Plasmablastic Lymphoma in HIV positive patients in the Free State Province of South Africa

by

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Rad Onc M.Med

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DECLARATION

I herewith declare that the work as submitted is the result of my own, independent, investigation. Support in this study is recognised in the acknowledgements.

I further declare that this work is submitted for the first time and in relation to the M Med degree.

Dedication

Firstly I want to thank my husband who has been my rock and support in writing my thesis. I would not have been able to complete this thesis without your support and love. My two basset hounds Fred and Grietjie, my parents and my brothers and sisters for all their words of encouragement and support. Thank you.

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1. <u>Abbreviation list in alphabetical order:</u>

aaIPI - Age- adjusted International Prognostic Index

AIDS - Acquired immune deficiency syndrome

ART - Antiretroviral Therapy

CART - Combination Antiretroviral Therapy

CDE - Cyclophosphamide, Doxorubicin, Etoposide

CHOP - Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisone

CODOX-M/IVAC - Cyclophosphamide, Doxorubicin, Vincristine, high dose Methotrexate / Ifosfamide, Etoposide and high dose Cytarabine

CVP - Cyclophosphamide, Vincristine, Prednisone

DA-EPOCH - Dose adjusted - Etoposide, Doxorubicin, Vincristine, Cyclophosphamide, Prednisone

DICE - Dexamethasone, Ifosfamide, Cisplatin, Etoposide

DLBCL - Diffuse large B-cell lymphoma

EBV - Ebstein-Barr Virus

ECOG - Eastern Cooperative Oncology Group

HAART - Highly active antiretroviral therapy

HB - Hemoglobin

HHV8 - Human Herpesvirus - 8

HIV - Human immunodeficiency virus

Hyper - CVAD - Cyclophosphamide, Vincristine, Doxorubicin, Dexamethasone (hyperfractionated)

IPI - International Prognostic Index)

LDH - Lactate dehydrogenase

NHL - Non- Hodgkin's Lymphoma

NHLS - National Health Laboratory Service

NNRTI - Non-Nucleoside Reverse Transcriptase Inhibitors

ORR - Overall response rate

OS - Overall survival

PBL - Plasmablastic Lymphoma

PI - Protease inhibitor

PR - Partial response

WCC - White cell count

2. Abstract

2.*1 Methods: The patient sample of this study consisted of all HIV-positive patients that were diagnosed with PBL in the period between 2005 and 2013 in the Free State Province of South Africa and who were treated by the Department of Oncology at the Universitas Hospital Complex. The study design is a retrospective study with descriptive and analytical components aimed at analysing the patient profile and the performance of a range of treatment regimes.*

2.2 Results: Fifty nine patients from one institution were evaluated after all exclusions. The mean age at diagnosis with PBL was 39,1 years with the gender distribution favouring males. Forty one point eight percent of patients presented with a performance status of ECOG 1. The amount of patients diagnosed with HIV prior the diagnosis of PBL was 59,3%. A third of patients were on HAART prior to diagnosis of PBL and 37% of patients were documented to be started on HAART with diagnosis of PBL. The median CD4 value on diagnosis of PBL was 108,5. The most popular extra nodal site was the oral cavity. According to our statistics only 38 of our patients received some form of treatment for their PBL, 21 patients were either too critical or lost to follow up to start treatment. Thirty seven patients received chemotherapy. Radiation therapy was part of the treatment for 12 of the patients. The median follow up time was 2,3 months. Progression free survival at 3 months for our study population was 90,8% (95%CI 83,1%-98,5%). The overall survival of patients according to treatment modality at 3 months calculated as follows: HAART prior to PBL (n=14) 71,43%, HAART with PBL (n=12) 91,66%, No HAART (n=9) 55,56%, patients receiving chemotherapy as treatment modality (n=27) 92,59%.

2.3 Conclusions: The importance of improved management of HIV is highlighted by the results of the study. If better control over HIV and a patient's general immunity can be achieved, more intensive chemotherapy regimes can be employed. Therefore, HAART is the mainstay and most important factor of the treatment of PBL. By starting HIV positive patients on HAART at an earlier stage in the disease (despite the CD4 value) might help in the survival of PBL patients or play a role in preventing PBL.

3. Introduction

In the 1980's an HIV–infected patient had a hundred fold increased risk of developing non-Hodgkin lymphoma (NHL) (Goedert, Coté et al. 1998). Consequently, NHL was added to the list of Acquired Immune Deficiency Syndrome (AIDS)-defining illnesses in 1985 (Hull 2009). Following Kaposi's sarcoma, NHL is the second most common HIV-associated neoplasm (Levine 1993). AIDS-related NHLs are mainly aggressive and high-grade, characterised by diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma, primary effusion lymphoma, and plasmablastic lymphoma (PBL) (Jaffe 2001). Plasmablastic lymphoma (PBL) (originally described in 1997) is an aggressive high grade NHL that was initially considered to be a variant of DLBCL. However, the World Health Organisation's classification of tumours of haematopoietic and lymphoid tissues (2008) separated it from the category of DLBCL and identified PBL as a distinctive mature B-cell lymphoma (Stein H 2008). PBL was originally described as involving the oral cavity of immune compromised patients. Recently, however, PBL has also been described in extra-oral sites including the nasopharynx,

maxillary sinus, lung, skin, soft tissues, heart, stomach, small bowel, anus, spermatic cord, bone marrow, testes, omentum, bone, soft tissue and CNS (Schichman, McClure et al. 2004, Dong, Scadden et al. 2005). Oral involvement is higher in HIV positive individuals than in HIV negative individuals, 58% versus 16% respectively (Castillo, Winer et al. 2010).

PBL has a strong association with AIDS (Delecluse, Anagnostopoulos et al. 1997) and accounts for 2,6% of all HIV-associated NHLs (Folk, Abbondanzo et al. 2006). Interestingly, it was documented that PBL can also arise form long-standing sacrococcygeal cysts in HIV positive patients (Ojanguren, Collazos et al. 2003). The majority of patients with PBL are men with a mean age of 39 years (Castillo, Pantanowitz et al. 2008).

Five pathogenic pathways were suggested in the development of AIDS-related lymphomas: EBV, HHV8, c-MYC, p53 and BCL-6 gene aberrations (Carbone 2003).

