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CASTLEMAN'S DISEASE AT CHIS HANI BARAGWANATH ACADEMIC HOSPITAL - A RETROSPECTIVE STUDY

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A research report submitted to the faculty of Health Sciences, University of Witwatersrand, in partial fulfilment of the degree of Master of Medicine (Internal Medicine)

Johannesburg, 2018

DECLARATION

I, Lebogang Mirriam Mmusi declare that this research report is my own work. It is being submitted to the University of the Witwatersrand, Johannesburg, in partial fulfilment for the degree of Master of Medicine in the branch of Internal Medicine. It has not been submitted before for any degree at this University or any other University.

Lebogang Mirriam Mmusi

Date: _____

DEDICATION

I dedicate this research report to my beloved mother and brother.

ABSTRACT

Castleman's disease (CD) is a rare B-cell lymphoproliferative disorder that manifests clinically as unicentric or multicentric disease and pathologically as hyaline vascular, plasma cell or mixed variants. Multicentric Castleman's Disease (MCD) is aetiologically linked to HIV and HHV-8 and has recently been classified into HHV-8 positive and HHV-8 negative or idiopathic MCD subtypes.

In this study, there was a total of 41 patients diagnosed with CD during the period 01/01/1990 to 31/12/2015, in the Division of Clinical Haematology, Department of Medicine, CHBAH. The mean age at presentation was 40 years, with a range of 18-69 years. There were 24 males (24/41 – 59%) and 17 females (17/41 – 41%), with a male to female ratio of 1.4:1. Seventy eight percent of the patients were HIV sero-positive.

Lymphadenopathy was the most common clinical finding, occurring in 97.5% (39/40 patients) of the patients at presentation. Other common findings at presentation included fatigue (26/39 – 66.7%), hepatomegaly (25/39 – 64.1%), and fever, night sweats, weight loss, and splenomegaly (all occurring in 24/39 – 61.5%) of the patients at presentation. The two most common comorbidities seen in association with CD were Tuberculosis and Kaposi's sarcoma.

Morphologically, the mixed variant of CD was most frequently encountered (52%), followed by the plasma cell variant (28%) and hyaline vascular variant (20%). HHV-8 positivity was seen in 92% of the biopsies in which the stain was performed.

Therapy included both supportive care and specific modalities of treatment. In UCD, the mainstay of treatment was surgical resection, with a favourable outlook in all the patients. In MCD, combination

chemotherapy was the mainstay of treatment, with CHOP as the backbone and the addition of etoposide and rituximab in a number of patients.

Nineteen patients were lost to follow up (19/41 – 46.3%), and six patients (6/41 – 14.6%) are reported alive at the last follow up visit, while a total of 16 patients (16/41 – 39%) demised. Of the nineteen patients that were lost to follow up, twelve were not in remission (12/19 – 63.2%), and six had achieved complete remission (6/19 – 31.6%) at their last follow up visit. Of the six patients that were alive, five were reported to have achieved a complete remission at the last follow up visit (5/6 – 83.3%). Sixteen patients have been reported dead (16/41 – 39%), of which thirteen (13/16 – 81.2%) of them were reported to have died from some form of sepsis. The mean survival time for the patients in this study was 78.6 months.

ACKNOWLEDGMENTS

I would like to thank all the medical, nursing and allied healthcare professionals who were involved in the diagnosis, management and follow up of patients with Castleman's disease. In particular, I would like to thank all the staff of the division of Clinical Haematology, the Department of Medicine and the National Health Laboratory Services at CHBAH. And most importantly, I would like to thank all the patients whose data has been used for the purpose of this study.

LIST OF ABBREVIATIONS AND SYMBOLS

18F-FDG:	Fluorodeoxyglucose F 18
AIDS:	Acquired Immunodeficiency Syndrome
ALP:	Alkaline Phosphatase
ALPS:	Autoimmune Lymphoproliferative Syndrome
ANA:	Anti-nuclear Antibodies
ART:	Antiretroviral Therapy
BSF-2:	Human B-cell Stimulatory Factor-2
CD:	Castleman's Disease
CHBAH:	Chris Hani Baragwanath Academic Hospital
CHOP:	Cyclophosphamide, Vincristine, Doxorubicin, Prednisone
CRP:	C - reactive protein
cART:	Combined Antiretroviral Therapy
CT:	Computed Tomography
CVAD:	Cyclophosphamide, Vincristine, Doxorubicin, Dexamethasone
EBV:	Epstein - Barr virus
ECOG:	Eastern Cooperative Oncology Group
ESR:	Erythrocyte Sedimentation Rate
Gy:	Gray
HHV-8:	Human herpesvirus-8
hIL-6:	Human Interleukin-6
HIV:	Human Immunodeficiency Virus
HL:	Hodgkin Lymphoma
IgA:	Immunoglobulin-A
IgG1κ:	Immunoglobulin-G1 kappa

IgM:	Immunoglobulin-M
IL-1:	Interleukin-1
IL-10:	Interleukin-10
IL-6:	Interleukin-6
iMCD:	Idiopathic Multicentric Castleman's Disease
iMCD-NOS:	Idiopathic Multicentric Castleman's Disease not otherwise specified
IPL:	Idiopathic Plasmacytic Lymphadenopathy
ISH:	Isotopic in situ hybridization
KS:	Kaposi Sarcoma
KSHV:	Kaposi Sarcoma-associated herpesvirus
LANA-1:	Latency-associated nuclear antigen-1
mAb:	Monoclonal antibody
MCD:	Multicentric Castleman's Disease
MRI:	Magnetic Resonance Imaging
NHL:	Non-Hodgkin Lymphoma
NHLS:	National Health Laboratory Services
PCR:	Polymerase Chain Reaction
PEL:	Primary Effusion Lymphoma
PET:	Positron Emission Tomography
POEMS:	Polyneuropathy, Organomegaly, Endocrinopathy, Monoclonal proteins, Skin changes
SLE:	Systemic Lupus Erythematosus
SUV:	Standardised Uptake Value
TAFRO:	Thrombocytopenia, Anasarca, Fever, Reticulin myelofibrosis, Organomegaly
UCD:	Unicentric Castleman's Disease
USA:	United States of America

VEGF:	Vascular Endothelial Growth Factor
vIL-6:	Viral-analogue of Interleukin-6
λ:	Lambda

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1. CHAPTER 1: INTRODUCTION

1.1. Literature Review

Castleman's disease (CD) is a rare lymphoproliferative disorder. It is also known as angiofollicular lymph node hyperplasia and giant lymph node hyperplasia. It was first described in a case report in 1954, wherein a 40-year old male presented with fever, night sweats, fatigue and a non-productive cough. In addition, the patient was found to have an anterior mediastinal mass and laboratory investigations revealed anaemia and a raised erythrocyte sedimentation rate. He was treated empirically for tuberculosis prior to complete resection of the mediastinal mass. Benjamin Castleman, after whom the disease is named, described the histology of the mediastinal mass as lymph node hyperplasia with regressed germinal centres. Two years later, Castleman described a case series of 13 patients, with localized mediastinal lymph nodes. Clinically, most of these patients were asymptomatic and the mediastinal lymph nodes were found on routine chest roentgenographs. Histologically, two prominent features were noted, lymph node hyperplasia and marked capillary proliferation. This description of the lymph nodes later became known as Unicentric Castleman's Disease (UCD) (1-4).

1.1.1. Unicentric Castleman's Disease

Castleman's disease (CD) has been classified clinically into UCD and Multicentric Castleman's Disease (MCD). However, recently, the nomenclature has changed to further describe MCD with reference to its association with human herpesvirus-8 infection (HHV-8). As a result, MCD can now be classified into HHV-8 positive MCD and HHV-8 negative or idiopathic MCD (iMCD) (4,5).

As previously mentioned, early studies often described CD as a localized disease which we now know as UCD. UCD is rare, and is characterized by involvement of one lymph node area and often follows an indolent course. The majority of the patients are asymptomatic at presentation and often come to clinical attention when lymphadenopathy is found incidentally, either on clinical examination or on

imaging. However, a few patients may present with obstructive symptoms as a result of the enlarging mass compressing adjacent structures or causing impingement. Systemic symptoms such as fever, fatigue, drenching night sweats and significant loss of weight are not common in UCD (and the hyaline vascular morphological variant of CD), but have been described more commonly in patients with the plasma cell morphological variant of CD (4, 6).

There are no epidemiological studies that have explored the incidence of UCD outside of the United States of America (USA). The estimated approximate annual incidence of UCD is reported to be between 4900 to 5900 cases (7). It is often described as a disease affecting a younger population, with a median age of 35 years at presentation. With regard to gender, there is either an equal incidence between genders, or a slight female predominance (4, 6, 8).

An important characteristic of UCD, unlike MCD, is that it is not associated with HHV-8 infection or human immunodeficiency virus (HIV) infection (4, 9). However, a number of conditions have been noted to be associated with UCD such as paraneoplastic pemphigus, lymphoma – including Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), and follicular dendritic cell sarcoma (3, 4, 10, 11).

1.1.2. Multicentric Castleman's Disease – HHV-8 associated

In contrast to UCD, MCD is a 'systemic' disease that involves lymphadenopathy at multiple lymph node sites; systemic inflammatory symptoms such as fever, night sweats, weight loss, weakness and fatigue; hepatosplenomegaly; cytopenias and multi-organ system dysfunction due to the presence and production of excessive pro-inflammatory cytokines, especially interleukin (IL)-6. As previously mentioned, MCD is now further subclassified according to the presence of HHV-8 infection. Hence, with the new classification MCD can be divided into HHV-8 associated MCD and HHV-8 negative or idiopathic MCD (iMCD). Previously, MCD was classified according to the presence of HIV

infection, however, this is no longer the case as we now know that the HIV virus simply plays its role in rendering the patient immune-deficient, thus making the patient susceptible to HHV-8 infection (12). This state of immunodeficiency, allows for the replication of the HHV-8 virus within the plasmablastic cells of the lymph nodes and the release of cytokines leading to the clinical and pathological features which characterize the entity of MCD (5). A recent institutional case series by Oksenhendler et al. (2018), found that HHV-8 associated MCD was associated with a more aggressive clinical and biological presentation when compared to iMCD, and that in the context of concurrent HIV-infection, was associated with the worst prognosis. These patients were found to have a higher prevalence of splenomegaly, severe cytopenias, haemophagocytic syndrome and a higher incidence of lymphoma (13). This observation has been well documented in other studies as well (14-16).

The annual incidence of CD has been reported to be between 6500 to 7700 cases and approximately 75 percent are UCD and 25 percent are MCD (7). However, with the advent of the HIV pandemic, the prevalence of MCD, especially HHV-8 associated MCD has dramatically increased in HIV prevalent areas. A study looking at the incidence of HIV-associated MCD through a prospective HIV database of 56 202 patient-years of follow-up compared to that of Kaposi's sarcoma (KS), demonstrated an increased incidence of HIV-associated MCD and a decreased incidence of KS, since the introduction of antiretroviral therapy (ART) as part of the management of HIV. On multivariate analysis, the study also demonstrated that well preserved immune function, increased age, African race, a short known duration of HIV infection and no prior ART exposure were all risk factors for the development of HHV-8 associated MCD (17).

In a recent review of CD at Chris Hani Baragwanath Academic Hospital, the median age at presentation of MCD (in a predominantly HIV sero-positive population) was 36 years (range 18-64

years), with a male to female ratio of 1.2:1, demonstrating a younger age at presentation in keeping with the HIV sero-positivity (14).

