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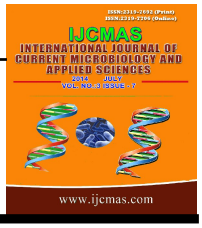


**Original Research Article**

# Spectrum of various fungal & bacterial respiratory tract opportunistic infections in relation to mean CD4 count profile among HIV patients of Gujarat, India

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**A B S T R A C T**

In the present study we tried to co-relate mean CD4 count of HIV patients with the causative agents of various bacterial and fungal RTIs . At which CD4 count to initiate HAART therapy has continued to be a core controversy within the medical community. The development of a stable consensus is hampered by the lack of randomized controlled studies with many guidelines and consensus statements basing their recommendations on observational studies. In present study it was found that irrespective of itiology of RTI(viral, bacterial, fungal or parasitic) in HIV sero positive patients, the mean CD4 count observed in them was 339.10+84.0, which means most RTI which are the first secondary infections in HIV patients, sets in when the patients immunity is deteriorated with mean CD4 count of 339.10 + 84.0, which indirectly support the WHO's recommendation of early initiation of HAART at the CD4 count of 350 cells/µl and recently even 500 rather than 200 cells/µl as given in our country.

**Keywords**

CD4 count, RTI, HAART, standard HAART and early HAART

## Introduction

The HIV infection is alarming due to the unique pathogenesis of the virus that decreases the CD4 cells, signalling the emergence of opportunistic infections in the host (Rewari BB). Among the various opportunistic infections, respiratory infections account for upto 70% of AIDS defining illness (Walker PA, White DA.1996). Their relative importance differs in different parts of the world (Joshi PL, Joshi PL, Mishra SN).

HIV is now in pandemic form and treatment of HIV with HAART has decreased morbidity and delayed the mortality up to 20-30 times. WHO recommends HAART should be started early at the CD4 count of around 350 cells/µl. But in ART centre of NACO, HAART usually starts at standard CD4 count of around 200 cell/µl. In the present study we collected sputum samples and blood samples at the same time for CD4 count, to co-relate isolation or finding

of micro-organisms with that of patients CD4 count, to evaluate at which degree of deterioration of CD4 count which bacterial or fungal RTI infections are more common. Thus mean CD4 count for each and every individual micro-organism was calculated with their standard deviation. Same way the mean CD4 count with its standard deviation was calculated for all HIV patients with respiratory tract infections, which was found out to be 339.10 $\pm$ 84.0 , which it self favoured initiation of early HAART which starts at CD4 count of around 350 to minimize or avoid the morbidity of this respiratory tract infections.

## Materials and Methods

Patients clinically diagnosed to have lower respiratory tract infections attending ICTC and ART Centres, Civil Hospital, Surat were included in the study. All these patients were initially screened for anti HIV antibodies before being enrolled in the study. Nine hundred sixty one HIV reactive [UBI HIV 1/2 EIA (United Biomedical, Inc., Beijing) and HIV TRIDOT (J.Mitra & Co. Ltd., New Delhi) confirmed] patients with lower respiratory tract infection formed the study groups.

### Specimens

An early morning expectorated sputum as well as induced sputum collected separately in sterile containers from all patients included in the study. Induction of sputum was done using a Nebulizer (model - Medel Aero Family) and 3% hypertonic saline for 15 minutes. In addition to sputum 5 mL of blood was collected from all patients included in the study.

### Processing of specimens

Microscopic examination of sputum Induced sputum was examined for the presence of trophozoites and cysts of *P.carinii* while the expectorated sputum was examined for bacterial and fungal pathogens. 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(Koneman EW et al.,1997). 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.

### Culture of sputum

The sputum specimens were inoculated into blood agar with 10% sheep blood, Chocolate agar with 10% sheep blood, McConkey's agar and Brain Heart Infusion (BHI) agar. Any significant bacterial growth was further processed as per the standard procedure to identify the pathogens(Garcia LS et al,1998).

The species identification of *Enterobacteriaceae* was performed using the Entero rapid identification kit (Mikro LA test kit - Entero Rapid 24). A portion of the specimen was concentrated by the modified Petroff's method and inoculated in the Lowenstein Jensen's medium[ Frederick S.et al,1995] and in the Bactec-12B vials for culturing Mycobacteria. The latter was processed in the BACTEC 460 TB system (Becton Dickinson, USA).

