Kaposi sarcoma, the Chris Hani Baragwanath Academic Hospital experience: demographics of Kaposi sarcoma and HHV8 immunohistochemical expression in a retrospective cohort of cases

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CANDIDATE’S DECLARATION

I, Reena Dhansukh Mohanlal (Student number: 295331) am a student registered for the degree of MMed (Anatomical Pathology) in the academic years 2013/2014.

I hereby declare the following:

I am aware that plagiarism (the use of someone else’s work without their permission and/or without acknowledging the original source) is wrong.

I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.

I have followed the required conventions in referencing the thoughts and ideas of others.

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Signature: [Signature]

Date: 27/5/2014
DEDICATION

I dedicate this research report to my three beautiful daughters,

Neha; Tejal and Deepika

and to

my parents who instilled in me the value of a good education.
ABSTRACT

According to the UNAIDS global report 2013, an estimated 6.1 million people are living with human immunodeficiency virus (HIV) in South Africa. The incidence of Kaposi sarcoma (KS) has increased dramatically since the Acquired Immunodeficiency Syndrome (AIDS) epidemic. Of the estimated 66 200 cases of KS worldwide, 58 800 are thought to have occurred in SSA (Parkin 2002). However, there remains a paucity of published data about KS from South Africa. This retrospective study was conducted to describe the epidemiology of KS at Chris Hani Baragwanath Academic Hospital (CHBAH) and to determine possible links among the CD4 counts, intensity and distribution of human herpes virus 8 latency-associated nuclear antigen 1 (HHV8 LNA-1) immunohistochemical staining and the stage of KS.

Nine hundred and thirty eight histopathology reports of KS diagnosed in 901 patients at CHBAH between 2005 and 2009 were reviewed and demographic data (age, gender, topographic site, CD4 count, HIV status, KS stage, HHV8 LNA-1 staining, concomitant pathology) were recorded. The H&E stained sections and HHV8 LNA-1 immunostains of a cohort of 127 cases were subsequently reviewed and categorised with regard to intensity and distribution of staining.

The male:female ratio was 1,2:1. The mean age was 36,8 years (standard deviation (SD) 10,2 years) and the median CD4 count 127,5 cells/mm$^3$ (quartile range (QR) 184,5 cells/mm$^3$). Lower limb skin biopsies accounted for 49,6% of cases. Concomitant pathology was seen in 4,6% of cases. Infections and inflammatory dermatoses were the most frequently diagnosed concomitant pathology in cutaneous biopsies. Paediatric, visceral and endemic KS accounted for only limited proportions of cases (1,44% of patients; 1,4% and 1,3% respectively). There was a significant difference in the distribution of HHV8 LNA-1 staining in patch versus nodular KS ($p = 0,011$). The CD4 counts were not predictive of KS
stage (p = 0.701) or the intensity (p = 0.877) and distribution (p = 0.846) of HHV8 LNA-1 immunohistochemical staining.

This study highlights the epidemiology of KS and the variability in HHV8 LNA-1 immunohistochemical staining across CD4 counts and stages of KS.
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<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTG</td>
<td>AIDS Clinical Trials Group</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
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<tr>
<td>CHBAH</td>
<td>Chris Hani Baragwanath Academic Hospital</td>
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<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly active antiretroviral therapy</td>
</tr>
<tr>
<td>HHV8</td>
<td>Human herpes virus 8</td>
</tr>
<tr>
<td>HHV8 LNA-1</td>
<td>Human herpes virus 8 latency associated nuclear antigen 1</td>
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<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<tr>
<td>HIV-ELISA</td>
<td>Human immunodeficiency virus enzyme-linked immunosorbent assay</td>
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<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>IRIS</td>
<td>Immune reconstitution inflammatory syndrome</td>
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<tr>
<td>KS</td>
<td>Kaposi sarcoma</td>
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<tr>
<td>KSHV</td>
<td>Kaposi sarcoma herpes virus</td>
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<tr>
<td>LNA-1</td>
<td>Latency associated nuclear antigen 1</td>
</tr>
<tr>
<td>M:F</td>
<td>Male–to-female ratio</td>
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<tr>
<td>ORF</td>
<td>Open reading frame</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PDGF</td>
<td>Platelet derived growth factor</td>
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<tr>
<td>PDGFR</td>
<td>Platelet derived growth factor receptor.</td>
</tr>
<tr>
<td>PMTCT</td>
<td>Prevention of mother to child transmission</td>
</tr>
<tr>
<td>QR</td>
<td>Quartile range</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SSA</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td>STIs</td>
<td>Sexually transmitted infections</td>
</tr>
<tr>
<td>SA</td>
<td>South Africa</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
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</table>
vCYC D: Viral cyclin D
vFLIP: Viral FLICE-inhibitory protein
Chapter 1: INTRODUCTION

1.1. Background

Kaposi sarcoma (KS) is a disease that was first described by Moritz Kaposi in 1872. Four forms have since been described - classic KS, African/endemic KS, acquired immunodeficiency syndrome (AIDS) related KS and transplant related KS. Although regarded as a neoplasm, KS in many regards is not a typical cancer. It is usually multifocal, may regress with immune restoration and the cells are dependent on exogenous growth signals in vitro (Ganem 2006). KS is characterised by violaceous patches, plaques or nodules most commonly in mucocutaneous sites. However, lymph nodes; visceral organs and unusual sites such as bone may also be involved (Radu & Pantanowitz 2013).

Diagnosis is made on histological examination of submitted biopsies and may be confirmed with relevant ancillary investigations eg. human herpes virus 8 latency associated nuclear antigen 1 (HHV8 LNA-1) immunohistochemistry (IHC).

According to the UNAIDS global report 2012; 23,5 million people are living with the human immunodeficiency virus (HIV) in sub-Saharan Africa (SSA). Of the estimated 66 200 cases of KS worldwide, 58 800 are thought to have occurred in SSA (Parkin 2002). The disease burden in SSA is therefore enormous. The majority of KS cases seen in SA are AIDS related.

1.2. Problem statement

AIDS related KS is a diagnosis made on a daily basis at the Chris Hani Baragwanath Academic Hospital (CHBAH) histopathology laboratory, yet published data from this hospital are lacking. There are minimal data from SA regarding the frequency of cutaneous versus extracutaneous KS, the mean age at diagnosis of KS, the gender ratio, the most common stage of lesions at biopsy diagnosis, the mean CD4 count at time of biopsy, the prevalence of paediatric KS, the prevalence of endemic KS and the prevalence of dual pathology i.e. the finding of an additional pathological process in addition to KS in the same biopsy.
1.3. Rationale
The purpose of this study is to address some of the abovementioned deficiencies in the literature as it pertains to patients at CHBAH, the largest hospital in the Southern Hemisphere.

In addition to highlighting the epidemiology of KS at CHBAH, the variability of HHV8 LNA-1 immunohistochemical staining will also be addressed. The diagnosis of KS is made histopathologically by examination of routine haematoxylin and eosin stained sections and confirmed by IHC for HHV8 LNA-1. Interestingly, the HHV8 LNA-1 immunoreactivity pattern may be variable in intensity and distribution. There is limited literature addressing this variability of HHV8 LNA-1 immunohistochemical staining and the potential association with the patient’s CD4 count. Are the different patterns of staining possibly related to the CD4 count of the patient at time of biopsy? Is staining more diffuse in nodular stage KS when very low CD4 counts are present and less so with earlier lesions when high CD4 counts are present? In the South African context, where the vast majority of cases of KS are AIDS associated, the relationship of HHV8 LNA-1 staining to the CD4 counts and various stages of KS would be interesting to determine. In contrast to the limited number of AIDS associated KS cases used by researchers (Hong et al 2003; Patel et al 2004; Pak et al 2005; Ramos da Silva et al 2007), this study will incorporate a greater number of cases due to the high prevalence of AIDS associated KS in the sample population group.

1.4. Specific aims and objectives

- Aims of the study
  1. To describe the epidemiology of KS at CHBAH from 2005 to 2009.
  2. To describe the variability of HHV8 LNA-1 staining across the three stages of KS and CD4 counts in a retrospective cohort of patients.

- Objectives
  a. To determine the male-to-female (M:F) ratio of KS
b. To determine the mean age at diagnosis of KS  
c. To determine the median CD4 count at time of diagnosis of KS  
d. To determine the frequency of KS at various topographic sites including the frequency of visceral KS  
e. To determine the prevalence of KS in the paediatric age group  
f. To determine the prevalence of endemic KS  
g. To determine the frequency of the patch, plaque and nodular stages of KS at the time of histological diagnosis  
h. To determine if the CD4 count is predictive of the stage of KS  
i. To determine the prevalence of special histomorphological variants  
j. To determine the prevalence of concomitant pathology in KS biopsies  
k. To determine if there is relationship between the CD4 count and the distribution and/intensity of HHV8 LNA-1 immunohistochemical staining.  
l. To determine if there is a relationship between the stage of KS and the distribution and/intensity of HHV8 LNA-1 immunohistochemical staining.

1.5. **Significance**

CHBAH provides health care services to a population that bears the brunt of HIV infection and KS. The findings of this research project may highlight the vast extent of KS for allocation of much needed resources. Documentation of the various demographic aspects of the disease will be of historical, clinical and pathologic interest as the long term effects of highly active antiretroviral therapy (HAART) begin to emerge.

The literature review that follows will highlight available data from Africa and more recent developments in KS research that have emerged from abroad.
Chapter 2: LITERATURE REVIEW

The literature review contains background literature on the clinical classification, epidemiology, risk factors, pathology and treatment of KS.

2.1. Clinical classification

Four distinct types of KS are recognised. These include the classic, African/endemic, iatrogenic/transplant associated and HIV associated/epidemic types.

The classic type KS occurs typically in elderly Mediterranean males. The African/endemic type occurs in young African males and was first described in 1914. The lymphadenopathic variant of endemic KS affects young children. The iatrogenic type is seen in patients on long term immunosuppressants and in recipients of organ transplants. In 1981, epidemic KS was first described in homosexual males who were HIV positive (Ruocco et al 2013).

2.2. Epidemiology

The majority of cases of KS seen in SSA are AIDS-associated. HIV infection is associated with a 50 fold increased risk of developing KS (Mosam et al 2010).

2.2.1. M:F ratio

KS is considered to have a gender bias, being commoner in men since its appearance in homosexual males in the United States of America (USA) (Aboulafia 2000). There are several hypotheses for this gender bias. Some investigators believe that androgens may play a role (Christeff et al 1995). However, Ziegler et al (1995) confirmed that KS lesional cells lack sex hormone receptors.

The “iron hypothesis” suggests that the lower incidence of endemic KS in females may be due to their lower iron reserves than men as iron is needed for the development of KS (Simonart 2004).
A study by Ramos da Silva et al (2007) which included 25 cases of AIDS associated KS from Brazil, demonstrated a M: F ratio of 24:1. Although the sample size was limited, this is in stark contrast to the African experience.

In Africa, KS was found to be the most common neoplasm in adult Ugandan men (Orem et al 2004).

Sitas & Newton (2000) demonstrated a male to female ratio of 2:1. This gender ratio reflected an increase in incidence in women from 7:1 in 1988. Data from the National Cancer Registry between 1992-1996 was used in the abovementioned study.

Pitche et al (2007) described a M:F ratio of 1.4:1 in cases of HIV KS compared to a ratio of 9:1 in cases of African KS. Mosam et al (2008) showed a M:F ratio of 1:1 and furthermore demonstrated that women presented earlier, with more disseminated disease and had a poorer prognosis compared to men.

2.2.2. Age

A Nigerian study revealed a mean age of 36.3 years (Onunu et al 2007).

Mosam et al (2008), in a study of 152 patients with HIV associated KS, demonstrated that the mean age in males was 37 years (range 25-64 years) and in females 34 years (range 19-64 years).