1.1.3. Multicentric Castlemans Disease – HHV-8 negative/idiopathic

Idiopathic MCD (iMCD) is a relatively new entity, with a paucity of information regarding the condition. The diagnosis is made once the clinical and histological features of CD are noted and other causes known to present with a similar clinical picture have been excluded. These causes include infectious, autoimmune and neoplastic diseases (5). Of importance in making the diagnosis of iMCD, is that the patients must be negative for HIV or HHV-8 infection (5). iMCD accounts for 33-58% of all cases of MCD and typically affects individuals between the ages of 40 to 50 years (although it may occur at any age), with a slight male predominance (5, 18-20). The clinical presentation can range from mild lymphadenopathy with classical 'B' symptoms to more severe disease with systemic features involving extensive inflammation, hepatosplenomegaly, capillary leakage syndrome causing anasarca, pleural effusions and ascites, organ failure and eventually death (5, 18). Skin manifestations in the form of cherry haemangiomas have also been described (5, 18, 21). In a systematic review that included 127 patients with iMCD the following clinical and laboratory features were observed: fever, night sweats, unintentional weight loss, hepatomegaly or splenomegaly, features of fluid overload such as oedema and ascites, elevated inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), anaemia, thrombocytopenia, elevated blood urea and creatinine as well as proteinuria, hypoalbuminaemia, elevated IL-6 and vascular endothelial growth factor (VEGF) levels, positive Coomb's test, positive anti-nuclear antibody (ANA) test and hypergammaglobinaemia (20).

Idiopathic MCD is also associated with POEMS syndrome - characterized by polyneuropathy, organomegaly, endocrinopathy, a monoclonal immunoglobulin spike and skin changes. It is believed that the malignant plasma cells are responsible for secreting VEGF and other cytokines thereby

resulting in a clinical and histopathological picture similar to iMCD, including volume overload and renal disease (5). Approximately 37 to 60% of POEMS reported cases have histopathological features of CD and many patients with CD have demonstrated clinical features of POEMS syndrome, such as immunoglobulin-A (IgA) monoclonal gammopathy of undetermined significance as well as a sensory glove-and-stocking neuropathy (5, 22, 23). As a result of the great overlap between these two disorders, CD is recognized as a major criterion in the diagnostic criteria of POEMS syndrome (22).

Another association of iMCD is with regard to the TAFRO syndrome. This syndrome is characterized by thrombocytopenia (T), anasarca (A), fever (F), reticulin myelofibrosis (R), organomegaly (O) and normal or slightly elevated immunoglobulin levels. A study by Iwaki et al (2016), proposed that iMCD be classified clinically into two groups, TAFRO-iMCD and iMCD-NOS (not otherwise specified) (24). In this study, 25 patients diagnosed with iMCD and demonstrating clinical features of TAFRO syndrome were analysed. They noted that these patients usually presented acutely, with approximately three quarters of the patients (77%) having an Eastern Cooperative Oncology Group (ECOG) performance status of greater than one. Of importance was that the patients also had an aggressive clinical course; demonstrated refractoriness to corticosteroid therapy, had moderate to severe thrombocytopenia, high frequencies of anasarca, raised serum alkaline phosphatase (ALP) levels and normal gammaglobin levels (18, 24). In contrast, patients with iMCD-NOS had no clinical features of TAFRO, and their general condition at diagnosis was much better, with an ECOG performance status of 0 or 1. In addition, they had thrombocytosis, polyclonal hypergammaglobinaemia, less severe fluid accumulation, and plasma-cell type histopathological features (24). In the literature, this group of patients with iMCD-NOS or none-TAFRO have also been called idiopathic plasmacytic lymphadenopathy with polyclonal hypergammaglobinaemia, otherwise known as, IPL-type (19, 25).

In the diagnostic criteria of TAFRO-iMCD, there is a requirement for histopathological criteria to be fulfilled, involving histological findings in keeping with the diagnosis; three major criteria which could be either thrombocytopenia, anasarca, fever, reticulin fibrosis or organomegaly, absence of hypergammaglobulinaemia and small volume lymph nodes; and one or more minor criteria which could be either hyperplasia or normoplasia of megakaryocytes in the bone marrow or high levels of serum ALP without markedly raised serum transaminases (24). Furthermore, in order for the diagnosis to be made, certain medial conditions need to be excluded, viz., systemic lupus erythematosus (SLE), Epstein-Barr virus (EBV) infection, and neoplastic diseases such as lymphoma, POEMS syndrome and other malignancies (24).

1.1.4. Histological variants

Several histological (morphological) variants of CD have been described in the literature; hyaline-vascular, plasma-cell and mixed variants (6, 11). The hyaline-vascular variant is characterized by prominent vascular proliferation and hyalinization of the central portion of the follicles, surrounded by prominent mantle zones containing lymphocytes which are arranged in concentric layers thus giving the so-called impression of an ‘onion-skin’ appearance. Associated with this variant are sclerotic blood vessels which are often seen penetrating the central portion (germinal centre), and imparting a classical ‘lollipop’ appearance morphologically. The surrounding interfollicular stroma is made up of numerous hyperplastic vessels, plasma-cells, eosinophils and immunoblasts. This histological variant is commonly associated with UCD (2, 6, 11, 14).

In contrast, the plasma-cell variant has less distinctive features. It is characterized by diffuse proliferation of plasma-cells in the interfollicular stroma, together with immunoblasts, lymphocytes and histiocytes. The lymph node architecture remains relatively intact and there is variable germinal centre hyperplasia. This histological variant is commonly associated with MCD and a few cases of UCD. As most of these histological features are non-specific, it is important that conditions that may

give a similar appearance be excluded. These conditions include: B-cell lymphoma, such as marginal zone B-cell lymphoma with reactive follicles and plasmacytic differentiation; plasmacytomas; reactive lymphadenopathies associated with infection, autoimmune diseases such as rheumatoid arthritis and other causes of primary and acquired immunodeficiencies (6, 11, 26).

Human herpes virus-8 (HHV-8) associated MCD has its own characteristic histological features, which is now known as the plasmablastic variant of CD. Unlike the plasma-cell variant of CD, there are increased numbers of immunoblasts or plasmablasts in the outer mantle zones of the hyperplastic follicles and sometimes in the germinal centres as well. The interfollicular stroma is hypervascular with diffuse proliferation of plasma cells that are similar to those seen with the plasma-cell variant of CD. The nodal architecture remains well preserved. These plasmablastic cells demonstrate HHV-8 positivity by immunohistochemistry using monoclonal antibodies specific for viral proteins, such as the latency-associated nuclear antigen-1 (LANA-1). They are also found to express cytoplasmic immunoglobulin-M (IgM) and demonstrate lambda (λ) light chain restriction. Of particular interest regarding these cells is how some of them would coalesce to form microscopic collections, referred to as ‘microlymphomas’, which might transform into frank HHV-8 associated plasmablastic lymphomas (11, 16).

Lastly, there is the mixed variant, which is characterized by a combination of morphological features of hyaline-vascular and plasma-cell variants of CD, and is the classical histological variant associated with MCD (14, 27).

1.1.5. Pathophysiology

The pathophysiology of CD is poorly understood but it is believed to be multi-factorial. The role of viruses, namely HHV-8 and HIV, cytokines such as IL-6 and interleukin-10 (IL-10), and growth factors such as VEGF have all been incriminated in the pathogenesis of CD (28).

When CD was first described in 1954 and subsequently in 1956 by Benjamin et al., the entity was described as a benign lymph node hyperplasia, with histological features characteristic of a 'chronic non-specific inflammatory process', similar to a lymphadenitis. It was also suggested that, as many of the lymph nodes resected were from the thoracic cavity, they probably resulted from 'some low-grade, non-specific, chronic inflammatory process' (2).

Later on, Kellar et al. (1972), reviewed similar cases and discovered that CD was characterized by two distinct histological variants: hyaline-vascular and plasma-cell variants. They also described the associated systemic features that are often seen with the plasma-cell variant CD, including anaemia, raised ESR, hypergammaglobinaemia and hypoalbuminaemia; which were uncommon in the hyaline-vascular variant of CD (6). Of interest, was their theory that the histological description of the lymph nodes dissected, suggested an underlying inflammatory or infective process, similar to viral lymphadenitis. This coupled with the systemic features often associated with the plasma-cell variant of CD and, since most of the lymph nodes examined were from the thoracic cavity, an infectious agent that had a mode of transmission through the respiratory tract was postulated to play a pathogenic role in the development of CD. However, unfortunately none of the cultures from the specimens collected yielded positive results. It was these clinical and histological discoveries that lead to the search for viral pathogens as aetiological factors for CD (28).

Initially, the viral pathogen that was investigated was Epstein Barr Virus (EBV). It is well known that infection with EBV gives rise to several medical conditions including, infectious mononucleosis, lymphoproliferative syndromes, nasopharyngeal carcinoma, Burkitt's lymphoma and B-cell lymphomas in immunocompromised patients (29, 30). However, none of the studies in the literature were able to demonstrate infection with EBV as having any causal effect in the pathogenesis of CD (28).

The discovery of the KS-associated herpesvirus-like (KSHV), also known as Human Herpes virus-8 (HHV-8), by Chang et al. (1994), in KS lesions of patients infected with HIV, marked the beginning of exploring the association between HHV-8 and MCD (31). HHV-8 is a gamma herpesvirus that has been found in both endemic and HIV-associated KS (32). In the literature, there are numerous reports about KS being reported in patients with MCD (14, 27, 28, 33, 34). This discovery led to further interest in exploring the association between HHV-8 and CD. A study done by Soulier et al. (1995), demonstrated the association between HHV-8 and MCD (27). The study looked at 31 cases with all histological variants of CD. Of these patients, 14 were HIV-infected and 17 were HIV-negative. The study also included 34 HIV-negative cases with reactive lymph nodes that did not demonstrate any histological features suggestive of MCD, which were used as controls. A polymerase chain reaction (PCR) and Southern blot analysis was used to determine the frequency with which HHV-8 was detected from excised lymph nodes of the patients. The results showed that 7 of the 17 HIV-negative participants (41%) demonstrated evidence of HHV-8 infection and all 14 of the HIV-infected patients (100%), had HHV-8 detected in the lymph nodes. In the control group, only 1 of the 34 participants (3%) had HHV-8 detected in the lymph nodes. Following this landmark study, other studies have also confirmed the association between HHV-8 and MCD (27, 28, 35, 36).

Human Herpes virus-8 (HHV-8) infection has also been shown to be associated with a spectrum of lymphoproliferative disorders. Apart from HHV-8 associated MCD, there is also HHV-8 positive diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS), which often arises in the background of MCD; and HHV-8 positive germinotropic lymphoproliferative disorder (GLPD), which unlike the other two disorders is commonly seen in patients that are not HIV infected, but can occur in other immunosuppressed states such as post-transplantation (37).

The role of cytokines and growth factors in the pathogenesis of CD has been well documented in the literature, especially with regard to IL-6, IL-10 and VEGF (Vascular Endothelial Growth Factor). IL-

6, also known as Human B-cell stimulatory factor-2 (BSF-2), is a cytokine produced by various cells and is involved in a variety of functions. These include amongst others, inducing B-cell differentiation to antigen-producing cells, promoting the growth of plasmacytomas and acting as an autocrine growth factor in multiple myeloma, regulating the biosynthesis of acute phase proteins such as CRP and having a direct influence on haematopoietic progenitor cells in the bone marrow (26, 38). In a study by Yoshizaki et al. (1989), 2 patients were reviewed, one had UCD with a localized solitary mediastinal mass and a few systemic symptoms and laboratory abnormalities such as hypergammaglobulinaemia, raised ESR and increased levels of acute phase proteins, while the other patient had MCD (38). Interestingly, once the solitary mediastinal mass of the first patient was removed, all the clinical and laboratory abnormalities resolved and completely normalized within 4 weeks, while the patient with MCD did not show any improvement after the removal of one of the largest nodes in the abdomen. The study demonstrated a significant correlation between the serum IL-6 levels, lymph node hyperplasia, hypergammaglobulinaemia and raised acute phase reactants, suggesting that this cytokine is responsible for the clinical manifestations of CD (38). In the literature, there a number of mechanisms that have described the stimulation of IL-6 production (28). One mechanism described is that of the possibility that IL-6 production may be from HHV-8 infected cells, in CD. A study by Moore et al. (1996), demonstrated that HHV-8 can produce a viral analogue of IL-6 (vIL-6) which has a similar molecular structure as human IL-6 (hIL-6) and as a result, behaves in a similar way to the biological activities known to be due to hIL-6 (39).