Mussaed et al. 2015 summarised recent studies as follow: the contribution of HIV to PBL pathogenesis might be the degree of immunosuppression, chronic B cell proliferation/exhaustion by chronic antigen stimulation, a decrease in immune control of oncogenic herpesviruses such as EBV, and an incomplete immune reconstitution or factors not related to immune dysfunction (Elyamany, Al Mussaed et al. 2015).

PBL shows little to no expression for B-cell markers – CD20, PAX5, CD79a or leukocyte common antigen- CD45. Plasma cell markers VS38c, CD38, CD138 and MUM1 (multiple myeloma oncogene-1) though seem to be expressed in PBL (Folk, Abbondanzo et al. 2006), (Castillo, Pantanowitz et al. 2008) and Ki67 expression is usually more than 80% in PBL (Elyamany, Al Mussaed et al. 2015).

A bimodal distribution is seen in HIV+ patients with 80% presenting with Ann Arbor Stage 1 or 4 disease (32% and 49% respectively). Interestingly it has been observed that only 33% of HIV-infected patients present with B-symptoms. In the same literature review comparing 112 HIV associated PBL patients (including pre-and post- HAART eras), the average CD4 count on presentation was 178 cells/mm^3 with an average viral load of over 86000 copies/mL. In the study 5 years had lapsed between the diagnosis of HIV and the diagnosis of PBL (Castillo, Pantanowitz et al. 2008). Regardless of the site of origin PBL is known to have a very aggressive clinical course and a very poor prognosis with most patients dying in the first year after diagnosis (Dong, Scadden et al. 2005), (Carbone, Gloghini et al. 2004). The addition of HAART to chemotherapy has, however, significantly improved the prognosis in cases of PBL (Rafaniello Raviele, Pruneri et al. 2009). In a case study of 3 HIV positive paediatric patients done by Pather et al., one patient received HAART prior to diagnosis of PBL whereas the other two patients commenced HAART only on diagnosis of

PBL. The observation was that the patient who followed the HAART regime displayed a higher CD4 (592 cells/mm³ at diagnosis of PBL) compared to those who only commenced HAART upon the diagnosis of PBL (221 and 237 cells/mm³ respectively at diagnosis of PBL). The conclusion that was made was that the patient already on HAART prior to the diagnosis of PBL displayed a better overall response to treatment compared to those patients that commenced with HAART only on diagnosis of PBL (Pather, MacKinnon et al. 2013).

A large literature review of treated PBL cases shows a 77% overall response rate (ORR) to chemotherapy, with a partial response (PR) of 31% and a complete response of 46% (Castillo, Winer et al. 2010). If not treated with chemotherapy patients died with a median survival of 3 months (Castillo, Winer et al. 2010). Surprisingly, spontaneous remissions in cases with HAART without chemotherapy were also observed (Armstrong, Bradrick et al. 2007),(Gilaberte, Gallardo et al. 2005). Despite a variety of therapeutic approaches, the median overall survival (OS) in HIV-positive patients is 14 months with a 5 year OS rate of 31% (Castillo, Pantanowitz et al. 2008). This was compared to HIV-negative patients who were found to have an OS of only 9 months (Castillo, Winer et al. 2010). Mussaed et al explained the finding as the restoration of immune surveillance to combat the tumour more efficiently by the use of HAART (Elyamany, Al Mussaed et al. 2015). Since the mid-1990s, when combination antiretroviral therapy (CART) was introduced, HIV associated lymphomas have fallen in incidence and improved in outcome, mainly because of improved immune function and better control of HIV replication (Dunleavy and Wilson 2012). NNRTI (non-nucleoside reverse transcriptase inhibitor) - or PI (Protease inhibitor)-based HAART regimens (and both in combination) appear equally effective at protecting against NHL, and the combination of two antiretroviral classes conferred significantly more protection than nucleoside analogs alone (Stebbing, Gazzard et al. 2004).

Interestingly survival can also be influenced by the site of presentation. In a small study reviewing 13 patients with PBL oral presentation had a significantly increased OS compared to extra-oral presentation (Hansra, Montague et al. 2010). Factors influencing the prognosis can include the International Prognostic Index (IPI) score, the CD4 cell count and the possible association of MYC/IgH gene rearrangement with a poor prognosis (Mounier, Spina et al. 2006, Valera, Balagué et al. 2010). Interestingly the MYC translocation was identified in near 50% HIV positive patients with PBL (Shaffer III, Young et al. 2012). Other factors affecting the outcome are reported as achieving complete remission, clinical stage performance status, comorbidities and the extent and destruction of the tumour at diagnosis (Barta, Xue et al. 2013, Bibas and Castillo 2014).

CHOP chemotherapy was the mainstay of treatment with good response but a poor survival and more intensive therapies are currently suggested (Elyamany, Al Mussaed et al. 2015). Intensive regimens as CODOX-M/IVAC, Hyper - CVAD or DA-EPOCH are recommended (NCCN version 2.2014).

Autologous bone marrow transplantation (ABMT) has also been shown to be of benefit in HIV positive patients (Dunleavy and Wilson 2012, Al-Malki, Castillo et al. 2014). Bortezomib alone or in combination with chemotherapy seems to have an antitumor effect in PBL and Lenalidomide as a single agent has been reported to have an effect in relapsed or refractory PBL (Wiernik, Lossos et al. 2008, Bibas, Grisetti et al. 2010). Interestingly, according to Castillo et al HIV positive patients with PBL had an overall survival of 11 months regardless of the intensity of chemotherapy and therefore did not have a better survival with more intensive chemotherapeutic regimens. (Castillo, Furman et al. 2012). This study however looked at 50 patients from 13 institutions with 37% receiving more intensive regimens. Only 21 patients in this study were on HAART with diagnosis and the median CD4 count was 206. Receiving chemotherapy with a low immunity could explained the worse outcome to more intensive chemotherapeutic agents.