In a subsequent publication, Mosam et al (2009) highlighted that KS now occurs at a much younger age. The mean age of patients with KS decreased from 59.7 years in 1983 (pre-AIDS era) to 36.5 years in 2006.

2.2.3. Paediatric KS

Ziegler & Katongole-Mbidde (1996) described KS in childhood in their analysis of 100 cases from Uganda. The mean age reported was 4 years. The commonest topographic region of involvement was the head and neck and the M:F ratio was 1:1.7.
Amir et al (2001) compared paediatric KS before and during the AIDS epidemic in Tanzania. Boys were more commonly affected than girls especially in the 0-5 year age group with a ratio of M:F of 11:1. Twelve per cent of cases seen in the AIDS era involved sites not usually involved in the pre-AIDS era. These sites included genitalia, eyelid, ear, lip and face. There was also an increase in upper limb involvement in contrast to decreased numbers of lower limb and lymph node cases.

In 2010, Gantt et al, described KS in 73 patients who were younger than 18 years of age. Included in their study were mostly clinically diagnosed cases with only 36% (n=26) being biopsy confirmed KS cases. Although 40% of cases showed lymph node involvement, the reliability of this data is questionable as some of these cases were not histologically confirmed and the causes of lymphadenopathy in HIV positive patients are myriad. The mean age recorded was 10.1 years of age and the M:F ratio; 1.03:1.

Similarly, Cox et al (2013) described 81 cases of paediatric KS in Malawi and Botswana with biopsy diagnosis in only 25% of cases. The lack of pathology expertise was cited as the reason for the failure to obtain histological confirmation.

Tukei et al (2011), in a large retrospective study of HIV associated malignancies in children presenting at the Baylor-Uganda Paediatric HIV Clinic in Kampala; Uganda, recorded 97 cases of KS in 6530 patients between the ages of 6 weeks to 18 years. Seventy six of these cases were in children ≤12 years of age. Thirty two per cent had lymph node involvement, 26% had cutaneous lesions and 5% had visceral KS.

There have been two recent publications of paediatric KS from SA. In a series of 9 cases of KS in children diagnosed between January 2003 and December 2007, Cairncross et al (2009) documented oropharyngeal and inguino-scrotal involvement most commonly. Three cases involved the gastrointestinal tract (GIT). The study revealed that the incidence of paediatric KS had increased to 2 patients per year from 1 child every 4 years before the HIV epidemic at Red Cross Children’s Hospital, Cape Town, South Africa. The sample size was,
however, limited. In a recent study, Stefan et al. (2011) described 70 cases of HIV associated paediatric KS from patients between 0 and 14 years which were diagnosed at 3 large hospitals in SA. A M:F ratio of 1.59:1 and a median age of 72 months were highlighted. Skin involvement was seen in the majority of cases (57.14%); nodal involvement in 30.15% and visceral involvement in 38.09% of cases. The mean CD4 count was 440 cells/mm$^3$. This data obtained from the South African Children's Cancer Registry confirmed a substantial increase in paediatric KS from only one case reported in 1998 to 26 cases reported in 2008.

2.2.4. Topographic sites of involvement

KS occurs in the skin, oral mucosa, lymph nodes, lungs, GIT, genitalia, oropharynx, tonsils, nasal cavity, liver, spleen and less commonly bone marrow. Mucocutaneous KS is most common (Radu & Pantanowitz 2013) with lower limbs being most commonly affected (Onunu et al. 2007).

In literature cited by Bunn et al. (2013), the oral cavity may represent the initial site of KS in up to 22% of cases and is usually the first sign of HIV infection. Involvement of buccal mucosa can be seen in up to 50% of cases of AIDS associated KS (Pitche et al. 2007).

In an autopsy study by Leimlich et al. (1987) involving 24 patients with HIV associated KS, 29% of patients showed visceral involvement in the absence of skin lesions. The most common viscera involved were lung (37%) and those of the GIT (50%). Nodal KS was seen in 50% of cases.

GIT involvement may be found in 40% of cases at initial diagnosis and in up to 80% of patients at autopsy (Dezube 1996). The stomach is most frequently involved followed by the oesophagus, colon and small bowel (Slavik 2012).

Miller et al. (1992) showed an 8% prevalence of bronchopulmonary Kaposi sarcoma in their study involving 361 HIV positive patients who underwent bronchoscopy. Mitchell et al. (1992)
in a similar study found 19 patients with bronchopulmonary KS in the 140 patients who presented for bronchoscopy. Interestingly, all 19 patients also had cutaneous lesions.

Unusual sites of involvement have also been reported in the literature. These include brain, peripheral nerves, spinal cord, heart, eye, ear, pancreas, breast, wounds, blood clots, thoracic duct and heart. Involvement of these unusual sites has not been widely documented except for random reports which formed the basis of the review article by Pantanowitz & Dezube (2008). As KS is thought to derive from endothelium, the rare occurrence of KS in the brain and eye is thought to be due secondary spread of KS from other sites (Rahman & Mazumber 2002).

2.3. Risk factors

The risk of developing Kaposi sarcoma is related to human herpes virus 8 (HHV8) seropositivity. HHV8 is also known as Kaposi sarcoma–associated herpesvirus (KSHV). Viral DNA is always found in KS lesions (using PCR) and HHV8 infection rates are high in groups in which KS is frequent (Ganem 2006).

Whitby & Howard (1995) described that HIV positive patients with HHV8 in their peripheral blood are more likely to have KS and detection of HHV8 in HIV positive patients without KS predicts progression to KS. In an interesting study, Rezza et al (1999) demonstrated that 10 years after HIV seroconversion, 30% of HHV8 positive individuals developed KS and that the risk of KS increased with increasing anti-HHV8 antibody titres.

HIV predisposes to KS by infecting and depleting CD4 expressing T lymphocytes which are responsible for the helper function of the immune response. This in turn impairs defense against HHV8. The CD4 count is widely regarded as a specific marker for immunodeficiency (Clifford & Fransceschi 2009).

Portsmouth et al (2003) suggested that nadir CD4 cell counts were an independent factor associated with KS development. Similarly, Biggar et al (2007) agreed that the incidence of
KS was strongly associated with declining CD4 counts. In contrast to these findings, it has recently been highlighted that severe immunosuppression is not required for the development of KS. The minimum CD4 count recorded by Mosam et al (2008) in their study was 1 cell/mm$^3$ and the maximum 1406 cells/mm$^3$. Thirty eight per cent of patients had CD4 counts of >200 cells/mm$^3$ and 21% had CD4 counts more than 350 cells/mm$^3$. Similarly, Cassol et al (2005) demonstrated that 50% of KS patients in their cohort had CD4 counts >150 cells/mm$^3$ and 38.5% had CD4 counts >400 cells/mm$^3$. More recently, Mani et al (2009) and Crum-Cianflone et al (2010) confirmed that KS occurs in HIV positive patients with undetectable viral loads and CD4 counts of >300 cells/mm$^3$ and >350 cells/mm$^3$ respectively. This trend has also been observed in HAART naïve patients (Daly et al 2014) and is strong motivation for the initiation of HAART at higher CD4 counts than previously. De Souza et al (2007) confirmed that latency associated nuclear antigen (LNA) antibody detection in serum by immunofloourescence assay (IFA) correlated with CD4 counts of patients with KS. When CD4 counts increased, previously seronegative patients became seropositive when follow-up IFA-LNA was performed. Therefore, studies involving LNA detection by IFA should include patients with CD4 counts of more than 300 cells/mm$^3$.

2.4. Pathology

2.4.1. Cell of origin

The cell of origin of KS is controversial. However, many researchers favour an endothelial origin (Horenstein et al 2008). As early as 1985, Beckstead et al convincingly demonstrated resemblance of KS cells to lymphatic endothelium using immunohistochemical and lectin histochemical procedures with markers like Ulex europaeus 1 lectin and 5´-nucleotidase. The lymphatic origin of these endothelial cells has also subsequently been supported by positive D-240 immunohistochemical expression (Rosado et al 2012). Using oligonucleotide microarrays, Wang et al (2004), postulated two possible theories 1: that HHV8 infects both
lymphatic and blood endothelial cells and drives gene expression profiles of each type closer to that of the other and 2: that HHV8 infects endothelial cell precursors in vivo and drives these cells toward a lymphatic endothelium genotype.

Therefore, the downstream effect of HHV8 infection results in reprogramming of host’s endothelial cells and subsequent resemblance to lymphatic endothelium. Lymphatic vessel endothelium receptor 1, and vascular endothelial growth factor 3 are also all upregulated in KS lesions (Radu & Pantonowitz 2013).

2.4.2. HHV8/KSHV

HHV8 is a gamma herpes virus with a long latency period. The virus was first identified by Chang et al (1994) using representational difference analysis to identify DNA sequences uniquely present in KS tissue compared to non diseased tissue obtained from the same patient. Since their pioneering work, other independent researchers have confirmed that HHV8 is associated with all forms of KS.

Boshoff et al (1995) used polymerase chain reaction (PCR) to demonstrate that HHV8 was present in spindle cells and endothelial cells of KS lesions. Similarly, Smith et al (1997) identified HHV8 DNA in 8 of 9 KS biopsies using PCR and furthermore demonstrated higher immunofluorescence antibody titres against HHV8-lytic and latent antigens in the sera of their KS positive cohort compared to those patients without KS. Kennedy et al (1998) subsequently used PCR in 16 cases of early KS and detected HHV8 in 87% of cases. They also confirmed that HHV8 amplicons were localised to endothelial and spindle cell proliferations.

Bezold et al (2001) described no significant differences in HHV8 DNA copies in paraffin embedded biopsies from HIV negative versus HIV positive KS cases. Quantitation of copy numbers was done by competitive PCR. The HIV negative cases used were of the classic type. This research group used 14 biopsies from only 4 HIV negative patients in the study
whereas the HIV positive cases originated from 13 separate patients. CD4 counts and the stage of the lesions were not considered. The study implied that CD4 count and other factors related to HIV may not impact on the quantity of HHV8 DNA in KS lesions.

The seroprevalence of HHV8 is high (83%) in patients with KS (Sitas et al. 1999).

HHV8 is also associated with primary effusion lymphoma, Castleman disease and large B-cell lymphoma arising in the setting of HHV8 associated multicentric Castleman disease. In vitro studies with HHV8 have been performed with primary effusion lymphoma cell lines. Only a handful of endothelial based models for HHV8 study are available as spindle cells cultured from KS do not maintain the HHV8 genome (Moses et al. 2002). This is a limiting factor in all forms of research relating to HHV8 and the pathogenesis of KS. Consequently, there remain many unanswered questions regarding the pathogenesis of KS.

- **Prevalence**

There is geographic variation in the prevalence of HHV8 seropositivity with more than 50% seropositivity in SSA, 20-30% seropositivity in Mediterranean countries and less than 10% in Europe, Asia and USA (Uldrick & Whitby 2011). Gao et al. (1996) showed that HHV8 seroprevalence is higher in Ugandans compared to Americans and Italians.

Two studies published in 1999 based in SA revealed an HHV8 seroprevalence of 32% and 34.5% respectively (Wilkinson et al., Sitas et al.). The prevalence of HHV8 antibodies increased with increasing age, increased number of sexual partners and decreased with increasing levels of education. In addition, HHV8 serum antibodies were more prevalent in Black patients compared to White patients (Sitas et al. 1999).

Maskew et al. (2011) confirmed an HHV8 seroprevalence of 48% in their study based in a Johannesburg clinic involving 440 HIV positive patients. The KSHV positive group was found to have a higher body mass index (>18.5kg/m²) and higher CD4 counts.
• **Mode of transmission**

Modes of transmission of HHV8/KSHV include sexual transmission among homosexual males, oral exposure to infectious saliva, vertical transmission and blood transfusion (Wilkinson *et al* 1999).

HHV8 has been detected in the saliva of 75% of HIV infected patients. The presence of HHV8 in saliva may be due to its affinity to localise in B lymphocytes in tonsillar tissue (Koelle *et al* 1997).

