HUMAN IMMUNODEFICIENCY VIRUS AND CANCER IN CHILDREN IN SOUTH AFRICA

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ABSTRACT

Background. Infection with human immunodeficiency virus-1 (HIV) increases the incidence of certain cancers in adults. Much less is known about the impact of infection on the risk of cancer in children.

Aim. This study aimed to: 1) investigate the association between HIV infection and various cancers in children; and 2) compare the outcomes after cancer therapy between children with and without HIV.

Patients and methods. In the first study, the occurrence rates of various malignancies were analyzed on a group of 882 children with cancer, where 38 were HIV positive. The association between each cancer type and HIV was analyzed using all other cancers as controls – excluding those already known to be HIV-associated: Kaposi sarcoma (KS) and lymphomas. The association between HIV and outcome after treatment with standard cytostatic protocols and anti-retroviral medication was evaluated in a second study, on a group of 669 children with cancer out of which 99 were living with HIV. The data were processed with Student's t-test and chi-square test in order to discern the statistical significance of the differences recorded. Kaplan-Meyer survival curves were constructed and compared by means of log rank (Mantel-Cox) tests.

Results. In the first study, HIV infection was positively associated with KS - all 10 cases were HIV infected, $p<0.001$- and Burkitt lymphoma (BL) (OR=46.2, 95% CI 16.4–130.3, based on 13/33 infected cases). In the second study, the Kaplan-Meyer survival curves do not differ significantly by HIV serostatus. However, the proportion of children alive disease-free at the end of follow-up, to the total of children in the respective category, is significantly smaller in the HIV positive subjects: 32.1% vs. 47.7% (chi-squared, 1 degree of freedom, two-tailed $p=0.01$). Additionally, the proportion of children who died from toxicity (including infection due to marked leucopoenia), to the total in their respective group, is

significantly larger in the HIV positive subjects: 11.9% vs. 0.2% (chi-squared, 1 degree of freedom, two-tailed p<0.0001).

Conclusions. HIV is associated with KS and BL among children with cancer in South Africa. The proportion of children disease-free at end of follow-up is significantly lower in children infected with HIV while the proportion of deaths due to toxicity is significantly higher.

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ACKNOWLEDGEMENTS

AUTHOR'S DECLARATION

The epidemiological research presented in this dissertation is comprised of two studies. The first study was undertaken as part of a collaborative project between Tygerberg Hospital / Stellenbosch University, as coordinating unit for the South African team, and the Epidemiology and Genetics Unit, Department of Health Sciences, University of York. I played a key role in all parts of the overall project. I collected data from the Department of Paediatric Oncology at Tygerberg hospital, while coordinating the study at all other participating South African centres. I collaborated in submitting the project proposal for ethical approval and subsequently attended the meeting of the Research Ethics Committee at Stellenbosch University, to present the project and answer any questions from the panel. I performed the literature review and the interpretation of the results. The statistical analysis for this first study was done by W. Tom Johnston at York University. I wrote the paper under the guidance of Dr R. Newton, paper which was published in Pediatric Blood and Cancer 2011: 56: 77-79, under the title: "Infection with HIV among children with cancer in South Africa".

The second part of the study was initiated entirely by me. It was my own idea and concept. I personally carried out the literature review presented at the beginning of the thesis. I designed the study, developed the protocol, designed the tables and submitted the project proposal for ethical approval (which included writing the protocol and obtaining the permission to use patients' data). I collected personally the data from Tygerberg Hospital. The data from Universitas Hospital in Bloemfontein was kindly made available by Prof. David Stones.

I entered the study data in the Excel program and ran statistical tests under the supervision and with the support of Prof. Martin Kidd, Department of Statistics & Actuarial Sciences, Stellenbosch University.

I was entirely responsible for the analysis and interpretation of the results and for writing up this dissertation.

CHAPTER 1

INTRODUCTION

The AIDS epidemic remains a pervasive threat to the health of the world population. Its presence is most intensely felt in Sub-Saharan Africa, which is home to more than two-thirds of all persons infected with HIV. Within this region, South Africa has the world's largest population living with HIV in one single country (UNAIDS 2009).

The existing data document without doubt a higher incidence of a number of malignant diseases in adults living with HIV (Engels EA et al, 2008). Some studies found that, among the HIV positive population, as many as 40% will be affected by cancer at some moment in time (Flint SJ 2009). It is significant that the most frequently encountered cancers in this population group are caused by viral infections: Kaposi sarcoma – Human Herpes Virus 8 (HHV-8), Burkitt lymphoma – Epstein-Barr virus (EBV) and cervical carcinoma – Human Papilloma Virus (HPV). It is conceivable that other cancers appear more frequently in people with HIV than in those without and might also be associated with viral infections.

An increase in the incidence of malignancies has been observed not only in adults, but also in children with HIV. There is, however, a notable difference in the relative incidence of various cancers in these children, between Europe and USA on the one hand, and Africa, on the other hand. In resource-rich countries, non-Hodgkin lymphoma (NHL) is found most frequently, while leiomyomas and leiomyosarcomas, associated with EBV infection are second with regard to incidence increase (McClain CL 1996). In Africa, Kaposi's sarcoma is by far the most frequent malignancy found in children living with HIV, for reasons which will be detailed in the next chapter.

It is incomparably more difficult to conduct research on cancer in children with HIV than it is to do it on adults with HIV. To start with, cancer is much less frequent in children than in adults. Further, there are fewer children than adults, living with HIV. Children are mostly infected by vertical transmission from their mothers and the transmission rate without antiretroviral prophylaxis can be as high as 30%; however, the use of antiretrovirals during

pregnancy, combined with other measures, reduces the transmission rate to less than 5%. Furthermore, the majority of children infected with HIV would die during the first two years of life, if not treated with combined antiretroviral therapy, and thus would not live long enough to develop certain malignant diseases. Finally, in Africa, the paucity of cancer registries, the often deficient hospital records and the patchy availability of histological diagnosis make it difficult to obtain the necessary data. For this reason, most of the studies in Africa are based only on small series of cases that may not be representative of the totality of cases in the population.

By collating data from several South African hospital paediatric oncology databases, this dissertation assembles a relatively large series of children with cancer and HIV and compares them with even larger groups of children with malignant disease but without HIV.

1.1   Aims of this study. Research questions

The purpose of the study was to investigate the relative frequency of occurrence of various childhood cancers, as well as the characteristics of their prognosis, in children with cancer infected with HIV in comparison with children with cancer not infected with HIV

The study was undertaken with the intention to answer two research questions:

a) What is the relative risk for the occurrence of certain cancers in children living with HIV by comparison with children not infected with HIV?

It is already established that a number of childhood cancers occur more frequently in children living with HIV. Notwithstanding that, a number of differences were noted, with regard to the types of cancer encountered more frequently in children with HIV in Africa, compared with Europe and US. They will be detailed in the next chapter. This study will contribute further data towards defining the association of childhood cancers with HIV in African children.

It is important to note that cancers like KS and BL are closely associated with viral infections, as shown in Chapter 2; similarly, a confirmation of strong association between any other particular cancer and HIV infection may single out that cancer as potentially being induced by a viral infection and indicate a possible benefit of conducting research, both

epidemiologic and in laboratory, aimed at identifying the responsible microorganism, as a target for cancer prevention.

The second question to be answered is:

b) Is the prognosis of cancer significantly different in children living with HIV, from that of HIV-negative children?

While the immune system impairment due to HIV is worsened by the administration of cytostatics, thus predisposing the patient to even more severe opportunistic infections and death, the effect of HAART may mitigate this immunity deterioration. Certain small studies, which will be presented in the next chapter, have reported good results with cytostatic therapy and HAART in children with cancer.

1.2   Chapter plan

The following chapter of this thesis describes the setting in which this research took place, with focus on the HIV-AIDS epidemic in South Africa, the paediatric cancer burden of disease and the healthcare institutions which are tasked with the diagnosis and treatment of childhood cancers. Chapter 3 is a review of the related literature which summarizes the findings on the subject of HIV and malignancy, starting with epidemiological data, continuing with the study of oncogenesis in HIV infection and going on to explore the knowledge regarding the clinical characteristics and the treatment of cancers in children with HIV.

The fourth chapter describes the characteristics of the series of children studied and the statistical methods used to analyze the data. Two datasets were analyzed in this study, each of them containing findings from a series of children with cancer some of whom also had HIV and the majority of whom did not. The first dataset served mainly to identify the association of HIV infection and individual cancers in children, while the second dataset provided also information on the prognosis of the two groups.

The results are presented in the fifth chapter and are discussed in the sixth. The distribution of various malignancies is analyzed comparatively in children with and without HIV. The prognosis is described by means of comparative rates of various adverse outcomes as well as by Kaplan − Meyer survival curves. The discussion focuses on the significance of the

findings for the management of similar cases, with reference to data already published. This study presents, for the first time in Africa, as far as I could determine, comparative data obtained from relatively large series, where all cases were tested for HIV and their diagnoses of cancer were confirmed by histopathology.

CHAPTER 2

BACKGROUND

2.1 Status of the HIV epidemic in South Africa

The Republic of South Africa is situated at the southernmost part of the continent, covering a surface comparable with that of Central Europe. Its total population was estimated, for mid-2011, at 50.6 million inhabitants. Out of these, 31.3% corresponding to 15,8 million, are children under 15 years of age. The infant mortality rate is 38/1000.

The diverse ethnicity of the South African people is best described by the qualification of "rainbow nation", proposed by the Peace Nobel Prize laureate Archbishop Desmond Tutu. Approximately 40 million inhabitants are black, belonging to nine major ethnic groups, with the remaining 10 million made up mainly of white, coloured (officially used term for persons of mixed ethnicity) and Indian people.

The HIV-AIDS epidemic started relatively late in South Africa, by comparison with other African countries. In 2011 it was estimated that the overall HIV prevalence was 10.6%. This figure corresponds to 5.3 million infected persons, the largest number of people living with HIV in any single country. Out of the adult population (15 to 49 years of age), 16.6% were HIV-positive. Among women attending antenatal clinics the overall prevalence of HIV infection was 20% (Shisana O et al 2009).

The high prevalence of HIV infection among women of reproductive age constitutes a major risk for vertical (mother to foetus) transmission of the virus. Without intervention, the rate of transmission is as high as 30%. In South Africa, where an estimated 98% of pregnant women attend antenatal care clinics, a nation-wide program of prevention of mother-to-child transmission (PMTCT) of HIV, by administering zidovudine from 13 weeks of gestation, was implemented. It is estimated that the rate of vertical infection was thus reduced to 5% of newborns of infected mothers. A further reduction is achieved by prescribing HAART to all pregnant women whose CD4+ lymphocyte count is 350 or lower. However, this remains the main pathway of acquiring the infection HIV in children: it is estimated that 63.600 new infections have occurred in children, in 2011 (DOH SA 2011). According to UNAIDS, in

2009, some 330,000 children were living with HIV in South Africa (UNAIDS 2009). The figure might be in reality much higher.

2.2 Data on cancer in South African children

Statistical information regarding cancer in South African children is provided by The South African Paediatric Tumour Registry, the only dedicated children's cancer registry on the continent. The annual incidence of tumors in the 0 - 14 years age group varied between 33,4 and 47,2 per million from 2003 to 2007, a figure much lower than that found in studies from other countries. By comparison, in Europe, the annual incidence of cancer in this age group was 140 per million in 1990, and increased slowly for the last 3 decades. The contributors to this marked discrepancy are, among others: failure to diagnose the cancers, lack of referrals to the oncology units and failure to report the cases to the SACCSG registry (Stefan DC et al 2012). The combined statistics of HIV and cancer in children suggest that an increasing number of South African children with malignant disease are also living with HIV. Their exact numbers are not reflected by the available data. A synopsis of the diagnoses reported to the registry is presented in Table 2.1.

2.3 The South African public healthcare network and childhood cancer

In South Africa, the majority of children with cancer are treated in the public healthcare sector, at tertiary (subspecialist) level. A few patients are in the care of private oncologists or surgeons. As there is no paediatric oncologist practising exclusively in private, the number of children treated in the private sector is small. There are seven paediatric oncology centres covering the whole country:

- Tygerberg Hospital, academic tertiary unit situated in Cape Town, serves the eastern part of the municipality and a number of districts over a radius of 250 km to the east of Cape Town, with a population of around 700.000 children, mainly coloured and black. An estimated 300.000 children from the Northern Cape Province are also covered by Tygerberg Hospital.

- The Red Cross Memorial Children's Hospital, also in Cape Town, serves the western part of the city and the Atlantic Coast up to 200 km north of the city; it also receives

children from the Eastern Cape Province. The hospital's catchment area has estimated 2.800.000 children, mainly coloured and black.

- Universitas Hospital, situated in the centre of the country, is an academic tertiary institution serving a children population of over 800.000, mainly black.

Type of cancer	Year											Total
	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	
Leukemia	146	157	142	177	162	140	162	206	154	139	150	1737
Lymphoma	69	83	66	98	85	83	88	86	98	78	89	923
Central nervous system tumours	62	61	100	78	80	85	106	104	94	82	69	921
Nephro-blastoma	64	85	75	84	104	62	72	94	71	73	61	845
Neuroblas-toma	38	25	3	44	29	30	27	41	29	31	41	372
Rhabdomyo-sarcoma	40	44	35	55	48	41	42	42	57	52	33	487
Liver	12	7	13	17	17	14	12	8	13	14	16	143
Sarcoma	58	59	44	73	54	65	64	73	65	43	53	651
Bone	22	25	20	22	26	3	25	28	34	48	28	309
Other malignant diseases	15	19	16	44	25	26	22	22	16	19	15	238
Germ cell tumours	20	27	27	24	20	21	20	17	19	15	13	223
Total	546	592	575	716	648	598	640	723	650	594	568	6850

Table 2.1: Cases reported to the Children's Tumour Registry, 1997-2007 (Stefan DC et al 2012)

- Chris Hani – Baragwanath Hospital, main teaching base of the Faculty of Health Sciences, University of Witwatersrand in Johannesburg, is situated in the north-eastern quadrant of the country and serves mainly the black population of Soweto, but also the Nort-West province, with a total estimate of 1.600.000 children.

- Charlotte Maxeke Hospital, also associated with the University of Witwatersrand, serves mainly the inner Johannesburg population, with an estimated children number of 500.000, mainly black.

- Steve Biko Academic Hospital in Pretoria, offers health care for the children living in the capital, but also for those coming from Mpumalanga and Limpopo provinces, adding up to 3 million children, from various black ethnic groups.

- Inkosi Albert Luthuli Hospital, in Durban, receives referrals from Kwa-Zulu Natal province, mainly black children, totalling 2.660.000.

The paediatric oncologists form the South African Children's Cancer Scientific Group (SACCSG), with regular meetings where scientific and clinical practice issues are discussed. The management protocols of various childhood cancers are identical in the various public centres, although practice in the private sector is not standardised or monitored.

The diagnoses are based on the pathology tests and histology evaluations done by the National Health Laboratory Services. This nation-wide institution works according to uniform standards, audited regularly by national and international accrediting organizations, thus ensuring the uniformity in the diagnostic criteria required by studies such as this one.

CHAPTER 3

REVIEW OF THE LITERATURE

A most comprehensive review of the published data on the role of HIV as an oncogenic factor was published recently by the International Agency for Research on Cancer (WHO/IARC 2011). This chapter, however, is not a summary of that valuable monograph. It is the result of independent research and evaluation of the literature, with emphasis on the studies published in the last 10 years.

3.1 Changes in the incidence of cancers in Africa associated with the HIV epidemic

The apparently inexplicable apparition of cases of pneumonia caused by *Pneumocystis jiroveci* and of Kaposi's sarcoma (KS), mainly among homosexual men in San Francisco and New York, led to the discovery of AIDS and HIV, at the onset of the ninth decade of the last century. It became evident soon that KS and non-Hodgkin lymphoma (NHL) are strongly associated with advanced stages of the HIV infection. Statistics from Western Europe and USA have since clearly indicated not only a substantial increase of the two types of malignancy already mentioned, but also a higher incidence of cervical and anal cancers as well as an increased frequency of conjunctival tumours, Hodgkin lymphoma and leiomyosarcoma in children (Biggar RJ 2000 in Feigal EG).

Studies of adult populations in resource-rich countries illustrate convincingly the excess of malignancies related to the infection with HIV. A community-based study done in Texas, by means of linkage between the cancer register and the HIV register data (Cooksley CD et al 1999), found an increase in the incidence of cancers associated with the AIDS epidemic, by comparison to the general population, of 16.7 times in males and 2.9 times in females (age-adjusted). Fifteen per cent of the HIV infected group were found to have a malignant disease, with an overwhelming predominance of males. While KS, NHL and cancer of the uterine cervix were encountered most often, other cancers were also seen more frequently (4 to 5.6

times the expected incidence for the general population): Hodgkin lymphoma, non-melanocytic skin cancer in males and cancer of the colon in females.

The largest study to date, The HIV/AIDS Cancer Match Study, based on the linkage of HIV/AIDS registries with cancer registries in several states of USA, has yielded similar findings (Engels EA et al. 2008). Another research group, who looked at a cohort of over 13,000 people with HIV infection or AIDS in Australia, added to the list of cancers appearing more frequently in HIV patients myeloma, leukaemia and lip cancer (Grulich 2002). Other authors quoted even higher figures, of 40%, for the incidence of cancer in the HIV infected population subgroup (Flint SJ 2009). These statistic findings have the advantage of being supported by reliable and comprehensive registers and, as a consequence, they are a credible representation of the influence of the HIV epidemic on the incidence of cancers in adults, in resource-rich countries.