4. Problem statement

PBL in HIV- positive patients continues to have a very poor prognosis and poor overall survival. Greater understanding of the complexities of the disease is, therefore, critical to advancing the treatment of PBL in HIV- positive patients. However, the approach to treating PBL in HIV- positive patients is constrained by the relatively rare occurrence of the disease which makes it difficult to interrogate the treatment regime and consequent outcomes for a large number of patients in a homogenous context while being treated. Current literature attempts to overcome this shortcoming by combining and jointly analysing data from fragmented studies to then draw generalisable conclusions from these synthetically created "large" samples. There was, therefore, an opportunity to analyse a relatively large sample of patients presenting with HIV associated PBL and their treatment. This dissertation sought to address this shortcoming in the associated literature by undertaking a study of HIV associated PBL treatment in the Free State province of South Africa and then compare the results of this unfragmented study to previous studies in this field.

South Africa is believed to have more people living with HIV/AIDS than any other country in the world and consequently HIV/AIDS and its complications is a prominent health concern in the country. Midyear population estimates of the total number of South Africans living with HIV is estimated at approximately 5,26 million and it affects 15.9% of the adult population aged 15 to 49 (see Table 1 below). (p0302 may 2013) Dynamics in the prevalence of HIV in South Africa among various categories of the population are illustrated in Figure 1. The prevalence of HIV in South Africa highlights the need for research in HIV and related ailments and specifically emphasizes the unique and topical theme of this dissertation and the importance of the research in the general discourse of HIV and PBL occurrence and treatment.

Table 1: HIV prevalence estimates and the number of people living with HIV in South Africa

	Prevalence				Incidence (15-49)	HIV Population (millions)
	Women (15-49)	Adults (15-49)	Youth (15-24)	Total population		
2002	16.69	14.50	6.75	8.80	1.65	4.02
2003	16.85	14.58	6.35	9.00	1.63	4.14
2004	16.93	14.62	6.07	9.10	1.65	4.25
2005	17.01	14.65	5.91	9.20	1.67	4.35
2006	17.22	14.82	5.82	9.40	1.65	4.51
2007	17.52	15.10	5.76	9.70	1.58	4.71
2008	17.81	15.39	5.71	10.00	1.50	4.93
2009	18.09	15.66	5.69	10.20	1.43	5.13
2010	18.29	15.87	5.70	10.40	1.38	5.32
2011	18.42	16.01	5.64	10.60	1.34	5.48
2012	18.53	16.14	5.61	10.70	1.31	5.65
2013	18.67	16.29	5.60	10.90	1.28	5.83
2014	18.85	16.46	5.59	11.10	1.23	6.02
2015	18.99	16.59	5.59	11.20	1.22	6.19
Change	14%	14%	-17%	27%	-26%	54%

Source : StatsSA (2015) http://www.statssa.gov.za/publications/P0302/P03022015.pdf

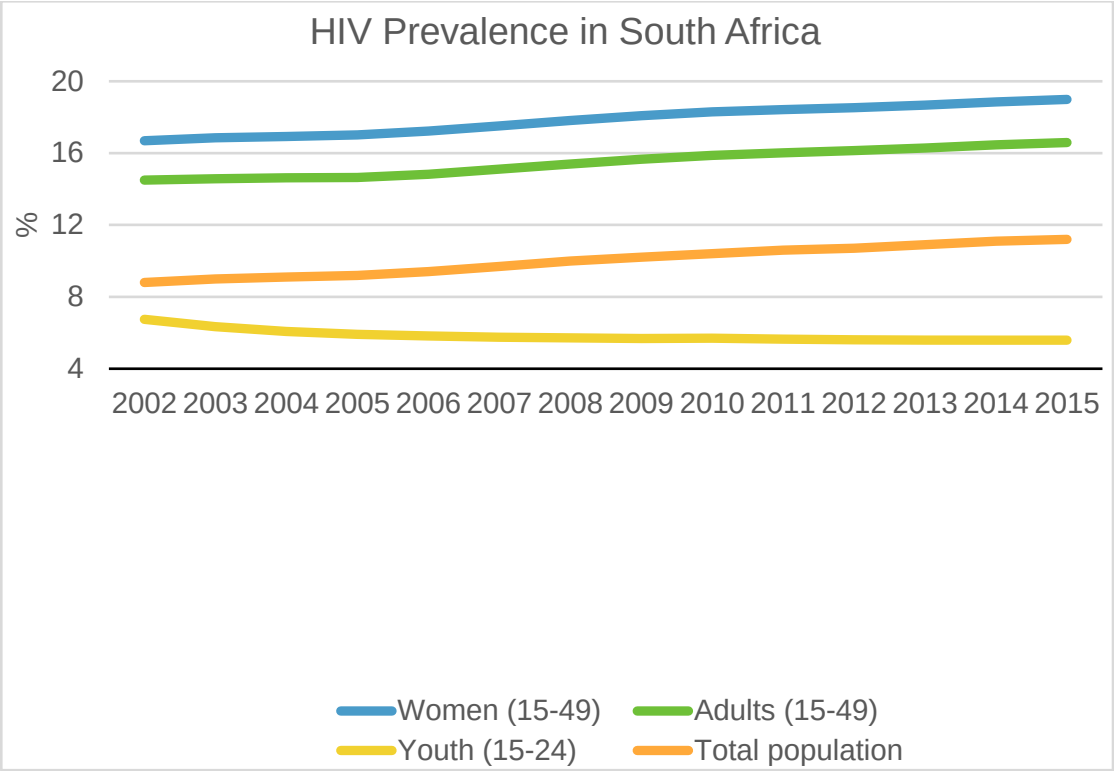


Figure 1: HIV prevalence estimates and the number of people living with HIV in South Africa (2002 – 2015)

Source : StatsSA (2015) http://www.statssa.gov.za/publications/P0302/P03022015.pdf

Several studies, as cited earlier, identify prognostic factors for survival and describe the distinct variant of lymphoma in the HIV positive population. Usually studies are approached by combining cases published globally and then comparing and making inferences from the combination of cases from different studies. However, given the prevalence of HIV in South Africa, this study of patients with HIV associated PBL (receiving treatment at the same institution) is of great value in identifying the clinicopathological characteristics with which PBL presents in the South African HIV population. The influence of HAART/HAART–chemotherapy/chemotherapy/radiotherapy regimes on the study population and the outcome and influencing factors determining the outcome were an additional dimension that added value to the existing knowledge in this regard. Comparing the research with findings of previous literature is also beneficial to advance knowledge of the presentation of these patients as well as the benefit and outcome of certain treatment regimes and HAART.