The sputum was also inoculated onto Sabouraud dextrose agar (SDA) with antibiotics, SDA without antibiotics in duplicate (incubated at 37°C and 25°C) and BHI agar (incubated at 37°C). Any significant growth of a fungal species was further identified as per standard protocol (Procop GW, Robert GD ,1998).

## CD4 Monitoring

The blood for CD4 detection had been collected within one week of collection of sputum for culture of bacteria and fungus. The CD4 count is the number of CD4 cells per microliter (µL) of blood. It is used to stage the patient's disease, determine the risk of opportunistic illnesses, assess prognosis, and guide decisions about when to start antiretroviral therapy (ART). CD4 count was calculated by FACScount, (Becton Dickinson) method.

## Results and Discussion

In the present study M. tuberculosis was detected only by AFB staining and had been detected in 209 (21.75%) patients other bacterial infections were observed in 177 (18.41%) fungal infections were observed in 66(6.87%) while polymicrobial infections were observed in 80 (8.32%). Total 532 (55.36%) patients had been detected with either fungal or bacteerial pathogens.

In present study the corelation of mean CD4 of the patients from whom the different pathogen were isolated or detected, was carried out and found that mean CD4 to be about 29cells/µl for the Aspergillus with ±2.16 SD, in the HIV sero-positive patients. In the same group other mean CD4 was found to be 193.26 cells/µl for the E.coli infections with ±70.77 SD value. While mean CD4 was found to be 198.52 cells/µl with standard deviation value of ±32.25 in the HIV patients of group T for the *Mycobacterium tuberculosis* infections.(Table). For the *Strept. pyogens* RTI infections the mean CD4 was found to be 547.72cells/µl(SD±248.25), while patients infected with *K.pneumoniae* it was found to be about 288.22 (SD=±90.61). In the HIV sero-positive patients the mean CD4 was found to be 399.10(SD=±84.0) for the all 961 HIV patients with RTI .

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In the present study mean CD4 calculated from the all patients of respiratory tract infection with HIV was 339.10±84.0 cells/µl, while in the study of Hiroyuki Yoshimine et al (2001), found 400 cells/µl mean CD4 lymphocyte, which means HIV-infected patients in Sub-Saharan Africa may, therefore, be at risk for RTI/CAP even in the early stage of HIV infection. In the present study mean CD4 cell count observed in TB patients was 198.52 ± 32.25 cells/µl in the HIV-sero-positive T group patients, which almost matches with the study of E.J.Peters et al.(2007), as they found mean CD4 cell count 173.85±5.4cells/µl in their study. In fact, if we calculate mean CD4 cell count of only 207 monomicrobial TB patients in the present study, it was found around 187, which is very close to the mean CD4 cell count observed by other workers. In addition, it is likely that demographic and regional differences will also affect the spectrum of illness seen in our environment.

In the present study mean CD4 count of the patients with Klebsiella infections was found to be 407.93 ± 115.34 cells/ µl, while Hiroyuki Yoshimine et al.(2001), found

415±270 cells/µl CD4 count for Klebsiella in their study. In the present study we have seen 201.57±52.88 cells/µl CD4 count for S. pneumoniae while they found 260±212 cells/µl in their study in 2001. This difference might be due to demographic and regional differences which also affect the spectrum of illness seen in different environment along with exposure to various predisposing factors. Moreover, in the present study most of all the mean CD4 were cumulatively calculated both from the patients with monomicrobial as well polymicrobial infections. Whereas, other worker with whom comparison has been made, their mean CD4 were calculated purely from isolates from monomicrobial infections. In the present study S.aureus was seen to be isolated from the HIV patients with mean CD4 lymphocyte count of 115.71 ± 54.91 cells/µl, which correlates well with the findings of Hiroyuki Yoshimine et al.(2001), in which they found 127 ±127 cells/µl mean CD4 in S. aureus. These data support the view of Boschini A et al.,(1998) that S.aureus is a causative pathogen of RTI in HIV-infected persons with an advanced stage of immunosuppression.

