

Original Research Article

Effects of CD4 count level on patterns of respiratory tract infections of HIV-infected patients in Western India

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Abstract

Background: Individuals with HIV infection are at increased risk for tuberculosis (TB) and other respiratory tract infections (RTIs). The altered CD4 T-cell homeostasis induced by HIV infection may play a key role in the development of respiratory tract infections in HIV-infected patients.

Aim: Finding out of mean CD4 count of HIV patients at which they were at higher risk of developing various RTI and accordingly when HAART is to be started in this part of the world.

Material and methods: All the 961 HIV infected patients and 300 HIV sero-negative patients' three early morning sputum were screened for routine bacterial and fungal pathogens and even examined for AFB and few of the samples were even cultured on LJ medium. All sputum samples' smears were also examined for PMNLs in Gram's staining.

Results: Out of all these 961 HIV patients, in 349 patients with probable viral RTI etiology, the mean CD4 count was found to be 474.62 ± 114.89 , followed by mixed polymicrobial RTI (80 patients) with mean CD4 about 392.26 ± 87.14 . The patients with pure fungal etiology (66), the mean CD4 count was found to be 377.29 ± 268.29 followed by 466 patients with pure monomicrobial bacterial RTI the mean CD4 count was about 223.07 ± 83.21 .

Conclusion: Very vague co-relationship between pattern of RTIs and CD4 counts had been attempted. Only Fungal and Bacterial RTIs were seen first to establish in even HIV infected patients at very high mean CD4 counts of about 377 ± 268.29 and 223.07 ± 83.21 respectively, but in both very high prevalence rate had been observed when compared with HIV non-infected patients with probability values of <0.05 and <0.001 respectively. Probable viral etiology of RTI was significantly high in HIV-non infected subjects when compared to HIV-infected RTI patients with probability value $P < 0.001$.

Key words

HIV, CD4 count, Pulmonary TB, RTI, Atypical bacterial RTI, Viral RTI.

Introduction

The World Health Organisation has estimated that 25 million people have died of HIV/AIDS, including 2 million people who died in 2007 [1]. A significant proportion of these deaths were due to opportunistic pneumonias. The majority of new HIV infections are in adults aged 20 to 39 years old [2]. With the introduction of HAART antiretroviral therapy, patients are living longer and the incidence and severity of opportunistic pneumonias have decreased. However, HIV-associated opportunistic pneumonias remain a major cause of morbidity and mortality. Many patients in developing countries are unaware of their HIV infection until they present with an opportunistic pneumonia. Patients whom are aware of their HIV status but have poor adherence to antiretroviral treatment and prophylaxis are also at risk of opportunistic pneumonias. The range of HIV associated opportunistic pneumonias is broad and includes bacterial, mycobacterial, fungal, viral and parasitic pneumonias.

Tuberculosis, *Pneumocystis* pneumonia (PCP) and recurrent bacterial pneumonias (defined as two episodes occurring within a 12 month period) are frequent causes of AIDS-defining diseases [3, 4]. Doctors are frequently faced with challenges in making an accurate diagnosis for these 3 conditions. These 3 conditions often require prompt treatment decisions before microbiological confirmation is available. The majority of new cases in Malaysia (70% of cases in 2003 and 53.2% of cases in 2008) were intravenous drug users [2] and this group have been, found to be at higher risk of TB and bacterial pneumonias compared to other HIV infected groups [5].

Upper respiratory tract infections are loosely divided by the areas they affect, with the common cold primarily affecting the nose, the throat (pharyngitis), and the sinuses (sinusitis),

occasionally involving either or both eyes via conjunctivitis. Symptoms are mostly due to the body's immune response to the infection rather than to tissue destruction by the viruses themselves. The common cold may occasionally lead to pneumonia, either viral pneumonia or secondary bacterial pneumonia.

Symptoms include coughing, sore throat, runny nose, sneezing, and fever which usually resolve in seven to ten days, with some symptoms lasting up to three weeks. Well over 200 virus strains are implicated in the cause of the common cold; the rhinoviruses are the most common.