HHV8 can also be transmitted sexually. In support of this, the virus has been found, to varying degrees, in the genital tract of HIV positive women (Calabro *et al* 1999; Whitby *et al* 1999) and in the semen of 8% of HIV infected males (Pellet *et al* 1999). A 35% seroprevalence was observed in bisexual and homosexual HIV positive males (Kedes *et al* 1996). The practice of oral-genital and receptive anal intercourse may account for the high transmission rates of HHV8 in homosexual men (Dukers *et al* 2000; Melbye *et al* 1998).

In a large study from Carltonville, Malope *et al* (2008) argued that there is no evidence that KSHV is sexually transmitted. Their results showed an overall prevalence of KSHV of 47.5%. Although the prevalence of HHV8 in the peripheral blood was higher in sex workers, this was not significantly different from other groups in their study. The prevalence of KSHV did not follow the pattern of sexually transmitted infections (STIs). Sexual intercourse and the number of sexual partners were not risk factors. The prevalence of KSHV was found to be similar in HIV negative and HIV positive groups and in individuals with and without STIs.

As the HHV8 virus is detectable in peripheral blood, transmission via blood and blood products is possible (Whitby *et al* 1995).

The possibility of transmission via intravenous drug use has not been not clearly established.

Vertical transmission especially in HIV positive mothers is an important potential mode of transmission especially in SA where the rate of vertical transmission of HIV is also high. Bourbolia *et al* (1998) highlighted that approximately one third of HHV8 positive mothers
infect their children. Other investigators have disputed vertical transmission of HHV8 arguing that children born to HHV8 positive mothers have passively transmitted antibodies and sero-revert by age 2 years (Calabro et al 2000).

- **Replication**

HHV8 has both lytic and latent phases of replication. In situ hybridisation studies have shown that most spindle cells in KS are latently infected. Only 1-3% of cells show lytic replication.

Four major proteins are involved in latency, LNA; viral cyclin D (vCYC D); viral FLICE-inhibitory protein (vFLIP) and Kaposin B. Lytic genes include K1, vIL-6, viral interferon regulatory factors, viral Bcl-2, viral macrophage inflammatory proteins and four interferon regulatory factors (Horenstein et al 2008).

### 2.4.3. Pathogenesis of KS

Douglas et al (2007) suggested that the pathogenesis of Kaposi sarcoma is complex and involves 4 main steps including initiation, proliferation, inflammation and tumourigenesis. Initiation involves infection of endothelial cells with KSHV. These cells then home and proliferate in response to anti-apoptotic genes and pro-angiogenic molecules. The inflammation component involves secretion of cytokines and finally there is activation of autocrine and paracrine signalling with subsequent spindle cell proliferation to produce the lesions of KS.

HHV8 is necessary for KS development (Dittmer & Krown 2007).

- **HHV8 Latent genes**

HHV8 latent genes, LNA; vCYC D; v-FLIP and Kaposin are able to deregulate cellular growth. LNA will be discussed due to its application in IHC.
LNA is consistently expressed in KS across all stages. Open reading frame (ORF) 73 encodes LNA which mediates episome persistence. LNA interacts with p53 resulting in resistance to p53 mediated apoptosis (Komatsu et al 2001). This in turn promotes cell survival. LNA binds to the tumour suppressor, retinoblastoma, resulting in loss of retinoblastoma function. LNA also acts directly on host-gene expression. Cells that express LNA, dysregulate host genes such that when LNA is fused to GAL4, it inhibits expression of GAL4-dependant reporters.

Once endothelial cells are latently infected, they undergo a conformational change to resemble spindle cells (Ganem 2006). This accounts for the presence of spindle cells in KS lesions.

- **HHV8 Lytic genes**

HHV8 lytic genes eg. Kl and v-MIP-1 provide strong paracrine signals to adjacent latently infected cells (Horenstein et al 2008).

- **C-kit**

The role of c-kit in KS has been demonstrated by Moses et al (2002) by showing enhanced expression of c-kit. Indirect evidence has been CD117 immunohistochemical expression in these tumours and response to anti-c-kit therapy (Gleevec). C-kit is a proto-oncogene that is induced by HHV8. C-Kit activation increases the response of tumour cells to growth factors and thereby accelerates cell proliferation and conformational changes (Kandemir et al 2009a).

- **PDGFR**

Platelet derived growth factor receptor (PDGFR) is upregulated but not mutated in KS (Dittmer & Krown 2007). PDGFR induces angiogenesis through vascular endothelial growth factor (VEGF) (Koon et al 2005). The process of angiogenesis to increase blood supply is
thought to be a response to injury, further in support of the theory that KS is an inflammatory lesion (Douglas et al 2007).

HHV8 infection alone is not sufficient to cause KS as not all HHV8 positive patients will develop KS (Miles et al 1996). Other factors such as HIV infection, decreased immunity due to other causes, as well as inflammatory cells and iron are also thought to play a role.

- **HIV and host immunity**

HIV is a co-factor in AIDS related KS. This is supported by a decrease in the incidence of KS with reduction in HIV viraemia and regression of KS lesions with HAART treatment. The mechanisms by which HIV promotes the development of KS are unclear. Some researchers propose that HIV tat protein is a growth factor for KS. HIV infection upregulates cytokines which then promote spindle cell survival and growth. HIV may induce T cell depletion and immunosuppression thereby facilitating KS progression (Ganem et al 2006). Patients with HIV are at increased risk for all cancers that have an infectious aetiology (Grunich et al 2007) as HIV lowers the immune response to infectious agents like HHV8 (Clifford & Francheschi 2009).

Dysregulation of host immunity is thought to be important in the pathogenesis of KS as the disease manifests in immunosuppressed patients ie. HIV positive patients and transplant recipients. It is thought that in these individuals, HHV8 infected spindle cells escape immune surveillance and are able to progress to KS. The risk of developing KS is linked to both cellular and humoral immunity. It has been verified that both decreasing CD4 and CD19 counts are associated with an increased risk for developing KS (Stebbing et al 2004; Biggar et al 2007).

- **Inflammatory cells**
Inflammatory cells are observed in most KS lesions. They are sparse in early lesions and are well established in the tumour stage. The inflammatory cells comprise plasma cells, lymphocytes and dendritic cells. Constant signalling from KS lesional tissue acts as a stimulus for infiltration by inflammatory cells. These inflammatory cells in turn, secrete cytokines, chemokines and other growth factors that stimulate growth and progression of KS lesions (Pantanowitz et al 2009a). There is hyperactivation of the humoral response (Th2-mediated) and a decrease in the cellular response (Th1-mediated) (Douglas et al 2007).

- **Iron and KS**

Haemosiderin is an integral component of all KS lesions. Using in vitro studies, Simonart et al. (1998) demonstrated that iron may be a co-factor in KS development for the following reasons: addition of iron salts stimulated growth of KS cells and addition of iron chelators reduced cell growth, iron decreased host defenses by inhibiting CD4 lymphocytes and macrophages and iron loading was found to enhance production of HHV8 viral nucleic acids.

The high incidence of endemic KS in places like Uganda and Sudan may be explained by the absorption of iron through bare feet from soil in iron oxide rich volcanic clays (Simonart 2004).

Aluminosilicates may also be absorbed by walking bare feet. These cause obstruction of lower limb lymphatics resulting in lymphoedema and chronic lymphostasis which result in local immunodeficiency and predispose to opportunistic infections and tumours (Ruocco et al 1984; Ruocco et al 2013).

- **Other factors**

Use of nitrite inhalants among homosexual men with AIDS has also been linked to KS, as have quinine (and analogues) and ACE inhibitors. The link to quinine is supported by the high prevalence of KS in malaria areas that use quinine and is thought to be due to the drugs’ well known immunosuppressive effects (Ruocco et al 2013).
2.4.4. Histopathological features

Histopathologically, Kaposi sarcoma is a vasoformative lesion comprising a proliferation of spindle cells with formation of slit-like vascular spaces. Extravasated red blood cells, haemosiderin, hyaline globules and plasma cells are also usually present. Hyaline globules are derived from fragmented erythrocytes (Radu & Pantanowitz 2013).

Three stages are morphologically recognised, patch; plaque and tumour/nodular. Patch stage KS is the earliest phase of KS and may be subtle and easily missed. It is characterised by a vasoformative process centred around native blood vessels. Slit-like vascular spaces may also be seen dissecting the dermal collagen. The promontory sign is characterised by protrusion of newly formed blood vessels into an existing vessel. Plaque stage lesions demonstrate a more diffuse cellular proliferation. The cells are more spindled and may be arranged in fascicles. Nodular KS lesions are typified by a circumscribed proliferation of spindle cells arranged in fascicles.

Several special morphologic variants have been comprehensively described by Grayson & Pantanowitz (2008). Anaplastic KS is locally aggressive, displays a significant degree of nuclear pleomorphism, is more mitotically active and may show necrosis. Lymphoedematous variants include lymphangioma-like (lymphangiomatous) KS, lymphangiectatic KS and bullous KS. The lymphangioma-like variant may show two patterns. The first comprises a conventional patch or plaque stage KS with irregular, ectatic vessels dissecting dermal collagen. The second pattern shows larger vessels in the superficial dermis mimicking lymphangioma circumspectum. The lymphangiectatic variant has large, dilated vessels and the bullous variant (usually a clinical diagnosis), comprises a subepidermal or intraepidermal bullous. The telangiectatic variant of KS shows large, congested ectatic vascular spaces with features of nodular KS. Hyperkeratotic or verrucous KS shows verrucous acanthosis and hyperkeratosis overlying a typical KS lesion. Keloidal KS shows the spindle cell proliferation of KS amongst thick, glassy collagen. Micronodular KS comprises a small nodule of KS in the mid to deep dermis. Pyogenic granuloma-like KS
comprises superficial nodular KS that has undergone ulceration and mimics a classic pyogenic granuloma. In ecchymotic KS, there is marked red cell extravasation. Intravascular KS shows a spindle cell proliferation within the lumen of a vessel.

Biopsy of regressing lesions may show subtle features including an increase in dermal capillaries, perivascular plasma cells, stromal sclerosis, haemosiderin laden macrophages and spindle cells around native vessels (Grayson & Pantanowitz 2008).

Occasionally, concomitant pathology may be identified during light microscopic examination of biopsy specimens with KS. Concomitant pathology may include cryptococcosis, cytomegalovirus or granulomatous inflammation (Radu & Pantanowitz 2013). Grayson (2011) estimated that a combination of lesions is encountered in less than 2% of biopsies from HIV positive patients. In his comprehensive review, he cites cases of KS in combination with infections, non infective dermatoses and dual neoplasia in the same biopsy. Cryptococcus, Histoplasma, Candida, Molluscum contagiosum, Mycobacterium Avium Intracellulare, Mycobacterium tuberculosis and Cytomegalovirus have all been recorded in combination with KS. Interface dermatitis (inflammation at the dermo-epidermal junction) may occasionally be seen as part of “AIDS interface dermatitis”, due to a drug or with no identifiable underlying aetiology. KS of the penis with concomitant squamous cell carcinoma in situ of the surface epithelium has also been recorded.

Classic KS with sarcoïd like granulomas has also been described and is thought to be a reaction to the tumour and tumour antigens akin to the granulomatous response documented in carcinomas (Kandemir et al 2009b). Fifteen biopsies of concomitant KS and Mycobacterium tuberculosis-associated granulomatous inflammation were described by Ramdial et al 2010. Investigation of concomitant granulomatous inflammation required special stains to detect an infective organism and molecular studies. Confirmation of Mycobacterium tuberculosis infection may serve as a clue to undiagnosed HIV infection, systemic tuberculosis, non compliance to treatment or multidrug resistance. Inflammatory
oncotaxis and Koebner phenomenon have been proposed as mechanisms for co-lesional KS and tuberculosis.

