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Hiv and lymphadenopathy

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1.0 Introduction

1.1 The Epidemic

The first Acquired Immunodeficiency Syndrome (AIDS) cases were described in 1981. Human Immunodeficiency Virus (HIV) was described by Luc Montagnier of France, and proposed as the causative agent of AIDS by Robert Gallo of the United States in the early 1980's. Initially the virus was called Human T-lymphotropic Virus type III (HTLV III) or Lymphadenopathy-Associated Virus (LAV). The name HIV has been in use since 1986 (HIV-Wikepedia, 2005).

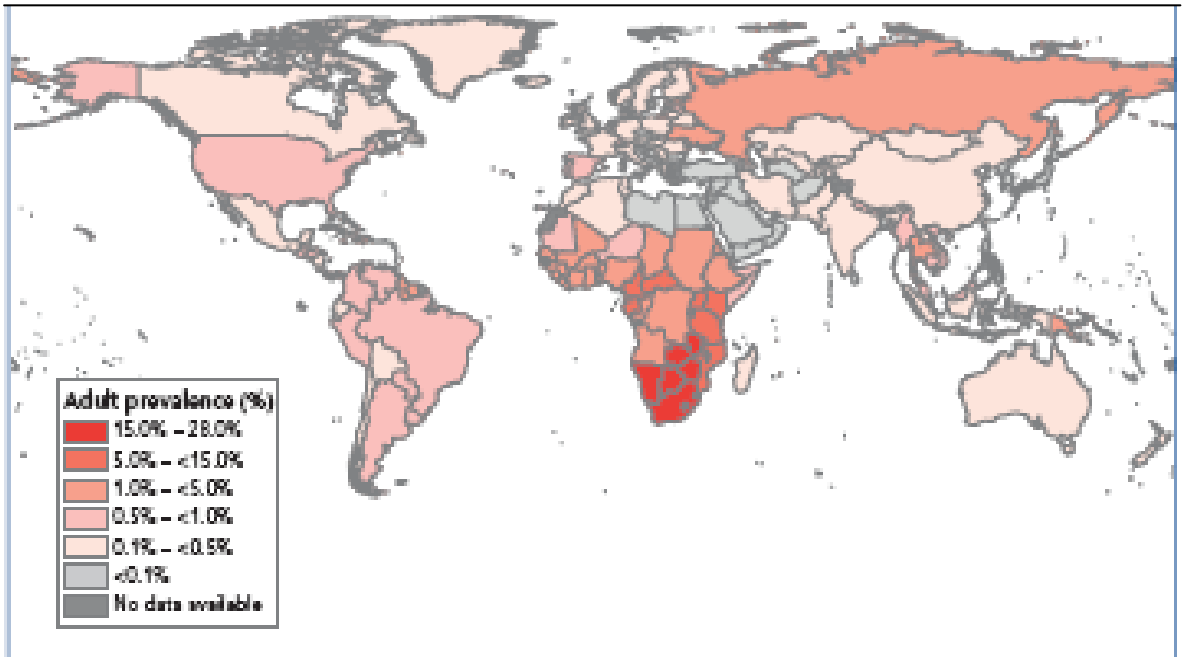


Figure 1 A global view of HIV infection, 2007- 33 million people living with HIV http://data.unaids.org/pub/GlobalReport/2008/jc1510_2008_global_report_pp29_62_en.pdf (accessed 12th November 2008)

The UNAIDS Estimation and Projection Package (EPP) was developed to aid in country-level estimations and short term projections of the HIV/AIDS epidemic (Brown et al., 2008). In 2007 alone, 33 million people were living with HIV, 2.7 million people became infected with the virus, and 2 million people died of HIV

related causes (see figure 1). Southern Africa continues to bear a disproportionate share of the global burden of HIV: 35% of HIV infections and 38% of AIDS deaths in 2007 occurred in this sub- region (see figure 2) (UNAIDS).

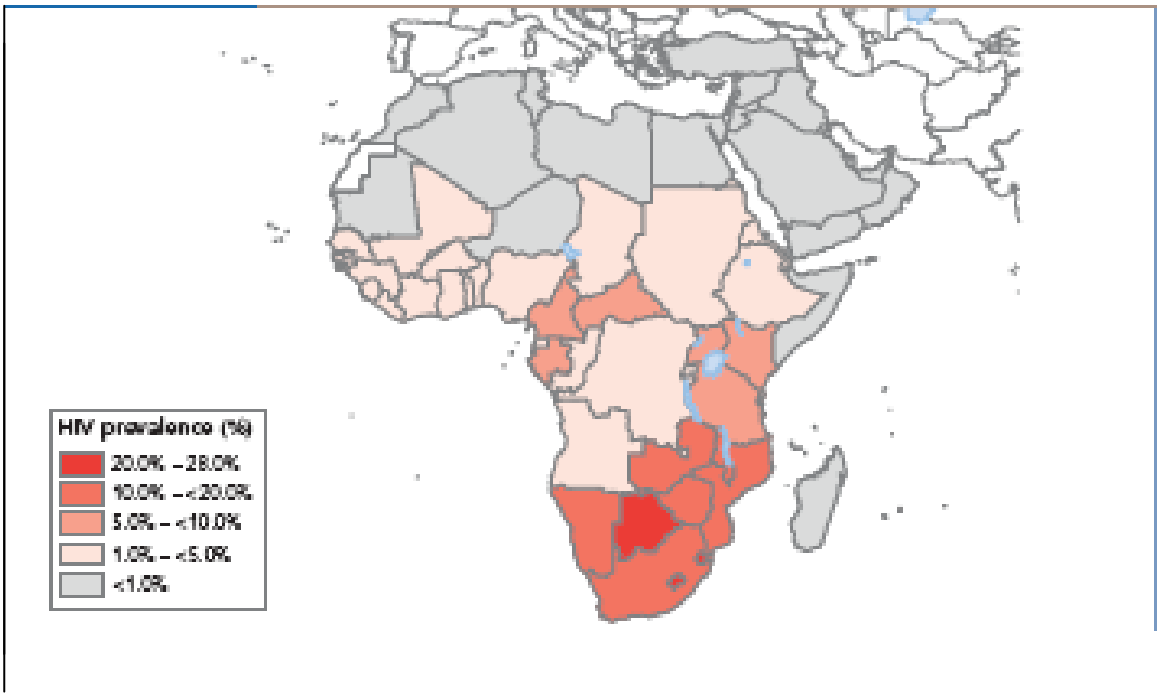


Figure 2: HIV prevalence in adults (15-49) in Africa, 2007

http://data.unaids.org/pub/GlobalReport/2008/jc1510_2008_global_report_pp29_62_en.pdf (accessed 12th November 2008)

AIDS has provoked panic and stigmatized in the same way as other plagues of historical dimensions, but in many ways it is different. It is caused by a persistent infection and has a silent period of many years between infections to the onset of serious symptoms. HIV is integrated into the very genome of the cell it attacks (Kallings, 2007).

1.2 The Virus

HIV is a lentivirus belonging to the retrovirus family (Nielsen, 2005). It is an enveloped RNA virus, 120nm in diameter, containing two copies of the RNA genome. Replication of the virus is dependent on reverse transcriptase. HIV has several major genes coding for structural proteins that are found in all retroviruses, and several non-structural or "accessory' genes that are unique to HIV (see figure 3). The general retrovirus genes include gag, pol and env. The gag-derived protein makes up the cone-shaped viral capsid. The pol gene codes for the virus enzymatically active proteins. Most important is the so-called reverse transcriptase (RT) which performs the unique transcription of the viral RNA into the double-stranded DNA. Proteins derived from the env gene are a surface (gp120) and a trans-membrane protein (gp41). Specific regulatory HIV genes include tat, rev, nef, vif, vpr and vpu, which contain information essential to produce proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease. For example, the protein coded by the nef gene is necessary for the virus to replicate efficiently, and the vpu-encoded protein influences the release of new virus particles from infected cells (Trono, 1995, Emerman, 1998).

1.2.1 Types of HIV and their origin

It has been recognized since 1986 that there are two species of the virus, HIV-1 and HIV-2. HIV-2 infections are mostly found in Western Africa and India. HIV-1 is endemic in the rest of the world(Cohen, et al. 2000)..

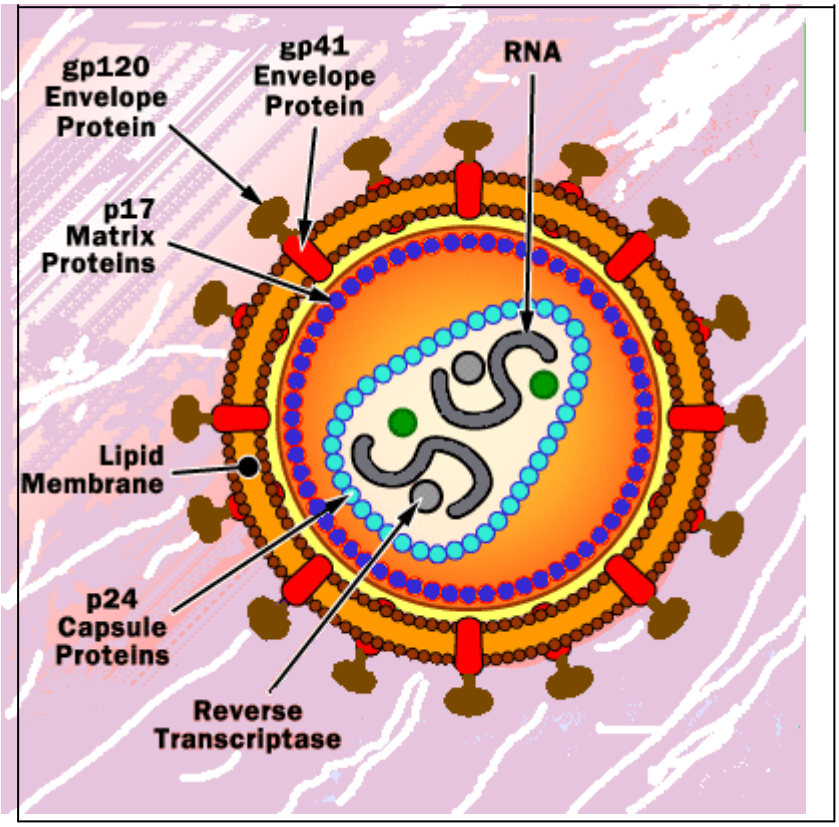


Figure 3 The Human Immunodeficiency Virus

Modified from http://static.howstuffworks.com/gif/aids-hiv-anatomy.gif-accessed 30 june 2009

1.3 Lymph node

The architecture of the lymph nodes is of highly organized centers of immune cells that filter antigen from the extracellular fluid. Directly interior to the fibrous capsule is the sub-capsular sinus. This allows lymph, an ultra filtrate of blood, to traverse from the afferent lymph vessels, through the sinuses, and out the efferent vessels. In addition to supplying lymph with a channel for lymph flow, the sinuses are populated with macrophages, which remove 99% of all delivered antigen.

Interior to the sub-capsular sinus is the cortex containing primary follicles, secondary follicles, and the inter-follicular zone. Follicles within the cortex are major sites of B-

cell proliferation, whereas the inter-follicular zone is the site of antigen-dependent T-cell differentiation and proliferation. The deepest structure within the lymph node is the medulla, which consists of cords of plasma cells and small B lymphocytes that are strategically arranged near the sinuses to facilitate immunoglubin secretion into the existing lymph (Guyton, 1992)

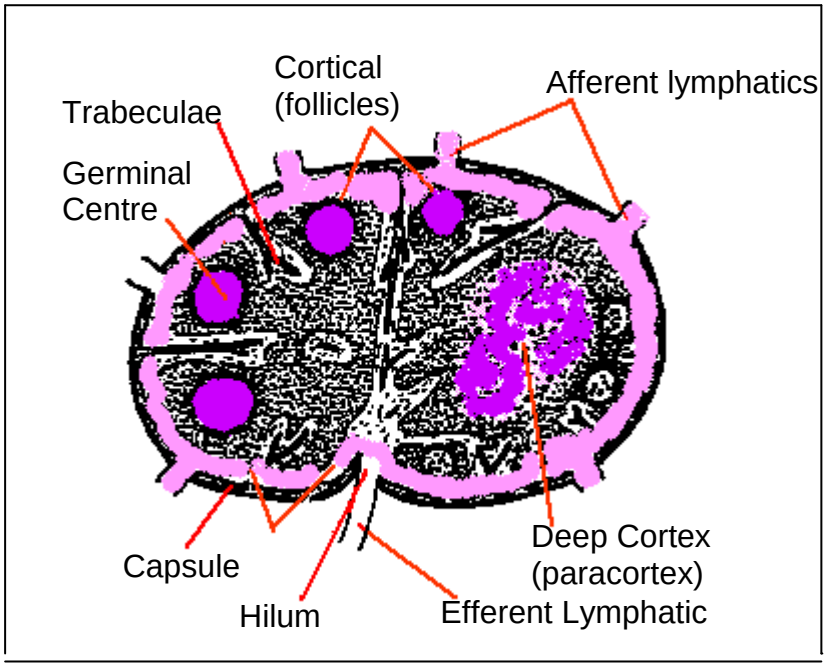


Figure 4: Anatomy of a Lymph node

Modified from http://altered- states.net/barry/newsletter195/LymphNode.gif-accessed 30 June 2009