On the background of the above findings, the effect of the HIV epidemic on the apparition of cancer in children (0-14 years) from the above geographical areas was found to be, predictably, an enhancing one: non-Hodgkin lymphomas, soft tissue tumours, cancer of the uterine cervix in adolescent girls, thyroid and pulmonary cancers were found to appear significantly more often in children with HIV than in non-infected children (Mueller BU, 1996, 1998). While NHL was found most frequently, leiomyomas and leiomyosarcomas, associated with Epstein-Barr virus (EBV) infection were second with regard to incidence increase (McClain CL 1996). Kaposi sarcoma remained, in children, rare by comparison with adults living with HIV (Mueller BU 1996, McClain 1996).

Before considering the influence of the HIV epidemic on the incidence of malignant disease in Africa, it is important to acknowledge the characteristics of cancer incidence on the continent prior to the advent of the AIDS epidemic. Two peculiarities, with substantial epidemiologic consequences, exist in Africa, by comparison with the rest of the world. The first one relates to the incidence of KS. This disease was known, initially, to affect only a few elderly people in geographic areas around the Mediterranean basin. Later, with the advent of organ transplantation procedures, which required pharmacological immune suppression, the disease was seen occasionally in the organ recipients. However, in Africa, before the onset of the AIDS epidemic, the incidence of KS was completely different from the rest of the world. In large geographical areas situated in Central Africa, extending to Cameroun in the west and to Malawi in the south, KS was found to be endemic, with incidence rates greater than

6/1000. In these regions, the KS incidence was found to be comparable to that of the cancer of colon in Europe (Cook-Mozaffari, 1998). These relatively high rates of pre-existing, endemic KS were exacerbated to uniquely high levels under the influence of the HIV epidemic. The pre-AIDS endemic KS would also explain why it is much more frequent in African children than in children from other regions of the world.

The second African characteristic is the relatively high incidence of Burkitt lymphoma, which is one of the non-Hodgkin lymphomas, pre-dating the AIDS era. Rates as high as 4 to 5/1000 were seen in parts of Uganda and Tanzania, while in other geographic areas the rates were lower: 0.2/1000 in males and 0.04/1000 in females (Cook-Mozaffari, 1998). These figures are considerably higher than European standardized incidence rates, even during the AIDS epidemic, which were only 3.94/million (Marcos-Gragera R. et al 2010).

Any epidemiologic study on cancer in Africa has to find ways of overcoming the paucity of statistical data: The International Association of Cancer Registries counts only 60 members in Africa, out of which only 15 are national level institutions, the remaining being hospital or city registries (www.iacr.com.fr). These are by no means permanent, their activity being often suspended for various lengths of time, due to lack of means or personnel. Only a few registers, from 12 African countries in total, reported their data to CI5 (Cancer Incidence on 5 Continents) database held by IARC and only 5 accepted in group C (2 from Sub-Saharan Africa).

There is definite under-reporting even where registries exist, due to restricted accessibility of rural patients to health facilities and limited availability of histopathology services. Further, the poor quality of the population census data casts a shadow over the accuracy of any rates calculated on the basis of population numbers. With regard to children's cancer, this is usually reported to the adults' register, thus raising the question whether some data specific to children are actually recorded or maybe left aside. The only children's cancer register in Africa is kept by the South African Children's Cancer Study Group.

In African adults, the HIV infection has exacerbated, in the first place, the occurrence of KS. Comparing the Kampala Cancer Register entries before and after the onset of the AIDS epidemic, an increase in the KS incidence from 2.6 to 30.1/100,000 was found in men, while in women the increase was from 0 to 11/100,000. The incidence of the cancer of the uterine cervix doubled; that of NHL changed only little (Wabinga HR et al 1993). Similar findings were published 8 years later, this time in a synopsis of data from Sub-Saharan Africa: the

incidence of KS increased the most, with a dramatic reduction in the male/female ratio from 19:1 to 1.7:1. The risk of NHL in HIV-infected people was lower in the area studied than in the developed world. The changes in incidence of the cervical cancer were not significant, but the incidence of human papilloma virus infection of the cervix and that of the high-risk squamous intra-epithelial lesions were increased. The author noted also the significant increase in the incidence rate of the squamous cell carcinoma of the conjunctiva (Thomas JO et al 2001). These findings were reinforced in a later case-control study, based on histopathological records, done in South Africa (Stein L. et al 2008). A recent review of the literature regarding the incidence of cancer in HIV-infected subjects in Sub-Saharan Africa found, in case-referent studies, odds ratios ranging from 21.9 (95% confidence interval –CI - 12.5-38.6) to 47.1 (31.9-69.8) for Kaposi sarcoma and from 5.0 (2.7-9.5) to 12.6 (2.2-54.4) for non-Hodgkin lymphoma (Sasco AJ et al 2010). The other findings fell in line with the above.

Data on the influence of the HIV epidemic on the incidence of cancers in children are, relative to adults, patchy, with regard to geographical area and time intervals studied. In Uganda, the spreading of AIDS has increased the incidence of KS in children by a factor of 40 (Ziegler 1996). Another case-control study in the same country found that children with HIV had a significantly increased probability of acquiring KS (OR = 94.9, 95% CI 28.5-315.3, based on 36 cases) and Burkitt lymphoma (OR = 7.5, 95% CI 2.8-20.1, based on 33 cases) but no increase in risk was seen for other cancers (Newton R et al 2001). In Tanzania too, a comparison of cancer register records before and after the AIDS era indicate a significant increase of the cases of paediatric KS (Amir H et al 2001). In Zambia, a comparative review of the histopathological records of cancer in children (less than 14 years of age) during the pre-AIDS interval 1980-1982 with the 1990-1992 interval when the epidemic was established, found also an increase in the incidence of KS and of retinoblastoma. Non-Hodgkin lymphoma, nasopharyngeal carcinoma and rhabdomyosarcoma were seen more frequently in the second interval studied but their incidence increase was not statistically significant (Chintu C et al 1995).

In Malawi, the proportion of HIV-positive children in cohorts diagnosed with the same malignancy was determined during the periods 1998-2003 and 2005-2008 in two separate studies. Sinfield RL et al (2007) reported a review of hospital records, while Mutalima N et al (2010) performed a case-control study. According to their research, the infection with HIV was associated significantly with KS (Odds Ratio - OR = 93.5, 95% CI 26.9 to 324.4) and

with non-Burkitt, non-Hodgkin lymphoma (OR = 4.4, 95% CI 1.1 to 17.9). Burkitt lymphoma was endemic in Malawi, with relatively high incidence rates, before the advent of AIDS and its occurrence did not appear to be affected by the epidemic. This is an as yet unexplained finding, as in South Africa, where Burkitt was relatively rare before the AIDS era, it was seen much more often afterwards: the odds ratio for the occurrence of this cancer among HIV positive children - versus HIV negative - was calculated at 46.2 with 95% CI= 16.4-130.3 (Stefan DC 2011). This figure is even higher than the increase in risk found in the Ugandan study mentioned above (Newton R 2001).

The effect of the HIV infection on the incidence of cancer in children in Africa, as depicted by the findings from the above studies, is that of a possible modest increase in the incidence of a certain number of cancers such as retinoblastoma, nasopharyngeal carcinoma and rhabdomyosarcoma, coupled with a substantial increase of KS; Non-Hodgkin (and non-Burkitt) lymphoma seem to have increased modestly. The findings on the incidence of Burkitt lymphoma in children with HIV are contradictory and this contradiction awaits further verification and a convincing explanation. There is a clear distinction between the patterns of increase in cancer incidence in African HIV infected children and children from the rest of the world and the main difference is that KS is much more frequent in Africa than elsewhere.

3.2 Oncogenesis in HIV infection

The list of malignancies which appear more frequently in people infected with HIV is extensive. However, only a few cancers, as highlighted in the previous chapter, are highly prevalent in this population group: KS, Non-Hodgkin lymphomas and invasive cancer of the uterine cervix. They are so often encountered in association with HIV infection that they are considered to be AIDS-defining conditions, together with infections like tuberculosis, candidiasis, *Pneumocystis jiroveci* pneumonia and others. It is significant that the above cancers are also, almost always, caused by infections: KS is the result of infection with Human Herpes-Virus 8 (HHV-8), also known as Kaposi Sarcoma Herpes Virus (KSHV) (Chang 1994), Burkitt lymphoma and other AIDS-associated lymphomas are caused mainly by the Epstein-Barr virus (EB) (Epstein MA 1964) and the Human Papilloma Virus (HPV) is the agent of the cancer of the uterine cervix (Zur Hausen H 1983).

The mechanisms by which viruses may trigger cancers are not entirely elucidated, but essentially they would alter the structure of cellular DNA or the expression of genes involved in the control of cell division. Viral oncogenes may be added to the cellular DNA, leading to excessive expression of the respective encoded proteins and consequent disturbance of cell multiplication. Other viruses may activate cellular oncogenes or inactivate tumour suppressor genes.

It has been established that HIV does not have any of the above properties, but promotes the oncogenesis induced by the other viruses mentioned. This effect is due, on the one hand, to an inefficient immune surveillance against both oncogenic viral agents and the tumour cells they may produce. On the other hand, the HIV-associated chronic hyperactivity of the immune system mediated by cytokines stimulates the immune cells' proliferation, which in turn enhances the replication of oncogenic viruses within those cells. Secondarily, the cytokines promote the growth of blood vessels in the tumour tissue. The inefficient immune surveillance may also explain why other cancers, not known to be associated with infectious agents, appear slightly more frequently in HIV-infected children (Flint SJ, 2009).

As the cancer of the uterine cervix may first be seen towards the end of adolescence, only the oncogenic processes associated with KSHV and EBV would be responsible for most of the excess of paediatric malignancies associated with HIV. Since its characterization by Chang in 1994, KSHV was found in transformed epithelial cells in all specimens of KS, but not in healthy tissue. A number of viral genes were identified, which may be responsible for the tumoural transformation of the vascular epithelium induced by KSHV. Some of these are entirely viral genes (i.e. without correspondent in the target cells): K1, kaposin and viral G protein-coupled receptor. Others are generating proteins similar to those found in the cell, thus deregulating the processes of cell multiplication and apoptosis: viral IL-6, viral interleukin 10, viral cc-class chemokines and viral FLICE*-inhibitory protein. A third group of genes are involved in maintaining the persistence of the virus through the process of cell replication: the gene for Latency-Associated Nuclear Antigen and K-15 (Angeletti 2008).

The Epstein-Barr virus infects mainly the B lymphocytes. It is one of the most widely spread

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* FLICE stands for FADD-Like Interleukin 1β-Converting Enzyme, where FADD is the acronym for Fas-Associated protein with Death Domain, a protein involved in the initiation of the apoptosis process.

viruses in humans, as it infects around 95% of adults worldwide. For most of the time, the virus is present in latent form, with its genome integrated in the cell DNA and replicating with it when the cell divides. Sometimes the lytic form of the virus will be generated, and it would spread to infect other susceptible hosts. Some of the viral genes are active during the latency phase, producing proteins which have the potential to induce malignant transformation. The genetic lesions identified in the resulting malignant cells include c-myc gene rearrangement, bcl-6 gene rearrangement, ras gene mutations, and p53 mutations/deletions (Angeletti 2008). Three patterns of gene expression were identified, corresponding to three latency types, and to three different malignancy groups of the B cells: Burkitt lymphoma, some AIDS-associated lymphomas (primary central nervous system lymphomas, diffuse large cell lymphomas, Hodgkin lymphoma, primary effusion lymphomas) and leiomyosarcomas (Kutok JL 2006, Bajaj BG 2007).

Both KSHV and EBV have considerable potential to induce malignancies, but their influence is probably not sufficient, as only a fraction of those infected will ultimately develop cancers. The concomitant infection with HIV enhances the oncogenetic activity of these viruses. Some of the mechanisms responsible for this process have been presented at the onset of this chapter; there may be other interactions involved, which will be revealed by future research.

3.3 The influence of the HIV infection on the clinical presentation of malignancies in African children

The immune response deterioration in AIDS might result, in theory, in faster progression of the malignant disease. This could be expressed by more advanced or widespread cancers at the first presentation, a poorer response to treatment and a reduced survival rate. It is also possible that the symptoms and signs of the cancer would be altered by the immune suppression, as the immune response is often contributing to generating the signs and symptoms of a disease. There is a dearth of information in the literature on these aspects. An explanation may reside in the relative rarity of cancer in children coupled with a relative rarity of HIV in this age group, with the resulting difficulty of finding enough cases to comply with the power requirements of the studies. In most of the world, even those cancers whose incidence is increased by AIDS were relatively rare in children before the epidemic (and remain rare in those not infected with HIV) and therefore it is not easy to compare their AIDS and non-AIDS presentation. In Africa, this comparison might be possible, at least for

KS and Burkitt lymphoma, which were endemic even before the advent of the HIV epidemic. This endemic presence continues unabated in HIV-negative children, alongside to the epidemic form, which is associated with AIDS. In the following paragraphs, the published evidence on the effect of AIDS on the clinical presentation of KS and Burkitt lymphoma (BL) will be reviewed.

There are only a few studies that evaluate the clinical presentation of KS in African children with HIV and not all of them relate the findings to HIV-negative controls. As their methodologies and presentation of results differ substantially, they will be presented one by one in this and the following paragraphs. Ziegler and Katongole-Mbidde (1996) analyze 100 Ugandan children, below 15 years of age, with KS. Out of 63 tested, 49 were found to be HIV-positive, but they are not compared with non-infected children. The median age of the group at diagnostic was 4 years and the male/female ratio was 1.7:1. The most frequent localization of the lesions was in the lymph nodes: 70 cases, versus only 45 cases with skin lesions. The radiographs showed chest lesions in 56 children; abdominal lesions were found in 11 cases. The authors distinguished three patterns of distribution of the lesions: oro-facial, frequently associated with disseminated lymphadenopathy; inguino-genito-anal and isolated visceral lesions.

Amir H. et al (2001) looked at the clinical presentation of KS in 150 Tanzanian children, registered in the national cancer register. Out of them, 73 presented with the disease between 1968 and 1982, before the onset of the AIDS epidemic in Tanzania. The remaining 77 were registered during the epidemic, from 1983 to 1995. However, the HIV testing was sporadic and the results are not presented. The median age was 2 years in the first group and 3 years in the second. The respective male/female ratios were 5.1:1 and 5.4:1. During the first period, patients seen were having almost equally often lymph node lesions (47.9%) and cutaneous lesions (49.3%), while in the AIDS period, the lymph node localization was less frequently seen (31.2%) and the skin was involved more often (53.7%). Further, the skin manifestations of KS were mainly limited to the lower limbs in the pre-AIDS era, while thereafter they were often disseminated, covering also the upper limb and ears, eyelids and lips.

Mwanda OW et al (2005), in the context of a retrospective analysis of 91 cases of KS of all ages, reports on 15 children included in the study group; 7 were infected with HIV whereas 8 were not. The children were aged between 6 and 10 years. The HIV-infected children had oral/mucosal lesions more frequently (p=0.007) and more often abnormal chest radiographs

(p=0.01) than their counterparts. The same subgroup showed an "apparent" trend, but not significant, towards respiratory system and skin involvement.

Sinfield RL et al (2007) compared the clinical presentation in 52 HIV-positive Malawian children with KS that of 4 HIV-negative. The median age at the initial consultation was 7.2 years in the HIV subgroup and 2.3 in the negative subgroup. The male/female ratio was 1.7:1 in the HIV-positive whereas all 4 non-infected children were male. Lymphadenopathy was the presenting complaint in the non-HIV group, while the children with AIDS complained on admission of symptoms related to the HIV infection and KS was an incidental finding. All pulmonary (2 cases) and abdominal (2 cases) KS was seen in the HIV-positive group. The rest of the group had cutaneous lesions.

Gantt S. et al (2010), in a series of 73 HIV-positive children with KS in Uganda, found a male/female ratio of 1.02:1. The median age was 10.1 years; lymph node localization was more frequent than skin involvement (25 cases – 59.5% versus 20 cases – 47.6% respectively). Those children with lymph node disease were 3.7 years younger than those with other distribution of lesions (p=0.01) and their CD4+ count and percentage was significantly higher, although remaining in the immune deficiency range of values, even after adjusting for age-corresponding CD4+ decrease. The researchers included children up to 18 years of age in this study and this makes it difficult to compare its results with the other analyses summarized above.

While it is difficult to distil a characteristic clinical picture of KS in HIV-positive children from the findings described above, there is evidence pointing to a more disseminated disease at presentation (similar with KS in adults) in HIV-infected versus non-infected, including more frequent visceral involvement. The question whether HIV does correlate with more frequent lymph node involvement than skin involvement by the malignancy cannot be answered conclusively from these data. It was clearly seen from studies in adults that in the pre-AIDS era, males were predominantly affected, while the epidemic KS shows only a slightly higher incidence in males. This is due to the considerable increase of the reported incidence of KS in females, who are also comparatively more often infected with HIV than males are. The same kind of male/female distribution ratio is seen in children infected with HIV, probably reflecting the equal risk of acquiring HIV by vertical transmission at birth. The only discordant data regarding this issue are found in the study by Amir et al (2001),

with ratios of 5.1:1 pre-AIDS and 5.4:1 afterwards, but in that retrospective series the children were only sporadically tested for HIV.

In contrast with the sparse data on the influence of HIV on the clinical picture of KS, its effect on the presentation of BL was analyzed in detail in a well-conducted retrospective study in Uganda (Orem J et al, 2009). They looked at the records of 228 children with BL, out of which 70 were HIV positive and 158 negative. The mean age was 6.9 years, with no statistical difference between the subgroups. The male/female ratio was 1.33:1 in the HIV-positive subgroup and 1.67:1 in the HIV-negative children (not statistically significant). In both groups the most frequent localization of the tumour was facial (71.4% in HIV-positive, 76.6% in HIV-negative); however, children with retroviral infection had significantly more frequent liver and thoracic involvement, as well as lymphadenopathy. As a consequence, there were significantly more children presenting in advanced stage (stage D) in the HIV-positive subgroup compared with the HIV-negative (37.15 versus 20.3%, respectively), with the corresponding effect on the survival rates.