Bunn et al (2013) documented concomitant pathology in 25 of 135 cases of oral KS. The majority of cases showed superficially invasive candidiasis in addition to KS. This is not surprising as HIV positive patients frequently have oral candidiasis due to immunosuppression. One case showed Cytomegalovirus and another showed necrotising granulomatous inflammation.

KS has been noted in 13% of patients with unicentric Castleman disease and 75% of patients with multicentric Castleman disease (Bowne et al 1999). Oksenhendler et al (1996) reported on KS and multicentric Castleman disease in 20 HIV positive patients. KS was present 5 – 54 months before the diagnosis of Castleman disease in 6 patients and developed simultaneously in an additional 6 patients. Histological examination of the 20 lymph node biopsies revealed subtle KS lesions co-existing with Castleman disease in as many as 8 cases. The plasmablastic variant is most commonly seen in the setting of HIV infection and is characterised by plasmablasts in the mantle zone (Stebbing et al 2008). HHV8 LNA-1 IHC highlights positively staining cells in the mantle zone (Dupin et al 1999).

2.4.5. Diagnosis:

The diagnosis of KS is based on the clinical context (eg. presence of immunosuppression) recognising the clinical features (eg. violaceous mucocutaneous lesions) coupled with the abovementioned morphological and relevant immunophenotypic characteristics.

- Clinical features

Patients may present with minimal or disseminated disease (Douglas et al 2007). Clinically, early skin and mucosal lesions may be difficult to recognise as they appear as faint, red brown to violet macules. Usually, they present as violaceous papules, plaques or nodules.
There may be associated lymphoedema (Dezube 1996). Punch biopsies of skin are often taken to confirm the diagnosis.

Patients with bronchopulmonary KS usually present with respiratory symptoms such as cough and breathlessness with synchronous skin lesions (Mitchell et al. 1992). Chest X-ray and bronchoscopy can reveal lymphadenopathy, pleural effusion, peripheral nodules, endobronchial lesions or an interstitial infiltrate (Aboulafia 2000).

Patients with GIT KS may present with abdominal pain, nausea, weight loss, gastrointestinal bleeding, intestinal obstruction or diarrhoea. Endoscopy may reveal macules with submucosal haemorrhage or nodules. There may be a low diagnostic yield of biopsy due to possible submucosal or deeper locations of KS. As such, deeper biopsies may need to be performed in suspected cases of GIT KS (Friedman et al. 1985).

There are several clinical staging schemes for KS. Mitsuyasu divided KS into 4 stages with stage I being localised nodular KS in elderly men in North America and Europe and stage IV representing disseminated KS with visceral involvement. A 4 tier staging system originally proposed by Schwartz in 1984 has since been modified to include the presence of opportunistic infections, HIV seropositivity and cutaneous anergy into categories A, B and C respectively (Schwartz et al. 2008).

The AIDS Clinical Trials Group (ACTG) classification devised in 1988, classifies the tumour extent, immune system status (as determined by CD4 count) and systemic illness as good risk or poor risk. Nasti et al. (2003) recommended that this staging needs revision in the HAART era as the CD4 count does not seem to provide prognostic information.

- **Immunohistochemistry**

HHV8 can be detected by IHC, PCR and in situ hybridisation.

IHC is a readily available and timeous means of confirmation of KS. IHC is performed on formalin fixed paraffin embedded tissue using commercially available monoclonal antibodies
to different viral antigens. Monoclonal antibodies to KSHV LNA-1 are available. LNA-1 helps viral DNA attach to host chromosomes, therefore immunoreactivity appears as nuclear stippling. Lytic replication is only seen in a small percentage of cells, therefore it cannot be utilised in IHC (Pantanowitz *et al* 2009b). Application of HHV8 LNA-1 IHC may be required in cases where the morphological appearance mimics other vasoformative lesions such as pyogenic granuloma and angiosarcoma. HHV8 LNA-1 IHC is also diagnostically useful in subtle patch stage KS and when KS occurs in unusual topographic regions (Pantanowitz *et al* 2009b).

HHV8 IHC using LNA-1 anti-HHV8 antibody is sensitive (95%) and specific (100%) (Robin *et al* 2004). Hammock *et al* (2005) similarly found that 92% of KS lesions were positive with HHV8 LNA-1 IHC whilst none of the haemangiomas or epithelioid haemangiendotheliomas included in their study showed staining.

Of their 37 cases of KS, Hong *et al* (2003) detected HHV8 LNA-1 immunopositivity in 80% of patch, 88% of plaque and 74% of nodular KS. Interestingly, they asserted that HHV8 LNA-1 immunohistochemical staining was unrelated to age, gender, tumour recurrence, multiplicity or site of the lesions. Ramos da Silva *et al* (2007) applied HHV8 LNA-1 IHC to 64 cases, 41 (64%) of which revealed positive staining. Their sample included AIDS-KS (*n* = 25), classical KS (*n* = 20), iatrogenic KS (*n* = 2) and KS NOS (*n* = 17). Positive staining in the AIDS KS cases were seen in all nodular KS (which represented only 4 cases), in 6 of 9 patch and 7 of 8 plaque stage KS. The authors did not state their criteria for positive staining.

Positive immunohistochemical staining with HHV8 LNA-1 may show varying intensity and distribution. Some cases reveal only focal staining with isolated punctate staining in occasional nuclei whilst other cases stain diffusely within all lesional nuclei. Although Pantanowitz *et al* (2009b) has cautioned against comparing staining across the stages due to the greater number of cells in nodular KS compared to patch/plaque stage, there are
earlier studies that have assessed staining across the different stages. Patel et al (2004) and Pak et al (2005) demonstrated that more cells stained with HHV8 LNA-1 in nodular stage compared to patch/plaque stage KS. In early patch stage KS, only 10-30% of spindle cells may express latent genes whilst in later lesions, almost all cells express latent genes (Dupin et al 1999). A possible explanation proposed for this variability is that early lesions are thought to be hyperplastic and proliferative whilst the more advanced lesions of nodular and plaque stage are clonal (Miles 1996, Rabkin et al 1997).

Robin et al (2004) also alluded to the variability in staining and furthermore noted that the different epidemiologic forms of KS showed no specific variations.

Patel et al (2004) assessed HHV8 LNA-1 staining in 21 cases of KS. Stage was applied to 13 cases of cutaneous KS. Three were nodular and 10 were patch/plaque. This group of investigators defined positive staining as strong and diffuse staining in >10% of cells. Three cases of nodular KS showed positivity in ≥40% of cells. In their cases of patch/plaque KS, 1 showed positivity in 10%, 4 in 20% and the remaining 5 in ≥40% of cells. Interestingly, they regarded focal and weak staining in less than 10% of cells as negative. Other studies have regarded any nuclear staining as positive, with no quantitative criteria specified (Hong et al 2003).

CD31 and CD34 IHC may be also be used to confirm the diagnosis of KS. FLi1 and D-240 have also shown to be of use and are positive in 100% of AIDS related KS (Rosado et al 2012). Pantanowitz et al (2005) reported CD117 (C-kit) immunoreactivity in 43% of their cohort of classic, AIDS related and endemic KS cases. This CD117 positivity has been cited as a potential pitfall in the diagnosis of KS and gastrointestinal stromal tumours which are also CD117 and CD34 positive (Rossi et al 2009).

- Other ancillary tests
PCR is performed when IHC is negative, yet the suspicion for KS is high. PCR is subject to false positives as circulating lymphocytes may harbour HHV8. KSHV in situ hybridisation may also be used to detect the virus in Kaposi sarcoma lesions (Horenstein et al 2008).

2.5. Management

Despite the huge burden of disease in resource limited settings, application of simple treatment options is possible and effective.

According to Lorenzo et al (2007), treatment options for AIDS related KS include antiretroviral therapy, local treatment (radiation; topical agents), cytotoxic chemotherapy and targeted agents.

KS lesions regress with initiation of HAART. HAART inhibits HIV replication, decreases production of HIV-tat and ameliorates the immune response to HHV8 (Cattelan et al 2002).

Semeere et al (2012) reviewed literature to assess the impact of HAART on the incidence of KS in resource rich and resource limited settings. Data was cited from the Southern Africa International Epidemicologic Databases to evaluate AIDS (IeDEA) as showing an incidence of 624 per 100 000 person years among non-HAART users and 174 per 100 000 person years in HAART users, equating to a reduction of 72%. The most effective treatment modality remains HAART.

Besides side effects, HAART may be associated with immune reconstitution syndrome (IRIS). IRIS is an inflammatory reaction that occurs after initiation of HAART. It is due to reconstitution of the immune system which is now able to recognise pathogens that were previously asymptomatic. IRIS is associated with an increase in CD4 count and a decrease in HIV viral load. Progressive or recrudescent KS lesions may occur as part of IRIS (Ramdial 2010). Patients with IRIS-KS have a higher CD4 count and more tumour-associated oedema (Bower et al 2005).
Chemotherapy which includes anthracyclines is used for treatment of visceral disease and progressive mucocutaneous lesions. Local therapy such as radiotherapy is useful for palliation, for management of bulky lesions and for cosmesis. In this era of molecular medicine, targeted pathogenesis based treatments such as anti-angiogenic compounds, retinoic acid, hormonal agents and anti-herpes agents are also being used in the treatment of KS. Tyrosine kinase inhibitors eg. imatinib have been shown to induce regression as PDGF, PDGFR and c-kit play a role in KS pathogenesis (Lorenzo et al 2007).

Developed countries have shown a 24 month survival rate of 58% in patients living with AIDS and diagnosed with KS (Biggar et al 2005). But, in resource limited settings, such as SSA, weak infrastructure is a limiting factor in conducting clinical trials. Poverty, late diagnosis, lack of cancer treatment centres and trained professionals in combination with inconsistent availability of treating agents are barriers to rendering optimal care to patients (Krown 2011). Of the estimated 6.1 million people living with HIV in South Africa, only 2 010 340 adults are reported to be on HAART (UNAIDS global report 2013).
Chapter 3: METHODS

3.1. Study design

An observational retrospective cross sectional study design was used.

3.2. Target population and sampling technique

3.2.1. Target population:

The target population included all cases of KS diagnosed at the Histopathology Laboratory, Division of Anatomical Pathology, National Health Laboratory Services (NHLS) at CHBAH, Soweto, Gauteng, South Africa from 1/1/2005 to 31/12/2009.

3.2.2. Sampling technique:

The sampling technique was purposive. All cases diagnosed as KS, SNOMED code M-91403, at the laboratory were included. For the second component of the study, all cases of mucocutaneous KS with available CD4 counts and HHV8 LNA-1 immunohistochemical stains were included in the sample.

3.2.3. Ethics

An application for ethical approval of this study was submitted to the Wits Human Research Ethics committee. Institutional ethics approval is important for all human studies. Due to the retrospective nature of this research project, there was no contact with patients.

The laboratory numbers were recorded and subsequently coded on a datasheet, thereby ensuring that patient anonymity was preserved. Only linked-codes were used during statistical analysis. Ethics approval was obtained (clearance no. M130533) from the Wits Ethics committee (see annexure 3). Written permission to utilise the NHLS DISA system for the purposes of the study was obtained from the complex business manager (annexure 2).
3.3. Materials and data collection

3.3.1. Data collection:

The cases were retrieved using a SNOMED search of the DISA computer system at the NHLS laboratory at CHBAH. Cases of KS diagnosed in the histopathology department during the above-stated time period were retrieved using the SNOMED code, M-91403 and the SNOMED search program. Data were extracted from these laboratory reports and entered manually onto a datasheet (annexure 1).

The following variables were recorded:

- Age of the patient
- Gender of the patient
- HIV status (positive or negative)
- CD4 count (included only if the patient had retroviral disease and recorded if tested within one month of the confirmatory biopsy)
- Topographic region biopsied
- Type of specimen submitted/biopsy method (punch, incisional or excisional)
- Stage of KS (patch, plaque or nodular)
- Concomitant pathology identified in the same biopsy specimen

The information entered was cross checked on three occasions and subsequently transposed onto an Excel spreadsheet. Data for most of the variables except for CD4 count and HIV status were obtained from the histology reports. HIV status and CD4 counts were extracted in most cases from relevant serological investigations and CD4 reports available on the DISA laboratory system.