5. Objectives of the study

The objective of the study was to investigate the epidemiology, clinicopathological presentation, patient profile, influence of HAART, response to chemotherapy/radiation and general outcome of PBL in HIV positive patients of the Free State Province in South Africa in the period between 2005 and 2013.

The specific goals of the study, aligned to the overall objective, are:

- Profile the HIV associated PBL in the Free State Province of South Africa
- The role of HAART in the outcome of PBL
- Compare different treatment regimes of HIV associated PBL in the Free State Province of South Africa
- Compare and contrast the results of fragmented and un-fragmented studies
- Provide generalisable conclusions
- Provide views on the application of the research results to practice

Forming a conclusive idea regarding the impact of the disease and impact of different treatment modalities in our province, which can help to improve the overall survival and progression free survival in this patient population.

6 Methodology

6.1 *Study design*

The study design is a retrospective study with descriptive and analytical components aimed at analysing the patient profile and the performance of a range of treatment regimes. Patients were eligible for inclusion in the study if they suited the following inclusion criteria:

- They were HIV positive.
- They were diagnosed with plasmablastic lymphoma between 1 January 2005 and 31 December 2013 (Histological proof of diagnosis must have been obtainable).
- They were patients from the Free State Province of South Africa.
- Any age, gender, race.

HIV negative patients were excluded from the study.

A search was performed on the NHLS's DISA computer system to identify all cases of plasmablastic lymphoma in the Free State seen by the Department of Anatomical Pathology at the Universitas Hospital Complex. The Oncology files of all these patients, who were eligible for inclusion, were drawn followed by the gathering of the necessary data to conduct the study.

6.2 *Study participants/sample*

The patient sample included all patients that were diagnosed with PBL in the period between 2005 and 2013 in the Free State Province of South Africa and who were treated by the Department of Oncology at the Universitas Hospital Complex and who met the inclusion criteria, as noted, for eligible study participants. In the end fifty nine (59) patients were eligible for inclusion in the study.

6.3 ***Data***

The data required to meet the objectives of the dissertation was manually collected from the Oncology files of the eligible patient sample and transcribed for electronic processing. The following data were collected from each of the patient's files:

Patient's profile:

- Age
- Gender
- Race
- Performance status (ECOG)
- Number of years HIV + prior to being diagnosed with plasmablastic lymphoma
- HAART prior to PBL diagnosis
- HAART with PBL diagnosis
- Age-adjusted International Prognostic Index (aaIPI)

Clinicopathological characteristics:

- Presence of B-symptoms
- Associated opportunistic infections
- CD 4 count, Viral load
- Number and location of extra-nodal sites
- Clinical stage
- Laboratory data including HB, WCC, Absolute lymphocyte count, platelet count, LDH
- Immunohistochemical expression for CD20, CD45, CD138, CD3

Therapy

- Chemotherapy and regime used
- Radiation therapy(adjuvant or after progression on chemotherapy)
- Response to therapy (complete, partial or none)

Final outcome:

- Overall survival
- Progression free survival
- Cause of death or lost to follow up

A data form was created to note all the relevant information of each patient and was then entered into an Excel spread sheet for data analysis.

6.4 ***Measurement and method challenges***

A number of measurement and methodological challenges had an impact on the study. Firstly, undocumented data (eg. which B symptoms the patient presented with was not always recorded), not using the right terms in notes and not staging patients in the notes were some of the challenges in this study. In a number of instances notes did not always stipulate the ECOG status of the patients. Therefore the following was assumed: Good condition equaled ECOG1, fairly/relative good condition equaled ECOG 2, poor general condition equaled EGOG 3 and very poor condition equaled ECOG 4. Secondly, it was a challenge to find all the relevant information regarding the patient's condition, especially if the patient failed to follow up or where files of the periphery (regional hospitals) had to be extracted to find the necessary details. Thirdly if patients failed to follow up the date of death and cause of death was difficult to find. Only the date of death of the patients whose identity numbers were recorded could be extracted from the national population database held by the Department of Home Affairs. And after writing extensive letters to Statistics South Africa there was also delays in extracting the cause of death of these patients. Fourthly, random errors in the recording of the information could have been a difficulty in this study but these challenges was limited by using a single person to collect the information.

6.5 ***Pilot study***

The first ten cases were considered to be a pilot study to determine whether the data form was adequate to capture all of the necessary data or whether it needed adjustments.

6.6 ***Analysis of data***

Analysis of the data was performed by the Department of Biostatistics at the University of the Free State. Results were summarised by frequencies and percentages (categorical variables) and means, standard deviations or percentiles (numerical variables). Subgroup comparisons were done using 95% confidence intervals for differences in means, medians or percentiles as noted in the analysis and the discussion.

The Age adjusted International Prognostic Index was calculated by the following method. One point was given for each of the following characteristics present in the patient:

- Serum lactate dehydrogenase concentration above normal
- ECOG performance status ≥2
- Ann Arbor stage 3 or 4

Total scores ranging from zero to three.

Table 2: Prognostic index by risk group

Risk group	*IPI-score*
Low	0
Low intermediate	1
High intermediate	2
High	3

Overall survival was defined as date from initial histology report confirming the diagnosis, to the date of death and if death was not confirmed, then the last follow up date. Progression free survival was defined by date from histology report confirming the diagnosis to the date of first progression or death due to PBL.

6.7 ***Ethical aspects***

This study was subjected to the approval of the Ethics committee of the Faculty of Health Sciences, University of the Free State. Confidentiality regarding patients' names in the study was kept by assigning an identification number to each file and not recording any information that could identify the patient. Permission to perform the study was obtained from the Head of Department of Radiation Oncology, the CEO of Universitas Annex Hospital and the appropriate authorities at the NHLS.

7. Results

The study considered 59 patients, in total, after all exclusions. Exclusions consisted of: Three patients that were originally from the Northern Cape and followed up for treatment at another facility (in Kimberley) who were consequently excluded on the basis of not conforming to the geographical limits of the study. Two patients had their diagnosis changed to plasmacytoma and multiple myeloma. One patient was HIV negative, two patients did not have confirmation of their HIV status and one patient could not be traced despite a histology record.