In conclusion, KS and BL, whose endemic variants coexist with their epidemic variants associated with AIDS, offer a unique opportunity to assess the effect of HIV infection on the course of cancers. The evidence from retrospective studies on these two diseases in children with and without HIV in Africa point to a faster progression of the malignancies in the presence of immune deficiency, suggested by a larger proportion of more disseminated forms involving more often thoracic or abdominal organs. As it will be shown below, the consequence of this negative influence is a poorer prognosis.

3.4 Particularities of the management of malignant disease in children with HIV and cancer in Africa

Before discussing the specific issues related to malignancy treatment in children with HIV in Africa, it is necessary to review briefly two topics of universal interest in the management of such cases. The first one regards the use of standard cytostatic or radiotherapy protocols versus low-dose protocols. Cancer chemotherapy is associated with a severe immune suppression due to the destruction of leucocytes. In children with a healthy immune system, the leucopaenia induced by cytostatics is transient and will redress itself during the intervals between therapeutic doses. They are, however, susceptible to severe infections during the

neutropenic spells. In contrast, children with an already compromised immunity by HIV would not recover but would develop a progressive neutropaenia during the course of their treatment (Chanock PJ, Pizzo PA 1995). They are thus even more prone to developing infection during cancer chemotherapy. The answer to this complication is, however, not a low-dose cytostatic protocol (Spina M 2011, Re A et al 2009, Galicier L et al 2007) but rather an efficient infection prevention by controlling the child's environment, applying strict hygiene measures and using well-planned prophylactic antibiotherapy, guided by the oncology unit's cumulative antibiogram and by the knowledge of the infectious agents that are usually being encountered in AIDS (Chanock PJ-see above).

An important role in the management of patients with advanced malignant disease receiving chemotherapy is fulfilled by the peripheral stem cells transplant, which assists with the reconstitution of the immune system. There is also an increasing interest in using the bone marrow transplant with HIV-resistant (naturally or genetically modified) cells as a definitive treatment for the retroviral infection (Krishnan A et al 2010).

Radiotherapy should be given in standard doses, irrespective of the HIV status of the patient. It will be, however, associated with increased toxicity, mainly for the mucosa of the digestive tract. Further, radiotherapy was clearly shown to reduce the CD4+ cell counts, both in HIV-negative and positive patients. This reduction is persistent over several years. There are no published data yet to support the potential increase in risk for opportunistic infections in HIV positive patients who receive irradiation. While there is no evidence to support the initiation of HAART at the onset of radiotherapy or before it in such cases, the use of prophylactic antibiotics is common (Housri N et al 2010).

The second topic of general interest for review in this chapter is the role of HAART in the management of malignancies among people with HIV infection . Antiretroviral therapy reduces the viral load and allows for restoration of the depleted CD4+ lymphocytes and, consequently, the immune status of the patient improves. A number of case studies reported complete or partial remissions of Kaposi's sarcoma after initiation of HAART, mostly in adults but a few also in children (Feller L et al 2010, Niehues T et al 1999). The shortcomings of most of these reports are the small number of cases and short follow-up. Among the more substantial ones is the report by Cattelan AM et al (2005) who have followed up 22 adults with KS for a median duration of 40 months (17-78 months) after initiation of HAART. They observed a durable complete remission in 18 subjects (91%), a partial remission in 2 and a

progression of the disease in a further 2 patients. The complete remission coincided with an increase in the CD4+ cells count and a drop in the number of viral copies/ml. In view of such findings, it seems logical to consider HAART as the first line of treatment in childhood KS, while cytostatics constitute the second line. Caution should be used, however, in conducting antiretroviral therapy on patient with KS, as a substantial increase in the volume of the lesions may occur after a few weeks of treatment, due to the immune reconstitution inflammatory syndrome (IRIS). The reconstitution of the CD4+ cell number stimulates the immune system and leads to additional inflammatory reaction around the cancerous lesions. Depending on the localization of these lesions, the IRIS may be fatal; the addition of chemotherapy will control the process (Leidner RS et al 2005).

The effect of antiretroviral treatment in NHL including BL is not equally dramatic, but definitely beneficial: they enable the administration of a normal-dose cancer chemotherapy and thus improve considerably the prognosis of the patients (Kenkre WP and Stock W 2009, Magrath I 2009, Blinder VS et al 2008). Cornejo-Juarez P et al (2008) compares 28 patients on cytostatics for NHL who had not received HAART with 59 patients on both NHL therapy and antiretrovirals; all were treated in the same institution. Only 14.3% of the subjects not receiving HAART achieved complete response to chemotherapy, compared with 57.6% of those taking antiretrovirals (p≤0.0001). The mean survival time was similarly extended from 4.8 to 14 months respectively (p=0.01).

While there is a clearly documented, significant increase in incidence of cervical intraepithelial lesions in people infected with HIV, and while cervical cancer is an AIDS-defining disease, the evidence accumulated so far does not find any significant effect of HAART on the incidence of cervical pre-cancerous lesions or on the course of the cervical cancer, with or without treatment (Adler DH 2010). All the same, combined antiretroviral therapy does not constitute a remedy in non-AIDS-defining cancers. These appear in individuals who are infected with HIV but are not necessarily immune- compromised and have no indication for HAART. These cancers remain relatively rare in children (as shown in the first chapter), although their incidence increased slightly in the AIDS era.

Finally, the use of HAART in conjunction with cancer chemotherapy calls for increased vigilance and careful planning due to possible drug interactions, as both cytostatics and antivirals may be metabolized by the same hepatic enzymes (cytochrome P450 group), with

consequent increase or decrease in the actions of some of the agents involved (Mounier N et al 2009).

None of the issues presented so far are specific to Africa, but they apply nevertheless to all patients treated on the continent. More frequently seen in African children with malignancies and AIDS than anywhere else, are tuberculosis, malaria and malnutrition. Tuberculosis is one of the AIDS-defining infections, but it is difficult to circumscribe the contribution of the virus to the spread of tuberculosis in the absence of systematic HIV testing of children. In communities with a high prevalence of tuberculosis, this contribution may be minimal (Middelkoop K et al 2008). Irrespective of the potential influence of the HIV epidemic, studies have reported a high incidence of tuberculosis in children with cancer in Africa. The latest evaluation, done in a South African children's hospital, found an incidence of tuberculosis among children treated for cancer of 9117/100,000/year, which is 22 times higher than the overall TB incidence reported in children from a similar background (Stefan DC et al 2008). The particularities of the triad formed by tuberculosis, HIV and solid malignant tumours in children, as they appeared from an analysis of a series of 18 patients, were described by Hadley GP and Naude F (2009): the overall survival in their series was 33%; they had difficulty in using imaging, including positron emission tomography, to identify the tumour spread, as lymph nodes enlarged due to the tumour or tuberculosis or simply due to HIV, were generating similar images. The authors used neoadjuvant chemotherapy to gain more time to investigate their patients and to improve their nutritional status.

Malaria is widespread in Sub-Saharan Africa and the geographical distribution of the high prevalence areas of BL correspond to those of malaria. The co-operation of malaria and infection with EBV in the pathogenesis of BL is generally accepted but insufficiently studied. For instance, it has been shown that during malarial infection the replication of the virus is enhanced, and the treatment of malaria is significantly reducing the viral load (Donati D et al 2006). On a population level, interventions successful in reducing the incidence of malaria appeared to result in a reduction of the incidence of Burkitt (Geser A et al 1989). Malaria and BL coexist undoubtedly in numerous children and malarial cure should be achieved before chemotherapy; no studies have been found addressing this particular issue.

 Malnutrition in children with cancer is frequently seen and not necessarily related to AIDS. Studies done in Malawi, using anthropometric parameters such as arm circumference, arm

muscle area and arm skin fold, found that between 55.1 and 59.3% (depending on the measurement used) of the children admitted with malignancies were undernourished. Further, 44.5% of these children had a height for age below two standard deviations, indicating stunting due to chronic malnutrition (Israels T 2008). A further observation of the same children during cytostatic therapy showed that the malnourished children had a higher rate of profound neutropaenia, resulting in a higher risk of severe infections and death (Israels T 2009). These observations call for a thorough evaluation of the nutritional status of children with cancer on admission – even more so if they are infected with HIV - and a nutrition plan established by a dietician with experience in cancer management.

In conclusion, although HIV does not cause cancer by itself, it strongly facilitates the oncogenic action of other viruses: HHV-8, EBV and HPV. This explains the significant increase of the incidence of malignancies known to be induced by the above agents. The infection with HIV worsens the prognosis of cancers, mainly due to the profound neutropaenia during the treatment with cytostatics, which does not recover between the courses of therapy. HAART makes possible a better recovery from neutropenia, thus enabling the administration of standard chemotherapy protocols. Tuberculosis, malaria and malnutrition, much more prevalent in Africa, add to the complexity of cancer management in children with HIV.

CHAPTER 4

PATIENTS AND METHODS

The first question asked in this study was whether children infected with HIV who develop cancer have a higher risk of occurrence of any particular cancer, when compared with other children with cancer but without HIV. Such a finding, beyond the associations already known (e.g. Kaposi sarcoma), may suggest an underlying infectious origin of the respective cancer, by analogy with Kaposi sarcoma or Burkitt lymphoma, and, should this hypothesis be confirmed by future research, a possible avenue may open for preventing that particular malignancy. The second question asked was whether the prognosis of cancer in children with HIV is different from the prognosis observed in the absence of HIV.

4.1 Study of the association of HIV infection with particular cancers

The data on HIV and the risk of specific malignancies in children originated from an on-going study of the comparative odds of various cancers in children with and without HIV (please see also "Author's declaration").

 The data were collected from 2005 to 2009, in four South African paediatric oncology centres: Tygerberg Hospital in Cape Town, Chris Hani-Baragwanath Hospital and Charlotte Maxeke Central Hospital, both in Johannesburg, as well as Universitas Hospital in Bloemfontein. The children, aged from 0 to 14 years, had been either referred directly to the paediatric oncology unit by clinics, community health centres or private practitioners located in the drainage area of the study hospitals, or had been brought by their parents/relatives to the emergency rooms of those hospitals, from where their management was taken over by the oncologists.

A departmental admission register was kept, recording the name, age, sex and presumptive diagnostic on admission. This register was used to identify patients' records later for data capture. The age, sex, clinical diagnoses, histology results and HIV test results were

retrieved periodically from the archived clinical records of all children admitted with a diagnosis of cancer and were entered manually in an electronic database by study investigators in each of the above healthcare units. At the end of the study period, anonymous data from the databases were sent to The Epidemiology and Genetics Unit, University of York, UK, to be analyzed for demographic differences and distribution of various forms of cancer between groups with and without HIV infection. It was found subsequently that thirty children had benign neoplasms and 27 were diagnosed with non-neoplastic conditions – they remained in the statistical analysis, as there was no evidence that their conditions may be associated with HIV and their numbers contributed to the size of the comparison group. The age and sex distribution of cases was similar in all centers. Out of the 882 children, 38 (4%) were HIV infected.

Viral testing was done by rapid ELISA followed by confirmation with PCR, according to uniform national South African guidelines. Only HIV-1 was considered throughout this study, since HIV-2 is mostly present in Western Africa (Centers for Disease Control 2009). Further, viral clade differences were not considered, mainly because it was beyond the scope of this research, but also because there is no evidence that such differences impact on cancer risk in HIV-infected people. The children living with HIV were either on HAART at diagnosis or were prescribed HAART immediately thereafter.

All cancer diagnoses were confirmed by histopathological review as part of routine procedures at NHLS and coded to the International Classification of Childhood Cancer, 3rd edition. A central review of pathology specimens was not considered necessary: the diagnoses established by NHLS were considered adequate for the present study, as the laboratory practice is in accordance with uniform national standards (see also Chapter 2).

All eight hundred and eighty-two children newly admitted with suspected cancer, during the period indicated above, were entered into the study (for 12 the HIV status was unknown and their information was not entered in the statistical analysis). Odds ratios for the association between HIV and each cancer type were estimated using unconditional logistic regression with adjustment for age (by single year), sex, and centre (SAS software). For each cancer type, the comparison group comprised all other cancers, excluding Kaposi sarcoma and lymphomas, which are already known to be HIV-associated.

Ethical approval for this research was granted from each centre separately and from the Oxford Tropical Research Ethics Committee.

4.1.1 Definition of cases and controls

Cases were children with each one of the cancer types. In order to evaluate the effect of the exposure to HIV infection, the odds of HIV positivity in cases were compared with the odds in all other children with cancer, serving as controls. The cancers already known to be associated with HIV – Kaposi sarcoma and lymphomas - were excluded from controls. Controls were admitted to the same hospitals during the same time intervals as cases; their number was several times larger than the cases number, thus increasing the precision of the results (Grimes 2005).

4.2 Study of the impact of HIV infection on the prognosis of cancer in children

The second question asked in this dissertation aimed at exploring the effect of HIV infection on the prognosis of cancer in children. In order to answer this question, a retrospective cohort study was designed, using two cohorts of children admitted to hospital with malignant disease, one living with HIV and the other without HIV. It was thus possible to study the differences in prognosis only for children (0 to 14 years of age) admitted to hospital, with the intention to undergo treatment for their HIV infection and malignant disease.

The first cohort included eighty-four children HIV positive (the exposed group): these patients had been consecutively admitted from January 2002 to April 2010 to the paediatric oncology units of Tygerberg Children's Hospital in Cape Town and Universitas Hospital, Bloemfontein, both in South Africa. The children with HIV were exclusively of black African ethnicity: based on the results of previous studies indicating differences in the outcome of cancer therapy between various ethnic groups in South Africa (Stefan DC et al 2009), it was decided to consider only consecutively admitted black African children, without HIV, in the second cohort. This cohort (the unexposed subjects) was comprised of 570 children with malignant diseases, not infected with HIV, consecutively admitted at Tygerberg Children's Hospital as well as at Universitas Hospital, from January 2002 to December 2010. The HIV testing result was not registered in 9 cases, and the histology results were missing in 6 cases; these children were not entered in the study.

Similarly with the study described in the subchapter 4.1, the HIV testing was done by rapid ELISA followed by confirmation with PCR, according to uniform national South African guidelines. Only HIV-1 was considered throughout this study and clade differences were not

considered (see subchapter 4.1) Further, all cancer diagnoses were confirmed by histopathological review according to NHLS routine protocols and coded using the International Classification of Childhood Cancer, 3rd edition. The diagnoses established by NHLS were considered adequate for the present study, as the laboratory follows uniform national standards (see also Chapter 2).

All patients in both cohorts (exposed and unexposed) were followed up from the date of diagnosis until they died, were lost to follow up or to the end of the study (June 2011). While the chemotherapy protocols were identical in both centres for any given cancer, all children with HIV were additionally receiving HAART, either initiated prior to diagnosis or prescribed shortly (within 48 hours) after diagnosis.

Data were entered in each oncology unit's electronic database, by data capturers, after each discharge from hospital or after each follow-up visit. The following information was extracted by the author from the units' databases and entered onto a Microsoft Excel spreadsheet: date of birth, sex, date of diagnosis, diagnosis (confirmed by histopathology), HIV status, date last seen, outcome and therapy protocol. The information related to staging was not available in most patients. The CD4-positive leukocyte count or the viral load figures were not available for most subjects and could not be considered when performing the analysis of outcomes. Due to the retrospective nature of the study, no active or standardized follow-up procedures could be instituted.

The outcomes were defined similarly in both centres: alive disease free, alive with disease, dead of disease, dead of toxicity, dead of other causes, lost to follow-up in remission and lost to follow-up presumed dead. Deaths due to severe infection in children with neutropaenia were included, together with deaths from severe side-effects of the medication, under the heading "dead of toxicity".

Comparative analyses were then carried out on both cohorts, as follows. . Proportions for every outcome enumerated above were calculated, separately for HIV positive and negative children, for each cancer appearing in both cohorts (exposed and unexposed). Pearson's chi square tests were used to compare proportions in each group and t-tests were used to compare means between the groups

Comparative Kaplan-Meyer survival curves were constructed for each malignancy in both groups. Log rank tests were used to compare the time to mortality between the groups.

All calculations were done using STATISTICA software and SPSS version 19 (IBM SPSS Inc.).

Ethical approval for this research was granted from each centre separately and from the Oxford Tropical Research Ethics Committee.

CHAPTER 5

RESULTS

5.1 Association of HIV infection with specific cancers in children

The association of HIV infection with specific cancers in children was investigated using data from 882 children with suspected cancer diagnosed during the time interval indicated in the methods section: 84 from Tygerberg Hospital in Cape Town, 79 from Chris Hani-Baragwanath Hospital, 642 from Charlotte Maxeke Central Hospital in Johannesburg, and 79 from Universitas Hospital in Bloemfontein. The group consisted of 476 boys, 400 girls, while in 6 cases information on sex was missing. Thirty children had benign neoplasms and 27 were subsequently diagnosed with non-neoplastic conditions. Data on sex, age and HIV serostatus are shown in Table 5.1, together with estimated odds ratios (OR) and 95% confidence intervals (CI). The age and sex distribution of cases was similar in all centres. In Table 5.1, only cancers counting a minimum of 20 cases were included (with the exception of Kaposi sarcoma). The result is that numbers in the columns will not add up to the totals shown. The restriction to 20 cases is arbitrary, in order to retain a power of the study capable to better identify differences between the groups, but even with this precaution, some large odd ratios are not statistically significant, and that is an indicator of insufficient power (see Chapter 6: Discussion).