Cases of paediatric KS were extrapolated from the main dataset. For the purposes of the study "paediatric" was defined as children less than 14 years of age as per admission regulation at CHBAH. Nodal KS cases were reviewed.
The second component of the study involved selecting all mucocutaneous KS cases where CD4 counts and HHV8 LNA-1 immunohistochemical stains were available. The original haematoxylin and eosin stained slides and HHV8 LNA-1 immunohistochemically stained slides were retrieved from the histopathology archives. All cases with previously performed HHV8 LNA-1 IHC and available CD4 counts were reviewed by a single pathologist (the researcher) and entered onto a separate Excel data spreadsheet.

The distribution (either focal or diffuse) and intensity (either weak or strong) of HHV8 LNA-1 immunohistochemical staining were recorded on the datasheet together with the CD4 count of the patient and stage of KS (patch/plaque/tumour). The histological stage was also assessed and recorded in those cases where it was not originally reported.

The criteria for defining the histological stages were as those described by Grayson and Pantanowitz (2008). Patch stage KS shows a subtle spindle cell proliferation mostly around native blood vessels. Plaque stage KS comprises more spindle cells arranged in fascicles and tumour stage KS usually shows a nodular proliferation of tumour cells. For the purposes of this study, focal staining has been defined as a limited proportion of lesional cells (i.e. ≤10%) showing nuclear staining. Diffuse staining has been defined as most (>10%) of lesional cells showing nuclear staining. Weak staining has been defined as discrete, dot-like nuclear stippling. Strong staining has been defined as complete nuclear staining (multiple closely packed dots). Weak staining requires examination under high magnification (X400), whilst strong staining may be seen at low magnification (X100). Positive staining is detected by a brown colour due to the chromogen used in IHC.

Pantanowitz et al (2009b) have cautioned against comparing staining across the stages due to more lesional cells being present in nodular versus plaque versus patch stage. In considering the distribution of HHV8 LNA-1 staining, the proportion of lesional cells that are positive were assessed rather than considering absolute number.
3.3.2. Reliability of data

Demographic data entered onto the DISA system were obtained from the requisition slip completed by the submitting clinical doctor at the time of biopsy. The reliability of data for this study is dependent on the accurate submission of information especially with regard to variables such as age, gender and biopsy topographic sites. The accuracy of data obtained from the DISA system is also reliant on data capturers who entered details from requisition slips.

3.3.3. HHV8 LNA-1 immunohistochernistry

All the reviewed HHV8 LNA-1 immunostains were performed in the histopathology laboratory at the Charlotte Maxeke Johannesburg Academic Hospital. Where indicated, this stain was requested by the reporting pathologist at CHBAH.

The staining was performed by a technologist/technician using the mouse monoclonal antibody against HHV8-LNA (dilution 1:100 from Novacastra). Staining was done manually until the end of 2009, when an autostainer was acquired. Slides were incubated overnight at 37 degrees Celsius and then dewaxed in xylene for 10 minutes. They were blocked with peroxidase for 10 min and the antibody was applied for 20min. Chromogen was applied for 10min and the slides were then counterstained with haematoxylin. The slides were rinsed in buffer between all steps mentioned. Microwave and high pH/EDTA were used in antigen retrieval. Each test was run with the appropriate positive and negative controls as per quality assurance requirements and standards in the laboratory.

3.4. Data analysis:

All information collected was extrapolated onto an Excel spreadsheet. STATISTICA 12 was used for descriptive and analytic statistics. Descriptive statistics have been performed as a total. Continuous data (age, CD4 count) have been presented as
mean±SD if normal distributed and as median and quartile (QR) when the distribution was non-normal.

Categorical data have been summarised as frequencies and percentages.

The Mann-Whitney test was used when 2 independent variables (CD4 count and intensity of HHV8 LNA-1 staining, CD4 count and distribution of HHV8 LNA-1 staining) were compared and the Kruskell Wallace (median) test when multiple independent samples (CD4 count and nodular, patch, plaque stages) were compared.

The Fisher exact test was used to assess if there were any significant differences in the staining intensity and distribution across the 3 stages.

All data analysis was personally performed under the supervision of Prof Libhaber.

The following chapter presents results obtained when the above methodology was applied.
Chapter 4: RESULTS

A total of 901 patients with 938 biopsies were recorded. The number of cases diagnosed per year is shown in Figure 1, below.

Figure 1: Number of cases of KS histologically diagnosed in each year at CHBAH from 2005 and 2009.

4.1. Male:Female ratio

Of the total 901 patients that were biopsied, the gender was unknown/unspecified in one patient (n = 900). A total of 488 males and 412 females were biopsied. This equates to a male: female ratio of 1.18:1 for all cases of KS diagnosed.

Of the 901 patients that were diagnosed with KS, 731 patients were confirmed as being HIV positive either in the clinical history provided by the submitting clinician or on a positive serological test located on the DISA computer system. The gender was unknown in one
confirmed HIV positive patient \( (n = 730) \). There were 396 males and 334 females \( (M:F = 1.19:1) \).

**Figure 2:** Number of males versus number of females histologically diagnosed with KS.

### 4.2. Mean age at biopsy diagnosis

The mean age at biopsy diagnosis of KS was 36.79 years \( (SD 10.24 \text{ years}) \). Age was not recorded in 12 patients \( (n = 889) \). The youngest patient in this study was 1 year of age and the oldest patient 85 years.

For the confirmed HIV positive subgroup of 731 patients, age was available in 726 patients. The mean age for this group was 36.43 years \( (SD 9.66 \text{ years}) \) and median 35.50 years \( (QR 12.00 \text{ years}) \).

The mean age for confirmed HIV positive males was 38.08 years \( (SD 9.32 \text{ years}) \) and the median 37.00 years \( (QR 11.00 \text{ years}) \). The mean age for confirmed HIV positive females was 34.46 years \( (SD 9.72 \text{ years}) \) and the median 33.00 years \( (QR 12.00 \text{ years}) \).
Figure 3: Distribution of age at time of biopsy for all patients diagnosed with KS.

4.3. CD4 count at KS biopsy diagnosis

The mean CD4 count within one month of first biopsy diagnosis was 155,74 cells/mm$^3$ (SD 143,58 cells/mm$^3$) and the median CD4 count was 127,50 cells/mm$^3$ (QR 184,50 cells/mm$^3$).

The CD4 counts were unknown in 361 patients ($n = 540$). 382 patients had CD4 counts less than 200 cells/mm$^3$, 127 CD4 counts between 200 cells/mm$^3$ and 400 cells/mm$^3$ and 27 patients had CD4 counts between 400 cells/mm$^3$ and 600 cells/mm$^3$.

For the confirmed HIV positive group, CD4 counts were available for 536 patients. The median CD4 count was 126,00 cells/mm$^3$ (QR 184,50 cells/mm$^3$) and the mean 155,29 cells/mm$^3$ (SD 143,24 cells/mm$^3$).
The confirmed HIV positive males had a median CD4 count of 130,00 cells/mm$^3$ (QR 127,67 cells/mm$^3$) and the confirmed HIV positive females had a median CD4 count of 120,50 cells/mm$^3$ (QR 181,00 cells/mm$^3$).

**Figure 4:** Distribution of CD4 count at time of biopsy.

**Table 1:** CD4 counts of confirmed HIV positive patients, males and females diagnosed with KS and all cases diagnosed with KS.

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<td>CD4 count females</td>
<td>236</td>
<td>120,50 cells/mm$^3$</td>
<td>181,00 cells/mm$^3$</td>
</tr>
<tr>
<td>CD4 count males</td>
<td>299</td>
<td>130,00 cells/mm$^3$</td>
<td>182,00 cells/mm$^3$</td>
</tr>
<tr>
<td>CD4 total (HIV confirmed)</td>
<td>536</td>
<td>126,00 cells/mm$^3$</td>
<td>184,50 cells/mm$^3$</td>
</tr>
<tr>
<td>CD4 count of all cases</td>
<td>540</td>
<td>127,50 cells/mm$^3$</td>
<td>184,50 cells/mm$^3$</td>
</tr>
</tbody>
</table>

* The gender was unknown in one patient
4.4. Topographic sites

Topographic sites were recorded in 674 biopsies and were unspecified in 264 biopsies. Of the total 674 where topographic sites were specified; 81.45% of the biopsies were from skin; 6.97% of biopsies were from the oral cavity and 4.0% were from lymph nodes. Most skin biopsies (334/551) were from the lower limbs.

The one case recorded in the breast was reviewed and showed spindle cells infiltrating among terminal duct lobular units. HHV8 IHC performed on this case was positive.

Table 2 (overleaf) shows the specific topographic sites involved.
Table 2: Distribution of topographic sites involved by KS.

<table>
<thead>
<tr>
<th>Visceral</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oesophagus</td>
<td>1</td>
</tr>
<tr>
<td>Stomach</td>
<td>6</td>
</tr>
<tr>
<td>Small bowel</td>
<td>2</td>
</tr>
<tr>
<td>Colon</td>
<td>1</td>
</tr>
<tr>
<td>Anus</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oral cavity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonsil</td>
<td>5</td>
</tr>
<tr>
<td>Tongue</td>
<td>8</td>
</tr>
<tr>
<td>Palate</td>
<td>23</td>
</tr>
<tr>
<td>Oral cavity not otherwise specified</td>
<td>4</td>
</tr>
<tr>
<td>Gingiva</td>
<td>3</td>
</tr>
<tr>
<td>Alveolar</td>
<td>1</td>
</tr>
<tr>
<td>Lip</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lymph node</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen/trunk/back</td>
<td>17</td>
</tr>
<tr>
<td>Lower limbs</td>
<td>334</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>125</td>
</tr>
<tr>
<td>Head and neck</td>
<td>27</td>
</tr>
<tr>
<td>Chest/breast</td>
<td>40</td>
</tr>
<tr>
<td>Genital area</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>549</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eye</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyelid</td>
<td>18</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>9</td>
</tr>
<tr>
<td>Eye not otherwise specified</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

| Pharynx/larynx                | 7  |

| Breast                        | 1  |
4.5. Visceral KS

Thirteen cases of visceral KS were identified.

The organs involved are shown in the pie graph (figure 5) below. Most of the biopsies of visceral KS were from the stomach.

*Data is presented as absolute numbers

Figure 5: Distribution of visceral organs involved by KS.

4.6. HIV status

The HIV status was unknown in 161 cases. Seven hundred and thirty one patients (98.78%) were HIV positive and 9 were HIV negative confirmed on HIV enzyme-linked immunosorbent assay (HIV-ELISA) (n = 740).
4.7. HHV8 LNA-1 immunohistochemical staining

HHV8 LNA-1 immunohistochemical staining was performed in 289 biopsies. There were 271 (94%) positive results and 18 (6%) negative results.

Twelve biopsies from 9 HIV negative patients were recorded. Nine HHV8 LNA-1 immunohistochemical stains were performed on the total 12 HIV negative KS biopsies. One (11%) of the total nine HHV8 LNA-1 immunohistochemical stains performed on the biopsies from the HIV negative patients, was negative. The remaining 10 were positive.

4.8. Paediatric KS

Thirteen (1.44%) cases of paediatric KS (diagnosed in patients younger than 14 years) were identified.

Seven male children and 6 female (M: F= 1.17: 1) were noted.

The mean age was 6.46 years (SD 3.36 years) and the median 7.00 years (QR 4.00 years).
Of the 13 cases, HIV status was unknown in two patients, was negative in 1 and positive in 10 patients.

Six CD4 counts were available, with a mean of 209,15 cells/mm$^3$ (SD 141,85 cells/mm$^3$) and a median of 217,0 cells/mm$^3$ (QR 204,0 cells/mm$^3$).