1.4 Immunopathogenesis of Human Immunodeficiency Virus Infection

The most common mode of HIV infection is sexual transmission at the genital mucosa (Royce et al., 1997). The first cellular targets of the virus are Langerhans's cells and tissue dendritic cells, found in the lamina propria subjacent to the cervico-vaginal epithelium. These cells then fuse with CD4+ lymphocytes and spread to deeper tissues. Within two days after infection, virus can be detected in the draining

internal lymph nodes. Shortly, thereafter systemic dissemination occurs, and HIV can be cultured from plasma four to eleven days after infection. Studies of persons with acute HIV-1 infection demonstrate selective infection by certain populations of HIV-1 variants. Transmitted viruses are typically macrophage-tropic (not T-cell tropic) and lack the ability to induce multinucleated synctia in tissue culture**(**Zhu et al., 1993, Zhu et al., 1996)**.** Glycoprotein 120, the viral-envelope protein, binds to the CD4 molecule on susceptible cells, but cell entry requires the presence of a co-receptor (Feng, 1996). The co-receptor for macrophage-tropic strains is CCR5, a surface chemokine receptor**(**Alkhatib et al., 1996, Dragic et al., 1996). T-cell tropic viruses require CXCR4 for cell entry. Langerhan cells, the earliest target of the virus, express CCR5. The core of the virus is released into the cell cytoplasm. After the core disassembles, the viral genome is reverse transcribed into DNA by the virus own reverse transcriptase enzymes (Simon et al., 2006). As with all retroviruses and lentiviruses, HIV-1 must integrate into the DNA of the host cell. As a consequence, the activity of the integrated viral genome, or provirus is greatly influenced by the metabolic and activation state of the host cell, and the longetivity of the provirus are dictated by the life span of the cell that contains it. Nowhere else are extremes in proviral activity and longevity more evident than in the reservoir of CD4+ T lymphocytes (Stevenson, 2003). After infection, there is a rapid rise in plasma viraemia, with widespread dissemination of the virus associated with seeding of lymphoid organs (Cavert et al., 1997, Pantaleo et al., 1993a) and trapping of virus by follicular dendritic cells (Heath et al., 1995). The lymph nodes are the major anatomic sites for the establishment as well as the acute and chronic propagation of HIV infection. Lymph node involvement is a common denominator in virtually all patients with HIV infection, even those without easily detectable lymphadenopathy. HIV shows strong tropism for CD4+

lymphocytes, monocytes, macrophages and dendritic cells **(**Ioachim, 1994). Simultaneous examination of lymph node and peripheral blood mononuclear cells in the same patients during various stages of HIV disease including the early asymptomatic phase (when CD4+ T cells are generally > 500/ul), the intermediate stage (200-500 cells/ul) and the advanced stage of disease (<200cells/ul), has lead to substantial insights into the pathogenesis of HIV infection. Laboratory studies performed during the initial infection may show lymphopenia and thrombocytopenia, but atypical lymphocytes are in-frequent (Levy, 1993).

Early in the course of infection, there are copious amounts of extracellular virions, trapped in the germinal centres of the lymph node (Tenner-Racz et al., 1986). The architecture of the germinal centers is preserved and may even be hyperplastic due to in-situ proliferation of cells and recruitment of cells to lymph nodes. Electron microscopy demonstrates a fine network of follicular dendritic cells (FDC) with interdigitating processes that envelop virtually every lymphocyte in the germinal centre. As the disease progresses, the architecture of the germinal centres begin to show disruption and the trapping efficiency on the node diminishes. With progression to the advanced stage of the disease, there is complete dissolution of the FDC network and massive dropout of FDC's. The trapping function of the lymph node is lost and virus spills over freely into the circulation. At this point the lymph nodes are characterized as "burnt out". Thus the lymph node plays a vital role in the pathogenesis of HIV disease. They serve as an important reservoir of the virus, a filter or trapping apparatus of free virions, and an environment for the exposure of susceptible cells to large amounts of virus trapped among the processes of the FDC, which activate adjacent cells as well as present virus to these cells (Fauci and Lane, 1994).

Many of the pathogenetic mechanisms associated with HIV infection that lead to clinical disease have been established. CD4 T lymphocytes confined to lymphoid tissue appear to remain the major source of HIV-1 production in end stage disease (van de Ende et al., 1999). The functional abnormalities and quantitative depletion of CD4 T lymphocytes, that cause profound immunosuppression, are characteristic of advanced HIV infection **(**Pantaleo, 1993b).

In the acute HIV syndrome (acute seroconversion illness), which occurs approximately 3-6 weeks following primary infection, lymphadenopathy may be present. The lymph nodes are small, often generalized and transient. Lymph node biopsy shows a follicular infiltration and there is little activation and proliferation of the germinal centres.

The primary infection with or without the acute syndrome is followed by a period of clinical latency (asymptomatic stage).

1.5 The Clinical Stages

1.5.1 Seroconversion Illness

Acute infection with HIV is a specific syndrome, which is easily missed due to its similarity to infectious mononucleosis and other viral infections. Fever, fatigue and rash are the most common symptoms, and many develop lymph node enlargement. Pharyngitis, myalgia and several other symptoms also occur (Kahn and Walker, 1998). The long phase of clinical latency that follows primary infection conceals substantial virological and immunological activity (Ho, 1995).

1.5.2 Incubation Period

After primary infection, with viral dissemination, the appearance of HIV-specific immunity, and the apparent curtailment of viral replication, most patients have a

period of clinical latency that lasts for years. The term clinical latency is misleading, as during this period virtually all patients have a gradual deterioration of the immune system manifested particularly by the depletion of the CD4 T cells. HIV disease is clearly progressive during the so-called latent period. This is the period when the patient is completely asymptomatic, and can vary from a few months to ten years. The median incubation period is 8-10 years (Bird, 1992).

1.5.3 AIDS-related complex and/or PGL

The inevitable outcome of the progressive deterioration of the immune system that occurs in most patients with HIV infection is clinically apparent disease or an acquired immunodeficiency syndrome (AIDS) defining illness, either severe and persistent constitutional signs and symptoms or an opportunistic infection or neoplasm. At the end of the incubation period, a number of signs and symptoms may appear which do not fulfill the definition of AIDS or other HIV associated syndromes. These include mild immunological, dermatological, haematological and neurological signs. Constitutional symptoms such as fever, weight loss, night sweats and diarrhea may develop. Laboratory findings may show a decrease in the CD4 count, hyperimmunoglobulinaemia and cytopenias. AIDS-related complex is defined as fever, weight loss, night sweats, or chronic diarrhea of more than 1 month duration in the presence of disturbances of the cell mediated immunity (CMI) and in the absence of any other recognizable cause other than HIV infection. These definitions may in part overlap and are not mutually exclusive and they can be described generally as pre-AIDS.

The first manifestation of HIV infection may be noted at any disease stage and the different stages may not occur consecutively. The transition to AIDS may occur rapidly or slowly. The disease progression is probably influenced by cofactors, such

as other viral infections, stress, genetic make-up of the individual etc. Poor prognostic factors include the serial decrease in the number of CD4 lymphocytes, the reappearance of HIV antigens in the blood, the decline or disappearance of anti-core antibodies, and increased levels of ß2 microglobulin and neopterin. The diagnosis of AIDS is established with the appearance of opportunistic infections or of certain neoplasms, such as Kaposi's sarcoma, primary lymphoma of the brain and other Non-Hodgkin's Lymphomas. The diagnosis of AIDS is also established by the finding of a CD4 count of less than 200 cells/mm^3.

Exceptions to the direct correlation between deteriorating immune function and clinically apparent disease is the progressive generalized lymphadenopathy that some patients have early in the course of infection and that may be caused by a vigorous immune response against HIV in the lymph nodes. Lymphadenopathy is a common feature and may manifest at any stage in the course of HIV/AIDS. Indeed, in 1983, HIV was isolated from a patient with lymphadenopathy and the disease was formerly also called lymphadenopathy associated virus (LAV).

Progressive generalized lymphadenopathy (PGL) syndrome may manifest in the early symptomatic phase of the disease. PGL is defined as the presence of enlarged lymph nodes (> 1cm in diameter) in two or more extra-inguinal sites for more than three months without an obvious cause, and the presence of reactive hyperplasia in a lymph node, if a biopsy is performed (MMWR, 1982). Three distinct histopathological patterns have been recognized: i. follicular hyperplasia with or without follicular fragmentation, ii. follicular involution, and iii. follicular depletion. Histology and immunohistochemistry support the possibility that HIV infection represents, at lymphoid tissue level, an aberrant immune reaction to several stimuli still incompletely known (Baroni et al., 1990). PGL is generally not indicative of

progression of disease. With the often late presentation of patients it is difficult to differentiate PGL from other causes of lymphadenopathy.

1.6 Laboratory diagnosis of HIV infection

1.6.1 Serology

a) Antibody tests

Enzyme linked-immunosorbent assays (ELISAs) are the most frequently used method for screening blood samples for the HIV antibody. The sensitivity and specificity of the presently available commercial systems approaches 100%, but false positive and false negative reactions occur. Other test systems available include passive particle agglutination, immunofluorescence, Western blots and Radioimmunoprecipitation (RIPA) assays. Western blot assays are regarded as the gold standard. Seropositivity is diagnosed when antibodies against both the env and the gag proteins are detected. The sensitivity of the test systems are currently being improved by the use of recombinant antigens.

b) Antigen tests

HIV antigens (e.g. p24) can be detected early in the course of HIV infection before the appearance of antibody. It is undetectable during the latent period (antigen antibody complexes are present) but become detectable during the final stages of the infection(Grande et al., 2010).

1.6.2 Virus Isolation

Virus isolation is accomplished by the co-cultivation of the patient's lymphocytes with fresh peripheral blood cells of healthy donors or with suitable culture cell lines such as in T-cell lymphomas. The presence of the virus can be confirmed by reverse transcriptase assays, serological tests, or by changes in the growth pattern of the

indicator cells. However virus isolation is tedious and time consuming (weeks) and is successful in only 90% of cases. Therefore virus isolation is only used for the characterization of the virus(Jackson., 1988).

1.6.3 Demonstration of viral Nucleic Acids

This can be accomplished by probes or PCR techniques. The latter may be useful because of its extremely high sensitivity.

1.6.4 Prognostic tests

The following may be useful prognostic tests:

-HIV antigen

-Serial CD4 count

-Neopterin

-B2 Microglobulin

-Viral Load

Of these, only serial CD4 count and viral loads are still routinely used.

1.6.5 HIV viral load

The HIV viral load has the greatest prognostic value and can be used to predict the risk of the development of opportunistic infections (Swindle et al., 2002). HIV viral load in serum may be measured by assays which detect HIV-RNA e.g. RT-PCR, NASBA, or bDNA. Patients with a low viral load during the incubation period have a better prognosis than those with a high viral load. Patients whose viral load decreased significantly following the commencement of antiviral therapy, had a better prognosis than those who did not respond.

1.6.6 CD4 counts

The CD4 counts are valuable in monitoring disease progression and response to antiviral chemotherapy. The CD4 count gives an indication of the WHO clinical

stage of the disease (Kassa et al., 1999). The measurement of HIV viral load tells us where the disease is going, whereas the CD4 count tells us where the disease is at this current moment.

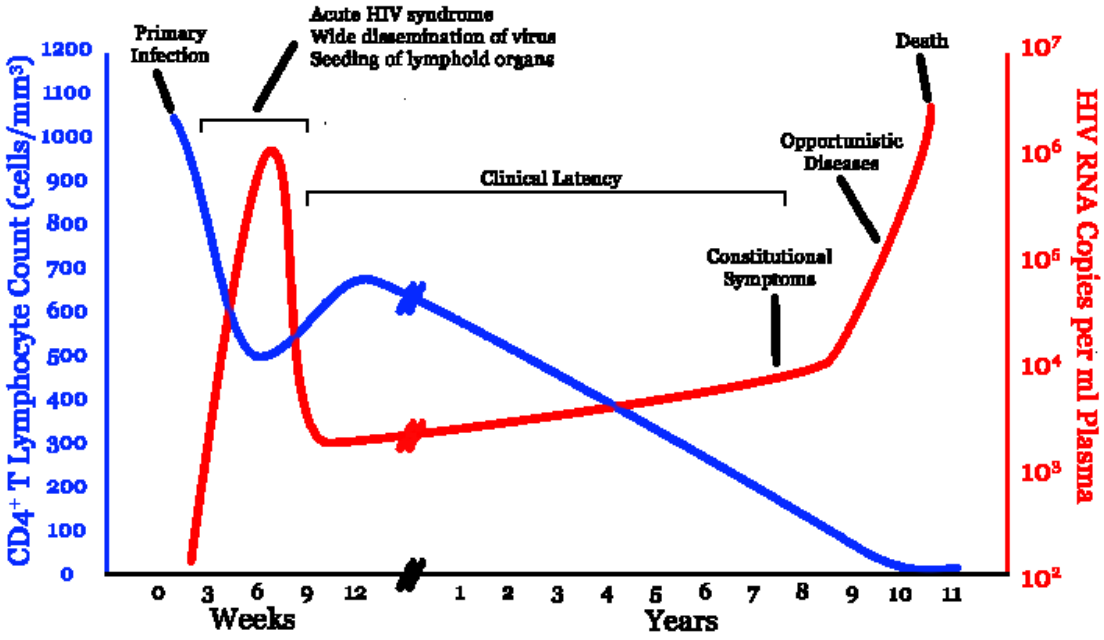


Figure 5: Graph: HIV copies and CD4 counts during the course of HIV infection

http://upload.wikimedia.org/wikipedia/commons/a/a4/Hiv-time course.png

(accessed 18th February 2009)

1.6.7 Antiviral susceptibility assays

Phenotypic and genotypic assays are currently available.