Of the 882 children, 38 (4%) were HIV infected. HIV was associated with Kaposi sarcoma (all 10 cases were HIV infected; P<0.001) and with Burkitt lymphoma (OR=46.2, 95% CI 16.4–130.3; based on 13/33 infected cases). For non-Burkitt NHL, 2/39 were HIV infected (OR=5.0, 95% CI 0.9–27.0). Out of 172 children with lymphoid leukemias, only one had evidence of concurrent HIV infection (OR=0.4, 95% CI 0.04–2.9). No other cancer type was significantly associated with HIV, however, with the small numbers of cases for many types of malignancy, the power of the study was insufficient to detect differences for a level of confidence of 95% (see Chapter 6: Discussion).

Cancers	Total number	Sex			Age (years)		HIV status			Odds ratio (95% CI)
		M	F	Missing	Mean	Missing	+	-	Missing	
Total patients in the study	882	476	400	6	6.6	13	38	832	12	
Kaposi's sarcoma	10	7	3	0	6.4	0	10	0	0	Infinite (*P* < 0.001)
Non-Burkitt non-Hodgkin lymphoma	40	19	20	1	9.4	1	2	37	1	5.0 (0.9–27.0)
Burkitt lymphoma	33	27	6	0	6.2	0	13	20	0	46.2 (16.4–130.3)
Hodgkin lymphoma	44	26	18	0	10.5	0	1	43	0	2.3 (0.3–21.4)
Lymphoid leukemias	172	105	65	2	6.8	3	1	171	0	0.4 (0.04–2.9)
Nephro-blastoma	95	49	45	1	3.5	3	2	89	4	1.2 (0.2–6.1)
Myeloid leukaemias	65	27	38	0	6.5	1	2	61	2	2.4 (0.5–11.9)
Rhabdomyo-sarcoma	56	27	29	0	7.7	0	1	54	1	1.2 (0.1–10.0)
Neuroblastoma	49	27	22	0	3.2	0	0	49	0	—
Osteosarcoma	26	18	8	0	12.5	0	0	26	0	—
Retinoblastoma	25	17	8	0	2.7	0	1	24	0	2.5 (0.5–34.0)
Astrocytoma	22	13	9	0	6.7	1	1	21	0	3.9 (0.5–34.0)
Cranial embryonal tumour	21	12	9	0	6.5	0	0	21	0	—

Table 5.1: Characteristics of cancer patients and association with HIV infection (Stefan DC et al 2011)

5.2 Outcome after treatment in HIV positive versus HIV negative children with cancer

The differences in outcome after treatment in HIV positive and negative children with cancer, were explored in the second study, described in 4.2, by comparing 570 HIV negative children with cancer (87.2%) with 84 HIV positive children with cancer (12.8%). The analysis of the outcomes was performed first on the entire study cohort, followed by a comparative analysis of HIV positive and negative groups and lastly by a comparison of HIV positive and negative subgroups with the same malignancy: Burkitt lymphoma, leukaemia and (non-Burkitt) lymphoma.

5.2.1 Characteristics of the study cohort

a) Age and sex

The mean age in the group studied was 76.6 months and the median 67.5 months. The interquartile range was 33 to 118 months. There was a slight difference in the distribution of children by sex with 364 males (55.7%), compared to 290 females (44.3%).

b) Ethnicity

As discussed in Chapter 4, given that the children with HIV were all black and previous studies addressing the outcome of the treatment for cancer in South Africa indicated differences associated with ethnicity, the children entered in the group without HIV were also black.

c) Distribution of cancers in the study group

The whole group included 654 subjects, diagnosed with: leukemia 127 cases (19.4%), brain tumors 118 (18%), nephroblastoma 74 (11.3%), lymphoma 60 (9.2%), retinoblastoma 44 (6.7%), neuroblastoma 34 (5.2%), Burkitt lymphoma 33 (5%) and rhabdomyosarcoma 24 (3.7%). Kaposi sarcoma was only present in the HIV positive group in 36 cases (5.5%). Some of the unclassified tumors as well as cancers observed in small numbers (below 10) were grouped in a separate group under "others" (see Table 5.2 and Figure 1).

Malignancy	Number of cases	Percent
Brain tumor	118	18.0
Burkitt lymphoma	33	5.0
Kaposi sarcoma	36	5.5
Leukemia	127	19.4
Lymphoma	60	9.2
Nephroblastoma	74	11.3
Neuroblastoma	34	5.2
Osteosarcoma	27	4.1
Other	77	11.8
Retinoblastoma	44	6.7
Rhabdomyosarcoma	24	3.7
Total	654	100.0

Table 5.2. Distribution of cancers in the whole study cohort
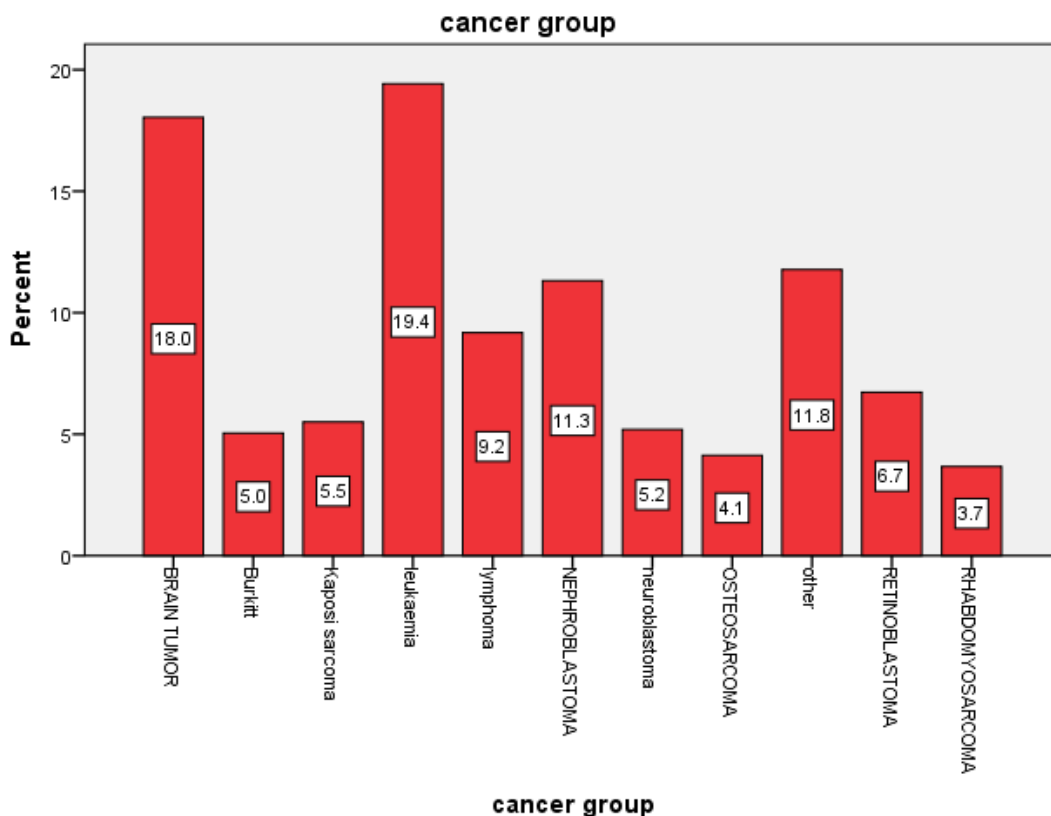
Figure1. The distribution of cancers in the whole study cohort

d) Length of follow-up of subjects studied

The average follow-up was 21.5 months and the median 11 months, with a standard deviation of 25 months). The interquartile range was of 3 to 31 months.

5.2.2 Study of outcomes for the whole group

The outcome was defined as: alive disease free, alive with disease, died due to toxicity, died due to disease, died of unknown or unrelated causes, lost to follow-up presumed dead lost in remission. However, due to the small numbers, for comparison purposes, the 7 groups above were also merged into 3: alive, dead and lost to follow up. The final outcome was defined as the status of the subject at the end point of the study. Most of patients were still alive (around 53%) with a small number lost to follow up (little above 6%) and just over 41% were dead at the end of the study (Tables 5.3, 5.4 and Figure 2).

Outcome	Frequency	Percent
Alive with disease	45	6.9
Alive without disease	299	45.7
Dead from disease	245	37.4
Dead from toxicity	11	1.7
Dead from unknown	14	2.1
Lost in remission	20	3.1
Lost presumed dead	20	3.1
Total	**654**	**100.0**

Table 5.3. Detailed outcomes for the study group

Merged outcomes	Number of cases	Percent
Alive	344	52.6
Dead	270	41.3
Lost	40	6.1
Total	**654**	**100.0**

Table 5.4. Final merged outcomes for the whole group
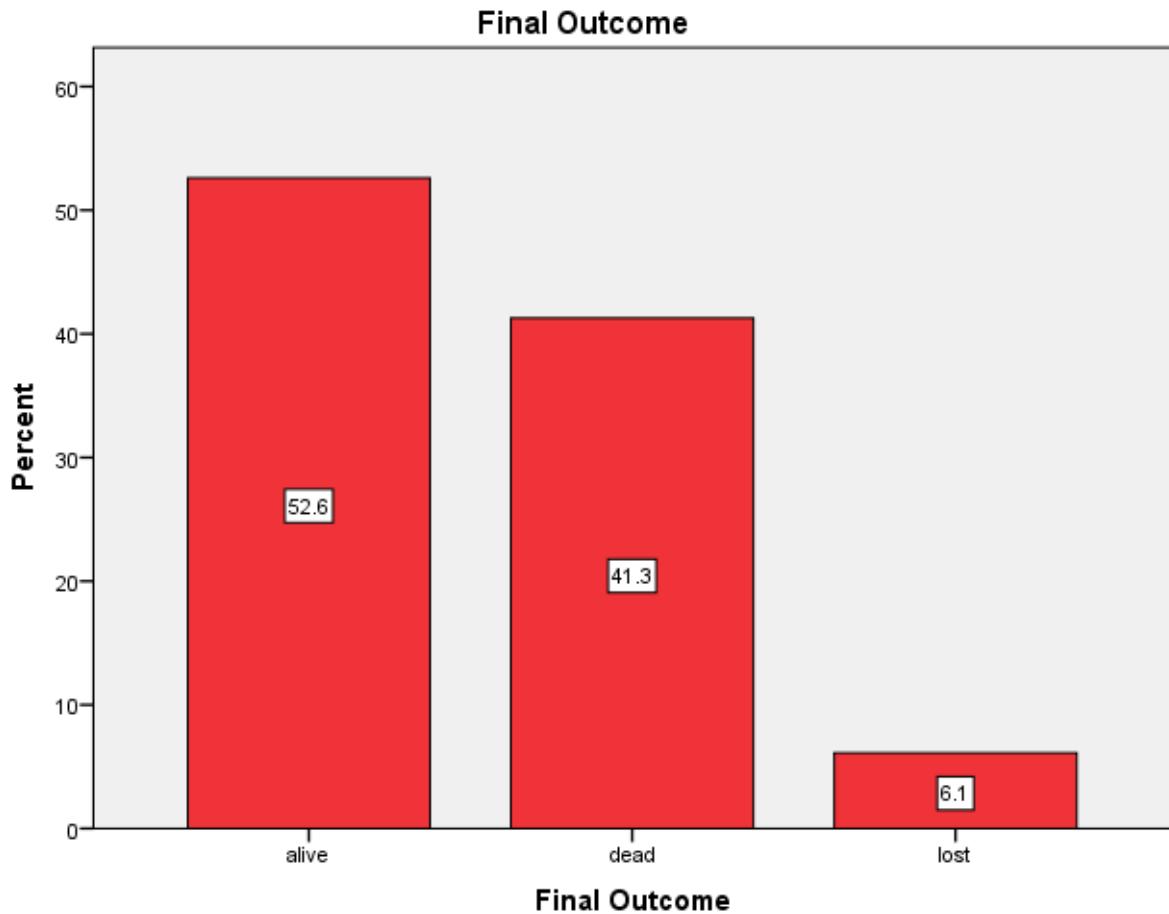
Figure 2. Final merged outcomes for the whole group

5.2.3 Comparative analysis of HIV positive and negative groups

a) Distribution of cancers

The distribution of the different kinds of malignancies in the two groups is far from identical. Kaposi sarcoma was the most frequently encountered malignancy it the HIV positive group, followed by Burkitt lymphoma, whereas in the HIV negative group leukemia is the most frequent, followed by brain tumours, each amounting to around one fifth of all cases . Kaposi sarcoma was exclusively seen in HIV positive children while the retinoblastoma, osteosarcoma, germ cell tumor, liver tumor and chronic myeloid leukaemia were only encountered in HIV negative cases (Table 5.5 and Figure 3).

CANCER GROUP	HIV NEGATIVE		HIV POSITIVE		TOTAL	
	NUMBER CASES	%	NUMBER CASES	%	NUMBER CASES	%
Brain tumours	118	20.7	0	0	118	18
Burkitt lymphoma	16	2.8	17	20.2	33	5
Kaposi sarcoma	0	0	36	42.9	36	5.5
Leukaemia	121	21.3	6	7.1	127	19.4
Lymphoma	45	7.9	15	17.8	60	9.2
Nephroblastoma	71	12.5	3	3.6	74	11.3
Neuroblastoma	32	5.6	2	2.4	34	5.2
Osteosarcoma	27	4.7	0	0	27	4.3
Retinoblastoma	44	7.7	0	0	44	6.7
Rhabdomyosarcoma	23	4	1	1.2	24	3.7
Other	73	12.8	4	4.8	77	11.7
TOTAL	570	100	84	100	654	100

Table 5.5. Distribution of the various cancers in the 2 groups (HIV positive versus HIV negative children)
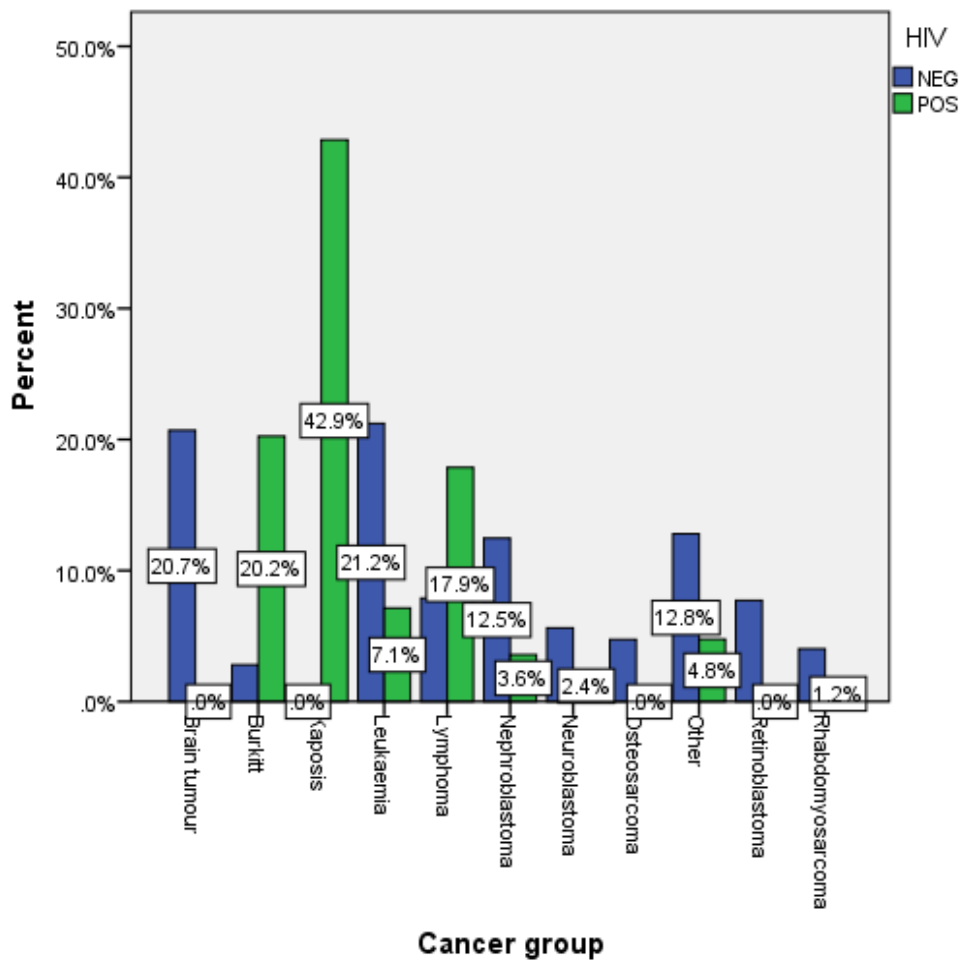
Figure 3. Distribution of cancers in relation to HIV

b) Distribution by sex

The distribution of subjects by gender shows a male numeric predominance, compared with female numbers in the HIV group (Table 5.6 and Figure 4). This difference is statistically significant (chi-squared, 1 degree of freedom, two-tailed p=0.02).