The topographic sites for the paediatric cases are shown in figure 7 below. The one child who was HIV negative had an incisional skin biopsy from the arm. The diagnosis of KS was confirmed with a positive HHV8 immunohistochemical stain.

![Figure 7: Topographic sites involved by KS in children younger than 14 years.](image)

*Data is presented as absolute numbers

4.9. **Endemic KS**

Twelve biopsies of KS from 9 confirmed HIV negative patients were seen. There was no history of transplantation in these patients. No further clinical history of immunosuppression was provided. Five HIV negative patients were males, whilst 4 were females (M:F = 1,25:1).
1). The mean age was 55.44 years (SD 19.72 years). The topographic sites involved are shown below (figure 8).

![Topographic sites of endemic KS](image)

*Data is presented as absolute numbers

**Figure 8: Topographic sites of endemic KS.**

All nine patients with endemic KS had serological investigations for HIV performed, see table 3 below.

<table>
<thead>
<tr>
<th>Patient</th>
<th>HIV ELISA x 1 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>HIV ELISA x 1 negative</td>
</tr>
<tr>
<td>Patient B</td>
<td>HIV ELISA x 1 negative</td>
</tr>
<tr>
<td>Patient C</td>
<td>HIV ELISA x 1 negative</td>
</tr>
<tr>
<td>Patient D</td>
<td>HIV ELISA x 5 negative</td>
</tr>
<tr>
<td>Patient E</td>
<td>HIV ELISA x 1 negative</td>
</tr>
<tr>
<td>Patient F</td>
<td>HIV ELISA x 2 negative</td>
</tr>
<tr>
<td>Patient G</td>
<td>HIV ELISA x 2 negative</td>
</tr>
<tr>
<td>Patient H</td>
<td>HIV ELISA x 2 negative</td>
</tr>
<tr>
<td>Patient I</td>
<td>HIV ELISA x 1 negative</td>
</tr>
</tbody>
</table>
4.10. Stage of KS at biopsy diagnosis

Stages of Kaposi sarcoma ie. patch, plaque or nodular were recorded in the histopathology reports of 708 biopsies and were not recorded in 230 biopsies.

109 cases were of patch stage, 380 cases were of plaque stage and 219 cases showed nodular stage KS.

![Pie chart showing stages of KS at diagnosis.](image)

**Figure 9: Stages of KS at diagnosis.**

Figure 10 overleaf shows the differences in histomorphology among patch, plaque and tumour/nodular stage KS.
Figure 10: A Patch stage KS, B Plaque stage KS & C Tumour stage KS (X100 magnification).
**Figure 11:** Patch stage KS, “busy dermis” (X200 magnification).

**Figure 12:** HHV8 LNA-1 immunohistochemical staining in subtle patch stage KS (X200 magnification), same case as figure 10 (A) above.
Figure 13: Plaque stage KS (X200 magnification).

Figure 14: Tumour stage Kaposi sarcoma with ulceration of overlying mucosa (X100 magnification).
4.11. Relationship of median CD4 count to stages of KS

No statistically significant correlation was demonstrated between the median CD4 count and the stage of KS \((p = 0.701)\).

<table>
<thead>
<tr>
<th>Stage (n)</th>
<th>Nodular (31)</th>
<th>Patch (40)</th>
<th>Plaque (56)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 count (median, IQR)</td>
<td>146.0 cells/mm(^3) (193 cells/mm(^3))</td>
<td>168.5 cells/mm(^3) (230.5 cells/mm(^3))</td>
<td>129.0 cells/mm(^3) (169.5 cells/mm(^3))</td>
<td>0.701</td>
</tr>
</tbody>
</table>

4.12. Morphological variants of KS

Classic histopathological features of KS are a spindle cell proliferation, formation of slit-like vascular spaces, extravasated red blood cells, haemosiderin pigment, hyaline globules and plasma cells as shown in the figures (15-23) below.

*Figure 15: Fascicles of spindle cells with red cell extravasation.*
**Figure 16:** Spindle cell proliferation with extravasated red blood cells.

**Figure 17:** Slit like vascular spaces (black arrow) and haemosiderin pigment (white arrow).
Figure 18: Conspicuous plasma cells (white arrow).

Figure 19: Hyaline globules.
Figure 20: Haemosiderin pigment.

Figure 21: Plasma cells (white arrow), extravasated red blood cells (black arrow) and spindled cells (blue arrow).
**Figure 22:** Hyaline globules (white arrow) and extravasated red blood cells (black arrow).

**Figure 23:** Promontory sign.
Special morphological variants were recorded in 17 cases. All 17 of these patients were HIV positive. Lymphangioma-like/lymphangiomatous, lymphangiectatic and telangiectatic variants were documented as shown in table 5 below. The lower limb biopsies accounted for the most of the cases diagnosed with a special morphological variant.

**Table 5: Morphological variants of KS and topographic sites of these biopsies.**

<table>
<thead>
<tr>
<th>Site</th>
<th>Lymphangioma-like</th>
<th>Lymphangiectatic</th>
<th>Telangiectatic</th>
<th>Total for specific site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower limbs</td>
<td>4*</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Eyelid/conjunctiva</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total for specific variant</td>
<td>11</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Data is presented as absolute numbers*
**Figure 24:** Lymphangiectatic variant (arrows indicate dilated lymphatics).

**Figure 25:** Lymphangiomatous variant of KS (X200 magnification).
4.13. Concomitant pathology

Concomitant pathology was noted in 43 (4.6%) of 938 biopsies. Infections, inflammatory dermatitis and panniculitis were most commonly documented as dual pathology in cutaneous KS biopsies as shown in table 6 overleaf.

Seven cases of granulomatous inflammation and KS were seen in the same biopsy. Five were recorded in skin punch biopsies, 1 was from a lymph node and an additional case was from the breast. Ziehl-Neelsen and Periodic Acid Schiff special stains performed on all 7 cases were negative for micro-organisms. PCR for *Mycobacterium* genus was performed on 1 case of cutaneous KS with granulomatous inflammation and was positive. Acid fast bacilli were detected in the sputum of another patient with cutaneous KS and granulomatous inflammation.

*Figure 26*: Granulomatous inflammation and KS (X200 magnification).
Table 6: Concomitant pathology in biopsies in which KS was diagnosed.

<table>
<thead>
<tr>
<th>Type of concomitant pathology</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td></td>
</tr>
<tr>
<td>• Cytomegalovirus</td>
<td>3</td>
</tr>
<tr>
<td>• Candida</td>
<td>3</td>
</tr>
<tr>
<td>• Cryptococcus</td>
<td>1</td>
</tr>
<tr>
<td>• Possible Syphilis</td>
<td>2</td>
</tr>
<tr>
<td>• Herpes</td>
<td>1</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>10</td>
</tr>
<tr>
<td>Dermatitis</td>
<td></td>
</tr>
<tr>
<td>• Seborrheic dermatitis</td>
<td>1</td>
</tr>
<tr>
<td>• Spongiotic dermatitis</td>
<td>1</td>
</tr>
<tr>
<td>• Interface dermatitis</td>
<td>4</td>
</tr>
<tr>
<td>• Granulomatous inflammation</td>
<td>7</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>13</td>
</tr>
<tr>
<td>Panniculitis</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>• Papilloma</td>
<td>1</td>
</tr>
<tr>
<td>• Verruca</td>
<td>1</td>
</tr>
<tr>
<td>• Chalazion</td>
<td>1</td>
</tr>
<tr>
<td>• Intussusception</td>
<td>1</td>
</tr>
<tr>
<td>• Gastritis</td>
<td>1</td>
</tr>
<tr>
<td>• HIV lymphadenitis/Castleman disease-like</td>
<td>10</td>
</tr>
<tr>
<td>• Castleman disease</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>43</td>
</tr>
</tbody>
</table>

The 3 cases of concomitant candidiasis were recorded in oral cavity biopsies.

There were 27 cases of nodal KS, 12 of which showed concomitant pathology. One showed granulomatous inflammation (noted above), 10 showed HIV lymphadenitis/Castleman disease-like features and 1 showed concomitant Castleman disease. There were 2 cases diagnosed as showing Castleman disease-like features. These were stained with HHV8.
LNA-1 and the histology although diagnostic for KS fell short of the criteria for true Castleman disease. These two cases were reviewed by multiple pathologists at the time of sign out. The eight cases showing HIV lymphadenitis demonstrated follicle-lysis, expansive or atrophic germinal centres and interfollicular plasma cells. The features in two skin punch biopsies raised the possibility of concomitant syphilis due to the dense lymphoplasmacytic infiltrate. In one case, Warthin Starry special stain was performed and was negative. Confirmatory serological investigations for syphilis were advised by the reporting pathologist/s. However, no records of syphilis serological investigations were subsequently found.

A total of 132 cases were initially selected for review of HHV8 LNA-1 immunohistochemical staining. Only 127 cases could be reviewed. The slides could not be located in 4 cases and one of the cases had a poor section with extensive folding of the tissue rendering it un-interpretable. Of the total 127 cases reviewed, there were 40 (31.5%) patch stage, 56 (44.09%) plaque and 31 (24.41%) nodular stage cases.

4.14. Distribution of HHV8 LNA-1 immunohistochemical staining across the stages of KS

Of the total 127 biopsies reviewed, 110 showed diffuse (>10% of lesional cells showing nuclear staining) and 17 showed focal (≤10% of lesional cells showing nuclear staining) HHV8 LNA-1 immunohistochemical staining.

The distribution of HHV8 LNA-1 staining across each of the stages, patch; plaque and nodular are shown in table 7 below. Most cases that stained diffusely were plaque stage and only one case of nodular KS showed focal staining.
Table 7: Distribution of HHV8 LNA-1 staining, number of cases that showed diffuse and focal staining in each of the 3 stages.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Distribution</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diffuse</td>
<td>Focal</td>
</tr>
<tr>
<td>Patch</td>
<td>30 (27.3%)</td>
<td>10 (58.8%)</td>
</tr>
<tr>
<td>Plaque</td>
<td>50 (45.5%)</td>
<td>6 (35.3%)</td>
</tr>
<tr>
<td>Nodular</td>
<td>30 (27.3%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>110 (100%)</td>
<td>17 (100%)</td>
</tr>
</tbody>
</table>

*Data is presented as frequencies and percentages

No statistically significant differences were noted as a total but when individual stages were compared, a statistically significant difference was noted in the distribution of staining between patch and nodular stage KS ($p = 0.011 = <0.016$). No significant differences were calculated for patch vs plaque ($p = 0.06$) or plaque vs nodular stages ($p = 0.21$).
Figure 27: A & B: Strong and diffuse HHV8 LNA-1 immunohistochemical staining (X100 magnification).
Figure 28: A & B: Weak HHV8 LNA-1 immunohistochemical staining. X400 magnification indicated by arrows.
4.15. **Intensity of HHV8 LNA-1 immunohistochemical staining across the stages of KS**

Of the 127 biopsies reviewed, 102 showed strong staining (complete nuclear staining with multiple closely packed dots seen at low magnification) with HHV8 LNA-1 immunohistochemical stain and 25 showed weak staining (discrete, dot-like nuclear stippling that can only be seen at high magnification).

Table 8 overleaf shows the intensity of staining across the three stages. Only 3 cases of nodular KS showed weak staining.

No statistically significant difference was noted in the intensity of staining across the 3 stages of KS ($p = 0.30$). When the intensity of combined patch and plaque stage were compared against nodular stage there was no statistical significance ($p = 0.08$).