Age

In terms of the patient profile the mean age at diagnosis with PBL was 39.1 years with the eldest in the study being 67 and the youngest 10 years old.

Gender and race

The gender distribution was weighted in favour of males (55.9%). Of the patients studied 57 of the 59 patients were African, while the other 2 patient's racial origin was not specifically stated.

Performance status

Patients were mostly seen with a performance status of ECOG 1 (41.8%) and ECOG 2 (29.1%). In the case of 4 patients no indication of a performance status was mentioned.

HIV diagnosis and HAART

Fifty nine point three percent of patient were documented to be diagnosed with HIV prior the diagnosis of PBL. The amount of years being positive prior PBL diagnoses could not be used in our study as we only had these numbers documented for 10 patients in this study therefore it would not have been representative. A third of patients were on HAART prior to diagnosis of PBL (20) and 37 % of patients were documented to be started on HAART when diagnosed with PBL.

The Age adjusted International Prognostic Index (AAPI)

The AAPI could not be calculate for 37 of the patients as a result of undocumented information needed to calculate the index. Therefore we decided to omit this index in our study.

B- symptoms

The presence or absence of B-symptoms with presentation was only stated in 39 of the patients' files. Of these 39 patients 30 patients presented with B-symptoms, with weight loss being the most common symptom present in 90% of patients.

Associated opportunistic infections

Associated opportunistic infections were only documented in 5 patients.

CD4

Forty patients had a CD4 on diagnosis with a mean value of 177,4 and a median value of 108.5.

Site

Patients presenting with one extra nodal site was in the majority reaching 55,9%. Eighteen point six percent of patients had involvement of 2 nodal sites followed by 10,2% and 3,4% having 3 and 4 extra nodal sites respectively. Eleven point nine percent only had nodal involvement. Interestingly as in other studies the most popular extra nodal site was the oral cavity that was involved in 37,3% (22) of our patients followed by the maxillary sinus (18,6%), nasopharynx and lung (both 10,2%), anus and soft tissue (6,8%), liver and skin (5,1%) and stomach(1,7%). There were no involvement of the spermatic cord, small bowel and heart as seen in previously studies. The other extra nodal sites (28,8%) that were seen in our study included the omentum, breasts, paravertebral, orbit, kidney, vaginal, bone, rectum, brain and spine.

Clinical stage

Nineteen patients were staged as clinical stage 1 on presentation, fifteen patients were staged as stage 2, thirteen patients were staged as stage 3 and 12 patients were staged as stage 4 which emphasises the fact that patients really present late to us already falling into a poorer clinical stage category.

Blood profile

Variability in the blood counts prior and after chemotherapy could not come to any conclusion due to the poor documentation and loss to follow up of patients.

Immunohistochemistry profile

Investigation on the immunohistochemistry profile revealed a CD20 positivity in 4 patients, CD45 positivity in 34 patients, CD138 positivity in 54 patients followed by 12 patients being CD3 positive.

Treatment

According to our statistics only 64,4% (38) of our patients received some form of treatment for their PBL, 35,6% (21) were either too critical or lost to follow up to start treatment.

Chemotherapy:

Thirty seven patients received chemotherapy. Fourteen patients received CHOP versus 21 patients receiving CDE as first line regime, this highlights the fact that with patients presenting either with a CD4 of below 200 or in a poor condition in our institution our regime of choice is CDE. One patient received CVP as first line regime (Cyclophosphamide, Vincristine and Prednisone) and in one patient the specific chemotherapy regime received was not stated. Five patients were started on a second line chemotherapy. Two patients received CDE as second line chemotherapy and one received DICE.

Radiation:

Radiation therapy was part of the treatment for 12 of the patients being part of our study. Eight receiving adjuvant radiation and 5 receiving palliative/salvage radiation.

Response of treatment

The response of treatment was only evaluated in 30 of the patients as the other were lost to follow up. From this 30 patients, 8 had complete response, 19 had partial response and 3 patients had no response on treatment.

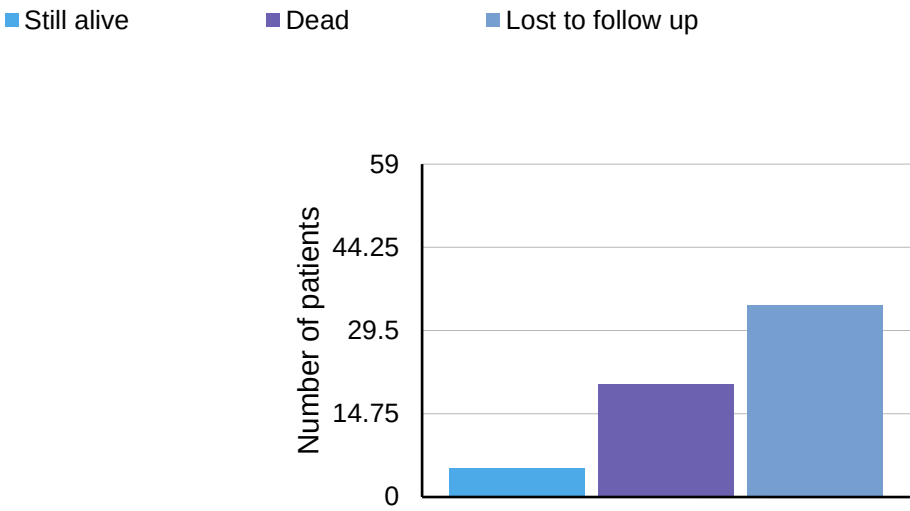
Follow- up

The median overall follow up time was 2,3 months.

Overall outcome

From our 59 patients, 9 patient died from PBL that we know of, 11 patients died of other causes (pneumonia and natural causes). As natural causes can not be specified as PBL or other we decided to only look at the number of patients that died, (whichever cause). Therefore 20 patients died. Five patients are still alive and following up, and 34 patients lost to follow up. We calculated the overall survival of these patient on the date of last visit.