Interleukin 10 (IL-10) is another cytokine that is believed to play a role in the pathogenesis of CD. Its role as a growth factor in the pathogenesis of acquired immunodeficiency syndrome (AIDS)-related B-cell lymphoma as well as its involvement in the proliferation of HHV-8 infected cells in primary effusion lymphomas (PEL) is well documented in the literature (40-42). A prospective study by Oksenhendler et al. (2000), demonstrated that a strong correlation exists between plasma IL-6 and

IL-10 as well as high HHV-8 viral loads in HIV-infected patients with MCD (43). The exact mechanism of action of IL-10 in the pathogenesis of CD remains unknown.

There is also a role for angiogenesis-promoting cytokines in CD. This was suggested by the increased vascularity associated with CD. The most common and relevant angiogenesis-promoting cytokine is VEGF, which specifically promotes growth and proliferation of vascular endothelial cells and can also increase blood vessel permeability (44). A study by Foss et al. (1997), showed that 5 out of 8 cases of CD (63%), demonstrated VEGF positivity by isotopic in situ hybridization (ISH) in the vascularized germinal centres, compared to cases of tonsillar tissue, some of which were infected with infectious mononucleosis, as controls. None of the controls demonstrated the presence of VEGF (45). Nishi et al. (1999), also arrived at a similar conclusion showing elevated levels of VEGF in the sera and supernatants of cultured lymph nodes of 2 patients with CD, one with UCD and the other with MCD, compared to normal controls (44). Both these studies, highlight the role of VEGF in the pathogenesis of CD (44, 45).

In conclusion, with regard to the pathogenesis of CD, it is evidently clear that it is a multifactorial process of which little is known. However, it can be summarized as initially involving B-cells in the lymph nodes, with the production of IL-6 and to some degree IL-10 from the mantle zones. This process is stimulated by HHV-8 infection in the majority of cases, but in some cases the cause remains unknown. The release of these cytokines and in turn the production of VEGF leads to the characteristic features associated with CD. The systemic features that are associated with MCD are a result of increased levels of IL-6, as well as widespread HHV-8 infection in immunocompromised individuals (28).

However, the entity of iMCD, which represents those cases of CD which are HIV-negative and HHV-8 negative also needs some explanation with regards to its pathogenesis. The dilemma with regard to

iMCD, is that it is a diagnosis of exclusion. The characteristic clinical and histopathological features that describe CD are often driven by the hypercytokinaemia, as previously mentioned. However, in the absence of HIV and HHV-8 infection, other causes of viral, inflammatory and neoplastic origin have to be sought. This was well described by Fajgenbaum et al. (2014), who suggested three hypotheses that would explain the pathogenesis of the hypercytokinaemia in iMCD (5). The following three mechanisms were proposed: i) the systemic inflammatory disease mechanisms, through the production of autoantibodies or inflammatory gene mutations; ii) the paraneoplastic syndrome mechanism, whereby malignant or benign tumour cells within the lymph nodes or at extranodal sites secrete cytokines and iii) the viral driven mechanism by a non-HHV-8 virus (5). It is believed that either one or more of these mechanisms is responsible for initiating the events that lead to the hypercytokinaemia in iMCD. However, further research is needed to understand the pathogenesis of iMCD in greater detail (5).

1.1.6. Investigation and Management

Unicentric Castleman's Disease (UCD) is a rare disease, and as such, most of the literature regarding its management is based on systematic reviews and case series and reports, without there being any randomized control trials (46). The diagnosis of UCD can be very challenging, because no diagnostic criteria have been proposed to date and secondly, there are a number of conditions that can mimic the histopathological and clinical features of UCD. Such conditions include infectious diseases such as toxoplasmosis lymphadenitis and HIV lymphadenitis; malignancies such as lymphoma, follicular dendritic sarcoma and plasmacytoma; autoimmune diseases such as rheumatoid arthritis and other conditions such as follicular hyperplasia and autoimmune lymphoproliferative syndrome (ALPS) (4, 46). Once a diagnosis of UCD is suspected based on histopathological and clinical findings, imaging is the next modality to assess the extent of the disease, especially in assessing whether the involved lymph node(s) are resectable or not. Various imaging modalities can be used including plain chest radiographs, ultrasonography, contrast-enhanced computed tomography (CT), magnetic resonance

imaging (MRI) scans or positron emission tomography (PET) scans (3, 4). The role of the PET scan includes providing additional information regarding the metabolic status of the lymph nodes. Fluorodeoxyglucose F 18 (18F-FDG)-avid lymph nodes have been observed in patients with CD, and of interest is that the standardised uptake values (SUV) demonstrated in these patients are often less than those found in patients with malignancies such as lymphoma or metastases (47, 48). Another benefit of the FDG-PET scan is that it can identify abnormal uptake in lymph nodes that are not enlarged and therefore is more sensitive than the contrast-enhanced CT in assessing and monitoring patients with CD (4). Once a diagnosis of UCD has been confirmed and a site identified as resectable through imaging, complete surgical resection of the involved lymph node(s) in most cases is curative. This is considered to be the gold standard approach for the treatment of UCD (13, 46, 49, 50). As mentioned previously, the majority of patients with UCD are asymptomatic at presentation, however, the few that are symptomatic, interestingly, have demonstrated complete resolution of symptoms after surgical resection of the involved lymph node(s) (6, 51). In a systematic review by Talat et al. (2012), of 404 published cases of CD, approximately 69% (278/404) had UCD and of this, up to 94% (262/278) had undergone resective surgery or diagnostic lymph node biopsy. Of interest was that UCD was associated with a significantly higher overall survival rate than MCD, with disease-free survival rates at 3 and 5 years of 90% and 81%, respectively. Failure to treat UCD by complete surgical resection was associated with a mortality rate of approximately 18% (49).

There are a few patients with UCD who have disease that is not amenable to complete surgical resection. This could be as a result of the lymph node(s) involving vital structures such as major blood vessels or the main bronchus if there is mediastinal involvement, or due to the increased size of involved lymph node(s). In such cases debulking the lymph node mass either by surgery, or embolization, or neoadjuvant therapy with Rituximab may be considered, to reduce the local symptoms or compression on vital structures, thereby allowing for complete surgical resection of the involved lymph node(s) to be undertaken (4, 49, 50, 52, 53).

Another alternative treatment option for unresectable UCD is the use of radiotherapy. A number of studies have reported favourable responses to radiotherapy, especially at radiation doses of 30 to 45 Gray (Gy) (54, 55). However, Neuhof and Debus (2006), analysed the clinical outcome of 5 patients with UCD during the period between 1991 and 2005 (56), and reported serious acute and late toxicities such as paraneoplastic pemphigus vulgaris and stenosis of the oesophagus and trachea.

In some patients who are asymptomatic and have a low burden of disease, and cannot be treated with surgery or radiotherapy, close observation can be the treatment modality of choice, given the indolent course of the disease (3). However, follow-up is recommended for all patients with UCD due to the reported cases of disease reoccurrence (4, 49, 54, 55).

The management of HIV and HHV-8 associated MCD often requires both specific and supportive treatment modalities. Due to the aggressive nature of the disease, systemic therapy is often favoured. As a result of the paucity of randomized control trials, treatment options are based on a few non-randomized prospective studies, small case series and expert opinions (3).

The supportive treatment modalities used for patients with HIV associated MCD include aspects such as transfusion of blood or blood products, analgesia, tumour lysis prophylaxis with allopurinol and HIV antiretroviral therapy (14).

Specific treatment modalities include the use of anti-herpesvirus agents, single-agent and combination chemotherapy and monoclonal antibody therapy (57). A number of studies have demonstrated that a rituximab-based treatment is the mainstay of treatment for HIV associated MCD either alone or when used with combination chemotherapy (15, 58-60). However, a few case reports have reported failure of rituximab as monotherapy, in patients who have a more aggressive disease

with clinical evidence of organ failure (61, 62). A review by Bower, 2010 (57), demonstrated a stratified approach in managing HIV associated MCD by initially assessing patients on the basis of disease severity by looking at parameters such as poor performance status (ECOG-PS > 1), and clinical evidence of organ failure. He demonstrated that by using a combination of weekly rituximab (375mg/m^2) together with intravenous (IV) etoposide (100mg/m^2) for a period of 4 weeks for patients with aggressive HIV associated MCD, there was an overall 2 year survival of 85% and using rituximab monotherapy (375mg/m^2 weekly for 4 weeks) in patients without any organ failure and with good performance status, there was an overall 2 year survival of 100% (57). In HIV sero-positive patients, in addition to chemotherapy, concomitant antiretroviral therapy should form the cornerstone of management in these patients (14).

Antiretroviral therapy forms part of the treatment modalities for managing HHV-8 associated MCD, in patients who are HIV infected. In the literature, the increased incidence of HIV and HHV-8 associated MCD since the introduction of antiretroviral therapy is well described (17, 63). However, antiretroviral agents assist in reducing the HIV viral load and improving the immune function, thereby preventing other opportunistic infections and reactivation of KS, as well as providing better tolerance to chemotherapy (57).

The rationale for using anti-herpesvirus agents in the management of HIV and HHV-8 associated MCD is based on the understanding that the clinical manifestations present have been shown to correlate with increased serum viral loads of HIV suggesting underlying viral replication (43). In vitro studies have demonstrated that KSHV replication is sensitive to ganciclovir, foscarnet and cidofovir (64). However, there is very limited data on their efficacy (65, 66). A case series by Casper et al. (2004), demonstrated both clinical and virological response in 3, HIV-infected HHV-8 associated MCD cases, with intravenous and oral ganciclovir (67).

Human Immunodeficiency Virus (HIV) associated MCD is well recognized for being a relapsing and remitting illness, with approximately 25% of patients showing a relapse at 3 years of follow-up (57). Fortunately, second remissions can be achieved by re-treatment with rituximab-based therapy (68).

The management of iMCD has been based on 3 treatment strategies: i) anti-inflammatory and immunosuppressive therapies, ii) cytotoxic elimination of cells that are responsible for the hypercytokinaemia and iii) blocking the IL-6 signalling pathway by using monoclonal antibodies (5).

The use of corticosteroids in the management of MCD is well described, especially for their anti-inflammatory properties in improving symptoms during acute exacerbations of iMCD (5). However, when used as monotherapy they have demonstrated very limited efficacy (28, 34, 51). Other immunosuppressive therapies such as cyclosporine A, have also been used for treating iMCD as the clinical presentation can lean more towards that of a systemic disease (5).