*Figure 29: Strong HHV8 LNA-1 immunohistochemical staining indicated by brown nuclear staining (X400 magnification).*
Table 8: Intensity of HHV8 LNA-1 staining, number of cases that showed strong and weak staining in each of the stages.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Intensity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
<td>Weak</td>
</tr>
<tr>
<td>Patch</td>
<td>30 (29,4%)</td>
<td>10 (40%)</td>
</tr>
<tr>
<td>Plaque</td>
<td>44 (43,1%)</td>
<td>12 (50%)</td>
</tr>
<tr>
<td>Nodular</td>
<td>28 (27,5%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Total</td>
<td>102 (100%)</td>
<td>25 (100%)</td>
</tr>
</tbody>
</table>

*Data is presented as frequencies and percentages

Figure 30 below allows for comparison of HHV8 LNA-1 immunohistochemical staining.

**Figure 30:** Number of biopsies showing weak/strong and focal/diffuse HHV8 LNA-1 immunohistochemical staining across the three stages of KS.
4.16. Relationship of CD4 count to intensity and distribution of HHV8 LNA-1 immunohistochemical staining

The study showed that CD4 count was not predictive of the intensity or distribution of HHV8 immunohistochemical staining (p value = 0.878 and 0.846 respectively).

Table 9: Relationship of intensity (weak/strong) and distribution (focal/diffuse) of HHV8 LNA-1 immunohistochemical staining to CD4 count

<table>
<thead>
<tr>
<th>Intensity (n)</th>
<th>Weak (25)</th>
<th>Strong (102)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median CD4 count (IQR)</td>
<td>121 (221)</td>
<td>148 (191)</td>
<td>0.878</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distribution (n)</th>
<th>Focal (17)</th>
<th>Diffuse (110)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median CD4 count (IQR)</td>
<td>121 (199)</td>
<td>147 (194)</td>
<td>0.846</td>
</tr>
</tbody>
</table>

CD4 counts were log transformed and the distribution was not normal. Using the same statistical tests to compare, there was no statistically significant difference found for stage, distribution and intensity of HHV8 LNA-1 staining.
Chapter 5: DISCUSSION

Of the tens of thousands of cases of KS diagnosed worldwide, the vast majority are thought to have occurred in SSA (Parkin 2002). Much has changed since HIV associated KS was first recognized in homosexual males in the 1980’s. The pathogenesis of KS has been extensively investigated and HHV8 has been linked to KS. While developed countries are experiencing a decline in the incidence of KS due to timeous HAART initiation, in developing countries, like South Africa, KS remains a cause of significant morbidity and mortality due to lack of optimal availability of HAART (Semeere et al 2012).

SSA is home to approximately 80% of the world’s HIV-infected female population and heterosexual transmission of HIV remains the main mode of spread worldwide (De Cock et al 2012). In SSA, women younger than 20 years of age are eight times more likely to be HIV infected compared to males in the same age range (Abdool Karim 2013). The UNAIDS global report 2013, estimated that approximately 13,9% of females (vs 3,9% of males) between the ages 15 and 24 years were living with HIV in South Africa. The male female ratio of patients diagnosed with confirmed HIV associated KS was 1,2:1 in this study, in keeping with the trend of KS shifting from a male predominant disease (Sitas & Newton 2000) and is reflective of the high prevalence of HIV in young women who bear the brunt of HIV in South Africa. This is in stark contrast to the initial recognition of KS as an overwhelmingly male predominant disease in homosexual men in the USA in the 1980’s (Biggar & Rabkin 1996).

Patients diagnosed with KS at CHBAH showed a mean age in males of 38 years and in females of 35 years. This is similar to findings by Mosam et al (2008). The mean age for all cases of confirmed HIV associated KS at CHBAH was 36 years; congruent with findings by Mosam et al (2009) of a mean of 36,5 years in 2006. This is in contrast to the mean of 59,7 years which was recorded (by Mosam et al) in cases from 1983 (pre-AIDS era). The mean age of mid thirties may once again change as the long term effects of the antiretroviral roll-
out programmes begin to emerge. It is predicted that with HAART, HIV positive patients will live longer and thus may present later with KS (Daly et al 2014).

Confirmed HIV positive males with KS at CHBAH during the study period had a mean CD4 count of 157.40 cells/mm$^3$. A mean CD4 count of 153.22 cells/mm$^3$ was demonstrated in the female patients. The mean CD4 counts recorded were lower than that documented by other comparative studies. Mosam et al (2008) demonstrated a mean CD4 count in males of 230 cells/mm$^3$ and a mean CD4 count in females of 205 cells/mm$^3$. The mean CD4 count for the entire cohort used by Mosam et al (2008) was 218 cells/mm$^3$ compared to the CHBAH mean CD4 count recorded of 155.74 cells/mm$^3$. The mean CD4 count recorded by Onunu et al (2007) was 127 cells/mm$^3$. The wide range of CD4 counts recorded in the present study (minimum <1 cell/mm$^3$ and maximum 1465 cells/mm$^3$) is in keeping with the suggestion that in Africa severe immunosuppression is not a pre-requisite for the development of KS (Cassol et al 2005). In fact, results of this study show that 158 patients at CHBAH diagnosed with KS (ie. 29.26% of the total 540 patients with known CD4 counts) had CD4 counts in excess of 200 cells/mm$^3$.

Mucocutaneous sites are most commonly involved in KS (Radu & Pantanowitz 2013). Six hundred and thirty three (64.5%) biopsies of the total 938 received at the CHBAH Histopathology department originated from mucocutaneous sites. Most of these biopsies were from the lower limbs (334 of 674 cases with recorded topographic sites). Only 9 cases of visceral KS were found. Ramos da Silva et al (2007) documented 6/64 (9.3%) cases of visceral KS. However the specific viscera involved were unspecified. Results of this study show that stomach and lung were most frequently involved followed by oesophagus, anus and small bowel. This limited number may be attributed to under sampling as synchronous easily accessible mucocutaneous lesions in these patients may have been biopsied. KS occurring in topographic sites considered unusual by Pantanowitz & Dezube (2008) that have been noted at CHBAH include larynx (2 cases), conjunctiva (9 cases) and breast (1 case).
KS may be localised to the breast or may be part of more disseminated disease (Pantanowitz & Conolly 2002). There have been only isolated reports of KS occurring in the breast (Hamed et al 2000, Michelow et al 2010). This study contributes one additional case of KS which presented as a mass in the lower outer quadrant of the breast. The patient was female, however the HIV status was not known. Furthermore, there was no additional history regarding the presence of disseminated KS or KS confined exclusively to the breast parenchyma. Four additional cases in the present study were of KS involving skin of the breast and one case derived from an intramammary lymph node presenting as a breast mass.

 Conjunctival and ocular adnexal KS have been reported by Schmid et al 2003 and Curtis & Durairaj 2005. Nine cases of conjunctival KS (from 7 patients) were detected in this study. CHBAH laboratory provides histopathology service to the neighbouring St John’s Eye Hospital thus accounting for the relatively high number of this topographic location being represented.

 Review of the clinical history in the reports showed that one of the two patients who was diagnosed with laryngeal KS at the CHBAH histopathology laboratory presented with complete airway obstruction. Pantanowitz and Dezube (2008) cited 25 cases of laryngeal KS from the literature in their review with complete airway obstruction being one of the presenting signs. No brain, spinal cord, heart, endocrine or urinary tract lesions were identified at CHBAH during the period of study.

 Results of our study show that the majority of patients (731 of 901; 81.13%) who were diagnosed with KS on biopsy were also confirmed to be HIV positive on serological testing. The HIV status was not available in 161 of 901 patients. This may due to patients possibly refusing consent for retroviral testing. Furthermore, an HIV test may have been performed at peripheral hospitals/clinics that are not linked to the DISA database at CHBAH and therefore these results could not be documented. In some instances, clinicians may have failed to offer HIV testing to the patient at time of biopsy or failed to specify the patients’
status in the clinical history section of the requisition form. KS is an AIDS-defining condition and clinical suspicion of the lesion should prompt verification of the HIV status. Nine patients from our cohort were HIV negative. Biopsies from these patients have been regarded as endemic KS. No history of organ transplantation was specified in these patients. In addition, the clinical files were not available for thorough review. Unknown HIV test results in 161 cases are likely to underestimate the burden of true endemic/African KS seen at CHBAH.

Ninety four percent of HHV8 LNA-1 immunohistochemical stains performed on KS biopsies at the CHBAH histopathology laboratory during the study period were positive, similar to findings by Hammock et al (2005) and in excess of the 78% positivity and 64% positivity recorded by Hong et al (2003) and Ramos da Silva et al (2007) respectively. Interestingly, HHV8 LNA-1 staining was only applied in 289 of the 938 (30.8%) of biopsies at CHBAH during the study period. This is reflective of variable individual pathologists' practice as in the appropriate clinical context, the diagnosis may be based on the typical morphological features.

Results show that only one of 9 HHV8 LNA-1 immunostains performed in the endemic group was negative. Schwartz et al (2003) investigated HHV8 LNA-1 expression in 16 cases of endemic KS from the 1960’s. Five cases (31.25%) were recorded as negative and the age of the tissue blocks was thought to influence the staining of these endemic cases.

Thirteen (1.44%) cases of paediatric KS (diagnosed in patients younger than 14 years) were identified in this study and a median age of 7 years was demonstrated. Of the 10 paediatric patients with HIV-associated KS recorded, the majority of cases (5/10) were from biopsies of lymph nodes and one case was of gastrointestinal origin. Tukei et al (2011), in a study in which children were defined as aged between 6 weeks and 18 years, revealed a similar nodal predominance of KS. In contrast, Cairncross et al (2009) reported only 1 case of nodal KS and 3 cases of gastrointestinal KS. Despite being inclusive of cases of paediatric KS from CHBAH (in addition to cases from other South African tertiary hospitals), the
findings by Stefan *et al* (2011) contrasted those of the present study in that their median age was younger, the average CD4 count was higher and the most commonly affected site was skin. Further studies to determine the incidence of paediatric KS may be useful in light of the vigorous Prevention of Mother to Child Transmission (PMTCT) antiretroviral programmes initiated at local clinics and CHBAH. In data cited by Hankins (2013), new HIV infections in children over 2 has decreased by 24% due to the PMTCT. The UNAIDS global report of 2013 has estimated 21 000 new infections in children as of 2012 with a reported 234 952 pregnant females living with HIV who received HAART for preventing mother to child transmission. As such, the impact of reduced vertical transmission of HIV infection may result in a decline in the incidence of KS in children.

Results of this study show that though very rare, KS in HIV negative patients (probable endemic KS) does present to CHBAH. The patients regarded as having endemic KS were noted to have tested negative for HIV by means of HIV ELISA on one or multiple occasions (see table 3). According to the South African Department of Health’s HIV counselling and testing policy guidelines of 2010, confirmation of HIV infection by means of an HIV-ELISA test is reserved for those cases where the rapid/screening test is positive. Pitche *et al* (2007), in a study of 20 cases of African/endemic KS, found a mean age of 49,5 years and M:F ratio of 9:1. Their cases were mostly localised to the lower limbs (78,9%). The mean age of patients with HIV-negative/endemic KS at CHBAH was 55,4 years with a M:F ratio of 1,25:1. Similarly, 50% of cases were localized to the lower limbs. The prevalence of endemic KS may be under-estimated due to absence of a recorded HIV status in 161 patients in this study. Endemic KS, although rare in comparison to HIV associated KS, does still occur and as such patients who have KS should not be assumed to be HIV positive. Formal serological tests for confirmation/exclusion of HIV infection are warranted.

Results of this study show that most lesions of mucocutaneous KS biopsied were of plaque stage, followed by nodular stage and then patch stage KS. Patch stage KS may be a subtle manifestation, both clinically and histopathologically. The subtle nature of patch stage KS
may account for more obvious plaque and nodular stages being biopsied for confirmation of KS. Patients are more likely to recognise and be concerned about well-established and/or larger lesions; hence their presentation with plaque and nodular stage lesions. From a clinical viewpoint, larger lesions are thought to be more representative and diagnostically yielding. Therefore these lesions are targeted by the biopsying clinician. The stages of KS diagnosed were not specified in 230 of the 938 histopathology reports reviewed. Reporting of the histological stage is dependent on the preference of the reporting pathologist. The ACTG staging for KS is not reliant on any histological parameters (Nasti et al 2003).