Material and methods

The present study was approved by the institutional ethical committee. The present prospective study was conducted in between 9th August 2009 to 23rd January, 2012.

A predesigned and pretested questionnaire was used to collect data on socio-demographic profile. Blood samples of these subjects were tested for HIV. The HIV-infected patients were all diagnosed as HIV reactive as per the NACO guidelines [6]. In the patients found HIV sero-positive even, CD4 count was calculated on FACS count, by flow cytometry method (Becton Dickinson) method from their blood samples.

Three consecutive early morning sputum samples were collected and even samples were concentrated before reporting negative for AFB from 961 HIV infected patients and 300 HIV sero-negative subjects who had complaint of cough and fever for more than one week. Sputa samples were collected in a sterile wide mouthed container. The quality of the expectorated sputum was assessed both by macroscopic and microscopic examination. Any sample that was thin, watery and with no purulent matter was considered unsuitable for further processing. Bartlett's scoring method was used for

microscopic evaluation of the expectorated sputum [7]. A sputum was considered unsuitable if it had a final score of 0 or less. All unsuitable specimens were discarded and a repeat specimen was collected.

Case definition for T

Cases were defined as patients with both HIV sero-positive as well as having complaints of cough and fever for more than one week or in other words suffering from respiratory tract infections (RTI) at the time of sputum and data collection. One patient was included only once. If HIV infected patient with sign and symptoms of RTI but having history of allergic common cold, were excluded from the study.

Definition for C1 (Control group)

C1 Control group was defined as patients with respiratory tract infection (RTI) but seronegative for HIV at the time of sample and data collection.

Exclusion criteria

Patients having history of allergic common cold or allergic respiratory tract infections.

Sputum smear microscopy and culture

The most frequent method of TB detection involved microscopic examination of sputum for acid-fast bacilli (AFB) [8]. Microscopy had the advantage of being inexpensive, relatively rapid to perform, and specific in most settings. However, to be considered smear positive a specimen needs to contain approximately 10^5 Mycobacteria per milliliter. The sensitivity of sputum microscopy in HIV infection ranges from 43 to 51 per cent [9] and in many resource-limited settings with high rates of co-infection, the sensitivity may be much lower [10]. Methods that improved speed or sensitivity include fluorescence microscopy [11] and alternative specimen processing methods, such as concentration, bleach sedimentation and same-day sputum collection (so called front loading) strategies [11, 12]. Any procedure for digestion or liquefaction followed by centrifugation, prolonged gravity sedimentation, or filtration

increased sensitivity by 13 to 33 per cent over direct microscopy, when culture was used as the reference standard [11].

Culture of *Mycobacterium tuberculosis* is much more sensitive than smear microscopy and has been recommended to assist in the diagnosis of TB in HIV-infected individuals. But in the present study it had been used in the limited number of the patients, which were clinically strongly suggestive of pulmonary TB but negative by ZN staining even after concentration of sputum samples.

Expectorated sputum was used to detect the bacterial and fungal pathogens and induced sputum was used for detection of trophozoites and cysts of *P. carinii*. Quality of the expectorated sputum was assessed both by macroscopic and microscopic examinations. Specimens which were clear, thin, and watery with no purulent material were rejected. Microscopically, Bartlett's scoring method was used to assess the quality of the sputum.

Smears were prepared and subjected to Gram's staining, Ziehl-Neelsen staining (20% H_2SO_4 and 1% H_2SO_4), and Toluidine O stain. KOH mount was done for fungi.

The specimens were cultured on 5% Sheep Blood agar, MacConkey agar, heated Blood agar, Lowenstein Jensen Media (only in limited number of cases), and Sabouraud's dextrose agar. The plates were incubated at 37°C for 18 to 24 hours in humid air plus 5 to 10% CO_2 . Sabouraud's dextrose agar slopes were incubated in duplicates at 28°C and another at 37°C for 4 weeks and observed for growth at intervals. Lowenstein Jensen media was incubated at 37°C for 4 weeks and observed for growth. Identification of the organisms was conducted according to standard microbiological procedures [13, 14]. Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion methods according to CLSI guidelines [15, 16]. Antibiotic discs were selected as per CLSI (Clinical and Laboratory Standards Institute).