SEX	HIV NEGATIVE		HIV POSITIVE		TOTAL	
	NUMBER CASES	%	NUMBER CASES	%	NUMBER CASES	%
Female	263	46.1	27	32.1	290	44.3
Male	307	53.9	57	67.9	364	55.7
TOTAL	570	100	84	100	654	100

Table 5.6. Distribution of HIV positive versus HIV negative children, by sex
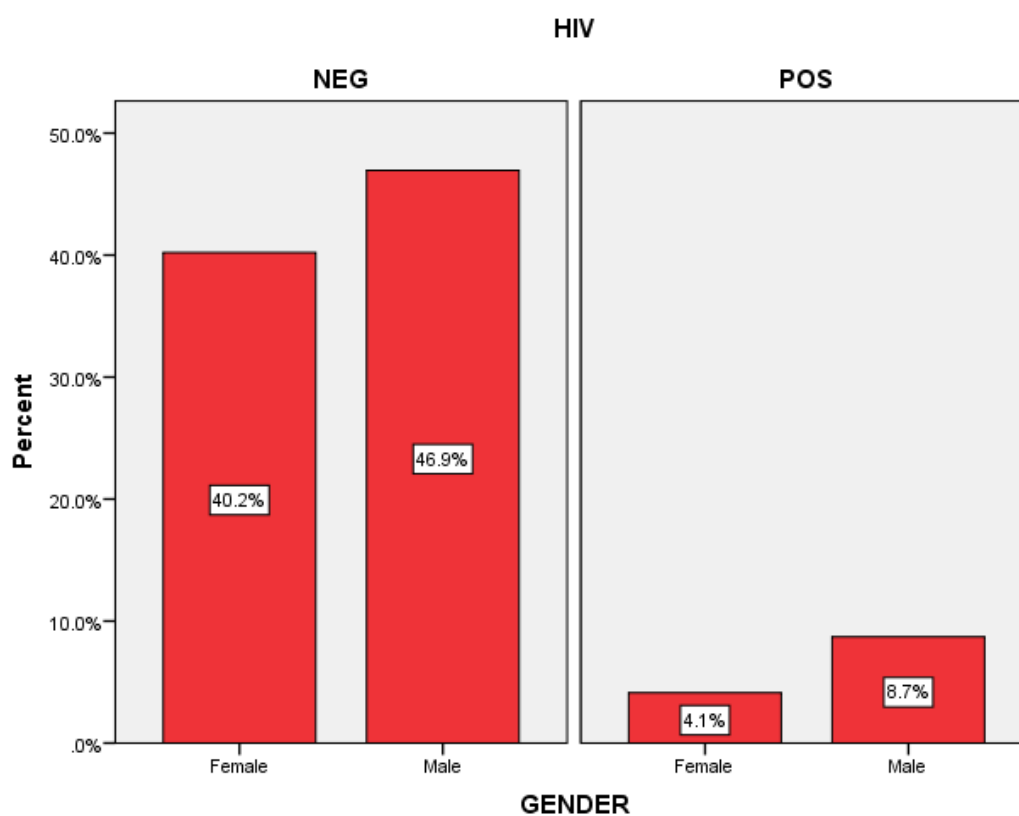
Figure 4. Distribution by sex of the HIV negative and positive groups (columns represent percentages out of the total of 654 children)

c) Age at diagnosis of subjects in HIV negative versus positive group

HIV positive patients with cancer tend to be younger (70.6 months average) than HIV negative patients with cancer (77.5 months) but the difference is not statistically significant (unpaired t-test, two-tailed p-value = 0.186; Table 5.7).

HIV STATUS	NUMBER CASES	MEAN AGE (MONTHS)	STANDARD DEVIATION
NEGATIVE	570	77.53	51.421
POSITIVE	84	70.62	43.250

Table 5.7. Mean age in HIV negative versus positive groups

d) Comparison of outcomes in HIV negative versus HIV positive groups

There was an excess mortality in the HIV positive group (Table 5.8). The differences noted are, however, not statistically significant (Pearson's chi square test, two-tailed p-value = 0.495).

FINAL OUTCOME	HIV NEGATIVE		HIV POSITIVE		TOTAL	
	NUMBER CASES	%	NUMBER CASES	%	NUMBER CASES	%
Alive	304	53.3	40	47.6	344	52.6
Dead	233	40.9	37	44.0	270	41.3
Lost to follow-up	33	5.8	7	8.3	40	6.1
TOTAL	570	100	84	100	654	100

Table 5.8. Final merged outcomes in HIV negative group versus positive group

FINAL OUTCOME	HIV NEGATIVE		HIV POSITIVE		TOTAL	
	NUMBER CASES	%	NUMBER CASES	%	NUMBER CASES	%
Alive disease free	272	47.7	27	32.1	299	45.7
Alive with disease	32	5.6	13	15.5	45	6.9
Died from disease	230	40.3	15	17.8	245	37.5
Died from toxicity	1	0.2	10	11.9	11	1.7
Died of unknown cause	2	0.4	12	14.3	14	2.2
Lost presumed dead	15	2.7	5	5.9	20	3.0
Lost in remission	18	3.1	2	2.5	20	3.0
TOTAL	570	100	84	100	654	100

Table 5.9. Detailed outcome comparison between HIV positive and negative children with cancer

However, when analyzing the detailed outcomes in the two groups, it appears that the ratio of children alive disease-free at the end of follow-up, to the total of children in the respective category, is significantly smaller in the HIV positive subjects (chi-square, 1 degree of freedom, two-tailed p=0.01). Additionally, the ratio of children who died from toxicity (which include children with infection due to marked leucopoenia), to the total in their respective group, is significantly larger in the HIV positive subjects (chi-squared, 1 degree of freedom, two-tailed p<0.0001). The data are presented in Table 5.9 above.

e) Survival analysis in HIV negative versus HIV positive subjects

The comparative survival analysis over the entire follow up period and in all cancer types using Kaplan-Meyer curves is shown in Figure 5. The HIV-positive group demonstrates a higher death rate in the first year but the difference observed over the whole duration of follow-up is not significant (Table 5.10).
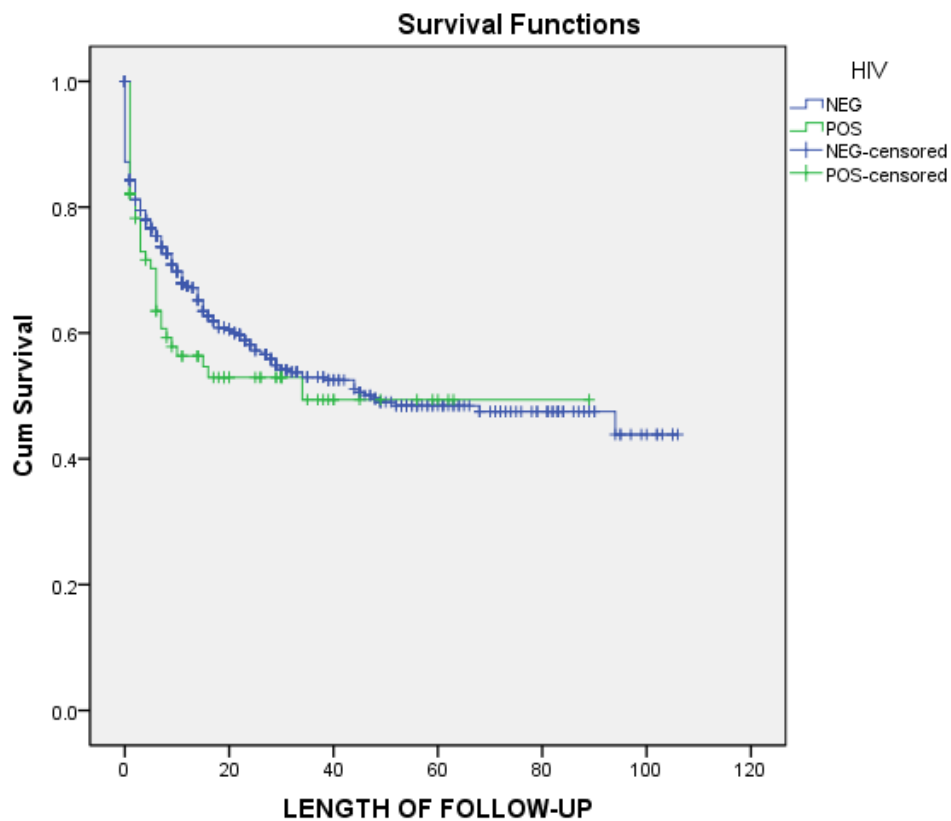
Figure 5. Comparative survival (in months) of HIV-negative versus HIV-positive children with cancer

SURVIVAL CURVES COMPARED	CHI-SQUARED	P
Overall survival	0.791	0.374
Survival during the first year	3.165	0.075
Children with leukaemia	0.181	0.670
Children with lymphoma	0.056	0.812
Children with Burkitt lymphoma	1.130	0.288

Table 5.10. Test of equality of survival distributions for the different exposures to HIV.

Even when the test for equality of the Kaplan-Meyer curves was used only for the first year of follow-up, where the difference is most obvious, borderline non statistically significant values were obtained (Figure 6 and Table 5.10).
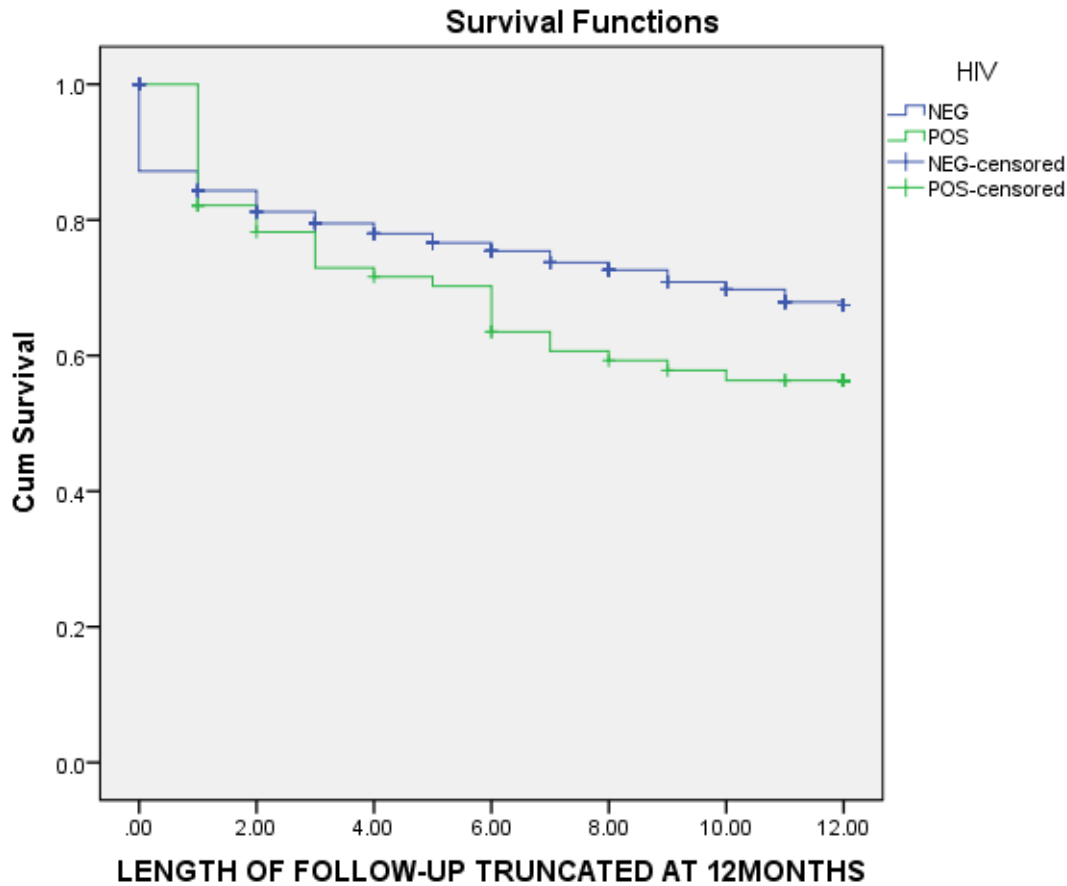
Figure 6. Comparative survival of HIV-negative versus HIV-positive children with cancer in the first year of follow-up

f) Survival analysis of HIV negative versus HIV positive subjects for single malignancies

Several cancers appeared exclusively in one of the groups studied: Kaposi sarcoma was present only in the HIV-positive children, while brain tumours, osteosarcoma and retinoblastoma appeared only in the HIV-negative group. For these cancers, no comparison analysis of survival was possible. Further, no such comparative analysis was done for those cancers with only one or two cases in either of the groups: rhabdomyosarcoma and neuroblastoma. Finally, all 3 cases of nephroblastoma in HIV-positive children were lost to follow-up, thus their outcome is not known. Thus, a comparative analysis of survival was

performed only for leukaemias (Table 5.10 and Figure 7), lymphomas (Table 5.10 and Figure 8) and Burkitt lymphoma (Table 5.10 and Figure 9). When compared by means of log rank tests, the differences noted between curves did not attain statistical significance due to small sample size and lack of power (see Chapter 6: Discussion)
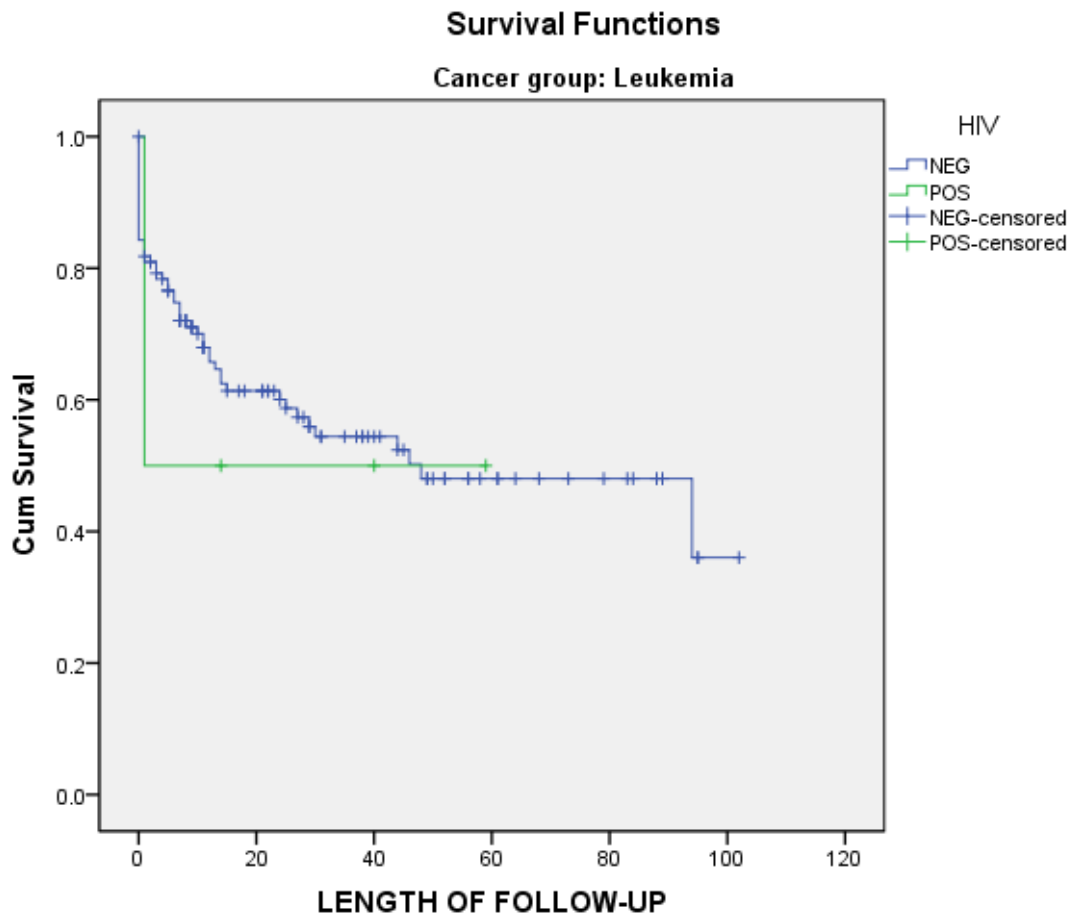
.