The use of cytotoxic chemotherapy for the management of MCD is based on models used to treat NHL (28). Single-agent chemotherapy such as cyclophosphamide, vinblastine and etoposide have been used to induce remission in the treatment of iMCD, but with very limited efficacy (20). Rituximab monotherapy, on the other hand, is well known for its treatment in HIV associated MCD, however, there is very limited data on its role in the treatment of iMCD (20). The use of combination chemotherapy with cyclophosphamide, vincristine, doxorubicin and either prednisone (CHOP) or dexamethasone (CVAD), in severely ill iMCD patients have demonstrated good responses (5, 69). However, relapses and side-effects are frequently encountered (5).

Treatment targeted at blocking the IL-6 signalling pathway includes tocilizumab, an anti-IL-6 receptor monoclonal antibody (mAb), and siltuximab, an anti-IL-6 mAb (5). Tocilizumab is a humanized anti-IL-6 receptor mAb that inhibits the activity of IL-6 by improving symptoms and

biochemical abnormalities that are encountered in patients with MCD (70, 71). An open label, prospective study by Nishimoto et al. (2005), evaluated the safety and efficacy of tocilizumab in 26 patients with iMCD (70). The initial treatment dose was 8mg/kg every 2 weeks for 16 weeks, with an extension phase allowing variable dosing, being permitted thereafter. The study reported significant improvement in nutritional status and fatigue scores as well as lymphadenopathy and inflammatory markers such as CRP, after 16 weeks of therapy. In addition, there was also a significant increase in haemoglobin levels compared to baseline. Tocilizumab is approved for the treatment of MCD in Japan only (5, 72).

Siltuximab, is a chimeric, human-murine immunoglobulin (Ig) G1κ mAb against hIL-6 (72-75). In a multicentre, randomised, double-blinded, phase II trial, the safety and efficacy of siltuximab was assessed in 79 HIV negative patients with symptomatic iMCD (73). Siltuximab demonstrated durable tumour and symptomatic response at a significantly higher rate compared to placebo (34% vs 0%, p = 0.0012) (5, 72, 73, 74). Based on these results, Siltuximab is approved for the treatment of iMCD in the US, Europe and many other countries (72). While both of these mAbs (siltuximaband tocilizumab) have demonstrated clinical efficacy, they require lifelong administration and are not effective in all patients (5).

The use of other treatments such as bortezomib, a selective proteasome inhibitor that targets plasma cells, thalidomide an immunomodulatory agent, and anakinra, an interleukin (IL)-1 receptor inhibitor, have been reported in a few case reports of the treatment of iMCD. However, larger studies are needed to assess the reported efficacy of these agents (5, 28, 76, 77).

As our understanding of the definition of subtypes and new entities related to and associated with CD become clearer and more defined, the therapeutic landscape and modalities of treatments to manage

these entities will become clearer and easier to apply in everyday practice. However, currently this remains an interesting and ongoing challenge.

2. CHAPTER 2: PATIENTS AND METHODOLOGY

2.1. Study design

A retrospective record review of adult patients with a confirmed histological diagnosis of CD during the period 01/01/1990 to 31/12/2015, in the Division of Clinical Haematology, Department of Medicine, at CHBAH.

2.2. Sample population

The sample population included all patients known to the Division of Clinical Haematology, Department of Medicine, with CD, during the period 01/01/1990 to 31/12/2015. Additionally, only demographic details were included of patients diagnosed histologically (as determined by records in the pathology department) with CD.

2.3. Inclusion criteria

- Patients ≥18 years of age
- Histological diagnosis of CD
- Patient seen in the Division of Clinical Haematology, Department of Medicine at CHBAH, during the period 01/01/1990 to 31/12/2015
- Lymph node biopsies or tissue biopsies done at CHBAH, during the period 01/01/1990 to 31/12/2015 that confirmed CD, even though the patients may have not been seen or followed-up by the Division of Clinical Haematology, Department of Medicine.

2.4. Exclusion criteria

- Patients < 18 years of age
- Patients without a histological diagnosis of CD on lymph node or tissue biopsy

2.5. Collection of data

The data collection was done retrospectively by reviewing the records of all adult patients with a histological diagnosis of CD, during the period 01/01/1990 to 31/12/2015. The information from the patient records was then entered into a data collection sheet (see Appendix A). In addition, after gaining permission from the NHLS Anatomical Pathology Department, CHBAH, histological and demographic data of patients with a diagnosis of CD was entered onto a prepared data sheet (See Appendix B).

2.6. Data analysis

Once the data was collected using a questionnaire, it was captured onto a spreadsheet using Microsoft Excel®. A line listing format was used to enter the different variables. These variables included patient demographics, clinical presentation, histological diagnosis of Castleman's disease, treatment and final clinical outcome. The variables were then analysed into frequency distributions which allowed for the calculation of the arithmetic mean, median and mode. Calculations for the range and standard deviations were performed and association between two variables were measured using the chi-square test and corresponding p-values were determined.

3. CHAPTER 3: RESULTS

3.1. Demographics

The data obtained was from 41 eligible patients, who were diagnosed with Castleman's Disease (CD), during the period of 01/01/1990 to 31/12/2015. The patients were all known to the Division of Clinical Haematology at Chris Hani Baragwanath Hospital (CHBAH). The age distribution of the patients is summarised in table 3.1 below.

Table 3.1 Age at presentation

Analysis of Age	
N	41
Mean (±SD)	40.8 (±11.52) years
Median (IQR)	40 (33-47) years
Range	18 - 69 years

The mean age at presentation was 40 years (range: 18-69). There were 17 females (17/41 41%) and 24 males (24/41 – 59%), with a male to female ratio of 1.4:1. The gender distribution is summarised in the pie chart below.

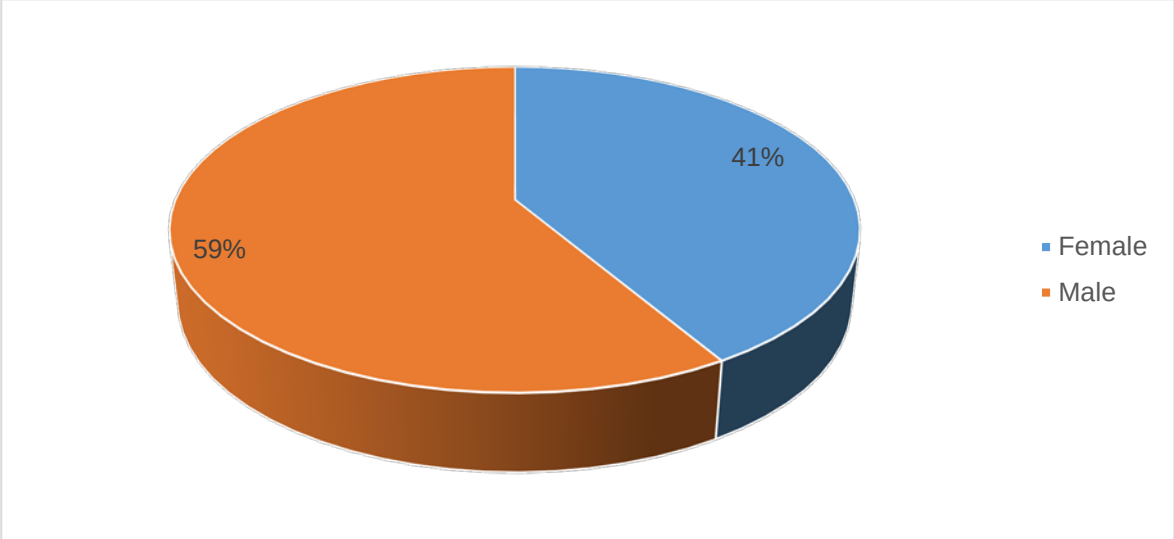


Figure 3.1 Gender distribution of patients with CD

The ethnic distribution of the patients is summarised in the chart below.

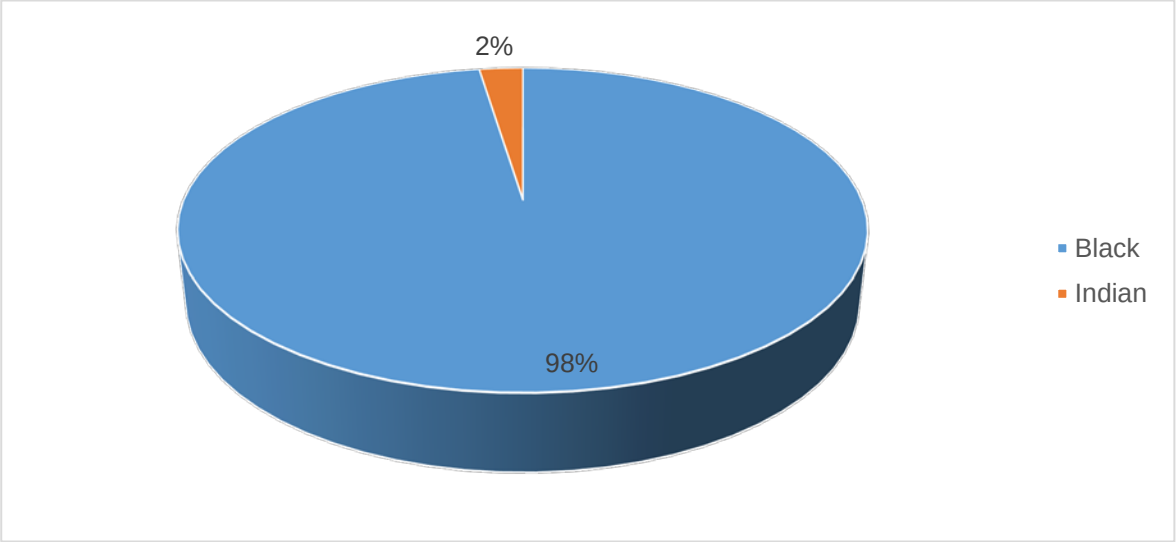


Figure 3.2 Ethnic distribution of patients with CD

Most of the patients were Black Africans (98%, 40 patients) while the other 2% (1 patient) was Indian.

Figure 3.3 shows the number of patients seen per year from 1990 to 2015, indicating an increase in the number of patients in the latter years.

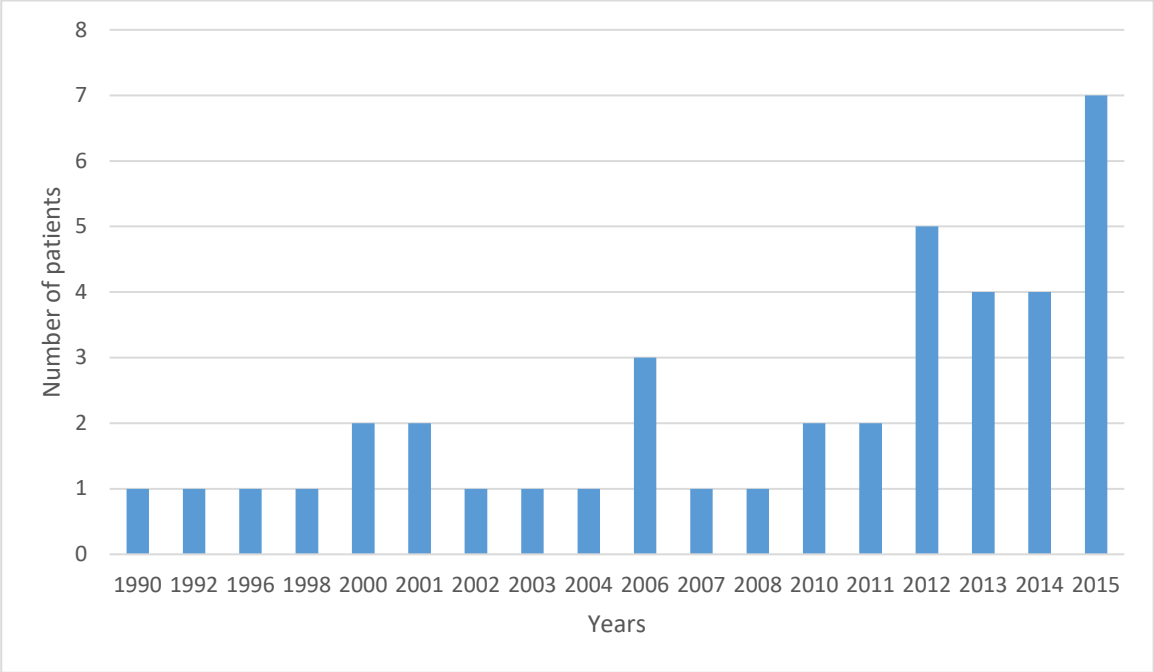


Figure 3.3 Bar graph which depicts the number of patients with Castleman's disease at Chris Hani Baragwanath Academic Hospital from 1990 to 2015

3.2. Clinical presentation

Thirty-two patients (32/41 - 78%) were HIV sero-positive and nine (9/41 - 22%) were HIV sero-negative at presentation. Of those that were HIV sero-positive, twenty-five (25/32 - 78%) were known to be on combination antiretroviral therapy (cART), while seven (7/32 – 22%), were newly diagnosed with HIV. Approximately half of the patients (53.1%), were diagnosed with HIV less than a year from the time of diagnosis of CD. Information regarding the association with HIV in this study cohort is summarized in table 3.2 below.