HIV positive PBL patient's outcome in the Free State South Africa between January 2005 to December 2013



Progression free survival

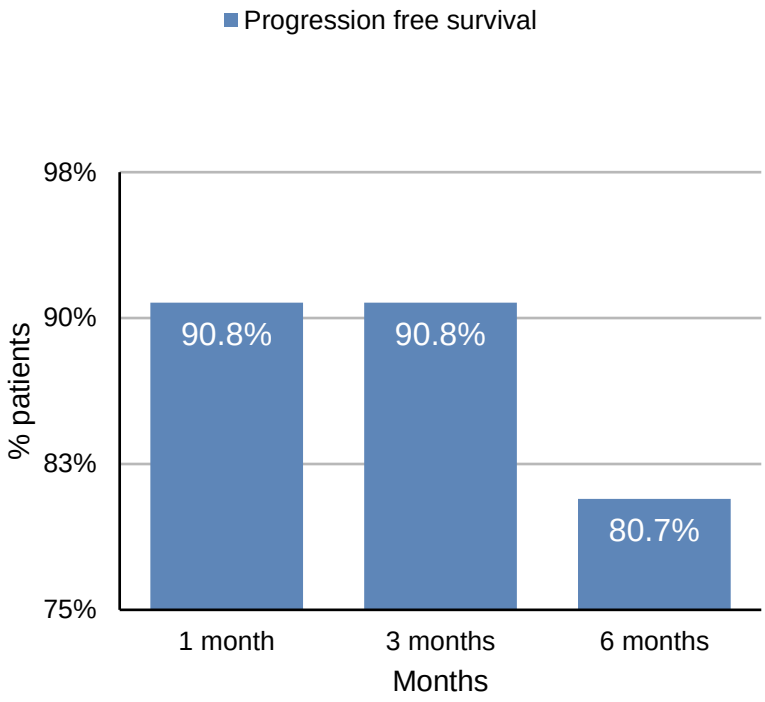
The progression free survival was calculated from diagnosis (on histology) to the date of first progression or death of PBL. Death of PBL only included the 9 patients of which we had proof being the cause of death).

Progression free survival

Months	Percentage	95% CI
1	90,8%	83,1%-98,5%
3	90,8%	83,1%-98,5%
6	80,7%	65,8%-95,6%

Progression free survival for HIV+ patient with PBL in the Free State South Africa 2005-2013

Overall survival



Survival of patients still alive

Months	Percentage	95%CI
1	83,7%	(73,7%-93,7%)
3	78,6%	(66,8%-90,4%)
6	71,7%	(57,5%-85,9%)

Overall survival of patients according to treatment modality at 3 months

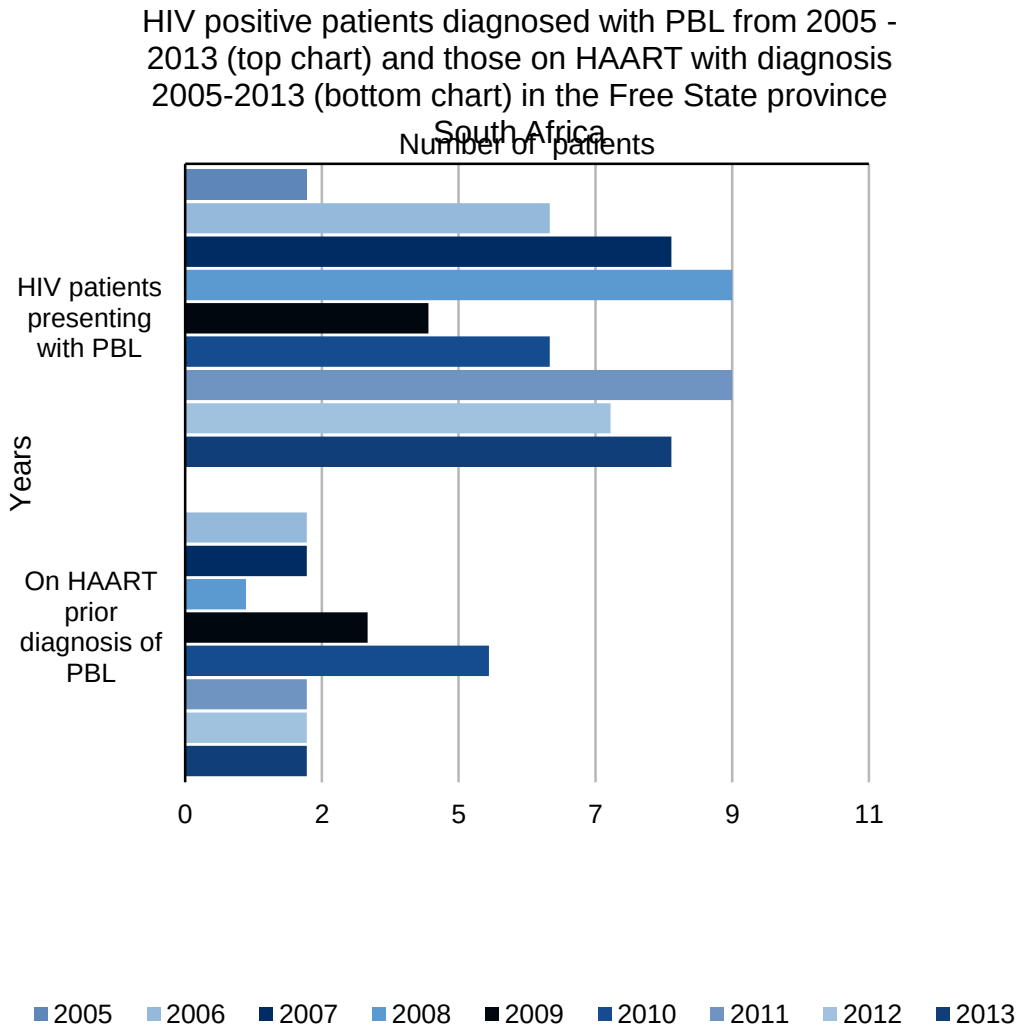
	Patients that died	Patients still alive
HAART prior PBL (n =) 14	28,57%	71,43%
HAART with PBL (n = 12)	8,33%	91,66%