Candida in the sputum was regarded as pathogen only after obtaining the same strain in repeated samples in pure growth, observing numerous polymorphonuclear leucocytes on Gram's stain of the sputum samples along with pseudohyphae. All *Candida* obtained in Sabouraud's dextrose agar was then processed for identification of species. Germ tube test was done. All *Candida* were inoculated on corn meal agar and incubated at 25°C to demonstrate chlamydo spore formation and to look for typical morphology. Sugar assimilation test was done on Yeast Nitrogen Base agar for further speciation, using the following sugars: glucose, maltose, sucrose, lactose, trehalose, raffinose, and galactose.

All Gram stained smears from samples of sputum were even examined for Poly Morpho Nuclear Leucocytes (PMNL) cells to co relate pathogenicity and avoid contamination. The CD4 count of the patients included in the study was done. The method used was Flow cytometry method and done by FACS count.

Results

Prevalence of pathogenic bacterial and fungal isolates from both HIV seropositive (T) and HIV seronegative (C1) groups was as per **Table – 1**. Comparison of mean CD4 of patients of various groups was as per **Table – 2**. Comparison of mean CD4 values of patients suffering from respiratory tract infections by various organisms was as per **Table – 3**.

Table - 1: Prevalence of pathogenic bacterial and fungal isolates from both HIV seropositive (T) and HIV seronegative (C1) groups.

Identified Pathogens	HIV +VE RTI +VE (T) Patients (n=961)		HIV -VE RTI +VE (C1) patients (n=300)		X ² Values	P Values
	(n)	%	(n).	%		
<i>M. tuberculosis-infecs.</i>	244	24.45	27	9.00	6.80	<0.02
Other bacteria	466	26.74	44	14.67	15.38	<0.001
Fungal agents	163	11.75	10	2.33	7.36	<0.02
Poly-Microbial	80	8.32	7	2.33	2.83	<0.1
Total Patients with identified Pathogens	612	63.68	78	23.67	14.12	<0.001
PMNLs seen but No isolate	87	9.05	23	7.67	0.12	NS
No Patients with Viral RTI	262	27.26	199	66.33	22.73	<0.001

Discussion

Although HIV infection is most closely associated with altered cell-mediated immunity (which is manifested by a decrease in CD4 count), a number of additional immune deficits may occur in association with HIV infection [17, 18, 19], including a poor antibody response due

to B cell dysfunction and defects in chemotaxis, phagocytosis, and intracellular killing by monocytes, macrophages, and neutrophils [17, 19]. In addition, HIV-infected individuals may experience impairment of local defenses, manifested by a depression of specific IgA at the mucosal surfaces [18, 19]. These immune

abnormalities all contribute to an increased risk of bacterial infection among HIV-infected persons, particularly by encapsulated bacteria, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Table - 2: Comparison of mean CD4 of patients of various groups.

Organisms	Mean CD4 cells/ μ L	Standard Deviation	Cases	%
Pulmonary tuberculosis	198.52	\pm 32.25	244	25.39
Extra-pulmonary TB	104.89	\pm 47.09	64	06.66
All TB cases	151.71	\pm 72.62	308	32.05
Patients excluding only pulmonary TB	408.40	\pm 202.23	653	67.95
Candida albicans	257.12	\pm 82.96	63	6.55
Candida (NCAC)	499.73	\pm 196.24	97	10.09
All patients with identified organism	288.62	\pm 94.47	612	63.68
Only bacterial RTI	223.07	\pm83.21	466	48.49
Only fungal RTI = Candida albicans + Candida(NCAC) + Aspergillus	377.29	\pm268.29	66	6.87
Atypical bacterial RTI (No isolates with PMNLs seen)	389.22	\pm89.93	87	09.05
Polymicrobial RTI	392.26	\pm87.14	80	08.32
All RTI patients without identified pathogens (Probable Viral RTI)	502.97	\pm114.89	262	27.26
Total patients	339.10	\pm84.0	961	100

Table - 3: Comparison of mean CD4 values of patients suffering from respiratory tract infections by various organisms.