Table 3.2 Summary of HIV infection and cART

		Frequency	Percent
HIV	Positive	32	78.0
	Negative	9	22.0
	Total	41	100.0
cART	Yes	25	78.1
	No	3	9.4
	Unknown	4	12.5
	Total	32	100.0
cART in relation to HIV diagnosis	< 1 Year	13	40.6
	≥ 1 - < 2 years	2	6.3
	≥ 2 - < 5 years	1	3.1
	Unknown	16	50.0
	Total	32	100.0
Duration of HIV prior to CD diagnosis	< 1 Year	17	53.1
	≥ 1 - < 2 years	1	3.1
	≥ 2 - < 5 years	3	9.4
	≥ 5 years	5	15.6
	Unknown	6	18.8
	Total	32	100.0

The median CD4 count at presentation was 287 μ/ℓ, with a range of 12-1111 μ/ℓ (see table 3.3 below). The HIV viral load is shown in figure 3.4. Only 12.5% of all the HIV seropositive patients had suppressed viral loads (<20 copies / ml). This is summarized in table 3.3 and figure 3.4 below.

Table 3.3 CD4 count at time of clinical diagnosis of CD

Mean (± SD)	287.08 (±279.12) μ/ℓ
Median (IQR)	213 (57.5-213) μ/ℓ
Range	12 – 1111 μ/ℓ

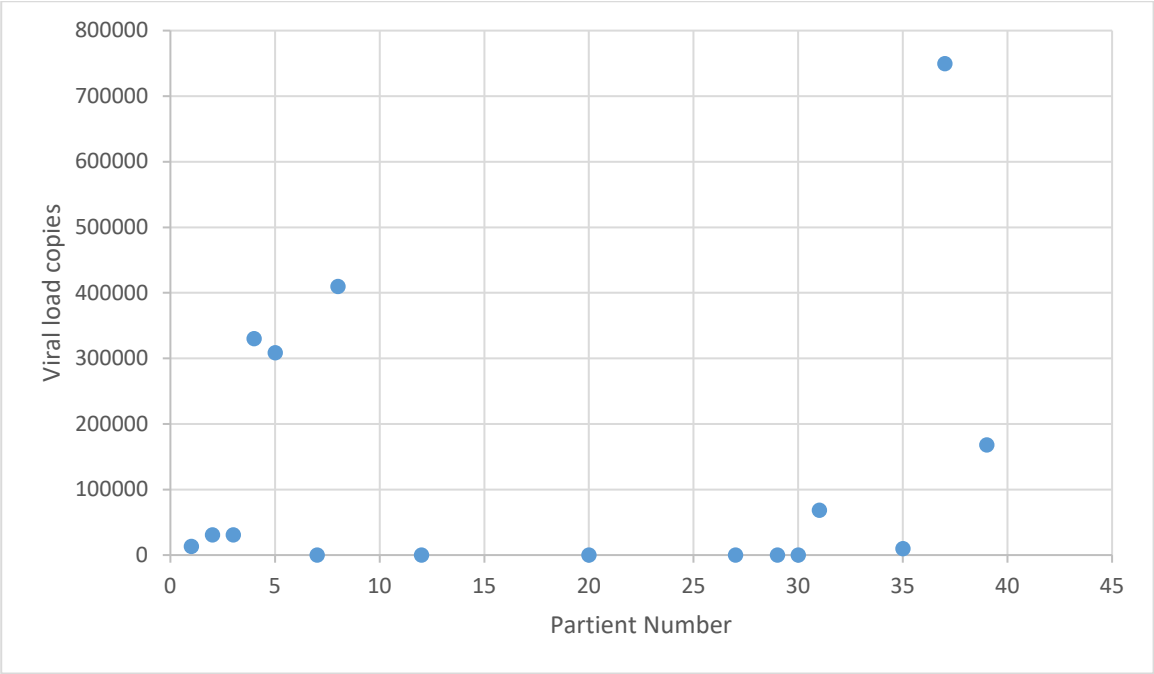


Figure 3.4 HIV Viral load at clinical presentation

The ECOG (Eastern Cooperative Oncology Group) Performance Status (PS) was used to ascertain the impact of the disease had on the patient's daily living and functional abilities. Of the patients with a documented PS, 73% presented with a PS of <2, (16/22 – 73%).

Table 3.4 ECOG Performance Status

<2	**≥2**
73% (16/22)	27% (6/22)

The clinical findings of the patients with CD at presentation are presented in table 3.5 below.

Table 3.5 Summary of clinical findings of patients with CD

Clinical finding	n	Missing	Frequency	Percentage
Lymphadenopathy	40	1	39	97.5
Fatigue	39	2	26	66.7
Hepatomegaly	39	2	25	64.1
Fever	39	2	24	61.5
Night sweats	39	2	24	61.5
Weight loss	39	2	24	61.5
Splenomegaly	39	3	24	61.5
Pallor	38	3	17	44.7
Bone pain	39	2	4	15.4
Jaundice	38	3	4	10.5
Bleeding	39	2	4	10.3

Lymphadenopathy was the most common clinical finding, occurring in 97.5% (39/40 patients) of the patients at presentation. Other common findings at presentation included fatigue (26/39 – 66.7%), hepatomegaly (25/39 – 64.1%), and fever, night sweats, weight loss, and splenomegaly (all occurring in 24/39 – 61.5%) of patients at presentation.

Table 3.6 details the full blood count of the patients with CD at presentation.

Table 3.6 Full blood count

Variable	No.	Missing	Mean	Std Dev	Median	Lower Quartile	Upper Quartile	Min	Max
Initial Haemoglobin (Hb)	41	0	8.73	2.83	8.30	6.60	9.95	4.00	15.10
Initial White cell count (WCC)	41	0	7.46	4.19	6.62	4.85	9.17	1.11	22.45
Initial Platelets (Plts)	40	1	218.30	156.21	195.00	130.25	276.50	22.00	748.00
Initial Mean Corpuscular Volume (MCV)	39	2	88.66	10.42	87.30	82.70	92.40	65.10	128.00
Post treatment Hb	21	20	10.69	2.39	9.90	8.65	12.80	7.20	16.00
Post treatment WCC	21	20	7.06	4.93	5.50	3.71	8.29	2.28	20.73
Post treatment Plts	21	20	214.10	153.90	209.00	65.00	329.00	19.00	504.00
Post treatment MCV	21	20	89.97	7.69	91.80	86.60	94.40	72.70	103.70

The mean haemoglobin level at presentation was 8.73 g/dl (range 4.00-15.10 g/dl) and post treatment it increased to a mean of 10.69 g/dL (range 7.20 – 16.00 g/dl). The mean platelet count was 218 x 10^9/l (range 22.00 – 748 x 10^9/l), while the mean white cell count was 7.46 x 10^9/l (range 1.11 - 22.45 x 10^9/l) at presentation.

Paired samples t-test was performed to assess whether there was a significant difference in the full blood count results for patients prior to and post treatment. The results are shown in table 3.7 below.

Table 3.7 Comparison of the full blood count before and after chemotherapy

		N	Mean	Std. Deviation	t	P-value
Pair 1	Post chemotherapy Hb	21	10.69	2.39	3.200	0.005
	Initial Hb	21	8.25	2.34		
Pair 2	Post chemotherapy WCC	21	7.06	4.93	0.341	0.737
	Initial WCC	21	6.79	4.28		
Pair 3	Post chemotherapy Plts	21	214.10	153.90	0.126	0.901
	Initial Plts	21	209.29	131.27		
Pair 4	Post chemotherapy MCV	21	89.97	7.69	-0.785	0.442
	Initial MCV	21	91.74	11.70		

The results show that the mean haemoglobin level had increased significantly from 8.25 g/dL at presentation to 10.69 g/dL after chemotherapy (p-value = 0.005). There were no significant differences in the white cell count (p-value = 0.737), platelet count (p-value = 0.901) and mean corpuscular volume (p-value = 0.7442).

A number of co-morbidities were seen in patients with CD. The most common co-morbidities that were encountered, included concomitant TB (tuberculosis) and KS (Kaposi's sarcoma), in 47.1% and 32.4% of patients, respectively. Other associated co-morbidities are shown in table 3.8 below.

Table 3.8 Co-morbidities associated with CD

Association	Frequency	Percentage
Tuberculosis	16	47.1
Kaposi's sarcoma	11	32.4
Primary effusion lymphoma	2	5.9
Autoimmune haemolytic anaemia	3	8.8
Immune thrombocytopenia	2	5.9
Microlymphoma	1	2.9
Bullous pemphigoid	0	0
Adenocarcinoma	1	2.9
Pure red cell aplasia	1	2.9
Hypoglycaemia	2	5.9
Lymphoma	2	5.9

In addition to the above, the chest X-ray and CT (Computerized Tomographic) scan were reviewed for evidence of mediastinal involvement in patients with CD. Based on the findings at imaging, mediastinal involvement was documented in 65.2% (15/23 of patients) of the patients with CD. This is represented in table 3.9 below.

Table 3.9 Radiological evidence of mediastinal involvement

Mediastinal involvement	Frequency	Percentage
No	8	34.8
Yes	15	65.2
Total	23	100.0
Missing	18	

The histological subtype of CD was reviewed and documented in all except one patient. The predominant histological subtype was mixed variant CD, which accounted for 52.5% (21/40) of the cases. This was followed by the plasma-cell variant CD in 27.5% (11/40), and the hyaline-vascular variant CD accounting for 20% (8/40) of the patients. This is summarized in table 3.10 below.

Table 3.10 Morphological subtype of the lymph node biopsy

Morphology	Frequency	Percentage
Hyaline vascular	8	20
Mixed variant	21	52.5
Plasma cell variant	11	27.5
Total	**40**	**100.0**
Missing data	1	

Human Herpes virus-8 staining only became available from 2006 onwards at our histopathology laboratory at CHBAH. As such, there was no report on the staining of previous lymph node biopsies. As a result of this, staining was only done on twenty-four of the lymph node biopsies. Twenty-two (22/24 – 91.7%) of the lymph node biopsies stained positive for HHV-8. This is shown in the table 3.11 below.