Figure 7. Survival analysis of HIV-negative, versus HIV-positive children with leukaemia
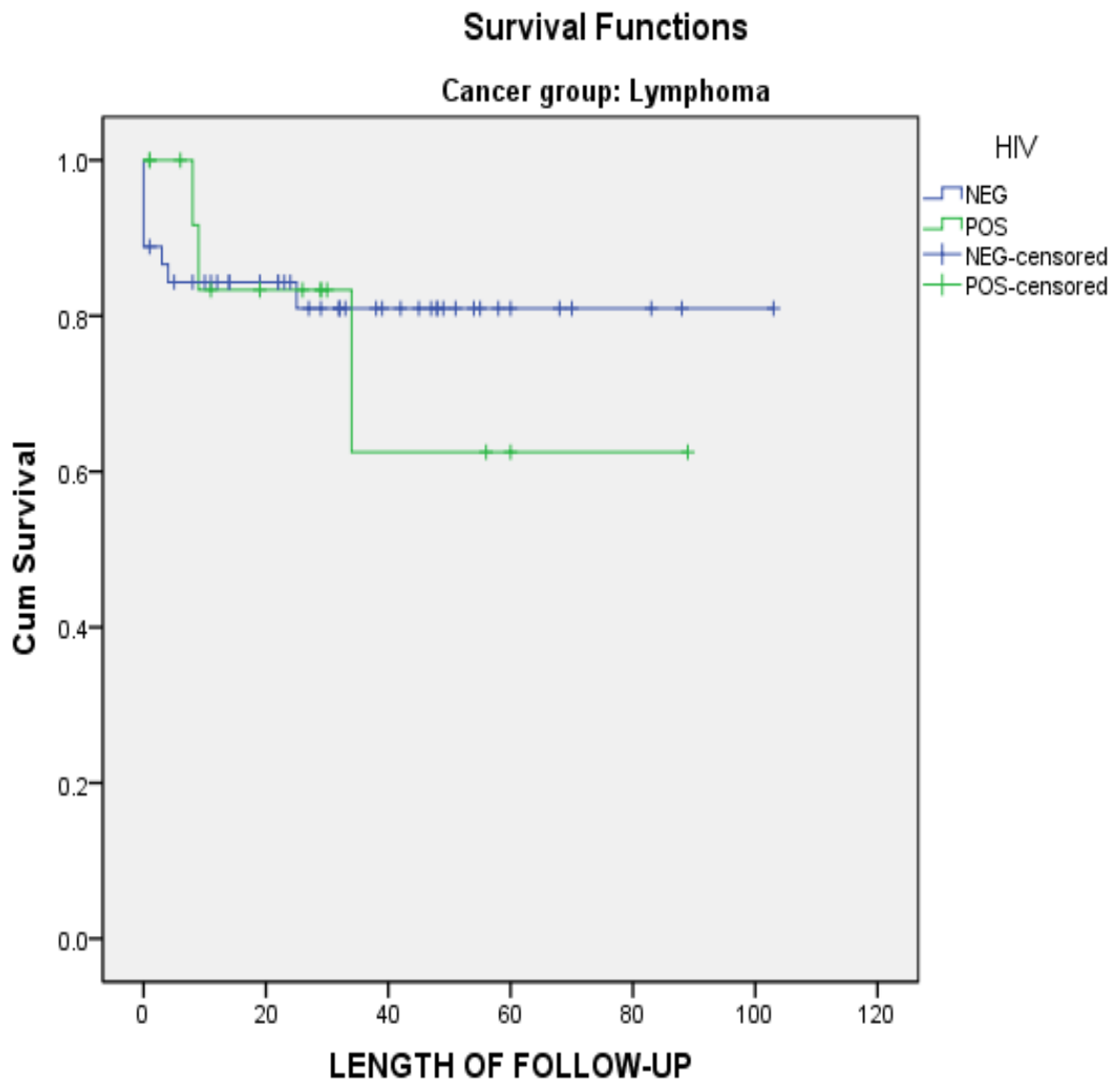
Figure 8. Comparative survival analysis between HIV-negative and positive children with lymphoma
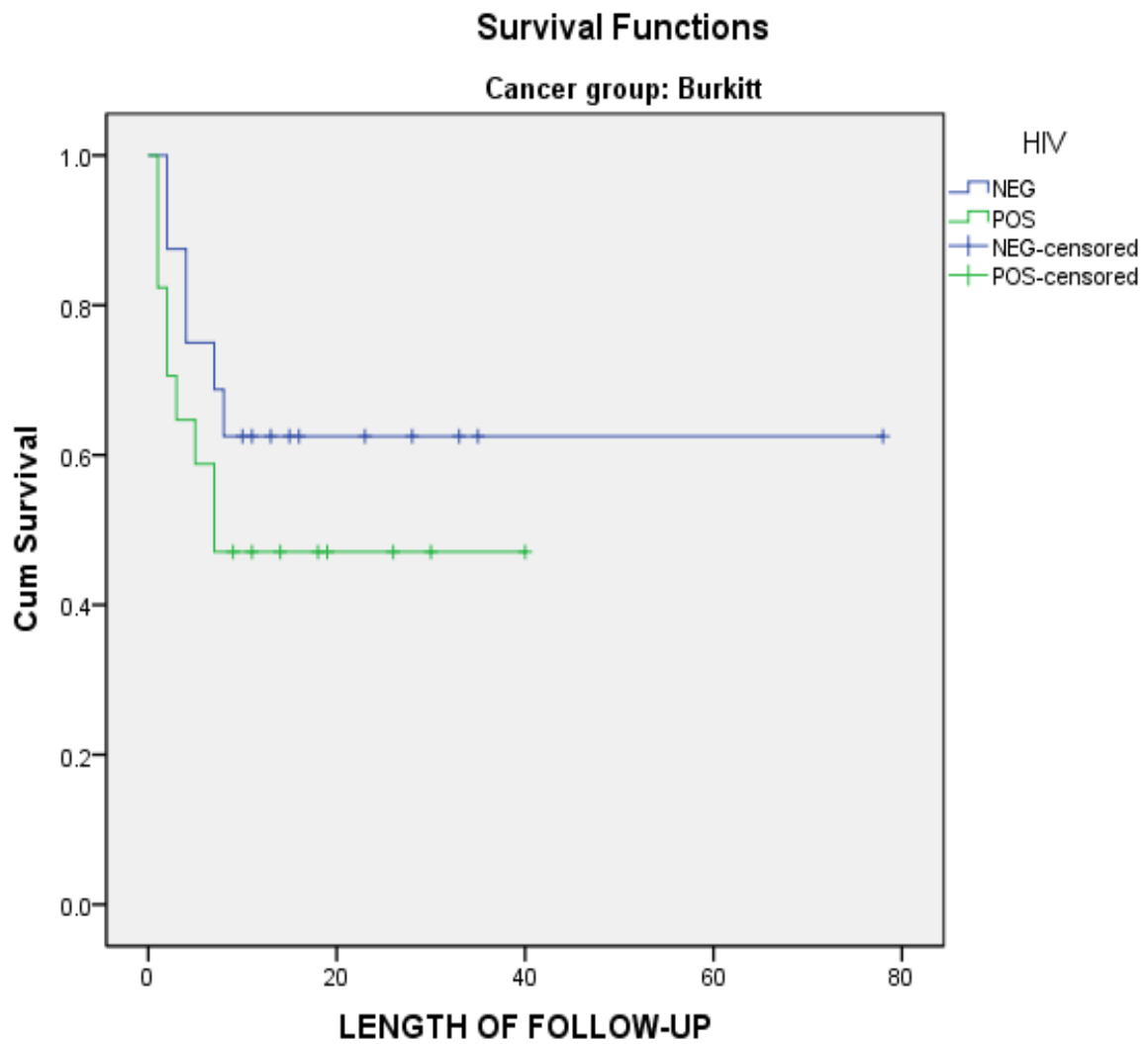
Figure 9. Comparative survival analysis between HIV-negative and positive children with Burkitt lymphoma

CHAPTER 6

DISCUSSION

The data presented above add a few details to the understanding of the complex impact of the HIV infection on the risk of cancer, the type of cancer and its prognosis in children. The literature in this field is far from exhaustive, partially due to the fact that most of the children with HIV live in Africa, where it is incomparably more difficult to compile accurate statistics and to perform epidemiologic studies, than it is in western populations. In the following paragraphs, an attempt will be made to discern the meaning of this study's findings, with reference to other works published to date.

6.1 The association between HIV and certain cancers in children

The evidence accumulated so far supports the association between several cancers and HIV infection in adults: Kaposi sarcoma, NHL, Hodgkin lymphoma, cancers of the uterine cervix and anus, and conjunctival squamous cell carcinoma were identified as clearly being more frequent in people with HIV than in those without (Bouvard V. et al 2009). They are, however, not directly induced by the immunodeficiency virus: other viruses, such as EBV, HHV-8 and papillomaviruses are responsible for the malignant process, as described in Chapter 3. The evidence for the primordial role of these latter viruses is that their association with the cancers mentioned was also detected in people not infected with HIV (Beral V et al, 1998). HIV, therefore, facilitates the development of certain cancers with other underlying infectious causes and so identifying HIV-related cancers provides a way of identifying new cancers that may be caused by infections. This was the purpose of the first study described in this dissertation.

The role of HIV in the oncogenic process in children is not as well defined as in adults, mostly because the epidemic was not so strong in the countries which have the resources and

skills to research this issue adequately. As a result, the findings are based on a limited number of cases. For example, Biggar RJ (2000) found that children living with HIV were at an increased risk of NHL (based on 42 cases) - including sporadic Burkitt lymphoma, immunoblastic lymphoma and primary lymphoma of the brain - Kaposi sarcoma (based on four cases), and leiomyosarcoma (based on three cases).

The vast majority of children with HIV are living presently in Africa, where the human and infrastructural capacity for research is considerably limited. Moreover, in numerous populations on the continent, Kaposi sarcoma and Burkitt lymphoma were endemic before the onset of the HIV epidemic, and this disease background may interfere with the efforts of evaluating the effect of HIV-AIDS on childhood cancer. Only two epidemiological studies have addressed so far the question of the excess risk of cancer in HIV-positive children when compared with HIV-negative children. Newton R.et al (2001), in Uganda, found that HIV was associated with a higher risk of Kaposi sarcoma and Burkitt lymphoma, against a background of an endemic presence of these two diseases. In Malawi, Mutalima N.et al (2010) found that in children with HIV, the incidence of Kaposi sarcoma and non-Burkitt-non-Hodgkin lymphoma was increased, while the results for Burkitt lymphoma were statistically not significant. The explanation may be that this disease existed in endemic form before the HIV-AIDS epidemic, and the study might not have had the power required to detect the change due to HIV infection.

The endemic form of Burkitt lymphoma does not occur in South Africa, but in the present study, "sporadic" Burkitt lymphoma was clearly associated with HIV as it is among adults and children in the west (Engels EA et al 2008). Non-Hodgkin, non-Burkitt lymphoma deserves a special mention: this group of cancers were observed significantly more often in association with HIV infection (Mueller BU 1996, 1998); however, the odds ratio of 5.0 for this association was marginally non-significant (95% CI: 0.9-27.0), suggesting that the study might not have the required power to reproduce the findings presented in the literature. Other cancer types in children show no significant association with HIV in our study; similar data were produced so far by all studies done in Africa.

Leiomyosarcoma, which was found to show an increase in frequency in children with AIDS in SUA, did not appear related to HIV in this study, neither in other African research published so far. This is even more intriguing when considering that leiomyosarcoma is associated with the Epstein-Barr virus, as is Burkitt lymphoma, and consequently an

increased frequency of observation of this tumour would be expected. It is, however, a rare tumour in children and probably a larger series of subjects would be necessary in order to clarify its association with HIV infection.

The lack of support for the association between HIV infection and acute lymphoblastic leukaemia (ALL) may throw a particular light on the hypothetical connection of infections and immunity with this haematological malignancy. Kinlen LJ et al (1993, 1995) proposed that leukaemia might be the consequence of an infection, aided possibly by the population mobility and mixing. Such infectious etiology was demonstrated clearly for leukaemias in other mammals (cats, cattle) and in chickens, but not yet in people. Greaves MF et al (1997) thought that a relative protection from infections in early childhood, due to higher standards of hygiene (the "hygiene hypothesis") leave the immune system unprepared for later infections, which may trigger an inadequate proliferative response leading in some cases to leukaemia. Others (Roman E et al 2007, Cardwell CR et al 2008) have, however, found that children with leukaemia register an excess of infections in early childhood. It would appear, from our findings, that there may not be any correspondence between HIV infection or the associated impaired immunity and leukaemic malignancy. In fact, this lack of association of poor immunity and leukaemia was noted in immune suppression of other etiologies (Beral V, Newton R 1998, Grulich AE et al 2007).

It is conceivable that children infected with HIV would die before the leukaemia develops; this would still leave place for considering an infectious factor in the etiology of leukaemia. However, considering the relatively small and statistically insignificant difference in age between HIV negative and positive children, found in the second study forming this dissertation (Table X), such an asymmetrical distribution of leukaemia cases would suppose that HIV positive children who were at risk of leukaemia died at a much higher rate than those who were not. So far, there is no evidence to support such death rates.

The distribution of various cancers in the other study (Table IX and Figure 4) is markedly similar to that in the first dataset. Almost 2/3 (60%) of the children living with HIV had either Kaposi sarcoma or Burkitt lymphoma. Kaposi sarcoma was exclusively encountered in the HIV positive group, while Burkitt lymphoma was 7.2 times more frequent in the children with HIV. In contrast to the above, germ cell tumours, hepatic tumours (grouped under "others") and osteosarcoma were recorded solely in the HIV negative children, although this

may simply have been due to chance, given the relatively low prevalence of HIV among cancers other than KS and NHL.

6.2 The impact of HIV infection on the prognosis of children with cancer

6.2.1 Demographic findings

a) The average age at diagnosis

The age at diagnosis average was lower by 6.9 months in the HIV positive group compared to the uninfected group; this difference did not attain statistical significance, but the study might have been under-powered to detect differences of that magnitude. The available literature contains minimal data on the age of onset of malignant disease in HIV positive children by comparison with HIV negative. Sinfield et al (2007) found an average age of 2.3 years in 52 HIV-negative children with Kaposi sarcoma, compared with 7.2 years in 4 HIV-positive children with the same malignancy. For Burkitt lymphoma, the average age found by Orem J. et al (2009) was 6.9 years, with no statistic significant difference between 158 children not infected with HIV and 70 positive children.

b) The male / female ratio

The ratio of 1.16:1 in the group not infected with HIV (see Table X) probably reflects the generally higher incidence of cancer in males at all ages, which is well documented (Cook MB et al 2009). However, this ratio is significantly higher in the children with cancer and HIV, at 2.1:1. Considering the published evidence for a 2:1 ratio of vertical transmission of HIV to female foetuses versus males (Taha TE et al 2005), this imbalance appears even larger. It is possible that the predominance of malignancies known to occur more often in boys, in the HIV group, accounts for the unequal representation of the sexes here. Considering that Kaposi sarcoma represents almost 40% of the HIV and cancer cases in this study and Burkitt lymphoma constitutes over 20%, and that both these cancers are registering a male preponderance of 2.3:1 and 3-5:1 respectively, (World Bank 2006, p.294, Boerma EG et al 2004), the difference in sex ratios observed might be due to these two diseases.

c) The ethnicity

The HIV-positive children with cancer were exclusively black in this series. The main explanation for this may be the high prevalence of HIV in black women of reproductive age: 30.2%, versus between 3 and 7.1% in women belonging to the other ethnic groups in South Africa (SA DOH survey, 2011). As the main modality of acquiring HIV in children is from vertical transmission from their mothers, a predominance of black children with HIV will result.

6.2.2 Outcome of treatment

a) Survival rates

The overall survival rate in the study of outcome after treatment was 52.6% (HIV positive and negative children together). While this figure is much lower than the 79 to 82% survival reported from countries like USA, UK or Canada, it is close to published figures from China (55.7%) and compares favourably with the 40% survival rate published in a study from India (Bao PP 2012, Basta NO 2011).

The comparison of crude survival figures found a slightly lower probability of survival for the HIV infected children than for HIV negative, but the difference is not statistically significant. The interpretation of this finding is complicated by the considerable discrepancy in the types and distribution of various cancers in the two groups. Notwithstanding that, it is important to note that there were comparatively fewer children alive and disease-free in the HIV positive group, at the end of follow-up: 32.1% versus 47.7% in HIV negative children (χ^2=6.544, 1 degree of freedom, two-tailed p=0.01). The percentage of children dead due to the toxicity of the treatment is almost 60 times greater in the series with HIV than in the one without HIV: 11.9% versus 0.2% respectively (Fisher's exact test, two-tailed p<0.0001). Both these differences are statistically significant, but the numbers of toxicity deaths are small. As far as it could be determined, there are no similar findings published in the literature.

In spite of the small numbers, the higher rate of deaths due to toxicity of cancer chemotherapy in HIV patients deserves attention. In these children, chemotherapy induces a much longer lasting leucopoenia than in HIV negative subjects and thus exposes them to

potentially fatal infections. HAART was seen to promote faster remission from leucopoenia, thus allowing for a full dose chemotherapy regimen in these patients. The extent to which HAART restores the immune response to a level comparable with HIV negative children, during cancer chemotherapy is insufficiently studied. In adults, there are several small studies indicating comparable results of chemotherapy in HIV negative and HIV positive patients on HAART (Suzuki K et al 2010, Blinder VS et al 2008).

Further, the potential interactions between cancer chemotherapy drugs and HAART are not fully understood: both drug categories are metabolized by the same liver enzymatic systems (mainly cytochrome P450) and their competing actions on the liver enzymes may result in either stronger or weaker systemic effects of some of the drugs (Mounier N et al 2009). All children with cancer and HIV in our series received HAART either before or at the onset of the cancer therapy. The discrepancy in rates of deaths due to toxicity between the two subgroups indicates that, even in the context of present medical progress, HIV infected children with cancer remain at very high risk of complications and require close monitoring with immediate intervention, should such complications be detected. There is an imperious need to broaden our understanding of this kind of drug interactions, as indicated above, if we are to improve the outcome of children with cancer and HIV.

The analysis of the Kaplan-Meyer graphs of survival indicates a comparatively higher probability of death in the HIV positive children, in the first months after presentation, before the curve joins, even crosses, the HIV negative survival graph; these differences lack statistical significance. The survival in both groups stabilizes by about 50 months. However, when considering the distinct pattern observed in the first year, separately from the rest of the curve, the difference is just close to attaining statistical significance (Table XV and Figure 8). This early surplus of deaths in HIV positive children was also signalled by Tukei VJ et al (2011). Galicier L. et al (2007) provide a possible explanation of this observation: in a mortality analysis of Burkitt lymphoma and AIDS, the deaths occurring early after the onset of therapy were due mainly to failure of treatment or to the toxic effects of the medication.

b) Survival analysis in single malignancies

It was explained in Chapter 5 why only three malignant diseases were suitable for comparative survival analysis: leukaemia, lymphoma (non-Burkitt) and Burkitt lymphoma.

The analysis was done by means of Kaplan-Meyer curves; while a lower survival for HIV positive children was noted in all 3 diseases, none of the differences observed are statistically significant.

As far as it could be ascertained, there are no published data on survival of children with HIV and leukaemia, while the data on non-Burkitt lymphoma are obtained on series of less than 10 cases (Gandemer V et al 2000). This study brings thus an original contribution, with compared survival data on larger series of children with these malignancies.