1.7 Treatment

1. Nucleoside analogue reverse transcriptase inhibitors, for example. AZT, DDC, DDI and lamivudine
2. Non-nucleoside analogue reverse transcriptase inhibitors e.g. Nevirapine

3. HIV protease inhibitors e.g. Ritonavir, Indinavir

A simple and commonly used regimen includes two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor

1.8 Markers predicting progression of HIV-related disease

Because the likelihood and timing of development of clinical AIDS following sero-conversion, for any particular individual, is not readily predictable, the use of non-clinical disease markers has become critically important in patient management. These markers should fulfill the following criteria:

(i) permit identification of patients at highest risk of disease progression,

(ii) aid in estimating the duration of infection,

(iii) assist in disease staging,

(iv) predict development of indicator disease (opportunistic infections of AIDS), and

(v) follow in vitro, the therapeutic efficacy of immunomodulating or antiviral treatment

When the prognostic values of cellular and serologic surrogate markers in HIV infection were evaluated, CD4+ T-cell levels expressed as either absolute number, percentage of lymphocytes, or CD4+/CD8+ T cell ratio were found to be the best single predictor of progression to AIDS. Lymphocyte count categories could not be substituted for CD4+ T-cell count categories in the laboratory axis of the WHO staging, except for the highest category where sensitivity and specificity of the highest lymphocyte counts ($>2000x10^6/l$) for use in diagnosing CD4+ T-cell counts ($>500x10^6/l$) were above 80%. For other categories, risk of misclassification would be high (Tsoukas and Bernard, 1994).

1.9 Lymphadenopathy

Lymphadenopathy, which is defined as an abnormality in the size or character of lymph nodes, is caused by the invasion or propagation of either inflammatory or neoplastic cells into the node. The association of persistent lymphadenopathy and HIV has been well described in a longitudinal study (Mathur-Wagh et al., 1984). Generalized lymphadenopathy is defined as involvement of two or more non-contiguous sites of lymph nodes. Generalized lymphadenopathy is more likely than localized lymphadenopathy to result from serious infections, autoimmune disease and disseminated malignancy. Hodgkin's lymphoma and most metastatic carcinomas typically progress through the lymph nodes in anatomic sequences. Differentiating between localized and generalized lymphadenopthy is important in the differential diagnosis.

Lymphadenopathy that lasts less than two weeks or more than one year with no progressive size increase has a very low likelihood of being neoplastic, the exceptions to the latter include low grade non-Hodgkin's lymphoma, Hodgkin's lymphoma and occasionally chronic lymphocytic leukemia.

1.9.1 Clinical presentation of lymphadenopathy

The principal lymph nodes are distributed to the occipital, submandibular, cervical, supraclavicular, axillary, epitrochlear, para-aortic, inguinal, femoral and popliteal areas. These areas should be examined and the following points about the lymph nodes should be considered:

1. How many nodes are palpable?
2. What is the approximate diameter in centimeters?
3. What is their consistency?
4. Are they discrete or confluent or matted?

5. Are they fixed or mobile?
6. Is the skin in the vicinity of the nodes or overlying the nodes abnormal?

In general, lymph nodes larger than 1 cm in diameter are considered significant (Ferrer, 1998). In the series of Slap et al., 1984, a maximum diameter of more than 1.5cm was reported as an appropriate starting point for a high suspicion of malignant or granulomatous disease. Increasing size and persistent over time are of greater concern for malignancy than a specific level of nodal enlargement. Tender lymph nodes with a soft consistency are suggestive of an infection or a reactive process (Kunitz, 1985). A lymph node which is 'rock hard' is suggestive of a neoplastic process. The nodes of lymphoma are firm, often circumscribed, non-tender and rubbery. Viral infections, which typically produces hyperplastic nodes that are bilateral, mobile, tender or non-tender, and clearly demarcated. Painful or tender Lymphadenopathy is nonspecific but typically represents nodal inflammation/ infection. In rare cases, painful or tender lymphadenopathy can result from haemorrhage into the necrotic center of a neoplastic node or from pressure on the nodal capsule caused by rapid tumour turnover. The largest lymph nodes are more often seen in patients with malignancy, particularly the lympho-proliferative disorders such as Hodgkin's disease, non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. Striking lymph node enlargement may also occur in some reactive conditions such as HIV infection (e.g. PGL), Castleman's disease and SHML (Sinus Histiocytosis and Massive Lymphadenopathy). However, malignancy may occur in lymph nodes which are only slightly enlarged.

Essential to identifying the infrequent but serious causes of peripheral lymphadenopathy are the following: an awareness of lymphatic anatomy, drainage patterns, and regional differential diagnosis, a thorough history including key factors

such as age, location, duration and patient exposures, and a focused physical examination according to the location of adenopathy. Lymph node involvement is a common denominator in virtually all patients with HIV infection, even those without easily detectable lymphadenopathy (Bazemore and Smucker, 2002).

1.9.2 Head and neck lymphadenopathy

Palpable cervical lymph nodes, which are common, were noted in more than 50 percent of adult physical examinations in some outpatient studies. The most common cause of cervical lymphadenopathy is infection. The most common head and neck manifestation of mycobacterial infections (tuberculosis) is cervical adenopathy. Occasionally, this manifestation provides a diagnostic and therapeutic challenge because it mimics other pathologic processes and yields inconsistent physical and laboratory findings (Bayazit, 2004). Some entities such as atypical mycobacteria, cat scratch disease, toxoplasmosis, Kikuchi's lymphadenitis, sarcoidosis, and Kawasaki's syndrome can create persistent lymphadenopathy for many months, and may be confused with granulomatous disease and malignancy. Among the different groups of head and neck lymph nodes, supraclavicular nodes are the most likely to be malignant and should always be investigated. Overall the prevalence of malignancy with supraclavicular adenopathy is variable, but rates of 54 to 85 percent have been reported in biopsy series.

1.9.3 Axillary and Epitrochlear Lymphadenopathy

Infectious causes of prolonged lymphadenopathy such as toxoplasmosis, tuberculosis, mononucleosis rarely manifest with lymphadenopathy alone and persistent lymph-adenopathy is less commonly found in the axillary nodes than in the inguinal chain. Hodgkin's and non-Hodgkin's lymphoma rarely manifest solely or initially in the axillary nodes.

Epitrochlear lymphadenopathy of > 0.5 cm in diameter must be viewed with suspicion (Malin et al., 1994). It may occur with infections (e.g. HIV, syphilis), sarcoidosis, malignancy (e.g. NHL and CLL) as well as potentially any other cause of generalized lymphadenopathy. However, importantly, epitrochlear nodes are not a feature of Hodgkin's disease.

1.9.4 Inguinal Lymphadenopathy

Inguinal lymphadenopathy is common, with nodes enlarged up to 1 to 2 cm in diameter in many healthy adults, particularly in those who spend time barefoot outdoors. Benign reactive lymphadenopathy and infections are the most common etiologies, and inguinal lymphadenpathy is of low suspicion for malignancy.

1.9.5 Generalized Lymphadenopathy

Localized adenopathy should prompt a search for an adjacent precipitating lesion and an examination of other nodal areas to exclude generalized lymphadenopathy. Patients who are immuno-compromised and those with AIDS have a wide differential for generalized lymphadenopathy, including early HIV infection, activated tuberculosis, cryptococcosis, cytomegalovirus, toxoplasmosis, bacillary angiomatosis, Epstein Barr virus infection, Kaposi's sarcoma, multi-centric Castleman's disease, and lymphoma.

1.10 Fine needle Aspiration and Lymphadenopathy

Fine needle aspiration/cytology (FNA/C) offers an accurate, sensitive, inexpensive, and rapid method for the evaluation of cervical adenopathy or masses (Nasuti et al., 1999, Gleeson, 2000). In 218 patients with supraclavicular lymph nodes, FNA showed a sensitivity of 92.7%, specificity 98.5%, positive predictive value of 97.3% and a negative predictive value of 94.8%. The results were further supported by

preparations from needle washings and cell blocks (Gupta et al., 2003) In a study done in Nigeria, at a FNA clinic reviewing FNA's between 1995 to 1997 in 96 patients, the most common diagnosis was reactive changes/nonspecific inflammation in 33.4%; tuberculosis and metastatic lesions made up 25,7% and 22,4% respectively, while lymphoma constituted 16,9% of cases (Thomas, 1999). FNAC aspirated material may be used in ancillary investigations such as immuno-cytochemistry and flow cytometry to support the diagnosis of a benign or malignant process (Lioe et al., 1998, Nasuti et al., 2001, Beaty and Geisinger, 2005). Some studies have revealed a correlation between absolute CD4 and FNA cytology findings. Evidence of tuberculous lymphadenitis was detected in 22 (41%) with CD4 counts varying between 113 and 422 cells/ul (median value 212 cells/ul). Non-Hodgkins lymphoma was diagnosed in 2 cases (3.7%) with CD4 counts of 79 and 13 cells/ul respectively (Shobhana et al., 2002). However, this study did not look at the correlation with lymph node biopsies.

A study of the cytological features and role of FNAC in tuberculous lymphadenitis (TBL) of 21 patients with HIV and 21 patients without HIV infection showed four cytological patterns. Necrotizing lymphadenitis and necrotizing suppurative lymphadenitis was more common in the HIV positive group, whereas necrotizing granulomatous lymphadenitis and granulomatous lymphadenitis was more common in the HIV negative group. No pattern was found to be specific for either group. In addition Ziehl-Nielsen stained cytology smears of the HIV positive group showed a much higher percentage of positivity (61.9%) and a higher density of acid fast bacilli (Nayak et al., 2004).

FNAC is a reliable diagnostic technique; its diagnostic utility for malignant lymphomas has been controversial as a result of problems related to sampling errors and inability of cytology to demonstrate histological growth patterns (Bezabih, 2003).

1.11 Lymph node biopsy

Despite several attempts to create a scoring system to identify which patients with lymphadenopathy should require a biopsy, it remains an inexact science. Different models using age, tenderness on palpation, size, generalized pruritus, supra-clavicular site and texture, ear-nose and throat symptoms and chest x-ray findings have shown differing predictive value in deciding which patients should have a lymph node biopsy (Vassilakopoulos and Pangalis, 2000, Tokuda et al., 2003, Slap et al., 1986). These models have not been analyzed in the context of HIV and have not taken other clinical features into consideration.

Ideally the largest, most suspicious and most accessible node is selected for biopsy. Inguinal nodes offer the lowest yield and supraclavicular nodes have the highest yield. Although the advent of new immuno-histochemical analytic techniques has increased the sensitivity and specificity of FNA, excisional biopsy remains the diagnostic procedure of choice. The preservation of the nodal architecture is critical to the proper diagnosis of the cause of lymphadenopathy, particularly when differentiating lymphoma from benign reactive hyperplasia.

Excisonal biopsy has few complications such as vessel injury and rare spinal accessory nerve injury, local sepsis and fungation **(**Battista, 1991, Gooder and Palmer, 1984).

1.12 HIV and Lymphadenopathy

Opportunistic infections from protozoa, fungi, bacteria and viruses occur in patients with AIDS. Infections usually occur as part of the symptomatic phase of the disease. The most important cause of significant lymphadenopathy in our setting is mycobacterium tuberculosis. However, a multitude of other infective causes have been described.

1.12.1 Viral Infections

These include cytomegalovirus, Epstein-Barr virus, HSV (Herpes simplex virus) and VZV (Varicella Zoster Virus) **(**Jayaram, 2000, Fauci, 1994). A triad of fever, pharyngitis, and lymphadenopathy is suggestive of mononucleosis. Lymph node involvement is typically symmetrical and involves the posterior rather than the anterior chain of cervical lymph nodes. Lymphadenopathy may also be present in axillary and inguinal areas.

1.12.2 Fungal Infections

Pneumonia caused by pneumocystis jiroveci is the most frequent opportunistic infection in AIDS patients in North America and Western Europe. The typical symptoms are shortness of breath on exertion, dry cough and fever. Definitive diagnosis requires bronchoscopy and the demonstration of pneumocystis in the lavage fluid, although in many patients empiric treatment may be initiated based on the clinical picture, chest radiograph and arterial blood gases. Pentamidine or co-trimoxazole and corticosteroids are the treatments of choice PCP. Candida albicans is the second most frequent opportunistic infection in AIDS patients. Thrush of the oral cavity is frequently seen in patients with pre-AIDS. Candidiasis of the oesophagus is a CDC criterion defining AIDS. Therapy is usually with an amphomoronal solution/lozenges or a ketoconazole (e.g. fluconazole) or nystatin. Of further

importance is infection with the fungus Cryptococcus neoformans which if untreated is fatal in all cases. Early diagnosis may be made by the demonstration of the fungus or fungal antigens in the CSF.

1.12.3 Parasitic infections

Toxoplamosis is particularly relevant because it causes CNS disease. It can be treated by a combination of pyrimethamine and a sulfonamide. Crytosporidiosis frequently causes intractable diarrhea for which no specific therapy is available.