In the present study 325.45±81.73cells/µl mean CD4 was seen in male, while in the female 360.33±108.96 cells/µl mean CD4 was found, this difference with high mean CD4 in females, might be due to early detection of HIV in female as when their counterpart husband/male' found HIV positive, they were tested and HIV was detected in comparative very early stage of infection than males, who mostly only came to know about HIV-positivity when they found some AIDS related secondary infections in the later stage of HIV. So, majority females got HAART started well in time before their CD4 declined below 200 cells/µl. This difference in fact proved effectiveness of HAART on maintaining CD4 level high in AIDS patients. The cumulative mean CD4 of both genders seen in the present study was 339.10±84 cells/µl. The CD4 lymphocyte counts of the most (73.47%) of the HIV-1-infected patients were less than 400/µl, which matches well with the study of Hiroyuki et al(2001),who also found the CD4 lymphocyte count of 73% in their study with less than 400/µl in HIV-1-infected patients. This findings suggests that a close relationship between HIV infection and the incidence of respiratory tract infections.

The main effect of HAART is to suppress viral replication, allowing the individual's immune system to recover and protecting him/her from the development of AIDS and death. The decision of when to start treatment in an HIV-infected individual has always been problematic. On the one hand, treatment should be initiated at an early point in the individual's course of disease, prior to a time when CD4 cell loss is such that there is substantial risk of clinical progression. On the other hand, the original antiretroviral drugs were often inconvenient to take, of limited efficacy, and were associated with substantial toxicities(Sabin, Caroline A, Phillips, Andrew N, 2009). Thus, clinicians balanced the risks of delaying treatment (potentially placing the patient at risk of serious illness and death from AIDS) with the inconvenience and possible long-term effects of taking treatment. On the basis of evidence that clinical progression rates were low while the CD4 cell count remained above 200 cells/µl but increased rapidly at lower levels, most early treatment guidelines recommended that treatment be delayed until the CD4 cell count have low 200 cells/µl. Over time, however, as treatments have improved and the number of treatment options available to patients has increased, this threshold has increased; most treatment guidelines now

recommend that all individuals with a CD4 cell count less than 350 cells/µl should be treated (Hammer SM et al,2008) and even according to latest concept it is about 500cell/µl.

In 1995, David Ho (1995) promoted a "hit hard, hit early" approach with aggressive treatment with multiple antiretrovirals early in the course of the infection. Later reviews noted that this approach of "hit hard, hit early" ran significant risks of increasing side effects and development of multidrug resistance, and this approach was largely abandoned(Harrington M, Carpenter C.C.2000).

The timing of when to initiate therapy has continued to be a core controversy within the medical community. The development of a stable consensus is hampered by the lack of randomized controlled studies with many guidelines and consensus statements basing their recommendations on observational studies. More recently, the trend has been in favour of earlier treatment of asymptomatic HIV patients, with more studies analyzing various treatment regimens in progress(Jain V, Deeks SG ,2010).

HIV-infected persons who adhere to a regimen of HAART are likely to enjoy suppression of HIV replication, and preservation or improvement in immunological function, with a reduced incidence of opportunistic events and mortality(James M Beck, 2001). Major cohort studies demonstrate conclusively that mortality is higher for individuals who begin ART when their CD4 cell counts are <200cell count(James M ,20001).

Specifically, the risk of death was 1.4 times higher for those who initiated ART with CD4 cell count of 200-350 cells/µL, compared with those who initiated ART with a CD4 cell count >350 cells/µL (P <0.05)(Timothy J Wilkin & Roy M. Gulick, 2008). Enhanced immunity is reflected both in the decline in rates of PCP and other infections, and the fact that prophylaxis against PCP and other infections may be discontinued successfully in patients who have a sustained increase in $CD4^+$ lymphocyte counts to > 200/µl(Currier J, et al. 2000). However, HAART does not appear to reduce the risk of all HIV-associated disorders, including lymphoma and invasive cervical cancer(Jones JL et al.,1999).

The increased risk of bacterial pneumonia with decrease CD4 cell count, recognized in earlier cohort studies (*Hirschtick, et al, 1995;* Sullivan JH, Moore RD et al 2000; Wallace JM,et a,1997). In the study of Gordin FM et al,. (2008)[23], they found that the risk of bacterial pneumonia was 3.3 per 100 person-year for those with fewer than 250 CD4 cells, compared with risk of 1.4 per 100 person-year for those with 500 or more CD4 cells.