The median CD4 counts calculated across the three stages of KS were similar (168 cells/mm$^3$ for patch stage, 129 cells/mm$^3$ for plaque stage and 146 cells/mm$^3$ for tumour stage) in this study. The premise at the outset was that the lower the CD4 count, the greater the degree of immunosuppression and therefore the more advanced the KS lesion. Patients with low CD4 counts were therefore assumed to present with nodular KS and those with higher CD4 counts with patch stage KS. However, we have demonstrated that the CD4 count is not predictive of the stage of KS ($p = 0.701$). Patients who have HIV infection may have synchronous KS lesions of different stages. The lesion biopsied and therefore the stage recorded by the pathologist may not produce a true reflection of the severity of the disease. Clinical records regarding the number of lesions and the stages of each lesion may have been more useful for comparison to the CD4 count.

This study revealed that infections represented 23.26% of the concomitant pathology diagnosed in KS biopsy specimens. *Herpes simplex* (1 case) and possible syphilis (2 cases) were two concomitant infections diagnosed at CHBAH in addition to the more commonly described *cryptococcus, cytomegalovirus* and *candida*. Interface dermatitis as described by Grayson (2011) also occurred concomitantly. In addition, spongiotic dermatitis not otherwise specified and seborrheic dermatitis have also been diagnosed as concomitant pathology in this laboratory. Granulomatous inflammation was seen in 7 biopsies. PCR for *Mycobacterium* genus was positive in one case. Ziehl neelsen special stains performed on
all 7 cases were negative. Kandemir et al (2009b) reported sarcoid-like granulomas in a case of classic KS. Special stains for infective causes were negative and the reaction was postulated to be similar to that observed in carcinomas. Ramdial et al (2010) stressed the importance of adequately investigating granulomatous inflammation. Confirmation of concomitant Mycobacterium tuberculosis may be crucial in diagnostic and management decisions by the treating clinician especially with regard to multidrug resistance and possible non compliance.

One case of penile KS was confirmed at the CHBAH histopathology laboratory during the study period. However, no associated squamous dysplasia or special morphologic variants of KS were diagnosed in this genital region. The review article by Grayson (2011) focused on cases of cutaneous KS and concomitant pathology thereof. The present study contributes and documents concomitant pathology occurring in the setting of extracutaneous KS.

Concomitant HIV lymphadenitis/Castleman disease like features occurred in 10 of the 27 cases of nodal KS and 1 case showed Castleman disease. As concomitant pathology was noted in approximately a half of all lymph nodes biopsies, reporting pathologists should be vigilant. In lymph nodes which display morphological features of KS, routine application of HHV8 LNA-1 immunohistochemical stains should be considered as HHV8 positive cells may be detected in the mantle layer of follicles thereby confirming the diagnosis of concomitant HHV8 associated Castleman disease. One must be wary though of isolated circulating lymphocytes that may also be HHV8 positive. Large intestinal intussusception and gastritis were also documented.

Variability of HHV8 LNA-1 immunohistochemical staining was noted on review of the retrospective cohort of cases. This variability was noted both in the distribution (focal vs diffuse) and intensity (weak vs strong) of immunohistochemical staining. Reasons for this variability were explored by assessing if there was a relationship among the intensity and/or
the distribution of HHV8 LNA-1 immunohistochemical staining, CD4 counts of the patients at
time of biopsy and the stages of the lesions (ie, patch, plaque or nodular).

It was found that most biopsies in this study across all 3 stages stained strongly (102 of the
total 127 biopsies reviewed). No statistically significant difference was noted in the intensity
of staining across the 3 stages of KS (p = 0.30) in this study. When the intensity of
combined patch and plaque stage were compared against nodular stage there was no
statistical significance (p = 0.08).

Most KS biopsies across all the stages stained diffusely (110 of the total 127 cases
reviewed). No statistically significant differences were noted as a total but when individual
stages were compared, a statistically significant difference was noted in the distribution of
staining between patch and nodular stage KS (p = 0.011 = <0.016). No significant
differences in distribution were calculated for patch vs plaque (p = 0.06) or plaque vs nodular
stages (p = 0.21).

A possible explanation for the variable staining was proposed by Dupin et al (1999) when
they demonstrated immunohistochemical staining of 10-30% of cells in patch stage KS and
attributed this limited staining in patch stage KS to the possibility that early KS is not a
monoclonal expansion of HHV8 infected cells. Several authors who have employed the use
of limited KS case numbers, have attempted to address the variability of HHV8 LNA-1
immunohistochemical staining (Hong et al 2003; Pak et al 2005; Patel et al 2004; Ramos da
Silva et al 2007). However, until now, there has not been a large study which has addressed
this variability.

The detection of focal and weak HHV8 LNA-1 immunohistochemical staining in 13% and
20% respectively of biopsies examined at the histopathology laboratory at CHBAH,
highlights that close scrutiny of the stained section is required. The presence of subtle forms
of nuclear HHV8 LNA-1 immunoreactivity can be easily overlooked resulting in an
unnecessary repeat biopsy and delay in initiation of treatment.
Furthermore, the findings of this study are that the CD4 count is not predictive of the intensity ($p = 0.878$) or distribution ($p = 0.846$) of HHV8 LNA-1 immunohistochemical staining. Factors other than the immune status and CD4 count may be responsible for the variability in HHV8 LNA-1 immunohistochemical staining. Bezold et al (2001) demonstrated that HHV8 copy numbers (as assessed by PCR) were similar in HIV negative and HIV positive patients and surmised that the immune status therefore had no bearing on the HHV8 copy number. At present, there is no published English literature which has addressed the issue of whether the intensity or distribution of HHV8 LNA-1 immunohistochemical staining is reflective of HHV8 copy number.

**Limitations:**

As this was a retrospective study, patients’ files were not available and clinical data could not be reviewed. Data regarding CD4 counts in some of the cases were not available and additional information regarding whether patients were on HAART at the time of biopsy could not be retrieved.

Automated immunohistochemical staining was introduced towards the latter half of 2009. Some of the HHV8 LNA-1 IHC stains reviewed were automated, the majority were manually stained. In addition, controls were external rather than internal and were therefore not available at time of review of the stains for comparison. However, the controls were checked prior to being issued to the reporting pathologist.

Some cases fulfilling criteria for review of HHV8 LNA-1 immunostaining could not be located in the archives and thus had to be excluded from the study.

There was an element of sampling bias as CHBAH is a tertiary hospital. Patients treated at this hospital will have greater burden of disease (i.e. lower CD4 counts and more advanced HIV infection) than those treated at local clinics. HHV8 LNA-1 immunohistochemical stains
showed predominantly strong and diffuse staining. This may be a reflection of the study population.

The proportion of patch, plaque and nodular stage KS for the total number available versus the proportion of the 3 stages in the second component of the study, ie. cases for review did not match. This lack of correlation with regard to proportions was due to selection being limited by available CD4 counts and HHV 8 LNA-1 immunohistochemical stains.

The criteria for defining focal (≤10%) versus diffuse staining (>10%) were arbitrarily chosen. It may be, that statistically significant findings may result from adjusting this cut off. This aspect may form the basis of a future prospective study.
Chapter 6: CONCLUSION

Kaposi sarcoma is a pathogenetically and morphologically diverse disease. It is thought to be both an inflammatory and neoplastic process. KS may present at almost any topographic site and may mimic other vasoformative lesions both clinically and histopathologically. The epidemiology has changed dramatically since HIV associated KS was first diagnosed in the 1980’s. With the introduction of HAART, the demographics may once again shift as the effects of therapy become apparent.

This study highlighted the burden of KS at CHBAH and addressed the spectrum of histopathology that may be seen including the interesting aspects of concomitant pathology and variability in HHV8 LNA-1 immunohistochemical staining.

The documentation of the features of Kaposi sarcoma and HHV-8 staining in this study provides a foundation on which further comparative research could be based in future.

The demographics of KS diagnosed at CHBAH are similar to those described by others (Mosam et al 2008; Sitas & Newton 2000 and 2009; Tukei et al 2011). The male:female ratio, mean age at presentation and CD4 counts are comparable.

The finding of concomitant pathology in 4,6% of biopsies is of value from both clinical and pathologic perspectives. Furthermore, there has been no formal previous documentation of concomitant pathology in extracutaneous KS.

Paediatric, endemic and visceral KS accounted for only limited proportions of cases. The typical microscopic characteristics of KS may be masked or absent in the special morphological variants.

The CD4 counts of patients were not predictive of the stage of KS or the intensity and distribution of HHV8 LNA-1 immunohistochemical staining. A statistically significant difference was noted in the distribution of staining between patch and nodular stage KS (p = 0,011).
Recommendations for future research:

- The effect of antiretroviral therapy on KS incidence at CHBAH both in adults and the paediatric age group.

- Comparison of HHV8 viral load in the peripheral blood, quantity of HHV8 viral DNA in tissue biopsies and intensity/distribution of HHV8 LNA-1 immunohistochemical staining.

- Immunohistochemical staining of cases with CD117 and cytogenetic analysis for C-kiit mutations to support treatment with Gleevec.
REFERENCES


HIV counselling and testing policy guidelines, 2010, Department of Health: 
http://www.sanac.org.za/resources/cat_view/1-resources


trial group staging system in the HAART era- the Italian co-operative group on AIDS and
tumours and the Italian cohort of patients naïve from antiretrovirals. *Journal of Clinical
Oncology, 21*:2876-2882.


### ANNEXURE 1: DATA COLLECTION SHEET

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MEMO

Date: 6/02/2013

To whom it may concern:

I hereby grant permission for Dr Reena D Mundial to utilise the National Health Laboratory Services database at Chris Hani Baragwanath Hospital for the purpose of her MMed research project in Kaposi sarcoma.

[Signature]

Dr Agnes Mapwete
Business Manager
NHLS, Chris Hani Baragwanath Hospital
ANNEXURE 3: ETHICS CLEARANCE CERTIFICATE

R14/49 Dr Reena D Mohanial
HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M130533

NAME: Dr Reena D Mohanial
(Principal Investigator)

DEPARTMENT: School of Pathology/Anatomical Pathology
National Health Laboratory Services

PROJECT TITLE: Kaposi Sarcoma, the Chris Hani Baragwanath
Academic Hospital Experience: Demographics of Kaposi Sarcoma and HHV8 Immunohisto-
chemical expression in a Retrospective Cohort of Cases

DATE CONSIDERED: 31/05/2013

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Dr S Pather

APPROVED BY: Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 23/07/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and ONE COPY returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report.

Principal Investigator's Signature

Date 2/06/2013

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES
Dear Dr Mohanlal,

PO Box 1740
Mabopana
2020

Johannesburg, South Africa

Dear Dr Mohanlal,

Master of Medicine: Approval of Title

We have pleasure in advising that your proposal entitled "Kaposi sarcoma, the Chris Hani Baragwanath Academic Hospital experience: demographics of Kaposi sarcoma and HCV/ HIV immunohistochemical expression in a retrospective cohort of cases," has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely,

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences
Dear Dr Mohanial,

PO Box 1740
Mubarton
2659
Gauteng

Johannesburg, South Africa

Dear Dr Mohanial

**Master of Medicine: Change of title of research**

I am pleased to inform you that the following change of title of your research report for the degree of Master of Medicine has been approved.

**FRCM:** The demographics of Kaposi sarcoma at Chris Hani Baragwanath Academic Hospital and CD117 immunohistochemical staining in a retrospective cohort of cases.

**TO:** Kaposi sarcoma, the Chris Hani Baragwanath Academic Hospital experience: demographics of Kaposi sarcoma and HHV8 immunohistochemical expression in a retrospective cohort of cases.

Yours sincerely,

[Signature]

Mrs Sandra Mann
Faculty Registrar
Faculty of Health Sciences