Table 3.11 HHV-8 staining

HHV-8 staining	Frequency	Percentage
Positive	22	91.7
Negative	2	8.3
Total	**24**	**100.0**
Not done	10	
No data	7	

Table 3.12 Histology of lymph node

	n	Frequency	Percentage
Kaposi's sarcoma on lymph node	41	10	24.4
Microlymphoma	41	1	2.4
Lymphoma	41	1	2.4

Of the lymph node biopsies, in addition to establishing the diagnosis of CD, 24.4% (10/41) of the patients had features of KS, one patient showed features of a microlymphoma (1/41, 2.4%) and one patient had evidence of lymphocytic lymphoma (1/41, 2.4%). This is presented in table 3.12 above.

In relation to multicentric and unicentric CD, the following breakdown was noted (see table 3.13 below).

Table 3.13 Comparison of MCD and UCD

	Frequency	Percentage
MCD	36	90.0
UCD	4	10.0
Total	**40**	**100.0**
Missing data	1	

The presence of MCD and UCD was further compared with the morphological subtype of CD. This comparison is detailed in table 3.14 below.

Table 3.14 Morphological comparison of MCD and UCD

		MCD/UCD		Total	P-value
		MCD	UCD		
	number	36	4	40	
Morphology of lymph node	Mixed variant	21 (58.3%)	0 (0%)	21 (52.5%)	.013
	Plasma cell variant	10 (27.8%)	1 (25%)	11 (27.5%)	
	Hyaline vascular variant	5 (13.9%)	3 (75%)	8 (20%)	

Three quarters of the UCD had a histological subtype of hyaline-vascular, whereas MCD had a predominantly mixed variant MCD subtype on histology (58.3%, 21/36) and plasma-cell variant (27.8%, 10/36).

A comparison between MCD and UCD in HIV sero-positive patients, as well as the different morphological subtypes as seen in HIV sero-positivity, is detailed in table 3.15 below.

Table 3.15 Comparison between MCD and UCD in HIV-seropositive patients

Histological subtype	MCD	UCD	Total	P-value
	32	0	32	
Mixed variant	20 (62.5%)	-	20 (62.5%)	-
Plasma cell variant	8 (25%)	-	8 (25%)	
Hyaline vascular variant	4 (12.5%)	-	4 (12.5%)	

None of the HIV sero-positive patients had UCD, all of them presented with MCD and the most common histological subtype was the mixed variant CD, at a frequency of 62.5% (20/32).

Among the thirty-six patients with MCD, the differences between HIV sero-positivity/negativity with regards to demographics, clinical presentation and outcome was established. Independent sample t-tests were conducted for matric variables such as age and initial haemoglobin, while the chi-square test was used for categorical variables such as gender. This is summarized in table 3.16 below.

Table 3.16 Comparison HIV sero-positivity versus HIV sero-negativity among MCD patients

		HIV		Total	Test	P-value
	n	Positive (32)	Negative (4)			
Age	36	39.34±43.75	8.09±21.34	39.83±9.95	T-test	0.709
Gender						
Female	36	11 (34.4%)	2 (50%)	13 (36.1%)	Chi-square	.609
Male		21 (65.6%)	2 (50%)	23 (63.9%)		
Race - Black	36	32 (100%)	4 (100%)	36 (100%)	Chi-square	-
Fever	36	20 (62.5%)	4 (100%)	24 (66.7%)	Chi-square	.278
Night sweats	36	21 (65.6%)	3 (75%)	24 (66.7%)	Chi-square	1.000
Weight loss	36	21 (65.6%)	3 (75%)	24 (66.7%)	Chi-square	1.000
Fatigue	36	21 (65.6%)	4 (100%)	25 (69.4%)	Chi-square	.290
Bleeding	36	4 (12.5%)	0 (0%)	4 (11.1%)	Chi-square	1.000
Bone pain	31	3 (9.4%)	2 (50%)	5 (13.9%)	Chi-square	.084
Pallor	34	15 (50%)	1 (25%)	16 (47.1%)	Chi-square	.604
Jaundice	34	3 (10%)	1 (25%)	4 (11.8%)	Chi-square	.409
LAD	35	30 (96.8%)	4 (100%)	34 (97.1%)	Chi-square	1.000
Hepatomegaly	34	22 (73.3%)	3 (75%)	25 (73.5%)	Chi-square	1.000
Splenomegaly	34	21 (70%)	2 (50%)	23 (67.6%)	Chi-square	.580
Initial Haemoglobin	36	8.24±8.88	2.45±3.2	8.31±2.5	T-test	.637
Initial White cell count	36	7.29±8.56	4.13±4.02	7.43±4.08	T-test	.564
Initial Platelet count	36	189.91±35.00	123.23±169.38	207.69±136.06	T-test	.024
Initial MCV	35	89.89±82.95	11.23±5.27	89.09±10.9	T-test	.236
Outcome						
Alive	36	5 (15.6%)	0 (0%)	5 (13.9%)	Chi-square	0.731
Dead		13 (40.6%)	2 (50%)	15 (41.7%)		
Lost to follow-up		14 (43.8%)	2 (50%)	16 (44.4%)		

Mean ±Standard Deviation for metric data and n (%) for categorical data

LAD – lymphadenopathy

Except for the mean platelet count for the patients with MCD and HIV sero-positivity that was 189.91 x 10^9/l compared to patients with MCD and HIV sero-negativity (123.23 x 10^9/l) (p-value = 0.024), none of the other parameters as shown in table 3.16 was statistically significantly different. This is likely to be unreliable in view of the very small number of patients with HIV sero-negativity.

3.3. Treatment

Treatment was divided into supportive care and specific modalities of treatment. Supportive care included analgesia, tumour lysis prophylaxis with allopurinol and the use of blood and blood products (see table 3.17 below).

Table 3.17 Comparison of supportive treatment modalities

	n	Missing	Frequency	Percentage
Analgesia	40	1	31	77.5
Allopurinol	41	0	31	75.6
Blood transfusions	41	0	19	46.3
Blood products	41	0	14	34.1

Specific treatment included standard combination chemotherapy (CHOP), and chemotherapy with or without the addition of rituximab and etoposide. Seventeen patients (17/41 – 41.5%) received CHOP, fifteen (15/41 – 36.6%) received CHOP with etoposide (CHOEP) and only three patients received rituximab (3/41 – 7.3%) (see table 3.18 below). None of the patients received any anti-herpes viral agents such as valganciclovir. However, all the patients had a biopsy of a lymph node or at times total removal of the tumour tissue for diagnostic purposes. All HIV sero-positive patients who were not on cART were commenced on antiretroviral therapy.

Table 3.18 Chemotherapy

Treatment option	N	Missing	Frequency	Percentage
CHOP	41	0	17	41.5
CHOEP (CHOP with etoposide)	41	0	15	36.6
Rituximab	41	0	3	7.3

3.4. Outcome

Nineteen patients were lost to follow up (19/41 – 46.3%), and six patients (6/41 – 14.6%) are reported alive at the last follow up visit, while a total of 16 patients (16/41 – 39%) demised. This is shown in table 3.19 below.

Table 3.19 Outcomes

Total 41	Frequency	Percentage
Alive	6	14.6
Lost to follow up	19	46.3
Demised	16	39

Of the nineteen patients that were lost to follow up, twelve were not in remission (12/19 – 63.2%), and six had achieved complete remission (6/19 – 31.6%) at their last follow up visit. Of the six patients that were alive, five were reported to have achieved complete remission at the last follow up visit (5/6 – 83.3%). Sixteen patients have been reported dead (16/41 – 39%), of which thirteen (13/16 – 81.2%) of them were reported to have died from some form of sepsis. This is summarized in table 3.20 below.

Table 3.20 Summary of the outcomes

Total 41	Frequency	Percentage
Alive	6 (5 – remission) (1 – Not remission)	14.6
Lost to follow up	19 (6 – In remission) (12 – Not in remission) (1 – Unknown)	46.3
Demised	16 (13 – sepsis) (1 – tumour lysis syndrome) (1 – pulmonary oedema) (1 – unknown, died at home)	39.0

Table 3.21 depicts the average survival of 78.6 months for the patients in this study.

Table 3.21 Survival analysis (months)

Means and Medians for Survival Time			
Mean[a]			
Estimate	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
78.624	13.623	51.923	105.325
a. Estimation is limited to the largest survival time if it is censored.			

The Kaplan-Meier survival curve for all the patients in the study is shown in figure 3.5 below.

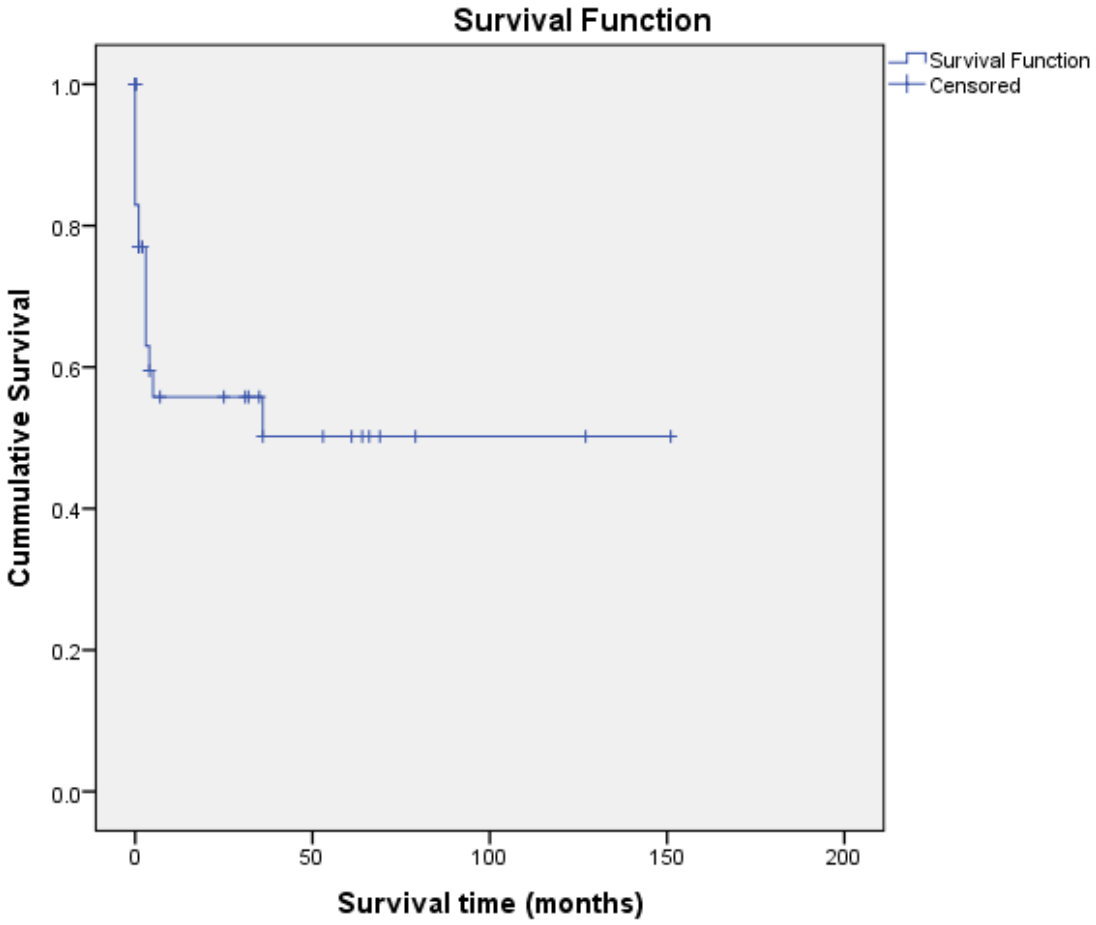


Figure 3.5 Survival time of patients with CD

The mean survival time was 78.6 months for the patients in this study.