With regard to Burkitt lymphoma, the analysis of larger series has shown indeed significant differences in survival. Orem J. et al (2009) analyzed the outcome of treatment in Burkitt lymphoma on 158 HIV negative children compared with 70 HIV positive subjects. Their study indicated significantly lower survival odds for the HIV positive group: p=0.023, OR=2.22, 95% CI: 1.11- 4.42. Another notable finding was that there were more children alive with disease at the end of observation in the HIV positive group (OR 4.84, 95% CI: 0.99-30.6, p=0.03).

6.3 Original contribution to the knowledge in the domain of childhood cancer and HIV

a) The findings reinforce the known association between HIV infection and Kaposi sarcoma as well as Burkitt lymphoma. The previous two studies which addressed the issue had found different results: Newton et al (2001) found a positive association between HIV and Burkitt lymphoma, but Mutalima et al (2010) could not confirm it.

b) The study results cannot support an association between HIV infection and leukaemia. The hypothesis that leukaemia has an infectious origin in humans would have been strengthened by the finding that the weak immune response induced by HIV increases the risk of that haematological malignancy; further research needs to be done on the issue.

c) Leiomyosarcoma, found significantly more often in children with cancer in studies from Europe and USA, was not observed in association with HIV in the two studies described here.

d) The second study presented gives a rate of mortality for black children with cancer admitted to hospital in South Africa, based on a relatively large cohort of 882 cases. The rate

observed is comparable with similar figures from China and India, but about 30% lower than that recorded in resource-rich countries.

e) The second study found, for the first time in literature, a significantly lower rate of survival free of disease for black children with cancer and HIV, compared with other black children with cancer but not infected with HIV, while treated with the same protocols.

f) Also for the first time in literature, a significantly higher risk of death from toxicity of therapy was described in black children with cancer and HIV, by comparison with black children with cancer but not infected with HIV.

6.4 Considerations on study design and methods

6.4.1 Referral bias

It was not intended in the studies presented, to obtain data applicable to the whole population of children with cancer. The findings of this research are based only on data from children with cancer and HIV admitted to several hospitals in South Africa. They are not representative for all children with cancer and HIV in South Africa, for reasons which will be detailed here. The following considerations apply to both studies described. To start with, as shown at the beginning of this study, the annual incidence of cancers in children, according to the South African Children's Cancer Registry, was between 33.4 to 47.2 per million, whilst in Europe in the last decade of the last century it was around 140/million and increasing slowly. Children cancer rates were shown to be relatively constant all over the world, mainly because they are less determined by environmental and lifestyle factors (which are geographically different) but by genetic factors. It follows that the cancer in children in South Africa is likely to be significantly under-reported. In this country, children with cancer are referred mostly to paediatric oncology units at tertiary hospitals and these units report their cases correctly. It is possible thus that the majority of children with cancer do not reach these oncology units. Probably the main reason for this lack of referral is either incorrect diagnosis or delayed diagnosis, with a number of children dying before they reach the referral centres.

Other reasons for referral bias may be related to the fear of losing status in the social group once the diagnosis of AIDS (associated with a cancer) is revealed. Conversely, children known to the healthcare network as living with HIV may be given regular check-ups and thus

they may have a higher chance of being diagnosed with cancer and be admitted to hospital before dying

Existing research (Newton et al 2001) found a similar likelihood of seeking medical attention for children with various cancers, so the probability of a referral bias by admitting to hospital certain cancers to the detriment of others is small. However, it is possible that children with the more advanced stages of malignancy are so severely ill that they do not live always to be admitted (a variant of the "healthy worker" effect). Finally, families with medical insurance (representing 15% of the population) would prefer to admit their children to private hospitals, which did not contribute information to the studies presented here. This may also introduce an ethnic bias in referrals, as the medical insurance members are predominantly white.

Another reason for not extrapolating the findings to the whole paediatric population is that the data in the second study were obtained exclusively on black children. It was shown earlier in this study that there are differences in outcome of treatment between various ethnic groups, so the results may not entirely apply to coloured, Indian or white children.

6.4.2 Other sources of bias, random errors and confounding factors

Both studies reported in this dissertation are based on data abstracted from medical records. In the first study, data were obtained directly from the records, while in the second study databases were first created by abstracting data from the records in a contemporary manner, during the patient's hospitalization or follow-up, and the study data were extracted retrospectively from these databases. Records used in the studies were created primarily for patient care and they are often not sufficiently accurate for research. For instance, it is possible that the clinical notes were more accurate in cases of patients with severe diseases. Aside from the above, data were incomplete for many patients, e.g. not mentioning the stage of the cancer on admission.

A random error may have originated in the rates of detection by the medical service of various childhood cancers, of cancers in HIV positive versus HIV negative children, and the specific rate of detection in each centre's territory. These are unknown and thus impossible to adjust for. With regard only to the second study, due to its retrospective nature, it was not possible to standardize the follow-up procedures. This may have introduced further random errors, as in absence of a planned follow-up procedure, and due to the succession, over years,

of several doctors who evaluated the patients, unknown variations might have occurred in the evaluation of the outcomes.

Tuberculosis, often associated with HIV infection but sometimes difficult to diagnose, could have been a confounding factor for death from infectious complications due to leucopoenia induced by chemotherapy, which in this study is included under "death from toxicity".

6.4.3 Adequacy of sample size; power of the study

The sample size was dictated by the availability of hospital databases and therefore it may have been inadequate to detect all differences between the HIV positive and negative groups.

For example, in the study of outcomes described in 5.2, a difference of 7 months was found in the duration of follow-up of children with Burkitt lymphoma and HIV when compared with children with the same malignancy but without HIV. However, this difference was not statistically significant. Power calculation for a $p = 0.05$, with the standard deviation found for the Burkitt subgroup indicate that 105 subjects would have been required in each arm to detect such an effect with a precision of 95%. These subjects were not available in this study and consequently the observed difference cannot be supported under the study circumstances.

There is controversy regarding the usefulness of post-study power calculations with the purpose of finding the meaning of a non-significant result. Hoenig and Heisey (2001), discussing this issue, point out that it is more useful to compute the power of the study to detect the minimum clinically useful difference, rather than the power to detect the observed difference, called "observed power"(in this research, however, the aim is not to detect the minimum clinically useful difference). The authors demonstrate further that the observed power is directly related to the P value. They conclude that the observed power does not contribute new information.

6.4.4 Cancer subjects as controls

The use of subjects with cancer as controls in studies of cancer was analyzed by Linet and Brookmeyer (1987) and later reviewed by Lasky and Stolley (1994), with similar conclusions. According to Linet and Brookmeyer, the advantages of using cancer controls in

studies of cancer cases consist of minimizing recall bias and interviewer bias, the capacity to identify specificity of exposure and practicality. While minimizing the bias due to recall or to the interviewer did not play any role in the choice of controls in this study, the other two characteristics were essential. Linet and Brookmeyer (1987) found that cancer controls may be suited for studies where "the question being addressed is whether a specific factor is uniquely associated with one or more specific cell types of a particular cancer, one or more specific anatomic locations of a particular primary cancer, or a particular cancer versus 'cancer' in general". In the present study, the "specific factor" is HIV infection, which may be associated with a "particular cancer". Practicality was the other determinant of the choice of controls: the data on childhood cancers was readily available, while finding controls with other diseases was logistically difficult, as the clinical information on such cases is not stored in databases. Moreover, children with other diseases admitted to hospital may have a higher ratio of HIV infection than children outside of hospital (some of their diseases, such as tuberculosis, may be in fact facilitated by HIV) and thus would introduce a bias in the study. Using subjects with cancer on both arms of the study has the additional advantage that the same set of data would be found in the records for all subjects.

The main disadvantage of using cancer controls is that the odds ratios for a particular exposure reflect the association of that exposure with a specific cancer by comparison with other cancers and not versus the odds of remaining healthy In this study, where the focus is on the association between HIV and certain childhood cancers, but not others, the meaning of the odds ratio is the desired one. Other disadvantages of using cancer controls presented by Linet and Brookmeyer (1987) may result from the unequal distribution of other, known or unknown, carcinogenetic factors in the two groups, thus confounding the effect studied. Also, the possibility that controls and cases might not originate from the same catchment area may be the consequence of more complex cases (or specific types of cancer) being referred from the whole country while less difficult ones only from the hospital catchment area. While the biases introduced by different catchment areas do not apply to this study, the statistic effect of unknown carcinogens was minimized by using a diversity of cancers as controls.

Other examples of cancer research using cancer patients as controls are the studies done by Smith et al (1988), Newton R et al (1996) and Sitas F et al (1999).

6.4.5 Strengths and limitations of the presented studies

The first study has the advantage of a complete ascertainment both of cancer diagnosis and of HIV serostatus within the participating centers. This is in contrast with other African studies where this completeness is not attained. Also, as far as it could be established, the series of children with cancer, with and without HIV, are among the largest ever analyzed.

The second study contributes data to areas less researched such as the survival profile of children with cancer and HIV compared with that of children with malignancies but not infected with HIV. The study brings an original contribution by providing compared survival data on the largest series, to date, of children with leukaemia as well as non-Burkitt lymphoma. Additionally, until now, no other figures were published to quantify the lower disease-free survival, as well as the higher death rate due to treatment toxicity, in HIV positive children with cancer.

Nevertheless, a number of limitations encountered here may have left their mark on the results. To start with, the data on outcome were obtained retrospectively and this made it impossible to consider the effect of potential confounders such as the stage of cancer and the incidence of tuberculosis, in both groups. However, other studies done in South Africa indicate that most of the children enter the health care system in advanced stages of disease (Stefan DC et al 2011). Another factor which could not be accounted for, due to missing information in the records, was the influence of the HIV stage on the prognosis of infected children.

It is impossible to ascertain in what way the real outcome of children lost to follow-up, should it have been known, would have modified the results. While most of losses to follow-up could be realistically ascribed to death, these numbers cannot be added to the deceased numbers.

Although South Africa is inhabited by a number of ethnic groups, this research was done exclusively on black children, limiting thus the extrapolation of findings to the whole of the population. A study to address possible differences between children with cancer and HIV versus cancer without HIV across different ethnic groups has not yet been done in South Africa.

Finally, the small size of subgroups with individual cancers restricted the analysis of outcome to only three malignancies and impacted on the power of the study. Other barriers to

extrapolation were discussed in 6.4.1.; other limitations of the studies were analyzed in 6.4.2 and 6.4.3.

6.4.6 Alternative approaches to answering the study questions; suggestions for further research

a) The study of association of HIV with particular cancers

The design used, while having the advantage of being better suited to the analysis of relatively rare diseases such as cancer in children with HIV, had insufficient power, with only 38 cases in the HIV and cancer arm. As a result,the non-Hodgkin, non-Burkitt lymphoma could not be significantly connected to HIV infection, although elsewhere in the literature there is enough evidence for the association. However, as this is an ongoing study, more cases will accumulate in time.

An alternative approach to the first study would have been a prospective study of two large cohorts of children, one without HIV and the other HIV-positive, followed up from birth, over 1 ½ decades. Such a design would enable a more precise observation of the incidence of various cancers in children with and without HIV infection. The children with cancer, from both cohorts, could be entered in prospective studies where the investigations done, the treatments instituted and the follow-up protocols could be standardized, to enable a better comparison of the outcomes. The results obtained in this manner could have been generalized to the whole paediatric population. However, cohorts are not particularly suited to the study of rare diseases, and cancer in children is one of the latter. The main disadvantages of such a design are the required large number of participants, with the associated cost, and the long time to completion of study.

Another design which could have been considered was the linkage between the Children's Cancer Registry and the HIV Registry, retrospectively over a number of years, in order to analyze the frequency of observation of various malignancies in children with cancer and HIV versus children with cancer without exposure to HIV. This design was used with success elsewhere (Cooksley CD et al 1999, Engels EA et al 2008); however, the Children's Cancer Registry in South Africa is known to be incomplete, due to underreporting, and it is quite possible that the HIV registry is in a similar situation.

b) The study of association of HIV infection with various outcomes after treatment.

A major shortcoming of the second study was the relatively small number of children with cancer and HIV, which resulted in insufficient power in the analysis of the outcomes of children with the same malignancy. The problem could have been addressed by gaining access to databases of more paediatric oncology centres. Another major shortcoming was the absence of certain data such as cancer stage or HIV infection stage, and the consequent impossibility to estimate their impact on the outcome.

The outcome after treatment in children with cancer is influenced by the following independent variables:

- Age

- Sex

- Ethnicity

- Nutrition status

- Associated diseases (e.g. tuberculosis)

- Type of cancer

- Stage of cancer

- Therapy protocol for cancer

- Supportive therapy (blood transfusion, granulocyte colony growth factor, antibiotics)

- Follow-up standards

Further, when assessing the impact of the HIV infection – the exposure - on the outcome of cancers after treatment, other variables to consider are the stage of retroviral infection and whether it is being treated or not.

The ideal design of such a study would be a case – control prospective study. Cases could be identified from a population-based cancer registry, by linkage with the HIV registry. Depending on the availability of cases, the study may be restricted to one or maybe only a few cancers, represented in sufficient numbers in both HIV infected and not infected children with cancer. The number of participants in the study would be determined by power calculations. The selection of cases should minimize the role of confounders, by example excluding children with tuberculosis or running a separate analysis for that group. Children

receiving only palliative therapy or no therapy should be included. By recruiting more than one control (up to four) for each case, the effect of random error in the control group would be minimized.

In order to reduce the number of independent variables, identical therapy protocols, and standardized supportive therapy, should be applied in all participating centres. Further, cases and controls could be matched for cancer stage, age, sex and ethnicity. Otherwise, the effect of these variables on mortality may be evaluated by logistic regression. The results would be expressed in odds ratios for the association of HIV and death or other outcomes considered. The outcomes could include the categories used in this study (alive without disease, alive with disease, dead from disease, dead from toxicity, dead from unknown cause, lost to follow-up presumed dead, lost to follow-up in remission). Kaplan-Meyer curves could be obtained after a number of years of follow-up and compared.

The disadvantage of such a study, assuming that the required registers used for recruiting would exist, is the need to follow the participants for a sufficiently long time to assess survival. Additionally, the simple inclusion in the study may improve the outcome in children with cancer and HIV, who may receive more attention and better care.

An intriguing finding is the absence of the leiomyosarcoma from the study cohorts, whilst in the literature it is quoted as being one of the neoplasms encountered much more frequently in children with HIV. The finding could be due to a much smaller incidence of leiomyosarcoma in black African children and this is an issue awaiting further investigation. Another area for investigation could befinding novel ways of mitigating the toxic effects of cancer therapy on children with HIV. The second study presented here found a significantly higher proportion of deaths from toxicity, including infection on a background of leucopoenia, in children with cancer and HIV

CHAPTER 7

CONCLUSIONS

a) HIV infection increased the odds for Kaposi sarcoma and Burkitt lymphoma but not the odds for leukaemia.

The first two associations were well known from literature; however, in Africa the increase in Burkitt lymphoma in children was not observed by previous studies. The lack of increase in the odds for leukaemia in children with HIV does not support the hypothesis that it may have an infectious origin.

b) The overall survival figure in black children, of 52.6% is lower than the 79 – 82% survival rates in resource-rich countries

This figure is close to data found in studies from China and better than survival figures in studies from India.

c) Overall survival rates are marginally lower in HIV-positive black children, without attaining statistical significance

Although marginally lower in HIV positive children than in HIV negative – 47,6% versus 53.3% respectively - the difference in survival rates is not statistically significant. The analysis of the Kaplan-Meyer survival curves indicates that in the first year of follow-up there is a surplus of deaths in HIV positive black children compared with HIV-negative, which was borderline non-significant. The literature attributes this finding to the failure of therapy in some children or to toxic complications (including infections due to leukopaenia).

d) Disease-free survival rates in black children with cancer are lower in those infected with HIV, while deaths from toxicity are around 60 times higher.

The rates of disease-free survival are significantly lower in black children with cancer and HIV (32.1% versus 47.7%). Additionally, deaths from treatment toxicity are significantly higher (almost 60 times) in the HIV positive group, although the numbers in both groups are relatively low.

e) Kaplan-Meyer survival curves of black children with HIV were not found to differ significantly from those of black children without HIV, for the same type of cancer

Kaplan-Meyer survival curves were calculated for leukaemia, lymphoma and Burkitt lymphoma. When compared by means of the log-rank test, the differences between the curves for HIV positive and negative children were not statistically significant.

f) Overarching conclusions

The first study reinforces the finding that children with cancer and HIV have an excess of Kaposi sarcoma and Burkitt lymphoma, when compared with other children with cancer but not infected with HIV. The study did not support other correlations between particular cancers in children and HIV infection, which, if found, might have indicated a presumptive infectious aetiology of the respective cancers. Differences observed when comparing the survival of HIV positive black children with cancer, with that of HIV negative black children with same malignancies, treated with the same protocols of cytostatics and HAART, were not statistically significant. Notwithstanding that, HIV positive black children with cancer are more susceptible to treatment toxicity and infections and require a sustained effort to prevent these complications during treatment. Less black children with HIV will survive disease free than their HIV negative counterparts with same cancers.

REFERENCES

1. Adler DH. The impact of HAART on HPV-related cervical disease. *Curr HIV Res*. 2010, 8(7):493-7.

2. Amir H, Kaaya EE, Manji KP, Kwesigabo G, Biberfeld P. Kaposi's sarcoma before and during a human immunodeficiency virus epidemic in Tanzanian children. *Pediatr Infect Dis J.* 2001, 20(5):518-21.

3. Angeletti PC, Zhang L, Wood C. The viral etiology of AIDS-associated malignancies. *Adv Pharmacol*. 2008, 56:509-57

4. Bajaj BG, Murakami M, Robertson ES Molecular biology of EBV in relationship to AIDS-associated oncogenesis. *Cancer Treat Res*. 2007, 133:141-62.

