaching hospital in the country. It is a tertiary referral hospital for the country but in practice it functions as a second and sometimes a first referral level hospital. The hospital is located in the city of Dar es Salaam with an estimated population of 2,497,940 (2002 census). There are 3 districts in Dar es Salaam, namely Ilala, Kinondoni, and Temeke. Each has a district hospital, which takes care of HIV patients both on in- and out-patient basis. MNH runs a HIV care and treatment clinic with an enrolment of about 2000 patients. These are patients from the city as well as few patients referred from other clinics in Dar es Salaam and other parts of the country.

6.3 Target population

HIV infected patients with cough in Dar es Salaam who are more than 18 years old.

Accessible population: HIV infected patients complaining of cough with or without other respiratory complaints attending Muhimbili National Hospital's HIV clinic.

Inclusion criteria:

1. The presence of cough for two weeks or more

2. Age between 18 and 65 years.
3. Ability to consent

Exclusion criteria

1. Patients with chronic respiratory diseases like chronic obstructive airway diseases and bronchiectasis.
2. Patients with cardiac conditions

6.4 Sample size and sampling procedure

Sample size

The sample size was obtained using this formula

$$n = \frac{Z^2 p\,(100 - p)}{\varepsilon^2}$$

Where, n is the minimum sample size required,

Z, the 95% critical value of the normal distribution, equals to 1.96,

p, prevalence of *Pneumocystis jirovecii* pneumonia in Tanzania estimated to be 3.6%.[34]

ε, maximum likely error, is 3.3%

Therefore, at least 122 HIV infected patients with respiratory symptoms were to be included in the study. The observed sample in this study was 125 HIV infected patients.

Sampling procedure

Sampling Technique

Between May and November 2006, a total of 125 eligible patients were consecutively recruited from the HIV clinic. Recruitment was based on first-come-first served.

6.5 **Data collection methods**

Patients who presented with respiratory symptoms were consecutively recruited. All the information regarding study objectives and procedure were explained to the eligible patients. After a written or a witnessed informed consent subjects were interviewed through a face to face interviewer-assisted questionnaire after which clinical examinations were done. Findings were then documented in the questionnaires.

Sputum

Sputum samples were obtained by induction in all patients involved in the study. Sputum induction was done using an ultrasonic nebulizer (ATOM Soniclizer 205). This was done in an open space using a 3% saline as an inducing fluid. From each induced patient, one sputum sample was collected and divided into three parts which were all stored at -20 ^{0} C before they were processed. The first part was Toluidine blue stained for the diagnosis of PCP; the second part was used for PCR diagnosis of PCP and the third part for Gram and Ziehl–Neelsen staining diagnosis of sputa bacterial and pulmonary Mycobacterium tuberculosis infections respectively.

Polymerase Chain reaction was done at Bergen University in Norway for on the first 83

samples. These were shipped while frozen at -20 ^{0} C.

Staining for *Pneumocystis jirovecii* with toluidine blue involved the following steps

1. Addition of sputolysin to the sputum (10% solution).
2. Waiting for 15 – 20 minutes.
3. Addition of 70% alcohol
4. Waiting for 15 – 20 minutes (Inactivates HIV).
5. Centrifugation for 5 minutes at 1000 rpm.
6. Sucking off supernatant
7. Addition of 7.1 pH buffer
8. Centrifugation again for 5 minutes at 1000 rpm.
9. Putting the sediment on slides.
10. Drying in oven at 37^{0}C for 15 minutes.

Staining

1. Putting slides in staining jar
2. Addition of acid + ether and cover – 5minutes
3. Rinsing in tap water carefully
4. Staining in Toluidine for 3 mins
5. Washing quickly in propanol.
6. Putting xylene (few minutes)
7. Mounting in DPX (Put DPX on cover slip)
8. Microscopic examination.

Other Laboratory Investigations

$CD4^+$ lymphocytes cell counts within the preceding three months were taken for the study. Venous blood was drawn from the forearm of study subjects whose $CD4^+$ cell counts were older than three months to determine their $CD4^+$ lymphocytes cell counts. New counts were also determined in all newly diagnosed HIV-patients. CD4 cell count was determined using Becton Dickson Facs Count Machine in the MNH main laboratory.

6.6 Data entry, cleaning and analysis

Information obtained above was collected using questionnaire. All questionnaires were daily checked for completeness and consistencies. Then data were entered into computer using SPSS version 10 for further cleaning, categorizing of continuous variables and eventually analyses. Univariate analyses included summary statistics and frequency tables. Bivariate analyses included comparison of different categories by the use of χ^2 and fischer's exact t tests especially when comparing different proportions. Furthermore, I estimated risks of PTB in different categories were estimated by Odds ratios (unadjusted because of small samples) and their stability by the 95% confidence intervals. All these procedures were performed using bivariate logistic regression analysis. The cut-off point for rejecting the null hypotheses was when p-values were less than 5%.

6.7 Ethical Consideration

The study received ethical clearance from the Research and Publication committee of MUHAS and the Ethics department of MNH. Patients were included after informed verbal and written consent. Patients with PTB, PCP or bacterial pneumonia were treated accordingly.