Organisms	Mean of CD4 Cells/ μ l	Standard Deviation (SD)	Total Isolates (monomicrobial + polymicrobial)	
			n=961	%
Klebsiella	407.93	\pm 115.34	92	9.57
Acinobacter	254.73	\pm 231.77	11	1.14
C. albicans	257.12	\pm 82.86	115	11.97
Candida	499.73	\pm 196.24	63	06.56
E. coli	193.26	\pm 70.77	24	02.5
Proteus	208.67	\pm 111.74	06	0.62
Pseudomonas	252.29	\pm 72.78	59	06.14
S. aureus	115.71	\pm 54.91	20	02.08
S. pneumoniae	201.57	\pm 52.88	35	03.64
S. pyogens	547.72	\pm 248.25	14	01.46
M. tuberculosis	198.52	\pm 32.25	244	24.35
Aspergillus	29.00	\pm 2.16	03	0.31
Male	325.45	\pm81.73	577	60.05
Female	360.33	\pm108.96	383	39.85
Total	339.10	\pm84.0	961	100

Although bacterial pneumonia often occurs in the early stages of HIV infection, the risk of bacterial infection increases steadily with declining CD4 lymphocyte counts [20]. For example, HIV-infected individuals with CD4 counts of less than 200 cells/mm³ have a fivefold increased prevalence of bacterial pneumonia compared with infected persons with CD4 counts greater than 500 cells/mm³.

Recent trends in the prophylaxis and treatment of HIV-infected individuals have influenced the relative frequency of various pulmonary infections. For example, the widespread use of prophylaxis for *Pneumocystis carinii* pneumonia has dramatically decreased the incidence of this infection [21]. The use of trimethoprim-sulfamethoxazole as a *P. carinii* pneumonia prophylactic agent also provides a lesser degree of protection from bacterial infection [20]. In the present study attempt had been made for even detecting *P. carinii* by Giemsa staining but not a single *P. carinii* was detected.

Bacterial pneumonia is a common complication of HIV infection, occurring at all stages of HIV disease, but more frequently as immune function declines [20]. The pathogens and clinical features of bacterial pneumonia are generally similar in patients with and without HIV infection, although the role of atypical pathogens (*Mycoplasma*, *Chlamydia*, and *Legionella*) has not been studied systematically. In the present study 87 patients (09.05%) were found with probable atypical bacterial RTI, as in all these patients significant PMNLs were seen in Gram's staining but no bacterial or fungal etiology was either seen or isolated subsequently.

Many patients in developing countries are unaware of their HIV infection until they present with opportunistic RTI or pneumonia, in the present study also, the mean CD4 associated with respiratory tract infections found was, to be around 339.10 ± 84.0 cells/μl, which means RTI were the first opportunistic infections to occur in AIDS patients. The range of HIV associated opportunistic pneumonia/RTI is broad and

includes bacterial, mycobacterial, fungal, viral and parasitic pneumonias/RTI [22]. The HIV infection decreases the CD4 cells, signaling the emergence of opportunistic and non opportunistic type of pulmonary infections [23].

Early in the HIV epidemic, researchers noted that bacterial pneumonia was a common cause of morbidity [24]. Decreasing CD4+ lymphocyte count, injection drug use, prior sinusitis, and prior lower respiratory tract bacterial infection are risk factors for bacterial pneumonia in patients with HIV infection [25]. In the present study we found 5 patients who were injection drug users, all of them had RTI, while in RTI negative but HIV positive patients none was injection drug user. A multicentre study showed the incidence of bacterial pneumonia to be 5.5 per 100 person-years among HIV seropositive individuals; this incidence was higher than that of *P. carinii* pneumonia [26]. An autopsy study from two medical centers has confirmed bacterial pneumonia to be the most common pulmonary complication in patients with HIV [27]. The cumulative incidence of bacterial pneumonia in hospitalized patients with HIV infection may be as high as 12.5 per 100 person-years [25].