As we know that lower RTI are always very serious and even fatal diseases, with even healthy individuals without having HIV. So when in HIV with suppressed immunity naturally mortality increases to a peak. In the present study one patient expired due to bacterial Pneumonia during the study period. The pulmonary TB itself is highly mortal itself, combination with HIV with suppressed immunity worsen the scenario. The thing get worsen due to firstly immuno suppression, secondly due to over lapping toxicities of both AKT and HAART and increased resistance of tuberculus bacterium in HIV. In the present study three patients expired due to pulmonary TB even before AID had appeared in one patients with as high as 330 CD4 counts.

In the present study RTI were observed in 587(61.08%) HIV patients, while they were

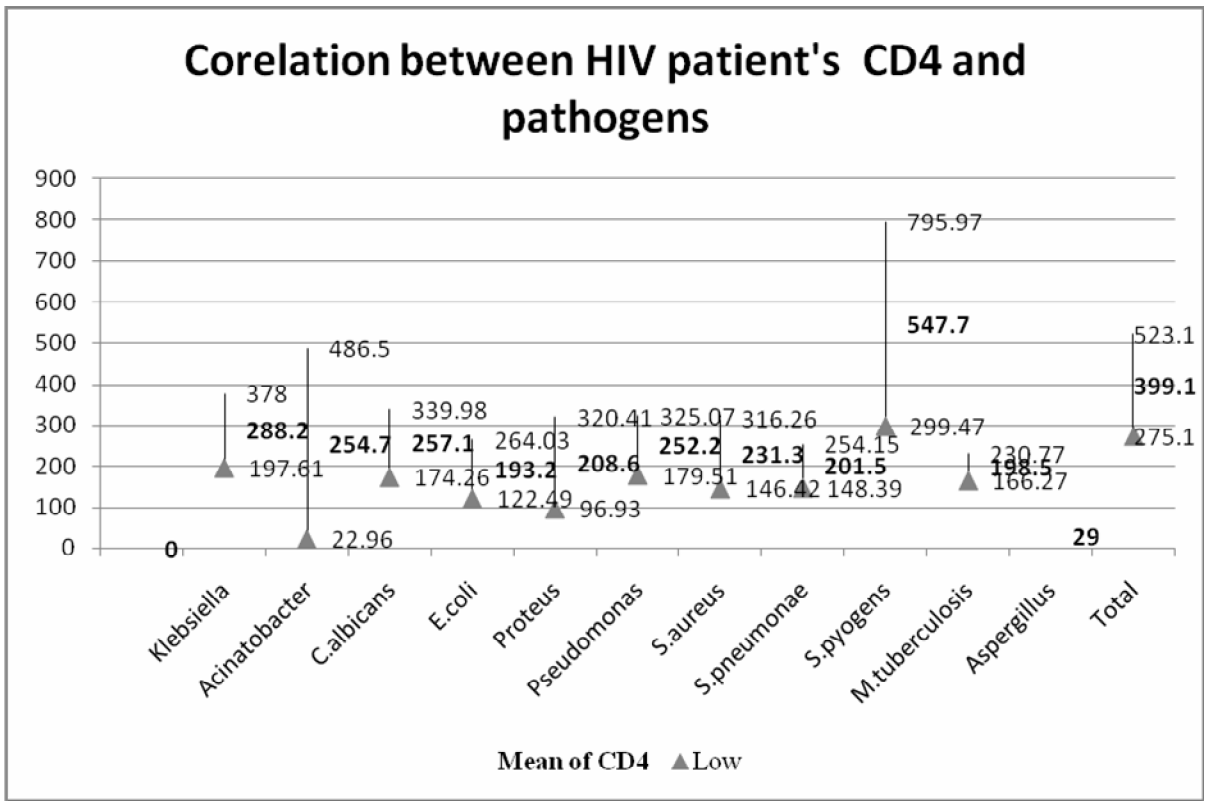
observed in 374(38.92%) full blown AIDS patients, which signified that most RTI occurred early before any other secondary infections were seen or AIDS had been established. These result matches well with the statement that RTI are the first secondary infections observed even before full blown AIDS start appearing with many secondary infections. Starting HAART early would delay in appearing AIDS late which would certainly extend life span of HV patients, as we know that HIV patients do not die of HIV itself, but they die due to fatal secondary infections appear in late AIDS.