7 RESULTS

7.1 Characteristics of study population

Socio-demographic and anthropometric characteristics of the study population

The study involved 125 HIV patients who had cough and were attending the HIV clinic between May and November 2006. The Muhimbili HIV clinic has a mean attendance of 93 adult patients per day and an enrolment of 3928 patients aging 15 years or older by the end of 2006. Fifty- seven of these clinic attendees were females and 2932 patients were already on ART.

Of the studied patients, 48 (34.8%) were from Temeke district, 35 (28%) from Ilala while others resided in Kinondoni district. A total of 88 (70.4%) patients were females and 37 (29.6%) were males. About three quarter (72.8%) of all patients enrolled for the study aged between 30 and 49 years with the majority (48.0%) being in the age group 30 – 39 years. The mean age of the study population was 38.5 ± 9.0 years with males being significantly older (41 ± 9.3 years) than females (37.5 ± 8.8 years) ($p = 0.046$)

Fifty five patients (44%) were married while 43 (34.4%) were single. The remaining 27 were widowed, divorced, separated or cohabiting. The mean body mass index (BMI, kg/m^2) of the studied population was 22.2 ± 3.9 and a body mass index of less than 18.5 kg/m^2 was seen in 22 (17.6%) patients.

$CD4^+$ T cell counts checked within three months were documented. The mean $CD4^+$ cell count was 186 ± 169 cells/μL and ranged between three and 789cells/μL. About two thirds of the patients 80(64%) had $CD4^+$ cell count below 200 cells/μL.

About half 67(54.6%) of patients were not on antiretroviral drugs for HIV. These included newly registered patients in the HIV clinic as well as patients who did not qualify for antiretroviral (ART) treatment according to the National guidelines.[83] Fifty eight (45.4%) patients were already on antiretroviral therapy for a mean duration of 10 ± 9.3 months. The duration ranged between half and 42 months

The use of prophylactic cotrimoxazole was reported in less than half (43.2%) of the patients. Most patients who had not started ART were also not on cotrimoxazole prophylaxis except for a few patients whose clinical condition and CD4 counts qualified them for ART but had to wait for pre-treatment counselling. Some patients were not on cotrimoxazole because it was stopped following development of side effects. One patient (0.8%) had started empirical cotrimoxazole treatment for a diagnosis of pneumocystis pneumonia at recruitment. The duration of cotrimoxazole prophylaxis ranged between half and 48 months with a mean duration of 9.09 ± 8.98 months.

Table 1. Socio-demographic and anthropometric characteristics of HIV-patients with cough at MNH HIV clinic,2006. (N =125)

Characteristic	Number (%)
Female	88 (70.4)
Male	37 (29.6)
Age group	
< 30	16 (12.8)
30 – 49	91 (72.8)
50+	18 (14.4)
Marital status	
Single	43 (34.4)
Married	55 (44.0)
Other	27 (21.6)
Age, years (mean ± SD)	38.5 ± 9.0
BMI, kg/m^2 (mean ± SD) Height, m (mean ± SD)	22.2 ± 3.9
BMI, kg/m^2 (mean ± SD)	

Clinical characteristics of recruited patients

Cough was the main presenting symptom, all patients reported to have cough of at least two weeks at the time of interview. Productive cough was reported in more than three quarters 105 (84%) of patients while 20 (16%) patients had a history of dry cough. Of patients presenting with a productive cough, hemoptysis was reported in 19 (15.2 %) patients.

Table 2. Presenting symptoms and signs of recruited HIV-patients with cough at MNH HIV clinic, 2006 (N =125)

Clinical characteristics	Number (%)
Symptoms	
Weight loss	68 (54.4)
History of pulmonary tuberculosis in past 2 years	52 (41.6)
Difficulty in breathing	48 (38.4)
Fever	45 (36.0)
Chest pain	30 (24.0)
Night sweats	25 (20.0)
Dry cough	20 (16.0)
Hemoptysis	19 (15.2)
Painful swallowing	8 (6.4)
Signs	
General examination	
Oral thrush	10 (8.0)
Lymphadenopthy	3 (2.4)
Severe pallor	2 (1.6)
Kaposis sarcoma	2 (1.6)
Respiratory findings	
Crackles	51 (40.8)
Rhonchi	1 (0.8)
Pleural effusion	1 (0.8)
None	72 (57.6)

More than half of the patients (54.4%) reported to have lost weight while 52 (41.6%) had a history of previously diagnosed and treated pulmonary tuberculosis. Difficulty breathing was reported in 48 (38.4%) patients whereas chest pain and excessive night sweats were reported in 30 (24%) and 25 (20%) respectively. A history of fever was reported in about one third (36%) of all patients while eight (6.4 %) had pain during swallowing.

Common general physical signs were wasting, severe pallor, oral thrush, peripheral lymphadenopathy, crackles and rhonchi in the chest; and cutaneous Kaposis sarcoma (Table 2).