1.12.4 Tuberculosis

Mycobacterium tuberculosis is an ongoing and increasing problem in the HIV/AIDS population. In Western Europe and North America, the atypical M. avium-intracellulare and M. Kansaii predominate for which therapy is difficult. In the developing world Mycobacterium tuberculosis is more frequent and is treatable (Collins, 1989).

In many developing countries, tuberculosis has emerged as the most common opportunistic infection associated with HIV. Up to 54% of AIDS patients in Africa have clinical tuberculosis during the course of HIV infection (Raviglione et al., 1995). Miliary TB or disseminated tuberculosis is an important consideration in patients with generalized lymphadenopathy. As the level of immunosuppression increases in HIV-infected individuals, mycobacteraemia and extra-pulmonary tuberculosis becomes progressively more common (Jones et al., 1993). In HIV-infected patients with pyrexia of unknown origin, diagnostic studies should be done to exclude extra-pulmonary tuberculosis. In HIV-infected individuals intra-abdominal tuberculosis is characterized by visceral lesions, and intra-abdominal lymphadenopathy with necrosis, which is best visualized by computed tomography (Havlir and Barnes, 1999).

Clinical studies have shown the detrimental effects of tuberculosis on the course of HIV infection. The risk of death in HIV-infected patients with tuberculosis was reported to be twice that in HIV-infected patients without tuberculosis, independent of the CD4 count (Whalen et al., 1995). The high mortality rate among patients with tuberculosis appeared to be due to progressive HIV infection rather than tuberculosis. The degree of immunosuppression is the most important predictor of survival in HIV-infected patients with tuberculosis, since negative tuberculin skin tests, prior opportunistic infections, and low CD4 counts are associated with increased mortality (Whalen et al., 1997).

1.12.5 Malignancy and Lymphadenopathy

Since 1980, NHL incidence in individuals aged 25-54, has undergone a dramatic escalation, mostly related to the HIV epidemic (Fisher., 2004). Data from 125,691 persons with AIDS from 17 Western countries indicates that AIDS-defining cancers remained as the fourth and sixth leading AIDS-defining illnesses at AIDS diagnosis among males and females respectively, in 2001 (Ebrahim et al., 2004). HIV disease, complicated by malignancy may also manifest with lymphadenopathy. This may occur in the setting of lymphoma and Kaposi's sarcoma. After Kaposi's sarcoma, Non-Hodgkin's lymphoma (NHL) is the second most common malignancy in patients with AIDS. The incidence of NHL is increased 60-200 fold in patients with AIDS (Biggar, 1996). AIDS related NHL are typically high grade, mostly of B-cell lineage, present with advanced stage disease, with frequent involvement of extra-nodal sites and have an unfavorable prognosis (Broder and Karp, 1992). They present more commonly in patients with advanced stage of HIV/AIDS. Histologically, they are most commonly diffuse large B-cell lymphoma (including centroblastic, immunoblastic and other subtypes of diffuse large B cell lymphoma) and

Burkitts/Burkitts-like lymphoma (Ioachim et al., 1991). Primary cerebral (brain) lymphomas and primary effusion lymphomas are unique entities that are largely confined to individuals with immunodeficiency (typically HIV/AIDS) (Ansari et al., 1996**,** Gaidano et al., 1996, Knowles, 1996)**.** The CD4 cell count has been the major factor used to establish prognosis of patients with HIV-NHL (Little et al., 2001). Malignant lymphomas of HIV-infected patients differ from other known lymphomas by their localization, degree of malignancy and response to therapy. HIV-associated lymphomas are frequently found outside the lymphatic system, particularly in the brain, bone marrow, GI tract and skin. Their response to therapy is much poorer than that of classical lymphoma, i.e. lymphoma occurring in seronegative individuals.

1.12.6 Kaposi's Sarcoma

The most frequent opportunistic tumour is Kaposi's sarcoma (KS), observed in 20% of patients with AIDS (the incidence has decreased in the post HAART era). Kaposi's Sarcoma, can occur before the onset of severe immunosuppression. The causative agent in KS is HHV-8 (Human herpes virus-8) / KSHV (Kaposi's sarcoma herpes virus) and the pathogenesis involves an interaction of this virus with HIV as well as a complex interplay of growth factors (Youree et al., 2003)**.** KS is a vascular tumour, and in its characteristic form is readily identified. First described in 1872 by Moritz Kaposi, KS was classically noted in elderly men of Eastern Europe or Mediterranean descent (Antman and Chang, 2000). However, in the early 1980's, this rare tumour came to be associated with AIDS – in homosexual men, as an AIDS defining illness (Safai et al., 1992). The clinical presentation of KS varies widely, but the majority of patients manifest with mucocutaneous disease. KS presenting with generalized lymphadenopathy with no mucocutaneous involvement is much less common.

Visceral involvement may also occur in KS, with the lungs and GIT being the most common sites of involvement.

1.12.7 Other causes of Lymphadenopathy

Castleman's disease (CD), also known as angiofollicular lymph node hyperplasia, is an uncommon lymphoproliferative disorder with an increased prevalence in HIV. In particular, the multicentric form of Castleman's disease (MCD), which histologically has features of the hyaline vascular type and plasma cell variety of Castleman's disease, has been linked to both HHV-8 and HIV-infected individuals (Collins et al., 2006; Mylona et al., 2008). The clinical presentation of the MCD is similar to the plasma cell type with constitutional symptoms, generalized lymphadenopathy (involving the inguinal, paratracheal, retroperitoneal, axillary, pelvic and other regions), and hepatosplenomegaly (Izuchukwu, 2003**).** The clinical presentation often resembles that seen in lymphoma. Additionally, the disease may co-exist with Kaposi's sarcoma and NHL, or lymphoma may complicate MCD.

A parotid gland lymphoepithelial cyst is considered a precursor to AIDS. It is usually associated with lymphadenopathy, fatigue, night sweats, diarrhoea and weight loss. It responds well to HAART (Craven et al., 1998).

1.13 Neurological manifestations

A high percentage of HIV infected patients show neurological changes that are not explained by opportunistic infections or tumours. The spectrum of symptoms ranges from slight neuropsychological abnormalities (disturbances of memory, mood and behavior) to organic psychosis and complete dementia. In almost all cases a continuous deterioration is observed. The most frequent neurological disorder is sub-acute encephalitis (AIDS encephalopathy, AIDS dementia complex) which is seen in

two thirds of cases. Other clinical manifestations include acute meningoencephalitis, aseptic meningitis and peripheral neuropathy. Primary CNS lymphoma may manifest with non-specific neurological symptoms and signs or present with focal neurological signs consequent on the mass lesion/s in the brain, which is a classic feature of the disease.

1.14 Dermatological manifestations

HIV specific conditions include oral hairy leucoplakia and a pruritic maculopapular eruption. Seborrhoeic eczema occurs in 70% of AIDS patients and allergic exanthemas and acne like eruptions are less common. Viral induced skin eruptions are also frequently seen, such as herpes zoster, condylomata accuminata, verruca vulgaris and molluscum contagiosum.

1.15 Gastrointestinal Manifestations

Persistent diarrhoea is a frequent problem in these patients. Giardia Lamblia, Entamoeba Histolytica, Shigella, Salmonella and Campylobacter all cause symptomatic disease. However, appropriate therapy does not always eliminate the watery diarrhea. Cryptosporidium, mycobacterium tuberculosis, mycobacterium avium intracellulare, lymphoma and Kaposi's sarcoma may also be associated with diarrhoea. It has been suggested that HIV itself may be enteropathic through infection of the mucosal cells and /or through interfering with the nerve supply to the gastrointestinal tract.

1. 16 Haematological Manifestations

The pathogenesis of peripheral blood cytopenias in AIDS patients is clearly multi-factorial. HIV infected patients often suffer from multiple haematopoetic

abnormalities which include anaemia, thrombocytopenia, lymphocytopenia, monocytopenia, neutropenia and myelodysplastic/hyperplastic alterations of the bone marrow microenvironment (Koka and Reddy, 2004). Differential blood counts may demonstrate the benign atypical lymphocytes of infectious mononucleosis, cytomegalovirus, and other viral disease or the malignant cells of Burkitts leukemia or lymphoma. Less commonly, acute monocytic or myelo-monocytic leukemia may be associated with lymphadenopathy.

Mild anaemia, leukocytosis with or without eosinophilia and thrombocytosis are suggestive of Hodgkin's disease, especially if associated with an isolated increased serum alkaline phosphatase (Weinstock, 2001).

1.16.1 Leucopenia

Leucopenia is common in patients with HIV infection and seems to correlate with the stage of the disease (Costello, 1988).

1.16.2 Anaemia

With disease progression, patients infected with HIV develop a moderate to severe hypoproliferative anaemia. The anaemia is characteristically an anaemia of chronic disorder. Other contributors to the anaemia include haemolysis (microangiopathic and Coomb's positive auto-immune haemolytic anaemia, pure red cell aplasia, hypoplastic anaemia, secondary myelodysplasia, haemophagocytic syndrome, opportunistic infections such as TB, complicating malignancy such as lymphoma and KS, drugs such as AZT, bactrim and chemotherapy, nutritional deficiencies, hypersplenism and blood loss. Anaemia is the commonest cytopenia and manifests in over 70-80% of individuals with AIDS and is almost invariable in patients with advanced/terminal disease.

1.16.3 Thrombocytopenia

Thrombocytopenia may be an early consequence of HIV infection. Approximately 3% of patients infected with CD4 cell counts of greater than 400/uL have platelet counts of less than 150, 000/uL. Of patients who have CD4 cell counts less than 400/uL, 10% also have platelet counts of less than 150,000/uL. 30-50% of patients will manifest with thrombocytopenia during the symptomatic, full blown disease. Causes of thrombocytopenia include secondary immune thrombocytopenia, thrombotic thrombocytopenic purpura, DIC, secondary anti-phospholipid syndrome, opportunistic infections, complicating malignancgy (e.g. lymphoma), nutritional deficiency, haemophagocytic syndrome, hypersplenism and drugs

1.17 Biochemical Abnormalities

Liver biochemical abnormalities may be seen with the disease or its complications such as infectious mononucleosis, cytomegalovirus infection, viral hepatitis, TB, malignancy etc. Appropriate culture, serology or antigen testing may diagnose an infection

Antinuclear antibody and rheumatoid factor may suggest a connective tissue disease or angio-immunoblastic lymphadenopathy. A positive direct Coombs' test, a polyclonal increase in immunoglobuliins, and sometimes even cold agglutinins are seen in the latter disorder.

2.0 Objectives of the study

2.1 The primary objectives of the study are:

a) To define the causes of lymphadenopathy and the clinical patterns of presentation

2.2 The secondary objective of this study are:

a) To review the results of investigations that may suggest or exclude a possible cause of lymphadenopathy

b) To correlate the results of FNA and /or lymph node biopsy when this is performed.

c) To correlate the lymphadenopathy with other clinical features and HIV parameters where possible e.g. CD4 count and viral load.

d) To identify criteria for those patients who should have an FNA and/or lymph node biopsy.

3.0 Patients and Methods

3.1 Ethical approval and patient consent

Ethical approval was obtained from the University of the Witwatersrand Ethics committee (Protocol Number Mo1-10-1). The informed consent was given to patients to read, and a verbal explanation of the consent form was carried out. Prior to consent being obtained, patients received an explanation regarding:

- Aims and objectives of the study
- Collection of clinical data
- Lymph node biopsy

3.2 Patient Profile

All patients were recruited from the adult medical wards at Chris Hani Baragwanath hospital. A consultation service was offered to fellow colleagues, in aiding and obtaining a diagnosis on the patients. Only patients that tested HIV positive on a confirmatory ELISA and found to have significant lymphadenopathy of greater than 1 cm in diameter by the referring physician were consented for the study. In the single patient who was sixteen years old, consent was obtained from her mother.

Each patient was fully examined and all routine investigations performed by the referring doctor were reviewed and documented

3.2.1 Inclusion Criteria

- HIV seropositivity
- Informed consent
- Adult patients (generally above the age of 18 years, where the age is < 18, consent will be obtained from the parents)
- Significant lymphadenopathy (as per the definition above)

3.2.2 Exclusion criteria

- Inability to give informed consent or unwillingness to participate in the study
- Does not fulfil the inclusion criteria stipulated above

3.3 Investigations

3.3.1 Patient Demographics

The patient demographics were noted. Patients were asked to complete a standard questionnaire

3.3.2 Clinical History

A detailed clinical history was taken

3.3.3 Physical Examination

Patients were examined fully with particular attention to the details of the lymphadenopathy and underlying clinical disease

3.3.4 Special Investigations

The chest x-ray and other relevant investigations such as the Full Blood Count, urea and electrolytes, liver function tests and CD4 counts were noted and documented

3.3.5 Fine needle aspiration

FNA was performed using a small–gauge (usually 22- to 25-gauge) needle and negative pressure to withdraw cells or fluid from the palpable lymph node. After the sample was taken, it is smeared onto two separate slides. One slide is air dried and the other is sprayed with a cyto fixator. Thereafter, the samples are examined under a light microscope. The results of the FNA were documented.