3.5. Data from National Health Laboratory Services (NHLS)

Data from the NHLS at CHBAH for the period 01/01/1990 to 31/12/2015, was obtained to ascertain the number of lymph node biopsies that were seen with a diagnosis of CD. A total of sixty-four cases were identified during this period. Sixty one percent (39/64 – 61%) of the cases were male and 39% (25/64) were female, with a male to female ratio of 1.56:1. The predominant histological subtype was the plasma cell variant of CD which was identified in 81% (52/64) of the lymph nodes examined. Human herpes virus-8 immuno-histochemical staining was positive in 87.5% of the lymph nodes (and in 95% where the stain was performed), while 73% of the lymph nodes were from patients who were documented as having HIV sero-positivity (25% were unknown and 2% negative). Kaposi's sarcoma was also concomitantly diagnosed in 37.5% (24/64) of the lymph nodes and histological evidence of TB was only identified in 3 of the lymph node biopsies. A diagnosis of plasmablastic lymphoma was confirmed in two cases and a large B-cell lymphoma in one patient. This is summarized in table 3.22 below.

Table 3.22 Data from the NHLS

Variable	Options	Frequency	Percent
Gender	Female	25	39.1
	Male	39	60.9
Lymph node morphology	HV	7	10.9
	MV	5	7.8
	PC	52	81.3
HHV-8	Positive	56	87.5
	Negative	3	4.7
	No data	5	7.8
KS	Yes	24	37.5
	No	40	62.5
Lymphoma	Yes (plasmablastic lymphoma)	2	3.1
	Yes (Large B-cell lymphoma)	1	1.6
	No	61	95.3
TB	Yes	3	4.7
	No	61	95.3
HIV	Positive	47	73.4
	Negative	1	1.6
	No data	16	25.0

HHV-8=Human Herpes Virus 8; KS=Kaposi's sarcoma; TB=Tuberculosis; HIV=Human Immunodeficiency Virus;

HV=Hyaline vascular; MV=Mixed variant; PC=Plasma Cell variant

Ninety two percent (43/47) of the patients were HIV sero-positive and had HHV-8 positivity on lymph node biopsy. Only one case was reported as being HIV and HHV-8 negative on lymph node biopsy.This could represent a patient with HHV-8 negative or idiopathic MCD. The relationship between HIV and HHV-8 is represented in table 3.23 below.

Table 3.23 Comparison of HIV infection and HHV-8 positivity

		HIV			Total	P-value
		Positive	Negative	Unknown		
		47	1	16	64	
HHV-8	Positive	43 (91.5%)	0 (0%)	13 (81.3%)	56 (87.5%)	<0.001
	Negative	1 (2.1%)	1 (100%)	1 (6.3%)	3 (4.7%)	
	Unknown	3 (6.4%)	0 (0%)	2 (12.5%)	5 (7.8%)	

A comparison between HIV infection, Kaposi sarcoma and HHV-8 immuno-histochemical staining of the lymph node biopsies is presented in table 3.24 below.

Table 3.24 Comparison between HIV, Kaposi sarcoma and HHV-8 positivity

	Frequency	Percent
HIV and Kaposi sarcoma only	0	0.0
HIV and HHV8 only	26	40.6
HIV, HHV8 and Kaposi sarcoma	17	26.6

The most common histological subtype among HIV sero-positive patients was the plasma cell variant at a frequency of 83% (39/47).

Table 3.25 Comparison between various histological subtypes and HIV infection

		HIV			Total	P-value
		Positive	Negative	Unknown		
	n=	47	1	16	64	
Lymph node Morphology	Hyaline vascular variant	4 (8.5%)	1 (100%)	2 (12.5%)	7 (10.9%)	.053
	Mixed variant	3 (6.4%)	0 (0%)	2 (12.5%)	5 (7.8%)	
	Plasma cell variant	40 (85.1%)	0 (0%)	12 (75%)	52 (81.3%)	

Of the lymph nodes that stained positive for HHV-8, 91.1% (51/56) had the plasma cell variant of CD (see table 3.26 below).

Table 3.26 Comparison between HHV-8 and various histological subtypes

		HHV-8			Total	P-value
		Positive	Negative	Unknown		
	n=	56	3	5	64	
Lymph node Morphology	Hyaline vascular variant	4 (7.1%)	2 (66.7%)	1 (20%)	7 (10.9%)	<0.001
	Mixed variant	1 (1.8%)	0 (0%)	4 (80%)	5 (7.8%)	
	Plasma cell variant	51 (91.1%)	1 (33.3%)	0 (0%)	52 (81.3%)	

4. CHAPTER 4: DISCUSSION

Castleman's disease (CD) is a rare B-cell lymphoproliferative disorder, first described by Benjamin Castleman in 1954 (1, 2). It is a heterogenous disorder, which manifests itself clinically as a unicentric (solitary; localized) or multicentric disease and pathologically as hyaline vascular, plasma cell or mixed variants (6). Multicentric Castleman's Disease (MCD) is aetiologically linked to human herpes-virus 8 (HHV-8) infection, and more recently has been classified into HHV-8 positive and HHV-8 negative or idiopathic MCD (iMCD) (4, 5, 27). Furthermore, MCD is also associated with immunosuppression, being encountered more frequently in HIV positive populations (14, 17).

Sub-Saharan Africa is the epicentre of the HIV pandemic, with over 7 million individuals being affected/infected with the disease in South Africa (78). HIV and the resultant acquired immunodeficiency syndrome (AIDS) is associated with an increased risk of infection and malignancy, primarily as a result of the immunosuppression that underlies and is a major consequence of the infection (9, 17). HIV significantly increases the risk of specific malignancies, with the major impact in South Africa being in relation to the increased incidence of lymphoma (both Non-Hodgkin and Hodgkin lymphoma) (79, 80). As such, it is not surprising that CD, which is a B-cell lymphoproliferative disorder is gradually increasing in our patient population, as the burden of the HIV pandemic continues to manifest and unfold. This assumption and impression underpins the reason why this study was undertaken – to document the epidemiology, demographics and clinical profile of CD at CHBAH.

Although CD is rare in South Africa, there is indeed a noticeable increase in the incidence of the disease, as observed over the 26 year period of this study. This is particularly true of the number of patients seen in the last 5 years (22/41 – 54%), which is more than double the number of patients seen over the preceding 21 years (19/41 – 46%). This increase is mainly attributable to the ongoing burden

of HIV, with a sero-positivity rate of 78% in the current study. Furthermore, this trend of an increasing number of newly diagnosed patients with CD is continuing at CHBAH (personal communication with the Division of Clinical Haematology).

In this study, there was a total of 41 patients diagnosed with CD during the period 01/01/1990 to 31/12/2015, in the Division of Clinical Haematology, Department of Medicine, CHBAH. The mean age at presentation was 40 years, with a range of 18-69 years. There were 24 males (24/41 – 59%) and 17 females (17/41 – 41%), with a male to female ratio of 1.4:1. While the age is similar to that described in the literature, there is no marked male predominance as seen in MCD, as the major risk factor for the acquisition of HIV in our patients (i.e. heterosexual contact), is different from other cohorts in the Western world, with homosexuality and intravenous drug use being the dominant risk factors (15, 17).

The majority of the patients (40/41 – 98%) in this study were of Black African descent, in keeping with the demographic of the patients seen and treated at CHBAH. Almost three quarters of the patients (73%) had a good ECOG performance status of < 2. In contrast to other studies, MCD was the predominant clinical subtype, being present in 36/40 - 90%, of the patients, while UCD only accounted for the remaining 4/40 – 10%, of the patients (7, 8, 10, 13). However, the clinical features as seen in our patients is similar to that described in the literature.

Lymphadenopathy was the most common clinical finding, occurring in 97.5% (39/40 patients) of the patients at presentation. Other common findings at presentation included fatigue (26/39 – 66.7%), hepatomegaly (25/39 – 64.1%), and fever, night sweats, weight loss, and splenomegaly (all occurring in 24/39 – 61.5%) of the patients at presentation. The mean levels of the blood counts at presentation were as follows: Haemoglobin – 8.7 g/dl; white cell count – 7.46 x 10^9/l; platelets – 218 x 10^9/l.

In addition, a number of co-morbidities were noted in our patients in this study. Tuberculosis (TB) was the major co-morbidity, seen in 47% of the patients. This is likely to be reflective of the high prevalence of TB in our population and more particularly, the patients who are HIV sero-positive (81). In addition, TB is well documented as being seen more frequently in association with the lymphoproliferative disorders (82). Kaposi's sarcoma was present in 32% of the patients at presentation. The high association with KS is due to the common aetiological association of KS and CD to HHV-8 (27). Similarly, 6% of the patients had primary effusion lymphoma, which is also aetiologically linked to HHV-8 (83). Other associations in this study included auto-immune haemolytic anaemia, immune thrombocytopenia, microlymphoma, bullous pemphigoid, adenocarcinoma, pure red cell aplasia and hypoglycaemia (see table 3.8).

In CD, there are two distinct morphological variants that have been described. These include the hyaline vascular and the plasma cell variant. The mixed type or variant refers to the presence of both the hyaline vascular and plasma cell varieties in the same biopsy specimen (6). The hyaline vascular variety is most commonly associated with UCD, while the plasma cell and mixed variants are classically seen in MCD (6). The morphological subtypes encountered in our study were: mixed variant (52%), plasma cell variant (28%) and hyaline vascular (20%).

The immunohistochemical stain for HHV-8 only became available from 2006 onwards at the Anatomical Pathology Department, National Health Laboratory Services (NHLS). As a result of this, the stain was only performed in 24 of our patients. However, a significant number of the biopsies were positive for HHV-8 (22/24 -92%), while 2/24 -8% were negative.

Of the 41 patients in the current study, 32/41 – 78% were HIV sero-positive. Of those that were HIV sero-positive, 25/32 - 78%, were known to be on combination antiretroviral therapy (cART), while 7/32 – 22%, were newly diagnosed with HIV. Approximately half of the patients (53.1%), were

diagnosed with HIV in a period less than a year from the time of diagnosis of CD. The mean CD4 count in the sero-positive patients was 287 u/l (range: 12 – 1111 u/l). One hundred percent of the HIV sero-positive patients had MCD clinically, while morphologically the mixed variant (20/36 – 62.5%) was most commonly encountered (see table 3.15).

A comparison was made between the HIV sero-positive and HIVsero-negative patients with MCD (see table 3.16). Except for the mean platelet count at presentation, there were no statistically significant differences in the various parameters examined between the two groups (see table 3.16). The lack of a difference in these two groups is likely to be due to the very small number of HIV sero-negative patients compared to the sero-positive patients.

In addition to retrospectively reviewing the patients known to the Division of Clinical Haematology, Department of Medicine, CHBAH with CD, data from the Anatomical Pathology Department, NHLS, at CHBAH was obtained for the period 01/01/1990 to 31/12/2015. This was done in order to ascertain the number of lymph node biopsies that were seen with a histological diagnosis of CD. Where other information was documented on the histology report, this was collated and is shown in table 3.22. However, as only the histology (biopsy/pathology) report was reviewed, and additionally, as this was performed retrospectively, the information obtained is incomplete.

A total of 64 cases with a confirmed diagnosis of CD were identified during the above period. This figure is higher than the 41 patients known to the Division of Clinical Haematology for the same period. The additional cases could have comprised of the following: i) Paediatric cases, ii) Patients not referred to the Division of Clinical Haematology, iii) Patients who were non-compliant and patients who may have died prior to referral.