Respiratory examinations in more than half of the patients 72 (57.6%) found nothing abnormal. However, the commonest respiratory finding was chest crackles that were found in 40.8% (51 patients). The remaining two patients had rhonchi and signs of pleural effusion.

7.2 Prevalence of pneumocystis pneumonia (PCP)

7.2.1 Prevalence of pneumocystis pneumonia by Toluidine Blue stain

Of the 125 patients studied, *Pneumocystis jirovecii* stained positive in 13 (10.4%) patients by Toluidine blue staining. More than two third 9/13 (69%) of these patients were females and all patients aged less than 50 years. More than half (61.5%) of patients not on cotrimoxazole prophylaxis developed pneumocystis pneumonia. (Table 3)

Table 3. Baseline characteristics in relation to PCP diagnosed by Toluidine Blue stain among HIV-patients with cough at MNH HIV clinic, 2006.(N =125)

Characteristics	*Pneumocystis jirovecii*		P-value
	Positive (%) (N=13)	Negative (%) (N=112)	
Females	9 (69.2)	79 (70.5)	0.82
Males	4 (30.8)	33 (29.5)	0.78
Marital status			
Single	7 (53.8)	36 (32.1)	0.21
Current married	3 (23.1)	52 (46.4)	0.16
Others	3 (23.1)	24 (21.4)	0.86
Age			
<30	4 (30.8)	12 (10.7)	0.06
30 – 49	9 (69.2)	82 (73.2)	0.98
50+	0	18 (16.1)	0.25
Not on Prophylactic cotrimoxazole	8 (61.5)	63 (56.3)	0.72

Table 4. Clinical features of patients with PCP diagnosed by Toluidine Blue among HIV-patients with cough at MNH HIV clinic, 2006. (N =125).

Reported symptom	PCP+ (N=13)	PCP- (N=112)	P-value
Symptoms			
Cough			
Productive	12 (92.3)	93 (83.0)	0.64
Dry	1 (7.7)	19 (17.0)	0.69
Weight loss	6 (46.2)	62 (55.4)	0.53
previous pulmonary tuberculosis	6 (46.2)	46 (41.1)	0.73
Difficult in breathing	7 (53.8)	41 (36.6)	0.36
Fever	2 (15.4)	43 (38.4)	0.13
Chest pain	4 (30.8)	26 (23.2)	0.79
Night sweats	1 (7.7)	24 (21.4)	0.46
Hemoptysis	1 (7.7)	18 (16.1)	0.69
Signs			
BMI(kg/m^2)			
< 18.5	2 (15.4)	20 (17.9)	0.87
≥ 18.5	11 (84.6)	92 (82.1)	0.99
General examination			
Oral thrush	0	10 (8.9)	0.60
Lymphadenopthy	1 (7.7)	2 (1.8)	0.28
Respiratory findings			
Crackles	1 (7.7)	50 (44.6)	0.23
None	12 (92.3)	60 (53.6)	0.01

Several clinical symptoms were reported by patients involved in the study as seen in Table 4. Cough, difficult in breathing, chest pain, peripheral lymphadenopathy and a

previous diagnosis of pulmonary tuberculosis although found higher among patients with PCP, they were not statistically significant. However, a clear chest on physical respiratory examinations was found in a statistically significant proportion of patients (16.7%) who had a diagnosis of pneumocystis pneumonia (P=0.01).

7.2.2 Prevalence of pneumocystis pneumonia by a Polymerase Chain Reaction (PCR).

Of the 125 patients studied,83 patients' sputum samples underwent PCR for a diagnosis of Pneumocystis jirovecii. These patients had the same characteristics as the remaining 42 patients whose sputa did not undergo PCR because of logistic problems.They also included the 13 sputum samples which were positive on Toluidine Blue staining. Three (3.6 %) patients were diagnosed to have PCP by PCR. These were different from the above mentioned positive Toluidine stain. The three patients were all females and their ages ranged between 18 and 32 years.

The first patient was a young lady, single, aged 18 years whose $CD4^+$ cell count was 41cells/μL prior to coming to our clinic she was on ART and cotrimoxazole prophylaxis for two years without her knowledge. All medications were prescribed from a private health facility through her relatives. At our clinic she only complained of a productive cough for more than three months. She weighed 53 kg and her body mass index (BMI) was 21.2 kg/m^2. Respiratory examination revealed crackles in the chest.

The second patient was a married lady aged 32 years and whose $CD4^+$ cell count was 23cells/μL. She started ART and prophylactic cotrimoxazole a year back with initial $CD4^+$ cell count of 6 cells/μL and a body weight of 73 kg (BMI = 29.2 kg/m^2). At inclusion for the study she complained of a dry cough, difficult in breathing and weight loss. She also weighed 61 kg and her body mass index (BMI) was 24.4 kg/m^2. Respiratory examination revealed a clear chest.