3.3.6 Lymph node Biopsy

A lymph node biopsy was performed by the surgeons. The specimens were immersed in formalin and labelled for histology. The specimens were sent to the Anatomical Pathology Laboratory at Chris Hani Baragwanath hospital. All specimens were examined after staining with Haematoxylin and Eosin. Some of the specimens were subjected to flow cytometry. The findings of the pathologists were then documented

3.4 Statistical analysis

The available data was analyzed using the SAS System and the NPAR1WAY procedure. Wilcoxon Scores (Rank Sums) for the different variables were classified according to the biopsy result. The Kruskal-Wallis and the Fisher's exact Test were used to determine statistical significance

4.0 Results

4.1 Demographics and study population

A total of 43 black patients were assessed between 2004 and 2005 at Chris Hani Baragwanath Hospital. There were 21 males (49%) and 22 females (51%). The median age of the patients was 33 years (range 16-52 years). 39/43 (91%) of the patients were currently single (5/39 - 13%) having being married previously. Four patients were married. 26/43 (60%) of the patients were unemployed (see table 1).

Table 1: Demographics of study population

	Males	Females
Number	21	22
Age (median)	34 (range 24-43)	33 (range 16-52)
Marital status (single)	18	21
Unemployed	9	17
Employed	12	5

4.2 Presenting complaints

Patients presented with a variety of symptoms (see figure 6), with 22/43 (51%) of them listing "swollen glands"-S/G as one of their complaints (on biopsy: Malignancy – 7; TB – 8; PGL/Reactive – 6; Lymphoepithelial cyst – 1). Constitutional symptoms were not readily volunteered. A total of 37/43 (86%) patients could give a rough estimate of the duration of their lymphadenopathy, with a median duration of 16 weeks (3-52 weeks). There was no statistical significance between the cause and duration of the lymphadenopathy (p=0.84).

4/43 (9%) of the patients complained of fever (on biopsy: NHL - 1; TB – 1; Reactive – 2). 11/43 (26%) of the patients had night sweats (on biopsy: Malignancy – 3; TB – 6; Reactive – 2). 13/43 (30%) of the patients reported significant weight loss (on biopsy: Malignancy – 2; TB – 8; Reactive – 2; no biopsy – 1).

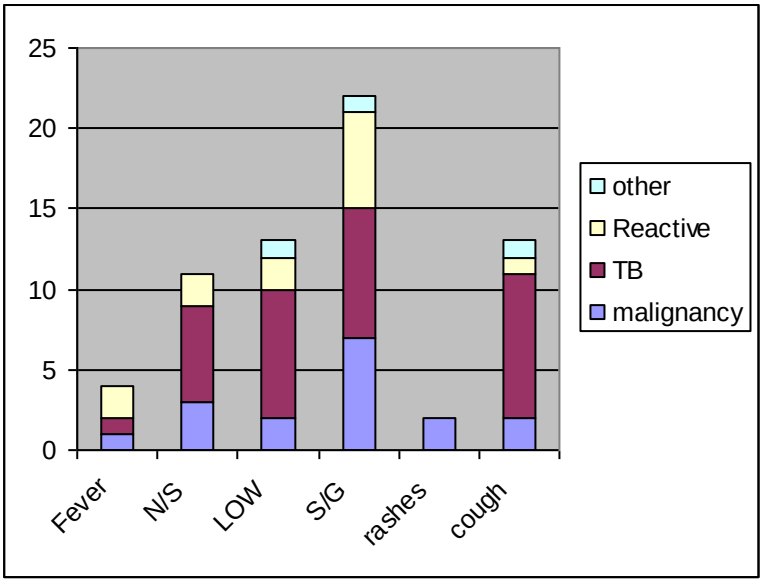


Figure 6: Presenting complaint with lymph node biopsy diagnosis
N/S=night sweats; LOW=loss of weight; TB=tuberculosis

4.3 Clinical Examination

4.3.1 Temperature

The normal temperature range is 37±0.5^{o}C. Pyrexia is defined as a temperature >37.5^{o}C. 19/43 (44%) of the patients were pyrexial on admission (on biopsy: Malignancy – 2; TB – 11; Reactive – 2; no biopsy – 2; other – 2).

4.3.2 Assessment of Lymphadenopathy

In general, lymph nodes ≥ 1x1 cm in diameter was defined as being significant (with the exception of epitrochlear nodes where nodes >0.5x0.5cm was regarded as being significant). In this study, significant lymphadenopathy was palpable in the anterior cervical, posterior cervical, axillary, inguinal, femoral and epitrochlear regions of 35 (81%), 33 (77%), 30 (70%), 18 (42%), 16 (37%) and 4 (9%) of the patients respectively. The breakdown of the nodes in the different regions based on the aetiology is as follows: Anterior cervical lymphadenopathy (on biopsy: Malignancy – 7; TB – 15; Reactive – 8; no biopsy - 1; other - 4). Posterior cervical lymphadenopathy (on biopsy: Malignancy – 7; TB – 13; Reactive – 5; no biopsy - 3;

other - 5). Axillary lymphadenopathy (on biopsy: Malignancy – 7; TB – 10; Reactive – 6; no biopsy - 3; other - 4). Inguinal lymphadenopathy (on biopsy: Malignancy – 4; TB – 3; Reactive – 6; no biopsy - 2; other - 3). Femoral glands (on biopsy: Malignancy – 5; TB – 4; Reactive – 4; no biopsy - 0; other - 3). Although epitrochlear nodes were evident in 22 patients, only 4 patients had nodes >0.5x0.5 cm in diameter. The breakdown of epitrochlear adenopathy is as follows (on biopsy: Malignancy – 2; TB – 2). The finding of lymphadenopathy in the cervical and axillary regions did not differentiate between malignancy, TB and reactive nodes. However, the presence of these nodes was suggestive of significant pathology i.e. malignancy or TB (see figure 7). In the 10 patients diagnosed with malignancy, 3 had significant peripheral lymphadenopathy at only a single site. The lymph node biopsy on these 3 patients confirmed Non-Hodgkin's lymphoma. In assessing the characteristics

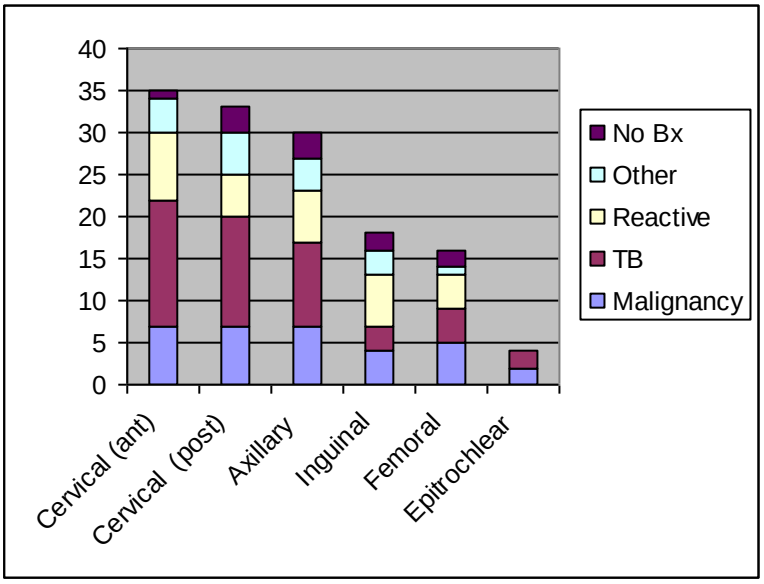


Figure 7: Sites of Lymph nodes and diagnosis
No Bx=No Biopsy; TB=Tuberculosis

of the lymph nodes, whether nodes were hard or rubbery, was inconclusive in determining the exact pathology. Thirty four patients were assessed as having hard lymph nodes (on biopsy: Malignancy – 8; TB – 15; Reactive – 5; no biopsy - 3; other

- 3). Seven patients had nodes that were rubbery in consistency (on biopsy: Malignancy – 2; Reactive – 4; other - 1). Two patients had soft nodes (on biopsy: TB – 1; other - 1). Matted nodes were seen in eight patients, seven of whom had TB (on biopsy: Malignancy – 1; TB – 7). Twenty one of the patients had nodes assessed as discrete (on biopsy: Malignancy – 8; TB – 4; Reactive – 6; no biopsy - 2; other - 1) (see figure 8).

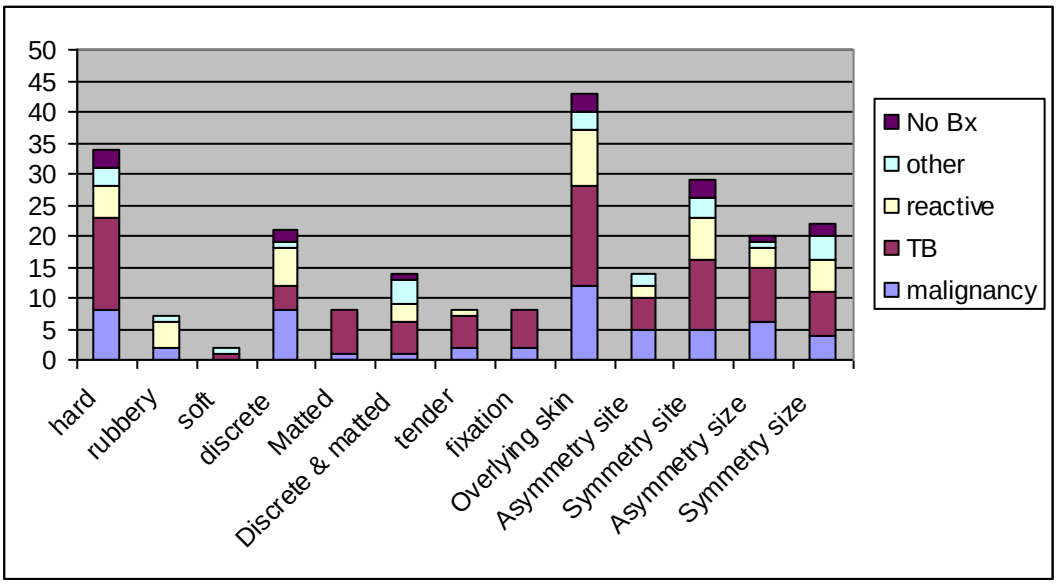


Figure 8: Lymph node characteristics and diagnosis
TB=Tuberculosis; No Bx=No biopsy

Fourteen patients had both discrete and matted lymph nodes in different nodal areas (on biopsy: Malignancy – 1; TB – 5; Reactive – 3; no biopsy - 1; other - 4). Tenderness was present in 8 of the patients (on biopsy: Malignancy – 2; TB – 5; Reactive – 1). Tenderness of lymph nodes by the Fisher exact Test (p=0.4293) was not statistically significant. Fixation of the lymph nodes was highly suggestive of TB, occurring in 6/8 (75%) of the patients. Malignancy accounted for the other two patients with fixed lymphadenopathy.

The overlying skin was intact in all forty three patients. None of them had evidence of ulceration, breakdown of the lymph nodes or draining sinuses.

Assessment of symmetry or asymmetry of the size and site of lymph nodes was not statistically significant, in relation to establishing a diagnosis (see figure 8).

4.4 Investigations

4.4.1 Chest x-ray

Table 2: Chest x-ray findings and biopsy results

CXR findings	Number	Malignancy	TB	Reactive	Other	No Bx
Normal	3	1	1	0	1	0
Mediastinal/intrathoracic L/N	6	3	1	0	2	0
Lung pathology	9	1	4	2	0	2
Mediastinal/intrathoracic L/N & lung pathology	11	1	6	3	1	0

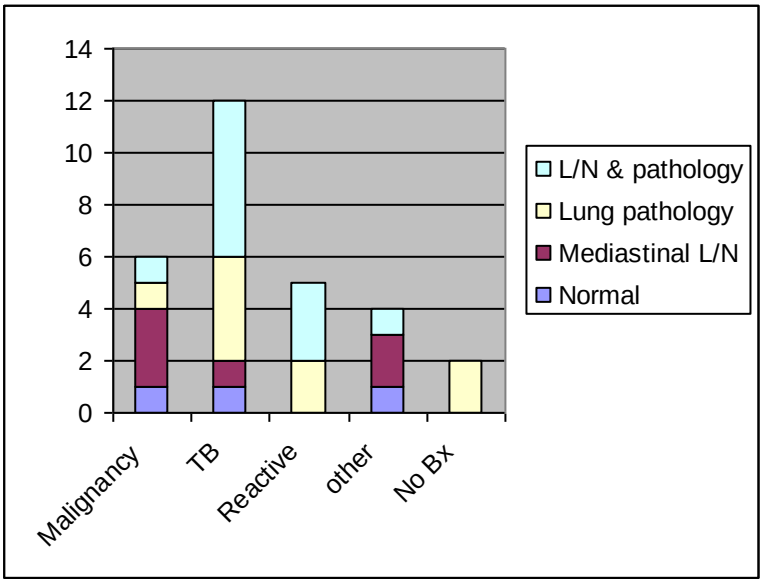


Figure 9: Chest x-ray findings and biopsy results

The chest X-ray was available in 29 of the forty three patients. 6/29 patients had mediastinal/intrathoracic lymphadenopathy only. In 11 of the 29 patients, mediastinal adenopathy was accompanied by lung pathology. Importantly, the finding of lung pathology (i.e. either an infiltrate or pleural effusion), or lung pathology with

mediastinal/intrathoracic adenopathy is suggestive of a diagnosis of tuberculosis (see table 2 and figure 9).

4.4.2 Haematology

Forty one patients had a full blood count available for analysis, with only thirty one having a differential white cell count. Leucopenia with or without a lymphopenia, in relation to the cause of lymphadenopathy was not of statistical significance (p=0.1474 and 0.053 respectively).