5. Bao PP, Zheng Y, Wu CX, Peng P, Gong YM, Huang ZZ, Fan W. Population-based survival for childhood cancer patients diagnosed during 2002-2005 in Shanghai, China. *Pediatr Blood Cancer*. 2012 Feb 2. Epub ahead of print]

6. Basta NO, James PW, Gomez-Pozo B, Craft AW, McNally RJ. Survival from childhood cancer in northern England, 1968-2005.*Br J Cancer*. 2011;105(9):1402-8

7. Beral V, Newton R. Overview of the epidemiology of immunodeficiency associated cancers. *Monogr Natl Cancer Inst* 1998 23: 1–6.

8. Biggar RJ, Frisch M, Goedert JJ, et al. Risk of cancer in children with AIDS. *JAMA* 2000;284:205–209.

9. Biggar RJ: Epidemiology of malignancies in HIV/AIDS. In: Feigal EG, Levine AM, Biggar RJ. *AIDS-related cancers and their treatment.* 2000, CRC Press, New York, NY

10. Blinder VS, Chadburn A, Furman RR, Mathew S, Leonard JP. Improving outcomes for patients with Burkitt lymphoma and HIV. *AIDS Patient Care STDS*. 2008, 22(3):175-87.

11. Boerma EG, van Imhoff GW, Appel IM, Veeger NJ, Kluin PM, Kluin-Nelemans JC. Gender and age-related differences in Burkitt lymphoma--epidemiological and clinical data from The Netherlands. *Eur J Cancer*. 2004,40(18):2781-7.

12. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens—Part B: Biological agents. *Lancet Oncol* 2009 10:321–322**.**

13. Cardwell CR, McKinney PA, Patterson CC, et al. Infections in early life and childhood leukaemia risk: A UK case-control study of general practitioner records. *Br J Cancer*

2008;99:1529–1533.

14. Cattelan AM, Calabrò ML, De Rossi A, Aversa SM, Barbierato M, Trevenzoli M, Gasperini P, Zanchetta M, Cadrobbi P, Monfardini S, Chieco-Bianchi L. Long-term clinical outcome of AIDS-related Kaposi's sarcoma during highly active antiretroviral therapy. *Int J Oncol*. 2005 27(3):779-85.

15. Centers for disease control and prevention: Human Immunodeficiency Virus Type 2. www.cdc.gov/hiv/resources/factsheets/ hiv2.htm (accessed on 02.03.2009).

16. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science*. 1994, 266(5192):1865-9.

17. Chanock SJ, Pizzo PA. Infection prevention strategies for children with cancer and AIDS: contrasting dilemmas. *J Hosp Infect*. 1995, 30 Suppl:197-208.

18. Chintu C, Athale UH, Patil PS. Childhood cancers in Zambia before and after the HIV epidemic. *Arch Dis Child*. 1995, 73(2):100-4;

19. Cook MB, Dawsey SM, Freedman ND, Inskip PD, Wichner SM, Quraishi SM, Devesa SS, McGlynn KA. Sex disparities in cancer incidence by period and age. *Cancer Epidemiol Biomarkers Prev*. 2009, 18(4):1174-82.

20. Cook-Mozaffari P, Newton R, Beral V, Burkitt DP. The geographical distribution of Kaposi's sarcoma and of lymphomas in Africa before the AIDS epidemic. *Br J Cancer*. 1998, 78(11):1521-8.

21. Cooksley CD, Hwang LY, Waller DK, Ford CE. HIV-related malignancies: community-based study using linkage of cancer registry and HIV registry data. *Int J STD AIDS*. 1999, 10(12):795-802.

22. Cornejo-Juárez P, Volkow-Fernández P, Avilés-Salas A, Calderón-Flores E. AIDS and non-Hodgkin's lymphoma. Experience at an oncological center in Mexico. *Rev Invest Clin*. 2008, 60(5):375-81.

23. Department of Health, South Africa. The national antenatal sentinel HIV and syphilis prevalence survey 2010. 2011, Pretoria, Department of Health.

24. Donati D, Espmark E, Kironde F, Mbidde EK, Kamya M, Lundkvist A, Wahlgren M, Bejarano MT, Falk KI. Clearance of circulating Epstein-Barr virus DNA in children with acute malaria after antimalaria treatment *J Infect Dis*. 2006, 193(7):971-7.

25. Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, Finch JL, Grigg R, Hylton T, Pawlish KS, McNeel TS, Goedert JJ. Cancer risk in people infected with human

immunodeficiency virus in the United States Int J Cancer 2008 123(1):187-94

26. Epstein MA, Henle G, Achong BG, Barr YM. A second virus-carrying tissue culture strain (eb2) of lymphoblasts from Burkitt lymphoma. *Pathol Biol (Paris)*. 1964, 12:1233-4.

27. Feller L, Essop R, Wood NH, Khammissa RA, Chikte UM, Meyerov R, Lemmer J. Chemotherapy- and radiotherapy-induced oral mucositis: pathobiology, epidemiology and management. *SADJ*. 2010, 65(8):372-4.

28. Flint SJ, Enquist LW, Racaniello VR, Skalka AM. *Principles of virology. Infection of a susceptible host.* p. 191. 2009 ASM Press, Washington DC.

29. Galicier L, Fieschi C, Borie R, Meignin V, Daniel MT, Gérard L, Oksenhendler E. Intensive chemotherapy regimen (LMB86) for St Jude stage IV AIDS-related Burkitt lymphoma/leukemia: a prospective study. *Blood*. 2007, 110(8):2846-54.

30. Gandemer V, Verkarre V, Quartier P, Brousse N, Blanche S. Lymphomas in children infected with HIV-1. *Arch Pediatr*. 2000, 7(7):738-44.

31. Gantt S, Kakuru A, Wald A, Walusansa V, Corey L, Casper C, Orem J. Clinical presentation and outcome of epidemic Kaposi sarcoma in Ugandan children. *Pediatr Blood Cancer*. 2010, 54(5):670-4.

32. Geser A, Brubaker G, Draper CC. Effect of a malaria suppression program on the incidence of African Burkitt lymphoma. *Am J Epidemiol*. 1989, 129(4):740-52.

33. Greaves MF. Aetiology of acute leukaemia. *Lancet* 1997;349:344–349.

34. Grimes DA, Schultz KF. Compared to what? Finding controls for case-control studies. *Lancet* 2005, 365:1429-1433.

35. Grulich AE, Leeuwen MT, Falster MO, et al. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: A meta-analysis. Lancet 2007;370:59–67.

36. Grulich AE, Li Y, McDonald A, Correll PK, Law MG, Kaldor JM. Rates of non-AIDS-defining cancers in people with HIV infection before and after AIDS diagnosis. *AIDS*. 2002, 16(8):1155-61.

37. Hadley GP, Naude F. Malignant solid tumour, HIV infection and tuberculosis in children: an unholy triad. *Pediatr Surg Int*. 2009, 25(8):697-701.

38. Hoenig JM, Heisey DM, The abuse of power, *The American Statistician*. 2001, 55(1): 19-24.

39. Housri N, Yarchoan R, Kaushal A. Radiotherapy for patients with the human immunodeficiency virus: are special precautions necessary? *Cancer*. 2010, 116(2):273-83.

40. Israëls T, Chirambo C, Caron HN, Molyneux EM. Nutritional status at admission of children with cancer in Malawi.*Pediatr Blood Cancer*. 2008, 51(5):626-8.

41. Israëls T, van de Wetering MD, Hesseling P, van Geloven N, Caron HN, Molyneux EM. Malnutrition and neutropenia in children treated for Burkitt lymphoma in Malawi. *Pediatr Blood Cancer*. 2009, 53(1):47-52.

42. Kenkre VP, Stock W. Burkitt lymphoma/leukemia: improving prognosis. *Clin Lymphoma Myeloma*. 2009, 9 Suppl 3:S231-8.

43. Kinlen LJ. Childhood leukaemia and non-Hodgkin's lymphoma in young people living close to nuclear reprocessing sites. *Biomed Pharmacother* 1993 47:429–434.

44. Kinlen LJ. Epidemiological evidence for an infective basis in childhood leukaemia. Br J Cancer 1995;71:1–5.

45. Krishnan A, Forman SJ.Hematopoietic stem cell transplantation for AIDS-related malignancies. *Curr Opin Oncol*. 2010, 22(5):456-60.

46. Kutok JL, Wang F. Spectrum of Epstein-Barr virus-associated diseases. *Annu Rev Pathol*. 2006, 1:375-404.

47. Lasky T Stolley PD Selection of controls. *Epidemiol Rev* 1994, 16(1):6-17

48. Leidner RS, Aboulafia DM. Recrudescent Kaposi's sarcoma after initiation of HAART: a manifestation of immune reconstitution syndrome. *AIDS Patient Care STDS*. 2005, 19(10):635-44.

49. LinetMS, Brookmeyer R. Use of cancer controls in case-control cancer studies. *Amer J Epidemiol* 1987, 125(1):1-11

50. Magrath I. Lessons from clinical trials in African Burkitt lymphoma. *Curr Opin Oncol*. 2009, 21(5):462-8.

51. Marcos-Gragera R, Cervantes-Amat M, Vicente ML, de Sanjosé S, Guallar E, Godoy C, Calvo C, Giraldo P, Sant M, Peris-Bonet R, Carmen Martos M. Population-based incidence of childhood leukaemias and lymphomas in Spain (1993-2002). *Eur J Cancer Prev*. 2010, 19(4):247-55.

52. Mbelle N, Van Zyl J, Parker W, Zungu NP, Pezi S & the SABSSM III Implementation Team 2009. *South African national HIV prevalence, incidence, behaviour and communication survey 2008:A turning tide among teenagers?* Cape Town: HSRC Press

53. McClain KL, Joshi VV, Murphy SB. Cancers in children with HIV infection. *Hematol*

Oncol Clin North Am. 1996, 10(5):1189-201.

54. Middelkoop K, Bekker LG, Myer L, Dawson R, Wood R. Rates of tuberculosis transmission to children and adolescents in a community with a high prevalence of HIV infection among adults. *Clin Infect Dis.* 2008, 47(3):349-55.

55. Mounier N, Katlama C, Costagliola D, Chichmanian RM, Spano JP Drug interactions between antineoplastic and antiretroviral therapies: Implications and management for clinical practice. *Crit Rev Oncol Hematol* 2009, 72(1):10-20

56. Mueller BU, Pizzo PA. Malignancies in pediatric AIDS. *Curr Opin Pediatr.* 1996 8(1):45-9.

57. Mueller BU. Cancers in human immunodeficiency virus-infected children. *J Natl Cancer Inst Monogr.* 1998, (23):31-5.

58. Mutalima N, Molyneux EM, Johnston WT, Jaffe HW, Kamiza S, Borgstein E, Mkandawire N, Liomba GN, Batumba M, Carpenter LM, Newton R. Impact of infection with human immunodeficiency virus-1 (HIV) on the risk of cancer among children in Malawi - preliminary findings. *Infect Agent Cancer.* 2010, 5:5.

59. Mwanda OW, Fu P, Collea R, Whalen C, Remick SC. Kaposi's sarcoma in patients with and without human immunodeficiency virus infection, in a tertiary referral centre in Kenya. *Ann Trop Med Parasitol.* 2005, 99(1):81-91.

60. Newton R, Ziegler J, Beral V, Mbidde E, Carpenter L, Wabinga H, Mbulaiteye S, Appleby P, Reeves G, Jaffe H; Uganda Kaposi's Sarcoma Study Group.A case-control study of human immunodeficiency virus infection and cancer in adults and children residing in Kampala, Uganda. *Int J Cancer.* 2001, 92(5):622-7.

61. Newton R, Ngilimana PJ, Grulich A, Beral V, Sindikubwabo B, Nganyira A, Parkin DM. Cancer in Rwanda. *Int J Cancer.* 1996 66(1):75-81.

62. Niehues T, Horneff G, Megahed M, Schroten H, Wahn V. Complete regression of AIDS-related Kaposi's sarcoma in a child treated with highly active antiretroviral therapy. *AIDS.* 1999, 13(9):1148-9

63. Orem J, Maganda A, Mbidde EK, Weiderpass E. Clinical characteristics and outcome of children with Burkitt lymphoma in Uganda according to HIV infection. *Pediatr Blood Cancer.* 2009, 52(4):455-8.

64. Re A, Michieli M, Casari S, Allione B, Cattaneo C, Rupolo M, Spina M, Manuele R, Vaccher E, Mazzucato M, Abbruzzese L, Ferremi P, Carosi G, Tirelli U, Rossi G.High-

dose therapy and autologous peripheral blood stem cell transplantation as salvage treatment for AIDS-related lymphoma: long-term results of the Italian Cooperative Group on AIDS and Tumours (GICAT) study with analysis of prognostic factors. *Blood*. 2009, 114(7):1306-13.

65. Roman E, Simpson J, Ansell P, et al. Childhood acute lymphoblastic leukaemia and infections in the first year of life: A report from the United Kingdom Childhood Cancer Study Investigators. *Am J Epidemiol* 2007;165:496–504.

66. Sasco AJ, Jaquet A, Boidin E, Ekouevi DK, Thouillot F, Lemabec T, Forstin MA, Renaudier P, N'dom P, Malvy D, Dabis F. The challenge of AIDS-related malignancies in sub-Saharan Africa. *PLoS One*. 2010, 5(1):e8621.

67. Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Pillay-van-Wyk V,

68. Sinfield RL, Molyneux EM, Banda K, Borgstein E, Broadhead R, Hesseling P, Newton R, Casabonne D, Mkandawire N, Nkume H, Hodgson T, Liomba G. Spectrum and presentation of pediatric malignancies in the HIV era: experience from Blantyre, Malawi, 1998-2003. *Pediatr Blood Cancer*. 2007, 48(5):515-20.

69. Sitas F, Carrara H, Beral V, Newton R, Reeves G, Bull D, Jentsch U, Pacella-Norman R, Bourboulia D, Whitby D, Boshoff C, Weiss R. Antibodies against human herpesvirus 8 in black South African patients with cancer. *N Engl J Med*. 1999 340(24):1863-71.

70. Smith AH, Pearce NE, Callas PW. Cancer case-control studies with other cancers as controls. *Int J Epidemiol*. 1988,17(2):298-306.

71. Spina M, Carbone A, Gloghini A, Serraino D, Berretta M, Tirelli U. Hodgkin's Disease in Patients with HIV Infection. *Adv Hematol*. 2011, Epub 2010 Sep 23.

72. Stefan DC, Kruis AL, Schaaf HS, Wessels G Tuberculosis in oncology patients. *Ann Trop Paediatr* 2008, 28(2):111-6.

73. Stefan DC, Stones D, Dippenaar A, Kidd M. Ethnicity and characteristics of Hodgkin lymphoma in children. *Pediatr Blood Cancer*. 2009 52(2):182-5.

74. Stefan DC, Stones D. The South African children's cancer registry: 25 years of activity. *SAMJ*, in press.

75. Stefan DC, Siemonsma F. Delay and causes of delay in the diagnosis of childhood cancer in Africa. *Pediatr Blood Cancer*. 2011, 56(1):80-5.

76. Stefan DC, Wessels G, Poole J, Wainwright L, Stones D, Johnston WT, Newton R. Infection with human immunodeficiency virus-1 (HIV) among children with cancer in South Africa. *Pediatr Blood Cancer*. 2011, 56(1):77-9.

77. Stein L, Urban MI, O'Connell D, Yu XQ, Beral V, Newton R, Ruff P, Donde B, Hale M, Patel M, Sitas F. The spectrum of human immunodeficiency virus-associated cancers in a South African black population: results from a case-control study, 1995-2004. *Int J Cancer*. 2008, 122(10):2260-5.

78. Suzuki K, Nakazato T, Sanada Y, Mihara A, Tachikawa N, Kurai H, Yoshimura Y, Hayashi H, Yoshida S, Kakimoto T. Successful treatment with hyper-CVAD and highly active anti-retroviral therapy (HAART) for AIDS-related Burkitt lymphoma. *Rinsho Ketsueki*. 2010 51(3):207-12.

79. Taha TE, Nour S, Kumwenda NI, Broadhead RL, Fiscus SA, Kafulafula G, Nkhoma C, Chen S, Hoover DR. Gender differences in perinatal HIV acquisition among African infants. *Pediatrics*. 2005, 115(2):e167-72

80. Thomas JO. Acquired immunodeficiency syndrome-associated cancers in Sub-Saharan Africa. *Semin Oncol*. 2001, 28(2):198-206.

81. Tukei VJ, Kekitiinwa A, Beasley RP.Prevalence and outcome of HIV-associated malignancies among children.AIDS. 2011 Sep 10;25(14):1789-93.

82. UNAIDS Fact sheet, http://www.unaids.org/en/media/unaids/contentassets/dataimport/pub/factsheet/2009/20091124_fs_ssa_en.pdf (accessed 3.08.2011)

83. Wabinga HR, Parkin DM, Wabwire-Mangen F, Mugerwa JW. Cancer in Kampala, Uganda, in 1989-91: changes in incidence in the era of AIDS. *Int J Cancer*. 1993, 54(1):26-36.

84. WHO/IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 100B. A Review of Human Carcinogens: Biological Agents. Lyon 2011.

85. www.iacr.com.fr Accessed 19 Nov. 2010

86. Ziegler JL, Katongole-Mbidde E. Kaposi's sarcoma in childhood: an analysis of 100 cases from Uganda and relationship to HIV infection. *Int J Cancer*. 1996, 65(2):200-3.

87. Zur Hausen H. Similarities of papillomavirus infections with tumour promoters. *Princess Takamatsu Symp*. 1983,14:147-52.