The third patient was a young lady, single, aged 20 years and whose CD4 cell count was 134 cells/μL. She was naive to ART and had never been on cotrimoxazole prophylaxis for PCP. She complained of a dry cough, difficult in breathing and weight loss. She weighed 44.5 kg and her body mass index (BMI) was 18.4 kg/m^2. Respiratory examination revealed crackles in her chest.

7.3 Prevalence of sputum positive pulmonary tuberculosis

Acid fast bacilli (AFB) positive smears were found in 9 (7.2%) of the 125 patients studied. A statistically significant greater proportion of these patients 6 (66.7%) were males. (P= 0.03). Al patients were in the age group, 30 and 49 years (Table 5).

Table 5. Demographic characteristics among HIV-patients with cough at MNH HIV clinic in relation to Sputum positivity by Acid Fast Staining. (N =125)

Characteristics	PTB + (N=9)	PTB-(N=116)	P- value
Sex			
Females	3 (33.3)	85 (73.3)	0.03
Males	6 (66.7)	31 (26.7)	0.03
Marital status			
Never married	3 (33.3)	40 (34.5)	0.77
Current married	4 (44.4)	51 (44.0)	0.75
Others	2 (22.2)	25 (21.6)	0.71
Age			
<30	0	16 (3.8)	Not applicable
30 – 49	9 (100)	82 (70.7)	0.13
50+	0	18 (15.5)	0.43

Clinical characteristics in relation to Sputum positivity by Acid Fast Staining.

Smear positive pulmonary tuberculosis was commonly found in patients who complained of productive cough, chest pain, weight loss and those who had previous diagnoses of pulmonary tuberculosis. However, the finding was not statistically significant. (Table 6).

Patients with smear positive pulmonary tuberculosis had statistically more prevalent haemoptysis, difficulty in breathing, chest pain, fever than those who were smear negative. On examination they were more likely to have oral thrush and crackles in their chests ($P< 0.05$). All these tuberculosis cases had $CD4^+$ T lymphocytes counts below 200 cells/μL with a mean count of 67 $CD4^+$ T- lymphocytes//μL.

Table 6. Clinical characteristics in relation to Sputum positivity by Acid Fast Staining among HIV-patients with cough at MNH HIV clinic. (N =125)

Reported symptom	PTB+ (%) n=9	PTB-(%) n=116	P – value
Symptoms			
Cough			
Productive	8 (88.9)	97 (83.6)	0.96
Dry	1 (11.1)	19 (16.4)	0.96
Weight loss	7 (77.8)	61 (52.6)	0.27
History of pulmonary tuberculosis	6 (66.7)	46 (39.7)	0.22
Difficult in breathing	8 (88.9)	40 (34.5)	0.004
Fever	7 (77.8)	38 (32.7)	0.02
Chest pain	6 (66.7)	24 (20.7)	0.01
Night sweats	1 (11.1)	18 (15.5)	0.90
Hemoptysis	7 (77.8)	18 (15.5)	< 0.01
Signs			
BMI(kg/m^2)			
< 18.5	4 (44.4)	18 (15.5)	0.08
≥18.5	5 (55.6)	98 (84.5)	0.08
General examination			
Oral thrush	3 (33.3)	7 (6.0)	0.02
Respiratory findings			
Crackles	7 (77.8)	44 (37.9)	0.046
None	2 (22.2)	70 (60.3)	0.06

Table 7. Clinical predictors for a diagnosis of AFB positive TB among HIV-patients with cough at MNH HIV clinic. (N =125)

Characteristics	Cases (%)	Unadjusted OR (95% CI)	P- Value
Sex			
Females (88)	3 (3.4)	Reference	
Males (37)	6 (16.2)	5.5 (1.3 – 23.3)	0.02
Difficult in breathing (20)	8 (40)	15.2 (1.84 – 125.9)	0.01
Fever (45)	7 (15.6)	7.2 (1.4 – 36.3)	0.02
Chest pain (25)	6 (24)	7.7 (1.8 – 32.9)	0.01
Hemoptysis (19)	7(36.8)	0.68 (0.1 – 5.8)	0.7
Respiratory examination			
Clear chest (72)	2 (2.8)	Reference	
Crackles (51)	7 (13.7)	5.6 (1.1 – 28.0)	0.04
$CD4^+$ cell count (125)	9 (7.2)	0.99 (0.98 – 1.0)	0.05
BMI kg/m^2 (125)	9 (7.2)	0.7 (0.5 – 0.9)	0.01

Table 7 shows estimated predictors of PTB using logistic regression analyses. Among eight initially hypothesized variables to predict AFB smear positivity, seven factors were found to be associated with smear positive results. They were: a male gender whereby males were more than five times (OR=5.5; 95%CI=1.3-23.3) at risk of PTB as compared to females. Patients complaining of difficult in breathing had an elevated risk of more than 15 times (OR=15.2; 95%CI = 1.8-125.9) as compared to those with normal

breathing. Other risky characteristics are fever, chest pain and chest crackles. I further considered continuous CD4 cell counts and BMI (measured in kg/m^2) by assessing trend in the logistic regression model. Both CD4 cell counts and BMI were significantly decreasing with increasing risk of PTB (Table 7).