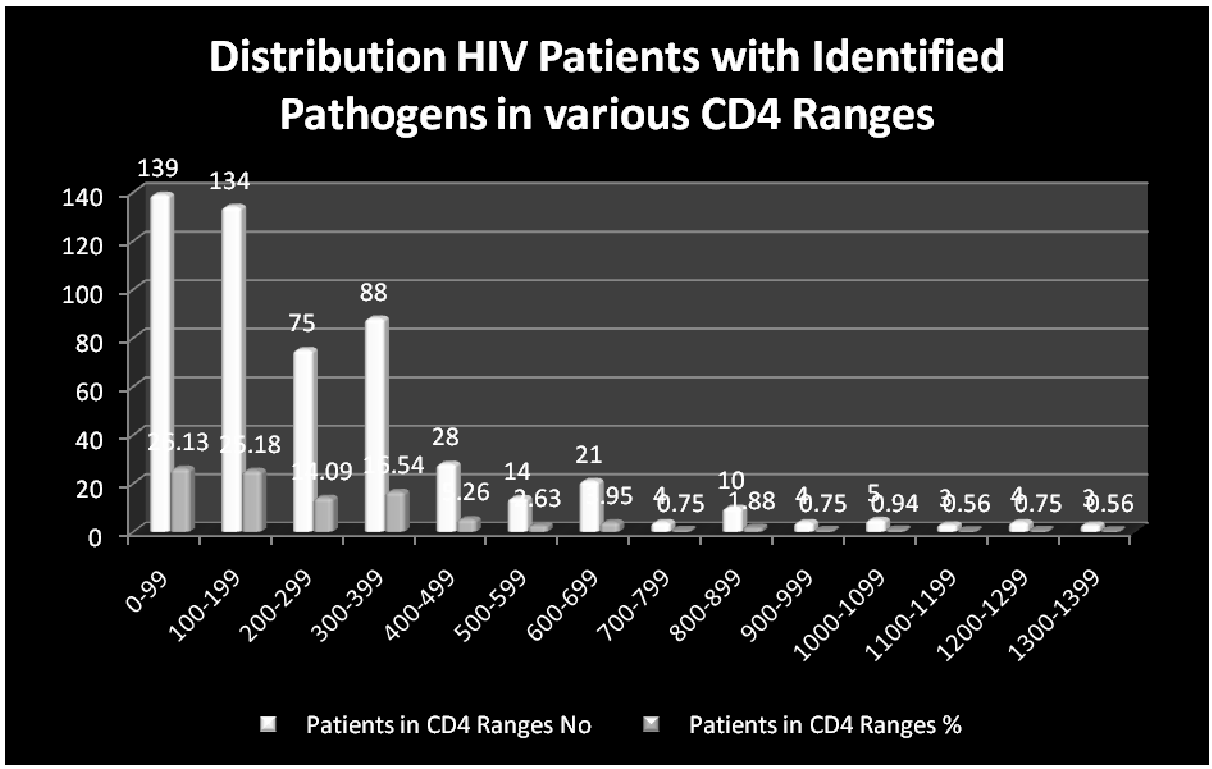
**Table.1** Prevalence of various RTI pathogens from HIV patients.

| | Pathogenic Isolates | HIV +VE RTI +VE patients(n=961) | |
|---|---|---|---|
| | | (n) | % |
| 1 | *Klebsiella pneumoniae* | 51 | 5.31 |
| 2 | *Pseudomonas aeuroginosa* | 42 | 4.37 |
| 3 | *Strepococcus pneumoniae* | 23 | 2.39 |
| 4 | *Escherichia coli* | 19 | 1.98 |
| 5 | *Staphylococcus aureus* | 17 | 1.77 |
| 6 | *Streptococcus pyogenus* | 14 | 1.46 |
| 7 | *Acinetobacter* | 11 | 1.14 |
| 8 | *Candia albicans* | 47 | 4.89 |
| 9 | *Candida (non-albicans)* | 63 | 6.56 |
| 10 | *Aspergillus* | 3 | 0.31 |
| | **Total Bacterial Pathogens** | **177** | 18.42 |