The pathology data set as shown in table 3.22 is in many respects similar to the findings in the current retrospective study, with respect to the gender ratio, HIV and HHV-8 sero-positivity and presence of concomitant KS. However, only a histological diagnosis of concomitant TB was ascertained and a history of past/current TB was not available. Additionally, the pathological variants are somewhat different, with the dominant histologically documented variety being the plasma cell variant, in contrast to the mixed variant in the current study. This could be explained by the evolving nomenclature in the literature, with only two varieties (hyaline vascular and plasma cell variant) being used in the literature initially, and subsequently, with the increasing association of HIV, the inclusion of the mixed variant (14, 15, 17). Moreover, all patients known to the Division of Clinical Haematology have their histology reviewed at a combined morphology meeting, following which a comprehensive/revised diagnosis is made.

Therapy for CD includes both supportive care and specific modalities of treatment. In UCD, surgical resection is the mainstay of treatment and is usually curative. This formed the backbone of the treatment in our patients with UCD, who after surgical resection have remained in long term remission. However, follow up is recommended in patients with UCD, as patients may rarely develop complications such as the development of lymphoma or relapse (8, 49, 55).

In addition to supportive care, a wide variety of specific modalities of treatment are available and are used in MCD. This includes antiviral (anti-herpesvirus) and antiretroviral drugs where a viral association is found, corticosteroids, chemotherapy, monoclonal antibodies, immunomodulatory agents, radiotherapy and splenectomy.

Specific treatment in our patients included standard combination chemotherapy (CHOP), and chemotherapy with or without the addition of rituximab and etoposide. Seventeen patients (17/41 – 41.5%) received CHOP, fifteen (15/41 – 36.6%) received CHOP with etoposide (CHOEP) and only

three patients received rituximab (3/41 – 7.3%) (see table 3.18). None of the patients received any anti-herpesvirus agents such as valganciclovir or monoclonal antibodies other than rituximab (anti-CD20 monoclonal antibody). All HIV sero-positive patients received concomitant combination antiretroviral therapy (cART).

Nineteen patients were lost to follow up (19/41 – 46.3%), and six patients (6/41 – 14.6%) are reported alive at the last follow up visit, while a total of 16 patients (16/41 – 39%) demised. Of the nineteen patients that were lost to follow up, twelve were not in remission (12/19 – 63.2%), and six had achieved complete remission (6/19 – 31.6%) at their last follow up visit. Of the six patients that were alive, five were reported to have achieved a complete remission at the last follow up visit (5/6 – 83.3%). Sixteen patients have been reported dead (16/41 – 39%), of which thirteen (13/16 – 81.2%) of them were reported to have died from some form of sepsis. The mean survival time for the patients in this study was 78.6 months.

5. CHAPTER 5: CONCLUSION

Since the discovery of CD more than sixty years ago by Benjamin Castleman, there has been significant progress in our understanding of the various aspects of the disease, including the aetiology, pathophysiology, morphology, clinical features and management of the disease.

From being a rarely diagnosed entity, the muticentric variety of CD, i.e. MCD has increased over the past decade, primarily as a result of the association of HIV, being highly prevalent in our patient population.

Multicentric Castleman's Disease (MCD) is the most common variety of CD seen in the current study. The prognosis of HIV-associated MCD has improved with optimization and control of the HIV (using antiretroviral therapy), prophylaxis and treatment of opportunistic infections as well as optimization of chemotherapy using drugs such as etoposide and rituximab.

Therefore, in the current HIV era, CD should be regarded as a potentially treatable disease with a reasonable long term outlook and not as a rare disease with a fatal outcome.

Study Limitations:

- In view of the retrospective nature of the study, information was incomplete or missing in some of the patients.
- Confounding factors such as environmental and immunological influences may have not been considered at the time the patients presented with the disease. Due to the retrospective nature of the study, these factors may have also influenced the outcome of the study.
- CD is a rare lymphoproliferative disorder and as a result, the sample size was small.

- There was also selection bias because only patients seen at CHBAH, were included in the study. This may have not been an entirely true reflection of the disease as seen in other sectors of the population and other parts of the country.
- Non-compliance and patients lost to follow up is not uncommon in our setting and has an impact on the therapeutic response and outcome in our patient population with CD.

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6. APPENDICES

6.1. Data Collection Sheet

Demographics of study						
Study No:						
Date of birth*	Date:	Month:	Year:	Age:		
Gender	Male ☐	Female ☐				
Date of diagnosis	Date:	Month:	Year:			
Date of referral to haematology	Date:	Month:	Year:			
Performance status at presentation	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
Race	B ☐	W ☐	C ☐	A ☐	Other:	
HIV status	Negative ☐	Positive ☐	CD4:	VL:		
Duration of HIV prior to diagnosis of CD	<1 year ☐	≥1 - <2 years ☐	≥2 - <5 years ☐	≥5 years ☐		
ARVs	No ☐	Yes ☐				
Commencement of ARVs in relation to HIV diagnosis	<1 year ☐	≥1 - <2 years ☐	≥2 - <5 years ☐	≥5 years ☐		
TB	No ☐	Yes ☐	Rx:			
Kaposi's sarcoma	No ☐	Yes ☐	Rx:			
Autoimmune haemolytic anaemia	No ☐	Yes ☐	Rx:			
Pure red cell aplasia	No ☐	Yes ☐	Rx:			
Primary effusion lymphoma	No ☐	Yes ☐	Rx:			
Microlymphoma	No ☐	Yes ☐	Rx:			
Adenocarcinoma	No ☐	Yes ☐	Rx:			
Bullous Pemphigoid	No ☐	Yes ☐	Rx:			
Immune thrombocytopenia	No ☐	Yes ☐	Rx:			
Hepatitis B	No ☐	Yes ☐	VL:		Rx:	
Hepatitis C	No ☐	Yes ☐	VL:		Rx:	
Hypoglycaemia	No ☐	Yes ☐	Rx:			
Nephrotic syndrome	No ☐	Yes ☐	Rx:			
Other, not stated above						
Details of other						

(* Date of birth is only to be used for calculating the patient's correct age and not as an identifying variable)

Clinical features					
Fever	Yes ☐	No ☐			
Night sweats	Yes ☐	No ☐			
Weight loss	Yes ☐	No ☐	Duration:	Quantity (kg):	
Fatigue	Yes ☐	No ☐			
Bleeding	Yes ☐	No ☐			
Bone pain	Yes ☐	No ☐			
Pallor	Yes ☐	No ☐			
Jaundice	Yes ☐	No ☐			
Lymphadenopathy	Yes ☐	No ☐	Region:	Size:	
Hepatomegaly	Yes ☐	No ☐			
Splenomegaly	Yes ☐	No ☐			
Other clinical features not stated					
Radiological features of mediastinal involvment	Yes ☐	No ☐	CXR:	CT:	
Other radiological features not stated					
Bone marrow infiltration	Yes ☐	No ☐			
Morphology of lymph node biopsy	HV ☐	PC ☐	M ☐		
HHV-8 immunostaining on biopsy	Yes ☐	No ☐	Positive ☐	Negative ☐	
Evidence of Kaposi sarcoma on lymph node biopsy	Yes ☐	No ☐			
Evidence of microlymphoma on lymph node biopsy	Yes ☐	No ☐			
Evidence of lymphoma on lymph node biopsy	Yes ☐	No ☐			
	Initial		**Post completion of Rx**		**Last visit**
Haemoglobin					
WCC					
Platelets					
MCV					
Differential count: (lymph/neutro/mono/baso/eosino					
Smear					
Na					
K					
Cl					
CO2					
Urea					
Creatinine					
CRP					

(HV: hyaline vascular; PC: plasma cell; M: mixed variant)

Treatment			
	No	**Yes**	**Duration**
Supportive treatment			
Analgesia			
Allopurinol			
Blood transfusion			
Blood products			
Specific treatment			
Herpesvirus agents: valganciclovir			
Antiretroviral therapy			
Corticosteroids			
Monoclonal antibodies: Siltuximab/toxilizumab			
Immunomodulatory agents			
Splenectomy			
Radiotherapy			
Surgery			
CHOP			
CHOEP (etoposide)			
Rituximab + CHOP			
Other, not stated			
Details of other			

Final clinical outcome				
	Yes	**No**		
Dead			Cause/s:	Survival time:
Alive				
Lost to follow-up			Date last seen:	Disease status:

6.2. Data Collection from pathology record

Demographics of study				
Study No:				
Date of birth*	Date:	Month:	Year:	Age:
Gender	Male ☐	Female ☐		
Date of diagnosis	Date:	Month:	Year:	
Morphology of lymph node biospy	HV ☐	PC ☐	M ☐	
HHV-8 immunostaining on biopsy	Yes ☐	No ☐	Positive ☐	Negative ☐
Other, not stated				
Details of other				

(HV: hyaline vascular; PC: plasma cell; M: mixed variant; *Date of birth is only to be used for calculating the patient's correct age and not as an identifying variable)

6.3. Case Recording Listing

(Information to be kept separately by study investigator)

Study No.	Haem file No.	Hospital No.	Initials	Contact No.	Physical address

6.4. Ethics Approval

R14/49 Dr Lebogang Mirriam Mmusi et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160728

NAME: (Principal Investigator)	Dr Lebogang Mirriam Mmusi et al
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Castleman's Disease at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	29/07/2016
DECISION:	Approved uncontionally
CONDITIONS:	
SUPERVISOR:	Prof Moosa Patel
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	17/10/2016

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 Research Office Secretary in Room 10004, 10th floor, Senate House/3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/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**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in July and will therefore be due in the month of July each year.

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

6.5. Turn-it-in report

0501206p:Final_-_MMed_-_Mmusi_(002).docx

ORIGINALITY REPORT

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1	www.intechopen.com Internet Source	3%
2	"2017 ACR/ARHP Annual Meeting Abstract Supplement", Arthritis & Rheumatology, 2017 Publication	1%
3	Moosa Patel, Vinitha Philip, Atul Lakha, Sugeshnee Pather, Muhammed Faadil Waja, Lucille Singh, Mohamed Arbee. "Chapter 11 Multicentric Castleman's Disease", InTech, 2015 Publication	1%
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5	Raymond S.M. Wong. "Unicentric Castleman Disease", Hematology/Oncology Clinics of North America, 2018 Publication	1%
6	"Rare Rheumatic Diseases of Immunologic Dysregulation", Springer Nature America, Inc, 2019	1%

Division of Haematology, Department of Medicine, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg
Chris Hani Road, Diepkloof, Soweto. Tel: +27 11 9339377, Fax: +27 11 9339449, email: moosa.patel@wits.ac.za

04 December 2018

The Chair

Postgraduate Studies Committee

Faculty of Health Sciences

University of the Witwatersrand

Re: Turn-it-in report: Dr Lebogang Mirriam Mmusi (0501206P) – MMed: 'Castleman's Disease at Chris Hani Baragwanath Academic Hospital: A retrospective review'

I have reviewed the Turn-it-in report of Dr Mmusi's MMed dissertation. The report identifies a similarity index of 25%. Much of this similarity relates to recurring terminology and

definitions which are standardized. The other information which bears a similarity has been appropriately referenced.

Thank you

Yours sincerely

Moosa Patel MBChB, FCP(SA), MMed(Wits), FRCP(Lond.), PhD(Wits)

Professor and Head of Clinical Haematology, Department of Medicine, Chris Hani Baragwanath Academic Hospital and the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

6.6. Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Lebogang Mirriam Mmusi (Student number: 0501206P) am a student registered for the degree of Masters in Medicine (Internal Med) in the academic year 2018.

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.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 06/12/2018