7.4 Other bacterial pathogens in the sputa

This study found different types of bacteria after sputa Gram staining as seen in Table 8. However in about one third (35.2%) of patients' sputa no organism was found on Gram staining. Gram positive cocci was the commonest organisms seen as it was seen in more than half (57.8%) of all patients' sputa. Gram positive rods were the least (1.6%) seen bacteria. There was no difference in the distribution of Gram-stain findings in relation to $CD4^+$ cell counts.

Table 8. Prevalence of other bacterial pathogens in patients' sputa by Gram- stain among HIV-patients with cough at MNH HIV clinic. (N =125)

Gram stain finding	Cases (%)
None	44 (35.2)
Gram positive cocci	66 (52.8)
Gram negative rods	7 (5.6)
Gram positive rods	2 (1.6)
Gram positive cocci + Gram negative rods	4 (3.2)
Gram positive cocci + Gram positive rods	2 (1.6)

5.1 *Pneumocystis jirovecii* co-infection with Smear-positive Pulmonary Tuberculosis and other pulmonary pathogens.

There was no co-infection of PCP and smear positive pulmonary tuberculosis. However, gram positive cocci were found in sputa of two patients with PCP whereas no organism was identified in one patient by Gram staining. Of the nine patients with smear positive pulmonary tuberculosis, Gram negative rods and Gram positive cocci were identified in one and two patients respectively. Six patients had smear positive mycobacteria tuberculosis alone.

8 DISCUSSION

Study population

The study population was drawn from patients who were attending the HIV clinic in Muhimbili National Hospital. The monthly attendance at the clinic has an average of about 2000 patients. This study involved patients who complained of cough with or without other respiratory complaints for at least 2 weeks. Most patients who were eligible and enrolled for the study were young and middle aged with a mean age of 38.6 years. About half (48%) of these patients were aged between 30 and 39 years. Similar findings have also been reported in a study done by Swai H, 2002; where the age group 30 – 39 comprised 40% of her HIV positive study population.[84] More females (70.2%) were involved in the study than males because more females (57%) are enrolled at the clinic than males.

Prevalence of PCP

Pneumocystis pneumonia has generally been regarded to be a rare opportunistic infection in HIV infected patients in sub-Saharan Africa. There is no clear reason for this but several postulates explain the situation. One explanation is that of limited resources for diagnosis for PCP, as bronchoscopy is expensive, and induced sputum requires specialized equipment and personnel to obtain adequate samples. All these are missing in many African settings and may have led to lower estimates of PCP. Experienced laboratory personnel are also required to prepare and interpret results of specimen.

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As *Pneumocystis jirovecii* cannot be routinely cultivated, histopathologic demonstration of organisms in tissue, bronchoalveolar lavage fluid, or induced sputum samples is required for a definitive diagnosis. Spontaneously expectorated sputum has low sensitivity and is not used to diagnose PCP. Chemical staining, direct immunofluorescent staining and polymerase chain reaction (PCR) are the commonly used diagnostic tests. However their sensitivity differs with PCR being the most sensitive and chemical staining the least sensitive.[13] In this study, *Pneumocystis jirovecii* pneumonia was diagnosed by a microscopic examination of induced sputum stained using Toluidine Blue from all 125 studied patients and by Polymerase Chain Reaction in 83 patients.

With Toluidine Blue staining, 13 (10.4%) patients were diagnosed to have PCP among all patients studied. Because of logistic problems, PCR was only done for specimen obtained in the first 83 patients enrolled in the study. This included the 13 patients who had tested positive on chemical staining. However, with a polymerase chain reaction only 3/83 (3.6%) patients were diagnosed to have pneumocystis. The reason for the discordant was not clear. However, lack of experience of the laboratory technician is a possible explanation for the disparity. Microscopic diagnosis of PCP is not routinely done in our setting.

Although chemical staining is a relatively cheap method as compared to PCR its reliability is questionable as it was obvious in this study. Microscopy is largely dependant on the experience of the laboratory person doing it.

Toluidine Blue staining has been used in different studies in Africa with prevalence rates ranging between 3.6 and 33%. [23, 28, 34, 37] However, most of these studies used Bronchoalveolar lavage which is a more sensitive method of sputum collection than sputum induction used in this study.[82] The PCP prevalence rate of 10.4% seen by chemical staining though higher than the previously known prevalence in Tanzania (3.6%), in a study done more than a decade ago,[34] it is however lower than the recent prevalence rates of 11 to 43% documented in studies done in sub-Saharan Africa. [23, 29, 35]

Discordant of the results obtained by chemical staining and PCR poses a question whether the recent increasing trend seen in African studies is a result of inexperience with laboratory diagnosis.

As PCR is the most sensitive test, the prevalence of PCP in this study is therefore considered to be 3.6%. By allowing for reduced sensitivity of induced sputum compared to BAL [82] but considering the high sensitivity of PCR the low prevalence of PCP found in this study possibly gives the picture in the HIV-infected population in general.