The overall rate of bacterial pneumonia in HIV-infected persons is approximately six times greater than that in the general population [20]. In the present study almost same 5.8 times (257/44) more bacterial RTI had been observed. The incidence of pneumococcal pneumonia is five to 18 times greater than that in the general population, and the development of pneumococcal septicemia is 100 times greater [28]. In the present study due to under diagnosed pneumococcal infections this difference had not been found. It has been estimated that greater than one third of all persons with AIDS will develop at least one episode of severe bacterial pneumonia over the course of their HIV infection [17]. Considering these data, Afessa, et al. [27] found bacterial pneumonia to be the most frequent pulmonary complication (42%) in a recent autopsy series of 233 HIV-infected individuals.

Although bacterial pneumonia often occurs in the early stages of HIV infection, the risk of bacterial infection increases steadily with declining CD4 lymphocyte counts [20]. For example, HIV-infected individuals with CD4 counts of less than 200 cells/mm³ have a fivefold increased prevalence of bacterial pneumonia compared with infected persons with CD4 counts greater than 500 cells/mm³. Eighty percent of cases of bacterial pneumonia occur with CD4 count lower than 400CD4/mm³, and recurrent pneumonia with less than 300 CD4/mm³ [29]. In the present study total 643(66.90%) bacterial (routine bacterial RTIs +Atypical Bacterial RTIs + Mixed Bacterial RTIs) were detected from HIV-infected patients with mean CD4 count of 223.07 ± 83.21 and 387.22 ± 98.93 respectively.

Pulmonary infection with more than one pathogen is not unusual in HIV infected patients. In one study a polymicrobial etiology was identified in 9% of all pulmonary infections [30]. In the present study all most the same result was seen as total 80 (8.32%) polymicrobial etiologies were detected.

Since the beginning of the AIDS epidemic, the lungs have continued to be a frequent site of organ complication. Traditionally, pulmonary infections in patients with HIV have been classified into opportunistic and non-opportunistic. Opportunistic infections are caused by organisms that do not cause disease in immunocompetent individuals. However, any human pathogenic organism can cause disease in patients with HIV, blurring this classic classification. The type of pulmonary infection occurring in an HIV-infected patient depends on the stage of the HIV infection, the individual history of prior infection, the virulence of the infecting organism, and other host-related factors, such as the disease exposure category and geographic location. Various defects in immunity have been described early in the disease process of patients with HIV infection, before we see a decline in the CD4+ lymphocyte count. In addition to the low CD4+ lymphocyte count, humoral immune dysfunction, depressed

IgA2 and IgG2 levels, and decreased CD4+ T-lymphocyte cell-mediated antibody-dependent cellular cytotoxicity are also present in HIV-infected patients, predisposing them to bacterial infections [28, 31, 32]. Bacterial infections are the most common respiratory complications in patients with HIV infection [33]. These infections occur at all levels of CD4+ lymphocyte count but become more frequent as the CD4+ lymphocyte count declines [20, 34].

Early in the HIV epidemic, researchers noted that bacterial pneumonia was a common cause of morbidity [24]. Decreasing CD4+ lymphocyte count, injection drug use, prior sinusitis, and prior lower respiratory tract bacterial infection are risk factors for bacterial pneumonia in patients with HIV infection [25]. In the present study we found 5 patients who were injection drug users, all of them had RTI, while in RTI negative but HIV positive patients none was injection drug user. A multicentre study showed the incidence of bacterial pneumonia to be 5.5 per 100 person-years among HIV seropositive individuals; this incidence was higher than that of *P. carinii* pneumonia [26]. An autopsy study from two medical centers has confirmed bacterial pneumonia to be the most common pulmonary complication in patients with HIV [27]. The cumulative incidence of bacterial pneumonia in hospitalized patients with HIV infection may be as high as 12.5 per 100 person-years [25].