Overall survival of patients according to treatment modality at 3 months		
	Patients that died	**Patients still alive**
No HAART (n = 9)	44,44%	55,56%
Chemotherapy (n = 27)	7,41%	92,59%
No Chemotherapy (n = 11)	81,82%	18,18%
Radiotherapy (n = 12)	8,33%	91.67%
No radiotherapy (n = 26)	38,46%	61,54%

8. Discussion and interpreting of data

The study population consisted of 59 patients, all from a single province in South Africa. This is in contrast to other similar studies that rely on combining a range of studies from multiple countries to compile a synthetic sample. This study represents almost 25% of the number of people investigated in other studies emphasising that further studies in South Africa and by including our country in further global studies, could be of value in future. This study's results are comparable to earlier studies noted in the review of literature. As in previously mentioned studies the oral cavity was the leading site of PBL extra nodal involvement. This study also concludes that PBL was definitely more likely to occur in male patients with a mean age of presentation of 39 years of age, this is the exact age compared to the age in the study by Castillo, Pantanowitz et al. 2008. The mean CD4 count value at presentation were exactly the same as previous studies at 177,4 versus a value of 178 previously mentioned. This similarity stresses the fact that PBL is a HIV defining illness by emphasising the link between PBL and a CD4 count value of less than 200. This outcome suggests that there is strong evidence to motivate commencement of ARV's in South Africa at higher CD4 levels. CD138 followed by CD45 were the two leading immunohistochemistry markers in this study.

In the following graph it appears that in the last 3 years (2013, 2012, 2011) 13,6 %, 11,9% and 15,3% of our study population respectively was diagnosed with PBL. These are more than 2005 and 2009 respectively. There was a decrease in diagnosis of PBL after a peak in 2008, but then a gradual increase again in 2011. The number of patients on HAART with diagnosis decreased in the years



where PBL diagnosis increased. These numbers were seen irrespective of the HAART initiation program in 2004. This emphasise the indirect relationship between HAART/immunity and PBL. This observation may justify further investigation of the role of compliance and resistance to HAART in the context of PBL management.

Approximately 370 000 people were initiated on HAART in the public sector between 2004 (the start of the national ART programme) and 2007. (Health. 2008) In a study done by Cornell et al 2010, the South African national ART programme has undergone rapid up-scale between 2003 and 2007. The recorded mortality has decreased, but the programme retention has deteriorated, as decreasing patient mortality has been greatly counteracted by high and increasing levels of failure to follow up treatment. The increasing difficulty in monitoring patients enrolling into care and the patients'

movement in and out of care as well as true loss of care leads to the increased failure to follow up seen in this study. The study therefore emphasises the importance to follow up and retain patients in large HIV treatment programs when the access to ART services rapidly expands. (Cornell, Grimsrud et al. 2010). Even though the prevalence of HIV is increasing and the incidence of HIV is decreasing, infrastructures and compliance programmes still need more exploration, to ensure adherence to HAART and prevent the development of HIV associated illnesses such as TB and PBL which result in multiplicative additional complexities in care.

The fact that 12 patients presented with stage 4 disease implies that a significant proportion of patients present themselves for treatment at very advanced stages of disease. Such patients are already in a poorer clinical stage and 35,6% patients were either to critical or failed to follow up to start treatment. Also the response to treatment was only evaluated in 30 patients as the rest failed to follow up. In my own clinical opinion (working at a state hospital): the fact that patients presented late, presented at an advanced stage and failed to follow up, highlighted 6 contextual aspects in South Africa that warrant further investigation:

- **Late referrals from the periphery,**
- **Late follow up of histology results,**
- **Problems with transport for patients to get to hospital,**
- **Patient education on health and diseases,**
- **The default and delay of ARV's**
- **Delay of diagnosis and**
- **Treatment seeking of a traditional healer before seeking modern treatment** (Kale 1995).

Our low median over all follow up time of 2,3 months and small study population allowed us only to look at the progression free and overall survival at 6 months. This is not comparable to previous studies which looked at a larger study population. However, it is important to keep in mind that this study population was not influenced by diversity where the unique contextual outcomes are lost through averaging. We looked at a patient population with uniformity of the underlying health system and a relative uniformity of the patient profile. This illustrates the importance of considering the contextual factors in the treatment of PBL in HIV + patients, whilst large studies across many contexts are arguably important in understanding the general disease dynamics and the interaction of PBL and HIV, these large studies do not capture the nuances of very specific context.

The results generated from this study was valuable in evaluating and illustrating the importance of HAART in the general treatment regime for PBL and what role radiation and chemotherapy plays in treating PBL HIV+ patients. The hypothesis is that prompt initiation of multimodality treatment may improve the outcome in HIV associated PBL patients. Patients that did not receive HAART before or on diagnosis did worse. Interestingly the patients starting HAART with PBL treatment did better than those starting HAART prior PBL in our study. This again emphasize the importance of the investigation of HAART compliance in our population group and starting HAART therapy at higher CD4 count values. Chemotherapy did have a drastic effect on survival at 1, 3 and 6 months ($p < 0.01$), despite not using intensive regimes. In our study 37 patients received chemotherapy, 14 patients received CHOP versus 21 patients receiving CDE (Cyclophosphamide Doxorubicin and Etoposide). We use CDE at our institution when the CD4 count is less than 200 or when a patient's general condition is unlikely to cope with the CHOP-regime. No comparison could be made to other studies using more intensive chemo regimes as our patient profile would definitely not tolerated these regimes. The difficulty for patients to get to hospitals early enough in neutropenic circumstances also makes these more intensive regimes challenging and not a favourable option in our province's setting. Due to a p value of 0,27 we did not draw any conclusion from radiotherapy as treatment model.

9. Conclusions and recommendations

Comparison of this research project's findings with other global studies to gain a better understanding of the HIV associated PBL patient profile in the Free State could lead to a different approach to the treatment of HIV positive PBL patients in South Africa. The importance of improved management of HIV is stressed by the results of the study. If better control over HIV and a patient's general immunity can be achieved, more intensive chemotherapy regimes can be employed. Therefore, HAART is the mainstay and most important factor of the treatment of PBL. By starting HIV + patients on HAART at an earlier stage in the disease (despite the CD4 value) might help in the survival of PBL patients or either play a role in preventing PBL. Further studies on Ki-67 and MYC rearrangement may indicate patients with a poorer prognosis and patients that should be started on a more intense chemotherapy regime despite a low CD4 count. Our low median follow up in the population of the Free State South Africa should open up our eyes to the 6 factors mentioned above that could make a difference in the outcome of our HIV positive PBL patients.