The sensitivity and specificity of PCR in the diagnosis of PCP is reportedly higher in several studies. Klinker et al [85] found a high degree of association of positive *P. jirovecii*

PCR results and clinical evidence of PCP in HIV-infected patients with AIDS. However, using PCR there is an increased analytical sensitivity but with a general risk of detecting colonization of *P. jirovecii* even in immunocompetent patients. [86] On the other hand, in HIV infected patients with progressively decreasing immune function, finding *P. jirovecii* is indicative of clinical PCP. This could be the case for the three patients diagnosed to have PCP in this study.

The three patients with PCP had CD4 + cell counts below 200 cells/μl. Two patients were already on ART and prophylactic cotrimoxazole for one year or more. One of the patients was noted to have poor adherence to both ART and cotrimoxazole for a period of two years. The second patient was considered to be adherent to ART but had unknown adherence to cotrimoxazole. They all had treatment failure to ARVs as indicated by their low CD4 counts, which may perhaps explain the increased risk to PCP. Occurrence of PCP in patients with lower CD4 cell counts in this study is similar to findings of other studies. In Chicago, Phair et al[13] found that CD4+ cell counts at base line of 200 per cubic millimeter or less is associated with an increased risk of *P. jirovecii* pneumonia. Analysis of lymphocyte subsets has shown that the risk of *P. jirovecii* pneumonia in HIV patients is inversely related to the number of circulating CD4 cells[13] this has led to the recommendation for *P. jirovecii* prophylaxis for CD4 counts in adults of less than 200/mm^3. The fact that adherence of the two patients to cotrimoxazole prophylaxis was poor and the third had not started on cotrimoxazole at recruitment made them susceptible

to PCP. As seen in a study done by Lundberg et al [16] patient non-adherence is the most common reason for the occurrence of PCP among patients in HIV care.

Although this prevalence rate of 3.6% is lower than the recently found prevalence rates in other African studies [23, 29, 31,40, 41] it is comparable to the known prevalence in Tanzania. [34,38] This persistently low rate of pneumocystis pneumonia in Tanzania may depict an actual prevalence of the disease. A low prevalence rate was also demonstrated in a Malawian prospective study which used immunofluorescent (IF) staining for *P.jirovecii* cysts and PCR on induced sputum for patients suspected to have PCP. [87] A final diagnosis of PCP was made in 6(1.0%) while *Pneumocystis* colonization was diagnosed in 9(1.5%) episodes. It is therefore likely that the prevalence of PCP is still low in our setting in contrast to increased clinical diagnosis and the recently documented higher rates in studies.

However, reported prevalence and incidence rates in adult African populations vary widely. The basis for this variation in reported PCP prevalence rates among African adults is not clear. Many of these reports were from cross-sectional studies in selected populations from tertiary hospitals, [29,42] and therefore an indication of selection bias that favors identifying higher rates of PCP. Apart from study design, other suggested explanations include differences in diagnostic approach and capability or regional environmental factors.[88] High prevalence rates are reported in patients whose sputum had already proven negative for Mycobacterium tuberculosis and/or had failed to

improve with antimicrobial treatment without activity against *P. jirovecii.* [23,40,42] For instance, reported Zimbabwean prevalence rate of 33% was from BAL in highly selected hospitalized patients with negative direct sputum smears for AFB, CXR verified acute diffuse pneumonia and no response to benzylpenicillin. [23] Similarly, a study from South Africa used 67 HIV-infected patients whose sputum smears were negative for TB and found a prevalence of PCP to be 43 % in their BAL[48]. Bronchoalveolar lavage (BAL) was the most common used method of sputum collection in these studies.

With the low prevalence seen among HIV patients in this study, the emerging trend of empirical treatment for PCP in our setting is probably over-treatment. Clinical improvement with TMP-SMX seen among these patients can partly be explained by the sensitivity of other causes of respiratory infection to the drug. This study dealt with patients with mild symptom who may not have been diagnosed to have PCP on clinical grounds but it gives estimate of the problem among HIV patients in our setting. A similar study among patients diagnosed to have PCP based on clinical characteristics is however needed to estimate the prevalence of pneumocystis in this group.

Prevalence of smear positive pulmonary tuberculosis.

In this study the diagnosis of pulmonary tuberculosis was done by sputum examination under the light microscope using Ziehl Neelsen (ZN) stain for Acid Fast Bacilli. However, the gold standard for the diagnosis of Tuberculosis involves the isolation and identification of *Mycobacterium tuberculosis* using culture.[89] Smear positive pulmonary

tuberculosis was diagnosed in 9/125 (7.2%) of patients. This prevalence rate was seen among HIV patients with mild symptoms attending their regular follow up scheduled HIV clinic. Although the above prevalence is lower than a prevalence rate of 21.6% found among asymptomatic HIV patients by Bakari et al, [90] it shows that tuberculosis is still a problem among HIV patients.