Table 3: Results of the Full blood count in relation to the diagnosis of lymphadenopathy

	Number	Malignancy	TB	Reactive	Other	No Bx
White cell count $x10^9$/l						
>3.92<9.88	27	6	10	7	3	1
<3.92	7	1	1	2	2	1
>9.88	7	1	5	0	0	1
Haemoglobin (g/dl)						
Hb <10	29	3	14	6	4	2
Hb> 10	12	5	2	3	1	1
Platelets $x10^9$/l						
<140	9	2	1	4	1	1
>400	5	1	3	1	0	0
>140<400	27	5	12	4	4	2
Neutrophils $x10^9$/l						
<2.0	9	2	1	4	2	0
>7.5	6	0	4	0	1	1
>2.0<7.5	16	4	6	3	2	1
Lymphocytes $x10^9$/l						
<1	14	1	9	1	2	1
>4.0	4	1	1	1	1	0
>1<4.0	13	4	1	5	2	1
Eosinophils $x10^9$/l						
<=0	30	6	11	7	4	2
>0.45	1	0	0	0	1	0
Basophils x1 0^9/l						
<=0	31	6	11	7	5	2
>0.2	1	0	1	0	0	0
Monocytes $x10^9$/l						
<0.18	8	0	6	1	1	0
>0.80	15	5	2	5	3	0
>0.18<0.80	8	1	3	1	2	1
CD4< 200/uL	19	3	8	5	1	2
CD4>200/uL	12	4	2	3	2	1

The majority of patients (66%) had a white cell count in the normal range. There was no relationship between the white cell count, haemoglobin and platelets to the lymph node biopsy result (see table 3). Two of the assessed patients were found to have a pancytopenia on their full blood count i.e. defined as a WCC less than $3.92x10^9$/l with a neutrophil count of less than $2x10^9$/l, Hb less than 10g/dl and platelet count of less than $140x10^9$/l. Unfortunately the one patient did not have a lymph node biopsy, but the FNA was suggestive of TB. The second patient was diagnosed with Castleman's disease on lymph node biopsy. However, on the bone marrow trephine, the patient had a granuloma suggestive of TB (see table 3) and indicative of possible dual pathology in the same patient.

A relative monocytosis was suggestive of a non-benign diagnosis (p=0.0168). Nine of the fifteen patients who had a relative monocytosis also had CD4 counts greater than 200/ul (see table 3)..

Of the forty patients that had a lymph node biopsy, thirty one had available CD4 counts for analysis. Using a CD4 count of less than or greater than 200/uL, there was no correlation with the biopsy results (p=0.2563) (see table 3).

4.4.3 TB Investigations

Table 4: TB investigations compared to Biopsy results

	Malignancy	TB	Reactive	Other	No Bx
Sputum positive	1 (1)	1 (4)	1 (2)	0 (1)	0 (0)
TB culture positive	0 (7)	6 (7)	0 (7)	0 (1)	1 (1)
ZN+ staining on Bx	0 (1)	10 (11)	0 (0)	0 (0)	0 (0)

The investigations performed to diagnose TB included analysis of sputum for AFB (acid-fast bacilli), body fluids (e.g. pleural fluid) and bone marrow for TB culture and biopsy material for positive Ziehl Nielson staining (e.g. bone marrow trephine). Additional investigations that were helpful in the diagnosis of TB included a CXR and FNA. A CXR and an FNA of the lymph nodes were suggestive of TB in 7 and 14

of the patients respectively, but generally required further definitive confirmation. 16/40 patients had TB diagnosed on a lymph node biopsy. Table 4 shows the TB investigations compared to the lymph node biopsy result. The investigations that yielded the highest evidence of TB were positive ZN staining on the biopsy material and TB cultures.

4.4.4 Fine needle Aspiration.

In 32 patients, 33 FNA's were performed. The breakdown of the FNA results was as follows (see table 5):

i) Tuberculosis – 14

ii) Malignancy – 5 (NHL-3, Neuroendocrine tumour-1, Spindle cell tumour-1)

iii) Reactive – 9

iv) Other – 5 (Lymphoepithelial cyst-2, suboptimal-3)

One patient had two FNA's, the first labelled as metastatic tumour and the second as TB.

Table 5 Diagnosis by FNA and Biopsy

	FNA	Biopsy
TB	14 (32)	16 (40)
Reactive	9 (32)	9 (40)
Malignancy	5 (32)	10 (40)
Other	5 (32)	5 (40)

( ) =total number of patients

4.4.5 Lymph node Biopsy

In the lymph node biopsy group, the breakdown of the results was as follows, (see figure 10):

i) Tuberculosis – 16

ii) Malignancy – 10 (NHL-7, Kaposi's sarcoma-2, Neuroendocrine tumour-1)

iii) Reactive – 9

iv) Other – 5 (Castleman's disease-1, Lymphoepithelial cyst-2, suboptimal-2)

Interestingly, one of the patients had a biopsy which showed Kaposi's sarcoma (KS) on one lymph node and reactive features in a second node. Another patient also had two different diagnoses, with one lymph node showing reactive features and the other node showing TB.

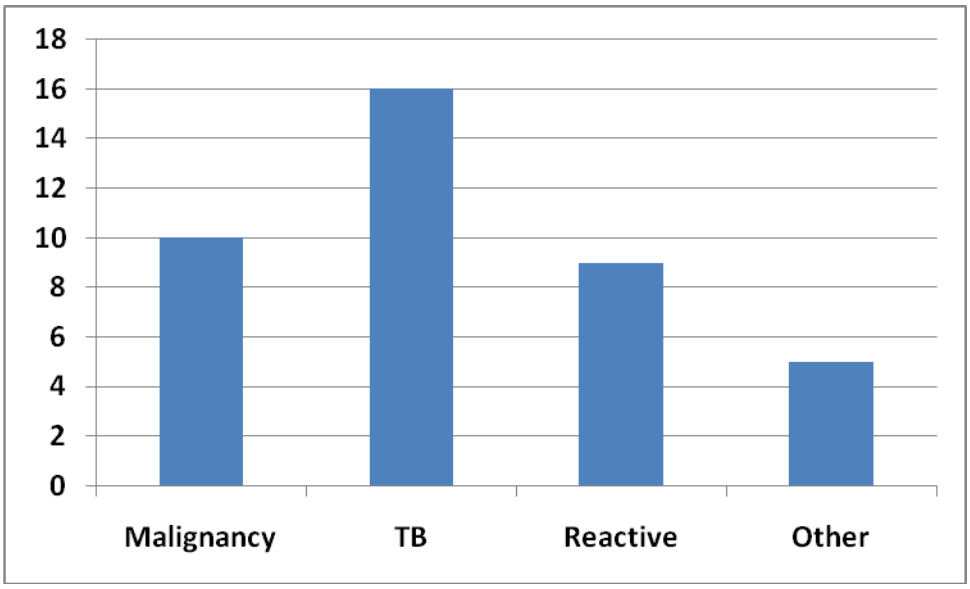


Figure 10: Diagnosis by Biopsy

4.4.6 Comparison of FNA to Biopsy

Thirty patients had both a FNA and a lymph node biopsy. One in each group was reported as suboptimal and therefore categorised under other. Comparing FNA with lymph node biopsy as the true positive, eleven patients were diagnosed with TB, four with malignancy, three as reactive and two as having other aetiologies of their lymphadenopathy. The patients in the other category included a patient with a lymphoepithelial cyst and a patient with fibrous tissue and skeletal muscle that was assessed as lymphadenopathy.

From the patients with a suboptimal FNA, one patient was confirmed to have NHL on biopsy, one to have a reactive lymphadenopathy and the third patient did not have a biopsy. The patient with the presumed diagnosis of spindle cell tumour had only fibrous tissue and skeletal muscle on biopsy, i.e. a suboptimal biopsy.

In the discrepant group of ten patients comparing FNA to Biopsy, five patients were diagnosed as having reactive lymph nodes on FNA, but the biopsy confirmed two with Castleman's disease, two with NHL and one with TB. One patient diagnosed as TB on FNA, had KS on biopsy. Another patient with a lymphepithelial cyst on FNA had reactive lymphadenopathy on biopsy. A further patient diagnosed with TB on FNA had a sub-optimal lymph node biopsy. Tables 6 and 7 show concordance and discordance between the FNA and lymph node biopsy diagnosis.

Table 6 FNA and Lymph node biopsy – concordant results

FNA and LNB diagnosis	FNA	Lymph node biopsy (LNB)
Tuberculosis	11	11
Malignancy	4	4
Reactive	3	3
Other	2	2

Table 7 FNA and Lymph node biopsy – discordant results

FNA diagnosis	Number of patients	Lymph node biopsy (LNB)
TB	1	KS
	1	Suboptimal
Reactive	2	Castleman's disease
	2	NHL
	1	TB
Spindle cell tumour	1	Suboptimal
Suboptimal	1	NHL
Lymphoepithelial cyst	1	Reactive

Using chi-square analysis, the specificity and sensitivity of FNA compared to lymph node biopsy for the diagnosis of malignancy was 100% and 43% respectively with wide confidence intervals (see table 8). This indicates that although a positive result for malignancy on FNA is highly specific, FNA for the diagnosis of malignancy is much less sensitive and a significant number of patients could be missed if the diagnosis is based on the FNA only.

The specificity and sensitivity for the diagnosis of TB was 86% and 95% respectively (FNA is less specific for TB, but more sensitive). Reactive lymphadenopathy had a lower specificity and sensitivity of 80% and 60% respectively. Similarly, the specificity and sensitivity for the diagnosis of other pathologies was 86% and 40% respectively. These results underline the importance of performing a lymph node biopsy, more particularly where malignancy is suspected or where a reactive FNA

result may not be sensitive enough to exclude a more definitive diagnosis for the lymphadenopathy.

Table 8: Specificity and sensitivity of FNA compared to Lymph node biopsy

	Specificity	CI	Sensitivity	CI
Malignancy	100%	95% CI {26%-174%}	43%	95% CI {61%-80%}.
TB	86%	95%CI {12%-104%}	92%	95% CI {77%-107%}
Reactive	80%	95% CI [64%-96%]	60%	95% CI {17%-103%}
Other	86%	95% CI [70%-98%]	40%	95% CI {-3%-83%}

CI=Confidence intervals

5.0 Discussion

The pandemic of HIV/AIDS, is an unrelenting scourge of our generation and continues to be a threat to the very survival of our young population. Sub-Saharan Africa remains the region most heavily affected by HIV accounting for approximately two-thirds of all people living with HIV and 75% of AIDS deaths in 2007. Young people aged 15-24 years make up an estimated 45% of new infections worldwide (UNAIDS, 2007). The median age of our study population was 33years with a range of 16 to 52 years.

Lymphadenopathy is common in HIV-infected individuals as lymphoid tissue is a major target and reservoir of the virus. Lymphadenopathy may occur at any stage of HIV infection, i.e. during the sero-conversion illness phase, the asymptomatic or latent phase and during the symptomatic phase of the disease. Lymphadenopathy in a HIV positive population is confounded by the presence of PGL. PGL is an important manifestation of HIV infection and has to be differentiated from other disease processes, especially infectious diseases and malignancies.

A good history is fundamental in determining the aetiology of lymphadenopathy. In our patient population, even on direct questioning, the presence of lymphadenopathy (swollen glands) was not readily volunteered. Only 22/43 (51%) of the patients interviewed were aware of the presence of "swollen glands.' A total of 37/43 (86%) of patients could give a rough estimate in weeks of the duration of their lymphadenopathy. The median duration of lymphadenopathy was 16weeks (3-52).

A history of constitutional symptoms such as night sweats and loss of weight was most common among the patients diagnosed with TB, but did not reach statistical significance (see figure 6). In all the patients with constitutional symptoms, it is

possible that in addition to the underlying diagnosis (e.g. TB or malignancy), HIV itself may cause or manifest with constitutional symptoms.

In a study of primary care physicians it was found that generalised lymph-adenopathy was identified in only 17% of the patients in whom lymphadenopathy was present (Paauw et al., 1995). Therefore, physicians have to be vigilant to the presence of lymphadenopathy in order to initiate the relevant investigations. This is further exemplified by a study in the United States, where an improvement of the general competence of physicians in the care of patients with known or suspected tuberculosis, resulted and formed an important strategy for re-establishing control of tuberculosis (Rao et al., 1999).

Lymph node significance may be assessed on their clinical characteristics such as location, size, tenderness, consistency, fixation and mobility, as a means of formulating a differential diagnosis. In predicting nodal malignancy from clinical data, variables that were identified include: age more than 40 years, males, generalized lymphadenopathy, presence of other signs and abnormal liver function tests to be independently associated with nodal malignancy (Abba and Bangboye, 2003).

The finding of lymphadenopathy in the cervical and axillary regions did not differentiate between malignancy, TB and reactive nodes. However, the presence of these nodes was suggestive of significant pathology i.e. malignancy or TB (see figure 7).

The presence of hard nodes was more in keeping with a diagnosis of malignancy and/or TB (see figure 8). The presence of matted nodes was classical of TB (see figure 8).

In the ten patients diagnosed with malignancy, three had significant peripheral lymphadenopathy at only a single site, emphasizing the point that malignancy may present with localised adenopathy as well as generalised adenopathy. The lymph node biopsy on the three patients with localised adenopathy showed non-Hodgkin's lymphoma.