**Table.2** Comparision of mean CD4 values among patients suffering from respiratory tract infections by various organisams

| Organisms | Mean of CD4 Cells/µl | Standard Deviation (SD) | Total Isolates (monomicrobial+polymicrobial) | |
|---|---|---|---|---|
| | | | n=961 | % |
| *Klebsiella* | 407.93 | ±115.34 | 92 | 9.57 |
| *Acinatobacter* | 254.73 | ±231.77 | 11 | 1.14 |
| *C.albicans* | 257.12 | ±82.86 | 115 | 11.97 |
| *Candida* | 499.73 | ±196.24 | 63 | 06.56 |
| *E.coli* | 193.26 | ±70.77 | 24 | 02.5 |
| *Proteus* | 208.67 | ±111.74 | 06 | 0.62 |
| *Pseudomonas* | 252.29 | ±72.78 | 59 | 06.14 |
| *S.aureus* | 115.71 | ±54.91 | 20 | 02.08 |
| *S.pneumonae* | 201.57 | ±52.88 | 35 | 03.64 |
| *S.pyogens* | 547.72 | ±248.25 | 14 | 01.46 |
| *M.tuberculosis* | 198.52 | ±32.25 | 234 | 24.35 |
| *Aspergillus* | 29.00 | ±2.16 | 03 | 0.31 |
| **Female** | **360.33** | **±108.96** | **383** | **39.85** |
| **Male** | **325.45** | **±81.73** | **577** | **60.05** |
| **Total** | **339.10** | **±84.0** | **676** | **70.34** |


Corelation between HIV patient's CD4 and pathogens
900
800
700
600
500
400
300
200
100
0
0
378
288.2
197.61
486.5
22.96
254.7
339.98
257.1
174.26
264.03
193.2
122.49
320.41
208.6
96.93
325.07
252.2
179.51
316.26
231.3
146.42
254.15
201.5
148.39
795.97
547.7
299.47
230.77
198.5
166.27
29
523.1
399.1
275.1
Klebsiella
Acinatobacter
C.albicans
E.coli
Proteus
Pseudomonas
S.aureus
S.pneumonae
S.pyogens
M.tuberculosis
Aspergillus
Total
Mean of CD4
Low


Distribution HIV Patients with Identified Pathogens in various CD4 Ranges
140
120
100
80
60
40
20
0
139
134
75
88
28
14
21
4
10
4
5
3
4
3
0.75
1.88
0.75
0.94
0.56
0.75
0.56
0-99
100-199
200-299
300-399
400-499
500-599
600-699
700-799
800-899
900-999
1000-1099
1100-1199
1200-1299
1300-1399
Patients in CD4 Ranges No
Patients in CD4 Ranges %

## References


Boschini A, Smacchia C, Di Fine M, et al.1996.Community - acquired pneumonia in a cohort of former injection drug users with and without human mmunodeficiency virus infection: incidence, etiologies, and clinical aspects.Clin Infect Dis 1996;23:107–113.

Currier J, Williams P, Koletar S, et al. 2000. Discontinuation of *Mycobacterium avium* complex prophylaxis in patients] with antiretroviral therapy-induced increases in CD4+ cell count. Ann Intern Med 2000;133:493–503.

Egger M, May M,Chene G et al.(2002). Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet;360:119-29.

E.J.Peters,O.E.Essien et al.,(2007). CD4 Count Levels and pattern of Respiratory complications in HIV sero-positive Patients in Calabar, Nigeria. Nigerian Journal of Physiological Sciences 22(1-2):93-97.

Garcia LS, Procop GW, Roberi GD, Thompson RB, Jr.(1998) Over view of conventional methods for bacterial identification, Chapter13. In : Bailey and Scott's diagnostic microbiology,10th edition. Forbes BA, Sahm DF, Weissfeld AS (Eds.) (Mosby). 167-181

Gordin FM, Mollie P Roediger et al,(2008), Pneumonia in HIV-infected Persons: Increased Risk with Cigarette Smoking and Treatment interruption. Am J of Resp Cri Care Med ;178: 630-636.