Because of the blunting effect of the HIV co- infection, the actual prevalence is likely to be higher than 7.2%. Co-existence of pulmonary Tuberculosis and HIV have been associated with a high proportion of sputum negative when examined under the light microscope using Ziehl Neelsen stain for Acid Fast Bacilli(AFB).[91] HIV is also associated with lower AFB concentration in sputum with AFB density falling with the falling CD4 cell count. [95] In a study done by Swai H among patients with suspected pulmonary tuberculosis whose sputum were AFB negative, more than a quarter (27.2%) of them had active TB evidenced by positive sputum culture. [84]

Patients with pulmonary TB presented with several clinical features such as, cough, weight loss, a previous diagnosis of pulmonary tuberculosis, difficult in breathing, fever, chest pain, night sweats, hemoptysis, oral thrush and crackles in their chests. However, there were differences of presenting clinical features among AFB positive and negative. Difficult in breathing, fever, chest pain, oral thrush and chest crackles were found to be significantly common in AFB positive patients than AFB negative. Similar findings were seen by in other studies.[84]

A decrease in CD4+ cell count was associated with an increased risk of pulmonary tuberculosis and most of patients who were AFB positive had counts less than 200 cells/μL. A similar finding was seen by Venkatesh et al[94] where patients with CD4+ cell counts below 200 cells/μL had about a four fold risk of pulmonary tuberculosis as compared to similar patients with higher counts. Akin to other studies, [96,97] in this study, TB infection was significantly seen in men.

Pulmonary TB was seen in patients whose CD4 cell counts were low and an increase in the count was associated with protection for the disease. As patients attending HIV clinic are immunosupressed; some severely transmission of the pulmonary TB is likely to occur at the clinic. Active searching for Tuberculosis and possible isolation of these patients from other HIV patients in the clinic is the way to reduce this spread.

In all patients with smear positive pulmonary tuberculosis, none had co-infection with pneumocystis pneumonia. The reason may be the small sample of the studied subjects as both infections affect patients with severe immunosupression and hence likely to coexist

With Gram staining different organisms were found in about two thirds of the studied patients. Gram positive cocci were the most common organisms identified, however species identification was not done because of limited funds. Normal upper airway tract flora are difficult to differentiate from pathogenic, especially when found in a symptomatic patient. Coexistence of these pathogens with both mycobacterial and

pneumocystis were found in this study. This suggests the possibility of co-infection of HIV- infected patients with several respiratory pathogens but more descriptive studies are needed.

Both mycobacterial and pneumocytis pneumonia were found to affect patients with severe immune depression as seen to occur at relatively low CD4 counts. All the three patients with PCP had $CD4^+$ T lymphocytes less than 200 cells /μL with two patients' counts less than 50 cells. The increased risk to PCP and tuberculous infection with progressive immune suppression is caused by the impaired cellular immunity associated with HIV/AIDS.

9 LIMITATION OF THE STUDY

In this study bronchoscopy and bronchoalveolar lavage were not done. These are the sensitive methods done especially when induced sputa are negative. Diagnostic PCR for PCP was also not done in all 125 patients because of financial and logistic constrains.

10 CONCLUSION AND RECOMMENDATION

In our setting the prevalence of pneumocystis pneumonia is still low among patients with HIV/AIDS presenting with cough. Mycobacterium tuberculosis infection is the common cause of respiratory infection among HIV- infected patients. Pulmonary tuberculosis should therefore be looked for and treated in patients with HIV/AIDS as it is still a problem and a risk to other patients especially in our clinic settings where patients come into contact. As immunosupression is a risk to acquisition of tuberculous infection, HIV patients co-infected with tuberculosis should be isolated from other HIV infected patients in the clinic to reduce chances of spreading tuberculosis to fellow risky patients. Before empirical treatment for pneumocystis is tried, other causes of pulmonary infection should be excluded. However, more studies are needed using more sensitive methods and large samples. Findings in this study should be taken as a baseline.

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12 APPENDIX I: QUESTIONNAIRE

THE PREVALENCE OF PNEUMOCYSTIS JIROVECII PNEUMONIA IN HIV-INFECTED PATIENTS WITH RESPIRATORY DISORDERS AT MNH, 2006

Q/N: _____

File No:_____

Name_____

Date_____

DEMOGRAPHIC INFORMATION

Date of Birth_____/_____/_____

Address _____

Sex F ☐ M ☐

Occupation_____

Marital status: single ☐ married ☐ other ☐ Specify _____.

CURRENT SYMPTOMS AND DURATION

	No	Yes	Duration
Cough	☐	☐	_____
Chest pain	☐	☐	_____
Difficult in breathing	☐	☐	_____
Fever	☐	☐	_____
Hemoptysis	☐	☐	_____
Night sweats	☐	☐	_____

Weight loss ☐ ☐ _____

previous history of TB ☐ ☐ _____

Is the patient on Cotrimoxazole prophylaxis No ☐ Yes ☐

Duration _____

Is the patient on -ART: No ☐ Yes ☐ Regimen _____

duration_____

PHYSICAL EXAMINATION

Weight: _____kg

Height: _____meters

General:	No	Yes
Pallor	☐	☐
Cyanosis,	☐	☐
Oral candidiasis	☐	☐
LN enlargement	☐	☐
Finger clubbing	☐	☐
Edema	☐	☐
Kaposis sarcoma	☐	☐