Lymphadenopathy in HIV positive patients does not seem to follow any specific clinical pattern. The nodes, in addition to the presence of malignancy, TB or other pathology may be distorted by the presence of PGL. This concept may still be true if one considers that some of the patients did indeed have more than one pathology in their lymph nodes. One patient was diagnosed as having a metastatic neuroendocrine tumour on the first FNA and TB on a second FNA. Lymph node biopsy was consistent with a neuroendocrine tumour. However, the patient also tested positive for acid fast bacilli on a sputum sample. Similarly, another patient had KS on one lymph node and reactive changes on another node from the same biopsy site. A third patient had TB and reactive lymphadenopathy from the same biopsy site.

As far as investigations are concerned, the chest X-ray should be a routine tool for any patient who presents with significant lymphadenopathy. The chest x-ray is important for the diagnosis of pulmonary tuberculosis. Upper lobe consolidation/infiltrates and cavitation are the typical findings in reactivation tuberculosis, whereas intra-thoracic lymphadenopathy and lower lobe disease are seen in primary tuberculosis. In the HIV infected patient with a CD4 T-cell count of greater than 200cells/mm^3, the radiographic patterns tend to be one of reactivation disease with upper lobe infiltrates with or without cavitation (Post et al., 1995). In HIV infected persons with a lower CD4 T-cell count i.e. less than 200cells/mm^3, a pattern of primary disease with intra-thoracic lymphadenopathy and lower lobe consolidation/infiltrates is seen. As chest

radiographs may appear normal in 7-14% of cases, a high index of suspicion must be maintained in evaluating patients with HIV, who have symptoms suggestive of TB (Long et al., 1991). Furthermore, sputum may be acid fast bacilli positive from these patients even with normal looking chest radiographs. Even though there was no correlation between chest radiographs and lymph node biopsy pathology in our study, the impression of intra-thoracic lymphadenopathy certainly suggested more significant pathology, such as TB or malignancy (see figure 9). Twelve of the patients with TB had a chest x-ray available for comment. The chest x-ray of these patients varied from normal to the presence of lung pathology only, to having intra-thoracic lymphadenopathy with lung pathology and only mediastinal lymphadenopathy. There was a similar variation in x-ray findings in the malignancy, reactive and other group of patients. Therefore, definite conclusions could not be drawn with regard to the aetiology of the lymphadenopathy from the available X-rays. The full blood count, CD4 count and possibly viral load is important in determining at what stage of disease the patient is, and will assist in determining what further intervention such as the introduction of highly active anti-retroviral therapy is required. The presence of cytopenias is common among HIV positive patients. Anaemia, neutropenia and thrombocytopenia can occur alone or in various combinations (Davis and Zauli, 1995). This can confound the presence of other pathologies. A patient with unexplained cytopenias especially with a pancytopenia should ideally have a bone marrow aspirate and trephine biopsy. Interestingly in our study, a patient diagnosed with reactive lymphadenopathy on FNA and subsequently shown to have Castleman's disease on lymph node biopsy, had a granuloma on bone marrow trephine consistent with TB.

A relative monocytosis was suggestive of malignancy in our study, but may also be an indication of viral latency as most of these patients also had a CD4+ lymphocyte count above 200cells/mm^3. Monocytes are a potential reservoir of HIV during viral latency (Coleman and Wu, 2009).

Tuberculosis and HIV have been closely linked since the emergence of HIV/AIDS epidemic. Although tuberculosis can be a relatively early manifestation of HIV infection, it is important to note that the risk of developing tuberculosis, and of disseminated infection, increases as the CD4 T-cell count decreases. Tuberculosis acts to accelerate the clinical course of HIV infection. Positive cultures for mycobacterium tuberculosis provide a definitive diagnosis of tuberculosis. However, culture results may not be available for 2-6 weeks, creating a need for more rapid diagnostic techniques. The finding of granulomatous infiltration in the lymph node with positive ZN staining for acid fast bacilli provides a definitive diagnosis of TB in patients in whom it is found.

Several authors have compared FNA to excisional biopsy in the diagnosis of lymphadenopathy with promising results. Steel et al, 1993, performed FNA on 1,103 patients with lymphadenopathy. A diagnosis was made in 90% using cytology alone. The false negative and false positive rates were 3.4% and 0.9 % respectively (Steel et al., 1993). Pilotti et al, 1993, report an overall accuracy rate of 99.1% using FNA on 285 outpatients with enlarged lymph nodes (Pilotti et al., 1993). Patients with HIV commonly have 1-2cm lymph nodes of unclear significance, and FNA can accurately diagnose infection from neoplasm. In a study of HIV patients with cervical lymphadenopathy the presence of unilateral adenopathy or lymph nodes greater than 2 cm predicted a successful diagnosis by FNA. Among the 26 patients studied, 10 had positive findings for toxoplamosis, histoplasmosis, tuberculosis, staphylococcus and

atypical mycobacteria (Shapiro and Pincus, 1991). In a further study of the diagnostic reliability of 350 aspiration biopsies of lymph nodes, a sensitivity of 85% and a specificity of 99% was achieved (Ramzy et al., 1985). The only aspirate giving a false positive result was from a reactive node, mistaken for a lymphoma, and of the nine aspirates that gave false negative results, only one was a carcinoma. A case can be made for performing FNA cytology regardless of the clinical findings. However, better results can be obtained if an experienced person aspirates the nodal mass. Slide preparation is critical for accurate diagnosis and immediate inspection in a specialised cytopathology unit is required. However, the limited material obtained by FNA will not provide information on the architecture of the cytological specimen and will not allow immunohistochemical staining of the specimen.

Also the lack of, or misleading clinical information, non-representative samples, contamination of sample by cells from tissues adjacent to the target lesion, artefacts caused by poor processing of samples, and too much reliance on and technical failure of ancillary tests, are all factors which can contribute to diagnostic errors in the interpretation of FNAC samples (Orell, 2003).

Disadvantages to FNAC include a high rate of non-diagnostic samples and incomplete classification of lymphoma. Ultrasound guided core biopsy has been proposed as a technique to obtain larger tissue samples that will permit the use of a range of histochemical and immunohistochemical stains. This factor, combined with preservation of tissue architecture in the core biopsy sample, may enable a more precise histological assessment to be made (Screaton, 2002).

In this study, FNA was reported according to cytological findings, presence of infection or organisms and background findings. In patients with reports consisting of for example numerous atypical cells of the lymphoid series, a diagnosis of NHL was

suggested. In patients with TB, a typical report was of no malignant cells, moderate numbers of lymphoid cells, necrotic material, epitheloid histiocytes and multinucleated giant cells, suspect for mycobacterial infection. Reactive lymphadenopathy was reported as no malignant cells, mature lymphoid cells, consistent with reactive lymphadenopathy.

In the thirty two patients that had a FNA, five patients were diagnosed with malignancy, fourteen with TB, nine with reactive lymph nodes and five with other pathologies (see table 5). Of the five patients classified under other pathologies, three had a suboptimal FNA. From the patients with a suboptimal FNA, one patient was confirmed to have NHL on biopsy, one to have reactive lymphadenopathy and the third patient did not have a biopsy.

Wilson et al, 2005, performed needle-core biopsies using a 16-gauge biopsy needle as a sterile procedure under local anaesthetic through a small incision using a biopsy gun. In the 26 HIV infected adults presenting with suspected tuberculosis, who were sputum smear negative, who underwent needle-core biopsies, a definite diagnosis was made on initial needle core biopsy in 22 subjects (85%) and in two of three subjects who underwent a second needle-core biopsy. Tuberculosis was the final diagnosis in 24 subjects (92%) (Wilson et al., 2005).

In these proposed more invasive techniques such as FNAB, vigilance should be exercised in the cases of cystic masses, which may show a high incidence of unsuspected carcinoma. There should be a low threshold for repeating the FNAB in cases where the initial aspiration has been negative for malignancy. The other common pitfall of FNAB concerns the distinction between reactive nodes and lymphoma (Sheahan et al., 2004).

A more cost effective and less invasive solution to increasing the specificity and sensitivity of FNA would be the use of cytological stains. The papanicolaou (PAP) stain has been the traditional and most frequently utilized stain for cytologic specimens. However, the increasing popularity of FNA as a primary diagnostic procedure has demonstrated the utility and adaptability of other stains such as the Romanowsky stains and H&E (haematoxylin and eosin). Personal preference and experience will dictate which stains are used on cytologic specimens, and more than one stain can be used (Powers, 1998). Considering that ten of eleven lymph node biopsy specimens stained AFB positive with the Ziehl Nielsen stain, it could be an improvement and add to the sensitivity and specificity of FNA, in the diagnosis of TB.

With respect to lymphoma, the diagnostic yield has improved when immunocytochemistry is combined with cytology (Schafermak et al., 2003).

In our study, thirty patients had both a FNA and a lymph node biopsy for comparison. One in each group was reported as suboptimal and therefore categorised under other. Comparing FNA with lymph node biopsy as the true positive, eleven patients were diagnosed with TB, four with malignancy, three as reactive and two as having other aetiologies of their lymphadenopathy. The patients in the other category included a patient with a lymphoepithelial cyst and a patient with fibrous tissue and skeletal muscle that was assessed as lymphadenopathy.

The patient with the presumed diagnosis of spindle cell tumour had only fibrous tissue and skeletal muscle on biopsy, i.e. a suboptimal biopsy.

In the discrepant group of ten patients comparing FNA to Biopsy, five patients were diagnosed as having reactive lymph nodes on FNA, but the biopsy confirmed two with Castleman's disease, two with NHL and one with TB. One patient diagnosed as

TB on FNA, had KS on biopsy. Another patient with a lymphepithelial cyst on FNA had reactive lymphadenopathy on biopsy. A further patient diagnosed with TB on FNA had a sub-optimal lymph node biopsy. Tables 6 and 7 show concordance and discordance between the FNA and lymph node biopsy diagnosis.

The differences in FNA and biopsy results could be explained as 'errors' of sampling. In other words the FNA and biopsy were done at different sites in the same patient, and therefore these patients potentially had more than one pathological cause for their lymphadenopathy. Also, less tissue was available for review from the FNA specimen and immunohistochemical stains were not possible on the paucity of FNA material available.

In the patients who had a lymph node biopsy, sixteen had TB, ten were diagnosed with malignancy (seven with NHL, two with KS and one with a neuroendocrine tumour). The other group included two patients with Castleman's disease, one with a lymphepithelial cyst, one with fibrous tissue and one as having a suboptimal biopsy. One patient had a biopsy which showed KS on one lymph node and a second node as having reactive features. A second patient had a diagnosis of TB and reactive lymphadenopathy on the same biopsy. Nine patients had reactive lymphadenopathy on biopsy (see figure 10).

Using chi-square analysis, the specificity and sensitivity of FNA was compared to the lymph node biopsy results. In general, the low specificity and sensitivity in the different groups with the wide confidence intervals may be due to the small sample size in our patient population. Nevertheless, when comparing FNA to lymph node biopsy for the diagnosis of malignancy, the specificity and sensitivity was 100% and 43% respectively, with wide confidence intervals (see table 8). This indicates that although a positive result for malignancy on FNA is highly specific, FNA for the

diagnosis of malignancy is much less sensitive and a significant number of patients could be missed if the diagnosis is based on the FNA only. The specificity and sensitivity for the diagnosis of TB was 86% and 95% respectively (FNA is less specific for TB, but more sensitive). Reactive lymphadenopathy had a lower specificity and sensitivity of 80% and 60% respectively. Similarly, the specificity and sensitivity for the diagnosis of other pathologies was 86% and 40% respectively. These results underline the importance of performing a lymph node biopsy, more particularly where malignancy is suspected or where a reactive FNA result may not be sensitive enough to exclude a more definitive diagnosis for the lymphadenopathy.

There are many models that have been proposed to aid in determining which patient should have a lymph node biopsy. Vassilakopoulos and Pangalis, 2000, proposed the application of a predictive rule using criteria of age, location of lymphadenopathy, size, texture, tenderness and the presence of pruritis to determine which patients should undergo lymph node biopsy. However, in applying this model to a sample of 151 patients, Tokuda et al, 2003, found that the model performs relatively poorly for the validation of the sample in terms of low specificity. The authors suggest that could well be related to the demographics and ethnicity of the patient groups.

In our study, there were certain pointers to specific pathology. For example, with respect to TB, the presence of constitutional symptoms, cervical and axillary lymphadenopathy, matted nodes, intrathoracic lymph nodes and lung pathology were more suggestive of the diagnosis. Similarly, the presence of peripheral lymphadenopathy at a single site with a relative monocytosis was suggestive of a non-benign diagnosis.

In general, excision biopsy remains the gold standard in the investigation of the cause of lymphadenopathy. However, it also has limitations. At times, excisional biopsy

yields a definitive diagnosis in only 40-60% of the patients. Several reasons for this failure exist, including inadequate specimen size, improper handling of/or preparation of the specimen and a poor choice of nodal sampling. Preparation of the specimen is crucial in that the specimen must be placed in saline and handed immediately to the pathologist after the lymph node is removed. A poor diagnostic yield may result from inattention to these factors. Additionally, in some malignancies such as Hodgkin's disease, there may be reactive changes in surrounding nodes. Therefore, sampling the most accessible (but non-pathological nodes) may miss the underlying malignancy. It is best to avoid sampling inguinal nodes because their architecture is frequently distorted by chronic inflammatory changes. If an excisional biopsy is unrevealing, a second biopsy may be indicated if symptoms and signs persist or worsen.

A suggested algorithm (based on our study findings) for the cause of lymphadenopathy, and indicating the need for biopsy confirmation, is shown in figure 11 below.