The annual incidence of pneumococcal bacteremia is estimated to be as high as 940 per 100,000 patients with AIDS. In certain regions, the majority of adults with pneumococcal infection and < 40 years of age are HIV seropositive [27]. Previous studies have shown *S. pneumoniae* to be the most common cause of bacterial pneumonia [25]. In the study of Bekele Afessa and Bethany Green [35], *S. pneumoniae* was not the most common pathogen causing bacterial pneumonia. *S. pneumoniae* is isolated in blood cultures of 60% of HIV-infected patients with pneumococcal pneumonia and 15 to 30% of patients without HIV infection [28]. In our present study *S. pneumoniae*

was isolated from 2.39% of HIV-infected patients with respiratory tract infections and 2.33% of patients without HIV infections, suggesting under diagnosis of *S. pneumoniae* RTI infections in HIV-infected patients. Because our study was purely observational, we did not have any control on the management plan of the individual patients.

The trends among persons dying of HIV infection in the United States show an increase in the percentage of deaths associated with bacterial respiratory tract infections [36]. An Italian study of 350 episodes of bacterial pneumonia reported a case-fatality rate of 27% [37]. The case-fatality rate was 21% in the study of Bekele Afessa and Bethany Green [35]. Compared with patients without bacterial pneumonia, patients with bacterial pneumonia had longer length of hospital stay and higher ICU admission and case-fatality rates in their study. This indicates the adverse impact of bacterial pneumonia on the morbidity and mortality of hospitalized patients with HIV infection [35].

The obvious result in the present study was significantly higher probable viral RTIs were seen in as many as 199 (66.33%) patients in HIV non-infected patients when compared with probable viral RTIs in HIV-infected 262 (27.26%) patients, with p value less than 0.001. The possible one reason for this phenomenon was might be due to early conversion of viral RTIs to secondary bacterial or fungal RTIs in HIV-infected patients, due to immune-suppression. But this is very vague indirect studies which cannot be more rely upon. For coming to any conclusion more direct study with viral culture/viral serology tests are required in this regards. The results of the present study proved that prevalence of pulmonary tuberculosis was significantly higher in HIV-infected 244 (24.45%) patients than HIV non-infected 27 (9.0%) patients with p value <0.02. The prevalence of bacterial RTIs in HIV-infected patients was when compared with that HIV non-infected patients, the p value was less than 0.05. In the present study we also tried to co-relate

various RTI with their mean CD4 counts and found poly-microbial infections in 80 patients (8.32%) with mean CD4 count 392.26 ± 87.14 . Probable atypical bacterial RTI were indirectly observed in 87 patients (9.05%) with mean CD4 count about 389.22 ± 89.93 . Last but not least fungal RTI were seen in 66 (06.87%) patients with mean CD4 count about 377.29 ± 268.29 followed by pure routine bacterial RTIs which were directly detected in 466 (48.47%) patients with least mean CD4 count of 223.07 ± 83.21 . The mean CD4 count for pulmonary tuberculosis was found to be 298.52 ± 32.25 .

Conclusion

Very vague co-relationship between pattern of RTIs and CD4 counts had been attempted. Only Fungal and Bacterial RTIs were seen first to establish in even HIV infected patients at very high mean CD4 counts of about 377 ± 268.29 and 223.07 ± 83.21 respectively, but in both very high prevalence rate had been observed when compared with HIV non-infected patients with probability values of <0.05 and <0.001 respectively. Probable viral etiology of RTI was significantly high in HIV-non infected subjects when compared to HIV-infected RTI patients with probability value $P < 0.001$, might be due to two factors, firstly secondary bacterial infections sets in early due to immunosuppression and secondly might be due to presence of high amount of interferon present due to HIV infection.

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