Other _____

Resp exam RR: _____/minute

	No	Yes
Crackles,	☐	☐
Rhonchi	☐	☐

Pleural effusion ☐ ☐

Other _____ _____

Abdominal exam

Ascites ☐ ☐

Hepatomegaly ☐ ☐

Splenomegaly ☐ ☐

Palpable masses ☐ ☐

Other: _____

CVS → HR: _____

Valvular heart disease ☐ ☐

Cardiomegaly ☐ ☐

Pericardial rub ☐ ☐

Pericardial effusion ☐ ☐

Other: _____

LABORATORY INVESTIGATIONS

$CD4^+$ count within 3 months _____

Induced Sputum results

- PCP Toluidine stain +ve ☐ -ve ☐
- AFB +ve ☐ -ve ☐
- Gramstain _____

13 APPENDIX II: CONSENT FORM

STUDY ON THE PREVALENCE OF PNEUMOCYSTIS JIROVECII PNEUMONIA IN HIV-INFECTED PATIENTS WITH RESPIRATORY DISORDERS AT MNH, 2006.

FOMU YA RIDHAA YA USHIRIKI KATIKA UTAFITI.

Habari za saa hizi,

Jina langu ni Dr Julius Mwita nafanya kazi katika hospitali ya taifa ya Muhimbili.

Ninafanya utafiti kwa watu wenye virusi vya UKIMWI wenye matatizo ya kifua kuangalia iwapo wana vijidudu vinavyoshambulia mapafu vya nimonia na kifua kikuu. Utafiti huu unafanyika kwa wagonjwa wanaohudhuria kliniki ya wagonjwa wa UKIMWI katika hospitali ya Taifa Muhimbili. Ningependa kukushirikisha katika utafiti huu kama utatoa ridhaa yako na kama utatimiza mashariti ya kushiriki.

MADHUMUNI YA UTAFITI

Umekaribishwa kujiunga na utafiti huu kwa sababu ya matatizo ya kifua uliyonayo. Madhumuni ya utafiti huu ni kuangalia iwapo watu wenye matatizo ya kifua waa magonjwa yanayosababishwa na wadudu mabalimbali ikwemo kifua kikuu. Dhumui la pili ni kuangalia uhusiano wa magonjwa hayo ya kifua a kinga ya wagonjwa wa UKIMWI.

NI NINI MSHIRIKI ANATAKIWA KUFANYA.

Ili kuweza kushiriki utafiti huu, utaombwa ukubali kutolewa makohozi kwa kutumia kifaa maalumu ili makohozi hayo yapimwe kuagalia uwepo wa wadudu waliotajwa hapo juu.Watakaokubali kushiriki itawabidi wasaini fomu kuonyensha wazi kukubali kwao.

Pia, tutawahoji maswali kadhaa na kuwapima kwa ajili ya utafiti huu.

USIRI

Kwa sababu habari zote tuazojaza kwenye fomu hii ni za siri, jina lako pamoja na habari zote zinazo kuhusu itakuwa siri kati ya mtafiti na wewe. Mtu mwigine hatashughulika, kuhusiska na majibu au habari za mgonjwa isipokuwa wafanyakazi maalumu wa utafiti huu.

MADHARA

Sitegemei madhara yoyote kutokea kwako kutokana na ushiriki wako katika utafiti huu.Isipokuwa utapata udhia kidogo wakati wa zoezi la kukuwezesha utoe makohozi kwa kutumia mashine.

HAKI YA KUJITOA KATIKA UTAFITI

Ushiriki wako ni kwa hiari kabisa na pia unayo haki ya kujitoa katika utafiti huu wakati wowote ule utakapojisikia kufanya hivyo. Uamuzi wako wa kushiriki ama la katika utafiti huu, hautaadhiri hata kidogo haki yako kama mgojwa ya kupata huduma na tiba.

FAIDA YA KUSHIRIKI

Faida utakayopata katika utafiti huu ni kujua kama umeambukizwa nimonia au kifua kikuu au la na hivyo kupata tiba.Kama una maswali yeyote kuhusu utafiti huu , uko huru kuwasiliaa na msimamizi wa utafiti huu Prof F. Mugusi katika idara ya Tiba ya Chuo kikii cha Afya Muhimbili.Kama una maswali kuhusu haki yako ya msingi kama mshirikikatika utafiti huu tafadhari wasiliana na mwenyekiti wa kamati ya utafitiya Chuo Kikuu cha Afya na Tiba Muhimbili.

Je una swali lolote?

Mimi _____Nimeelewa maelezo yaliyoandikwa hapo juu na kuridihika na majibu niliyopewa kwa maswali yangu yote. Mimi kwa hiari yangu mwenyewe, bila kushurutishwa na mtu, ninakubali kushiriki kwenye utafiti huu.

Sahii ya mshiriki:_____

Sahihi ya mtafiti:_____

Tarehe:_____

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