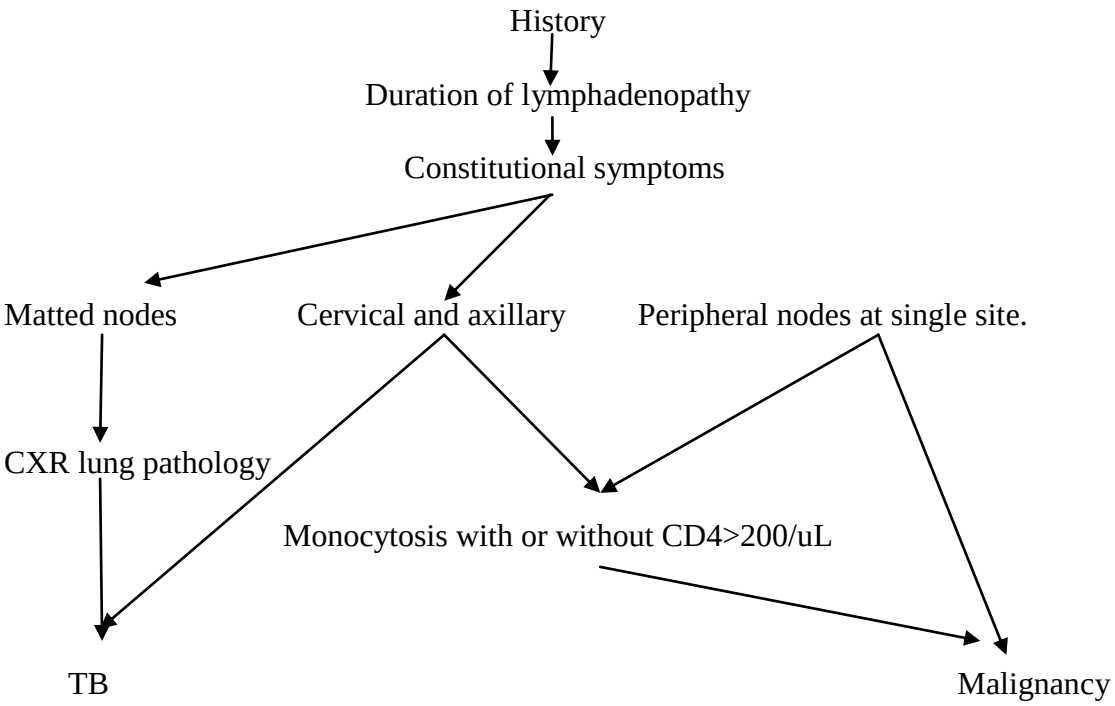


Figure 11: Algorithm suggesting approach to Lymphadenopathy

The introduction of highly active anti-retroviral (HAART) has been associated with a dramatic reduction in the incidence of opportunistic infections and both Kaposi's sarcoma (KS) and primary cerebral lymphoma. However, the effect on systemic lymphoma is less clear (Bower and Fife, 2000). There have been changes in the trend of the incidence of KS and NHL since 1996 as a consequence of both a decline in HIV/AIDS incidence and the introduction of HAART (Eltom et al., 2002) In patients with HIV-KS, HAART should be the treatment of choice (Paparizos et al., 2002)

However, despite dramatic declines in circulating virus and marked increases in CD4 cell counts; there was no equivalent reversal in lymph node hyperplasia activation during a 6 month study of the effects of HAART (Orenstein et al., 1999).

Therefore, even in the era of HAART, PGL may still be a confounding variable in our HIV positive population. The presence of more than one pathology in the lymph nodes has been clearly demonstrated in this study. When patients present with generalised significant lymphadenopathy from PGL, the chance of sampling errors might be increased especially if FNA's are done at a different site to the lymph node biopsy.

In the assessment of the aetiology of lymphadenopathy, there is no substitute for an adequate history and clinical examination of the patient, and maintaining a high index of clinical suspicion. The full blood count, CD4 lymphocyte count and viral load together with a chest x-ray should form part of the baseline investigations. Simple tests such as sputum for staining acid fast bacilli should not be ignored as it can aid in the diagnosis. FNA which is less invasive and more cost effective compared to lymph node biopsy should be directed at the most significant lymph nodes, based on their clinical characteristics. The addition of special cytological stains such as the Ziehl

Nielsen stain and immunoctyochemistry can aid in increasing the sensitivity and specificity of FNA. If the FNA result is not consistent with the clinical assessment, there should be a low threshold for a formal lymph node biopsy, particularly where malignancy is suspected.

Lymphadenopathy is a common clinical problem confronting the primary care physician. Although most often in the general population it is likely to be benign, other causes including infection and malignancy may predominate, where the lymphadenopathy (which is regarded as significant) occurs in the background of immunodeficiency related to HIV. As such, these individuals must be carefully assessed. Where a lymph node biopsy was performed, our study showed TB as the most common cause of the lymphadenopathy, occurring in 16/40 (40%) of the patients and malignancy occurring in 10/40 (25%) of the patients. However, reactive lymphadenopathy (whether related or unrelated to HIV) occurred in 9/40 (22.5%) of the patients.

6.0 Conclusions

It is interesting that during the same time that the study was performed (2004/2005), 80 – 100 new patients per year with lymphoma (approximately 75 – 80% with NHL and 20 - 25% with Hodgkin's disease - HD) were seen at the adult Clinical Haematology Unit at Chris Hani Baragwanath Hospital (CHBH) (information from Patel M, supervisor of the study). The percentage seropositivity during this time for NHL was 60 – 70%, and 30 – 40% for HD. This would mean that approximately 60 new patients/year would be having lymphoma and HIV. This is clearly not reflected in this study. The reason for this is the way in which the study was designed. For the purposes of our study, the patient had to have a known diagnosis of HIV, following which the lymphadenopathy was investigated. However, the usual way in which most lymphoma patients are diagnosed, is to perform the biopsy prior to knowing the HIV status. Although a minority of patients have a history of known HIV at the time of their lymphoma diagnosis, in most patients at CHBH, HIV is only diagnosed simultaneously with the diagnosis of the lymphoma, precluding referral of the vast majority of these patients onto our study. Moreover, a number of patients are referred to us (having had their biopsy already performed by the surgeons – general surgeons, ENT surgeons, neurosurgeons, urologists etc.). In addition, CHBH serves as a major referral centre for areas outside of Soweto, such as the Southern Gauteng, North West Province etc. from which patients are referred, having had a biopsy confirmation of lymphoma. This is a major limitation of the way in which our study was designed and therefore does not provide a true reflection of the causes of significant lymphadenopathy in an HIV positive population at CHBH. Rather, it reflects the causes of significant lymphadenopathy in a known HIV seropositive population.

Other shortcomings of this study include the small sample size, making statistical analysis less meaningful. Also, for technical and practical reasons, there was incompleteness of certain investigations such as the FNA. Also considering that the FNA and lymph node biopsy were often performed at different sites, the concordance and discordance of these investigations could not be effectively correlated. Lastly, this was a cross-sectional study with no long term follow up of the patients.

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7.0 References

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7.0 Appendices

Appendix A

SUBJECT INFORMATION SHEET

Hello! My name is Dr Sagren Naidoo. I am a registrar in the Department of Medicine. I am conducting a study on patients with HIV infection. Specifically; l would like to know whether you have swollen glands or lymph gland swelling (lymphadenopathy). I would like to briefly inform you about this study. You may decide whether you are willing to participate in this study or not.

As you may be aware of, HIV infection is very common in South Africa. The infection may present in different ways and can contribute to much suffering for both the patient and their care givers. As clinicians and researchers, it is important for us to know more about this disease and its complications .The purpose of this study is to find more about the lymphadenopathy that is associated with HIV infection. In particular, we would like to know the different causes of lymphadenopathy, the clinical presentation and the relationship of the lymphadenopathy with other clinical and laboratory findings. We would also like to determine which patients would benefit from a fine needle aspiration (FNA) or biopsy of the lymph nodes and whether the criteria we are using to decide when a patient should have a FNA or biopsy are correct or need modification.

I am offering a consultation service to the Hospital in aiding and obtaining a diagnosis in HIV positive patients who have lymphadenopathy. l have checked with your doctor that you have been counselled about HIV and its implications . This study will not affect the way you will be treated by your ward doctors. If you agree to participate in this study, I will interview you and examine you. I will then study your file and make a note of all your investigations.

The information obtained from you and your file will be strictly confidential. Your identity will remain confidential by assigning a study number to you.

If your lymphadenopathy is greater than 1 x 1 cm in diameter or the maximum diameter of the lymph node/s is greater than 1.5 cm, a FNA will be perfomed. If the FNA is non-diagnostic or suggestive of a lymphoid malignancy, a formal lymph node biopsy will be performed by the surgical unit. (The FNA and biopsy may have otherwise been done even if you were not participating in this study). The details of these procedures will be explained to you when the procedure is deemed necessary, suffice to say that a FNA entails aspirating cellular material with a needle and syringe from a lymph node, while a biopsy entails the removal of a lymph node surgically. This is usually performed under local anaesthetic cover.

I will follow you up until the cause of the lymphadenopathy is determined. There are no direct benefits to you if you enter the study. However, by taking part, you will be helping us to improve our understanding and knowledge of this aspect of the disease, which may have a positive impact on our future investigation and management of lymphadenopathy in association with HIV. Also, there should not be any untoward risks if you enter the study. The appropriate precautions will be observed when performing a FNA or lymph node biopsy.

Your participation in this study is entirely voluntary. If at any time during the study you wish to withdraw, we will respect your wishes and your care will in no way be affected or compromised.

Please feel free to ask any questions before you sign the consent form. Also if there are any further questions that you may have during the course of the study, please do not hesitate to contact me on 011 933 8000.
Thank you for allowing me your valuable time. If you decide to participate in this study, please sign the information consent form.
Thank you.

INFORMATION CONSENT FORM

STUDY TITLE: HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND LYMPHADENOPATHY

Name of patient: ……..…

Name of Hospital and Hospital number: ……...……

……

The aims and procedures of the clinical study that I have been asked to take part in, have been explained to me by Dr ……...

I have had the opportunity to ask questions and to consider the answers given.

I understand that participation in this study is entirely voluntary.

I hereby freely give my fully informed consent to take part in this study.

NAME: …… SIGNATURE:……

DATE:……..……

I confirm that I have explained the nature of the above study to the above named patient .

NAME OF DOCTOR: ……
SIGNATURE: ……
DATE…….…….

Appendix B
CH Baragwanath Hospital
HIV and Lymphadenopathy
Questionnaire and Clinical Findings
A: General details:

Patient Code:_____
Hospital no:__ _____
Age:_____
DOB:_____
Sex:_____
DOA:_____ DOD:_____
File no: _____

Address:_____
Tel no:_____
Marital Status:_____
Occupation:_____

B: Presenting Features :

Main Complaint (infection, fever , etc)

General:

Weight Loss:-
Amount _____ Duration:_____
Loss of energy: _____
Malaise_____
Proximal weakness_____
Distal weakness_____
Fever_____

	Yes	No
Wheezing?		

_____
Fever?_____
Pneumonia or TB ?_____
Recent chest X –Ray?:_____

Gastrointestinal system :

LOA/LOW/Indigestion?_____
Change of bowel habits?_____
Diarrhoea?_____
Malaena?_____
Frank blood in stool?_____
Dysphagia? _____
Heartburn ?_____
Yellow eyes/ skin? _____
History of hepatitis, PUD etc?_____

Genitourinary system:
Burning on micturition?_____
Urine colour?_____
Rashes or lumps on genitals?_____
Urethral / vaginal discharge?_____
UTI?_____
Contraception?_____

Yes **No**

Haematological System :
Bruising easily?_____
Excessive bleeding after trauma?_____
History of DVT or Pulmonary embolism?_____
Previous malignancies/treatment?_____
Blood transfusion?_____

Lumps	Neck	Axillae	Groin
Duration			
Increase in size			
Tenderness			
Discharge			

Musculoskeletal System :
Painful or stiff joints? _____
Joint swelling? _____
Recent skin Rashes? _____
Back or neck pain? _____
Dry mouth or ulcers? _____
Painful fingers - relation to weather?_____

Endocrine System :
Thyroid?_____
Diabetes?_____

Reproductive History:
Contraception?_____
Miscarriages?_____
No. of children?_____

Previous admissions to hospital:
_____-
_____
_____
_____
_ _____
_____

Allergies:

_____

Habits:

Smoking:_____

Ethanol:_____

Drugs:_____

Antiretroviral therapy-

Hydroxyurea_____ -

3TC/d4T/Effivarenz_____ -

AZT_____

Other_____

Clinical findings:

General appearance :

	Yes	**No**
Well looking/healthy/unwell:_____		
Mental state:_____		

	Yes	**No**
Cyanosis:_____		
Pallor:_____		
Jaundice:_____		
Clubbing:_____		
Oedema:_____		
Hair:_____		
Lymphadenopathy: (see Table 1and 2)_____		

Vital Signs:

BP:_____PR:_____

RR:_____T°:_____

Head and neck _____

Conjunctivae: inflammation:_____

Pupil size, equality, reaction to light:_____

Eye movements – nystagmus:_____

Fundi:_____

_____

Candida/Kaposis Sarcoma:_____

Buccal mucous membrane:-colour:_____

Pigmentation:_____

Veins:_____

Neck movements and pain: _____

Position of trachea:_____