Hammer SM, Eron JJ, Reiss P, *et al*. (2008) Antiretroviral treatment of adult HIV infection. 2008 recommendations of the International AIDS Society- USA Panel. J Am Med Assoc 300:555-570.

Harrington M, Carpenter C.C. (2000) "Hit HIV-1 hard, but only when necessary", The Lancet 2000;355(9221):2147.

Hiroyuki Yoshimine et al.,(2001) Hiroyuki Yoshimine, Kazunori Oishi et al.,(2001).Community-Acquired pneumonia in Ugandan adults: Short-Term Parenteral Ampicillin Therapy for Bacterial Pneumonia..Am J Trop. Med.Hyg.,64(3,4):172-177.

*Hirschtick, R, Glassroth, J, Jordan, MC, et al, (1995)* Hirschtick, R, Glassroth, J, Jordan, MC, et al(1995).The Pulmonary complication of HIV Infection. Study Group. Bacterial pneumonia in persons infected with human immunodeficiency virus. N Engl J Med ;333,845-851

Ho, D et al.,(1995) "Time to hit HIV , early and hard" The New England Journal of medicine 333(7):450-451.

Jain V, Deeks SG,(2010) "When to start antiretroviral therapy " Curr HIV/AIDS Resp 7(2):60-8.

James M Beck Mark J Rosen and Hannah H Peavy.(2001) Pulmonary complication of HIV infection. Report of the Forth NHBI workshop. Am J Res Crit Care Med (164) :2120-2126.

Jones JL et al, Hanson DL, Dworkin MS,Ward JM, Jaffle HW, (1999) Effect of antiretroviral therapy on recent trends in selected cancers among HIV persons. J Acquir Immune Defic Syndr 21:511-517.

Joshi PL, Joshi PL, Mishra SN. Opportunistic infections in HIV/AIDS Patients : An over view, Chapter 1. In : Manual on laboratory diagnosis of common opportunistic infections associated with HIV/AIDS. Baweja UK, Sokhey J (Eds.) Govt. of India. National Institute of communicable diseases

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr.(1997) The role of Microbiology laboratory in the diagnosis of infectious disease: Guidelines to practice and management, Chapter 2. In: Color atlas and text book of diagnostic microbiology, 5th edition. Lippincott, Philadelphia, New York (Pubs.) 1997:69.

Oneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. The role of Microbiology laboratory in the diagnosis of infectious disease: Guidelines to practice and management, Chapter 2. In: Color atlas and text book of diagnostic microbiology, 5th edition. Lippincott, Philadelphia, New York (Pubs.) 1997:69.

Procop GW, Robert GD.(1998) Laboratory methods in basic Mycology, Chapter 65. In: Bailey and Scott's Diagnostic microbiology, 10th edition. Betty A. Forbes Daniel F. Sahm, Alice S, Weissfied (Mosby) : 871

Rewari BB (Ed.) Virology and Immunopathology of HIV/AIDS, Chapter5. In: Specialist's training and reference module, State Pram (Delhi) National AIDS Control Organisation (New Delhi):34-48.

Sabin, Caroline A; Phillips, Andrew N, (2009) Should HIV therapy be started at a CD4 cell count above 350 cells/μl in asymptomatic HIV-1-infected patients? Current Opinion in Infectious Diseases: Volume 22 (2) April 2009 p 191-197.

Sullivan JH, Moore RD et al, (2000) Effect of antiretroviral therapy on the incidence of bacterial pneumonia in patients with advanced HIV infection. :Am J Respir Crit Care Med.162;64-67.

Timothy J Wilkin & Roy M. Gulick, (2008). When to start Antiretroviral Therapy ? Clinical Infectious Diseases;47:1580-6.

Walker PA, White DA.(1996) Pulmonary disease. Medical clinics of North America, ;80:1337-1362.

Wallace JM, Hansen NL et al. ,(1997) The pulmonary complications of HIV Infections . Study group respiratory diseases trends in the pulmonary complications of HIV infection study cohort. Am J Respir Crit Care Med 155:72-80.