10. Acknowledgements

I want to thank Dr Sherriff and Dr Goedhals in their support in my thesis, I also want to thank Prof Gina Joubert for all her input and help with the data processing and home affairs for providing the date of death for several patients.

11. Appendices

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17 December 2013

The Chairman
Ethics Committee
Faculty of Health Sciences
University of the Free State

STUDY: **PLASMABLASTIC LYMPHOMA IN HIV POSITIVE PATIENTS IN THE FREE STATE PROVINCE OF SOUTH AFRICA: A SYSTEMIC COMPARISON**

This is to certify that Dr J Venter and co-workers have my permission to carry out the above mentioned study in this department.

C Esterhuysen

DR C ESTERHUYSEN
ACTING HEAD: DEPT OF ANATOMICAL PATHOLOGY

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Example of the Data Form to collect all patient data **Patient number**		1-3
Data		
Patient's profile		
Age		4-5
Gender (Male =1, Female=2)		6
Race (White=1, African=2, Coloured=3, Asian=4)		7
HIV (+ = 1) (- = 2)		8
ECOG (1=1, 2=2, 3=3, 4=4)		9
HIV + prior to to PBL (yes = 1, no = 2, not stated = 3)		10
Years HIV+ prior to PBL (not stated = *)		11-12
HAART prior to PBL (yes = 1, no = 2)		13
Started HAART with PBL (yes = 1, no = 2)		14
aaIPI (Age-adjusted International Prognostic Index) (0-3)		15
LDH above normal (yes=1, no=2)		16
ECOG performance > or equal to 2 (yes=1, no=2)		17
Ann Arbor stage 3 or 4 (yes =1, no = 2)		18
Low/low intermediate risk (yes =1, no =2)		19
High/high intermediate risk (yes = 1, no =2)		20
Clinicopathological characteristics		
B symptoms (yes=1, no=2)		21
Fever (yes=1, no=2)		22
Night sweats (yes=1, no=2)		23
Weight loss (yes=1, no=2)		24
Associated opportunistic infection (yes=1, no=2)		25

CD4 on diagnosis (cells /microlitre(or mm^3)	26-29
CD4 prior to chemo/RT initiation(cells /microlitre or mm^3)	30-33
CD4 post chemo/RT(cells /microlitre(or mm^3)	34-37
Viral load on diagnosis (copies/ml)	38-44
Viral load prior chemo/RT initiation(copies/ml)	45-51
Viral load post chemo/RT(copies/ml)	52-58
Number of extra nodal sites	59-60
Location of extra nodal sites	
Oral(yes=1, no=2)	61
Nasopharynx(yes=1, no=2)	62
Maxillary sinus(yes=1, no=2)	63
Lung(yes=1, no=2)	64
Liver(yes=1, no=2)	65
Spleen(yes=1, no=2) Not extranodal	66
Heart(yes=1, no=2)	67
Stomach(yes=1, no=2)	68
Small bowel(yes=1, no=2)	69
Anus(yes=1, no=2)	70
Spermatic cord(yes=1, no=2)	71
Skin(yes=1, no=2)	72
Soft tissue(yes=1, no=2)	73
Other(yes=1, no=2)	74
Clinical stage (1=1, 2=2, 3=3, 4=4)	75
Laboratory data: (if not taken or stated *)	
HB on diagnoses (g/dl)	76-79
HB prior chemo/RT(g/dl)	80-83
HB post chemo/RT(g/dl)	84-87
WCC on diagnoses(10^9/l)	88-91
WCC prior chemo/RT(10^9/l)	92-95
WCC post chemo /RT(10^9/l)	96-99
ALC on diagnoses(10^9/l)	100-2
ALC prior chemo/RT(10^9/l)	3-5

ALC post chemo/RT(10^9/l)		6-8
PLT on diagnoses(10^9/l)		9-12
PLT prior chemo/RT(10^9/l)		13-16
PLT post chemo/RT(10^9/l)		17-20
LDH on diagnoses (IU/L)		21-25
LDH prior chemo/RT(IU/L)		26-30
LDH post chemo/RT(IU/L)		31-35
CD20 (positive=1,negative=2,not stated=3,aberrant=4)		36
CD45 (positive=1,negative=2,not stated=3,aberrant=4)		37
CD138 (positive=1,negative=2,not stated=3,aberrant=4)		38
CD3 (positive=1,negative=2,not stated=3,aberrant=4)		39
Therapy /Treatment (yes = 1, no = 2)		40
Chemotherapy (yes=1, no=2)		41
1st regime (line) (CHOP = 1, CDE = 2)		42
Date of first regime (ddmmjj)		43-48
2nd regime (line)(CDE = 1, DICE = 2, ESHAP= 3, Other = 4)		49
Date of second regime (ddmmjj)		50-55
Radiation therapy (yes=1, no=2)		56
Adjuvant (yes=1, no=2)		57
Date of adjuvant radiation (ddmmjj)		58-63
Salvage/Palliation(yes=1, no=2)		64
Date of Salvage/palliative radiation (ddmmjj)		65-70
Response:		
Complete (yes=1, no=2)		71
Partial(yes=1, no=2)		72
None (yes=1, no=2)		73
Final outcome		
Overall survival (months)		74-78

Progression free survival (months)		79-83
Cause of death		
PBL (yes=1, no=2)		84
Other(yes=1, no=2)		85
Lost to follow up (yes=1, no=2)		86
Date of first visit (ddmmjj)		87-92
Date of last visit (ddmmjj)		93-98
Date of death (ddmmjj)		99-104

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17 January 2014

For attention: Ethics Committee, Faculty of Health Sciences, UFS

Title of project:

Plasmablastic lymphoma in HIV positive patients in the Free State

Researcher:

Dr Jacoline Venter, Department of Oncology

I hereby confirm that I provided inputs on the protocol and approve the protocol with regards to: study design, sampling method, measurement, measuring instruments and statistical analysis. Our department will perform the statistical analysis.

Yours faithfully

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